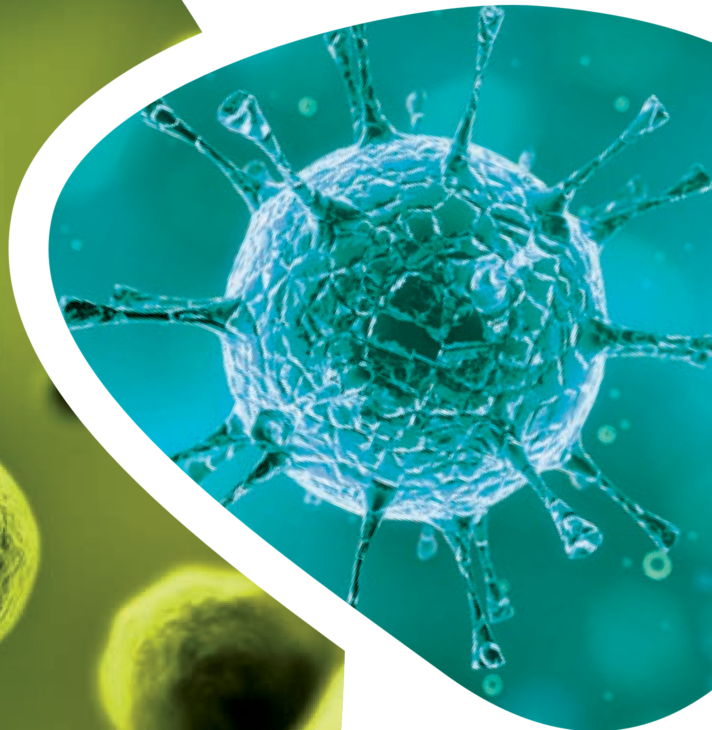
SOCIETY FOR GENERAL
MICROBIOLOGY

ANNUAL CONFERENCE

14-17 April 2014

Arena and Convention Centre, Liverpool

ABSTRACTS



(LI00Mo1210) – SGM Prize Medal Lecture

Climate Change, Oceans, and Infectious Disease

Dr. Rita R. Colwell

University of Maryland, College Park, MD, USA

During the mid-1980s, satellite sensors were developed to monitor land and oceans for purposes of understanding climate, weather, and vegetation distribution and seasonal variations. Subsequently inter-relationships of the environment and infectious diseases were investigated, both qualitatively and quantitatively, with documentation of the seasonality of diseases, notably malaria and cholera by epidemiologists. The new research revealed a very close interaction of the environment and many other infectious diseases. With satellite sensors, these relationships were quantified and comparatively analyzed. More recent studies of epidemic diseases have provided models, both retrospective and prospective, for understanding and predicting disease epidemics, notably vector borne diseases. The water borne disease, cholera can be predicted by monitoring sea surface temperature, salinity, rainfall, and chlorophyll, the latter as a tag for phytoplankton that precede zooplankton in abundance in coastal and river waters, since zooplankton carry cholera bacteria as a component of their natural flora. Recent studies of the cholera epidemic in Haiti provide evidence that an early warning system for infectious diseases can, indeed, be developed, providing a powerful tool for protecting public health and, more importantly, for measuring effects of climate change on human health. Genomic analyses of *Vibrio cholerae* isolated globally have provided additional evidence of multiple sources of cholera epidemics. Furthermore, metagenomic data show more than one pathogen in clinical samples collected during cholera outbreaks.

(LI00Mo1735) – Peter Wildy Prize for Microbiology Education

Science communication: a communicable disease?

Stephen Curry

Professor of Structural Biology, Director of Undergraduate Studies Department of Life Sciences, Imperial College, London UK

"I will share my experiences of science — such as they are and as frankly as I can." These are the words I wrote in my very first blogpost in 2008. They represent my mission statement but also, I now realise, mark the beginning of a kind of infection that has had unexpected sequelae. I had meant only to start writing about what it was like to work as a scientist in 21st century Britain but was surprised to find that my blog frequently took me outside the usual boundaries of a scientific life. Once you look up from the bench you soon find many interesting and important cultural growths that are not confined to petri dishes or baffled flasks. There are issues of careers, of funding, of public perceptions and of the politics inside and outside science, all of which affect its relationship with the rest of society. Many of us encounter and think about these matters on a regular basis; but doing something about them is easier than you might imagine. In my talk I would like to describe the pathology of my particular infection, which I believe to be mostly benign. I hope I might also be able to infect some of the members of the audience.

(LI00Tu1210) – Marjory Stephenson Prize Lecture

Understanding the basis of antibiotic resistance as a platform for early drug discovery

Laura JV Piddock

School of Immunity & Infection and Institute of Microbiology and Infection, University of Birmingham, UK

Antibiotic resistant bacteria are one of the greatest threats to human health. Resistance can be mediated by numerous mechanisms including mutations conferring changes to the genes encoding the target proteins as well as RND efflux pumps, which confer innate multi-drug resistance (MDR) to bacteria. The production of efflux pumps can be increased, usually due to mutations in regulatory genes, and this confers resistance to antibiotics that are often used to treat infections by Gram negative bacteria. RND MDR efflux systems not only confer antibiotic resistance, but altered expression influences the ability of the bacterium to infect its host and to form a biofilm. I will illustrate our studies on the mechanisms of antibiotic resistance - from phenotype to genotype and studies on gene regulation - in isolates of *Salmonella* from animals and a unique set of clinical isolates collected over the course of a complex infection in which the patient received numerous antibiotics, and yet died with a disseminated MDR infection. I will also describe how some of the information we have obtained informs those involved in drug discovery programmes.

(LI00We1210) – Fleming Prize Lecture

Multiple personalities of RNA polymerase active centre

Nikolay Zenkin

Centre for Bacterial Cell Biology,Newcastle University, Newcastle-upon-Tyne, UK

Transcription in all living organisms is accomplished by highly conserved multi-subunit RNA polymerases (RNAP). The view on the functioning of the active centre of RNAP has transformed recently with the finding that a conserved flexible domain near the active centre, the Trigger Loop (TL), directly participates in the catalysis of RNA synthesis and serves as a major determinant for fidelity of transcription. It also appeared that the Trigger Loop is involved in the unique ability of RNAP to exchange catalytic activities of the active centre. In this phenomenon the Trigger Loop is replaced by a transcription factor which changes amino acid content and, as a result, catalytic properties of the active centre. A number transcription factors that act through substitution of the Trigger Loop suggest that RNAP has several different active centres to choose from in response to external or internal signals.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI01

10 questions in virology

LI01Mo0900

Virus ecology: studying animal reservoirs to understand viral emergence

Christian Drosten

Institute of Virology, University of Bonn, Germany

Ten years after the SARS epidemic, zoonotic and emerging viruses have become a growing field of research. Some remarkable novel virus descriptions in animals have demonstrated how ignorant we are of the diversity of viruses around us. In our efforts to delineate viral origins we may have to re-asess our concept of reservoir. In many instances, we are mixing up ecological and epidemiological implications of viral evolution. Among the biggest challenges in this field is the integration of the concepts of virus-host codivergence, and viral host switching. In addition, assessments of viral reservoirs with the intention to predict future pandemic threats would have to take into account important host and virus traits which cannot be predicted merely from virus genes. For example, we need to know whether there are hosts which have a higher propensity to carry broader spectra or higher concentrations of viruses, potentially without being affected. Among the viruses borne in such reservoirs, there may be some that are more promiscuous in their choice of hosts than others, potentially due to the conservedness of their receptor structures or the way they interfere with conserved- or not-so-conserved immune properties. A synopsis of available approaches demonstrates how much work needs to be done before we will be able to assess functional, rather than genetic diversity of reservoir-borne viruses

LI01Mo0930

Offered paper – Harnessing the cellular microRNA machinery to probe norovirus tropism

Lucy Thorne, Ian Goodfellow

University of Cambridge, Cambridge, UK

MicroRNAs are small non-coding RNAs that regulate cellular gene expression in a cell-specific manner and play a role in the lifecycle of many RNA viruses. It has also been demonstrated that the cellular microRNA machinery can be harnessed to control viral replication and tropism by inserting cell-specific microRNA target sequences into a viral genome. Murine norovirus (MNV), the only norovirus which can replicate in cell culture, now provides a model to investigate the role of microRNAs in the norovirus lifecycle and the potential of using the microRNA machinery to probe norovirus tropism in vivo, which has not yet been fully defined. Here a panel of microRNAs was identified whose expression changed with MNV infection in two permissive cell lines, including a number known to contribute to antiviral immunity. To determine whether the microRNA machinery could be used to probe MNV tropism, a microglial-specific microRNA target sequence was inserted into the MNV genome, which restricted MNV replication only in cells expressing the cognate microRNA. Target sites for miR-142, highly expressed in cells of the hematopoietic lineage, were also introduced into the viral genome to allow the importance of MNV replication in macrophage and dendritic cells to be probed *in vivo.*

LI01Mo0945

Offered paper – Recombination in Enteroviruses is a Biphasic Process Involving the Generation of Greater-than Genome Length 'Imprecise' Intermediates

Andrew Woodman[1], Kym Lowry[2], David Evans[1]

[1]School of Life Sciences, The University of Warwick, Coventry, UK, [2]Biosecurity Sciences Laboratory, Queensland, Australia

Members of the Picornaviridae have error prone polymerases, short replication cycles, and high yields that together contribute to genetic diversity. However, far greater genetic variation is achieved by chance recombination events than is possible in a single round of genome replication. Genetic recombination is believed to be due to a 'copy-choice' mechanism and, at present, it is suggested that the RNA dependent RNA polymerase pauses or terminates at certain RNA sequence motifs, or secondary structures, promoting template switching.

We have developed a system that exploits two partially replication competent parental genomes to specifically select early recombination products from a co-transfected cell population. All viable progeny are recombinants and, strikingly, the majority contain in-frame duplications of up to ~400 nt. at the recombination junction. Mutagenic nucleoside analogues can lead to 'error catastrophe' amongst an enterovirus population. Interestingly, we have evidence to show that sub-lethal levels of the mutagenic compounds lead to a significant enhancement of recombination frequency. In contrast, a high fidelity polymerase mutant significantly reduces recombination frequency. This provides insights into the characteristics of the polymerase that are important for recombination and implies that antiviral therapy may, in addition to selecting for drug resistance, influence viral recombination in co-infected patients.

LI01Mo1000

Can we track viruses in real time, in the real world?

Jonathan Read

University of Liverpool, Liverpool, Merseyside, UK

A critical assumption within infectious disease epidemiology is that patterns of movement and contact between people drive observed patterns of viral infection. The importance of contact tracing in disease outbreak investigation is one example of how central this assumption is in our approach to epidemic management. Yet the evidence linking interactions between individuals and transmission of viruses is extremely limited. To track viruses accurately within a population requires a deeper understanding of where and how often encounters occur between people, improved identification of infected and infectious individuals, and how these aspects link together. This talk will summarise recent work on quantifying encounter patterns and how we can relate the findings to the transmission of respiratory viruses.

LI01Mo1100

In Vivo Veritas

Jonathan Yewdell

National Institutes of Health, Bethesda, MD 20892-0440, USA

Mammalian cells did not evolve to grow on a synthetic surface in synthetic media in air. This is just one of many barriers in trying to understand viral replication, transmission, and pathogenesis in the

real world. I will discuss others, including a marked tendency to oversimplify and focus on minutiae at the expense of more general principles.

LI01Mo1130

Offered paper – A unique secreted adenovirus E3 protein binds to the leukocyte common antigen CD45 and modulates leukocyte functions

Mark Windheim[1,2], Jen Southcombe[1,3], Elisabeth Kremmer[4], Lucy Chaplin[1], Doris Urlaub[5], Christine Falk[6], Maren Claus[5], Janine Mihm[7], Myles Braithwaite[1], Kevin Dennehy[8], Harald Renz[9], Martina Sester[7], Carsten Watzl[5], Hans-Gerhard Burgert[1]

[1]School of Life Sciences, Warwick University, Coventry, UK, [2]Institute of Biochemistry, Hannover Medical School, Hannover, Germany, [3]Nuffield Department of Obstetrics and Gynaecology, John Radcliffe Hospital, University Oxford, Oxford, UK, [4]Institute of Molecular Immunology, German Research Centre for Environmental Health, Munich, Germany, [5]Dept of for Immunology, eLeibniz Research Centre for Working Environment and Human Factors, Dortmund, Germany, [6]Institute of Transplant Immunology, Hannover Medical School, Hannover, Germany, [7]Department of Transplant and Infection Immunology, Saarland University, Homburg, Germany, [8]Institute for Medical Virology, University Hospital, Tübingen, Germany, [9]Institute for Laboratory Medicine and Pathobiochemistry - Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany

The E3 transcription unit of human adenoviruses (Ads) encodes immunomodulatory proteins. Interestingly, the size and composition of E3 differs considerably between Ad species, suggesting that distinct sets of immunomodulatory E3 proteins may influence their interaction with the host and the disease pattern. To date, only common species C E3 immune evasion functions were described. Here we report the first immunomodulatory activity of a species D-specific E3 protein, E3/49K. Unlike all other E3 proteins that act on infected cells, E3/49K seems to target uninfected cells. Initially synthesized as an 80-100 kDa transmembrane protein it is subsequently cleaved with the large ectodomain (sec49K) secreted/shed. Purified sec49K exhibited specific binding to lymphoid cell lines and all primary leukocytes, but not fibroblasts/epithelial cells. Consistent with this binding profile and the molecular mass, the sec49K receptor was identified as the cell surface protein-tyrosine-phosphatase CD45. sec49K binds to all CD45 isoforms. Functional studies showed that sec49K can suppress activation and cytotoxicity of NK cells and activation, signaling and cytokine production of CD4 T cells. Thus, we have discovered the first actively-secreted adenovirus protein and describe for the first time immunomodulatory activities of an E3 protein uniquely expressed by a single Ad species.

Windheim (2013) PNAS 110(50):E4884-93

LI01Mo1145

Offered paper – A novel mechanism inducing genome instability in Kaposi's sarcoma-associated herpesvirus infected cells

Brian Jackson, Marko Noerenberg, Adrian Whitehouse

University of Leeds, Leeds, UK

Like all herpesviruses, the oncogenic Kaposi's sarcoma-associated herpesvirus (KSHV) has a biphasic life cycle and both the lytic and latent phases are required for tumorigenesis. Recent evidence suggests that KSHV lytic replication is an important cause of genome instability in KSHV-infected cells, although no mechanism has thus far been described. A surprising link has recently been suggested between mRNA export, genome instability and cancer development. Notably, aberrations in the cellular transcription and export complex (hTREX) proteins have been identified in high-grade tumours and these defects contribute to genome instability. We have previously shown that the lytically expressed KSHV ORF57 protein interacts with the complete hTREX complex and therefore investigated the possible link between ORF57, hTREX and KSHV-induced genome instability. Herein, we show that lytically active KSHV infected cells induce DNA double strand breaks (DSB). Furthermore, we show that sequestration of the hTREX complex by ORF57 leads to significant levels of DSBs. Moreover, we describe a novel mechanism in KSHV infected cells showing that the genetic instability observed is a consequence of R-loop formation. Our data provide a model of R-loop induced DNA damage in KSHV infected cells and describes a novel system for studying genome instability caused by aberrant hTREX.

LI01Mo1400

Are there any 'good' viruses?

Michael R. Strand

Department of Entomology, University of Georgia, Athens, GA 30602, USA

Microorganisms form symbiotic associations with animals and plants that range from parasitic (pathogens) to beneficial (mutualists). Although numerous examples of obligate, mutualistic bacteria, fungi, and protozoans exist, viruses are generally thought to form parasitic associations and are also usually studied as pathogens. In this talk I will discuss viruses that have evolved beneficial associations with their hosts. Among the best examples of viruses that have evolved into mutualists is the *Polydnaviridae*, which are large DNA viruses that are associated with insects called parasitoid wasps. Recent studies show that polydnaviruses in the genus *Bracovirus* evolved approximately 100 million years ago and are most closely related to two other virus groups that are pathogens of insects and related arthropods. Recent results also identify several features of polydnavirus genomes that are essential for maintenance of their mutualistic associations with wasps. Other examples of beneficial associations between viruses and hosts such as bacteria, plants and vertebrates will also be presented.

LI01Mo1430

Offered paper – Genome segment selection and packaging in the Reoviridae

Mark Boyce, Paul Boyce, Malcolm McCrae

The Pirbright Institute, Surrey, UK

The genomes of members of the Reoviridae family are divided into 10-12 separate double stranded RNA segments. The process by which a complete set of genomic RNA segments is selected and encapsidated remains perhaps the most intriguing molecular mystery of this virus family. In the absence of a mechanism, it seems reasonable to expect that cis-acting sequences within the segments which regulate these processes should i) selectively identify the viral RNA over cellular RNAs and ii) strongly favour the packaging

Please note: Abstracts are published as received from the authors and are not subject to editing.

of a complete set of viral RNAs rather than partial sets. Using a combination of scanning mutagenesis and predictive algorithmic approaches, we have characterised for the first time sequences in the Reoviridae which identify specific genome segments for inclusion in the complete set of packaged segments. Reverse genetics was used to target specific bases in the identified motifs to elucidate the mechanism by which the motifs function in genome segment selection and to determine whether segment selection occurs prior to negative strand synthesis or after. Experimental data showing the mutational analysis of the identified sequences, their predicted structures and the mechanism of action in genome segment selection will be described.

LI01Mo1445

Offered paper – A system approach to analysis of protein-lipid modification in HSV infected cells

Remi Serwa, Fernando Abaitua, Ed Tate, Peter O'Hare

Imperial College, London, UK

Covalent lipid modifications of proteins represent major regulatory steps in diverse processes including membrane targeting and vesicular transport, signalling, cell structure and metabolic flux. In addition modulating function and transport of virus encoded proteins, alterations to acyl modifications of host proteins play keys role in infection and immune modulation. Novel chemical methods to study lipidation enable greater sensitivity and flexibility than traditional techniques. We constructed chemical reporters with functionalised azide or alkyne end groups to probe myristoylation, palmitylation, farnesylation and geranylgeranylation, enabling a broad systems approach to virus and host protein lipid modification during HSV infection and begun to identify and isolate modified proteins by chemical coupling to trifunctional capture reagents bearing combinations of fluorescent and affinity labels. Results from in-gel fluorescence imaging indicate a major suppression of host myristoylation and the appearance of several novel infected cell-specific species. New species were also observed in the palmitoylated population and while host prenylation was less affected a small number of select host species were suppressed. Furthermore SILAC experiments combined with mass spectrometry are now enabling a comprehensive and quantitative analysis of host and virus protein lipid modification that will be relevant to pathways of assembly, trafficking, signalling and immunity.

LI01Mo1530

Has the reductionist approach of reverse genetics had its day?

Richard Elliott

MRC-University of Glasgow Centre for Virus Research, Glasgow, UK

In the negative-sense RNA virus field, the term "reverse genetics" encompasses two major experimental approaches, firstly "minigenome" or "minireplicon" systems in which genome analogues, usually containing a reduced number of viral genes and the inclusion of an easily measurable reporter gene, are transcribed and replicated by co-expressed viral proteins, and secondly "rescue" systems whereby infectious viruses, containing specific genetic alterations, are recovered (rescued) from cDNA copies of the viral genomes. These approaches have had a revolutionary effect on the field, enabling functional dissection of viral proteins and genome sequences in fine detail, and highlighting the crucial importance of individual amino acids or nucleotides. Reverse genetics also has practical applications, for example in the design of attenuated viral vaccines. In speaking to the above title, which was supplied by the symposium organisers, some examples of the power of reverse genetics will be given. In addition, some thoughts on the future of reverse genetics will be aired

LI01Mo1600

Systems virology: a new "golden age" or a fishing exercise?

Angus Lamond, Armel Nicolas, Mark Larance, Tony Ly, Dalila Bensaddek and Aki Endo

Centre for Gene Regulation and Expression, MSI/WTB/JBC Complex, University of Dundee, Dundee, DD1 5EH, UK

Cell regulatory mechanisms and virus-cell interactions can now be studied in unprecedented detail using high throughput techniques, including mass spectrometry (MS) based proteomics and RNA seq. If properly designed, these new methods provide the opportunity for making unbiased, system-wide, quantitative measurements of gene expression. The opportunity here is clear: this can reveal unexpected new interactions and effects on both virus and cell physiology, thereby generating new hypotheses that can be evaluated systematically in downstream, follow-on experiments. This can provide the most detailed and informative molecular view thus far as to how exactly the sequential steps during viral infection occur within the cell. Importantly, it can also highlight the resulting effects of viral infection on the cell proteome, giving important clues as to how cells attempt to combat virus infection and the strategies by which viruses attempt to circumvent such host mechanisms. In particular, the latest proteomics methods have created a flexible suite of quantitative assays that we can use to characterize, system-wide, *'Protein Properties'*, including measurements of protein abundance, subcellular protein localization, turnover rates, post-translational modifications, cell cycle variation and specific protein complexes and protein-protein interaction partners etc (Lamond et al., Mol Cell Prot. 2012). Applying these assays allows us to determine how such *protein properties* vary during the course of a viral infection, for both cell and virus encoded proteins. However, a major issue in successfully applying these system-wide strategies is the resulting challenge of managing and analysing the very large data sets that are generated (Ahmad and Lamond, Trends in Cell Biology, 2013). Systems virology thus moves into the arena of 'big data', and demands that we develop new computational tools for the successful mining of the data harvest and new approaches at a community level for the effective sharing of the large data sets. Systems virology therefore presents both major challenges and major opportunities. Simply generating in future ever larger lists of poorly annotated and poorly designed protein and RNA identifications, which are difficult to share and compare between different laboratories, is not the way ahead. Instead, however, using good experimental design and with access to the requisite computational tools, systems virology has the potential to generate remarkable new insights into molecular regulatory mechanisms and thus to deliver a new golden age of virology and cell biology research. Achieving this golden age will depend upon action at the community level to agree on how to manage and share the data effectively. Undoubtedly, it will also rely on our funding bodies to support new initiatives in this area and to help to establish and recognise best practice in future funding applications.

LI01Tu0900

Can we link the dynamic development of viruses to what we see?

Sarah Butcher

Institute of Biotechnology, University of Helsinki, Finland

Although we structural biologists often think of a virus in one defined structural state, found extracellularly with the well-defined job of protecting the genome, actually these are metastable structures. Virus capsids must nucleate, assemble, exit from the cell, potentially undergo extracellular maturation, transfer to a new host (cell), attach to the cell, be engulfed, uncoat. In some cases this means that one can capture distinct virus structures as they undergo, for example, maturation or dissociation. In other cases, it may mean that the link between the virion structure and its function is not tightly regulated, giving rise to a multitude of different assemblies, described as pleomorphic. I will present a number of recent examples where the dynamic nature of viruses has been explored by integrating structural and functional analyses of the virus life cycle and suggest where we could go next.

LI01Tu0930

Offered paper – Enterovirus 71 Inhibitor Design and Drug Resistance

James Kelly[1], Luigi De Colibus[2], Xiangxi Wang[3], John Spyrou[2], Jingshan Ren[2], Jonathan Grimes[2,4], Gerhard Puerstinger[5], Thomas Walter[2], Zhongyu Hu[6], Junzhi Wang[6], Xuemei Li[3], Wei Peng[3], Zihe Rao[2,7], Elizabeth Fry[2], David Stuart[2,4], David Rowlands[1], Nicola Stonehouse[1]

[1]University of Leeds, Leeds, UK, [2]University of Oxford, Oxford, UK, [3]Chinese Academy of Science, Beijing, China, [4]Diamond Light Sources, Didcot, UK, [5]University of Innsbruck, Innsbruck, Austria, [6]National Institutes for Food and Drug Control, Beijing, China, [7]Tsinghua University, Beijing, China

EV71 is the main causative agents of hand, foot and mouth disease and is especially problematic in South Asia. Although usually associated with mild symptoms in children, occasionally it can result in fatal neurological and cardiovascular disorders. At present no therapies are available. Like most enteroviruses, the EV71 capsid harbours a hydrophobic "pocket factor"; a lipid buried within a pocket in VP1. Expulsion of this molecule following receptor binding allows capsid alterations required for release of the viral genome. Several low molecular weight hydrophobic compounds that tightly bind to the pocket and replace the "pocket factor" have been shown to inhibit viral uncoating and may be developed as antivirals. Using the skeletons of a class of imidazolidinones with anti-EV71 activity and the crystal structure of the EV71 particle, rational improvements of such inhibitors have been made. Beneficial substitutions within these molecules were predicted and two new compounds synthesized. One ligand (IC50 =25 pM) is an order of magnitude more potent than the best previously reported inhibitor, and is also more soluble. Here, we also show that resistance, associated with mutations around the VP1 pocket, can be selected rapidly *in vitro*. However, these mutations also result in reduced capsid stability.

LI01Tu0945

Offered paper – How does a virus tailor the infected cell to optimize viral replication?

Sharada Ramasubramanyan[1], Kay Osborn[1], Aditi Kanhere[3,2], Jianmin Zuo[2], Rajaei AlMohammed[1], Ijiel Barak Naranjo Perez-Fernandez[1], Martin Rowe[2], Richard Jenner[3], Alison Sinclair[1]

[1]University of Sussex, Brighton, UK, [2]University of Birmingham, Birmingham, UK, [3]University College London, London, UK

Epstein-Barr virus (EBV) is a wide-spread infection of mankind and it also causes infectious mononucleosis, lymphoid and epithelial cancers. Replication of the virus is vital for the spread of the virus and may also contribute to the growth and survival of infected cells. Many changes to the cell occur during viral replication, including a cessation of its own replication cycle and a decreased propensity to die by apoptosis. We hypothesize that EBV drives many of these changes by reprogramming the expression of the cell's own genes. Using genome-wide chromatin precipitation coupled to next-generation DNA sequencing and transcriptome approaches, we have identified that a single viral gene directly reprograms the expression of many of the cell's genes. This reveals that the master regulator of viral gene expression, Zta, (BZLF1, ZEBRA, EB1), is also a master regulator of the cell's gene expression. Investigating the frequency and location of Zta-binding sites revealed that Zta appears to use surprisingly different tactics to regulate the expression of cell compared to viral genes. Moreover, we have identified many novel cellular pathways that are regulated during viral replication; the impact of these on the replication of EBV will be discussed.

LI01Tu1000

Have viruses already told us everything they know about cells?

How Herpes Simplex Virus capsids probe the cytosol: Interactions with microtubule motors, nuclear import factors, and nuclear pores

Anna Buch, Jessica Janus, Fenja Anderson, Dagmar Bialy, Kerstin Radtke, Katinka Döhner and Beate Sodeik

Institute of Virology, Hannover Medical School, Germany

Herpes simplex viruses replicate in the skin and mucosa and establish lifelong latent infections in sensory ganglia innervating these areas. Incoming capsids traverse the cortical actin cytoskeleton, utilize microtubules transport, and dock at nuclear pore complexes, to release the viral genomes into the nucleoplasm for viral transcription and replication. There are approximately 25 tegument proteins and 2 capsid surface proteins that can interact with cytosolic factors after viral fusion.

To analyze nuclear targeting in living cells, we used GFP-tagged HSV1 strains and cells stably expressing YFP-tubulin. HSV1 particles accumulated at the nuclear envelope within 2 hpi, while at earlier time points they moved along the YFP-microtubules. Transport to the nucleus was more frequent, faster, and proceeded over longer distances than transport to the cell periphery. HSV1 capsids covered by inner tegument proteins, but lacking outer tegument and viral membrane proteins accumulated at the MTOC, and fewer capsids arrived at the nucleus when kinesin-1, but not kinesin-2 mediated transport had been inhibited. Using a unique biochemical approach, we could demonstrate that capsids coated with inner tegument

Please note: Abstracts are published as received from the authors and are not subject to editing.

proteins can recruit dynein, its co-factor dynactin, kinesin-1 and kinesin-2, as well as the nuclear import factors importin α1, α6, α7 and importin β.

LI01Tu1100

Will we really ever see a Hollywood style virus pandemic outbreak killing millions?

W. Ian Lipkin

Center for Infection and Immunity, Mailman School of Public Health, Columbia University, USA

The movie Contagion chronicled the identification, spread, investigation, and ultimate control of a globally emerging viral disease with unparalleled accuracy and penetrating authenticity. Columbia University professor, Dr. Ian Lipkin, a modern day microbe hunter and an acknowledged world expert in emerging infections, was recruited as a senior technical advisor by Contagion director Stephen Soderburgh and screenwriter Scott Z. Burns. Lipkin felt that "this was an effort to accurately represent the science and to make a movie that would entertain as well as educate." Over the course of the project, Lipkin assisted with story line development, set design and staging, even modeling a novel paramyxovirus. He worked closely with the cast and staff, coaching them on how to deal with lethal disease and to navigate a modern infectious disease lab. In his keynote address, Ian Lipkin will describe the challenges in translating science to cinema as well as the prospects for a pandemic in the style of "Contagion".

LI01Tu1130

Offered paper – Replication of Schmallenberg virus and related Orthobunayviruses is restricted by human Bst-2

Mariana Varela[1], Xiaohong Xi[1], Ilaria Piras[3], Catrina Mullan[1], Angela Mclees[1], Matthew Golder[1], Stuart Neil[2], Richard Elliott[1], Massimo Palmarini[1]

[1]Centre for Virus Research, Glasgow, UK, [2]Kings College London, London, UK, [3]Universita` degli Studi di Sassar, Sassari, Italy

Schmallenberg virus (SBV) is a novel Orthobunyavirus of ruminants that emerged in Europe in the summer of 2011. Although mild clinical signs are observed in infected adult animals, transplacental infection can cause congenital malformations, stillbirths and abortions. SBV has spread very rapidly across Europe and antibodies against the virus have been detected in a variety of animal species. However, no cases of SBV infection nor positive serology have been detected in humans.

We have recently developed molecular and serological tools, and an experimental *in vivo* model that were useful in identifying determinants of SBV virulence. Here, we show that human tetherin (hBst-2) is able to restrict replication of SBV and of related ruminant orthobunyaviruses. Interestingly, the sheep Bst-2 orthologue does not restrict SBV replication. We also show that the Bst-2 block of SBV replication is antagonized by HIV-1 Vpu, a known Bst-2 antagonist. In addition, we provide evidence that a SBV mutant, deleted of one of its non-structural proteins (NSm), is more sensitive to Bst-2 restriction. These results allow us to hypothesize that Bst-2 might be one of the host factors blocking cross-species transmission of orthobunyaviruses.

LI01Tu1145

Offered paper – Can you detect infection without sensing viral PAMPs?

Jerry C.H. Tam, Susanna R. Bidgood, William A. McEwan, Leo C. James

MRC Laboratory of Molecular Biology, Cambridge, UK

Virus infection can be sensed by the detection of viral genetic material, proteins or glycans as pathogen-associated molecular patterns. However, this requires the germline encoding of receptors against motifs that are under pathogen control. In contrast, we have demonstrated that pathogens can be detected when they alter the localisation of antibodies during infection by carrying them into the cytoplasm, initating immunity via TRIM21.

We have discovered that this mechanism is not unique to antibodies. Serum complement component C3, which can be deposited onto pathogens independent both of PAMPs or antibody, is taken into cells during infection. Intracellular C3 activates immune transduction pathways leading to secretion of interferon and other proinflammatory cytokines. Intracellular detection requires the translocation of C3-coated pathogens into the cytosol, is present in non-professional cells and is active against diverse non-enveloped viruses and bacteria. The importance of this pathway is highlighted by the finding of antagonism by certain viruses. We show that poliovirus and rhinovirus encode proteases capable of cleaving intracellular C3, enabling virus evasion from this immune detection. These findings highlight that, in addition to their known extracellular functions, serum proteins have an important intracellular role, by acting as danger-associated molecular patterns that activate innate immunity.

LI01Tu1400

Will virology ever have a 'penicillin moment'?

Leen Delang, Johan Neyts

University of Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium

In contrast to penicillin, which can kill different types of bacteria, the few antiviral drugs that are currently available on the market combat only specific viruses. This is due to the strategy of antiviral development involving the identification of a virus-specific target, screening for inhibitors, and optimizing lead compounds for maximal efficacy and minimal toxicity. The threat of emerging and re-emerging viruses emphasizes the need to develop broad-spectrum antivirals. The development of such drugs is very attractive because it implies a simplified clinical development and more practical aspects of treatment. As the two "broad-spectrum" antivirals currently in use, ribavirin and interferon-α, are both not very potent and treatment is associated with important side-effects, there is an urgent need for safer and more potent broad-spectrum antivirals. Different strategies are being employed to identify broad-spectrum antivirals of which we will discuss a few. For example, favipiravir, an inhibitor of influenza virus replication that is in clinical development, is able to block the replication of various other RNA viruses. Also nucleoside analogues such as 2'-C-methylcytidine have broad-spectrum antiviral properties. Drugs that are already FDA approved for other indications, and target host proteins required by multiple viruses, could be repurposed as for broad-spectrum antiviral therapy. The success of these and other currently explored approaches will affect the possibility of developing a broad-spectrum antiviral in the future.

LI01Tu1430

Why don't we have a vaccine against HIV yet?

Roger W. Sanders

Academic Medical Center, Amsterdam, Netherlands, and Weill Medical College of Cornell University, New York, USA

Most vaccines work by inducing protective humoral immunity and ideally an HIV-1 vaccine should induce broadly neutralizing antibodies. However, past and current HIV-1 vaccine candidates have thus far not been able to do so, because many features of the HIV envelope glycoprotein spike (Env), such as variability, flexibility, instability and glycosylation, limit the induction and binding of neutralizing antibodies. One vaccine approach is to create trimeric mimics of the native Env spike that expose as many neutralization epitopes as possible. Such trimeric mimics can be generated by introducing a disulfide bond between the gp120 and gp41 subunits, complemented with a trimer-stabilizing substitution in gp41 (SOSIP gp140). Soluble, cleaved (SOSIP) gp140 trimers based on the subtype A founder virus, BG505, are highly stable and homogeneous, expose most epitopes for neutralizing antibodies, closely resemble native virus spikes when viewed by negative stain EM, and their high-resolution structure has been determined by cryo-EM and X-ray crystallography. In contrast to previous Env-based vaccine candidates, these trimers induce strong and consistent neutralizing antibody responses against the autologous, neutralization-resistant virus, much more so than a traditional sequence-matched monomeric gp120 vaccine. Heterologous neutralization is limited to relatively neutralization sensitive virus isolates, indicating that future work should be aimed at broadening the neutralizing response. Combined with the detailed structural information on these Env trimers that is now available, these results will guide structure- and immunology-based improvements to the design of Env trimer-based vaccines.

LI01Tu1500

Offered paper – Flexible virus escapes antibody neutralization

Abimbola Kolawole[1], Ming Li[2], Chunsheng Xia[2], Chenchen Yu[1], Thomas Mehoke[3], Joshua Wolfe[3], Andrew Feldman[3], Thomas Smith[2], Christiane Wobus[1]

[1]University of Michigan, Ann Arbor, MI, USA, [2]Donald Danforth Plant Science Center, St. Louis, MO, USA, [3]Applie Physics Laboratory, Baltimore, MD, USA

Human noroviruses cause the majority of all non-bacterial gastroenteritis worldwide. New epidemic strains arise in part by mutations in the viral capsid leading to escape from antibody neutralization. Herein, we identify a series of point mutations in surface exposed regions in a norovirus capsid, which mediate escape from antibody neutralization in cell culture and in a small animal model. Fitting of the newly determined neutralizing antibody structure into the previously determined virion/antibody complex identifies two conformations of the antibody binding domain of the viral capsid, one with a superior and the other with an inferior fit to the antibody. These data suggest a unique mode of antibody neutralization. In contrast to other viruses that largely escape antibody neutralization through direct disruption of the antibody/virus interface, we identify mutations that act indirectly by limiting the conformation of the antibody binding loop in the viral capsid and drive the antibody binding domain into the conformation unable to be bound by the antibody. Thus, the flexibility in surface exposed loops in the norovirus capsid is an important prerequisite for evading antibody neutralization. These studies have important implications for vaccine design by increasing our understanding of the potential diversity of escape mutants.

LI01Tu1515

Can we track viruses in real time, in the real world?

Jonathan Read

University of Liverpool, Liverpool, Merseyside, UK

A critical assumption within infectious disease epidemiology is that patterns of movement and contact between people drive observed patterns of viral infection. The importance of contact tracing in disease outbreak investigation is one example of how central this assumption is in our approach to epidemic management. Yet the evidence linking interactions between individuals and transmission of viruses is extremely limited. To track viruses accurately within a population requires a deeper understanding of where and how often encounters occur between people, improved identification of infected and infectious individuals, and how these aspects link together. This talk will summarise recent work on quantifying encounter patterns and how we can relate the findings to the transmission of respiratory viruses.

LI02

Pseudomonas signalling, secretions and social interactions

LI02Mo0900

Quorum sensing sophistication: integrating signals new and old

Paul Williams

Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, Nottingham, U.K.

Bacteria employ quorum sensing (QS) systems for co-ordinating collective behaviours which depend on the production and actions of chemically diverse signal molecules to regulate for example, metabolism, motility, virulence and biofilm development. Multiple QS systems may be integrated with each other and within global transcriptional and post-transcriptional networks. QS signal molecules, although largely considered as effectors of QS-dependent gene expression are also emerging as multi-functional agents which influence life, development and death in single and mixed microbial populations in addition to their impact on host-pathogen interactions. In *Pseudomonas aeruginosa*, it is clear that there are at least seven different but overlapping QS pathways. Apart from the well characterized *las* and *rhl* QS systems that employ *N*-acylhomoserine lactones (AHLs) and the *pqs* system that employs 2-alkyl-4-quinolones (AQs), *P. aeruginosa* uses pyoverdine, unsaturated fatty acids and the 'gac' signal to co-ordinate population-dependent gene expression. While the *las* system hierarchically controls both *rhl* and *pqs*, it has recently emerged that it is also responsible for the biosynthesis of IQS, a thiazole signal molecule which links *las* to the phosphate stress response, *pqs* and *rhl* revealing the existence of a highly sophisticated QS regulatory network central to the adaptive lifestyle of P. *aeruginosa*.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI02Mo0930

The *Pseudomonas aeruginosa* *N*-Acylhomoserine lactone quorum sensing molecules target IQGAP1 and modulate epithelial cell migration

Elena Vikström

Division of Medical Microbiology, Department of Clinical and Experimental Medicine, Linköping University, Sweden

The human pathogen *Pseudomonas aeruginosa* communicate with each other using *N*-acylhomoserine lactones (AHL) as a quorum sensing (QS) signals. This is important for their virulence and biofilm formation. Also, eukaryotic cells “listen and respond” to QS signalling and I will show here how 3O-C12-HSL alters epithelial barrier functions and modulates the migration of epithelial cells. Further, I will focus my talk on our study to identify receptors for 3O-C12-HSL on human cells. Using newly designed and validated biotin- and fluorescein-based 3O-C12-HSL probes, mass spectrometry, affinity pull down assay and high-resolution microscopy, we identified the IQ-motif-containing GTPase-activating protein IQGAP1 as a human target of 3O-C12-HSL. We propose that the interaction between IQGAP1 and 3O-C12-HSL provides a novel mechanism for its mode of action on eukaryotic cells, and by affecting the distribution of IQGAP1 and phosphorylation of Rac1 and Cdc42, upstream effectors of cytoskeleton remodelling, also cell migration. I will also report our findings on the role of AHL in chemotaxis and phagocytosis in human neutrophils and macrophages and how water channels aquaporins are involved in these cellular events. Understanding the mechanisms of QS communication is fundamental for developing strategies to combat infection, virulence and biofilm formation.

LI02Mo1000

Regulation of the co-evolved HrpR and HrpS AAA+ proteins required for *Pseudomonas syringae* pathogenicity

Martin Buck, Milija Jovanovic, Christoph Engl, Joerg Scumacher, Christopher Waite

Department of Life Sciences, SAF Building, Imperial College London, SW7 2AZ UK.

The plant pathogenic *Pseudomonas syringae* DC3000 uses a tightly regulated T3SS as a part of its infection strategy. Expression of the T3SS genes is controlled via regulated expression of sigma factor HrpL through the direct action of a heteromeric HrpRS enhancer binding protein (EBP) complex HrpRS. Such heteromeric EBPs are rare in bacteria, and likely for Hrp regulation represent an evolved state with subunit specialisations useful for a more sophisticated regulation of transcription than is possible with a homomeric EBP complex. Progress in understanding the functionality of the heteromeric HrpRS complex by its in trans acting HrpVG co-regulators will be described, including its role in expressing a gene controlling c-di-GMP-dependent lifestyles behaviours Important for infection. Jovanovic et al (2011) Regulation of the co-evolved HrpR and HrpS AAA+ proteins required for *Pseudomonas syringae* pathogenicity. *Nature Communications* , Vol:2 Lawton et al (2014) Determination of the self-association residues within a homoeric and heteromeric AAA+ enhancer binding protein. *J Mol Biol.* in press.

LI02Mo1100

Global control of antibiotic resistance, virulence, stress survival, small RNA, and acute vs chronic infection by *Pseudomonas aeruginosa* AmpR

Kalai Mathee

Herbert Wertheim College of Medicine, Florida International University, Miami, USA

Pseudomonas aeruginosa is a major cause of many acute and chronic human infections, and its pathogenicity is determined by a tightly regulated expression of multiple virulence factors. Using genomics (microarray and RNA-Seq) and proteomics, AmpR, a member of the LysR family of transcription factors, is shown to play a major role in *P. aeruginosa* pathogencity. AmpR regulates both ß-lactam and non-ß-lactam resistance. In addition, AmpR positively regulates expression of the major QS regulators LasR, RhlR and MvfR, and genes of the *Pseudomonas* quinolone system. Importantly, AmpR affects many of the pathogenic phenotypes, in part, by regulating expression of non-coding RNAs, cyclic di-GMP levels and phosphorylation of Ser, Thr and Tyr. ChIP-Seq and ChIP-qPCR studies show that AmpR binds to the *ampC* and the *lasR* promoters encoding the QS master regulator. Additionally, using site-directed mutagenesis, amino acids that are (a) critical for the binding functionality and (b) important for transitioning between inactive and active states of AmpR have been identified. The studies show that AmpR is a unique global regulator that plays a critical role in P. *aeruginosa's* ability to cause debilitating infection.

LI02Mo1130

The YfiBNR signal transduction mechanism reveals novel targets for the evolution of persistent *Pseudomonas aeruginosa* in cystic fibrosis airways

Jacob G. Malone[1, 2], Tina Jaeger[2], Pablo Manfredi[2], Andreas Dötsch[3], Andrea Blanka[4], Guy R. Cornelis[2], Susanne Häussler[3, 4] and Urs Jenal[2]

[1] *University of East Anglia/John Innes Centre, Norwich, UK*

[2] *Biozentrum of the University of Basel, Basel, Switzerland*

[3] *Helmholtz Centre for Infection Research, Braunschweig, Germany*

[4] *Twincore, Centre of Clinical and Experimental Infection Research, a joint venture of the Hannover Medical School and the Helmholtz Centre for Infection Research, Hannover, Germany*

Small colony variants (SCVs) are hyper-adherent, auto-aggregative *Pseudomonas aeruginosa* morphotypes. SCVs arise during the morphological diversification characteristic of long-term cystic fibrosis (CF) lung infections and their appearance correlates with poor lung function and persistence of infection. The YfiBNR system is a key SCV phenotypic regulator, and consists of a membrane bound diguanylate cyclase (YfiN) under the tight control of a periplasmic repressor (YfiR) and an outer-membrane lipoprotein activator (YfiB). Our research shows that YfiN repression occurs via allosteric interaction between YfiR and the YfiN periplasmic domain. Release of repression induces structural rearrangements in YfiN that propagate through the protein, leading to GGDEF domain dimerisation, enhanced production of the second messenger cyclic-di-GMP and SCV phenotype generation. This may occur as a consequence of YfiB activation provoking YfiR sequestration to the outer membrane, or via YfiR misfolding under reducing conditions in the periplasm.

Our subsequent analysis of clinical CF P. *aeruginosa* isolates uncovered multiple strains with both SCV-inducing and loss-of-function mutations throughout the *yfiBNR* locus. We conclude that YfiBNR is important for the generation of clinical SCVs, and these SCVs can function as an environmental reservoir for the generation of new smooth morphotypes in the dynamic fitness landscape of the CF lung.

LI02Mo1400

The therapeutic challenge of *Pseudomonas aeruginosa* infections

Professor Susanne Häußler

Helmholtz Centre for Infection Research, Braunschweig, Germany

Since diagnosis of resistance prior to antibiotic treatment is essential to guide clinicians in their choice of anti-infective therapy, rapid results on antibiotic susceptibility are mandatory. Increasing rates of biofilm associated infections, emergence of multi-drug resistance and the urgent need of a timely reporting of antibiotic resistance calls for the development of modern molecular diagnostics for rapid detection of resistance. With the aim to identify novel genetic determinants of antibiotic resistance and biofilm formation in *Pseudomonas aeruginosa* we have recently screened the comprehensive Harvard Medical School transposon mutant library for resistance profiles towards several antibiotics and the capability to produce biofilms. From these studies it has become clear that many previously unidentified genes play a role in antimicrobial resistance and biofilm formation and that, depending on the antibiotic; there are many modifiers of the expression of resistance in *P. aeruginosa.* We will use extensive whole-genome sequencing approaches with the aim to identify novel genetic markers of antibiotic resistance and biofilm associated phenotypes via genome-wide association studies in clinical isolates and in antibiotic resistant strains that have been selected *in vitro*.

LI02Mo1430

Offered paper – Increased severity of *Pseudomonas aeruginosa* respiratory infections associated with elevated anti-LPS IgG2 which inhibits serum bactericidal killing

Timothy Wells[1], Deborah Whitters[2], Yanina Sevastsyanovich[1], Jennifer Heath[1], John Pravin[1], Margaret Goodall[1], Douglas Browning[1], Amy Cranston[3], Anthony De Soyza[3], Adam Cunningham[1], Calman Maclennan[4], Ian Henderson[1], Robert Stockley[2]

[1]University of Birmingham, Birmingham, UK, [2]Queen Elizabeth Hospital, Birmingham, UK, [3]Newcastle University, Newcastle, UK, [4]Novartis Vaccines Institute for Global Health, Sienna, Italy

Although specific antibody induced by pathogens or vaccines is a key component of protection against infectious threats, some viruses, such as dengue, induce antibody that enhances the development of infection. In contrast, antibody-dependent enhancement of bacterial infection is largely unrecognised. Here, we demonstrate that in a significant portion of patients with bronchiectasis and *Pseudomonas aeruginosa* lung infection, antibody can protect the bacterium from complement-mediated killing. Strains that resist antibody-induced complement-mediated killing produce lipopolysaccharide containing O-antigen. The inhibition of antibody-mediated killing is due to excess production of O-antigen-specific IgG2 antibodies. Depletion of IgG2 to O-antigen restores the ability of sera to kill strains with long-chain O-antigen. Patients with impaired serum-mediated killing of *P. aeruginosa* by IgG2 have poorer respiratory function than infected patients who do not produce inhibitory antibody. We suggest that excessive binding of IgG2 to O-antigen shields the bacterium from other antibodies that can induce complement-mediated killing of bacteria. As there is significant sharing of O-antigen structure between different Gram-negative bacteria, this IgG2-mediated impairment of killing may operate in other Gram-negative infections. These findings have marked implications for our understanding of protection generated by natural infection and for the design of vaccines, which should avoid inducing such blocking antibodies.

LI02Mo1445

Offered paper – Bile aspiration: molecular trigger of chronic respiratory infections

F. Jerry Reen[1], David Woods[1], Muireann Ni Chroinin[2], David Mullane[2], Claire Adams[1], Fergal O'Gara[1]

[1]University College Cork, Cork, Ireland, [2]Cork University Hospital, Cork, Ireland

Gastro-oesophageal reflux (GOR) is a significant complication of respiratory disease, including asthma, chronic obstructive pulmonary disorder, and Cystic Fibrosis (CF) in particular. Affecting approximately 40% of CF children and up to 80% of adults with CF, GOR is associated with increased morbidity and colonisation in these patients. Aspiration of gastric contents into the lungs has been confirmed in several studies and new data from the BIOMERIT Research Centre indicates that aspirated bile acids are a major host determinant triggering the establishment of dominant chronic biofilm forming microbial species, particularly *Pseudomonas aeruginosa*. Bile aspiration correlated with a significant reduction in the lung microbial biodiversity (Shannon index) and richness (Chao index) of paediatric CF patients and was associated with the emergence of dominant Proteobacterial pathogens. This is consistent with our previous findings that physiological concentrations of bile influence respiratory pathogens, and in particular *P. aeruginosa*, towards chronic behaviour, triggering a biofilm lifestyle. In addition, bile also impacted significantly on pathogen signalling systems while selectively repressing the host immune and hypoxic response. Transcriptional profiling and functional genomics analysis are providing molecular targets to counter what may be a major underlying cause of chronic respiratory disease.

LI02Mo1500

Offered paper – Stimulatory interactions between histidine kinases controlling the transition between acute and chronic infection in *Pseudomonas aeruginosa*

Vanessa Francis, Alan Brown, Steven Porter

University of Exeter, Exeter, UK

The GacS network plays a major role in controlling the transition between acute and chronic infection in *P. aeruginosa*. This network comprises several different kinases that work together to integrate signals and decide upon a mode of infection. Within this network, we have discovered a novel mechanism of signal

Please note: Abstracts are published as received from the authors and are not subject to editing.

integration that involves direct stimulatory interactions between kinases. Specifically, we have found two pairs of histidine kinases which when coincubated stimulate each others' ATP dependent phosphorylation activity by over ten-fold compared with their combined solo activities. These kinases play a major role in controlling the transition between acute and chronic modes of infection and we will present data demonstrating the *in vivo* importance of these stimulatory interactions. The ability of histidine kinases to directly stimulate one another is novel and has the potential to be widely found in other signalling networks employing multiple kinases.

LI02Mo1515

Offered paper – Regulation of two identical phenazine producing operons by quorum sensing in *Pseudomonas aeruginosa*

Steven Higgins[1], Stephan Heeb[1], Paul Williams[1], Natalio Krasnogor[2], Miguel Cámara[1]

[1]University of Nottingham, Nottingham, Nottinghamshire, UK, [2]University of Newcastle, Newcastle upon Tyne, Tyne and Wear, UK

The *Pseudomonas aeruginosa* Quorum Sensing (QS) systems; las, rhl and pqs play a key role in the adaptation of this bacterium to environmental changes and the control of virulence factor production. These three interlinked systems are central in the control of phenazine production. The biosynthesis of the pyocyanin precursor phenazine-1-carboxylic acid (PCA) is mediated by either of two nearly identical *phzA1B1C1D1E1F1G1and phzA2B2C2D2E2F2G2* operons. Due to high nucleotide sequence conservation between these operons, analysis of their individual expression by RNA hybridisation, qRT-PCR or transcriptomics is challenging.
To overcome this difficulty we constructed a new dual reporter system to simultaneously measure the transcriptional levels of both operons. Using this reporter we found that all three QS systems differentially regulate the two phenazine operons.
A significant delay in activation of these two operons was observed. Using a combination of computer modelling and transcriptional analysis of the *phz1* and *phz2* operons in a range of QS mutants we have established new links between QS and phenazine production and have identified the existence of a yet unknown regulator between the QS switches and the *phz* operons. A new model for the regulation of PCA production by QS will be presented.

LI02Mo1600

Offered paper – Interspecies signalling: *Pseudomonas putida* efflux pump TtgGHI is activated by indole to increase antibiotic resistance

Carlos Molina-Santiago[1], Abdelali Daddaoua[1], Juan Luis Ramos[2]

[1]Estación Experimental del Zaidín, Granada, Spain, [2]Department of Biotechnology, Abengoa Research, Sevilla, Spain

In Gram-negative bacteria, multidrug efflux pumps are responsible for the extrusion of chemicals. Some of these efflux pumps are induced by endogenous effectors, while abiotic or biotic signals induce the expression of other efflux pumps. In *Pseudomonas putida*, the TtgABC efflux pump is the main antibiotic extrusion system and is modulated by TtgR regulator. The plasmid-encoded TtgGHI efflux pump in *P. putida*, regulated by TtgV regulator, plays a minor role in antibiotic resistance in wild-type strain; however, its role is critical in isogenic backgrounds deficient in TtgABC. TtgV recognizes indole as effector which is not produced by *Pseudomonas*, therefore, indole-dependent antibiotic resistance seems to be part of an antibiotic resistance programme at the community level.
Transcriptomic analyses revealed that indole specific response involves the expression of TtgGHI pump but also a set of genes involved in iron homeostasis, as well as amino acid catabolism genes. In a ttgABC-deficient *P. putida*, background ampicillin and other bactericidal compounds lead to cell death. Co-culture assays of *E. coli* and *P. putida* ΔttgABC allowed growth of *P. putida* mutant in the presence of ampicillin because of induction of the indole-dependent efflux pump demonstrating that indole acts as an interspecies signalling molecule in antibiotic resistance.

LI02Mo1615

Offered paper – Combinatorial quorum-sensing allows bacteria to resolve their social and physical environment

Daniel Cornforth[1], Roman Popat[1], Luke McNally[1], James Gurney[2], Thom Scott-Phillips[3], Alasdair Ivens[1], Stephen Diggle[2], Sam Brown[1]

[1]University of Edinburgh, Edinburgh, UK, [2]University of Nottingham, Nottingham, UK, [3]University of Durham, Durham, UK

Quorum-sensing (QS) is a cell-cell communication system that controls gene expression in many bacterial species, mediated by diffusible signal molecules. While the intracellular regulatory mechanisms of QS are often well-understood, the functional roles of QS remain controversial. In particular, the use of multiple signals by many bacterial species poses a challenge to current functional theories. Here we address this challenge by showing that bacteria can use multiple QS signals to infer both their social (density) and physical (mass-transfer) environment. Analytical and evolutionary simulation models show that the detection of and response to complex social/physical contrasts requires multiple signals with distinct half-lives and combinatorial (non-additive) responses. We test these predictions using the opportunistic pathogen *Pseudomonas aeruginosa*, and demonstrate significant differences in decay between its two primary signal molecules as well as diverse combinatorial responses to dual signal inputs. QS is associated with the control of secreted factors, and we show that secretome genes are preferentially controlled by synergistic 'AND-gate' responses to multiple signals, ensuring the effective expression of secreted factors in high density and low mass-transfer environments. Our results support a novel functional hypothesis for the use of multiple signals and, more generally, show that bacteria are capable of combinatorial communication.

LI02Mo1630

Offered paper – Protist predation can favour cooperation within *Pseudomonas aeruginosa* bacteria

Ville-Petri Friman[2] ,[1], Stephen P. Diggle[3], Angus Buckling

[1]University of Exeter, Penryn, UK, [2]Imperial College London, Ascot, UK, [3]University of Nottingham, Nottingham, UK

The costs and benefits of bacterial cooperation and cheating are

dependent on environmental context. For example, recent studies have reported a benefit of cooperation in the context of antipredator toxin production. Here, we studied how protist predation affects cooperation in the opportunistic pathogen bacterium *Pseudomonas aeruginosa*, which uses quorum sensing (QS) cell-to-cell signalling to regulate the production of public goods. By competing wild-type bacteria with QS mutants (cheats), we show that a functioning QS system confers an elevated resistance to predation. Surprisingly, cheats were unable to exploit this resistance in the presence of cooperators, which suggests that resistance does not appear to result from activation of QS-regulated public goods. Instead, elevated resistance of wild-type bacteria was related to the ability to form more predation-resistant biofilms. This could be explained by the expression of QS-regulated resistance traits in densely populated biofilms and floating cell aggregations, or alternatively, by a pleiotropic cost of cheating where less resistant cheats are selectively removed from biofilms. These results show that trophic interactions among species can maintain cooperation within species, and have further implications for *P. aeruginosa* virulence in environmental reservoirs by potentially enriching the cooperative and highly infective strains with functional QS system.

LI02Mo1645

Offered paper – The differing biological fates of DNA minor groove binding (MGB) antibiotics in Gram-negative and Gram-positive bacteria

Leena Nieminen[1], Iain Hunter[1], Colin Suckling[2], Nicholas Tucker[1]

[1]University of Strathclyde, Strathclyde Institute of Pharmacy and biomedical Science, Glasgow, UK, [2]University of Strathclyde, Department of Pure and Applied Chemistry, Glasgow, UK

We have evaluated a collection of 49 novel synthetic antibiotic compounds (sMGBs), which draw inspiration from natural products such as distamycin - that have the intrinsic ability to bind to the minor groove of DNA. Some of these compounds are active against Gram-positive pathogens including *Clostridium difficile*, and *Staphylococcus aureus* (including MRSA), at comparable levels of activity to vancomycin. There is a good structure-activity relationship and resistance to sMGBs has not been an issue in these target organisms.

sMGBs appeared less effective against Gram-negatives such as *Pseudomonas aeruginosa.* However, efficacy improved considerably in the presence of an efflux pump inhibitor. We have screened over 20 efflux pump mutants in order to identify the relative contributions of different drug efflux systems. Certain efflux mutants exhibited only 10% growth relative to the wild type *P. aeruginosa* strain, demonstrating selectivity between pump and sMGB substrate and we are currently exploiting this information so that we can design better anti-pseudomonas drugs. RNA-seq transcriptome analysis of MGB-treated *S. aureus* has revealed that MGB antibiotics down regulate essential genes.

LI02Mo1700

The H2-T6SS of *Pseudomonas aeruginosa*: regulation and function during interaction with eukaryotic host cells.

Sophie Bleves

CNRS & Aix-Marseille Université, Laboratoire d'Ingénierie des Systèmes Macromoléculaires (UMR7255), Institut de Microbiologie de la Méditerranée, 31 Chemin Joseph Aiguier, 13402 Marseille cedex 20, France.

Three Type VI Secretion System (T6SS) loci called H1- to H3-T6SS coexist in *Pseudomonas aeruginosa*. H1-T6SS targets prokaryotic cells whereas H2-T6SS mediates interactions with both eukaryotic and prokaryotic host cells.

We demonstrated that the expression of H2-T6SS genes of strain PAO1 is up-regulated during the transition from exponential to stationary phase growth and is regulated by the Las and Rhl quorum sensing systems. In addition, we found that H2-T6SS transcription is negatively regulated by iron through Fur. While in many T6SSs, enhancer binding proteins (EBPs) act as RpoNγ54 activators to promote T6SS transcription, we found a RpoN-dependent repression of H2-T6SS mediated by Sfa2, the EBP encoded within H2-T6SS. Although considered an extracellular pathogen *Pseudomonas* is able to enter non-phagocytic cells. We have demonstrated that among the various factors facilitating the internalization, the H2-T6SS is involved in the uptake of the bacteria by epithelial cells. We recently discovered an evolved VgrG effector that is delivered into host cells by the H2-T6SS machinery. The VgrG2 host partners identified by TAP-Tag allow us to provide a model for H2-T6SS mediated internalization.

LI02Tu0900

Type VI protein secretion in *Pseudomonas aeruginosa*

Alain Filloux

Imperial College London, Department of Life Sciences, MRC CMBI South Kensington Campus, Flowers Building, SW72AZ, London, UK

Gram-negative bacteria have evolved robust nanomachines to transport proteins to extracellular compartments. The secreted proteins are usually released in the environment but some of these systems are specialized in bacteria-host interaction. As such they can for example inject effectors into eukaryotic cells thus subverting the host signaling process to their advantage.

The type VI secretion system (T6SS) brought several new concepts in the field. Most notably the structure of the machine is highly reminiscent of bacteriophage tails, which are specialized DNA injection devices. The similarities with the T6SS are promising and it is wise to follow the bacteriophage trail to perform structure-function analysis and elucidate mechanistic aspects. Some of the T6SS-related molecular mechanisms will be described and differences between systems highlighted. For example, studying the ClpV ATPase unveils how subtle changes contribute to the evolution and improvement of the mechanism.

Another important concept is the observation that while the T6SS is a potential device in the interaction with eukaryotic cells, it has also specialized in targeting other bacteria and can be seen as a very powerful weapon for bacterial warfare and competition. There are multi examples of bacterial toxins, which are transported in a T6SS-dependent manner, and *Pseudomonas aeruginosa* can use a

Please note: Abstracts are published as received from the authors and are not subject to editing.

remarkable collection of ammunitions, which make it a resilient and potent competitor when facing other organisms.

LI02Tu0930

Lipopeptide secretion from *Pseudomonas sp.* CMR12a

Nam Phuong Kieu[1], Chien-Jui Huang[1], Marc Ongena[2] and Monica Höfte[1]

[1]Laboratory of Phytopathology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Gent, Belgium
[2]Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés, 2, B-5030 Gembloux, Belgium

Pseudomonas sp. CMR12a is a tropical biocontrol strain, which is taxonomically positioned between *P. chlororaphis* and *P. protegens* in the *P. fluorescens* group. The strain has two quorum sensing systems and produces two different classes of cyclic lipopeptides (CLPs), orfamides and sessilins (which are related to tolaasins produced by mushroom pathogens), which are both involved in biocontrol. Orfamides are indispensable for swarming, while sessilin mutants showed reduced biofilm formation, but enhanced swarming motility. The presence of sessilins in wild type CMR12a interferes with swarming by hampering orfamide secretion. CLPs are believed to be secreted by ABC transporters similar to the *E. coli* macrolide efflux transporter MacA-MacB-TolC. MacA and MacB encoding genes are found downstream of both the orfamide and the sessilin biosynthetic gene clusters. A mutant of CMR12a in the macAB genes downstream of the sessilin gene cluster no longer produces sessilin, while mutation of *macAB* downstream of the orfamide gene cluster has no influence on orfamide production or secretion. Mutants in one of the two quorum sensing systems overproduce orfamides and sessilins, but only secrete sessilin in the medium, while orfamides are kept inside the cell, indicating that quorum sensing negatively regulates CLP biosynthesis, but positively regulates orfamide secretion.

LI02Tu1000

Early events in recycling of cell wall

Shahriar Mobashery

Department of Chemistry and Biochemistry University of Notre Dame, Notre Dame, IN 46556 USA

Many members of Enterobacteriaceae and *Pseudomonas aeruginosa* have the ability to sense damage inflicted to their cell wall by **β**-lactam antibiotics. A primary mechanism for this sensing involves the events of cell-wall recycling, and results in the induction of resistance mechanisms. These events have led to the obsolescence of many of the **β**-lactam antibiotics against these Gram-negative bacteria. These multiple complex steps are poorly elucidated, and are the subject of this presentation.

LI02Tu1100

The A-team: An autotransporter that helps *P. aeruginosa* establish a chronic infection

Hardie, K.R.

School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham, NG7 2RD. UK.

Autotransporters are the largest family of secreted proteins in Gram-negative bacteria, and those characterised in pathogens are virulence factors. The *P. aeruginosa* genome encodes at least three proteins exhibiting the characteristic three domain structure of autotransporters, but much remains to be understood about the functions of these three proteins or their role in pathogenicity. The AaaA autotransporter is a cell-surface tethered, arginine-specific aminopeptidase. AaaA provides a fitness advantage in environments where the sole source of nitrogen is peptides with an aminoterminal arginine, and the lack of AaaA led to attenuation in a mouse chronic wound infection, which correlated with lower levels of the cytokines TNFα, IL-1a, KC and COX-2. With the substrate for AaaA remaining elusive, the potential mechanisms by which AaaA contributes to the establishment of chronic infections caused by *P. aeruginosa* will be discussed.

LI02Tu1130

Tit-for-tat: The bacterial Type VI secretion system and bacterial cell-cell interactions

John J. Mekalanos

Department of mcirobiology and Immunobiology, Harvard Medical School, Boston, MA, USA

Type VI secretion systems (T6SS) are organelles that are encoded by gene clusters present in many bacterial species in including *Pseudomonas aeruginosa*, an opportunistic pathogen of Cystic Fibrosis (CF) patients. Our laboratory functionally defined T6SS in 2006 and has since determined that these nanomachines transport proteins into target host cells by a novel dynamic mechanism analogous to phage tail contraction. This organelle attacks prey cells by initially penetrating them with a large protein complex called the VgrG spike. In collaboration with Petr Leiman (Lausanne), we have recently determined new structural features of the spike that suggest a model for multiple effector molecule delivery in a single contraction event. Furthermore, we have shown that *P. aeruginosa* can use its T6SS to "counterattack" other aggressive T6SS+ organisms including *Acinetobacter* and *Vibrio* species leading to a remarkable phenomenon called the "T6SS dueling" counterattack response. The dueling counterattack is driven by a post-transcriptional regulatory response that apparently detects outer membrane perturbations. Recently we have found that the T6SS counterattack also occurs in response to formation of mating pairs between *P. aeruginosa* and other species carrying broad host range conjugative plasmids such as RP4. In contrast, *Vibrio cholerae* and *Acinetobacter baylyi* do not show this counterattack behavior but instead attack even nonaggressive cells such as *E. coli* K12. These data suggest that from an ecological perspective, T6SS play different defensive vs. aggressive roles in microbial communities. Indeed, for aggressive species such as *V. cholerae*, the up-regulation of T6SS *in vivo* (i.e., in the infected host) has been demonstrated and suggests that T6SS could target members of the host microbiome during the colonization process. Indeed, a comprehensive Tn-seq analysis has revealed a host colonization defect associated with TsiV3, a known immunity protein to a lysozyme-like effector called VgrG3. This defect is only apparent if the mutant is co-infected with either sister cells or another strain that expresses functional VgrG3 and T6SS. Together these data suggest sister cells attack each other during the intestinal colonization process. This insight suggests an opportunity for anti-microbial drug development that targets T6SS effector immunity proteins. Inhibition of these would enlist the T6SS of bacteria to participate in "self-destruction" of bacterial biofilms. Thus, our studies

offer novel opportunities for development of therapeutics that may benefit CF patients and others suffering from *P. aeruginosa* infections that are characterized by *in vivo* biofilm formation.

LI02Tu1400

Cooperation and competition in bacterial communities

Kevin Foster

University of Oxford, Oxford, UK

Since Darwin, evolutionary biologists have been fascinated by cooperative behavior. Honeybee workers labor their whole life without reproducing, birds make alarm calls, and humans often help one another. One major group that remains relatively unexplored, however, is the microbes whose full spectrum of sociality only recently came to light. Microbes often live in large dense groups where one cell can strongly affect the survival and reproduction of others. But do microbes typically help or harm those around them and what factors promote cooperation over competition? We study these questions using computer simulations and pseudomonad bacteria. We find that single-genotype patches naturally emerge in microbial groups, which creates favorable conditions for cooperation within a particular genotype. Experimental evolution in bacteria shows that this process drives extremely strong natural selection for cooperative adaptations that can be understood at the molecular scale. However, our work on interactions between different microbial genotypes suggests that, here, the evolution of competitive phenotypes is more likely than cooperation. This leads us to a simple model – the genotypic view – that predicts microbes will evolve to help their own genotype but harm most other strains and species that they meet.

LI02Tu1430

Pseudomonas aeruginosa population diversification during infections

Craig Winstanley

Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

Chronic lung infections due to *Pseudomonas aeruginosa* remain the main cause of the morbidity and mortality associated with cystic fibrosis (CF). Since its emergence in the mid 1990s, we have studied the transmissible Liverpool Epidemic Strain (LES), a CF-adapted clone that is widespread amongst CF patients in the UK and has been reported in North America. We have analysed populations of the LES from the sputa of multiple chronically infected adult CF patients. Following the establishment of a chronic infection in the lungs of CF patients, populations of *P. aeruginosa* LES adapt and diversify due to mutation. Hence, multiple single strain isolates from the same patient sample can exhibit diversity in phenotypes such as quorum sensing, mucoidy and antibiotic resistance. Using phenotypic and genome sequencing we have compared LES populations between patients and followed population changes over time, including during periods of exacerbation. Our data suggest that populations are highly variable between patients and dynamic within patients over time periods of several months. In parallel, we have used an artificial sputum medium (biofilm) model system to study potential factors driving diversification, such as biofilm lifestyle, antibiotics, bacteriophages and other microorganisms. The potential causes and implications of diversification will be discussed.

LI02Tu1500

Can't we just all get along: How polymicrobial interactions shape disease

Marvin Whiteley

The University of Texas at Austin, Department of Molecular Biosciences, USA

Most bacterial infections are polymicrobial, and the presence of multiple microbes in an infection is often associated with delayed healing, increased antibiotic resistance, and poor clinical outcomes. This is thought to be due in part to mutualistic and antagonistic interactions between bacteria; thus to fully understand these infections it is essential to view them through an ecological lens. Here I will discuss the use of high-throughput sequencing-based genomic methods such as RNA sequencing and transposon sequencing to study *Pseudomonas aeruginosa* polymicrobial communities *in vitro* and *in vivo*, both in animal models of infection and in human infections. These studies have yielded novel insights into bacterial behavior and community structure during infection. Our work suggests that metabolic interactions underpin the stability of disease-associated microbial communities, and point to "cornerstone" metabolic pathways as potential therapeutic targets for a variety of infections.

LI02Tu1600

Fitness costs, compensatory adaptation and the maintenance of antibiotic resistance: insight from *Pseudomonas aeruginosa*

Craig MacLean

University of Oxford, Oxford, UK

Antibiotic resistance comes at a fitness cost, and it is therefore challenging to understand how resistance can persist over the long term under sporadic exposure to antibiotics. In this talk, I will address two basic, but unresolved questions: (i) How do the molecular mechanisms of resistance dictate its costs? (ii) How do bacterial populations evolve compensatory adaptations that allow resistance to persist by overcoming its cost? To address these questions, we primarily rely on using experimental evolution of the pathogenic bacterium *P.aeruginosa* under tightly controlled laboratory conditions. Specifically, I will focus on experiments in which *P.aeruginosa* (a) evolves chromosomal antibiotic resistance by acquiring rifampicin resistance mutations in *rpoB* or (b) evolves plasmid-mediated by acquiring pNuk73, a small, non-conjugative plasmid. In these experiments, resistance carries a variable fitness cost, and I will show that it is possible to mechanistically dissect the cost of resistance by considering both the direct burden associated with resistance genes and the indirect, pleiotropic effects of resistance mutations on gene expression. I will argue that the ability of resistance to persist in the long term depends crucially on both the frequency of antibiotic use and the evolvability of populations of resistant mutants, which, in turn, depends on the molecular basis of resistance.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI02Tu1630

An experimental test of signalling theory using bacteria

Stephen P. Diggle

School of Life Sciences, University Park, University of Nottingham, Nottingham, U.K.

Animals use signals to coordinate a wide range of behaviours, from feeding offspring to predator avoidance. This poses an evolutionary problem, because individuals could potentially signal dishonestly to coerce others into behaving in a way that benefits the signaller. Theory suggests that honest signalling is favoured when individuals share a common interest and signals carry reliable information. However, whilst many studies have manipulated signals, to examine how this influences behaviour, it has not been possible to directly test how the behaviour of signallers and receivers evolve in response to manipulation. Here, we exploit the opportunities offered by signalling between bacteria ('quorum sensing') to show that: (1) a reduced relatedness, and therefore reduced common interest between interacting individuals, leads to the relative breakdown of signalling, via both reduced signalling and a reduced response to signal; (2) signal interference selects for lower levels of signalling. More generally, whilst our results provide clear support for signalling theory, we did not find evidence for the previously predicted coercion at intermediate relatedness, suggesting that mechanistic details can alter even the qualitative nature of specific predictions. Furthermore, the populations evolved under a lower relatedness caused less mortality and damage to insect hosts, showing how signal evolution leads to strain diversity influencing the evolution of virulence in the opposite direction to that usually predicted by theory.

LI02Tu1700

Metabolic regulation of community behavior in *Pseudomonas aeruginosa*

Lars Dietrich

Columbia University. 1212 Amsterdam Avenue, 1108 Fairchild Center, New York, USA

Studies of signaling cascades can reveal important mechanisms driving multicellular development, but the models that emerge often lack critical links to environmental cues and metabolites. We study the effects of extra- and intracellular chemistry on biofilm morphogenesis in the pathogenic bacterium *Pseudomonas aeruginosa*, which produces oxidizing pigments called phenazines. While wild-type colonies are relatively smooth, phenazine-null mutant colonies are wrinkled. Initiation of wrinkling coincides with a maximally reduced intracellular redox state, suggesting that wrinkling is a mechanism for coping with electron acceptor limitation. Consistent with this, provision of nitrate renders phenazine-null colonies smooth. Mutational analyses and *in situ* expression profiling have revealed roles for PAS-domain and other redox-sensing regulatory proteins, as well as genes involved in motility and matrix production, in colony morphogenesis. To characterize endogenous electron acceptor production, we have developed a novel chip that serves as a growth support for biofilms and allows electrochemical detection and spatiotemporal resolution of phenazine production *in situ*. We are further developing this chip for detection of various redox-active metabolites. Through these diverse approaches, we are developing a broad picture of the mechanisms and metabolites that exert an integrated influence over redox homeostasis in *P. aeruginosa* biofilms.

LI03

Cell Cycle

LI03Mo0900

The bacterial cell cycle: membranes, walls and division machines

David Adams, Ling Juan Wu, Romain Mercier, Yoshikazu Kawai and Jeff Errington*

Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, UK

Most bacterial cells dedicate a great deal of metabolic energy to synthesis of an external cell wall. The wall needs to expand to accommodate cell growth, and division requires the formation of a cross wall. Thus, the control of cell wall synthesis is a major element of cell cycle regulation in bacteria. Cell division is normally orchestrated by an essential and widely conserved protein FtsZ, which is a structural homologue of eukaryotic tubulin. FtsZ assembles to form a ring-like structure at the site of impending division and it controls the constriction of cell membrane and wall layers, as well as recruiting the various structural proteins needed for these events. Proper timing and positioning of the division machinery is controlled by 2 negative regulatory systems called the Min and nucleoid occlusion (NO) systems. Recent progress in understanding the NO system of *Bacillus subtilis* will be described. Recently, our lab has been investigating remarkable cell wall defective variants called L-forms. These cells loose many characteristic features of normal cell cycle progression including becoming completely independent of the FtsZ-based division machine. Proliferation of L-forms seems to be driven instead by simple biophysical processes largely involving membrane dynamics. The ability to switch into an L-form state appears to be widespread throughout the bacterial domain. Wu, L.J. and Errington, J. (2004) Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. *Cell* 117, 915-925. Wu LJ, Ishikawa S, Kawai Y, Oshima T, Ogasawara N, Errington J. (2009) Noc protein binds to specific DNA sequences to coordinate cell division with chromosome segregation. E*MBO* J. 28, 1940-1952. Mercier R, Kawai Y, Errington J. (2013) Excess membrane synthesis drives a primitive mode of cell proliferation. *Cell* 152, 997-1007.

LI03Mo0930

Control of cell cycle transcription during G1 and S phases in yeast

Robert de Bruin

University of London, London, UK

A regulatory network the general features of which are conserved from yeast to humans controls the eukaryotic cell cycle. It proceeds through tightly regulated transitions to ensure that specific events take place in an orderly manner. Our work investigates the G1/S transcriptional network involved in two crucial aspects of cell cycle regulation: cell division cycle control and maintenance of genome stability. This network comprise a large number of dosage-sensitive co-regulated genes, which encode proteins involved in many essential cellular functions such as cell division control, DNA

replication, cell growth and maintenance of genome stability. Our work and that of others shows that G1/S transcriptional regulation depends on evolutionary conserved mechanisms. Recently we uncovered an auto-regulatory negative feedback loop where transcriptional repressors, G1/S target themselves, accumulate and bind to G1/S promoters to turn off transcription when cells progress into S phase. Interestingly in response to replication stress checkpoint protein kinases directly target and inactivate these G1/S transcriptional repressors in order to keep transcription on to maintain genomic stability. So our work establishes that these G1/S repressors, lie at the nexus of the molecular mechanism involved in temporally confining G1/S transcription and the transcriptional response to DNA replication stress.

LI03Mo1000

Bactofilins as polar landmarks in *Myxococcus xanthus*

Lin Lin [1,2], Andrea Harms [1] , Lotte Søgaard-Andersen [1,3], Martin Thanbichler [1,2,3]

[1] Max Planck Institute for Terrestrial Microbiolology, [2] Faculty of Biology, Philipps-Universität, and [3] LOEWE Center for Synthetic Microbiology, Marburg, Germany

Bactofilins are a new group of cytoskeletal proteins that assemble into stable polymeric structures without the need for nucleotide cofactors. Although widespread among bacteria, their polymerization properties and biological roles are still incompletely understood. Here, we report a detailed analysis of bactofilin function in the social bacterium *Myxococcus xanthus*, a species containing four bactofilin homologs. Three of these proteins (BacNOP) are encoded in an operon and co-polymerize into filamentous structures that extend from both cell poles. These polymers were previously shown to interact with the small GTPase SofG, thereby ensuring proper cell polarity and type IV pilus-driven motility. In addition, we have now identified a role for BacNOP in chromosome segregation and arrangement. We show that the bactofilin filaments dynamically interact with both the chromosome partitioning ATPase ParA and the centromere-binding protein ParB to immobilize the chromosomal origin regions in the subpolar regions of the cell. In *M. xanthus*, bactofilins thus form multi-purpose cytoskeletal scaffolds that recruit distinct sets of proteins to defined subpolar locations, thereby possibly keeping the tips of the cell accessible to the complex motility machinery.

LI03Mo1100

Offered paper – Indole acts as an ionophore to regulate *E. coli* cell division

Hannah Gaimster, Jahangir Cama, Silvia Hernandez-Ainsa, Ulrich Keyser, David Summers

Cambridge University, Cambridge, UK

Indole is an aromatic heterocyclic signalling molecule produced by over 85 species of Gram-positive and Gram-negative bacteria. It has diverse and well-characterised roles, including modulation of biofilm formation, virulence and stress responses. These effects are induced by indole concentrations of 0.5-1.0 mM that are similar to those found in the supernatant of *E. coli* stationary phase culture. We have shown that indole also inhibits *E. coli* growth and cell division but these effects are not seen at concentrations of less than 4–5 mM. Under these conditions indole acts as a proton ionophore and, by reducing the electrochemical potential across the cytoplasmic membrane, deactivates MinCD oscillation and prevents formation of the FtsZ ring. Until recently it was questionable whether such high concentrations of indole are experienced by bacteria in the natural environment. However we have recently demonstrated that under circumstances such as the transition from exponential to stationary phase, *E. coli* cells experience a brief pulse of indole that transiently exceeds 50 mM. We propose that this novel mode of “pulse signaling” by indole plays an important role in the control of cell division during stationary phase entry and, potentially during a variety of stress responses.

LI03Mo1130

Bacterial cell division and morphogenesis

Waldemar Vollmer

Institute for Cell and Molecular Biosciences, The Centre for Bacterial Cell Biology, Newcastle University, Richardson Road, Newcastle upon Tyne, NE2 4AX, UK

The bacterial cell envelope has a complex structure and must be enlarged when the cell grows and divides. Gram-negative bacteria have in their periplasm a mainly single-layered peptidoglycan sacculus that protects the cell from lysis due to the turgor and that is required to maintain cell shape. During growth and cell division the sacculus is enlarged by the coordinated activities of peptidoglycan synthases (penicillin-binding proteins, PBPs) and hydrolases, which presumably form multi-enzyme complexes. The molecular mechanisms of peptidoglycan growth and its regulation are not well understood. Cytoskeletal proteins and associated cell morphogenesis proteins control peptidoglycan synthesis from inside the cell, within large cell envelope assemblies called elongasome and divisome. Recent work showed that peptidoglycan growth is also regulated from outside the sacculus in *Escherichia coli* and likely other Gram-negative bacteria. The outer membrane lipoproteins LpoA and LpoB are required for the functioning of the main peptidoglycan synthases, PBP1A and PBP1B, respectively. Lpo proteins interact with their cognate PBP and activate the transpeptidase function *in vitro*. The Lpo-interaction occurs with small, non-catalytic domains that have co-evolved with the outer-membrane activators. Presumably, Lpo proteins regulate peptidoglycan synthesis form outside the sacculus to maintain a homogenous peptidoglycan surface density and thickness, and to fine-tune peptidoglycan growth rate. The latest data on the activation of PBPs by Lpo proteins will be presented. PBP1A-LpoA are mainly active during cell elongation, and PBP1B-LpoB are members of the divisome and provide the main peptidoglycan synthesis activity during cell division.

LI03Mo1400

Using yeast functional genomics to explore pathways regulating cell cycle-dependent transcription

Brenda J. Andrews

The Donnelly Centre, University of Toronto, Toronto, Ontario, Canada M5S 3E1

To discover general principles of genetic networks and to define biological pathways, our group has focused on the systematic

Please note: Abstracts are published as received from the authors and are not subject to editing.

identification of genetic interactions in budding yeast. The Reporter Synthetic Genetic Array (R-SGA) method combines our standard SGA procedure, which automates the introduction of any marked allele of interest into an array of yeast mutants, with a dual-colour reporter system. In R-SGA screens, a GFP gene driven by a cell cycle-regulated promoter and an RFP gene driven by a control promoter are introduced in arrays of yeast mutants and fluorescence intensity is measured from colonies, allowing rapid assessment of the effect of several thousand genetic perturbations on promoter activity. We have generated a dataset covering 27 cell cycle-regulated promoters, which we have analysed to survey the effects of gene overexpression or deletion and to discover new regulators. For example, our screens using histone gene promoters, which are expressed during S-phase, identified a number of regulators, including a KEN box-containing protein of unknown function, Spt21, which has been linked to histone transcription due to its association with Spt10, a putative lysine acetyltransferase. We have used biochemical and genetic experiments to show that Spt21 is a key cell cycle oscillator that serves as a master regulator of histone gene expression.

LI03Mo1430

Identifying and characterising protein kinases regulating cell division in *Trypanosoma brucei*

Nathaniel G. Jones[1,2], Elizabeth B. Thomas[2], Elaine Brown[1,2], Nicholas J Dickens[1,2], Jeremy C. Mottram[1,2] and Tansy C. Hammarton[2]

[1]Wellcome Trust Centre for Molecular Parasitology and [2]Institute of Infection, Immunity & Inflammation, College of Medical, Veterinary & Life Science, University of Glasgow, UK

The *Trypanosoma brucei* kinome comprises 158 eukaryotic protein kinases (ePKs), 12 potential pseudokinases and 20 atypical protein kinases. To facilitate functional study of the kinome, we have generated a library of RNA interference (RNAi) cell lines targeting all 190 protein kinases. Screening the kinome library for cell lines displaying a growth defect following RNAi induction identified 42 protein kinases essential for normal proliferation of the *T. brucei* bloodstream stage in culture. Further screening showed 24 of these essential kinases to be required for cell cycle progression, including 15 protein kinases not previously linked to cell cycle control. These protein kinases were found to regulate kinetoplast replication/segregation, mitosis and/or cytokinesis, but surprisingly no kinases regulating G1 or S phase were identified in the screen. A separate screen also identified two protein kinases which negatively regulate bloodstream form to procyclic form differentiation. The challenges for the future will be to exploit the functional information gained to generate new therapeutics for Trypanosomiasis and to elucidate the protein kinase networks regulating cell cycle progression and differentiation throughout the life cycle of *T. brucei*.

LI03Mo1500

Offered paper – NrdR and the regulation of ribonucleotide reductase genes in *Escherichia coli*

Stephen Spiro, Brandon McKethan

University of Texas at Dallas, Richardson, Texas, USA

Escherichia coli expresses three ribonucleotide reductases (RNRs) encoded by *nrdAB, nrdDG and nrdEF*. The housekeeping enzyme is encoded by *nrdAB*, expression of which is regulated by the cell cycle, by a mechanism that has not been defined. All three *nrd* promoters are negatively regulated by NrdR, a small protein which has a zinc finger like DNA binding domain and a nucleotide binding domain. As-prepared NrdR contains a complex mixture of (deoxy) adenosine monophosphates, diphosphates and triphosphates, which cannot be completely removed by extensive dialysis. We have shown that (deoxy)adenosine phosphates bind to apo-NrdR with similar affinities, and that the binding of triphosphates (but not monophosphates) is negatively cooperative. Negative cooperativity provides a mechanism to allow NrdR to bind both ATP and dATP *in vivo*, despite the very large difference in their concentrations. NrdR binding to *nrd* promoter DNA *in vitro* is inhibited by tri and diphosphates, but not by monophosphates. We will describe our current understanding of the mechanism of transcriptional regulation by NrdR, and speculate about a possible link between NrdR and cell cycle regulation of the *nrd* promoters.

McKethan, B.L. and Spiro, S. (2013) Cooperative and allosterically controlled nucleotide binding regulates the DNA binding activity of NrdR. PMID: 23941567.

LI03Mo1600

Architecture of the FtsZ ring *in vivo* and *in vitro* indicates a sliding filament mechanism of constriction

Piotr Szwedziak, Qing Wang, Matthew Tsim, Jan Löwe

MRC Laboratory of Molecular Biology, Structural Studies Division, Francis Crick Avenue, Cambridge, CB2 0QH, United Kingdom

Cell division in most bacteria and archaea is facilitated by a constricting ring structure, containing the protein FtsZ. FtsA anchors FtsZ to the membrane and FtsZ recruits other proteins of the divisome, the machine that divides the inner and outer cell membrane and the cell wall. During division, the FtsZ ring decreases in diameter through an unknown mechanism, eventually separating the two daughter cells. Here we show the organisation of FtsZ filaments in constricting *E. coli* cells and in liposomes being constricted *in vitro* by a ring of FtsZ and FtsA filaments, all using electron cryotomography. In *E. coli* cells, mutant FtsZ(D212) forms bands of double filaments that form complete rings around the cell's perimeter. Over-expression of FtsZ and FtsA in *E. coli* leads to extra division sites that separate off minicells and hence function in division. We therefore performed in vitro reconstitution of constriction by encapsulating FtsZ and FtsA proteins on the inside of liposomes generated from *E. coli* lipids. This leads to spontaneous dumbbell-shaped membrane constrictions that co-localise with rings of filaments. Electron cryotomography of the ring structures formed allowed detailed visualisation of the constrictions as well as complete tracing of the helical paths of the filaments. Since *in vitro* reconstitution of FtsZ-based constriction does not require energy from nucleotide turnover and constrictions occur only at sites where rings have formed, we put forward a mechanism of FtsZ-based cell constriction that is driven by filament sliding and condensation.

LI03Mo1630

Molecular analysis of the quorum sensing signalling pathway in *Trypanosoma brucei*

Keith R. Matthews

Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh, UK

African trypanosomes are sustained in the bloodstream of their mammalian hosts by their extreme capacity for antigenic variation. However, for life-cycle progression, trypanosomes also must generate transmission stages called stumpy forms that are pre-adapted to survive when taken up during the bloodmeal of the disease vector, tsetse flies. These stumpy forms are non proliferative and morphologically distinct, and show particular sensitivity to environmental cues that signal entry to the tsetse fly. Interestingly, the generation of stumpy forms is also regulated and periodic in the mammalian blood, this being governed by a density-sensing mechanism whereby a parasite-derived signal drives cell-cycle arrest and cellular development both to optimise transmission and to prevent uncontrolled parasite multiplication overwhelming the host.

The presentation will detail recent developments in our understanding of the molecular mechanisms and signalling pathways that underpin the production of stumpy forms in the host bloodstream. These discoveries will be discussed in the context of conserved eukaryotic signalling and differentiation mechanisms, providing a molecular framework for the understanding of sociality and environmental sensing in a major eukaryotic pathogen.

LI03Tu0900

Visualizing genome instability in *Schizosaccharomyces pombe*

Susan L Forsburg

Program in Molecular & Computational Biology, University of Southern California, Los Angeles CA USA

Maintenance of genome stability during S phase is the first line of cellular defense against cancer. Using the fission yeast as a model system, we are investigating the response to genome stress using novel imaging methods. In live cells, we can distinguish different forms of replication fork collapse by the appearance of damage foci including RPA and Rad52, and monitor disruptions in chromosome segregation labeled histones. Strikingly, pedigree analysis of live cells under stress and during recovery identifies unique sub-populations with different behavior from the majority of cells. We have identified several cases where a sub-population of cells evades normal checkpoint control and continues to divide. In one case, this leads to an unusual phenotype of centromere fragmentation and mitosis with an unreplicated genome (also seen in some mammalian cell systems). In another case, we see evidence for a memory for DNA damage even after the genotoxic agent is removed. Because chemotherapeutic agents cause DNA damage, rare survivors can generate additional mutations that contribute to drug resistance in cancer cells.

LI03Tu0930

Evolution and Maintenance of Circular Chromosomes in bacteria

Gaëlle Demarre[1,2], Elisa Galli[1,2], Ariane David[1,2], Christophe Possoz[1,2], Evelyne Paly[1,2], and François-Xavier Barre[1,2]

[1] CNRS, Centre de Génétique Moléculaire, 91198 Gif-sur-Yvette, France

[2] Université Paris-Sud, 91405 Orsay, France

In all living cells on earth, genetic information is encoded on long double stranded DNA helices, the chromosomes, whose duplication results from the combined replication of the two complementary strands of the helix. Nevertheless, eukaryotes and bacteria employ different strategies to manage their genetic material. In particular, there is no apparent limit to the size and numbers of chromosomes in eukaryotic cells, in the evolution of which chromosome scission and whole genome duplication events are frequent. In contrast, most bacteria harbour a single chromosome and, in the rare case in which the genetic material is divided on several chromosomes, the extra-numerous chromosomes are related to plasmids and possess their own replication initiation machinery and their own segregation machinery.

Using a combination of fluorescent microscopy and genetic observations, we compared the choreography of segregation of the two *Vibrio cholerae* chromosomes. We found that they both adopted a longitudinal organisation. The replication arms of the larger of the two chromosomes *(chl)* extend from pole to pole, its *oriC* and terminus regions being anchored at the old pole and at the new pole, respectively. Interestingly, the smaller chromosome *(chll)* appears embedded in chl, its replication arms extending only over the younger cell half.

The *chl* partition machinery *ParAB/parS*1 was the main contributor to its arrangement within the cell and in particular, to the polar localisation of its oriC. However, *chl oriC* remained mobilised towards the old pole in the absence of *parABS1* and our results indicated that this was directy related to the replication program. The MatP/*matS* system was the main contributor to the septal localisation of *chll* sister termini. It also contributed to the positioning of the terminus region of *chl*. However *chl* sister termini remained together at midcell in its absence. Again, our results indicated that this was directy related to the replication program. Based on these results, we discuss the rules that govern the management of the genetic material in bacteria.

LI03Tu1000

Patterns of construction and inheritance during the proliferative and differentiation cell cycles of Trypanosomes.

Keith Gull

Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Kinetoplastid parasites such as the African trypanosome, *Trypanosoma brucei* and the various *Leishmania* species are important eukaryotic microbes with precise cell shapes and many single copy organelles. Their complex life cycle in an insect vector and a mammalian host is defined by a set of individual cell types produced by the ordered processes of both proliferative

Please note: Abstracts are published as received from the authors and are not subject to editing.

and differentiation cell cycles. We have studied the processes that explain how the microtubule cytoskeleton is ordered, replicated and inherited; also, how these events are coordinated by the regulatory molecules of the cell cycle but often in a manner that apparently differs to cells of those distant cousins, yeast and man.

LI03Tu1100

Regulation of DNA replication - insights from comparative genomics

Carolin A Müller and Conrad A. Nieduszynski

Centre for Genetics & Genomics, School of Life Sciences, University of Nottingham, Nottingham NG7 2UH, UK

Accurate and complete replication of the genome is crucial for life. DNA replication is initiated at discrete chromosomal sites called replication origins, with eukaryotic genomes replicated from hundreds or thousands of origins. These origins initiate replication at particular times during S phase, giving rise to a characteristic temporal order for chromosome replication. Aberrant DNA replication timing is associated with increased mutagenesis and genomic instability. We have compared genome replication in a range of yeast species to investigate the mechanisms regulating genome replication timing. Using deep sequencing to measure DNA copy number changes during S phase has allowed us to determine the temporal order of genome replication in eleven different yeast species. Comparisons between closely related species revealed that the location and activity of replication origins are highly conserved. Divergence in the activity of a minority of replication origins has allowed us to identify novel mechanisms regulating origin activity. In comparisons between more divergent species, we find that the location and activity of individual origins are rarely conserved. However, these comparisons identify conserved features of genome replication that reveal the fundamental requirements for regulating the temporal order of genome replication.

LI03Tu1130

Offered paper – Life Without DNA Replication Origins

Michelle Hawkins, Sunir Malla, Martin J. Blythe, Conrad A. Nieduszynski and Thorsten Allers

School of Life Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK

In 1963, François Jacob, Sydney Brenner and François Cuzin proposed the 'replicon model' to explain how DNA replication is regulated. They proposed that replication does not initiate at random points in the genome, rather it initiates at defined locations termed replication origins. Half a century later, this model has become a central tenet of biology. We have recently shown that genome replication does not require defined origins. In the archaeon *Haloferax volcanii*, deletion of all origins results not in cell death but in significantly faster growth. We show that origin-less cells use homologous recombination to initiate DNA replication, and that this occurs randomly throughout the genome. These results lead us to question the need for replication origins and why they have evolved – we suggest that origins may have arisen as selfish genes.

LI03Tu1145

Offered paper – When replication forks collide – how termination of DNA replication affects genomic stability

Christian Rudolph

Brunel University, Uxbridge, Middlesex, UK

That replication fork collisions threaten genomic stability appears to be a paradox because they are an intrinsic feature of replication termination. In the bacterial cell cycle termination is normally limited to one event. We were able to show that in *E. coli* fork collisions can have surprisingly severe consequences [1]. Both RecG helicase and 3' exonucleases are key players that defuse intermediates resulting from fork collisions. Without these proteins, a single termination event appears to be able to trigger significant amounts of over-replication in the termination area. Progression of synthesis is normally trapped by the *ter*/Tus replication fork trap. However, inactivation of the Tus terminator protein allows the forks established to continue, resulting in a level of synthesis that is sufficient to support chromosome duplication in the absence of a functional replication origin. Thus, in these cells we observed the paradoxical situation that termination of synthesis is priming new rounds of replication. The severity of the effects observed in bacteria poses the question of how the hundreds of collision events that occur in eukaryotic cells are coordinated.

[1] Rudolph *et al.* 2013. Nature 500 (7464):608-611

LI03Tu1400

The ciliate *Tetrahymena* uses unorthodox mechanisms to pair and recombine meiotic chromosomes

Josef Loidl

Department of Chromosome Biology, University of Vienna, Austria

During meiosis two homologous parental chromosome sets are reduced to a single mixed set. The mutual recognition and pairing of homologous parental chromosomes allow their crossover recombination and orderly segregation. The question of how homologous chromosomes find each other has occupied cell biologists for many decades. *Tetrahymena* offers an interesting solution to the pairing problem. Its meiotic nuclei are enormously elongated. Within these tube-shaped nuclei, chromosomes arrange in bundles with centromeres and telomeres clustering at opposite ends. This arrangement positions homologous chromosome regions near each other in the nucleus. Nuclear elongation is triggered by the deliberate induction of DNA double-strand breaks (DSBs), whose other, better understood, role is the initiation of crossing over. DSBs are sensed by ATR, and ultimately induce microtubule-dependent nuclear stretching. This sophisticated pairing mechanism contrasts with a simplified molecular recombination machinery compared to multicellular organisms. For example, *Tetrahymena* does not possess a synaptonemal complex, and meiotic recombination is largely dependent on a set of mitotic DNA repair proteins. The comparison of extant meioses of *Tetrahymena*, as the only protist representative studied so far, and the kingdoms of animals, plants, and fungi allows the identification of a core meiotic machinery and its adaptations during eukaryotic evolution.

LI03Tu1430

Starvation responses and cell cycle control

Sean Crosson

University of Chicago, Dept. of Biochemistry and Molecular Biology, Chicago, IL, USA

Caulobacter crescentus differentiates from a motile, foraging swarmer cell into sessile, replication-competent stalked cell during its cell cycle. This developmental transition is inhibited by nutrient deprivation to favor the motile swarmer state. We identify two cell-cycle regulatory signals, ppGpp and polyphosphate (polyP), that inhibit the swarmer-to-stalked transition in both complex and glucose-exhausted media, thereby increasing the proportion of swarmer cells in mixed culture. Upon depletion of available carbon, swarmer cells lacking the ability to synthesize ppGpp or polyP improperly initiate chromosome replication, proteolyze the replication inhibitor CtrA, localize the cell-fate determinant DivJ, and develop polar stalks. Furthermore, we show that swarmer cells produce more ppGpp than stalked cells upon starvation. These results provide evidence that ppGpp and polyP are cell-type specific developmental regulators.

LI03Tu1500

Offered paper – Overlapping functions of ADP-ribosyltransferases in signalling DNA damage in *Dictyostelium*

C. Anne-Marie Couto, Duen-Wei Hsu, Nicholas D. Lakin and Catherine J. Pears

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

ADP-ribosylation is the addition of mono- or poly-ADP-ribose units to proteins catalysed by ADP-ribosyltransferases (ARTs). Although vertebrates contain multiple proteins with predicted ART activity, these proteins are generally not apparent in single cell eukaryotes. The genetically tractable social amoeba *Dictyostelium discoideum* exhibits unusual conservation of certain DNA repair pathways previously identified only in vertebrates, including conservation of multiple ARTs. Similar to vertebrates, two ARTs (Adprt1b and Adprt2) are required for *Dictyostelium* to tolerate DNA single strand breaks (SSBs). In contrast, a third ART (Adprt1a) is required specifically for DNA double strand break (DSB) tolerance. Adprt1a is enriched in chromatin following induction of DSBs and regulates non-homologous end-joining (NHEJ)-mediated repair by promoting accumulation of Ku at DNA breaks, which is dependent on an ADP-ribose interaction domain in Ku. Although cells disrupted in Adprt2 are sensitive to SSBs, SSBs still cause robust ADP-ribosylation in these cells. We present data to suggest that loss of Adprt2 results in accumulation of DSBs that are subsequently signalled by Adprt1a to promote repair by NHEJ. These data indicate overlapping functions between different ARTs in signalling DNA damage and illustrate a critical requirement for NHEJ to maintain cell viability in the absence of an effective SSB response.

LI03Tu1600

Redox sensing mechanisms that control cell cycle progression

Brian Morgan

Institute for Cell and Molecular Biosciences, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4HH, Tyne and Wear, UK

Oxidative stress-induced cell damage caused by reactive oxygen species (ROS) is associated with common human diseases, such as cancer, and also with shortened lifespan. However, ROS are also used by the immune system to defend against disease-causing organisms and play important roles as signalling molecules. Thus, to understand how cells function in ageing and disease it is important to explore the mechanisms by which cells sense and respond to ROS. An important aspect of cell responses to oxidative stress is to inhibit cell cycle progression to prevent damage and to allow repair of damage and restoration of homeostasis. Ubiquitin (Ub)/ubiquitin-like (Ubl) protein modifications regulate fundamental biological processes including the cell division cycle and, excitingly, we recently demonstrated in the model yeast *Saccharomyces cerevisiae* that the specific sensitivity of a conserved ubiquitin pathway enzyme, Cdc34, to oxidation coordinates cell cycle delay to prevent oxidative stress-induced damage. We and others have also discovered that conserved antioxidants, previously thought to mainly act in restoring redox homeostasis, play key roles in sensing and signalling the presence of ROS. Here, work will be described about our investigations of regulation of the cell cycle by oxidative stress/antioxidants.

LI03Tu1630

Cell cycle control in response to proteotoxic stress

Kristina Jonas

LOEWE Center for Synthetic Microbiology, Philipps University Marburg, 35032 Marburg, Germany

The decision to initiate DNA replication is a critical step in the cell cycle of all organisms. Cells often delay replication in the face of stressful conditions, but the underlying mechanisms remain incompletely defined. Our recent work demonstrates that in response to unfolded protein stress the bacterium *Caulobacter crescentus* arrests the cell cycle by rapidly degrading DnaA, the conserved bacterial replication initiator. Under favorable conditions the protease Lon degrades DnaA at a slower rate. However, when unfolded proteins accumulate as a consequence of heat shock or defective chaperones, DnaA degradation is triggered in two ways. First, unfolded proteins induce Lon synthesis as part of the heat shock response. Second, unfolded proteins, which are Lon substrates themselves, can allosterically activate Lon to degrade DnaA. Together, these two modes of Lon activation help to ensure a cessation of DNA replication and cell cycle progression following heat shock or chaperone loss. We also find that DnaA degradation by Lon is required to remove the replication initiator when cells arrest growth during stationary phase or starvation. Altogether, these findings suggest that stress-induced proteolysis of replication factors provides an important adaptive mechanism that helps cells survive various stress conditions.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI04

Metabolic engineering for biotechnology: fundamental knowledge to societal benefit

LI04Mo0900

Engineering non-conventional yeasts for industrial biotechnology

Thomas W. Jeffries

University of Wisconsin-Madison, USA

There are about 1340 yeast species or varieties of yeasts1 with many more being added each year. Only 10 of these belong to *Sacharomyces*, which is by far the most prevalent genus for baking, brewing and wine making. Species from at least 18 other genera of *non*-conventional yeasts, however, are used in various biotechnological applications. The development of highly versatile methods for genetic transformation, widely applicable selection markers, rapidly falling costs for genomic sequencing and extensive databases on annotated enzymes and pathways have opened up many opportunities for metabolic engineering of yeasts with basic physiological properties extending far beyond those of *S. cerevisiae*. Many non-conventional yeasts are better for heterologous protein production *(Komagataella pastoris, Yarrowia lipolytica*), fermentation of cellulosic and hemicellulosic sugars to ethanol *(Scheffersomyces stipitis, Spathaspora passalidarum, Kluyveromyces marxianus)*, lipid production *(Lipomyces starkeyi, Rhodosporidium toruloides)*, carotenoids, isoprenoids, and astaxanthin *(Rhodotorula, Phaffia rhodozyma)*, omega-3 and omega-6 fatty acids *(Y. lipolytica)*, isobutanol *(Candida tropicalis)*, and metabolically engineered fuels, industrial and specialty chemicals. This lecture will explore the genomics and metabolic engineering of unconventional yeasts for biotechnological applications.

1. Kurtzman CP, Fell JW, & Boekhout T (2011) *The yeasts: a taxonomic study* (Elsevier) 5th Ed.

LI04Mo0930

Mining algal genomes for the biosynthesis of secondary metabolites

Natalie Oppenhäuser[1], Ekaterina Shelest[2], Arthur R. Grossman[3], Christian Hertweck[2], Maria Mittag, Severin Sasso[1]

[1] Institute of General Botany and Plant Physiology, Friedrich Schiller University, Jena, Germany [2] Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany [3] Department of Plant Biology, Carnegie Institution for Science, Stanford CA, USA

Over the last few years, full genome sequences of around 30 different algae have become available. Most sequenced species are microalgae. Genomic data help us to delineate biosynthetic routes to known compounds, but also give hints for the existence of unknown compounds. For example, many sequenced microalgae are predicted to contain large genes for type I polyketide synthases and non-ribosomal peptide synthetases. Such enzymes are well known from the secondary metabolism of bacteria and fungi, where they are involved in the biosynthesis of antibiotics and many other bioactive compounds. In sequenced microalgae however, the function of these enzymes and the resulting polyketides and non-ribosomal peptides remains enigmatic, and their existence has not been verified experimentally. First results of ongoing experimental investigations will be presented, together with a discussion of evolutionary implications of these findings.

LI04Mo1030

Towards Biotechnology 2.0: synthetic biology of bioactive molecules

Eriko Takano

Manchester Institute of Biotechnology, Faculty of Life Sciences, University of Manchester, UK

Our ability to readily sequence complete genomes and to manipulate/re-design them on a large scale enables the design and construction of organisms with new functionalities of unprecedented scope ("synthetic biology"). We explore these possibilities in the context of bioactive molecule production. Many microorganisms already have the machinery to produce diverse bioactive molecules. As a first step towards re-engineering bioactive molecule biosynthesis for enhanced productivity and diversity, we aim to understand the transcriptional circuitry controlling the native biosynthetic gene clusters so we can refactor it using orthogonal transcription and translation mechanisms while maintaining the subtle relationships necessary for optimal function, e.g. by use of promoter libraries, signalling molecules and ncRNA. In addition, we are expanding our collection of computational tools for the detection and analysis of secondary metabolite biosynthesis gene clusters, to enrich our library of parts and building blocks for pathway engineering. Furthermore, we are using computational modelling (constraint-based descriptions of bacterial metabolism) to identify suitable overproduction hosts and pinpoint biosynthetic bottlenecks to target for further cellular engineering in a synthetic biology strategy.

LI04Mo1100

Offered paper – Towards consolidated bioprocessing: Engineering *Geobacillus thermoglucosidasius* to secrete heterologous glycosyl hydrolases

Jeremy Bartosiak-Jentys, David Leak

University of Bath, UK

Reducing the dependence on fossil fuel derived petrochemicals is a significant global challenge. To this end, plant derived carbohydrate can serve as the starting material for fuel/chemical production by engineered microbes in a biorefinery. However, utilising accessible sugars from edible crops is surrounded by socio-political sensitivities; while non-edible lignocellulose derived carbohydrate is highly recalcitrant and requires expensive multistep processing. Consolidated bioprocessing (CBP), where the capability to convert lignocellulose to a useful product in one step is engineered into a single microorganism, has long been championed as a way of reducing these processing costs. However, CBP remains, at best, a partially tested hypothesis.

The thermophilic bacterium *Geobacillus thermoglucosidasius* has been engineered for commercial bioethanol production but lacks cellulolytic capability. Glycosyl hydrolase (GH) activities (exocellulase, endocellulase, xylanase and arabinofuranosidase) involved in the saccharification of the energy crop *Miscanthus x giganteus* were isolated and identified from compost inoculated cultures. Genes encoding these, as well as known thermophilic GHs,

ABSTRACTS

were assembled in a modular expression cassette, which allowed the facile interchange of parts. GH activities were measured in *G. thermoglucosidasius* culture supernatants, indicating successful secretion of active enzyme. Co-culture of GH expressing strains on M. giganteus resulted in 60-80% carbon utilisation within 48hrs

LI04Mo1115

Offered paper – Disrupting the acetate production pathway in the thermophilic ethanologen *Geobacillus thermoglucosidasius*

Christopher A. Hills, Kirstin Eley, Michael J. Danson

University of Bath, UK

The thermophile, *Geobacillus thermoglucosidasius*, is capable of producing ethanol from lignocellulosic derived sources. It has been demonstrated that an engineered strain of this organism, with disruptions to both the lactate dehydrogenase and pyruvate formate lyase encoding genes and an up-regulated pyruvate dehydrogenase complex, can produce ethanol in yields in excess of 90% of theoretical (1). However, small but significant quantities of certain organic acids, particularly acetate, are produced when grown under fermentation conditions.

The current project aims to study the metabolic flux of this strain, in particular the formation of acetate from acetyl-CoA in relation to ethanol production, in order to increase ethanol yields by further genetic manipulations. Acetate formation is a result of the action of phosphotransacetylase (PTA) and acetate kinase (AK), which convert acetyl-CoA to acetate via an acetyl-phosphate intermediate. This report describes the creation and characterisation of a *Geobacillus* strain in which the pta gene has been deleted in order to divert metabolic flux away from acetate, towards ethanol production. Upon characterisation of this strain a novel acetate producing pathway based on enzyme promiscuity has been discovered.

(1)Cripps R.E. *et al*. (2009) Metabolic engineering of *Geobacillus thermoglucosidasius* for high yield ethanol production. Metabolic Engineering. 11, 398-408

LI04Mo1130

Facing up to biological complexity: metabolic engineering though synthetic biology

Laurent Malivert, Michael Sadowski, Sean Ward and **Markus Gershater**

Synthace Ltd, London, UK

Biological systems have huge potential to produce high value products in a highly elegant and sustainable manner. However, the biological complexity presents great challenges when designing and implementing effective bioprocesses that are robust on a commercially relevant scale. Here, we present part of Synthace's strategy for using cutting edge methodology to pragmatically optimise effective biological processes for the production of high value specialty chemicals.

LI04Mo1400

Genome-sequencing based discovery of novel glycosyl hydrolases allows production of highly Man-6-phosphate substituted therapeutic lysosomal enzymes in glyco-engineered yeast

Nico Callewaert

Unit for Medical Biotechnology, Inflammation Research Center, VIB, Ghent, Belgium and Laboratory for Protein Biochemistry and Biomolecular Engineering, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium

At present, the biotechnological production of lysosomal enzymes for enzyme replacement therapy occurs in mammalian cells, at enormous cost. To lower the cost and increase the therapeutic efficacy, yeasts could be used as a production platform for these enzymes, but yeasts produces Man-Pi-6-Man glycotopes on their secreted and cell wall-associated mannoproteins, not the required Pi-6-Man structures. We were able to remove the mannose of these capped mannose-6-phosphate structures with the extracellular medium of a *Cellulosimicrobium cellulans* strain. To identify the uncapping activity, the genome of *C. cellulans* was sequenced and blasted for homologs of known alpha-mannosidases. After the identification of 5 genes of the GH92 family (*CcMan1-5*), they were synthesized and expressed in *E. coli*. Activity tests showed that CcMan4 was active as an alpha-1,2-mannosidase acting on phosphorylated high-mannose glycans, and CcMan5 as the desired novel uncapping enzyme. Crystallization of the N terminal domain gave more insight in the special uncapping activity of this enzyme and explained the absence of the typical mannosidase activity.

In combination with our biosynthetic engineering of yeasts to overproduce the Man-Pi-6-Man glycotopes, the discovery of this mannosidase and this uncapping enzyme opens perspectives to produce lysosomal enzymes in yeasts

LI04Mo1430

Engineering wine yeast for reduced ethanol production

Valentin Tilloy, Axelle Cadiere and **Sylvie Dequin**

INRA, UMR1083, 2 Place Viala, F-34060 Montpellier, France

The development of microbial strategies to develop *Saccharomyces cerevisiae* yeast strains with reduced ethanol yield is a major challenge in wine research. Various approaches based on engineering of central carbon and redox metabolism have been developed. As a proof-of-concept, low alcohol wine yeasts with carbons rerouted towards glycerol and 2,3-butanediol without accumulation of undesirable by-products have been developed, which can reduce alcohol in wine by up 3%. More recently, to overcome restrictions on the use of yeasts obtained by genetic engineering in the food industry, *in vivo* evolutionary approaches have been used to push genome evolution towards specific metabolic traits. Based on a better understanding of cell physiology, we imposed physiological constraints to promote the selection of strains with increased flux through alternative pathways to glycolysis, such as the the pentose phosphate or the glycerol pathways. New strains overproducing esters or producing more glycerol and less ethanol were obtained, representing new tools to improve the sensorial quality of fermented beverages. Besides, the elucidation of the genetic and metabolic bases of the evolved

Please note: Abstracts are published as received from the authors and are not subject to editing.

phenotypes will provide clues on the mechanisms controlling the orientation of metabolic networks

LI04Mo1500

Offered paper – Microalgae as a potential industrial expression platform: new genetic engineering tools

Rosanna Young, Saul Purton

University College London, UK

Due to their fast growth rates and ability to fix carbon via photosynthesis, unicellular microalgae are an attractive platform for the engineering of metabolic pathways and the introduction of transgenes to synthesize new bioproducts. To achieve this goal, it is necessary to first develop the genetic tools to understand and manipulate gene expression. We are currently working on systems that allow high-level constitutive or temperature-inducible transgene expression in the chloroplast of the model species *Chlamydomonas reinhardtii* and have also developed a negative selectable marker to study the nuclear factors that control chloroplast gene expression in this organism. Microalgae can be grown in open ponds for large-scale production (e.g. for biofuels) or in containment for the smaller scale generation of high-value products. We are investigating two possible approaches to prevent the release of functional transgenes: the first involves DNA degradation using a restriction enzyme expressed alongside a transgene, whilst the second makes use of non-native codon/tRNA combinations. Although the various genetic engineering tools are initially being developed for *C. reinhardtii*, the eventual aim is to expand the technology to other microalgal species

LI04Mo1515

Offered paper – Exploiting Fungal Diversity for Optimized Breakdown and Saccharification of Lignocellulosic Biomass

Craig Faulds, Marie-Noelle Rosso, Eric Record, Jean-Guy Berrin, Jean-Claude Sigoillot

INRA-Aix Marseille Université, France

Filamentous fungi are an invaluable source of enzymes able to deconstruct the complex lignocellulosic substrates embedded within plant cell walls and convert the breakdown products to useful platform chemicals. The wealth of genomic data obtained recently from filamentous fungi has revealed the diversity of lignocellulolytic enzymes they produce. The CIRM-CF collection is a unique tool to explore fungal functional biodiversity in various fields of biotechnology, including improved pretreatments and saccharification of biomass in existing processes, such as pulp and paper, wood products, lignocellulosic biorefineries, lignin modification and novel enzyme identification. The expanding numbers of sequenced fungal genomes provides an unprecedented framework for identifying their potential. The analysis of transcriptome profiles *in vivo* allows identification of the sets of genes encoding the enzyme machineries expressed according to the organisms' strategy for plant cell wall degradation/modification. A number of secretomes of fungal isolates improved the saccharification of lignocellulosic biomass compared to commercial enzyme cocktail of Trichoderma reesei. Genomic and secretomic analysis of a range of fungi, such as *Fusarium verticillioides*, *Trametes gibbosa* ,*Ustilago maydis, Pestalotiopsis sp.* from temperate and tropical forest environments allowed the identification and characterization of a number of novel cellobiose dehydrogenases, lytic polysaccharide monooxygenases, hemicellulases (including mannanases) and endoglucanases

LI04Mo1600

The Foundational Synthetic Biology of Amyris, from malaria cure to jet fuel

Jack Newman

Amyris, Inc., USA

Amyris established it's laboratory in 2005 with an aspiration to commercialize products made through synthetic biology. This year 70 million cures worth of artemisinin will be manufactured using the Amyris synthetic biology platform. Next year that figure will nearly double. The platform technologies developed in the non-profit anti-malarial project have been employed to produce dozens of high-performance molecules to create a sustainable business enterprise. We have applied the latest in synthetic biology to create industrial microbes producing Farnesene. This new molecule, abbreviated BioFene, is a high-performance olefin that is processed into Diesel and Jet Fuel as well as Squalane, a high performance lubricant and emollient. Farnesene is also in development as a polymer for use as an enhanced rubber. Last year we opened our first production plant in Brotas Brazil to manufacture Farnesene. As of March 2013, Amyris has produced over two million liters of Farnesene running this facility and other contracted fermentation capacity.
In this talk, I will discuss the evolution of the Amyris technology and engineering philosophy underpinning the successful commercialization of multiple products made through synthetic biology

LI04Mo1630

Engineering sustainable biofuels and novel chemicals in algae and non-food plants

PJ Harvey, D Bailey

University of Greenwich, School of Science, Central Avenue Chatham Maritime, Kent, UK

There has been a resurgence of global interest to exploit energy-rich, renewable bio-resources and replace fossil oil-based, bulk chemicals, prompted by concerns for energy supply security, oil price volatility and climate change mitigation. However there are no non-food bio-oil agricultural crops at a sufficiently mature stage of development with which to develop the necessary supply chains. Jatropha is still an undomesticated crop and now banned in many African countries. And, despite many years of effort and a wide range of initiatives few companies are cultivating algae on a commercial scale and none have large-scale units for biomass production for biofuels and carbon mitigation. Fuel-only algal systems are not economically feasible because yields are too low compared to agricultural crops and costs for producing microalgae for biofuel in raceways or PBRs for biofuel are too high compared to costs for producing agricultural residues for biofuel e.g. straw. These factors limit the scale of energy production and GHG abatement from algae by default to the amount of algal biomass that can be produced to support profitable applications. Biorefineries which integrate biomass conversion processes and equipment to produce fuels, power and chemicals from biomass, offer a solution, exemplified by the D-Factory, a 10 million Euro FP7-funded project

based on the microalga *Dunaliella*. The D-Factory serves as the springboard for exploring the chemical biology of Dunaliella as a direct source of bioactive compounds and for assessing its potential as a host within which to engineer new pathways for the production of novel, high-value biochemicals, specifically targeting pharmaceutical applications

LI04Mo1700

Bioengineering yeasts for generating bioethanol from biomass

Ursula Bond, William Kricka, James Fitzpatrick and Tharappel C. James.

School of Genetics and Microbiology, Trinity College Dublin, Dublin 2, Republic of Ireland

With dwindling fossil fuel resources and the necessity to reduce greenhouse gas emissions, there is a growing need to identify alternative environmentally sustainable energy sources. One potential green energy source is biomass derived from dedicated bioenergy plants, grasses or decaying or discarded biological waste, which can be converted to useable energy such as bioethanol. A goal of the bioethanol industry is the development of yeast strains capable of carrying out consolidated bioprocessing in which cellulose and hemicellulase-based biomass can be converted to bioethanol without the need to add exogenous enzymes. We examined the expression of the genes encoding for the three major classes of cellulases from the saprophytic fungus *Trichoderma reesei*, together with the genes required for xylose metabolism, in a several yeast species. We discuss the conditions required to degrade both cellulose and hemi-cellulose-based biomass and strategies for the conversion of released sugars into ethanol

LI04Tu0930

Modelling Yeast Metabolism

Paul Dobson

University of Sheffield, UK

Throughout engineering computer simulations allow rapid and cheap assessment of how modifications might improve system performance. Model-driven engineering is a key goal of Industrial Biotechnology but the scale and complexity of biochemical networks even in simple microbes makes building and even running cell simulations a daunting challenge. I will describe progress towards a model of yeast metabolism and set out a roadmap for capturing and integrating the extra information required for these models to be realistic enough to guide metabolic engineering

LI04Tu1030

Offered paper – Regulation of microbisporicin biosynthesis in the rare actinomycete *Microbispora sp.*

Lorena Fernández Martínez, Mervyn Bibb

John Innes Centre, Norwich, UK

Microbisporicin is a potent type AI lantibiotic produced by the rare actinomycete *Microbispora sp* that has proven very successful against multi-drug resistant infections in preclinical trials. The gene cluster for microbisporicin, which contains unusual post-translational modifications, including 5-chlorotryptophan and 3, 4-dihydroxyproline, has been cloned and analysed (Foulston and Bibb, 2010) revealing a novel regulatory mechanism that involves a pathway-specific extracytoplasmic function (ECF) sigma (MlbX)/anti-sigma (MlbW) complex. A model for the regulation of microbisporicin biosynthesis derived from mutational and quantitative RT-PCR analyses suggests that MlbR, a LuxR homologue, functions as an essential master regulator to trigger microbisporicin production while MlbX and MlbW induce feed-forward biosynthesis and producer immunity (Foulston and Bibb, 2011). Preliminary results show that over-expression of either of the two positive regulatory genes, *mlbR* or *mlbX,* leads to enhanced microbisporicin production. Further studies to unveil the interplay between the different components of the system have revealed the role of the stringent factor ppGpp and microbisporicin itself as triggers of this complex regulatory pathway. Understanding microbisporicin production and its regulation should result not just in increased yields, but also enable the development of variants with improved clinical activity and broaden our knowledge of lantibiotic biosynthesis in general.

LI04Tu1045

Conservation and divergence between enzymes involved in the biosynthesis of antibiotics mupirocin and thiomarinol

Mukul Yadav[1], Joe Hothersall[1], Zushang Song[2], Thomas J Simpson[2], Christopher M Thomas1

[1]University of Birmingham, Birmingham, UK, [2]The University of Bristol, Bristol, UK

Biosynthetic steps in the mupirocin (pseudomonic acids) biosynthetic pathway of *Pseudomonas fluorescens* NCIMB 10586 have already been deduced. Putative functions of most of the genes of *mup* cluster have been assigned although the exact sequence of tailoring steps and their timings is not yet known. Thiomarinols are a group of antibacterials produced by *Pseudoalteromonas* SANK 73390 that share striking structural similarity to pseudomonic acids in their polyketide and fatty acid moieties. This striking similarity is also reflected at the genetic level. Bioinformatic analysis reveals significant amino acid identity between products of 27 open reading frames in the two systems.

This project aims to learn more of biosynthetic steps in the biosynthesis of mupirocin and thiomarinol antibiotics by testing for cross complementation between pairs of genes or groups of genes whose products show significant homology. It involves creating and studying knock outs of these genes in the tml cluster and by inserting/swapping specific gene functions between two antibiotic systems. Surprisingly only two genes *tmlJ* and *tmlS* out of 9 studied so far have shown complementation in *mup* system suggesting protein-protein interactions limit interchangeability. By expressing genes as groups for complementation studies it should be possible to confirm specificities of such interactions.

LI04Tu1115

Lead optimisation of Polyketide-based natural products

Barrie Wilkinson

John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK

Phenotypic screening provides an alternative to high-throughput screening of large libraries of compounds against single isolated

Please note: Abstracts are published as received from the authors and are not subject to editing.

targets. In concert with this has been the realization that the typical small molecule libraries used in drug discovery are insufficient to effectively query these 'high-content' screens.
This observation has led to a renewed interest in more 'high-content' chemistries to provide appropriate screening libraries, an application for which microbe derived natural products are ideally suited. Results from phenotypic screening campaigns typically reveal prominent natural product hits, with bacterial polyketides well represented amongst these. However, these hits are often viewed as a challenge for further optimization using synthetic chemistry. Strategies and technologies for converting polyketide natural product leads into candidate drugs using natural product bioengineering will be presented using actual case studies.

LI04Tu1130

Cell factories: Engineering yeast for terpene production

Christine Lang

Organobalance GmbH Gustav-Meyer-Allee 25 13355 Berlin, Germany

The yeast *Saccharomyces cerevisiae* is a robust, well-established industrial production organism, it exhibits very good growth characteristics, a broad substrate spectrum and a high acid- and osmotolerance. Additionally, *S. cerevisiae* is genome-sequenced, genetically and physiologically well characterized, and tools for genetic optimization are established. These features make yeast particularly suitable for the biotechnological production of bulk and fine chemicals. Lipophilc Terpenes - e.g. with a size of C30 or higher - are often of high commercial relevance but not easily accessible in large scales due to limited natural resources for extraction. We addressed the fermentative production of this class of compounds by constructing strains of *Saccharomyces cerevisiae* for the biotechnological production of squalene and squalene deriviatives.. We engineered yeast strains, which contain a deregulated terpene biosynthetic pathway and deletions of genes responsible for the formation of triacylglycerols and sterylesters. While the deregulation of the pathway induces a strong increase in terpene productivity, the gene deletions lead to an abolishment of the formation of the neutral lipids triacylglycerols and sterylesters, which facilitates the downstream processing of lipophilic terpene compounds. The high productivity observed makes *Saccharomyces cerevisiae* a perfect host for the biotechnological production of valuable lipophilic terpenes.

LI05

Sexually transmitted and reproductive diseases in humans and animals

LI05Mo1400

Using novel diagnostic approaches to address the challenge of antimicrobial resistance in STIs

Tariq Sadiq

St Georgeís Healthcare NHS Trust, UK

Antimicrobial resistance (AMR) in a number of sexually transmitted infections threatens the ability to successfully treat patients empirically at diagnosis, which is a cornerstone of managing high risk, mobile sexually active populations. Failure to adequately treat some of these infections risks the emergence of serious reproductive and child health sequelae as well as increasing prevalence of infection.
Empirical therapy has becoming a particular problem for two infections, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*. Clinical services, however, increasingly diagnose gonorrhoea only by nucleic acid amplification tests without culture and susceptibility testing and do not routinely test for *M. genitalium* at all. Modern, nanotechnology and microfluidic based approaches to concurrently genotypically testing for both infection and antimicrobial susceptibility may enable personalised therapy in both laboratory-based diagnosis and at point of care settings. Such approaches will enable rational prescribing, offer new strategies for clinically managing drug resistance and potentially have impact on the spread of AMR.

LI05Mo1430

From syndromic to rational decision making in STI care- the unmet need in STI diagnostics

Jan Clarke

BASHH, Leeds Teaching Hospital, UK

The rapid development of molecular diagnostics has revolutionised STI management allowing a radical redesign of service delivery. However, it is becoming increasingly clear that current strategies of patient management (often based on syndromic care) may not be best suited to manage the range of new pathogens being identified. Management even in the developed world lags significantly behind the possibilities that new tests offer. Sexual Health services are increasingly finding it difficult to manage open access, greater service expectations and the need to prioritise serious STI within current financial limits. Making the case for new tests, looking for a wider range of pathogens and maintaining and extending surveillance in the climate of competitive service delivery will be a major challenge to Sexual Health services in the immediate future

LI05Mo1500

Trichomoniasis - when the villains team up

Raina N. Fichorova

Laboratory of Genital Tract Biology, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

Trichomoniasis, a sexually transmitted infection caused by the genitourinary parasite *Trichomonas vaginalis*, is often accompanied by deviations of the healthy vaginal microbiota, including the syndrome of bacterial vaginosis (BV). Both trichomoniasis and BV are highly prevalent in reproductive age women and associated with an increased risk of adverse pregnancy outcomes and viral infections, e.g. human papillomavirus and HIV, which thrive on mucosal lesions, activation of inflammatory cascades and suppressed innate immunity in the female genital tract mucosa. As many other protozoan parasites, *T. vaginalis* also carries endosymbionts as an evolutionary adaptation to survival in the human host. This lecture will provide emerging experimental and clinical evidence for reciprocal relationships between TV and BV, and the role of *T. vaginalis* and its endosymbionts, including mycoplasmas and *Trichomonas vaginalis* viruses, in the disturbance of the mucosal immune barrier and the healthy vaginal microbial environment

LI05Mo1600

A shot in the arm towards effective HIV vaccines

Robin Shattock

Imperial College, London, UK

The development of a safe and effective HIV-1 vaccine remains a pressing global public health priority. A protective prophylactic HIV-1 vaccine is thought to require the induction of broad neutralizing antibodies (bnABs) ideally at the mucosal portals of viral entry. However a critical gap currently impeding vaccine development against globally important pathogens is the lack of understanding in humans of how to induce and maintain long-lived protective immune responses. This includes how best to engage human germline encoded B and T cell receptors to drive initial steps required for induction and evolution of protective immunity. Recent advances in elucidating evolutionary pathways leading to the induction of HIV-1 broadly neutralizing antibodies (bnAbs) in infected subjects are now driving the broader vaccine discovery agenda. This is driving a shift in vaccine discovery to transition the focus from animal models to human clinical studies. However to date no vaccine candidate to date has been able to elicit such responses in humans. Furthermore, the recent RV144 'Thai' trial, utilizing a vector prime and subunit monomeric gp120 protein boost, that demonstrated a very modest reduction in HIV-1 acquisition (31%), with an apparent lack of nAb response suggests additional antibody function may contribute to vaccine induced protection. This presentation will review current progress in the induction of systemic and mucosal antibody responses against HIV illustrated by a range of pre-clinical and clinical studies.

LI05Mo1630

HPV immunisation in England

David Mesher

Public Health England, Centre for Infectious Disease Surveillance and Control, London UK

The national HPV immunisation programme in England was introduced in September 2008 offering the vaccine to 12-13 year old girls. A catch up programme was also implemented in the first two years of the programme which offered immunisation to women up to age 18 years.

Reported data on vaccine administration has shown coverage above 80% for all routine cohorts and ranging from 40-75% in the catch-up cohorts. A substantial impact on cervical abnormalities will not be seen for some time due to the relatively long interval between vaccination and the typical age that abnormalities develop.

Surveillance performed by Public Health England monitors the early impact of this high coverage HPV immunisation programme. I will present data on the seroprevalence for HPV antibodies for the HPV vaccine types which confirms high vaccine coverage in England.

I will also present some of the first results on the impact of the immunisation programme on type-specific HPV infections in sexually active young women in England which has shown a decrease in HPV-16/18 infection in the age-groups that would have been offered the HPV vaccine. Ongoing surveillance to consider the impact of the HPV immunisation programme on cervical abnormalities and disease will be discussed.

LI05Mo1700

Offered paper – A novel Quantum dot-based approach to visualize the enigmatic E5 oncoprotein during the human papillomavirus lifecycle and in cervical lesions

Christopher Wasson[1], Weili Wang[1], Yuan Guo[1], Christian Tiede[1], Iain Manfield[1], Nicola Stonehouse[1], Eric Blair[1], Michael McPherson[1], Sheila Graham[2], Nick Coleman[3], Dejain Zhou[1], Darren Tomlinson[1], <u>Andrew Macdonald</u>[1]

[1]University of Leeds, Leeds, UK, [2]University of Glasgow, Glasgow, UK, [3]University of Cambridge, Cambridge, UK

Human papillomaviruses (HPV) are the causative agents of cervical and oropharyngeal cancers, and are a major global health burden. Despite being studied for >25 years, E5 remains the least understood of the HPV oncoproteins. A paucity of reagents means that we still do not fully understand the contribution of E5 to HPV biology and pathogenesis. We developed a novel reagent to detect the highly hydrophobic, non-immunogenic E5 protein based on a protein scaffold called an Adhiron. Adhirons were conjugated to Quantum dots to increase their sensitivity. They showed remarkably high binding affinity and specificity against E5. Using cell staining we demonstrated that E5 localises to the ER within HPV-transformed cell lines. Staining of organotypic raft cultures, which recapitulate the HPV lifecycle, provides the first evidence that E5 is expressed at later stages of the HPV lifecycle, not at early stages as had been assumed. Finally, we screened an array of HPV cancer samples to ascertain the percentage that express E5 protein. Our aim is to correlate E5 expression with changes in cancer pathology. In summary, Adhirons overcome many of the limitations of antibody-based detection systems and their use will revolutionise our understanding of this enigmatic oncoprotein.

LI05Mo1715

Offered paper – Potent Serological AIDS Vaccine Protection Correlates with Complement Mediated Virus Neutralisation

Mark Page[1], Ruby Quartey-Papafio[1], Mark Robinson[1], Mark Hassall[1], Martin Cranage[2], Jim Stott[1], <u>Neil Almond</u>1

[1]NIBSC, Potters Bar, UK, [2]St George's, University of London, London, UK

In the simian model of AIDS, the most reproducible, potent vaccine protection was obtained by immunising with fixed inactivated virus vaccines. Protection was demonstrated to be transferable by serum alone and able to prevent detectable infection following mucosal or intravenous challenge with virus. However, it was determined that protection was NOT directed against VIRAL components BUT rather responses against HOST CELL antigens present in the vaccine and also expressed on virions. We investigated the properties of serological responses directed against virus and host cell antigens. Serum antibodies from macaques immunised with inactivated virus vaccines, prepared on human T cell lines, targeted HLA class I and class II framework epitopes common between multiple HLA alleles. Using an infectivity assay, we observed that virus neutralisation mediated through host cell antigens was enhanced by the addition of fresh serum as a source of complement, whereas neutralisation through viral antigens was not. These data indicate that the potent vaccine protection, mediated through anti-cell antibodies, was

Please note: Abstracts are published as received from the authors and are not subject to editing.

due to their ability to engage effectively complement to neutralise virions. Establishing how to generate anti-HIV envelope antibodies that similarly engage the complement system should result in the development of more potent AIDS vaccines.

LI05Tu0900

Antimicrobial resistant gonorrhoea: a public health challenge

Catherine Ison

Public Health England, Colindale, London, UK

Delivery of effective antimicrobial therapy and of prevention message are the mainstay of the public health control of gonorrhoea, due the lack of an apparent protective immune response and hence a suitable vaccine. Successful antimicrobial therapy is often administered before the results of laboratory tests are known, in order to break transmission. The choice of agent for this empirical therapy is recommended by national and international guidelines with the aim of achieving greater than 95% cure. Guidelines are informed by surveillance programmes that monitor trends in susceptibility and emergence of resistance. When resistance reaches 5% or greater, then an alternative antimicrobial agent is chosen to which, if possible, treatment failure is unknown. *Neisseria gonorrhoeae*, the causative agent of gonorrhoea, is highly genetically competent and has a propensity to become resistant. Hence, resistance and treatment failure has emerged to successive antimicrobial agents over decades. As drift in decreased susceptibility and emerging treatment failure occurs to the current treatment, the extended spectrum cephalosporins, cefixme and ceftriaxone, the lack of new alternative therapies is a concern. Action plans at global, regional and national level have been produced to raise awareness the gonorrhoea has the potential to be difficult to treat.

LI05Tu0930

Immune Evasion and Syphilis Pathogenesis

Sheila A. Lukehart

Departments of Medicine and Global Health, University of Washington, Seattle, WA, USA

Syphilis, caused by a corkscrew-shaped bacterium, *Treponema pallidum*, is one of the most fascinating of all infections. The organism has a miniscule genome and is so fragile that it dies within hours outside of the host, yet it is capable of evading host defenses to persist for many decades within the host. Syphilis is known as “the great imitator” because its clinical manifestations can range from an ulcer or rash to blindness and insanity. In contrast, the infection can lie smoldering for many years, without any clinical evidence. How does *T. pallidum* cause the many manifestations of syphilis and still evade immune clearance? Is there a biological basis for determining which patients can be re-infected? Molecular studies involving the laboratory and the clinic have shed insights into pathogenic mechanisms involved in lesion development and clearance, induction of host responses, and immune evasion by *T. pallidum.* Molecular epidemiology approaches have begun to unravel the movement of *T. pallidum* strains throughout communities. Our evolving knowledge about these issues will be discussed in the context of the natural history of syphilis infection.

LI05Tu1000

Mycoplasma genitalium: Microbiology, clinical disease and public health implications

David Martin

Louisiana State University Health Sciences Center, Dept. of Medicine, New Orleans Louisiana, USA

Among free living organisms *Mycoplasma genitalium* (MG) has the smallest known genome (580kb). Among this minimum set of genes are those encoding for a tip structure that is essential for cellular adherence and intracellular invasion of genital tract epithelial cells. The major proteins involved are highly antigenic but also highly plastic in that the genes encoding them frequently exchange DNA with a family of repetitive DNA elements known as MgPars which are located throughout the genome. The resultant variations allow the organism to evade the host immune response and, possibly, to adapt to differing environmental conditions contributing to the organism's ability to establish chronic infections in humans. Nongonococcal urethritis is the clinical syndrome most clearly attributable to MG. Antibiotic resistance explains why MG is the most common cause of persistent urethritis in men. Endocervicitis and pelvic inflammatory disease are also associated with MG. However, as yet, evidence that MG is a significant cause of ectopic pregnancy and infertility is not strong. As with many STIs, MG is associated with HIV transmission though data is lacking as to whether or not treating MG is preventive. Thus, screening asymptomatic individuals for MG even in high risk populations is not recommended currently.

LI05Tu1100

The genomics of *Chlamydia trachomatis:* a constant surprise

Nicholas Thomson

Wellcome Trust Sanger Institute, Genome Campus, Cambridge, UK

Chlamydia trachomatis is an important human pathogen, responsible for diseases ranging from trachoma to sexually transmitted infections that cause substantial morbidity in developed as well as developing countries. In the UK alone *Chlamydia* is estimated to cost the National Health System up to 100 million pounds every year [www.chlamydiascreening.nhs.uk]. ompA genotyping is the most widely used typing scheme for *Chlamydia* with the ocular genotypes represented by A to C, urogenital genotypes D to K and for LGV its L1-L3. Studies from all over the world show that the most common urogenital genotypes are E, F and D. This has lead to the pervasive view that that during the last few decades, the overall distributions urogenital of *C. trachomatis* genovars throughout the world has been relatively stable. Moreover, there are a large number of epidemiological studies that have used *ompA*-type as a marker to infer relatedness between isolates and compare disease presentations between genotypes. Looking across them all there is almost an equal number of epidemiological studies that have shown an association between genotype and the hosts: age, gender, number of sexual partners, or clinical symptoms, compared to the number of studies that have not.
We have been sequencing representative isolates of the trachoma (ocular and genital tract serotypes) and lymphogranuloma venereum (LGV) biovars to reveal their detailed phylogenies. In the light of this data its clear that typing protocols based on a single region or small number of genomic loci should be treated with caution. Until relatively recently *Chlamydia* were not thought to recombine

because it was thought that the barriers to this were too high. However, from the genomic data produced by us and others it is clear that *C. trachomatis* do recombine and in fact there are few barriers to this process. For *Chlamydia* we see examples of *ompA*-genotypes occupying unrelated positions on the phylogenetic tree with the *ompA* gene essentially being a chimera that can be exchanged in part or whole, both within and between biovars regardless of whether they cause ocular or sexually transmitted infections. This may explain why there is such as disparity in studies looking for correlations between *ompA*-genotype and disease presentation.

In essence genome wide analysis shows that predicting phylogenetic relationships using the small number of genetic targets traditionally used to type *Chlamydia* effectively masks our understanding of the true diversity of this species because of extensive recombination. Genomics data continues with the tradition of challenging the established wisdom that have marked the whole history of investigation into this fascinating bacterium.

LI05Tu1130

Offered paper – *Shigella flexneri* as a sexually transmitted infection in MSMs

Baker KS, Dallman T, Ashton P, Hughes G, Keddy K, Smith A, Kaiser, Bangladesh, Connor T, Baker S, Weill FX, Jenkins C, Thomson NR

[1] Wellcome Trust Sanger Institute, Hinxton, UK [2] Public Health England, Colindale, UK [3] National Institute for Communicable Diseases, Johannesburg, South Africa [4] International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh [5] Cardiff University, Cardiff, UK [6] Wellcome Trust Major Overseas Program, Ho Chi Minh, Vietnam [7] Institute Pastuer, Paris, France [8] London School of Hygiene and Tropical Medicine, London, UK

Shigella flexneri is a faeco-orally transmitted gram-negative bacterium with a low infectious dose that causes severe invasive enteric disease. The largest disease burden occurs in developing nations where locally persistent serotypes cause endemic disease. Comparatively in developed nations, *S. flexneri* infections are typically linked with travel, but there are also sporadic outbreaks of shigellosis in men who have sex with men (MSMs), in which the infection mirrors the epidemiology of sexually transmitted infections. In 2009, an MSM-linked outbreak of S. flexneri serotype 3a was identified in the UK, and this outbreak continues presently. We used whole genome sequencing on 323 S. flexneri setrotype 3a isolates from the UK outbreak and France, including both travel and confirmed MSM-associated strains. Endemic strains from 13 different countries were also used to contextualise the samples. Linking phylogenetic results with patient data demonstrated important relationships among the strains. There was clear phylogenetic fidelity of travel-related strains, as well as a monophyletic clade of strains linked with the UK MSM outbreak (including admixture of potential MSM-related strains from France). Together with epidemiological data, this demonstrates that S. flexneri is acting as a STI in MSMs across the UK and France.

LI05Tu1400

Taking the "Query" out of the Q fever bacterium, *Coxiella burnetii*

Robert Heinzen

Coxiella Pathogenesis Section, Hamilton, Montana, USA

Coxiella burnetii is an intracellular bacterium that causes the zoonosis Q fever. Dairy cows, sheep, and goats are important animal reservoirs where infections result in economic loss (e.g., abortion waves) and present a public health hazard as recently illustrated by a large outbreak (>4,000 cases) in the Netherlands associated with intensive dairy goat farming. Aerosol-transmitted *Coxiella* initially targets alveolar macrophages but in pregnant females can also heavily infect the placenta; thus, parturition can deposit tremendous numbers of stable and highly infectious bacteria into the environment. Enhanced environmental stability by *Coxiella* correlates with pathogen resistance to destruction within a phagolysosome-like intracellular niche. Understanding mechanisms of *Coxiella* extra- and intracellular resistance is central to understanding Q fever pathogenesis. Fortunately, host cell-free growth has recently rescued *Coxiella* from an obligate intracellular lifestyle that imposed severe experimental constraints. Now enabled by a complete set of genetic tools, virulence factors are being defined that allow *Coxiella* to subvert macrophage processes for successful parasitism, and to generate cell forms specifically adapted for intracellular replication and extracellular survival.

LI05Tu1430

Intra-cellular regulation of the innate immune response to bacteria in the bovine endometrium

Cronin JG, Thornton CA, Kanamarlapudi V, Sheldon IM

Institute of Life Science, College of Medicine, Swansea University, Swansea, SA2 8PP, UK

Postpartum uterine disease in dairy cows is characterized by persistent inflammation of the endometrium with sustained neutrophil infiltration. Pathogenic strains of *Escherichia coli* initiate the early phase of uterine disease, stimulating the secretion of the neutrophil chemoattractant IL-8, and the cytokine IL-6. Interleukin-6 orchestrates the transition from the initial recruitment of neutrophils to a more sustained mononuclear cell population, facilitating tissue regeneration and homeostasis. IL-6 signals via the IL-6 receptor *(IL6Rα)* and an associated transcription factor STAT3 (signal-transducer and activator of signaling 3). We hypothesized that IL-6 signaling may modulate IL-8 production by endometrial epithelial cells in response to lipopolysaccharide (LPS) from Gram-negative bacteria. *In vitro,* LPS stimulated IL-6 and IL-8 production by stromal and epithelial cells isolated from the bovine endometrium. The knockdown of IL-6 receptor expression, using siRNA, reduced LPS-induced IL-6 and IL-8 accumulation by stromal cells and epithelial cells. However, siRNA knockdown of STAT3 only reduced the secretion of IL-6 and IL-8 in response to LPS by stromal cells and not epithelial cells. In conclusion, a positive feed-forward through the IL-6 signaling pathway amplified the innate immune response to LPS in the endometrium, and this was STAT3 dependent in stromal but not epithelial cells.

LI05Tu1500

Offered paper – Mechanism of host-pathogen interactions between the bovine endometrium and *Trueperella pyogenes*

Sholeem Griffin, Martin Sheldon, Matt Amos

Swansea University, Swansea, Wales, UK

Please note: Abstracts are published as received from the authors and are not subject to editing.

Trueperella pyogenes is a Gram-positive facultative anaerobe that infects the bovine endometrium and causes tissue damage. All *T. pyogenes* isolates collected from the endometrium express the *plo* gene, which encodes pyolysin (PLO); a cholesterol dependent cytolysin. Pyolysin assembles into a macromolecular complex upon binding plasma membrane cholesterol to cause pore-formation in host cell membranes [1]. The growth and cytolytic activity of *T. pyogenes* were examined using 10 clinical isolates collected from animals on 3 continents. Bacterial growth characteristics were similar and functional PLO was expressed at the late exponential growth phase as determined by haemolysis assay. Maximal haemolytic activity for each isolate was at 16 hours of growth; however, the extent of haemolysis varied among isolates. Primary bovine endometrial epithelial and stromal cells challenged with recombinant PLO underwent concentration-dependent cell lysis. Stromal cells were six times more sensitive to PLO challenge compared to epithelial cells. A *plo*-deletion mutant did not cause haemolysis or cytolysis. These findings provide mechanisms linking *T. pyogenes* infection to tissue damage in the endometrium.

1.Jost, B.H., J.G. Songer, and S.J. Billington, An *Arcanobacterium (Actinomyces) pyogenes* mutant deficient in production of the pore-forming cytolysin pyolysin has reduced virulence. Infection and Immunity, 1999. 67(4): p. 1723-1728.

LI05Tu1515

Offered paper – Invasion of bovine caruncular epithelial cells by *Listeria monocytogenes*

Amy Glanvill, Sabine Tötemeyer

University of Nottingham, Nottingham, UK

Listeriosis is of major veterinary importance, impacting on animal welfare and its designation as a food-borne pathogen. Over the last 17 years, the percentage of bovine abortions caused by *Listeria monocytogenes* has risen by 2.9%, with every abortion costing the dairy farmer around £630. *L. monocytogenes* has particular tropism for the gravid uterus, however, the route of infection of the ruminant placentome is relatively unknown. We aimed to investigate the ability of a range of environmental and clinical *L. monocytogenes* to infect cells of the bovine foeto-placental barrier using two cell types: Caco2 (a human colon epithelial cell line used routinely to investigate Listeria pathogenesis) and Bovine Caruncular Epithelial Cells (BCECs, maternal cells of the placental foetal/maternal interface).
All *Listeria* isolates tested invaded Caco2 and BCEC cells. *L. monocytogenes* strain differences were only observed in Caco2 cells, however the level of invasion was significantly lower ($p<0.0001$) for BCEC cells. The majority of infected Caco2 cells contained >10 bacteria (52.3%), whereas the majority of infected BCEC cells had a single *Listeria* invading (81.8%). Our results demonstrate that *Listeria* is capable of invading BCEC cells, suggesting that these strains may also be capable of causing listeriosis at the placentome *in vivo*.

LI05Tu1600

Can viruses affect bovine fertility?

Joe Brownlie

Royal Veterinary College, Hatfield, UK

The very foundation of the dairy industry is an assured fertility; without calves there would be no milk! The quest for high yields makes considerable demands but so does the movement, marketing and intensive scale of today's farming. It is these factors that facilitate the transmission of viruses. One of these is a pestivirus, within the Flaviviridae, designated bovine virus diarrhea virus (BVDV); it is arguably the most serious pathogen of cattle in the developed world and can cause severe losses in reproduction. BVDV has a complex pathogenesis, which is exceptional amongst viral pathogens, and has recently been fully elucidated. The combination of two biotypes (one non-cytopathogenic and the other cytopathogenic in cell culture), the induction of a central tolerance during embryogenesis allowing a persistent infection during foetal life and thereafter into neonatal and adult life, permits this virus to infect and to remain within the majority of the British national dairy herds. The virus, being a single stranded RNA virus, can and does regularly mutate and, in so doing, can become highly pathogenic. More virulent strains can cause widespread fatalities following acute infection. Although pantropic, many strains have a tropism for the reproductive tissues. There are examples where strains of the virus sequester in the testis, following an acute generalized infection, even in the face of an active immune response. This appears to be an example of the virus 'high-jacking' a privileged site to remain a persistent infection within the testis. Such bulls will have infectious virus in their semen. It is unsurprising that a number of European countries have, or are presently, eradicating BVDV from their national cattle herd. In this lecture, I will discuss the virus and its fascinating pathogenesis.

LI05Tu1630

Role of BoHV-4 in bovine uterine infections

G.Donofrio, S. Jacca, V. Franceschi

Department of Medical-veterinary Science, University of Parma, Italy

Bovine uterine infections are the most important cause of economic losses in cattle industry. Although the etiology of uterine diseases is mainly ascribed to bacterial infection, they can also be associated with viral infection, such as bovine herpesvirus 4 (BoHV-4), which is often a secondary agent following bacteria. Bovine herpesvirus 4 (BoHV-4) has been most consistently associated with uterine disease in postpartum cattle. The first isolation of BoHV-4 from a case of bovine metritis was reported in 1973. Postpartum metritis has also been associated with BoHV-4 in the USA, Spain and Serbia. Several other isolates were associated with reproductive disorders and BoHV-4 seroprevalence was associated with postpartum metritis and chronic infertility in cattle. Like other herpesviruses, BoHV-4 can establish persistent infections in cattle, particularly in macrophages, and viral infection is often identified concurrently with bacteria that cause uterine diseases. It was suggested that there may be a vicious circle composed of bacterial endometritis, leading to secretion of prostaglandin E2 (PGE2) and then stimulation of viral replication by PGE2 and lipopolysaccharide (LPS), which causes further endometrial tissue damage and inflammation. In the present study, the interaction between BoHV-4 infected bovine endometrial stromal cells and tumor necrosis factor alpha (TNF-a) was investigated. Bovine herpesvirus 4 possess a special tropism toward endometrial stromal cells, for this reason a simian virus 40 (SV40) immortalized endometrial stromal cell line (SV40BESC) was established and proven to be stable and express Toll-Like Receptors (TLRs) (from 1 to 10), TNF-α Receptors I and II and to be responsive to exogenous TNF-α. Further, an increase of BoHV-4 replication and cytopathic effect was observed in BoHV-4 infected and TNF-α treated SV40BESCs. This increase of viral replication was associated with

BoHV-4 Immediate Early 2 *(IE2)* gene promoter trans-activation through the interaction of the nuclear factor KB (NFKB) with the putative NFKB responsive elements found within BoHV-4 IE2 gene promoter and this interaction was abolished when NFKB responsive elements were deleted. To summarize, a rather complex role for BoHV-4 as a cofactor for the development of bovine post-partum metritis may be hypothesized: in BoHV-4 persistently infected animals, BoHV-4 infection resides within the macrophages. During parturition, infection of the uterus can take place from environmental bacteria. However such infection in normoergic animals is cleared within 3 weeks whereas, in BoHV-4 persistently infected animals, the inflamed uterus attracts BoHV-4 persistently infected macrophages from the periphery to the site of inflammation. Inflammatory molecules produced by the inflamed endometrium and proliferating bacteria, such as PGE2 and LPS, induce the replication of BoHV-4 in persistently infected macrophages and endometrial stromal cells can become infected with newly replicating virus. Furthermore, TNF-α produced by LPS induced macrophages binds TNF-αR1 on the surface of BoHV-4 infected endometrial stromal cells, inducing BoHV-4 *IE2* gene expression and enhanced BoHV-4 replication. The *IE2* gene product ORF50/*Rta,* induces not only BoHV-4 replication but also IL-8 production, thus shifting the inflammation from a transitory and acute status (metritis) toward a chronic status (endometritis).

LI06
Mind-altering microbes

LI06We0930
Zombie ants: the precise manipulation of animal behaviour by a microbe

Lauren Quevillon

Penn State Department of Biology, USA

The microbe *Ophiocordyceps unilateralis* is an obligately parasitic fungus that infects ants. To transmit from one ant to another it has evolved the ability to precisely manipulate the behavior of worker ants making them go to precise locations to die. Following death the fungus grows from their cadaver and releases spores. In this talk I will present our work on the function, phylogeny, causation and ontogeny of manipulation. I will show how we integrate continental wide comparisons to single celled transcriptomics. I will also show how such examples of manipulation are relatively abundant and provide fertile ground for a close interaction between microbiologists and zoologists

LI06We1000
Infectious behaviour in a parasitic wasp or the story of a behaviour-manipulating DNA virus

Julien Varaldi, Anastasia Gardiner, David Lepetit, Marie-Christine Carpentier

Laboratory of Biometry and Evolutionary Biology – UMR CNRS 5558 University Lyon 1, France, Laboratório de Drosophila: Evolução de Caracteres Complexos Instituto de Biologia Universidade Federal do Rio de Janeiro, Brazil

The transmission of parasites often depends on the behaviour of their hosts. Thus, natural selection has selected genes in the parasites genomes that manipulate the behaviour of their hosts to their own benefit. However these genes and their functional impact on the hosts have rarely been identified. Here I will present the very first step towards their identification in a system involving an inherited virus and a parasitoid wasp. The parasitoid *Leptopilina boulardi* specifically parasitizes *Drosophila* larvae and one host allows the development of a single parasitoid. As expected, wasp females usually refuse to lay supernumerary eggs in already parasitized larvae, especially when unparasitized hosts are available. However, we have found that some *L. boulardi* lines often accept to do so (a behaviour called superparasitism). This superparasitism behaviour is in fact caused by an hereditary filamentous DNA virus (called LbFV). The virus directly benefits from this behaviour, since it allows it to colonize new parasitoid lineages. To address the question of the mechanisms underlying this behavioural manipulation, we have first sequenced the genome of this virus. In addition, we have compared the transcriptomes of infected and uninfected females using a combination of RNAseq and 454 sequencing

LI06We1100
How do psychedelics work on the brain?

Robin Carhart-Harris

Imperial College London, UK

This presentation will discuss the latest knowledge on how psychedelic drugs such as LSD work on the brain to produce their subjective effects. There will be a particular focus on psilocybin (magic mushrooms) and what has been learned from fMRI and MEG brain imaging studies with this drug. A model of how brain activity changes under psychedelics will be presented which takes inspiration from the statistical principle of entropy. There will also be some discussion of the therapeutic potential of psychedelics and the changes in brain activity that may be involved

LI06We1130
Alcohol and its role in the evolution of human society

Ian Hornsey

Nethergate Brewery, Suffolk, UK

Ethanol is the most widely used psychoactive agent in the world, and its use is embedded in human culture. With the exception of Oceania and most of North America, tribal peoples from all major parts of the world knew how to make alcoholic drinks, and there have been very few, if any, societies whose people knew about alcohol and yet paid little attention to it. Ethanol has thus been a fundamentally important social, economic, political, and religious artefact for millennia. Ethanologenesis requires a fermentable substrate and a microorganism capable of appropriately transforming it. Unicellular yeasts of the genus *Saccharomyces*, in particular *S. cerevisiae*, do the job better than anything else, but several other microbes can transform sugars into ethyl alcohol.

Ancient peoples used indigenous plants as a source of fermentable material and the 'first fermentations' were undoubtedly serendipitous and so involved spontaneous fermentations. Some processes still involve a degree of spontaneity, but most modern alcoholic beverages are the result of controlled fermentations.

I shall endeavour to outline the archaeological evidence for ancient fermented drinks; survey the major sources of fermentable material (rice, barley, agave, palm sap, etc.) and outline the drinks produced from them; look at the major ethanologenic microbes; indicate why *S. cerevisiae* is so important, and suggest why the 'genomic age' is

Please note: Abstracts are published as received from the authors and are not subject to editing.

so exciting for brewers, winemakers and their ilk.

LI06We1400

Diabolical effects of rabies encephalitis

Alan C. Jackson

Department of Internal Medicine (Neurology) and of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada

Rabies is an acute encephalomyelitis in humans and animals caused by rabies virus infection. Rabies vectors (e.g., dogs and foxes) frequently show behavioral changes. Aggressive behaviour with biting is particularly important for transmission of the virus to new hosts at a time when virus is secreted in the saliva. Behavioural changes include alertness, loss of natural timidity, aberrant sexual behaviour, and aggressiveness, suggesting limbic system involvement. Aggressive behaviour is associated with lesions in many locations in the brain: posterior olfactory bulbs, the ventromedial nucleus of the hypothalamus, and the septal area. Aggression is associated with low serotonergic activity in the brain and increased testosterone in human and animal studies. Few studies have been performed in natural models of rabies in which aggressive behaviour is exhibited. Charlton and coworkers performed studies in experimentally infected striped skunks with skunk rabies virus; the skunks showed aggressive responses to presentation of a stick in their cages. Heavy accumulation of rabies virus antigen was found in the midbrain raphe nuclei, suggesting that impaired serotonin neurotransmission from the brainstem may account for the aggressive behaviour. More research is needed in good natural models of rabies in order to better understand behavioural changes in rabies vectors.

LI06We1430

A review of Neurobrucellosis

Philip H. Elzer

Louisiana State University Agricultural Center, Baton Rouge, LA, USA

Brucellosis is an infectious bacterial disease of man and animals caused by the *Brucella* species. Human infection is generally due to contact with animals or animal products contaminated with the bacteria. The genus *Brucella* consists of six classical species, but human infection is generally due to *B. abortus*, *B. melitensis*, or *B. suis*. Several new species have been isolated recently and have the potential to infect humans.

Human brucellosis (undulant fever) manifests itself as a febrile illness that has a range of clinical symptoms and is diagnosed by obtaining a complete case history, physical exam, serological testing, and by culturing bacteria from blood, lymph, or cerebrospinal fluid. Patients suffering from brucellosis are routinely treated with a combination of antibiotics. Typically the acute phase of the disease causes flu-like symptoms, whereas the chronic phase manifests itself as crippling arthritis or in rare cases, meningitis, endocarditis and psychoneurosis. Neurobrucellosis is an extreme complication of untreated human disease that is estimated to affect 3-10% of patients. The clinical findings include headaches, depression, anxiety, nerve involvement, paraplegia, delirium, and abscess. Due to the wide range of neuropsychotic symptoms and the possible conflicting causes, a definitive evaluation is needed for diagnosis and treatment of neurobrucellosis.

LI06We1500

From bowel to behaviour: microbiota regulation of the mammalian gut-brain axis

John F. Cryan

Alimentary Pharmabiotic Centre University College Cork, Cork, Republic of Ireland

There is a growing appreciation of the relationship between gut microbiota, and the host in maintaining homeostasis in health and predisposing to disease. Bacterial colonisation of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Over the past 5 years substantial advances have been made in linking alterations in microbiota to brain development and even behaviour and the concept of a microbiota-gut brain axis has emerged. Animal models have been essential in moving forward this frontier research area. In order to assess such a role we use studies involving, germ free mice and early-life microbiota manipulations and finally probiotic administration in adulthood. We assess neurochemical, molecular and behavioural effects following these manipulations. Our data show that the gut microbiota is essential for normal stress, antidepressant and anxiety responses. Moreover, microbiota is essential for both social cognition and visceral pain. Finally, there are critical time-windows early in life when the effects of microbiota on brain and behaviour appear to be more potent. Our data also demonstrates that these effects may be mediated via the vagus nerve, spinal cord, or neuroendocrine systems. Such data offer the enticing proposition that specific modulation of the enteric microbiota by dietary means may be a useful "psychobiotic"-based strategy for both stress-related and neurodevelopmental disorders ranging from depression to autism

LI06We1600

The impact of *Toxoplasma gondii* on host behaviour: can this parasite play a role in some cases of human schizophrenia?

Joanne Webster

Imperial College, London, UK

The ability of parasites to alter the behaviour of their hosts fascinates both scientists and non-scientists alike. One reason that this topic resonates with so many is that it touches on core philosophical issues such as the existence of free will. If the mind is merely a machine, then it can be controlled by any entity that understands the code and has access to the machinery. One key example is the potential epidemiological and neuropathological association between some cases of schizophrenia with exposure to the protozoan *Toxoplasma gondii*. *T. gondii* establishes persistent infection within the CNS and can alter host behaviour. Altered dopamine levels have been reported for both *T. gondii* infection and schizophrenia. Moreover, several of the medications used to treat schizophrenia and other psychiatric disease demonstrate a anti-*T. gondii*, properties *in vivo* and *vitro*. Furthermore, it appears that the parasite itself may actually be a source of this neurotransmitter. Using the epidemiologically and clinically applicable rat-*T. gondii*

model system, I present a series of studies and discuss them in terms of their theoretical and applied implications for animal and human health.

LI06We1630

Clinical relevance of *Helicobacter* species and small intestinal bacterial overgrowth to idiopathic parkinsonism

Sylvia M Dobbs,[1,2,3] R John Dobbs,[1,2,3] Clive Weller,[1] André Charlett,[1,4] Andrew J Lawson,5 David Taylor,[1,2] Mohammad A A Ibrahim,[6] Ingvar Bjarnason.[2]

[1]Pharmaceutical Sciences, King's College London, UK, [2]Maudsley Hospital, London, UK; [3]Department of Gastroenterology, King's College Hospital, London, UK, [4]Statistics Unit, Centre for Infectious Disease Surveillance and Control, and [5]Laboratory of Gastrointestinal Pathogens, Public Health England, London, UK, [6]Diagnostic Immunology Laboratory, King's College and St Thomas's Hospitals, London, UK

Our aim is an 'aetio-pathogenic construct', for the Parkinson's disease (PD), overlap dementias/depression, and functional-bowel-disease complex. Systemic immuno-inflammatory processes may drive neuro-inflammation. The PD-complex is multi-step, multi-factorial: halting one driver may allow escape down subordinate-pathways, facets have different drivers.

Peptic ulceration is prodromal to PD. In our randomised placebo-controlled trial, hypokinesia improved over year following biopsy-proven *Helicobacter pylori* eradication, rigidity worsened, independent of any (stable, long t½) anti-parkinsonian medication. Surveillance confirmed hypokinesia-effect was indication-specific. There is increased proportional-mortality from PD amongst livestock-farmers. Using species-specific qPCR, backed-up by multi-locus-sequence-typing, relative-risk of having *H. suis* in PD-patients compared with gastroenterology-service-'controls' was 10-times greater than that of having *H. pylori.* H. suis-positivity increased all-cause, age/gender-adjusted, mortality 8-fold. Autoimmunity may underlie *Helicobacter-effect.*

During surveillance, successive antimicrobials courses, other than for *Helicobacter,* were associated with cumulative increase in rigidity. Bystander damage to enteric/central nervous system, perhaps driven by intestinal-dysbiosis, may accompany cross-reactivity, or be separated in time. There are biological-gradients of objective measures of PD-facets on circulating inflammatory-markers and leucocyte-subset counts. Lactulose-hydrogen-breath-test positivity for small-intestinal-bacterial-overgrowth (SIBO) is associated with the same subsets: higher natural killer and total CD4+ counts, lower neutrophils. SIBO, present in two-thirds of PD-patients, may be a major source of bystander damage.

LI07

Viruses in the respiratory tract

LI07Th0900

Why is RSV so prominent a cause of distal airway obstruction

Raymond Pickles

University of North Carolina at Chapel Hill, USA

Respiratory Syncytial Virus (RSV) is notorious at causing distal airway obstruction in human infants. Why RSV has such propensity for causing distal airway disease compared to other respiratory viruses is unknown. Post-mortem studies of RSV-infected infants show distal airway lumens clogged with accumulations of sloughed airway epithelial cells. Here, we identify the RSV Non-Structural 2 (NS2) protein as a unique viral genetic determinant for causing RSV-induced distal airway obstruction. Comparing consequences of RSV vs. parainfluenza virus (PIV) infection of human ciliated epithelial cells *in vitro,* we find ciliated cells infected by RSV *but not* PIV, become rounded and extrude from the epithelium. Using RSV deletion mutants, cell rounding and shedding was attributed to the NS2 protein. PIV engineered to express RSV NS2 (PIVNS2) also caused rounding of ciliated cells indistinguishable from cells infected by RSV. To determine the *in vivo* significance of NS2-induced cell rounding and shedding, hamster airways infected by PIVNS2 *but not* PIV caused epithelial cells to round and shed from the epithelium into the airway lumen. Cells shed into larger proximal airways were rapidly cleared presumably by mechanical clearance mechanisms. In contrast, epithelial cells shed into the distal airways rapidly accumulated within the narrower diameter lumens causing occlusion of the airway lumen. We propose a novel consequence of RSV NS2 expression in small diameter distal airways where it promotes sufficient shedding of epithelial cells to cause distal airway obstruction. Accumulation of shed cells likely serves as an inflammatory nidus for exacerbating distal airway inflammation and disease. These studies may explain why RSV has such a propensity for causing bronchiolitis in infants and identifies RSV NS2 as a novel therapeutic target for reducing RSV airway disease.

LI07Th0930

Understanding the symptoms of common cold and flu

Ronald Eccles

Common Cold Centre, School of Biosciences, Cardiff University, Cardiff, Wales UK

Acute upper respiratory tract viral infections (common cold and flu) are the most common infection in humans, as in a lifetime most will suffer from more than 200 illnesses. Common cold and flu are self-diagnosed on symptomatology, and treatments are mainly symptomatic, yet our understanding of the immune mechanisms that generate the familiar symptoms is poor compared to the amount of knowledge available on the molecular biology of the viruses involved. Symptoms will be discussed as caused by local and systemic mechanisms. The mechanisms of local symptoms caused by the local generation of inflammatory mediators such as bradykinin and prostaglandins; sore throat, runny nose, sneezing, nasal congestion, cough, watery eyes and sinus pain will be explained. Brain-immune interactions and of the effects of cytokines such as IL-1, IL-6 and INFa will be discussed as systemic symptoms of fever, anorexia, tiredness, chilliness, headache, mood changes and muscle aches and pains. The illness associated with colds and flu will be discussed in terms of 'sickness behaviour' and a case will be made for the adaptive significance of sickness as a means of diverting energy resources towards the immune response.

LI07Th1000

Influenza transmission: floating in the air

Please note: Abstracts are published as received from the authors and are not subject to editing.

Wendy Barclay
Imperial College London, London, UK

Viruses that infect the respiratory tract gain entry to the body through the air. Influenza virus causes seasonal respiratory disease and also has the potential to give rise to pandemic outbreaks when new viruses emerge from the animal reservoir. Understanding the requirements for influenza viruses to transmit efficiently between people will help us to estimate the risks of animal viruses sparking pandemics rather than dead end zoonoses. Understanding how and when seasonal human influenza viruses transmit through the air should lead to a better understanding of how to control them and how they evolve.
Studying transmission requires *in vivo* work since there is no *in vitro* model for this process at the moment. Experimental studies of influenza transmission in humans are limited, and confounded by the previous unknown influenza exposure of the subjects .Currently the ferret is a favoured model for studying influenza transmission, although guinea pigs also support transmission of human influenza viruses. Studies in ferrets have been used to predict the ability of avian or swine influenza viruses to cause pandemics. Understanding in more detail how this model works and how it relates to the situation in humans will help us to justify its use, even when the viruses studied are high risk viruses.

LI07Th1100

Airway responses to Respiratory Syncytial Virus in infants with bronchiolitis

Paul McNamara
Consultant Respiratory Paediatrician, Alder Hey Children's Hospital, Eaton Rd, Liverpool, UK

RSV is the leading cause of bronchiolitis, the second commonest medical cause for admission to hospital in children after asthma in the developed world. 80% of all infants are infected with RSV in their first year of life and 2-3% of all children under one are hospitalised with bronchiolitis. Following infection from this virus, protection is incomplete with most people getting infected every 1-2 years. Currently there are no effective treatments or vaccines available. Over the past decade, our group have studied infants with RSV bronchiolitis and used various models to better understand disease pathogenesis. In this presentation, the airway epithelial response to infection will be focused upon and its particular role in directing the subsequent adaptive immune response to RSV.

LI07Th1130

Mechanisms of rhinovirus induced exacerbations of airway disease

Sebastian L Johnston
Imperial College London, UK

Acute exacerbations of asthma and COPD are a major unmet medical need. Current therapies fail to prevent the majority of exacerbations, and new preventive/treatment approaches are needed.
Respiratory virus infections are the dominant cause of exacerbations with human rhinoviruses the major precipitant. Experimental studies and models of rhinovirus induced asthma exacerbations have identified impaired innate and Th1-mediated antiviral immune responses as important in asthma exacerbation pathogenesis. Virus induction of anti-viral interferons is also deficient in COPD. The mechanisms behind these impairments in anti-viral immunity are poorly understood.
Asthma is a Th2-mediated disease in the stable state, but Th1-mediated inflammation is induced following viral infections. It is not known whether respiratory virus infection in asthma leads to additional amplification of Th2 inflammation. IL-25 and IL-33 are epithelial-derived mediators identified as inducers of Th2 inflammation. The roles of IL-25 and IL-33 in asthma exacerbations are unknown.
Increased airway inflammation consequent upon oxidative stress is believed important in stable COPD. Their role in COPD exacerbations is unknown.
A series of studies investigating these mechanisms in models of asthma and COPD exacerbations will be discussed.
Therapeutic implications of these findings in asthma & COPD exacerbations will then be discussed.

LI07Th1200

Human Metapneumovirus: New Kid on the Block or Golden Oldie?

John V. Williams
Associate Professor Departments of Pediatrics and Pathology, Microbiology, and Immunology Vanderbilt, University School of Medicine, Nashville, USA

Respiratory infections are leading causes of morbidity and mortality worldwide. Several new viruses associated with respiratory infections have been discovered during the last decade. Human metapneumovirus (MPV, HMPV) is a paramyxovirus identified in 2001 by Dutch researchers. Epidemiologic studies in many countries show that MPV is a leading cause of serious respiratory infection in children and adults. Phylogenetic analysis of whole genome sequences obtained over decades from five continents show that MPV diverged from an avian metapneumovirus several hundred years ago. However, the fusion (F) protein is largely conserved and animal studies show that F is the sole protective antigen, unlike other paramyxoviruses. Prophylactic and therapeutic strategies targeting F, including monoclonal antibodies and vaccines, are effective in animal models. MPV F protein interacts with RGD-binding integrins as receptors, and uses integrins to enter cells by endocytosis. High-throughput screening has identified small molecule inhibitors of MPV that offer therapeutic potential. Finally, MPV and other acute respiratory virus infections induce impaired lung CD8+ T cells via Programmed Death-1 (PD-1), an immuno-inhibitory signaling pathway. Thus, in the decade since the discovery of MPV, remarkable progress has been made in our understanding of this newly recognized (but not truly new) human pathogen.

LI07Th1330

Pathogenesis of Middle East respiratory syndrome coronavirus infection

V. Stalin Raj[1], Huihui Mou[2], Saskia L. Smits[1], Ron A.M. Fouchier[1], Albert D.M.E. Osterhaus[1], Berend Jan Bosch[2] and Bart L. Haagmans[1]
[1] Department of Viroscience, Erasmus Medical Center, Rotterdam, the Netherlands
[2] Virology Division, Department of Infectious Diseases & Immunology,

Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Most human coronaviruses (CoVs) cause mild upper respiratory tract disease but may be associated with more severe pulmonary disease in immunocompromised individuals. SARS-CoV on the other hand caused severe lower respiratory disease with nearly 10% mortality and evidence of systemic spread. Recently, another CoV, Middle East respiratory syndrome (MERS)-CoV was identified in patients with severe and sometimes lethal lower respiratory tract infection. Genetically the virus bears resemblance to bat CoVs like HKU4 and 5 and - based on phylogenetic analysis using a small fragment of the virus – to a bat CoV found in *Pipistrellus pipistrellus* in the Netherlands. Coronaviruses have zoonotic potential due the adaptability of their S protein to receptors of other species, most notably demonstrated by SARS-CoV. We identified dipeptidyl peptidase 4 (DPP4) - also known as CD26 - as a functional receptor for HCoV-EMC. DPP4 specifically co-purified with the receptor binding S1 domain of the HCoV-EMC spike protein from lysates of susceptible Huh-7 cells. Antibodies directed against DPP4 inhibited HCoV-EMC infection of primary human bronchial epithelial cells and Huh-7 cells and expression of human and bat DPP4 in non-susceptible COS-7 cells enabled infection by HCoV-EMC. The use of the evolutionary conserved DPP4 protein from different species as a functional receptor provides clues about HCoV-EMC´s host range potential. In addition, it will contribute critically to our understanding of the pathogenesis and epidemiology of this emerging human CoV, and may facilitate the development of intervention strategies.

LI07Th1430

When is a respiratory virus not a respiratory virus?

W. Paul Duprex

Department of Microbiology, Boston University School of Medicine, MA 02118, USA

Studies into viral pathogenesis require access to clinical isolates of known provenance, the development of disease-relevant in vitro systems and the availability of animal models which closely mirror what is observed in natural human infections. We have developed a number of paramyxovirus reverse genetics systems based on the consensus sequences obtained from unpassaged clinical material. Insertion of genes encoding fluorescent proteins within an additional transcription unit (ATU) in the genome allows the viruses to be tracked *in vitro* and *in vivo* with unprecedented levels of sensitivity. Such recombinant paramyxoviruses grow efficiently and fluorescence intensity correlates with the position of the ATU in the genome. Since the fluorescent proteins are stably expressed and not fused to any viral protein they flood the cytoplasm of the cell providing insights into the spatial orientation of the infected cell and its neighbors. Application of these approaches to measles virus (MV) has helped to illuminate viral pathogenesis in new ways. We have shown that although MV enters the body via the respiratory route epithelial cells are not the primary targets. Rather the virus initially infects immune cells in the deep lung and then spreads systemically. Virus transmission ensues when sub-epithelial immune cells in the upper respiratory transfer the infectivity into epithelial cells. This cell-to-cell spread is facilitated by binding of the MV hemagglutinin (H) glycoprotein to a highly conserved, basolateral protein (PVRL4) which is a component of the adherens junction. Trafficking of the MV fusion (F) and H glycoproteins to the apical surface allows the virus to be assembled and released. Attaining such a comprehensive understanding of the processes involved in cell attachment, entry and egress of MV and other paramyxoviruses will facilitate the development of safe and efficacious vaccines.

LI08

Evolution of microbial populations within the host

LI08Th0900

Adaption of *Mycobacterium abscessus* to transmission and lung infection

Julian Parkhill

The Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambidge, CB10 1SA, UK

We have recently shown that *Mycobacterium abscessus*, a lung pathogen previously thought to be opportunistic, and primarily environmentally acquired, is capable of frequent transmission between Cystic Fibrosis patients in a hospital environment, despite strict infection control procedures. We studies a series of longitudinal isolates over several years at a single lung clinic, and found clear evidence for patient-to-patient transmission, as well as data suggesting the broader emergence of transmissible clones in the wider population. The analysis of the genomic data from these clones shows evidence of adaption to transmission. In addition, our samples from individual time points show evidence of within-patient variation, allowing us to study adaption to survival in the individual patient alongside the adaption to broader transmission in the community.

LI08Th0930

Evolution of antibiotic resistance in the host

Benjamin P Howden[1,2]

1Austin Centre for Infection Research (ACIR), Infectious Diseases Department, and Microbiology Department, Austin Health, Heidelberg, Australia; 2Department of Microbiology and Immunology, University of Melbourne, Parkville, Australia

Antimicrobial exposure, in addition to the *in vivo* milieu, provides strong selective pressures for microbial adaptation during bacterial infection. *Staphylococcus aureus,* a major pathogen of humans, frequently causes large burden infections that have a tendency to be clinically persistent. In these situations the *in vivo* evolution of antimicrobial resistance, particularly to the glycopeptide antibiotic vancomycin and other agents such as rifampicin, are well recognised. Recent genomic studies have uncovered a large range of mutations within key staphylococcal regulatory genes that result in low-level vancomycin resistance, as well as having other important effects on the bacterium that impact on host-pathogen interactions. Other genomic changes, including movements of IS*256* within the staphylococcal chromosome can also result in vancomycin resistance. While the range of mutations in *S. aureus* that result in rifampicin resistance are well established, it appears that these mutations result not only in antibiotic resistance, but other changes promoting innate immune system evasion that favour bacterial persistence. These studies highlight the complex relationship between the evolution of antimicrobial resistance and host-pathogen

Please note: Abstracts are published as received from the authors and are not subject to editing.

interactions in human bacterial infections.

LI08Th1000

Evolution of *Pseudomonas syringae* in planta

Dawn L. Arnold[1], Helen C. Neale[1], Scott A. C. Godfrey[1], Robert W. Jackson[2], John W. Mansfield[3]

[1]Centre for Research in Biosciences, University of the West of England, Bristol, UK [2]School of Biological Sciences, University of Reading, UK [3]Division of Biology, Imperial College London, UK

Pseudomonas syringae pv. *phaseolicola (Pph)* is the causative agent of halo blight in the common bean. Pph race 4 strain 1302A contains the effector gene *avrPphB*, which matches resistance gene R3 and causes a rapid resistance reaction call the hypersensitive response (HR) in bean cultivar Tendergreen. The evolution of virulent strains of 1302A occurs in Tendergreen due to the selection pressure on the pathogen to bypass the HR caused by *avrPphB* which resides on a 106kb genomic island designated PPHGI-1. PPHGI-1 is deleted from the genome of *Pph* 1302A infecting Tendergreen, causing a change in host range and the production of water-soaking lesions typical of disease. This is an example of host imposed stress leading to changes within the bacterial genome that favour bacterial colonisation and proliferation. Using florescent labeling and Q-RT-PCR we have observed that transcription of a number of genes, including *avrPphB*, was reduced during PPHGI-1 excision from the genome, which may allow the bacterium to elude further detection. We also discovered that the circular PPHGI-1 episome is able to replicate autonomously when excised from the genome. These results shed light onto the plasticity of the bacterial genome as it is influenced by conditions within the host.

LI08Th1100

Salmonella Enteritidis: rapid evolution within an immunocompromised patient

Robert A. Kingsley

The Wellcome Trust Sanger Institute, Cambridge, UK

An IL-12 beta-1 receptor deficiency patient presented with recurrent atypical *Salmonella* Enteritidis bacteraemia progressively acquiring more phenotypic antibiotic resistance over 16 years and with no identifiable clinical nidus. Phylogenomic analysis using whole genome sequence was used to compare *S*. Enteritidis isolates. All patient isolates were present on a rapidly evolving lineage within the *S*. Enteritidis clade. Whole genome comparison was used to predict the molecular basis of phenotypic variation of the patient isolates. Rapid evolution was due to an in-frame deletion in the *mutS* gene that encodes a component of the mismatch repair mechanism and SNP accumulation suggests that a common ancestor existed around the time the patient first presented with fever in 1996. Evolution has profoundly impacted coding capacity of the isolates genome resulting in phenotypic changes in susceptibility to antibiotics and host pathogen interaction. It is likely the patient became infected with an otherwise typical strain of *S*. Enteritidis around 1996. A hyper-mutator phenotype appears to have contributed to rapid evolution and changes in phenotype, some of which can be directly attributed to evasion of antibiotic treatment. Other phenotypic changes are consistent with a loss of selection for an enteropathogenic lifestyle.

LI08Th1130

Offered paper – Experimental evolution reveals extensive horizontal gene transfer during *Staphylococcus aureus* host adaptation

Jodi Lindsay

St George's, University of London, London, UK

Staphylococcus aureus is a commensal and major pathogen of humans and animals. Comparative genomics of *S. aureus* populations suggests adaptation to different host species is associated with carriage of mobile genetic elements (MGE), particularly bacteriophages and plasmids capable of encoding virulence, resistance and immune evasion pathways. Yet the process of bacterial adaptation to new hosts is poorly understood. To investigate the population genetics and evolution during this biological process, we utilized the powerful technique of experimental evolution. Gnotobiotic piglets were co-colonized with both human- and pig-associated variants of the lineage clonal complex (CC)398, and genetic changes investigated using whole genome sequencing. Extensive and repeated transfer of two bacteriophages and three plasmids from the pig to the human isolate was detected, resulting in colonisation with isolates carrying a variety of mobilomes. Whole genome sequencing of progeny bacteria revealed no core genome polymorphisms, highlighting the importance of MGE acquisition during adaptation. Our data show for the first time that MGEs transfer at high frequency *in vivo* and drive *S. aureus* evolution and adaptation to new hosts. Furthermore, adaptation involved selection for diverse populations rather than a single dominant variant, and bacteriophages played a key role in generating a variety of mobilome types.

LI08Th1145

Offered paper – Recombination drives extensive phenotypic and genotypic diversity of a *Pseudomonas aeruginosa* population within a single Cystic Fibrosis patient

Sophie Darch[2], Freya Harrison[2], Steve Diggle[2], Alan McNally[1]

[1]Nottingham Trent University, Nottingham, UK, [2]University of Nottingham, Nottingham, UK

We performed phenotypic testing on 44 colonies of *P. aeruginosa* isolated from a single CF sputum sample recovered from a chronically infected patient. Vast variation was observed in production of Quorum signalling molecules, pyocyanin, and in antibiotic resistance patterns. Genome sequencing of 22 haplotypes representing the full spectrum of phenotypic variation confirmed all of the variants were derived from a single infectious clone highly similar to LESB58, however SNP typing showed variation between haplotypes ranging from 7 to 70 SNPs difference. Recombination analysis highlighted that the vast majority of these mutations were in recombining regions with an r/m ratio of 9.7, and that these recombination events were intra-population events. None of the SNPs were in genes previously associated with the described phenotypic variations, and a statistical permutation GWAS analysis provided string associations between recombinations in specific loci and altered phenotypes. None of the loci have previously been associated with such phenotypes. Our data provides new information on the role of recombination in short term pathogen evolution, and

on the complexity of mutational effects on bacterial phenotypes

LI08Th1300

Offered paper – Evolution and Pathoadaptation of *Pseudomonas aeruginosa* in Cystic Fibrosis Patients

Rasmus L. Marvig[1,2], Helle K. Johansen[1], and Søren Molin[2]

[1] Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark [2] Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark

Advances in genome sequencing have made it feasible to sequence multiple genomes of the same lineage of bacterial pathogens as they evolve in their human hosts. Here, we analyzed the genomes of >500 longitudinally collected clinical isolates of *Pseudomonas aeruginosa* sampled from Danish cystic fibrosis (CF) patients. Our phylogenetic analysis reveals the patients to be infected by 53 different clone types of *P. aeruginosa.* Identification of the same clone type over time revealed that 36 of the clone types were causing persistent infections, enabling us to decipher the recent within-host evolutionary history of each of these lineages. We found the 36 lineages to exhibit mutational convergence in 56 pathoadaptive genes in which the host environment imposed a selection for mutations. Furthermore, our results showed that mutation of downstream transcriptional regulators in regulatory networks involved in adaptation was contingent upon the mutation of upstream regulators in the same regulatory network. In conclusion, we identified adaptive trajectories generic to *P. aeruginosa* in the CF environment, and elucidated how mutation of regulatory networks shows historical contingency. Knowledge of pathoadaptive mutations and evolutionary contingency may help the development of therapeutic strategies against *P. aeruginosa* infections.

LI08Th1330

Offered paper – Genetic Variation is Localised to Hypermutable Sequences During Persistent Meningococcal Carriage

Mohamed Alamro[1], Neil Oldfield[2], Odile Harrison[3], David Turner[2], Dlawer Ala'Aldeen[2], Martin Maiden[3], Christopher Bayliss[1]

[1]University of Leicester, Leicester, UK, [2]University of Nottingham, Nottingham, UK, [3]University of Oxford, Oxford, UK

Many bacterial pathogens and commensals exhibit phase variation or antigenic variation of surface antigens due to mutations in hypermutable sequences. Alterations in these surface structures mediate adaptation to fluctuations in the selective pressures encountered during colonisation and persistence in their hosts. *Neisseria meningitidis* is a major agent of meningitis and septicaemia but is usually found as an asymptomatic coloniser of the human nasopharynx. Several genes in this species are subject to phase variation due to alterations in simple sequence repeat (SSR) tracts. Multiple isolates of *Neisseria meningitidis* were collected from 21 individuals subject to persistence colonisation for up to six months. SSRs of nine outer membrane proteins were analysed for alterations in repeat number by fragment analysis. Two genes exhibited trends towards lower expression levels. Reductions in expression were also detected for combinations of these phase variable genes. The levels of phase variation were compared to recombination-mediated antigenic variation of pilE, encoding the major pilin sub-unit, and to allelic variation as detected from whole genome sequences of these isolates. We conclude that localised hypermutation in contingency loci is the major mechanism mediating adaptation of meningococci during persistent carriage in the upper respiratory tract of humans.

LI08Th1345

Offered paper – Multiple independent emergence and rapid expansion of pertactin-deficient *Bordetella pertussis* in Australia

Connie Lam[1], Sophie Octavia[1], Lawrence Ricafort[1], Vitali Sintchenko[2,3], Lyn Gilbert2, Nicholas Wood[3], Peter Mcintyre[3], Helen Marshall[4], Nicole Guiso[5], Anthony D Keil[6], Andrew Lawrence[4], Jenny Robson[7], Geoff Hogg[8], and Ruiting Lan[1]

[1]University of New South Wales, Sydney, Australia; [2]The University of Sydney, Sydney, Australia; [3]Westmead Hospital, NSW, Australia; [4]University of Adelaide, Adelaide, Australia; [5]Institut Pasteur, Paris, France; [6]Princess Margaret Hospital for Children, Perth, Australia; [7]Sullivan Nicolaides Pathology, Brisbane, Australia; [8]The University of Melbourne, Melbourne, Australia

The circulation of *Bordetella pertussis* isolates which do not express the vaccine antigen pertactin has recently emerged or has been increasing in highly vaccinated populations including France, Finland, Japan and the United States. To determine whether such isolates are present in the Australian *B. pertussis* population, isolates collected between 1997 and 2012 were tested for the expression of the 3 main vaccine antigens; pertussis toxin (PT), pertactin (PRN) and filamentous haemagglutinin (FHA). All isolates expressed PT and FHA. All isolates collected prior to 2007 also expressed PRN, however 30% (96/320) of *B. pertussis* isolates did not express the vaccine antigen, Prn. Multiple mechanisms of prn inactivation were documented, including IS481 and IS*1002* disruptions, a variation within a homopolymeric tract and deletion of the entire *prn* region. The mechanism of Prn non-expression in 16 (17%) isolates could not be determined at the sequence level. These findings suggest that Prn non-expressing *B. pertussis* arose independently multiple times since 2008 rather than by expansion of a single Prn-negative clone. All but one isolate had ptxA1/ prn2/ *ptxP3*, the alleles representative of the currently circulating strains in Australia, a pattern consistent with continuing evolution of *B. pertussis* in response to vaccine selection pressure.

LI08Th1400

Genomic evolution of *Helicobacter pylori* within its human host

Xavier Didelot

Department of Infectious Disease Epidemiology Imperial College, London, UK

Helicobacter pylori is a bacterial pathogen that infects the stomach of about half of the human worldwide population. It can be carried asymptomatically for many years, but can also lead to severe disease, including gastric cancer. To understand the evolutionary dynamics of *H. pylori* within its human host, we compare whole genome sequences of multiple isolates from the same carriers. Such

Please note: Abstracts are published as received from the authors and are not subject to editing.

comparisons reveal a complex evolutionary history. Point mutation happens at the highest rate reported for any bacteria so far, and estimating this rate allows to date evolutionary divergence between isolates. Co-infection with different strains is relatively frequent, and provides an opportunity for recombination which is the main force of diversification. Strong genetic drift is exerted on within-host *H. pylori* populations, probably driven by immune selection. Understanding and quantifying these within-host evolutionary properties is necessary to investigate epidemiological questions such as the importance of different transmission routes.

LI08Th1430

Within-host evolution of *Staphylococcus aureus* during asymptomatic carriage

Daniel Wilson

Nuffield Department of Medicine, University of Oxford, Oxford, UK

Staphylococcus aureus is a major cause of healthcare associated mortality, but like many important bacterial pathogens, it is a common constituent of the normal human body flora. Around a third of healthy adults are carriers. Recent evidence suggests that evolution of *S.aureus* during nasal carriage may be associated with progression to invasive disease. However, a more detailed understanding of within-host evolution under natural conditions is required to appreciate the evolutionary and mechanistic reasons why commensal bacteria such as *S. aureus* cause disease. Therefore we examined in detail the evolutionary dynamics of normal, asymptomatic carriage.
This investigation begins to paint a picture of the within-host evolution of an important bacterial pathogen during its prevailing natural state, asymptomatic carriage. These results also have wider significance as a benchmark for future systematic studies of evolution during invasive *S. aureus* disease.

LI09

RNA and riboswitches in bacterial regulation

LI09Th0900

The excludon and other new concepts in bacterial antisense RNAmediated gene regulation

Pascale Cossart

Institut Pasteur, Unité des Interactions bactéries-Cellules, Inserm U604, INRA USC2020, Paris, France

RNA-mediated regulation was until recently considered as occuring via either small non coding RNAs, 5'UTRs or antisense RNAs. We have recently shown that RNAmediated bacterial regulation is more sophisticated than that. Some messenger RNAs can act as antisense RNAs - in other words some antisense RNAs can act as messenger RNAs ! - hence the concept of excludons. Some 5 'UTRs called riboswitches not only control transcription or translation of mRNAs but can also regulate and be co-transcribed with antisense RNAs or small RNAs. Two examples of vitamine B12 riboswitches regulating expression of an antisense RNA and of a small RNA in the bacterial pathogen *Listeria monocytogenes* will be presented. Bioinformatic analysis indicates that these examples are probably the first of a long list of yet undiscovered riboswitch-regulated non-coding RNAs.

LI09Th0930

Offered paper – Small regulatory RNAs – a mechanism of introducing biological noise into a system

Ruben Mars[1], Pierre Nicolas[2], Uwe Völker[3], Ulrike Mäder[3], Jan Maarten van Dijl[1], Emma Denham[1,4]

[1]University of Groningen, Groningen, The Netherlands, [2]INRA, Paris, France, [3]Ernst-Moritz-Arndt-University Greifswald, Greifswald, Germany, [4]University of Warwick, Coventry, UK

In their natural habitat, bacteria constantly adapt to changing environments, while simultaneously anticipating further disturbances. To do this, intricately interlinked metabolic and genetic regulation has evolved. Small-regulatory RNAs (srRNAs) are part of this complex network of regulation and act by fine-tuning mRNA or protein levels to coordinate a cells behaviour. We identified one srRNA in the Gram-positive bacterium *Bacillus subtilis* that when deleted displayed a strongly increased final optical density on minimal medium with the relatively slowly metabolized carbon source sucrose. Inspection of evolutionary conserved predicted targets for this putative srRNA led us to test whether this aberrant growth was due to higher AbrB levels in this strain. Here we report that, under some growth conditions, *B. subtilis* employs an srRNA to post-transcriptionally regulate – and more specifically to modulate the protein expression noise – of AbrB, a protein encoded by a gene with relatively low transcriptional noise because of its autoregulation. These noise-generation dynamics of srRNA-regulation were subsequently tested and confirmed in a stochastic simulation model. Finally, we show that this srRNA-mediated diversity in AbrB levels generates diversity in growth speeds in the exponential growth phase.

LI09Th0945

Offered paper – Genome-wide identification of small RNAs in *Clostridium difficile*

Nimitray Joshi, Sarah A. Kuehne, Nigel P. Minton, Stephan Heeb, Klaus Winzer

University of Nottingham, Nottingham, UK

Clostridium difficile is a major hospital acquired pathogen that can cause symptoms ranging from mild diarrhoea to more serious, sometimes life-threatening inflammation of the colon. Despite its medical importance, very little is known about the regulatory networks that are operating in this organism, and almost nothing about the roles played by small regulatory RNAs (sRNAs). sRNAs have been found in many other bacteria where they accomplish important functions in the regulation of cellular processes such as sporulation, quorum sensing, metabolism, and virulence. Unravelling the sRNA regulatory networks in *C. difficile* would be an important step toward understanding how this pathogen survives in the gut environment and causes disease.
To identify and map *C. difficile* sRNAs on a genome-wide scale, total RNA was extracted from triplicate cultures at different time points during the growth cycle and subjected to RNA-Seq analysis. Numerous putative sRNAs were identified and the existence of several of these were independently confirmed by Northern blot analysis. The biological function and regulatory targets of selected sRNA candidates is currently being investigated.

LI09Th1000

Dual RNA-seq: the RNA complement of pathogen and host in one go

Jörg Vogel

University of Würzburg, Institute f. Molecular Infection Biology, Würzburg, Germany

RNA is eminently suited a class of molecules to capture the physiological state of a cell. While traditional, probe-dependent approaches such as microarrays are insufficient to analyze the full (i.e. coding and non-coding) transcriptome at high resolution, the probe-independent RNA-seq approach has begun to revolutionize transcriptomics. RNA-seq is becoming the method of choice to analyze selected classes of expressed RNAs in either eukaryotic or prokaryotic cells. Yet, can it also enable a new understanding of host-pathogen interactions by revealing expression changes in both the pathogen and the host, which would require the simultaneous analysis of many different classes of RNA? Using our previously established dRNA-seq approach [1], we have now taken global RNA profiling a step further and developed 'Dual RNA-seq' [2] to investigate the interplay of the invasive bacterial pathogen *Salmonella* Typhimurium with human host cells during infection. Our analysis of mixed total RNA from bacterial and eukaryotic cells has for the first time captured the RNA complement of an infected cell, without the typical need to physically separate pathogen and host, and revealed a new small RNA regulating the intracellular virulence programme of *Salmonella* and its impact on the human host response. Reference: [1] Sharma CM, Hoffmann S, Darfeuille F, Reignier J, Findeiß S, Sittka A, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PF, Vogel J (2010) The primary transcriptome of the major human pathogen *Helicobacter pylori* Nature 464(7285)250-255 [2] Westermann AJ, Gorski SA, Vogel J (2012) Dual RNA-seq of pathogen and host Nature Reviews Microbiology 10(9):618-30

LI09Th1100

Novel tuneable gene expression systems based on orthogonal riboswitches

Neil Dixon

Manchester Institute of Biotechnology Faculty of Life Sciences University of Manchester, UK

Strategies that permit precisely controlled, differential, and simultaneous expression of multiple genes would be extremely useful for a broad range of metabolic engineering, protein expression and synthetic biology applications. A new paradigm in genetic regulation emerged with the discovery of novel genetic regulatory elements within the 5' UTR of bacterial mRNA. Upon binding to a specific metabolite, these so-called 'riboswitches' change conformation, permitting differential gene regulation to occur. As these switches operate via a small molecule-dependent, protein-free mechanism, they present themselves as attractive targets for use as novel genetic control elements. New riboswitch-ligand pairs have been identified using a chemical genetic selection method. These new pairings have been shown to be orthogonally selective, highly responsive, and permit dose-dependent, tuneable regulation of recombinant gene expression in *E. coli* [Dixon et al *PNAS* 2010]. We have developed these into multi-component systems that permit fine-tuning over co-expression output stoichiometry, with potential applications in functional and structural analysis [Dixon et al *Angew Chem* 2012]. Finally, I will discuss the development of these cellular systems and molecular devices from simply proof-of-principle studies, into a tuneable modular recombinant expression system, and demonstrate the application of this technology for the production of proteins of biotechnological interest.

LI09Th1130

Big questions about small RNAs in *Mycobacterium tuberculosis*

Kristine B. Arnvig

University College London, UK

Tuberculosis, caused by infection with *Mycobacterium tuberculosis (Mtb),* remains a serious threat to global health; one third of humans carry a latent infection that can progress to active disease at any time. *Mtb* depends on an ability to adjust to changing environments within the host where it exists as multiple subpopulations, adapted to survival in different microenvironments. Previous efforts to elucidate the molecular basis of mycobacterial pathogenesis have been dominated by a protein-centric view, and an extensive literature documents transcriptional changes in response to environmental stresses. However, there is no doubt that small RNAs (sRNAs) play an important role in stress responses and host adaptation in other pathogens and it is therefore essential to include this so far poorly characterised aspect of *Mtb* gene regulation. We have identified numerous *Mtb* sRNAs by different approaches. In particular the application of RNAseq has uncovered an extensive repertoire of sRNAs, and the next step involves an in-depth characterisation of individual sRNAs: When and how are sRNAs expressed? What are their targets? What role do they play in *Mtb* pathogenesis?
I will present some of our recent data on selected sRNAs and discuss their putative roles in *Mtb* pathogenesis.

LI09Th1300

The choreography of transcription in *Salmonella enterica* serovar Typhimurium

Jay C. D. Hinton

Institute of Integrative Biology, University of Liverpool, UK

Bacterial transcriptional networks typically consist of hundreds of transcription factors and thousands of promoters. However, the true complexity of transcription in a bacterial pathogen remains to be established. We devised a suite of 22 different environmental conditions that reflects the pathogenic lifestyle of *Salmonella enterica* serovar Typhimurium strain 4/74 during infection of a host, and used RNA-seq to generate a compendium of *Salmonella* gene expression. The data can be accessed at http://tinyurl.com/HintonLabSalCom. Individual *in vitro* conditions stimulated characteristic transcriptional signatures, and the suite of 22 conditions induced expression of 86% of all S. Typhimurium genes. We found that the transcription of the majority of the 3825 S. Typhimurium promoters is environmentally-responsive. Our global approach revealed 112 new small RNAs, bringing the total number of small RNAs in S. Typhimurium to 280; these sRNAs were analysed in the context of chromosome localisation and Hfq-association. I will discuss the environmental conditions that stimulate expression of the *Salmonella* pathogenicity islands and will present a comprehensive expression landscape of sRNAs. We hope that

Please note: Abstracts are published as received from the authors and are not subject to editing.

this database of environmentally-controlled expression of every transcriptional feature of S. Typhimurium will be a useful resource for the bacterial research community.

LI09Th1330

Small RNAs as lifesaving suppressors in bacterial Type III toxin-antitoxin systems

George Salmond

Department of Biochemistry, University of Cambridge, UK

Bacterial toxin-antitoxin (TA) systems have been implicated in diverse phenotypes, including adaptation to stress responses, plasmid retention, and the phenomenon of persistence. The Type III TA class involves a complex of a toxic protein (an endoribonuclease) that is lethal in the absence of a small regulatory RNA that acts to suppress the toxic protein. The paradigm of the Type III TA class is the plasmid-encoded ToxIN system from the enterobacterial phytopathogen, *Pectobacterium atrosepticum*. The toxI gene encodes a small RNA that binds in a pseudoknot configuration to ToxN to inhibit toxic activity. In *Pectobacterium* the ToxIN complex is made constitutively but can be activated into a toxic form on infection by certain phages. These phages inadvertently induce precocious lethality by "releasing" the ToxN, leading to target bacterial mRNA degradation, bacterial stasis and eventually suicide, in a process arguably akin to a programmed cell death. The *Pectobacterium* system may have evolved to protect bacterial populations from phage infection through infected cell suicide that restricts viral replication. However, in addition to ToxIN, we have defined widespread subfamilies of the Type III TA system encoded in chromosomal, plasmid and phage genomes - and these might respond to different environmental or physiological cues.

LI09Th1400

The interactions of small non-coding RNAs and the RNA chaperone, Hfq, in the control of gene expression

Helen A. Vincent[1], Charlotte A. Henderson[1], Carlanne M. Stone[1], Jack O. Phillips[1], Peter D. Cary[1], Darren M. Gowers[1], Frank Sobott[2], James E. Taylor[1], Anastasia J. Callaghan[1]

[1]Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, King Henry 1st Street, Portsmouth, PO1 2DY, UK
[2]Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

The interactions of bacterial small non-coding RNAs (sRNAs) with their mRNA targets, mediated by the RNA chaperone protein, Hfq, are the subject of much current research. sRNAs play important, yet diverse roles, from regulating stress responses to conditions such as cold shock and iron depletion, though to regulating virulence gene expression. Biochemical and biophysical methods, including solution scattering, can be used to characterise the details of the molecular interactions of sRNAs with Hfq and their mRNA targets. In particular, we provide the first structural glimpses of sRNA-Hfq complexes, demonstrating the chaperone role of Hfq in remodelling sRNAs upon binding. An overview of our recent studies within this domain will be provided and the mechanistic insights gained discussed.

LI09Th1430

Streamlined identification of non-coding RNAs in routine DeepSeq transcriptomic results

Stephan Heeb

University of Nottingham, UK

RNA-Seq is revolutionising the ways we look at bacterial transcriptomes. From the not so distant era of probe-based microarrays as the only means to profile transcriptomes, technology has progressed to allow routine high-throughput transcript resolution down to the nucleotide level. Sophisticated protocols for the qualitative characterisation of transcriptomes have been developed to determine transcription start sites, mRNA processing points, transcription terminators, and have revealed the existence of an unsuspected number of non-coding RNAs. More frequently however, due to cost and simplicity considerations, RNA-Seq is used as a replacement to microarray hybridisation to compare annotated gene transcription levels between strains or populations growing under different conditions. Nevertheless, the data generated invariably contains additional information that generally remains unexploited, in particular about unannotated, non-coding RNAs, although using pre-existing data to extract this unplanned information a *posteriori* imposes specific constraints and limitations. To this purpose we have developed an algorithm that classifies every transcribed nucleotide according to its genetic context and compares its abundance between two sets. *Pseudomonas aeruginosa* and *Clostridium difficile* are used to illustrate the possibilities offered by this approach, with a user-friendly interface currently being developed for the facilitated mining of existing transcriptomic data.

LI11

Prokaryotic microbial infection forum

LI11We0900

Genomic interplay between host and pathogen

Gordon Dougan

Wellcome Trust Sanger Institute, Cambridge, UK

The genomic sciences have provided us with reference genomes for many pathogens and different hosts, including mice and humans. These genomes provide a blueprint to guide studies on host/pathogen interactions. We can use reference genomes as a framework to generate maps of genome variation within local or global populations. Data on natural genome variation provides a route into evolution and selection in populations, again for both pathogen and host. Thus, we are entering a period where studies on disease-associated populations will facilitate experiments that were previously impossible and that will encourage a move from the laboratory into the field. In this talk I will describe how information on pathogen and host genomes can be exploited to perform screens that can then be used to direct specific experiments on pathogenic mechanism.

LI11We0930

Offered paper – Artilysin®s: Novel antibacterials with low risk of resistance targeting multi-resistant *Pseudomonas aeruginosa*

Stefan Miller[1], Yves Briers[2], Rob Lavigne[2]

[1]Lisando GmbH, Regensburg, Germany, [2]K.U. Leuven, Leuven, Belgium

The increase of multi-resistant gram negative bacterial pathogens forms a global threat. Especially *P. aeruginosa* is well known for being highly resistant to antibiotics. Artilysins are novel recombinant fusion proteins providing a new mode of action. They are consisting of an efficient enzyme, which is based on a bacteriophage endolysin and a membrane penetrating peptide that facilitates passage through the outer membrane.

Minimal inhibitory concentration (MIC) experiments showed Artilysin Art-085 and Art-175 to be highly effective on *P. aeruginosa*. The MIC50 of 10µg/ml was independent of the strains being highly resistant to antibiotics. Resistance development against ciprofloxacin already within 7 cycles of MIC experiments was significant, especially for PAO1p and Br257 with more than 30-fold increase on Br257. Resistance development against Art-085 and Art-175 however was not observed within 20 experimental cycles on all strains investigated.

Artilysins are acting efficiently on *P. aeruginosa* with MIC values of few microgram also for strains being highly antibiotic resistant. The mode of action of Artilysins from outside of the bacterial cell leads to a reduced risk of resistance. Within the studies performed so far resistance was not developed. Thus Artilysins are an efficient tool to combat gram negative bacterial pathogens.

LI11We0945

Offered paper – DNA is an essential component of the *Campylobacter jejuni* biofilm extracellular matrix

Helen Brown[1], Mark Reuter[1], Roy Betts[2], Arnoud van Vliet[1]

[1]Institute of Food Research, Norwich, UK, [2]Campden BRI, Gloucestershire, UK

Introduction: The majority of bacteria exist in either single or multi-species biofilms. Biofilm growth allows increased tolerance to starvation, antimicrobials and survival in food chain relevant conditions. *Campylobacter jejuni* is one of the leading causes of infectious intestinal disease in the developed world. We have previously shown that *C. jejuni* biofilm formation is increased in aerobic conditions, however relatively little is known about *C. jejuni* biofilm formation and its extracellular matrix (ECM) composition.

Results: We have investigated the role of extracellular DNA (eDNA) as a component of the *C. jejuni* ECM. eDNA is present throughout the *C. jejuni* growth cycle, and DAPI-staining of GFP expressing *C. jejuni* showed that eDNA is a substantial component of the ECM. Degradation of eDNA in mature *C. jejuni* biofilms led to the rapid removal of the biofilm, without affecting *C. jejuni* viability. The extracellular DNase-expressing *C. jejuni* strain RM1221 was capable of degrading mature *C. jejuni* biofilms in co-culture assays. Molecular investigation of this mechanism is currently under investigation.

Conclusion: These results suggest that the ECM can be targeted to disrupt *C. jejuni* biofilms, which may lead to more efficient sanitisation of food processing equipment, and ultimately safer food for the consumer.

LI11We1000

Offered paper – A new secreted virulence factor of *Listeria monocytogenes*

Andrzej Prokop[1,3], Edith Gouin[1,2], Veronique Villiers[1,2], Marie-Anne Nahori[1,2], Renaud Vincentelli[4], Pascale Cossart[1,2], Olivier Dussurget[1,3]

[1]Pasteur Institute, Unit of Bacteria-Cells Interaction, Paris, France, [2]Inserm U604, Paris, France, [3]Paris Diderot University, Paris, France, [4]CNRS/Aix-Marseille University, Marseille, France

Listeriosis is a potentially fatal food-borne disease caused by *Listeria monocytogenes*. After crossing the intestinal barrier, *Listeria* spreads through the bloodstream to the liver and spleen where it multiplies. It can also cross the blood-brain and placental barriers leading to meningoencephalitis and abortions, respectively. We performed a genome-wide transcriptomic analysis of *Listeria* grown in human blood, which revealed a massive induction of transcripts activated by the major *Listeria* virulence regulator, PrfA. Among these transcripts, one was among the most highly induced in blood. The gene is specific to pathogenic *Listeria* strains and does not have any ortholog in other bacteria. We demonstrated that the gene encodes a small protein which is secreted by *Listeria* and contributes to virulence in a murine model of listeriosis. Importantly, it is required for bacterial survival in macrophages, cells that are critical for the control of infection. We next identified the eukaryotic partner of this new virulence factor. The interaction of the two partners takes place inside the nucleus. This is the second virulence factor of *Listeria* targeting nuclear proteins of infected cells after the nucleomodulin LntA.

LI11We1015

Offered paper – Host-pathogen interactions during uropathogenic *E. coli* (UPEC) infection

Rachel Floyd[1], Milena Lewanczyk[2], Disa Hammarlof[3], Susan Wray[1], Jay C. D. Hinton[3], Craig Winstanley[2]

[1]Dept of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, UK, [2]Dept of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [3]Dept of Functional and Comparative Genomics, Institute of Integrative Biology, University of Liverpool, Liverpool, UK

Urinary tract infections (UTI) are the second most common infectious disease resulting in widespread morbidity and mortality worldwide. Most UTIs develop when *Escherichia coli* colonize the bladder causing cystitis. Pyelonephritis can develop if bacteria ascend to the ureters, leading to kidney scarring and kidney failure. However, little is known about the cascade of events occurring during host-pathogen interactions in ascending infections. Previous studies in animal models have given some insight into the invasive potential of uropathogenic *E. coli* (UPEC) [1]. However there is poor understanding of whether these events occur during human infections due to the lack of a suitable laboratory model [2].Using

Please note: Abstracts are published as received from the authors and are not subject to editing.

a differentiated normal human urothelial cell (NHU) model that retains important characteristics of native urothelium, we showed that UPEC can invade host cells and replicate, causing exfoliation of the superficial layer of differentiated cells. RNA-seq-based transcriptomic analysis of UPEC gene expression during infection identified genes involved in adhesion and motility (e.g. *fimH, fliC*) that are regulated at critical checkpoints during invasion and replication. Collectively, these data shed light upon the complex host - pathogen relationship during infection.

1. Justice S, et al 2006
2. Rosen D, et al 2007

LI11We1100

Offered paper – Activity and inhibition kinetics of the essential enoyl-reductase (InhA) from *Mycobacterium tuberculosis*

Daniel Shaw[1,2], Kirsty Robb[1], Beatrice Vetter[1], Virginie Molle[3], Neil Hunt[2], Paul Hoskisson[1]

[1]University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, UK, [2]University of Strathclyde, Department of Physics, Glasgow, UK, [3]University of Montpellier, CNRS, Montpellier, France

InhA is an NADH-dependent enoyl reductase responsible for the biosynthesis of mycolic acids; an essential component of *Mycobacterium tuberculosis* (Mtb) cell walls. InhA is also the main target for the frontline anti-TB therapeutic Isoniazid. Although the inhibition of the wildtype (WT) InhA active site (hydrophobic pocket) is known to occur through oxidised isoniazid covalently binding to NADH to form an adduct, there is still much ambiguity about which residues within the hydrophobic pocket contribute to the mechanism of drug inhibition and why the clinically important S94A point-mutation confers isoniazid resistance. To this purpose, we employed site-directed mutagenesis (SDM) to generate a series of InhA single-point mutations (S94A, W222A, P193A, M155A, F149A) which, through time-dependent UV-Vis monitoring of NADH depletion, allows us to directly and unambiguously compare the dependence of the InhA activity and inhibition kinetics on to each specific residue. We found that the W222A and wildtype kinetics are almost identical, indicating that the Tryptophan residue plays no role in either enzyme activity or drug inhibition. Conversely, the P193A mutation results in a tenfold decrease in enzymatic activity highlighting its importance overall hydrophobic pocket architecture. These observations may aid in developing new anti-tuberculosis treatments and understanding the dynamics of inhibition.

LI11We1115

Offered paper – Deep Sequencing Approach for Dissecting nontypeable *Haemophilus influenzae* Transcriptome During *In Vitro* Colonization of Human Bronchial Epithelial Cells

Buket Baddal[1], Raffaele A Calogero[2], Scilla Buccato[1], Anna Rita Taddei[3], Alessandro Muzzi[1], John L. Telford[1], Stefano Censini[1], Marco Soriani[1], Alfredo Pezzicoli[1]

[1]Microbial Molecular Biology, Novartis Vaccines and Diagnostics, Novartis Vaccines and Diagnostics, Siena, Italy, [2]Bioinformatics and Genomics Unit, Molecular Biotechnology Center, University of Torino, Torino, Italy, [3]Centro Interdipentimentale di Microscopia Elettronica, University of Tuscia, Viterbo, Italy

Infection of host niches by pathogens is a complex and dynamic process that requires the fine-tuned expression of both bacterial and host regulatory pathways. A deeper understanding of the dynamics of this regulation could help decipher the interplay between invading bacterial pathogens and their host cells. We have set up an in vitro system based on primary normal human bronchial epithelial (NHBE) cells, representing a niche often preferred by nontypeable *Haemophilus influenzae* (NTHI) during colonization of the host. The epithelial cells are assembled in an air-liquid interface in which they undergo mucociliary differentiation, recapitulating the characteristic properties of the *in vivo* human airway. The bronchial epithelium was synchronously infected with an otitis media outbreak strain Hi176 and the infection progress was monitored at 1, 6, 24 and 72 hours post infection (hpi). Using RNA-seq, we have simultaneously profiled NTHi and host gene expression in infected epithelia during selected time-points. Comparison of expression profiles derived from infected and uninfected epithelia revealed dozens of factors with altered expression during the infection that can provide insights into the temporal progression of NTHi infections.
Taken together, our studies provide an understanding of the transcriptional responses underlying NTHi infections during a time-course via dual-sequencing approach.

LI11We1130

Offered paper – Development of an *in vivo* model to demonstrate the pathogenic role of superantigen *Y. pseudotuberculosis*-dervied mitogen A (YPMa) in Far East Scarlet-like Fever causing *Y. pseudotuberuclosis* strains

Alexander Amphlett[1], Sofia Filali[2], Elisabeth Carniel[2]

[1]Keele University, Staffordshire, UK, [2]Institut Pasteur, Paris, France

BACKGROUND: In Europe, human *Yersinia pseudotuberculosis* infection typically occurs sporadically, in the form of a self-limiting gastroenteritis. In Russia , outbreaks of infection occur causing severe systemic inflammatory symptoms. This disease variant is called Far East Scarlet-like Fever (FESLF). Geographical heterogeneity exists between virulence factors produced by European and Far Eastern strains, implicating superantigen *Y. pseudotuberculosis*-dervied mitogen A (YPMa) in the pathogenesis of FESLF. Previous research into FESLF using isogenic mutants in vivo has been limited, with no suitable *in vivo* model being identified.
METHODS: This work addresses these limitations by: Identifying a suitable in vivo model to assess the pathogenicity of FESLF causing strains and assessing the pathogenicity of YPMa *in vivo*.
RESULTS: This work found that: (i) FESLF causing strains are almost avirulent via the intragastric route of OF1 mice. (ii) The intravenous route is suitable to assess the pathogenicity of FESLF causing strains. (iii) Intravenous challenge with a ΔypmA mutant prolongs mean time to death of OF1 mice and increases LD50, relative to the wildtype.
DISCUSSION: My work will enable future researchers to use isogenic mutants *in vivo,* to differentiate between genomic elements associated with FESLF and those directly involved in the pathogenesis of the disease.

LI11We1145

Offered paper – Genotypic and Transcriptomic Characterization of a Small Colony Variant of *Pseudomonas aeruginosa* and its Parent Strain Isolated in a Murine Model of Chronic Bacterial Colonization

Sharon Irvine, Hannah Bayes, Tom Evans

University of Glasgow, Glasgow, UK

Objectives: Small colony variants (SCVs) of *P. aeruginosa* are often isolated in Cystic fibrosis that show a number of changes favouring chronic colonization. In a murine model of chronic pulmonary colonization with *P. aeruginosa*, a phenotypically stable SCV arose during the course of infection. We characterized the genomic and transcriptomic characteristics of the SCV compared to the parent strain, to establish the mechanism behind the switch to the SCV phenotype.
Methods: The SCV arose within 3 days of colonization. Genomic and transcriptomic analysis was performed by Illumina sequencing.
Results: The SCV showed a highly stable phenotype on repeated subculture. Analysis showed typical SCV colony morphology with markedly increased biofilm production. Pulsed field gel electrophoresis showed no difference between parent NH strain and the SCV. DNA sequencing revealed few consistent changes between the parent and SCV other than a single nucleotide mutation within the algD gene. Initial RNA-seq transcriptomic analysis suggests differential expression of over 400 genes, almost all upregulated in the SCV, and many involved in adaptations to adverse environments.
Conclusion: The observed upregulated genes give insight into the adaptations required for bacterial survival within the CF lung that could be novel therapeutic targets.

LI12

Environmental microbiology forum

LI12We0900

Molecular microbial ecology – a penny for your thoughts

Jim Prosser

University of Aberdeen, UK

Microbial ecology is often said to be driven by, and limited by techniques and the application of molecular techniques has certainly increased out ability to characterise the diversity and community composition of natural microbial communities. Technical advances, however, do not always lead to equivalent advances in understanding. In this respect, high-throughput sequencing and associated approaches, for example metagenomics, are providing enormous amounts of data, but arguably little increase in understanding of the major drivers of diversity, the links between community composition and ecosystem function and the ways in which microorganisms interact with each other and with their environments. Many ecological studies are descriptive, unstructured and rely on correlation, and other forms of *post hoc* data analysis, rather than adopting experimental approaches designed to test hypotheses. The bases for these different approaches will be discussed and critically explored and relevant developments in associated areas, in particular microbial evolution, that challenge traditional ecological ideas will be considered to exemplify the requirement for conceptual approaches, rather than merely descriptive research.

LI12We0930

Offered paper – Detoxification of hexavalent chromate by *Amphibacillus* sp. KSUCr3 cells immobilised in silica-coated magnetic alginate beads

Abdelnasser Ibrahim[1,2], Ali Al-Salamah[11], Ahmed Mohamed El-Toni[3], Mohamed El-Tayeb[1], Yahya Elbadawi[1], Garabed Antranikian[4]

[1]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, [2]National research Center, Cairo, Egypt, [3]King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia, [4]5Institute of Technical Microbiology, Hamburg University of Technology, Hamburg, Germany

Recently isolated Cr(VI)-reducing *Amphibacillus* KSUCr3 cells were immobilised in silica coated magnetic alginate beads. Magnetic magnetite (Fe3O4) nanoparticles were synthesised with an average particle size of 47 nm and 80 electromagnetic unit (emu)/g saturation magnetisation. Whole cells were immobilised by entrapment in alginate in the presence or absence of Fe3O4 nanoparticles for the preparation of both magnetic and nonmagnetic immobilised cells, respectively. Following optimisation of the entrapment process, the immobilisation yield reached 92.5%. In addition to the ease of separation for reuse of the magnetic cell-containing alginate beads using an external magnet, magnetically immobilised cells showed approximately 16% higher Cr(VI) reduction activity compared with nonmagnetic immobilised cells. Furthermore, to improve their mechanical properties, the magnetic alginate beads were successfully coated with a dense silica layer using sol–gel chemistry. *Amphibacillus* KSUCr3 cells immobilised in silica-coated magnetic alginate beads showed approximately 1.4- to 3.9-fold enhancement of thermal stability compared with free cells. Furthermore, after seven batch cycles, the Cr(VI) reduction activity of free cells decreased to 48%, whereas immobilised cells still retained 81.1% of their original activity. These results supported the development of a novel, efficient biocatalysts for Cr(VI) detoxification using a combination of whole cell immobilisation, sol-gel chemistry, and nanotechnology.

LI12We0942

Offered paper – Evaluation of Bioaugmentation and Biostimulation Using a Novel Reductive Approach for Remediation of Tetrachloroethene and Trichloroethene in a Chalk Aquifer

David Granger[1], Chris Tate[2], David Dyson[1], Richard Bewley[3]

[1]URS Infrastructure & Environment UK Ltd, Wimbledon, UK, [2]Environment Agency, Huntingdon, UK, [3]URS Infrastructure & Environment UK Ltd, Manchester, UK

Historical releases of chlorinated solvents (tetrachloroethene and trichloroethene) into a chalk aquifer from a tannery in Sawston, Cambridgeshire had resulted in a groundwater contamination plume in excess of 2km and closed a public water supply. A three-phase bioremediation pilot trial involved injection of (i) EHC-L® (a micro-emulsion of slow-release, food-grade carbon (lecithin), ferrous iron (Fe+2), and a redox buffer (cysteine) at 10-37m below ground level

Please note: Abstracts are published as received from the authors and are not subject to editing.

(bgl)), (ii) inoculation of *Dehalococcoides spp*. (DHC) and (iii) ELS® (EHC-L® without the iron component) at 5-10m bgl. Each injection was completed at a single location. Within 84 days of ELS® injection, evidence of complete dechlorination was observed across a minimum 13m width from 4-10m bgl, with complete dechlorination reaching 94-100% across a minimum 8m width in the underlying 5m. For the 15-37m bgl interval, an average of 62% complete dechlorination continued to be achieved across an 8m aquifer width 18 months after EHC-L® injection. In each case the DHC population increased by four orders of magnitude.In total, the pilot trial was estimated to have completely dechlorinated approximately 12% of the total chlorinated ethene mass flux migrating from the source and demonstrated that bioremediation represented a viable option for full-scale treatment.

LI12We0954

Offered paper – Impact of logging and forest conversion to oil palm on fungal communities in Borneo rainforest

Dorsaf Kerfahi[1], David P. Edwards[2], Jonathan M. Adams[1]

[1]Seoul National University, Seoul, Republic of Korea, [2]University of Sheffield, Sheffield, UK

The effects of tropical rainforest logging and clearance on soil fungal community composition and diversity are poorly known. We compared areas of unlogged, once-logged, twice-logged rainforest, and areas cleared for oil palm, in the Danum Valley, Sabah, Malaysia. The ITS1 region of extracted soil DNA was PCR-amplified and 454 pyrosequenced.

The community composition of soil fungi showed a difference with logging history, and between forest and oil palm plantation. The fungal diversity was highest in Primary and logged forests and lowest in oil palm. Basidiomycetes relative abundance was lower in logged forests and oil palm, while Ascomycetes were more abundant. Basidiomycetes families known to be important as mycorrhizal and wood decomposing fungi were less abundant in logged forest and in oil palm.

These results suggest a pervasive impact of logging on tropical rainforest soil community structure. Basiomycetes relative abundance is depressed, even decades after logging. Fungal diversity is also decreased. The strongest effects, with lowest abundance of the Basiomycetes and total fungal alpha and beta diversities, are seen for oil palm plantations.

Keywords: alpha diversity, beta diversity, fungal community, logging, ITS1 region

LI12We1006

Offered paper – Endolichenic bacteria and their roles in the *Peltigera membranacea* symbiotic association

M. Auður Sigurbjörnsdóttir[1,2], Ólafur S. Andrésson[2], Oddur Vilhelmsson[1]

[1]University of Akureyri, Akureyri, Iceland, [2]University of Iceland, Reykjavík, Iceland

Lichens are generally characterized as the symbiotic association of a fungus (mycobiont), green algae and/or cyanobacteria. Together the lichen symbiotic partners form a self-sustaining thallus that is able to sustain growth under hostile and dynamic environmental conditions where neither could survive alone. Lichens form a conspicuous and important part of the world's vegetation and often occur as the only visible vegetation where ecological conditions are extreme. Although generally described as bipartite mutualistic associations, species-specific communitites of endolichenic bacteria, typically dominated by Proteobacteria, can also be found within the symbiosis. In the present study, the functional roles and colonization mechanisms of endolichenic bacteria associated with *Peltigera membranacea* are elucidated using metagenomic and culture dependent methods.

Analysis of 28,000 bacterial contigs from the *Peltigera membranacea* metagenome yielded multiple hits on several genes involved in lichen secondary metabolite resistance, inorganic phosphate mobilization, biopolymer degradation and several other potentially important functions in thallus colonization and symbiosis. Phenotypic analysis of selected isolates indicated that biopolymer hydrolytic activity is common among these bacteria, as is inorganic phosphate mobilization and nitrogen fixation.

LI12We1018

Offered paper – Interaction mediated discovery of antimicrobial activity of bacteria

Olaf Tyc[1], Paolina Garbeva[1], Wietse de Boer[1,2]

[1]Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands, [2]Wageningen University & Research Centre (WUR), Wageningen, The Netherlands

Soil microorganisms are the richest source of natural products of human interest like e.g. antibiotics, enzymes and other important secondary metabolites. Many soil bacteria produce secondary metabolites to suppress microorganisms competing for the same nutrient resources. However, current screening techniques for secondary metabolites almost only targeting individual bacterial species without accounting for interactions that are vital in eliciting antimicrobial activities. Recent studies have shown that interspecific interactions between microbial species can be an essential trigger for antimicrobial activities (Garbeva *et al.*, 2011, Traxler *et al.*, 2013). We describe here a high-through-put method for screening interaction-triggered production of antibiotics by bacteria. In total 146 soil bacterial isolates were screened for the production of antibiotics in 2798 random combinations. Antimicrobial activity was recorded via an agar-overlay assay against two target organisms: E. *coli* WA321 and *S. aureus* 533R4.

We discovered many bacterial isolates that showed antimicrobial activity during interactions but not in mono-cultures. Interaction triggered antibiotic production was observed for phylogenetically different combinations of bacterial isolates like *Burkholderia* sp. & *Paenibacillus* sp. and *Janthinobacterium* sp. & Dyella sp. and many others. The identification of the antimicrobial compounds and the mechanism of antibiotic triggering are under current investigation.

LI12We1100

Offered paper – The role of siderophores in *Escherichia coli* host commensalism

Laura Searle[1], Guillaume Meric[1,2], Ida Porcelli[1], Samuel Sheppard[2,3], Sacha Lucchini[1]

[1]Institute of Food Research, Norwich, UK, [2]Swansea University, Swansea, UK, 3University of Oxford, Oxford, UK

Iron is essential for *Escherichia coli* survival and growth but is poorly available in both the host and external environments. To facilitate iron uptake, most *E. coli* export the siderophore enterobactin, however, some isolates can produce up to three other siderophores that are linked with pathogenesis; aerobactin, salmochelin and yersiniabactin. Population analysis of *E. coli* isolated from primary (GI-tract of mammals) or secondary (plants) environments revealed that siderophore production is increased in host-associated isolates. To assess whether specific siderophores are linked with life in the host or external environment, we compared the distribution and expression of siderophore biosynthetic genes in plant and faecal *E. coli* isolates. Our data show that compared to environmental isolates, host-associated *E. coli* are more likely to encode the genes for aerobactin and yersiniabactin production. High siderophore production was not simply linked with the number of encoded siderophore systems, but was also associated with strain-dependent differences in the transcription of enterobactin and salmochelin biosynthetic genes. Preliminary analysis of gene expression *in vivo* shows that siderophore genes are expressed within the murine GI-tract. This suggests that production of multiple siderophores could facilitate colonisation of the gut and, besides virulence, play an important role in *E. coli* commensalism.

LI12We1112

Offered paper – Developing new biocontrol agents with broad-spectrum antagonistic activity against phytopathogens: An efficient *in vitro* screening strategy

Faheem Uddin Rajer, Xuewen Gao

Key Laboratory of Integrated Management of Crop Diseases and Pests, College of Plant Protection, Ministry of Education, Nanjing Agricultural University, Nanjing, China

Biocontrol of phytopathogens using microbial antagonists is an eco-friendly and cost-effective alternative to chemical pesticides. However, selection of effective biocontrol agents (BCAs) requires an efficient screening strategy. Therefore, the aim of this study was to isolate and characterize potential BCA (s) with broad-spectrum antagonistic activity against multiple phytopathogens. We have developed an efficient screening strategy for the characterization of new BCAs by including top-ranked fungal and/or bacterial phytopathogens with other target phytopathogen (s) during *in vitro* antagonistic tests. Two well-studied BCAs, *Bacillus amyloliquefaciens* FZB42 and *B. subtilis* B3 were also included for better comparison of new strains. Among several isolated strains from the rhizospheric soils of Pakistan, Bacillus spp. strains FA12 and FA26 displayed broad-spectrum antagonistic activity against all fungal and gram-positive and -negative bacterial phytopathogens using dual-culture or agar diffusion techniques, and were positive for production of chitinase, protease, cellulase, glucanase and siderophores. Whole-cell MALDI-Tof-MS analyses further revealed production of surfactin and bacillomycin L types of antimicrobial lipopeptides by FA12 and FA26, respectively. Both strains showed potential against multiple phytopathogens, for the production of lytic enzymes and secondary metabolites, and produced major antibiotics compared with reference strains and therefore could be adopted as future prospects in ecological agriculture.

LI12We1124

Offered paper – Chronic Intake of Pesticides alters the Gut Viral Transcriptome and Metabalome: Impact on Health and Disease

G Velmurugan[1], M Pushpanathan[2], U Vishnu[2], J Rajendhran[2], Subbiah Ramasamy[1]

[1]Department of Molecular Biology, School of Biological Sciences Madurai Kamaraj University, Madurai, Tamilnadu, India, [2]Department of Genetics, School of Biological Sciences Madurai Kamaraj University, Madurai, Tamilnadu, India

The usage of synthetic pesticides all over the world increased tremendously and it enters the gut through food chain. This study is aimed to explore the chronic pesticide-induced functional variation of the gut virome and its impact on health and disease. Mice were fed independently with organochlorine (endosulfan), organophosphate (monocrotophos) and carbamate (carbofuran) pesticides at theoretical maximum daily intake dosage for six months directly via drinking water. Control set of animals were maintained. After the experimental period, the animals were sacrificed and pesticide residues, glucose and lipid profile in blood were studied. In addition, acetylcholine esterase level, oxidative stress, tissue damage were assessed in brain, heart, liver and kidneys. Subsequently, total RNA and metagenomic DNA were isolated from the cecum. The rRNAs and poly(A) mRNAs were depleted and the resulting RNAs were sequenced using Illumina platform. The reads were annotated with the reference human microbiome genome, which includes viral sequences and differential expression was studied. The variations in bacteriophage transcriptome were correlated with bacterial diversity studied by 16S rRNA amplicon sequencing. Further by analyzing the primary and secondary metabolome of cecum and correlating with toxicological data, the functional role of virome in pesticide-induced toxicity and its clinical implications was uncovered.

LI12We1136

Offered paper – Seasonal associations and atmospheric transport distances of Fusarium collected with unmanned aerial vehicles and ground-based sampling devices

David Schmale[1], Binbin Lin[1], Shane Ross[2]

[1]Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA, USA, [2]Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA, USA

Spores of fungi in the genus Fusarium may be transported through the atmosphere over long distances. Members of this genus are important pathogens and mycotoxin producers. New information is needed to characterize seasonal trends in atmospheric loads of Fusarium and to pinpoint the source(s) of inoculum at both local (farm) and regional (state or country) scales. Spores of Fusarium were collected from the atmosphere in Blacksburg, VA, USA using a Burkard volumetric sampler (BVS) 1 m above ground level and autonomous unmanned aerial vehicles (UAVs) 100 m above ground level. More than 2,200 colony forming units (CFUs) of Fusarium were collected during 104 BVS sampling periods and 180 UAV sampling periods over four calendar years (2009-2012). Spore concentrations ranged from 0 to 13 and 0 to 23 spores m-3 for the BVS and the

Please note: Abstracts are published as received from the authors and are not subject to editing.

UAVs, respectively. Spore concentrations were generally higher in the fall, spring, and summer, and lower in the winter. Some of the species of Fusarium identified from our collections have not been previously reported in the state of Virginia. This work extends previous studies showing an association between atmospheric transport barriers (Lagrangian coherent structures or LCSs) and the movement of Fusarium in the lower atmosphere.

LI12We1148

Offered paper – The metabolic composition of the anodic microbial community can predict the electrical power in microbial fuel cells

Andre Gruning, Nelli Beecroft, Claudio Avignone Rossa

University of Surrey, Guildford, UK

We analyse the relation of electric power production in MFCs with the composition of the anodic biofilm in terms of the metabolic capabilities of identified sets of species. MFCs inoculated with a natural microbial community were continuously fed with sucrose. The composition of the community, power output and other environmental variables were sampled over a period of a few weeks until a stable output was observed, and the community composition was determined down to the species level, including relevant metabolic capabilities.
We constructed mathematical models to correlate the power output of MFCs and the composition and the structure of the microbial communities with their metabolic function. The results confirm that an MFC with natural inoculum and fermentable feedstock is essentially a two-stage system with fermentation followed by anode-respiration.
Under identical starting and operating conditions, MFCs with comparable power output develop different anodic communities with no particular species dominant. Good power production requires that MFCs contain a sufficient fraction of low-potential anaerobic respirators that can use terminal electron acceptors with a low redox potential. These results are very relevant to understanding the microbial functioning of MFCs and to the development strategies to improve their reliability and yield.

LI13

Prokaryotic cell biology forum

LI13We1400

Building nanomachines in biological outer space: a chain mechanism for bacterial flagellum growth

Lewis D B Evans[1], Simon Poulter[1], Eugene M Terentjev[2], Colin Hughes[1] and Gillian M Fraser[1]

[1]Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP

[2]Cavendish Laboratory, University of Cambridge, JJ Thomson Avenue, Cambridge CB3 OHE

Bacteria build helical propellers, called flagella, on their surface. Biologists and physicists have long found flagella fascinating as they illustrate beautifully how complex structures are constructed to operate as tiny 'nanomachines' on the cell surface. During flagellum assembly, thousands of subunits destined for the growing structure are made inside the cell, then unfolded and exported across the cell membrane. Like other biological functions, this initial phase of export consumes energy produced by the cell. But then the subunits pass into a channel at the centre of the growing flagellum on the outside of the cell, and must transit a substantial distance to the flagellum tip where they assemble into the structure. In this way the flagellum grows at a constant rate to several times the length of the cell.
The mystery has been how are flagellar subunits passed down the long channel far outside the cell where there is no discernable energy source to propel them? I will describe a simple and elegant new mechanism that allows constant growth of the flagellum outside the cell by harnessing the entropic force generated by the unfolded subunits themselves as they link in a chain that is pulled to the flagellum tip.

Evans LDB, Poulter S, Terentjev EM, Hughes C and Fraser GM (2013) A chain mechanism for flagellum growth. Nature 504: 287-290

LI13We1430

Offered paper – Flagella interact with ionic plant lipids to mediate adherence of pathogenic *Escherichia coli* to fresh produce plants

Yannick Rossez[1], Ashleigh Holmes[1], Eliza Wolfson[1], David Gally[2], Arvind Mahajan[2], Henriette Lodberg-Pederson[3], William Willats[3], Ian Toth[1], Nicola Holden[1]

[1]The James Hutton Institute, Dundee, UK, [2]The Roslin Institute, Edinburgh, UK, [3]The University of Copenhagen, Copenhagen, Denmark

Bacterial flagella are best known for motility and responding to chemotactic signals. However, these multi-functional organelles don't just move the bacteria or help them to sense their environment, they can also interact directly with the host. This has been reported for flagella from various bacterial species. Our work focuses on the interactions between food-borne pathogens and plant hosts, to understand how the bacteria colonise plants and then go on to cause fruit- and vegetable-associated outbreaks. We have found that flagella from *Escherichia coli* interact directly and adhere to the plasma membrane (PM) of plant cells. The flagella target lipids in the PM through ionic interactions. We have shown adherence to sulpho- and phospho- lipids using a variety of biochemical techniques, including glycan array screening and TLC. Functional binding was demonstrated by manipulation of the lipids in the PM and with flagella mutants. The work focused on H7 flagella from *E. coli* O157:H7, and we also demonstrated similar interactions with *E. coli* H6 and H48 flagella-types. This work shows the mechanistic basis for bacterial interaction with plant hosts. Importantly, because these lipids are conserved in PM of eukaryotes, we expect that this mechanism of host-interaction is common across biological kingdoms.

LI13We1442

Offered paper – Transcriptional regulators of the LysR and TyrR family are involved in controlling the biosynthesis of indole-3-acetic acid in *Serratia plymuthica*

Xiaoguang Liu[1,2], Song Guo[2], Yan Wu[2], Yunfei Duan[2],

Stephan Heeb[1], **Miguel Cámara**[1]
[1]School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK, [2]Institute of Life Sciences, Jiangsu University, Zhenjiang, China

There is increasing evidence that the phytohormone indole-3-acetic acid (IAA) produced by plant-associated microorganisms can act as a reciprocal signal molecule in microbe-plant interactions with the potential to modulate the physiology of plants, by disturbing the auxin balance, and bacteria (Spaepen & Vanderleydan, 2011 Cold Spring Harb Perspect Biol; 3: a001438). Within the genome of the endophytic strain G3 of Serratia plymuthica, an organism with plant growth-promoting capacity, we have identified two copies of the indole-3-pyruvate decarboxylase ipdC gene (ipdC1 and ipdC2) required for IAA biosynthesis. We have also identified two novel LysR-type transcriptional regulators, LysR1 and LysR2, convergently transcribed from the ipdC1 and ipdC2 genes, respectively. Mutagenesis and transcriptional analysis revealed that whilst LysR1 and LysR2 only control the expression of their neigbour ipdC gene, TyrR, a transcriptional regulator involved in the transcriptional regulation of aromatic amino acid biosynthesis and transport genes controls the expression of both ipdC genes and IAA biosynthesis in strain G3. Interestingly, mutations in LysR and TyrR also have an impact on bacterial swimming motility and proteolytic activity. We are currently investigating the mechanisms by which IAA impacts on the physiology of G3.

LI13We1454

Offered paper – Nitropropionic acid is a signal influencing biofilm formation in *Pseudomonas aeruginosa*

Ken Vercammen[1], **Pierre Cornelis**[1], **Susanne Haussler**[2], **Qing Wei**[1], **Tim Tolker-Nielsen**[3]
[1]Vrije Universiteit Brussel, Brussels, Belgium, [2]Helmholtz Institute, Braunschweig, Germany, [3]University of Copenhagen, Copenhagen, Denmark

Pseudomonas aeruginosa genomes contain a conserved locus (PA4202-4203-4204 in PAO1 genome) with PA4202 encoding a putative nitropropane monooxygenase, PA4203 a LysR regulator and PA4204 a periplasmic gluconolactonase. Nitropropionic acid can be degraded by monooxygenases such as PA4202 to yield nitrate, nitrite, hydrogen peroxide and malonyl semialdehyde. The LysR regulator is a repressor controlling directly the expression of PA4202 and its binding to the short PA4202-PA4203 intergenic region was confirmed using the purified regulator protein in EMSA experiments. A chromatin immunoprecipitation assay revealed that PA4202 is the only gene the expression of which is controlled by PA4203 regulator. Accordingly, there is a de-repression of PA4202 transcription in a regulator mutant. However, the same mutant shows an important de-regulation of other genes, such as PA1225 encoding a dehydrogenase, and the hemO heme oxygenase gene. When the PA4202 gene is inactivated the mutant shows an increased sensitivity to nitropropionic acid while the formation of biofilms is strongly increased as assayed in the flow cell assay. We hypothesize that the reaction catalyzed by PA4202 generates NO which is known to be involved in biofilm dispersion. Accordingly, addition of a NO generating agent results in biofilm dispersion.

LI13We1506

Offered paper – The evolution of biofilm-forming Wrinkly Spreaders in glass bead columns and static microcosms leads to subtle differences in wrinkleality and fitness

Yvette Udall, Simona Hapca, Andrew Spiers
Abertay University, Dundee, UK

Adaptive radiation of bacteria has been investigated using *Pseudomonas fluorescens* SBW25 in static liquid microcosms where the biofilm-forming mutant known as the Wrinkly Spreader arises having significant fitness advantage over the ancestral strain. Wrinkly Spreaders also appear in evolving populations developing on partially-saturated glass bead columns (GBCs) constantly irrigated with nutrients, with competitive fitness (W) advantages on GBCs of 1.28 – 1.78. An environment effect was discernible in the wrinkleality (colony expansion, reversion rate, growth in static microcosms, biofilm attachment levels and strength) and W in static microcosms of isolates from GBCs and static microcosms ($P \leq 0.0496$), suggesting that the two environments had selected for subtly different classes of Wrinkly Spreader. An investigation of W using a GLM approach revealed a significant interaction between environment and growth, attachment and reversion ($P \leq 0.044$). Furthermore, when the interaction effects were investigated in more detail, differences in how each environment interacted with the covariates were found. This analyses suggest that these environments provide divergent pressures that result in the selection of subtly different classes of Wrinkly Spreader, illustrating how environmental differences may influence the evolutionary trajectories of adaptive lineages originating from the same ancestral population.

LI13We1518

Offered paper – The role of polyamines in bacterial growth and biofilm formation

Anthony Michael
Dept. of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas, USA

The polyamine spermidine is absolutely required for growth and cell proliferation in eukaryotes and archaea in part due to its role in the essential post-translational modification of the translation elongation factor eIF5A. Thus, eukaryotic and archaeal life is permenantly locked to spermidine biosynthesis or its acquisition. The analogous translation factor in bacteria (EF-P) is modified by lysine and therefore spermidine is not necessarily required for growth in bacteria. In some bacterial species such as *Pseudomonas aeruginosa* and *Campylobacter jejuni*, spermidine is essential for planktonic growth, whereas in *Bacillus subtilis*, spermidine is entirely dispensible for growth but essential for biofilm formation. The spermidine structural analogue norspermidine is required for normal planktonic growth of *Vibrio cholerae*, which is reduced 40% in its absence. It is, however, essential for biofilm formation and cannot be replaced by spermidine. There is some confusion about the role of polyamines in *B. subtilis* biofilm formation and disassembly. One report suggests that *B. subilis* synthesizes norspermidine to disassemble biofilms and that exogenous norspermidine inhibits biofilm formation without inhibiting growth. I will show that norspermidine is not synthesized by *B. subtilis*, and

Please note: Abstracts are published as received from the authors and are not subject to editing.

that increasing concentrations of exogenous norspermidine first promote biofilm formation and then inhibit planktonic growth.

LI13We1600

Offered paper – An Investigation into the Contributions of the Stressosome Sensor Proteins to the Growth and Survival of L. monocytogenes Under Stress Conditions

Beth O' Donoghue, Kerrie NicAogáin, Conor P. O' Byrne

NUI Galway, Galway, Ireland

Listeria monocytogenes is a ubiquitous food-borne pathogen that can be associated with mortality rates as high as 30% among humans who develop infections. Its success as a pathogen is due in part to an enhanced ability to withstand stresses, a number of which are commonly used in food processing environments. The alternative sigma factor σB is responsible for regulating the defence mechanisms of *L. monocytogenes* in response to stresses such as extremes of temperature, osmotic, acid and light stress. Various stress signals are thought to be integrated into the σB activation pathway via the stressosome, a large molecular complex consisting of multiple copies of signalling and sensor proteins. There are five RsbR sensor protein paralogues in the *Listeria* stressosome In order to determine the contribution of individual RsbR paralogues to survival and growth under osmotic, acid, light and other stress, Listeria monocytogenes EGD-e Δlmo0799 and ΔsigB strains were acquired and several mutant strains were constructed, each lacking one of the rsbR genes. The results of targeted phenotypic testing for each of the mutant strains will be presented. Interestingly, initial observations indicate that the absence of SigB enhances *L. monocytogenes* survival in the presence of light and osmotic stresses.

LI13We1612

Offered paper – Phospholipid trafficking in Gram-negative bacteria

Faye C. Morris[1], Timothy J. Knowles[1], Riyaz Maderbocus[1], Mark Jeeves[1], Jennifer Kirwan[1], Eva Heinz[2], Timothy J. Wells[1], Douglas F. Browning[1], Yanina R. Sevastsyanovich[1], Amanda E. Rossiter[1], Catherine A. Wardius[1], Daniel Walker[3], Trevor Lithgow[2], Mark R. Viant1, David A. Rasko[4], Adam F. Cunningham[1], Michael Overduin[1], Ian R. Henderson[1]

[1]Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK, [2]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia, [3]Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, [4]School of Medicine, University of Maryland, Maryland, USA

The Gram-negative outer membrane is an asymmetric lipid bilayer composed of lipopolysaccharide and phospholipid decorated with integral outer membrane proteins and peripheral lipoproteins. All the components of the outer membrane are synthesised in the cytoplasm, with the lipopolysaccharide and protein components trafficked to the outer membrane by dedicated proteinaceous complexes. However, a trafficking pathway for phospholipid has remained recalcitrant to identification.

During this study we have identified and characterised a conserved outer membrane lipoprotein, PlpA. Here we present the NMR structure of PlpA revealing it to be a dual BON-domain containing protein that binds anionic phospholipids. Loss of PlpA resulted in decreased levels of phosphatidylglycerol in the outer membrane, impaired outer membrane integrity and reduced virulence *in vivo*. Mutations that suppress the loss of PlpA map to the Mla phospholipid recycling pathway. Thus, PlpA is likely to play a role in trafficking anionic phospholipids to the outer membrane. The discovery of PlpA provides a new target for the development of therapies for Gram-negative bacterial infections.

LI13We1624

A bioinformatics study of signal peptides in Geobacillus thermoglucosidasius

Alexandria Holland [1], Michael J. Danson [2] and Albert Bolhuis [11] Department of Pharmacy and Pharmacology, [2]

Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, UK.

Geobacillus thermoglucosidasius (GT) is a thermophilic, ethanol-producing bacterium capable of utilising both hexose and pentose sugars for fermentation. Modification of GT strains to secrete hydrolases could increase the amount of available carbon sources from various feedstocks, which could in turn improve fermentation yields. As such, optimised protein secretion would be vital to achieve this. Secretion in the related mesophile Bacillus subtilis (BS) has been well studied, but it is conceivable that protein transport in GT has adapted to growth at higher temperatures. Initial studies were aimed at identifying abundantly secreted proteins in GT, which was performed using SDS-PAGE and mass spectrometry. This was combined with a bioinformatics study to identify secretory proteins in GT, and to explore potential differences of their signal peptides with those found in BS. The results indicate that although GT is a thermophile, the signal peptides in this organism do not differ significantly from those in BS, which could mean that signal peptides from either organism could be utilised to optimise secretion of hydrolases in GT. Ongoing work includes the analysis of secretion of model enzymes and their regulation, which will contribute to further our understanding of protein transport in GT.

LI13We1636

Offered paper – The role of siderophore secretion for iron competition

Konstanze Schiessl[1,2] Martin Ackermann[1,2]

[1]ETH Zürich, Zürich, Switzerland, [2]EAWAG, Dübendorf, Switzerland

Iron and successful iron uptake are essential for bacterial growth. Two kinds of iron uptake are known: Surface-based mechanisms rely on direct uptake via the cell surface. Secretion-based uptake, on the other hand, involves the exudation of a molecule binding iron. Only in a second step, iron is taken up in the bound form. A prominent example is the secretion of siderophores, strong iron chelators exuded by many bacteria. Secretion makes siderophores accessible to the environment, potentially leading to consumption by other cells or diffusion out of the producer's accessibility range. Considering these drawbacks, mechanisms not relying on secretion, i.e. surface-based uptake, seem superior.

However, secretion possibly also generates exclusive benefits. We investigate one potential benefit of secretion, the reservation of

iron in competition with other bacteria. Secreted siderophores can make iron unavailable to other cells, a reservation effect achieved by the specificity of the iron-siderophore uptake system. We explore whether secretion of siderophores conveys such exclusive benefits by competing *Pseudomonas aeruginosa* strains capable and incapable of siderophore secretion. Additionally, we test if the outcome of this competition depends on iron limitation, and whether such benefits can help explain the ubiquity of bacterial siderophore secretion despite its drawbacks.

LI14

Prokaryotic genetics forum

LI14We1430

Offered paper – Reducing ppGpp level rescues an extreme growth defect caused by mutant EF-Tu

Jessica Bergman[1], Disa Hammarlof[1,2], Diarmaid Hughes[1]

[1]Uppsala University, Uppsala, Sweden, [2]University of Liverpool, Liverpool, UK

Transcription and translation of mRNAs are coordinated processes in bacteria. We have previously shown that a mutant form of EF-Tu (Gln125Arg) in *Salmonella Typhimurium* causes ribosome pausing, resulting in increased rate of RNase E-mediated mRNA cleavage, causing extremely slow growth, even on rich medium. These phenotypes are reversed by mutations that reduce RNase E activity. Here we asked whether the slow growth phenotype could be compensated by overexpression of a wild-type gene. We identified *spoT* (encoding ppGpp synthetase/hydrolase) to do this. We found that the slow-growing mutant had an abnormally high basal level of ppGpp that was reduced when *spoT* was overexpressed. Inactivating *relA* (encoding the ribosome-associated ppGpp synthetase) also reduced ppGpp levels and significantly increased growth rate. Because RelA responds specifically to deacylated tRNA in the ribosomal A-site we measured the acylation levels of a set of tRNAs, showing that all three isoacceptors for proline are less aminoacylated in the slow-growing strain compared to the wild-type. The increased ppGpp level in the mutant was demonstrated to cause transcriptional changes that are inappropriate for rich media conditions, contributing to the slow growth. Reducing ppGpp levels removes one cause of the slow growth and reveals the interconnectedness of intracellular regulatory mechanisms.

LI14We1445

Offered paper – Horizontal gene transfer can rescue prokaryotes from Muller's ratchet: Benefit of DNA from dead cells and population subdivision

Nobuto Takeuchi[1], Kunihiko Kaneko[1], Eugene Koonin[2]

[1]University of Tokyo, Tokyo, Japan, [2]National Center for Biotechnology Information, Bethesda, MD, USA

Many prokaryotes absorb DNA from the environment and incorporate it into their genomes, the process known as horizontal gene transfer (HGT). What are the evolutionary consequences of HGT? One hypothesis posits that HGT can restore genes inactivated by mutations and thereby prevent stochastic, irreversible deterioration of genomes known as Muller's ratchet. However, incorporating DNA from the environment also could be expected to be harmful because DNA in the environment can come from cells that have died due to deleterious mutations, so that on average it contains more deleterious mutations than the DNA of recipient live cells. To address this issue, we analyzed a population genetic model of prokaryotes undergoing HGT. The result indicates that HGT can prevent the operation of Muller's ratchet even if on average HGT introduces more deleterious mutations than it removes. Moreover, if HGT is sufficiently frequent and diffusion of environmental DNA sufficiently rapid, a subdivided population is shown to be more resistant to Muller's ratchet than an undivided population of an equal overall size. These results suggest that HGT could be an important condition for the long-term maintenance of genomic information in prokaryotes through the prevention of Muller's ratchet.

LI14We1500

Offered paper – RNA polymerase recruitment is co-dependent on CRP and Fis at *Escherichia coli* promoters controlling cytotoxic autotransporter expression

Amanda Rossiter, Rita Godfrey, Jack Connolly, Stephen Busby, Ian Henderson, Douglas Browning

Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK

The plasmid encoded toxin (Pet) is a cytotoxic autotransporter protein secreted by the pathogenic Enteroaggregative *Escherichia* coli strain 042. Its expression is co-dependent on the CRP and Fis global transcription factors and at the pet promoter CRP binds to a single site, which is centred at position -40.5 upstream of the start site for transcription. Due to the suboptimal positioning of this site, CRP alone activates transcription poorly and requires Fis to bind upstream to promote full activation. Here, we investigate the mechanism of Fis-mediated co-activation and show that when bound at position -40.5, CRP recruits RNA polymerase inefficiently. Fis compensates for this defect by aiding polymerase recruitment using direct protein-protein interactions. Furthermore, we show that CRP and Fis control the expression of other important autotransporter toxins in both Uropathogenic *E. coli* and *Shigella sonnei*, and that this mechanism of co-activation by CRP and Fis has been maintained in different pathogens during evolution.

LI14We1515

Offered paper – The primary transcriptome of invasive S. Typhimurium D23580 reveals new mechanisms of gene regulation

Disa L. Hammarlöf[1,] Carsten Kröger[2], Sathesh K. Sivasankaran[2], Karsten Hokamp[2], Roy R. Chaudhuri[3], Melita Gordon[1], Jay C. D. Hinton[1]

[1]University of Liverpool, Liverpool, UK, [2]Trinity College Dublin, Dublin, Ireland, 3University of Sheffield, Sheffield, UK

A newly emerged pathovar and sequence type of non-typhoidal S. Typhimurium (ST313) is causing invasive disease in Sub-Saharan Africa. We are defining the primary transcriptome of a representative strain of ST313, D23580, using differential RNA-seq. This global gene expression analysis generated a map of all transcripts being made in the cell, including 5′ UTR, 3′ UTR, ncRNA and antisense transcripts.

Please note: Abstracts are published as received from the authors and are not subject to editing.

We define transcriptional start sites (TSS), which give us detailed information on the location of bacterial promoters, and thus aid in the dissection of gene regulation [1]. We have defined 3,489 TSS for D23580, expressed in a pooled sample generated from 16 infection-relevant growth conditions [2]. Ninety-eight percent of the TSSs are conserved in 4/74, which is a closely-related, gastroenteritis-associated strain of S. Typhimurium.
The prophage element BTP1 is unique to D23580 and has a distinct complex transcriptional structure that includes novel candidate regulatory small RNAs. The core genomes of D23580 and 4/74 differ by only ~1,000 SNPs. We will present an example of a SNP that is located in the promoter region of a D23580 virulence gene, and directly influences gene transcription.

1. Kröger et al (2012) PNAS 109:E1277-86
2. Kröger et al (2013) Cell Host Microbe. 14:683-95

LI14We1600

Offered paper – A new Serratia bacteriophage and related enterobacterial viruses are efficient horizontal gene transfer agents in biocontrol bacteria and plant and human pathogens

Miguel A. Matilla, George P.C. Salmond

University of Cambridge, Cambridge, UK

Transduction is one of the drivers of genetic diversity in bacteria, and transducing bacteriophages are very important tools in bacterial genetics. In this study we sequence and characterise a new phage that infects multiple environmental and clinical isolates of Serratia spp. and a rhizosphere strain of Kluyvera. The phage can transduce chromosomal markers at frequencies of up to 7 x 10^{-6} transductants per plaque forming unit (p.f.u.). We demonstrated transduction of a 77-kbp gene cluster encoding production of a potently-bioactive secondary metabolite and we have shown heterogeneric transduction, from Serratia to Kluyvera, of a Type III toxin-antitoxin encoding plasmid. Our genome comparative analyses suggested that related phages also may be capable of transduction in their respective bacterial hosts. Here, we show that four related phages, infecting plant and human pathogens, are also efficient generalised transducers and can transduce chromosomal markers and plasmids at frequencies of up to 10^{-4} transductants per p.f.u. We conclude that all bacteriophages taxonomically related to the new Serratia phage are likely to perform efficient horizontal gene transfer and so will have utility for functional genomics, bacterial engineering and synthetic biology studies. However, it may be undesirable and prudent to avoid their use in phage therapy.

LI14We1615

Offered paper – Influence on expression of the *Helicobacter pylori* virulence factor vacuolating cytotoxin A *(vacA)* by a potential stem-loop structure in the 5' untranslated region of the transcript

Karin Amilon, Darren Letley, Jody Winter, Karen Robinson, John Atherton

Nottingham Digestive Diseases Biomedical Research Unit, School of Medicine, The University of Nottingham, Nottingham, UK

The vacuolating cytotoxin, *VacA*, is an important virulence factor secreted by the gastric pathogen *Helicobacter pylori*. Certain *vacA* genotypes are strongly associated with disease risk, but the association is not absolute. Variation in toxin production exists and is likely to be important, but the determinants of *vacA* expression are unclear. We hypothesised that a potential stem-loop in the 5' untranslated region (UTR) of the *vacA* mRNA transcript was important for *VacA* production by stabilising the transcript, and have investigated this by site-directed mutagenesis.
Isogenic strains with altered *vacA* 5' stem-loop sequences were constructed. vacA mRNA levels and decay rates were measured using RT-qPCR. SDS-PAGE and western blotting were used to determine protein levels and toxin activity was assessed by epithelial cell vacuolation assay.
Disruption of the stem-loop structure in the vacA 5' UTR reduced mRNA levels two fold (p= 0.0011) and decreased mRNA half-life compared to wild type. Protein levels and toxin activity were similarly reduced. Restoring base-pairing in the disrupted stem-loop sequence resulted in wild-type *vacA* mRNA level and stability. Our results suggest that a stem-loop structure in the *vacA* 5' UTR is an important determinant of *vacA* expression, most likely through stabilisation of the *vacA* mRNA transcript.

LI14We1630

Offered paper – Comparative genomic and metabolomic analyses of *Pseudomonas aeruginosa* from the CF lung reveal signatures of host adaptation

Lewis Stewart[1], Amy Ford[3,1], Julie Jeukens[2], Irena Kukavica-Ibrulj[2], Brian Boyle[2], Paul Hoskisson[1], Roger Levesque[2], Nicholas Tucker[1]

[1]University of Strathclyde, Strathclyde Institute of Pharmacy and biomedical Science, Glasgow, UK, [2]Universite Laval, Institute for integrative and systems biology, Quebec City, Canada, [3]Queen's University Belfast, Centre for Infection and Immunity, UK

Pseudomonas aeruginosa undergoes genomic changes during chronic lung infections of cystic fibrosis patients. Perhaps the most notable phenotypic change is the switch to the mucoid phenotype caused by frameshift mutations in the *mucA* gene leading to the over-production of alginate. In the present work we used the mucoid phenotype as evidence of genetic adaptation to the CF lung and sequenced three mucoid strains and their non-mucoid progenitors along with four well known environmental isolates. A variety of comparative genomics software packages including IslandViewer, PanSeq and CCT were used to analyse the data allowing the determination of the core genome phylogeny and identification of pathogenicity islands. The mucoid strains exhibited decreased virulence in the *Galleria mellonella* infection assay as well as decreased twitching motility. All three mucoid strains showed evidence of genome shrinkage as part of the host adaptation process, typically involving the loss of genomic islands and phages. In order to determine the relative contributions of individual gene loss, we transduced the relevant mutation from the PA14 non-redundant mutant library into the isogenic non-mucoid progenitor strains using phage ϕPA3. We also applied HPLC-MS metabolomics analysis to the CF isolates grown in artificial sputum medium in order to interpret genomic differences.

LI15
Virology Workshop: DNA viruses

LI15We0900
Offered paper – Entry of adenovirus entry into skin-migrating dendritic cells

Efrain Guzman[1], Geraldine Taylor[1], Jayne Hope[2], Rebecca Herbert[1], Bryan Charleston[1]

[1]The Pirbright Institute, Pirbright, Woking, Surrey, UK, [2]The Roslin Institute University of Edingburgh, Edingburgh, Midlothian, UK

Human replication-deficient adenovirus 5 (AdV5) is a promising vaccine vector to deliver recombinant antigens. The mechanism of adenovirus attachment and penetration in permissive cells has been extensively studied. However, very little is known about the entry of AdV5-based vaccine vectors to immune cells. Dendritic cells (DC) are central to the initiation and maintenance of immune responses. Cannulation of lymphatic vessels allows for the collection of DC that migrate from the skin and which are involved in antigen uptake and presentation following vaccination through the skin. In this study, we investigated the interaction of bovine skin-migrating DC and replication deficient adenovirus-based vaccine vectors. We found that despite lack of expression of CAR and other known receptors, adenovirus readily enters skin-draining DC via actin-dependent endocytosis. Virus escape from endosomes is pH-independent and neutralizing antibodies do not prevent virus entry but prevent virus translocation to the nucleus. We also show that combining adenovirus with adjuvant increases the absolute number of intracellular virus particles but not the number of virus-containing DC. This, in turn, results in increased trans-gene expression and antigen presentation. We propose a new receptor-independent mechanism of adenovirus entry which occurs in skin-migrating DC and discuss its relevance in vaccination strategies.

LI15We0912
Offered paper – Herpes simplex virus type 1 targets the murine neuroepithelium for host entry

Maitreyi Shivkumar[1], Ricardo Milho[1], Janet May[1], Michael Nicoll[1], Philip Stevenson[2], Stacey Efstathiou[1]

[1]Department of Pathology, University of Cambridge, Cambridge, UK, [2]University of Queensland, Brisbane, Australia

Herpesviruses are important pathogens, and yet how they enter the host is not well-understood. Peri-oral ulcers and latency in the trigeminal ganglia (TG) have suggested that herpes simplex virus type 1 (HSV-1) enters by oral infection, while the distribution of HSV-1 encephalitis has suggested nasal entry. We tracked HSV-1 infection after non-invasive inoculation of mice. Nasal virus was 100-fold more efficient at establishing infection compared to oral virus. Live imaging showed that the virus spread from the nose to TG, then re-emerged in TG-innervated skin. Marking of infected cells by viral cre recombinase expression in floxed reporter gene mice showed that nasal virus consistently reached the TG, but rarely the olfactory bulbs. Nasal HSV-1 predominantly targeted the olfactory neuroepithelium, followed by rapid sub-epithelial spread of the virus. Immunohistochemical analysis revealed that in addition to sustentacular cells and olfactory neurons, a proportion of macrophages were also positive for HSV-1 antigens. Although infection of macrophages by HSV-1 is considered non-productive, preliminary studies with floxed virus marking in LysM-cre transgenic mice suggested that 12% of the virus reaching the TG had passed through LysM-expressing cells. The contribution of myeloid and other LysM-expressing cells to viral infection is still to be determined.

LI15We0924
Offered paper – TRAF2 facilitates *Vaccinia virus* replication by promoting rapid virus entry

Ismar Haga[1], Tali Pechenick Jowers[1], Samantha Griffiths[2], Juergen Haas[2], Philippa Beard[1]

[1]The Roslin Institute, University of Edinburgh, Edinburgh, UK, [2]Division of Pathway Medicine and Centre of Infectious Diseases, University of Edinburgh, Edinburgh, UK

Tumour necrosis factor receptor (TNFR)-associated factor 2 (TRAF2) is a pivotal intracellular mediator of signalling pathways with known pro- and antiviral effects. We investigated its role in the replication of the prototype poxvirus, Vaccinia virus (VACV). Loss of TRAF2 expression, either through siRNA treatment of HeLa cells or through genetic knockout in murine embryonic fibroblasts (MEFs), led to significant reductions in VACV growth following low multiplicity infection. We also observed delayed production of both early and late VACV proteins as well as accelerated virus-induced alterations to cell morphology, indicating that TRAF2 influences early stages of virus replication. Uncoating assays showed normal virus attachment but delayed virus entry in the absence of TRAF2. Treatment with an inhibitor of endosomal acidification greatly reduced virus entry into TRAF2 -/- MEFs, suggesting that VACV is reliant on the endosomal route of entry in the absence of TRAF2. Thus TRAF2 is a proviral factor for VACV that plays a role in promoting efficient viral entry most likely by facilitation of the plasma membrane entry route.

LI15We0936
Offered paper – The development and application of a robust system for the *in vitro* replication of a rodent anellovirus, RoTTV

Shoko Nishiyama, Bernadette Dutia, Peter Simmonds, Colin Sharp

The Roslin Institute, Edinburgh, UK

Infections with small DNA viruses of the genus Anellovirus are widespread in mammalian populations, often infecting over 90% of individuals in their host species including humans. Despite the widespread nature of these infections and over 15 years of study since the discovery of the prototype anellovirus, Torque teno virus (TTV), the details of anellovirus molecular virology (including replication, protein synthesis, virion assembly etc.) are still largely unknown. This is primarily due to the lack of an efficient cell culture system for viral replication and propagation. Here we describe the development of a system for the robust in vitro growth of a recently discovered rodent TTV homologue, RoTTV. The development of this

Please note: Abstracts are published as received from the authors and are not subject to editing.

system has allowed us to characterise the transcriptional profile of a member of a less common RoTTV genus (RoTTV2) and begin addressing the functions of virally encoded proteins. In addition, the generation and propagation of clonal virus using this technique has allowed for comparative studies into the mutation rates and evolution of single stranded DNA viruses in vitro and in wild hosts to further explore the nature of viral genetic diversity.

LI15We0948

Offered paper – Growing wild-type human cytomegalovirus *in vitro*

Isa Murrell[1], Gavin Wilkie[2], Andrew Davison[2], Gavin Wilkinson[1], Richard Stanton[1]

[1]Cardiff University, Cardiff, UK, [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK

Human cytomegalovirus (HCMV) is the leading infectious cause of congenital malformations, and a significant cause of morbidity and mortality in immunocompromised patients. To develop better therapeutics, it is important that researchers use viruses *in vitro* that faithfully represent clinical virus. However, clinical strains mutate rapidly when passaged *in vitro*, resulting in viruses with altered tropism and reduced pathogenicity. To circumvent this problem, HCMV genomes have been stabilized by cloning into bacterial artificial chromosomes (BACs), prior to transfection and passage. High-throughput DNA sequencing showed that viruses derived from BACs that retain vector sequences frequently underwent spontaneous deletions near these sequences, resulting in the loss of 2-6 HCMV ORFs plus the IRS inverted repeat. Viruses containing the wildtype HCMV genome, and derived from self-excising BACs, exhibited mutations in a similar manner to clinical viruses; genes in the UL128 locus (UL128L) mutated in fibroblasts but not in epithelial cells, whereas in epithelial cells mutations occurred in the UL/b' genome region. To circumvent these issues, fibroblasts allowing selective repression of UL128L were developed. This permitted passaging of virus derived from a self-excising BAC, containing a complete HCMV genome, *in vitro* without mutation. Determining the proteome of this virus has revealed 11 novel virion proteins.

LI15We1000

Offered paper – A systematic analysis of host factors involved in Herpes simplex virus type I (HSV-1) infection identified anti-viral cellular complexes and pathways

Samantha Griffiths[1], Julia Weber[1], Orland Gonzalez[2], Ralf Zimmer[2], Peter Ghazal[1], Juergen Haas[1]

[1]Univeristy of Edinburgh, Edinburgh, UK, [2]Ludwig-Maximilians Universität München, Munich, Germany

Herpes simplex virus type 1 (HSV-1) is a neurotropic virus causing vesicular oral or genital skin lesions, meningitis and other diseases particularly harmful in immunocompromised individuals. Like all herpesviruses, HSV-1 causes latent infections and a complex pathogen-host interplay enables HSV-1 to modulate and evade the host immune system. To comprehensively investigate the complex interactions between HSV-1 and its host we combined two genome-scale screens for host factors (HFs) involved in virus replication. A yeast two-hybrid screen for protein interactions and a RNA interference (RNAi) screen with a druggable genome siRNA library confirmed existing and identified novel HFs which functionally influence HSV-1 infection. Bioinformatic analyses found the 358 HFs were enriched for several pathways and multi-protein complexes, and the role of these in HSV-1 replication and pathogenesis will be discussed.

LI15We1012

Offered paper – Global analysis and modelling of real-time transcription and translation in lytic HSV-1 infection

Andrzej Rutkowski[1], Anne L'Hernault[1], Caroline Friedel[2], Florian Erhard[2], Philip Rosenstiel[3], Stacey Efstathiou[1], Ralf Zimmer[2], Lars Dölken[1]

[1]University of Cambridge, Cambridge, UK, [2]Ludwig-Maximilians-Universität München, Munich, Germany, [3]Christian-Albrechts-University Kiel, Kiel, Germany

Herpesviruses are very prevalent in humans and can cause a wide range of pathologies. Herpes simplex virus-1 (HSV-1) is an excellent model of lytic infection due to its fast replication. We analysed gene expression in the first 8 hours of HSV-1 lytic infection in human foetal foreskin fibroblasts, using Illumina sequencing on: (1) newly transcribed RNA (ntRNA) isolated by 4-thiouridine tagging and (2) ribosome-protected RNA fragments (ribosome profiling). ntRNA analysis showed that the synthesis of host mRNA and lincRNA is affected differently by HSV 1 infection: 91.9% and 6.4% of protein-coding genes were consistently down- and up-regulated, respectively, while for non-coding genes it was 40.0% and 52.1%, respectively. We observed that during the course of infection splicing of some host introns is continuously inhibited, while for others this inhibition becomes alleviated. Ribosome profiling revealed that host gene translation is shut down (with 4 identified exceptions), while that of viral genes mirrors the characteristic herpesvirus cascade of immediate early, early and late gene expression. Unlike for HCMV, in which multiple novel open reading frames were recently reported, current HSV-1 translatome seems to be annotated comprehensively. Further analysis, validation by SILAC and modelling of the results will extend our understanding of HSV-1.

LI15We1100

Offered paper – Using RNA aptamers as tools to study human papillomavirus (HPV) 16-mediated cell transformation

Ozlem Cesur, Clare Nicol, G. Eric Blair, Nicola J. Stonehouse

School of Molecular and Cellular Biology, University of Leeds, Leeds, UK

High-risk HPVs are responsible for around 90% of anogenital and oropharyngeal cancers, requiring the expression of E6 and E7 viral oncoproteins. The interaction of HPV16 E7 with the cell cycle control protein pRb has been extensively characterised and shown to promote pRb degradation, resulting in cell cycle misregulation. We are using RNA aptamers as tools to study E7 interactions. Aptamers are single-stranded oligonucleotides that can form complex structures and bind target molecules in a conformation-dependent manner. As aptamers are non-immunogenic, they have therapeutic potential; examples include the aptamer Macugen (also called Pegaptanib). The E7 aptamers have been stabilised by the

inclusion of modified pyrimidines. Several HPV16 E7 aptamers induced apoptosis in an HPV16-transformed cervical carcinoma cell line (SiHa) that actively expresses both E6 and E7. One E7 aptamer, A2, resulted in a loss of the E7 oncoprotein and a rise in cellular pRb levels in CaSki cells. Certain HPV16 E7 aptamers also appeared to target E7 from another high-risk HPV, HPV18. We are currently focusing on the pathway of E7 loss following aptamer transfections using inhibitors of protein degradation and co-localisation with cellular markers using immunofluorescence microscopy.

LI15We1112

A single amino acid in EBNA2 determines superior B cell transformation by type 1 Epstein-Barr virus

Stelios Tzellos, Paulo B. Correia, Claudio Elgueta Karstegl, and Paul J Farrell

Section of Virology, Imperial College Department of Medicine, St Mary's Hospital Campus, Norfolk Place, London W2 1PG, UK

Type 1 strains of Epstein-Barr virus are much better than type 2 strains at transforming human B cells into lymphoblastoid cell lines (LCLs). Replacement of the EBNA2 gene in type 2 EBV with type 1 EBNA2 confers a type 1 transformation efficiency on the type 2 strain, showing that EBNA2 is the functionally important gene. EBNA2 is a transcription factor for viral and cell genes.
We previously showed that sequences from the C terminal part of type 1 EBNA2 were sufficient to confer the maintenance of LCL growth when swapped into type 2 EBNA2. This part of type 1 EBNA2 contains the RG and transactivation domains. We also identified cell and viral genes that are differentially regulated by the EBNA2 types. Here we map the part of type 1 EBNA2 responsible for the superior growth transforming properties more precisely. Remarkably, changing a single amino acid (S442D) in type 2 EBNA2 confers a type 1 growth phenotype in LCL growth maintenance. This amino acid confers a stronger transactivation function but that does not seem sufficient to explain the different B cell transformation properties, which likely involve many of the differentially regulated genes.

LI15We1124

Offered paper – Epstein-Barr virus transcription factors control RUNX gene expression through long-range enhancers to regulate cell growth

Andrea Gunnell[1], Helen Webb[1], Sheryl Baptista[1], C. David Wood[1], Paul Farrell[2], Michelle West[1]

[1]University of Sussex, Brighton, UK, [2]Imperial College, London, UK

RUNX1 and RUNX3 genes are disrupted in myeloid and lymphoid cancers and RUNX expression is transcriptionally deregulated in Epstein-Barr virus (EBV)-immortalised cells by EBV nuclear antigens (EBNAs). EBNA2 upregulates RUNX3 expression leading to repression of the RUNX1 promoter by RUNX3, a requirement for growth of EBV-immortalised cells.
We have delineated the mechanism of RUNX3 activation by EBNA2 by identifying EBNA2-bound enhancer elements 50kb upstream of the RUNX3 promoter and have identified EBNA2-bound enhancer elements that may also contribute to the fine-tuning of RUNX1 expression. RUNX1 and RUNX3 enhancers are also targeted by EBNA 3 proteins implicating the involvement of multiple EBNAs in the control of RUNX gene expression.
Using chromosome conformation capture assays, we have detected looping interactions between RUNX enhancers and promoters and determined how these interactions are modulated by EBNA binding to regulate RUNX expression. Since EBNA2 and EBNA3 proteins do not bind DNA directly we are investigating the role of cellular transcription factors in EBNA binding to RUNX gene regulatory elements.
We have therefore begun to uncover the molecular mechanisms of RUNX gene expression control by EBV and cellular transcription factors improving our understanding of the role of RUNX regulation in cell growth control and tumourigenesis.

LI15We1136

Offered paper – NEDDylation is required for Kaposi's Sarcoma Associated Herpesvirus Lytic Reactivation and Represents a Novel Antiviral Target

David Hughes, Jennifer Wood, Adrian Whitehouse

University of Leeds, Leeds, UK

The ubiquitin-proteasome system and associated pathways are rapidly becoming accepted as major therapeutic targets for the treatment of malignancy, which potentially include those associated with oncogenic viruses. Additionally, small molecule inhibitors have been successfully used for dissecting the biological roles of these intriguing pathways. One such oncogenic virus that has shown promise in preclinical models when inhibiting the ubiquitin-proteasome system is Kaposi's sarcoma-associated herpesvirus (KSHV). Recently, a small molecule inhibitor (MLN4924) was developed that blocks NEDDylation, a Ub-like PTM that regulates cullin-RING Ub ligases (CRLs). Here we have investigated the importance of the NEDDylation cascade for KSHV biology. We found that treating KSHV-infected primary effusion lymphoma cells (PEL) with MLN4924 was cytotoxic by inhibiting NF-κB signalling. We also demonstrated that MLN4924 inhibited the KSHV lytic cycle. Intriguingly however, inhibition only prevented viral DNA replication but not lytic cycle-associated gene expression, highlighting a novel mechanism that uncouples these two features of KSHV biology. This suggests that CRL activity regulates a protein (viral or cellular) that governs viral DNA replication. Work is underway to identify this factor which may represent a novel restriction mechanism that could be exploited for anti-herpesvirus therapy.

LI15We1148

Offered paper – Modulation of myeloid differentiation by a viral miRNA during HCMV latency

Betty Lau[1], Emma Poole[1], Eain Murphy[2], Mark Wills[1], John Sinclair[1]

[1]University of Cambridge, Cambridge, UK, [2]Lerner Research Institute, Cleveland, Ohio, USA

Human cytomegalovirus (HCMV) encodes 24 miRNAs which target viral and cellular functions for efficient productive infection. However, whether viral miRNAs have any role in latency is unknown. Therefore, we profiled HCMV miRNAs expression in experimentally latent CD34+ cells and monocytes and routinely observed high levels of expression of a number of viral miRNAs including miR-UL148D. Using *in silico* predictions, we identified the cellular Activin

Please note: Abstracts are published as received from the authors and are not subject to editing.

A receptor 1B (ACVR1B) as one potential target of miR-UL148D and confirmed this experimentally.
Interestingly, ACVR1B is involved in regulating the differentiation of haematopoietic cells. As the state of myeloid differentiation determines whether or not HCMV undergoes latent or lytic infection, we predicted that latent HCMV infection influences differentiation by modulating ACVR1B expression. Consistent with this, we found that, whilst latent infection of monocytes slightly increased ACVR1B expression, this was prevented from reaching high levels by miR-UL148D. This moderate increase in ACVR1B expression during latent infection rendered latent monocytes more responsive to Activin A and only partially induced differentiation. In contrast, latent infection in the absence of miR-UL148D resulted in Activin A-independent terminal differentiation. Thus, miR-UL148D fine-tunes ACVR1B levels during latent infection to allow a restricted level of differentiation but preventing untimely induction of terminal differentiation.

LI15We1400

Offered paper – Epstein-Barr virus as a survival factor in Burkitt lymphoma: how does EBV tip the apoptotic balance?

Leah Fitzsimmons[1], Andrew Boyce[1], Rosemary Tierney[1], Martin Rowe[1], Gemma Kelly[2]

[1]University of Birmingham, Edgbaston, West Midlands, UK, [2]Walter and Eliza Hall Institute of Medical Research, Parkville, Melbourne, VIC, Australia

Epstein-Barr virus (EBV) can be lost spontaneously from certain EBV-positive Burkitt lymphoma (BL) cell lines, and these EBV-loss subclones have been reported to exhibit increased sensitivity to apoptosis. We have confirmed and extended those observations by performing extensive single-cell cloning on a panel of well-characterised BL lines displaying the Latency I pattern of virus gene-expression most commonly seen in the original tumours. Sporadic loss of EBV from BL cells is rare (0.03% of subclones) and such loss is associated with enhanced sensitivity to several apoptosis-inducing agents. Importantly, reinfection of EBV-loss clones with EBV can restore apoptosis protection. Additionally, our EBV-loss clones are less tumorigenic *in vivo*; the mean survival of immuno-compromised mice injected with EBV-loss BL cells being around half that of mice injected with EBV-positive counterparts.
Our drug sensitivity assays suggested that EBV protects BL cells via the intrinsic apoptosis pathway. We therefore used inducible pro-apoptotic BH3 ligands to investigate Bcl-2-family dependence in BL clones. Although EBV-loss was consistently associated with enhanced sensitivity to BH3-ligand-induced death, the dependence on individual anti-apoptotics varied between clones. To identify upstream factors, we are investigating the changes in gene transcript and protein expression induced in different subclones following treatment with apoptosis-inducing agents.

LI15We1412

Offered paper – Natural killer cell subsets that can slow down human cytomegalovirus dissemination

Kevin Chih-Chin Chen, Mark Wills

University of Cambridge, Cambridge, UK

HCMV encodes multiple genes which inhibit NK cell function. HCMV prevents expression of cellular proteins which engage activating receptors on NK cells, in addition to encoding a MHC Class I-like molecule (UL18) which has a high affinity for an inhibitory NK receptor (LIR1). While NK cells are a component of the innate immune system it is becoming increasingly clear that numerous different subsets exist within a single individual. There is some evidence that particular NK cell subsets might be positively selected and increase in frequency in response to cytomegalovirus infection. In this study we have developed a novel assay which measures the ability of NK cells to inhibit HCMV growth and dissemination in vitro and have used this technique to start to analyse particular defined NK cell subsets to recognise and inhibit viral dissemination. Our results show that LIR1+ NK cells control viral dissemination of a clinical strain of HCMV more efficiently than LIR1- NK cells, despite both populations of NK cells having similar cytotoxic and cytokine production capability. This is a counterintuitive result, given that LIR1 is an inhibitory receptor but is nevertheless intriguing as HCMV infection can lead to elevated LIR1+ NK cell frequencies.

LI15We1424

Offered paper – Merkel cell polyomavirus small T antigen (sT) targets the NEMO adaptor protein to disrupt inflammatory signalling

Hussein Abdul-Sada, David Griffiths, Laura Knight, Adrian Whitehouse, Andrew Macdonald

University of Leeds, Leeds, UK

Merkel cell Polyomavirus (MCPyV) is implicated in the pathogenesis of Merkel cell carcinoma (MCC), a highly aggressive skin cancer, through expression of two oncoproteins, (sT and LT). sT expression is essential for cell transformation, however, the mechanisms by which sT may contribute towards MCC are poorly understood.
Viruses disrupt the host innate immune response, the first line of immune defence, to establish a chronic infection. We demonstrate that sT is able to disrupt activation of the anti-viral response in Merkel cells. sT prevents activation of the NFkB transcription factor by binding to the NFkB adaptor protein NEMO and preventing phosphorylation of the IKK complex and nuclear translocation of the NFkB heterodimer into the nucleus. We mapped the regions of sT necessary for inhibiting this pathway, and show that sT interacts with two novel cellular phosphatases PP2A A(beta) and PP4C. The latter interaction is currently unique to MCPyV. Mutagenesis and inhibitor studies show that these phosphatases are necessary for the inhibition of NFkB.
This study provides the first evidence for immune evasion by MCPyV and may help to explain the chronic nature of this virus. Future studies will help to elucidate the contribution of these interactions towards MCPyV pathogenesis and transformation.

LI15We1436

Offered paper – Viral immune evasion gene is a direct target of Epstein Barr virus transcription factor

Rajaei Almohammed, Kay Osborn, Alison Sinclair

School of Life Sciences, University of Sussex, Brighton, UK

Activation of Epstein-Barr virus (EBV) lytic cycle results in the expression of viral genes that are excellent targets for immune recognition. One mechanism used by EBV to impede a robust

immune response is to express genes, such as *BNLF2a* that disrupts the presentation of viral antigens. *BNLF2a* protein acts by inhibiting the peptide transporter associated with antigen processing (TAP) leading to a reduced HLA class I antigen recognition by cytotoxic T cells. We identified *BNLF2a* as a potential target for regulation by the viral transcription and replication factor Zta during lytic cycle from our genome-wide ChIP-Sequencing analysis of Zta binding sites on the viral genome. This revealed 5 binding sites in close proximity to the *BNLF2a* transcriptional start site. Each peak contained a single consensus Zta binding site within. Analysis of promoter-constructs of this region revealed that the *BNLF2a* is a direct target for Zta regulation and that mutation of all five of the ZREs showed that direct DNA binding by Zta is required for activation. Further dissection of the relevance of the five binding sites revealed functional redundancy. The direct activation of *BNLF2a* by Zta will rapidly undermine immune presentation and so facilitate cell survival during viral replication.

LI15We1448

Offered paper – Dual mechanisms of interferon response inhibition by vaccinia virus protein C6

Jennifer Stuart, Rebecca Sumner, Geoffrey Smith

University of Cambridge, Cambridge, UK

The interferon (IFN) responses are critical innate immune signalling pathways that function to prevent viral replication and to activate cells of the adaptive immune response. Downstream of IFN receptor engagement, Janus kinase/signal transducers and activators of transcription (JAK/STAT) signalling pathways are initiated that culminate in the phosphorylation and subsequent nuclear translocation of the STAT proteins, from where they drive antiviral gene expression.

This study describes a novel VACV inhibitor of IFN-induced signalling encoded by the C6L gene. C6 was previously described as a virulence factor that inhibits the activation of interferon regulatory factor 3 and hence type I IFN production. In addition, we report that C6 inhibits the activity of type I IFN- and type II IFN-driven luciferase reporters. Furthermore, C6 does not inhibit the IFN-induced phosphorylation of the STATs, nor their nuclear translocation, indicating a novel and potentially nuclear mechanism of IFN inhibition by VACV. Additionally C6 interacts with STAT2 but not STAT1, an observation of much interest given its ability to inhibit both type I and II IFN signalling.

The role of viral immunomodulators within the nucleus is little studied and will likely lead to the identification of cellular proteins with novel roles in host innate immune responses.

LI15We1500

Offered paper – Expression of Type I and Type III IFN during ASFV infection, and the ability of ASFV to overcome the IFNα-induced antiviral state

Josephine Golding, Christopher Netherton, Linda Dixon, Geraldine Taylor

The Pirbright Institute, Woking, Surrey, UK

African swine fever virus (ASFV) is a large dsDNA virus that is the cause of a lethal haemorrhagic disease in domestic and wild pigs. The ASFV genome contains large length variations at its termini which are associated with the loss or gain of genes within multigene families (MGFs).

We have shown that pre-treatment with IFNα had no effect on the replication of virulent ASFV isolates. However the replication of a naturally occurring avirulent isolate OURT88/3, and a mutant Pr4Δ35, which both contain deletions of MGF 360/530, was reduced in IFNα pre-treated cells. This suggests a role for members of MGF 360/530 in overcoming the IFNα-induced antiviral state. We have also confirmed that biologically active IFN, as measured by a MxA-CAT ELISA, was detected in pigs infected with virulent ASFV, but not by avirulent ASFV. Plasmacytoid dendritic cells, a major IFN-producing cell population, were also shown to express IFN after infection with ASFV *in vitro*. A reduction of IFN activity was observed after acid treatment of *in vitro* and *in vivo* samples, suggesting ASFV can induce both Type I and Type III IFN. Subsequently, we have shown that Type III IFN is expressed during ASFV infection of macrophages *in vitro*.

LI15We1512

Offered paper – Human cytomegalovirus US18 and US20 direct the natural killer cell activating ligand MICA for lysosomal degradation

Ceri Fielding, Rebecca Aicheler, Richard Stanton, Sepehr Seirafian, James Davies, Brian McSharry, Song Han, Virginie Prod'homme, Daniel Sugrue, Peter Tomasec, Gavin Wilkinson

Cardiff University, Cardiff, Wales, UK

Natural killer (NK) cells are crucial role in controlling human cytomegalovirus (HCMV) infection. Their activity is regulated by cell surface-expressed activating and inhibitory receptors, which bind cognate ligands on potential target cells. NKG2D is an activating receptor, expressed on all NK cell subsets with 8 different ligands:MICA and B, ULBP1-6. Multiple HCMV immune evasion functions target target the NKG2D pathway: UL16 sequesters MICB, ULBP1, 2 and 5 in the ER while UL142 retains MICA in the Golgi. A viral microRNA also (miR-UL112) suppresses MICB expression. Using a series of 'block' deletion mutants, we identified two members of the HCMV US12 family, US18 and US20 that regulate MICA expression. Deletion of these genes from HCMV led to an increase in cell surface MICA and total MICA/B. US18 and US20 expression caused a reduction in MICA/B levels. Lysosomal inhibition increased MICA/B levels and restored expression of a MICA-YFP construct in US18- and US20 expressing cells. Although we found the HCMV was able to down-regulate the truncated MICA*008 allele, deletion of US18 and US20 did not alter this effect. These data suggest the US12 family may have developed as a virus gene 'accordion' and have roles in HCMV immune evasion.

LI15We1600

Offered paper – Crystal structure of vaccinia virus protein A49

Sarah Neidel[1], Carlos Maluquer de Motes[1,2], Geoffrey L. Smith[1], Stephen C. Graham[1]

[1]Department of Pathology, University of Cambridge, Cambridge CB21QP, UK, [2]Department of Microbial & Cellular Sciences, University of Surrey, Stag Hill, Guildford, GU27JG, UK

Vaccinia virus (VACV) is a large double stranded DNA virus that

Please note: Abstracts are published as received from the authors and are not subject to editing.

modulates innate immunity by multiple mechanisms. The VACV protein A49 was recently described as a virulence factor that blocks activation of the nuclear factor-κB by binding to the cellular E3-ubiquitin-ligase βTrCP. Here we report the crystal structure of A49, solved at 1.8 Å resolution. A49 lacking a short N-terminal extension was produced in bacteria and crystallised by vapour diffusion. Unexpectedly, A49 adopts an alpha-helical structure similar to that of members of the B-cell-lymphoma 2 (Bcl-2) protein family despite not sharing sequence similarity with other viral Bcl-2-like proteins. Structurally, A49 most closely resembles M11, a myxoma virus protein that blocks apoptosis by binding to the pro-apoptotic proteins Bak and Bax via a surface groove. In contrast to M11, the A49 surface groove is rather occluded and, consistently, no binding to Bak or Bax is observed.
Taken together, our data reveal A49 as an unpredicted member of the VACV Bcl-2-like family that now comprises at least 11 members. Our studies highlight the utility of the Bcl-2 fold in mediating pro-viral protein:protein interactions and of protein structure in characterising the evolution of viral proteins with undetectable levels of amino acid sequence conservation.

LI15We1612

Offered paper – Genotypic variation in the VZV R1 re-iteration region: Implications for evolutionary diversity and the structure of tegument protein VP13/14

Samantha Cooray[1], Stephen G. Graham[2], Daniel P. Depledge[1], Samit Kundu[1], Khidir Hawrami[1], Mark Quinlivan[1], Fiona T. Scott[1], Pietro G. Coen[1], Claire Depack[1], Judith Breuer[1]

[1]University College London, London, UK, [2]University of Cambridge, Cambridge, UK

Varicella-zoster virus (VZV) genomes display significant genetic stability with the exception of the origin or replication (OriS) and five tandemly-repeated re-iteration regions (R1-R5) (Davidson & Scott, 1986; Tyler et al. 2007; Breuer et al., 2010). The R1 region displays significant variation and is transcribed as a polypeptide of amino acid repeats that forms part of the ORF11 gene product. ORF11 encodes for the VZV tegument protein VP13/14, a homolog of herpes simplex virus (HSV) 1 and 2 UL47. These proteins have a conserved RNA binding domain and have been shown to shuttle between the nucleus and the cytoplasm of virus infected cells (Donelly et al. 2007; Che et al. 2009). In addition, VP13/14 has been shown to be required for VZV replication in human skin (Che et al. 2009).
In this study we have analysed R1 sequences from over 300 VZV isolates from varied geographic locations. We report geographical clustering of R1 variants and, strikingly, a distinct repeat pattern in clade 5 (endemic in African and Asia) compared to clade 1-4 viruses. We have also analysed the impact of R1 sequence variation on the structure of the VP13/14 protein and identified changes with important implications for protein function and stability.

LI15We1624

Offered paper – Quantitative analysis of the composition of the adenovirus particle

David Matthews[1], Jonathan Bramson[2], Hideyo Ugai[3], Ali Alqahtani[1]

[1]University of Bristol, Bristol, UK, [2]McMaster University, Hamilton, Canada, [3]Washington University, St Louis, USA

We have used high throughput quantitative proteomics to study the protein composition of the mature adenovirus particle. We have compared batches of highly purified wild type adenovirus particles to purified E1/E3 deleted adenovirus particles, a recently described protein V deleted adenovirus and to a replication defective recombinant adenovirus recently used in a clinical trial. By comparing the relative abundance of all the proteins present in each batch of virus we were able to show that all the known structural components of the virus can be readily detected and quantitated including those components whose copy number is only two per virion. Furthermore we were unable to detect evidence of systematic packaging of cellular proteins within the virus particle. Finally we were able to demonstrate that adenovirus particles deleted for viral core protein V are also deficient in core protein VII indicating an hitherto unknown link between packaging of these two proteins into the virus particle. This finding also indicates that protein VII (a viral histone like DNA binding protein) may play additional roles in the virus particle beyond condensing the viral DNA. This approach appears to be ideally suited to the study of wild type and recombinant virus particle composition.

LI15We1636

Offered paper – Identification of novel host-virus interactions through combined miRNA target analysis and siRNA screening

Jon Pavelin, Natalie Reynolds, Stephen Chiweshe, Finn Grey

Roslin Institute, Edinburgh, UK

Identifying targets of viral miRNAs not only establishes a basis for understanding the role of miRNAs in virus infection but also provides a means for discovering novel host-virus interactions. Using RISC immunoprecipitation and siRNA screening we have identified targets of HCMV miRNAs that play important roles in the biology of the virus. Here we show that ATP6V0C, a component of the vacuolar ATPase, is a target of miR-US-25-1 and that siRNA knockdown of ATP6V0C results in almost complete inhibition of infectious of virion production. Our results show that ATP6V0C knockdown does not drastically inhibit viral entry or DNA synthesis, which has led us to investigate the role of ATP6V0C in the assembly and egress of HCMV. A critical step in the HCMV replicative cycle is the formation of the viral assembly compartment (VAC). Using siRNA knockdown, immunofluorescence-microscopy, and western-blot analysis we have identified a role for ATP6V0C in the formation of the VAC. siRNA knock-down of ATP6V0C results in a failure of trans-golgi vacuoles and early-endosomes to reorganise and a failure of VAC formation. These findings demonstrate a crucial role for ATP6V0C during infection, and in doing so identify a novel host factor that is required for HCMV assembly.

LI15We1648

Offered paper – Identification of host plasma membrane proteins relocalised by HSV-1 glycoprotein M

Sheung-Yee Kathy Lau, Colin Crump

University of Cambridge, Cambridge, UK

Herpes simplex virus-1 (HSV-1) glycoprotein M (gM) is a multiple-spanning membrane protein that is highly conserved throughout the Herpesviridae family. We have previously shown that gM can function during virus replication to relocalise specific viral glycoproteins to sites for assembly. Furthermore, gM was also observed to relocalise certain co-expressed cellular membrane proteins, suggesting additional roles for gM during infection. In order to identify potential cellular membrane proteins that are targeted by gM, we performed plasma membrane profiling: a SILAC-based proteomic approach. Using this technique we compared the expression of plasma membrane proteins of cells infected with virus lacking gM (ΔgM) and its revertant (gMR). Several cellular membrane proteins were identified as candidates for gM-specific removal from the plasma membrane, including tetherin - a known target of gM activity. Further studies have now highlighted specific cellular plasma membrane proteins modulated by gM that may have diverse and important roles during herpesvirus infection.

LI15We1700

Offered paper – Experiments for modelling the African swine fever transmission

Claire Guinat[1,2], Linda Dixon[1], Dirk Pfeiffer[2]

[1]The Pirbright Institute, Pirbright, UK, 2The Royal Veterinary, London, UK

African Swine Fever (ASF) is a severe haemorrhagic disease of domestic pigs, causing high mortality and resulting in significant economic losses in affected countries. Since its introduction into Georgia in 2007, ASF virus (ASFV) has spread to neighbouring countries. In this context, understanding the mechanisms of spread of ASFV within and between pig herds is essential. Quantitative data of ASF transmission dynamics are still very limited and further studies are crucial to develop effective control and eradication measures. Transmission experiments have been conducted at the Pirbright Institute using the highly virulent strain, Georgia 2007/1. The ASF transmission process between experimentally inoculated and in-contact domestic pigs was expressed mathematically in a stochastic Susceptible-Exposed-Infected-Removed (SEIR) model. The ASF transmission dynamics were investigated in four experiments involving direct and indirect contact between domestic pigs. The within-pen (Bw) and between-pen (Bb) transmission rate parameters were estimated based on the measurement of virus in blood and oral swabs, and development of clinical sign. These parameter estimates were then used to perform simulations of the stochastic SEIR model in a large pig population. Results from these simulations will assist in improving our understanding of ASF epidemiology and in the design of more effective control measures.

LI15We1712

Inhibiting herpesvirus lytic replication by targeting a cellular interaction partner

Sophie Schumann, Richard Foster, Adrian Whitehouse

University of Leeds, Leeds, UK

Herpesviruses replicate in the nucleus of the host cell and require cellular factors to export viral mRNAs from the nucleus, in order to allow efficient translation of viral proteins in the cytoplasm. However, while mammalian mRNA export is coupled to splicing, many herpesviral mRNAs are intronless. To circumvent this step, all herpesviruses express a highly conserved SR protein, which interacts with the human transcription/export (hTREX) complex to form an export competent ribonucleoprotein particle. This allows for nuclear export of viral mRNAs and subsequent translation of viral proteins. Studying the conserved herpesviral SR protein, ORF57, encoded by the oncogenic gamma-2 herpesviruses Kaposi's sarcoma-associated herpesvirus (KSHV), we present a novel mechanism for specific disruption of the ORF57/hTREX interaction. Targeting a cellular ATPase, which is an essential hTREX component, using a small molecule inhibitor we prevent ATP-cycle dependent remodelling and formation of the viral ribonucleoprotein particle, while allowing endogenous hTREX formation. Bearing in mind the conserved mechanism for herpesvirus intronless mRNA export, we now present a series of compounds that are able to prevent KSHV late protein expression and disrupt virus lytic replication, with limited cytotoxicity.

LI16

Virology workshop: positive strand RNA viruses

LI16We0900

Offered paper – Identification of two additional norovirus genogroups able to recognize histo-blood group antigens

Sarah Caddy[1,2], Adrien Brieman[3], Jacques le Pendu[3], Ian Goodfellow[1]

[1]University of Cambridge, Cambridge, UK, [2]Imperial College London, London, UK, [3]INSERM Institut de Biologie, Nantes, France

Human noroviruses (HuNV) are the most common cause of viral gastroenteritis in man worldwide. HuNV attaches to cell surface carbohydrate structures known as histo-blood group antigens (HBGAs) prior to internalization, and HBGA polymorphism amongst human populations affects susceptibility to HuNV. Noroviruses are divided into 6 genogroups, with human strains grouped into genogroups I, II and IV. Canine norovirus (CNV) is a recently discovered pathogen in dogs, with strains classified into genogroups IV and VI. This study sought to determine the carbohydrate binding specificity of CNV, and compare this to the binding specificity of genogroup I and II HuNV. A panel of synthetic oligosaccharides was used to assess the binding of CNV virus-like particles (VLPs), and identified α1,2 fucose as a key attachment factor. CNV VLP binding to canine saliva and tissue samples using ELISAs and immunohistochemistry confirmed that α1,2 fucose-containing H and A antigens of the HBGA family were recognized by CNV. Phenotyping studies confirmed expression of these antigens in a population of dogs. The virus-ligand interaction was further characterized using blockade studies and enzymatic removal of candidate carbohydrates. Recognition of HBGAs by CNV provides new insights into evolution of noroviruses and raises concerns regarding potential zoonotic transmission to humans.

LI16We0912

Offered paper – Identification of novel capsid motifs involved in cell culture adaptation of FMDV

Kyle Chamberlain[1,2], Veronica Fowler[1], Nick Knowles[1], Paul Barnett[1], Sarah Gold[1], Terry Jackson[1]

Please note: Abstracts are published as received from the authors and are not subject to editing.

[1]The Pirbright Institute, Woking, Surrey, UK, [2]University of Surrey, Guildford, Surrey, UK

Field isolates of foot-and-mouth disease virus (FMDV) use integrins as receptors due to the presence of a conserved integrin-binding RGD motif located on the G-H loop of VP1, whereas cell-culture adapted variants can use other receptors such as heparan sulphate (HS). Recently a virus (A/Iran/87 (A-)) with a major deletion within the VP1 G-H loop was isolated from a vaccine stock. This virus is unable to bind to integrins (as it lacks the RGD), and lacks the known HS contact residues. Sequence comparison identified a limited number of surface exposed residue changes, including a LEK to SAR tri-peptide at VP2 78-80 and a KE to EK di-peptide at VP2 130-131. Here we have used reverse genetics to investigate a functional role for these motifs in infection, and have also used mutagenesis to introduce these motifs in to another type-A virus whilst simultaneously abrogating integrin-binding. Infectious virus could only be recovered when both the SAR and EK motifs were present suggesting that both the SAR and EK motifs are essential for infection, most likely by forming a novel receptor attachment site. These motifs may enable us to "engineer" cell-culture adaptation in to other type A FMDV's.

LI16We0924

Offered paper – Modelling Hepatitis C virus in an inflamed liver environment: A new role for stromal expressed VAP-1 in regulating HCV entry and replication.

Sukhdeep Kaur Galsinh[1], Chris Weston[2], Amy Barnes[1], Ian A Rowe[1,2], Gillian Muirhead[2], David Adams[1], Christopher Buckley[3], Peter Balfe[1], Jane A McKeating[1,2]

[1]Centre for Human Virology, School of Immunity and Infection, University of Birmingham, Birmingham, UK, [2]NIHR Centre for Liver Disease, University of Birmingham, Birmingham, UK, [3]Centre for Translational Inflammation Research, School of Immunity and Infection, University of Birmingham, Birmingham, UK

Hepatitis C virus (HCV) is a major cause of global morbidity and mortality. An estimated 170 million individuals are infected worldwide and a significant proportion will develop cirrhosis and hepatocellular carcinoma. While hepatocytes are the major site of viral replication the role of non-parenchymal cells in the viral lifecycle is poorly understood.

Stroma or liver myofibroblasts define the microenvironment of the liver and given their close proximity to hepatocytes *in vivo*, we established co-culture systems to study their role in the HCV lifecycle.

Myofibroblasts inhibit HCV replication in adjacent hepatocytes in a cell-contact dependent manner by limiting genome replication and particle entry. Importantly, this observation is not restricted to HCV as lentiviral pseudotypes expressing murine leukemia virus glycoproteins show limited infection of myofibroblast-hepatocyte co-cultures, suggesting a general perturbation of membrane protein dynamics.

Liver myofibroblasts express vascular adhesion protein-1 (VAP-1), a primary amine oxidase prevalent in the diseased liver, implicated in the establishment and persistence of hepatitis. We demonstrate that rVAP-1 induces a dose-dependent inhibition of HCV entry and replication, identifying a new paracrine pathway for myofibroblasts to regulate HCV replication. This novel mechanism provides new therapeutic avenues for treating both the underlying inflammatory response and viral replication in chronic hepatitis.

LI16We0936

Offered paper – The role of dynamin in bluetongue virus infection

Lisa Stevens[1,2], Katy Moffatt[1], Karin Darpel[1,2], Peter Mertens[1], Terry Jackson[1]

[1]Pirbright Institute, Pirbright, Surrey, UK, [2]University of Surrey, Guildford, Surrey, UK

Dynamin functions as a membrane scission protein and plays a role in the release of vesicles from the plasma membrane during a number of endocytosis processes. Bluetongue virus (BTV) has been reported to use more than one cell entry mechanism and it has been suggested that dynamin plays a role in BTV entry. Here we have further investigated a role for dynamin in BTV entry using a field isolate of BTV (BTV-1 GIB2007/01) and bovine endothelial cells (a cell type targeted by BTV *in vivo*). The dynamin-2 isoform exists as four alternative splice variants (termed Dyn2(aa), Dyn2(ab), Dyn2(ba) and Dyn2(bb)) which may function during different endocytic process due to targeting to different cellular locations. The role of these variants in BTV infection has been investigated. Dominant-negative mutants of the dynamin-2 splice variants aa and bb were shown to inhibit uptake of transferrin, consistent with a role in clathrin-mediated endocytosis. Dominant-negative mutants of aa, ba and bb did not appear to affect uptake of dextran. The dominant-negative mutant of ba but not aa or bb inhibited BTV infection suggesting that infection of BFA cells may be dynamin dependent but independent of clathrin-mediated endocytosis.

LI16We0948

Offered paper – Orthologues of the *Drosophila melanogaster* gene *cg4572* have an antiviral role in mosquito and tick cells

Claudia Rückert[1,2], Gerald Barry[3], Esther Schnettler[3], Alain Kohl[3], Lesley Bell-Sakyi[1], John K. Fazakerley[1], Rennos Fragkoudis[1,2]

[1]The Pirbright Institute, Woking, Surrey, UK, [2]The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Midlothian, Scotland, UK, [3]MRC - Centre for Virus Research, University of Glasgow, Glasgow, Scotland, UK

Arboviruses are transmitted to susceptible vertebrate hosts by vectors such as mosquitoes and ticks. In arthropods, the most important known antiviral defence is RNA interference, which is triggered by dsRNA molecules. It has been suggested that the Drosophila serine carboxypeptidase CG4572 is involved in cellular uptake of dsRNA and it was shown to be essential for an efficient antiviral response in *D. melanogaster* (Saleh *et al.* 2009, Nature, 458;346-350). In the present study orthologues of *cg4572* were identified in the genomes of the mosquito *Aedes aegypti* and the tick *Ixodes scapularis* and their putative antiviral role was investigated. Expression of three putative *cg4572* orthologues was silenced in mosquito cells by transfection of target-specific long dsRNA and cells were subsequently infected with the alphavirus Semliki Forest virus (SFV). Silencing of *cg4572* in mosquito cells resulted in significantly increased SFV replication and the highest increase was observed when all orthologues were silenced concurrently. In

the *I. scapularis* tick cell line IDE8, silencing of a putative orthologue of *cg4572* resulted in significantly increased replication of SFV and also of the tick-borne flavivirus Langat virus. In conclusion, our data suggests that *CG4572* orthologues of mosquitoes and ticks are involved in antiviral immunity.

LI16We1000

Offered paper – Bluetongue virus NS4 protein counteracts the host antiviral response

Maxime Ratinier[1], Marco Caporale[1,2], Yi Jin[1], Rick Randall[3], Gerald Barry[1], Massimo Palmarini[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [2]Istituto Zooprofilatico Sperimental dell'Abruzzo e Molise G Caporale, Teramo, Italy, [3]School of Biology, Centre for Biomolecular Sciences, University of St. Andrews, St. Andrews, UK

Bluetongue virus (BTV, genus *Orbivirus*) is the etiological agent of bluetongue, a major haemorrhagic disease of ruminants. BTV encodes NS4, a small non-structural protein with nucleolar localization. We showed that a NS4 deletion mutant (BTV8ΔNS4) replicates as efficiently as wild type BTV-8 in IFN-incompetent cell lines. However, BTV8ΔNS4 replicates inefficiently in primary endothelial cells and it is highly attenuated in sheep.
Using both an interferon (IFN) protection assay and a reporter cell line, A549/pr(IFN-β), we demonstrated that BTV8ΔNS4, unlike wt BTV-8, is unable to block IFN synthesis by the infected cells. Furthermore, using pulse metabolic radiolabeling we show that NS4 contributes to the general shut-off of protein synthesis observed in BTV infected cells. We confirmed this finding using an *in vitro* luciferase assay, which clearly showed that NS4 inhibits protein synthesis. The NS4 from a variety of BTV strains block protein synthesis with the exception of a particular BTV-1 strain. Interestingly, the NS4 of this strain also fails to localize in the nucleolus. Despite very poor amino acid conservation among the genus, a variety of other *Orbivirus* NS4 proteins are also able to inhibit protein synthesis in luciferase reporter assays. We conclude that the NS4 is a viral IFN antagonist.

LI16We1012

Offered paper – Dynamical control of Stress Granules formation during calicivirus infection

Majid NH Al-Sailawi, Elizabeth Royall, Lisa O Roberts, Nicolas Locker

University of Surrey, Faculty of Health and Medical Sciences, School of Biosciences and Medecine, Guildford, UK

Protein synthesis is a tightly controlled process and viruses which depend on the translation machinery of the host can manipulate it, altering the function of specific eIFs. The expression of host mRNAs can also be regulated through their storage and/or decay in subcellular RNA granules such as stress granules, to stall their translation, or processing bodies, for their further degradation. Recent studies have demonstrated that viruses can modulate the dynamic of RNA granules formation in the infected host, highlighting a new layer of host-virus interaction. Caliciviruses, which have a single-stranded, positive-sense RNA genome, are responsible for several important diseases and human norovirus is the main virus responsible for gastroenteritis in the western world. Using feline calicivirus and murine norovirus as models, we investigated the regulation of RNA granules formation during calicivirus infection. Our results are suggesting an intricate relationship between caliciviruses and stress granules. We will provide evidence that calicivirus infection impairs the formation of stress granules within infected cells, reflecting a survival strategy, while the infection foci impacts on the surrounding cells ability to form stress granules. This highlights a new and yet unidentified layer of gene expression regulation by viruses in the infected host.

LI16We1100

Offered paper – Modulation of downstream effectors of the Ras/ERK MAPK pathway by Theiler's virus leader protein

Frederic Sorgeloos[1,2], Didier Vertommen[1], Mark Rider[1], Thomas Michiels[1]

[1]de Duve Institute, Université catholique de Louvain, Brussels, Belgium, [2]University of Cambridge, Cambridge, UK

The Leader (L) of Theiler's virus is a multifunctional protein crucial for full viral pathogenesis. L was shown to antagonize type I interferon transcription, disrupt nucleocytoplasmic trafficking, inhibit stress granule formation and modulate cell apoptosis.
To better understand the mechanisms underlying these L-induced activities, identification of cellular targets of the Leader protein was undertaken. p90 ribosomal S6 protein kinases (RSKs) were identified as L-binding proteins during infection. RSKs are Ser/Thr kinases of the MAPK pathway that are involved in numerous processes, including transcription, translation, cell survival and proliferation. Interaction domain mapping identified the Leader N-terminal domain and the substrate-phosphorylating N-terminal Kinase Domain of RSK as the docking regions. Functionally, L induced RSK phosphorylation on residues important for kinase activity. Cellular ATP-depletion and *in vitro* phosphatase treatment demonstrated that L protected RSKs from phosphatases. This resulted in increased RSK activity as revealed by kinase assays. Our current hypothesis is that the Leader-RSK interaction allows RSK to phosphorylate non-canonical substrates, these being responsible for the pleiotropic activities of the Leader. Identification of these novel cellular substrates is ongoing.
Importantly, RSKs were shown to be targeted by others viral and bacterial pathogens suggesting a significant role of RSK in host-pathogen interactions.

LI16We1112

Offered paper – Screening for Attenuated FMDV RNA Replication Using a Novel Fluorescent Replicon and Rescue of Corresponding Viruses

Fiona Tulloch[1], Garry Luke[1], Martin Ryan[1], Uday Pathania[1], Sarah Gold[2], Toby Tuthill[2], Terry Jackson[2]

[1]Biological Sciences Research Complex, Uni. St Andrews, St Andrews, Fife, UK, [2]The Pirbright Institute, Pirbright, Woking, Surrey, UK

We have created FMDV replicon systems in which sequences encoding capsid proteins are replaced with those encoding fluorescent 'reporter' proteins. RNA replication can be monitored in cells transfected with transcript RNA by measurement of fluorescence in real-time. Maximal fluorescence was observed 6-8hr post-transfection, with a delay in signal of ~2hrs compared to RT-

Please note: Abstracts are published as received from the authors and are not subject to editing.

qPCR. Interestingly, the *Aequorea* GFP reporter protein is degraded by the FMDV L proteinase, although with much slower kinetics than degradation of eIF4G by Lpro. The system has been enhanced by the inclusion of ribozymes to generate authentic transcript RNA termini, and replacement of the Aequorea GFP with that of *Ptilosarcus gurneyi* (sea feather). Indeed, this form of fluorescent protein increases the replicon signal ~10 fold. FMDV replicons have been used to quantify RNA replication of genomic forms bearing deletions of sequences known to attenuate virus replication (L proteinase, stem-loop 1 in the 3'NCR) and genomes modified by codon de-optimisation. These attenuated forms have been further modified by deletion of sequences encoding the reporter proteins and insertion of sequences encoding the (missing) capsid proteins to the corresponding infectious copies. Viruses have been rescued and work is in progress to determine the relative replicative fitness of these recombinant viruses.

LI16We1124

Offered paper – MEK1/2 inhibitor (u0126) reduces *yellow fever virus* replication both *in vitro* and *in vivo*

Alice Torres, Leonardo Oliveira, Jonas Dutra, Rafael Palhares, Pablo Cardoso, Aryádina Ribeiro, Carolina Pacca, Paulo Ferreira, Erna Kroon, Maurício Nogueira, Cláudio Bonjardim

Universidade Federal de Minas Gerais, Belo Horizonte/Minas Gerais, Brazil

The *Yellow fever virus (YFV)* is a small enveloped virus with a single positive-stranded RNA genome. Currently, there is no specific treatment against the YFV and the available vaccine is not completely safe, leading to several complications. The goal of this work was characterize the MEK1/2 pharmacological inhibitor (U0126) effect on the vaccine YFV strain (YFV-17D) replication, both *in vitro* and *in vivo*. Our data showed a ~2log10 inhibition in the YFV-17D replication in Vero cells treated with 20µM of U0126. By electron microscopy, we saw a significant increase in the number of Endoplasmic Reticulum (ER)-associated vesicles, where viral replication machinery is located, as well as typical virions of ~40nm inside vesicles in YFV-17D-infected cells. In the U0126-treated cells, these structures were rarely observed. When intracranially inoculated with YFV-17D, BALB/c mice treated with U0126 (30µg/animal/day) had a significant decrease in the viral titers when compared to vehicle-treated infected mice. Taken together, our results strongly suggest that MEK1/2 plays a functional role during YFV life cycle both *in vivo* and *in vitro*, and it could be used as a therapeutic target for antiviral treatment of post-vaccination complications.

LI16We1136

Offered paper – Functional crosstalk between distant domains of chikungunya virus non-structural protein 2 is decisive for its RNA-modulating activity

Pratyush Kumar Das, Andres Merits, Aleksei Lulla

Institute of Technology, University of Tartu, Tartu, Estonia

Alphaviruses are positive-stranded RNA viruses that in recent years attracted significant public attention after chikungunya virus (CHIKV) outbreak started in the Indian Ocean region in 2005. CHIKV nsP2 is a multifunctional protein that is considered as a master regulator of the viral lifecycle and a main viral factor responsible for cytopathic effects and subversion of antiviral defence. The C-terminal part of nsP2 possesses protease activity, while the N-terminal part exhibits RNA and nucleoside triphosphatase (NTPase) activities, and was proposed to have helicase activity. In our studies recombinant CHIKV nsP2 demonstrated the unwinding of double-stranded RNA in a 5´-3´ directionally biased manner and RNA strand-annealing activity. Comparative analysis of NTPase and helicase activities of wild type nsP2 with enzymatic capabilities of different truncated or N-terminally extended variants of nsP2 revealed that the C-terminal part of protein is indispensable for helicase functionality and presumably provides platform for RNA binding, whereas the N-terminal-most region is apparently involved in obtaining conformation of nsP2 that allows for its maximal enzymatic activities. The establishment of the protocols for the production of biochemically active CHIKV nsP2 is expected to provide the starting point for further search of possibilities for therapeutic interventions to suppress alphaviral infections.

LI16We1148

Offered paper – Mapping of the norovirus VPg:eIF4G interaction

Eoin Leen[1], Frédéric Sorgeloos[2], Yasmin Goodfellow[2], Sam Correia[1], Melanie Stockhausen[1], Xulin Liu[1], Ian Goodfellow[2], Stephen Curry[1]

[1]Dept. of Life Sciences, Imperial College London, London, UK, [2]Dept. of Pathology, University of Cambridge, Cambridge, UK

Noroviruses are (+) sense RNA viruses that are responsible for ~20 million cases of acute gastroenteritis a year in the USA. Norovirus VPg (Viral Protein genome-linked), a ~15 kDa protein covalently attached to the 5' end of the RNA genome, has key roles in both the initiation of translation and replication of the viral RNA. We have recently determined the solution structures of murine norovirus (MNV) and the closely related feline calicivirus (FCV) VPg proteins. Both have small helical cores with extended flexible termini (1), raising intriguing questions about their modes of operation in translation and RNA replication. Recent work has revealed that the unstructured C-terminus of MNV VPg (but not FCV VPg) binds directly and specifically to the eukaryotic translation initiation factor eIF4G, an interaction that is critical for viral protein synthesis (2). To shed new light on the molecular mechanism on this unusual mode of translation initiation, we have now used mutagenesis coupled with biophysical and biochemical approaches to identify precisely the portions of MNV VPg and eIF4G that are responsible for this interaction.

1) Leen, E.N. et al., 2013. J. Virol. 87:5318-30; 2) Chung, L. et al., 2014. Submitted.

LI16We1400

Offered paper – Sapovirus translation requires an interaction between VPg and components of the eIF4F complex

Myra Hosmillo[1,2], Yasmin Goodfellow[1], Kyoung-Oh Cho[2], Ian Goodfellow[1]

[1]University of Cambridge, Cambridge, UK, [2]Chonnam National University, Gwangju, Republic of Korea

Sapoviruses of the *Caliciviridae* family have been recognized as emerging pathogens which contribute to the majority of gastroenteritis outbreaks in humans worldwide. However molecular studies of human sapovirus have been hampered due to the lack of a cell culture system. Porcine sapovirus (PSaV) can be recovered and grown in cell culture making it a good animal model for understanding the life cycle of sapoviruses and related-enteric caliciviruses. Here, we investigated the mechanism of translation initiation in PSaV and examined the role of the cellular initiation complex in virus translation and replication, employing a cap-independent translation initiation. PSaV, similar to other caliciviruses, was found to use a novel mechanism of protein synthesis that involves the covalently linked viral protein VPg at the 5' end. We have demonstrated that VPg linkage to the PSaV genome is required for the translation and infectivity of the virus. PSaV was found to hijack the cellular translation initiation mechanism by recruiting the eIF4F complex through interactions with VPg. Further inhibition assays demonstrated that binding of the eIF4F complex to VPg is functional. Overall our study showed that PSaV translation is VPg-dependent and requires eIF4E, the intact form of eIF4G and the interaction of eIF4E and eIF4G.

LI16We1412

Offered paper – Expresssion of Japanese encephalitis virus multi epitope gene in plants

Wipa Tangkananond[1,2], Paolo Lenzi[2], George P. Lomonossoff[2]

[1]Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Klongluang, Prathumtani 12121, Thailand, [2]Department of Biological Chemistry, John Innes Centre, Norwich NR4 7UH, UK

The E gene encodes E protein which protects the Japanese encephalitis virus (JEV) nucleocapsid and its immune response protects against JEV infection. Previous researchers unclosed that humoral immune response was elicited from immunised mice with inactivated JEV vaccine strain SA14-14-2 whereas both humoral and cellular immune responses were shown in immunised mice with recombinant multiepitope (MEP) peptide of JEV E protein expressed in *Escherichia coli*. Therefore, genetic engineering of JEV-MEP gene produced JEV-MEP protein in plants instead of culturing JEV in mice and animal tissue cultures, and expressing genetic engineered JEV-MEP protein in E.coli. Hence, this research is the pioneer for plant derived JE vaccine.
The various steps were construction of JEV-MEP from six B cell and two T cell epitopes from E gene of JEV vaccine strain SA14-14-2 and added the suitable promotor for cloning in E. coli and *Agrobacterium tumefaciens*. Second, *A. tumefaciens* was inoculated and expressed in *Nicotiana benthamiana* leaves using the pEAQ-HT vector. Then, the JEV-MEP protein was showed the bands which were detected by chemiluminescence immunoassay. Finally, it will be characterised and purified for the plant based vaccine and its immune response will be investigated further

Keywords: J*apanese encephalitis virus*, plant based vaccine, immune response

LI16We1424

Offered paper – Identification of functionally permissive insertion sites within the foot-and-mouth disease virus non-structural polyprotein by random transposon-mediated mutagenesis

Morgan Herod[1], Eleni-Anna Loundras[1], Fiona Tulloch[2], Garry Luke[2], Uday Pathania[2], Martin Ryan[2], David Rowlands[1], Nicola Stonehouse[1]

[1]University of Leeds, Leeds, UK, [2]University of St Andrews, Fife, UK

The picornavirus foot-and-mouth disease virus (FMDV) is the causative agent of a highly contagious and economically damaging disease of cloven-hoofed domestic livestock. The functions of the FMDV non-structural (NS) proteins required for viral genome replication are less well understood than for other picornaviruses (e.g. poliovirus). Insertion of genetic tags within viral NS proteins has shown to be a useful strategy for identifying viral proteins and their functional complexes within infected cells. Furthermore, genome-wide functional profiling by random insertional mutagenesis can reveal domains within NS that are non-essential for viral replication *in vitro*. To identify regions within the FMDV NS polyprotein which could potentially accommodate the insertion of genetic tags, random transposon-mediated mutagenesis was employed on the FMDV replicon 2A-3D region. This approach allowed us to generate a replicon library containing single 15 nucleotide random insertions within the FMDV NS polyprotein. Following isolation of replication-competent replicons from the library, genomes tolerating the 5 amino acid insertion can be readily identified. Work is on-going to confirm that these insertion sites may be utilised for the incorporation of genetic tags and markers, which will be used in dissecting the molecular interactions within the viral replication complex.

LI16We1436

Offered paper – Insights into the relationship between hepatitis C virus replication and virion assembly using complementation assays

Morgan Herod[1,3], Vera Schregel[2], Chris Hinds[2], Mengya Liu[1], John McLauchlan[2], Christopher McCormick[1]

[1]University of Southampton, Southampton, UK, [2]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [3]University of Leeds, Leeds, UK

HCV virion assembly depends on the replicase non-structural proteins NS3, NS4A, NS4B, NS5A and NS5B, indicating that assembly and replication are linked. To investigate this relationship, we sought to establish the minimum polyprotein cassette required to rescue NS5A defects in replication and packaging, as well as examine the consequence of polyprotein expression on NS5A distribution. In a trans-complementation assay, NS3-5A was the minimum component necessary to efficiently rescue replication and facilitate incorporation of NS5A into membrane associated foci (MAF), the site where the replication complex (RC) resides. Similar observations were made using an intragenomic complementation assay; here modified replicons expressed NS5A or the NS3-5A polyprotein from an open reading frame (ORF) adjacent to the NS3-5B replicase ORF. In contrast to replication, only limited rescue of virion assembly could be achieved by trans-complementation, and little benefit was gained from expressing NS5A within an NS3-5A polyprotein, or even a functional NS3-5B replicase. However, using an intragenomic complementation assay, expression of NS5A alone supported virion production far more effectively than trans-complementation. Our results point to NS3-5A playing a functional role in targeting NS

Please note: Abstracts are published as received from the authors and are not subject to editing.

proteins to MAF, and suggest that translationally active viral RNA can transit down a virion assembly pathway without RC involvement.

LI16We1448

Offered paper – Structural rearrangements within an HCV RNA pseudoknot modulate virus replication and translation

Andrew Tuplin[1], Madeleine Struthers[2], Jonathan Cook[2] Kirsten Bentley[2], Peter Simmonds[3] and David J. Evans[2].

[1] School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK: [2.] School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK: [3.] Roslin Institute, The University of Edinburgh, Edinburgh, UK.

The NS5B region of the HCV genome encodes a phylogenetically conserved RNA stem-loop (designated SL9266), which is a cis-replicating element (CRE) required for genome replication. SL9266 forms a pseudoknot structure (designated SL9266/PK), which includes a 'kissing-loop' interaction between its terminal loop and that of SL9571 (previously SLII) within the X-tail.

Using a combination of structural mapping and reverse genetic analysis we previously showed that SL9266/PK is a dynamic tertiary structure. It forms alternative 'closed' and 'open' conformations, both of which are required for efficient virus replication. In the closed conformation the terminal loop of SL9266 forms a kissing interaction with the terminal loop region of SL9571 and the predicted stem of SL9571 is predominantly unwound as a single stranded molecule. In the closed conformation the kissing loop interaction is absent and the base paired duplex stem of SL9571 forms.

Here we present evidence that SL9266/PK functions as a dynamic ribo-switch, in which different aspects of virus replication are favoured by alternative switch conformations. We propose that SL9266/PK functions as a temporal switch, modulating mutually incompatible translation and replication events and show that inhibitors of conformational switching significantly inhibit HCV replication in multiple virus genotypes.

LI16We1500

Offered paper – High-fidelity mutations in the vaccine TC-83 increase immunogenicity and attenuation

Mathilde Guerbois, Tiffany Kautz, Fanping Kong, Ruimei Yun, Rose Langsjoen, Maria Alcorn, Heidi Spratt, Bruce Luxon, Scott Weaver, Naomi Forrester

University of Texas Medical Branch, Galveston, TX, USA

All replication of RNA viruses has the potential to incorporate mutations that affect virulence or pathogenesis. For live-attenuated vaccines that utilize the virus' own replication machinery, this ability to incorporate mutations has implications for stability, as any replication may result in mutations that either restore the wild-type phenotype via reversion or compensate for the attenuating mutations (pseudoreversion) by increasing virulence. To validate the potential of high-fidelity mutations to prevent such reversion, several mutations were tested in the live-attenuated vaccine strain TC-83 against Venezuelan equine encephalitis virus. Mutations were validated for high-fidelity using a combination of resistance to 5'fluorouracil and next generation sequencing. One mutant, TC-83 3x, showed increased fidelity and a second mutant,TC-83 4x, showed decreased fidelity. Both mutants showed increased attenuation in an infant mouse model and increased immunogenicity and complete protection against lethal challenge compared to the original TC-83 when tested in an adult murine challenge model. Under serial passaging in a highly permissive model, the mutants increased in virulence but were less virulent than the original TC-83. These results suggest that incorporation of high-fidelity mutations into live-attenuated vaccines results in increased stability and a lower rate of mutations, as well as increase immunogenicity whilst being further attenuated.

LI16We1512

Offered paper – Identification of RNA aptamers specifically targeting the HCV Core protein provides evidence for packaging signals within the HCV genome

Hazel Stewart[1], Simon White[2], Eric Dykeman[3], Andrew Tuplin[1], Reidun Twarock[3], Peter Stockley[2], Mark Harris[1]

[1]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK, [2]Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK, [3]York Centre for Complex Systems Analysis, Departments of Mathematics and Biology, University of York, York, UK

The assembly of hepatitis C virus particles is poorly understood and therefore represents a novel target for therapeutic intervention. During this process the capsid protein (Core) must recognise and package nascent viral RNA, whilst excluding cytoplasmic RNAs; a process which is achieved in other RNA viruses through the recognition of specific packaging signals.

To identify which structural motifs were essential for HCV RNA packaging, we performed SELEX (Systematic Evolution of Ligands by Exponential Enrichment) with Core, to identify a library of RNA aptamers which bound specifically to the protein. A significant proportion of these aptamers displayed nucleotide homology to multiple regions of the HCV genome. In silico models of these regions indicated that their selection from the cellular RNA milieu may be aided by the presence of conserved secondary structural motifs. Using both *in vitro* SHAPE (selective 2'-hydroxyl acylation analyzed by primer extension), and mutagenesis of these motifs in the context of the JFH-1 infectious clone, we have analysed the effects of altering these structures upon both genome replication and virus assembly. These results indicate that novel RNA secondary structures may cooperatively drive specific encapsidation of the HCV genome, allowing us to map the structural requirements for HCV RNA packaging.

LI16We1600

Offered paper – Identification of inhibitors of the hepatitis C virus NS2 autoprotease

Joseph Shaw, Colin Fishwick, Mark Harris

University of Leeds, Leeds, UK

Treatment of hepatitis C virus (HCV) infection has recently been augmented with direct acting anti-virals targeting viral genome replication. However, the rapid emergence of viral resistance suggests future treatments will rely on a combination therapy.

There is therefore a need for additional inhibitors of viral replication, particularly those with a novel mechanism of action.
The autoprotease activity at the boundary of non-structural proteins NS2 and NS3 is known to be essential in the virus lifecycle for release of the NS3 protease and subsequent processing of the viral non-structural proteins responsible for genome replication. We therefore sought to assess the viability of inhibiting the NS2 autoprotease activity with small molecule compounds as a route to blocking viral replication.
Screening a subset of compounds optimised *in silico* and subsequent structure activity relationship analysis identified a series of potent small molecules capable of blocking NS2-NS3 autoproteolysis *in vitro*. These first-in-class inhibitors were also capable of blocking NS2-dependent HCV replication in a modified sub-genomic replicon system. Taken together these results suggest inhibitors of the NS2 autoprotease could offer an attractive additional component to future combination therapies.

LI16We1612

Offered paper – Combined anti-viral and anti-tumour therapy for virus-associated liver cancer

Adel Jebar[1,2], Matthew Bentham[1,3], Fiona Errington-Mais[1,3], Adam Peckham-Cooper[1,4], Rajiv Dave[1,4], Karen Scott[1,2], Giles Toogood[4], Peter Selby[1,2], Alan Melcher[2,3], Stephen Griffin[1,3]

[1]University of Leeds, Leeds, West Yorkshire, UK, [2]CRUK, Leeds, West Yorkshire, UK, [3]Leeds Institute of Cancer and Pathology, Leeds, West Yorkshire, UK, [4]Leeds Teaching Hospitals NHS Trust, Leeds, West Yorkshire, UK

Infection with hepatitis C virus (HCV) and hepatitis B virus (HBV) causes over 70% of hepatocellular carcinomas (HCC) worldwide, creating significant disease burden in both developing and developed countries. Therapy for HCC is both highly ineffective and often clinically separated from antiviral treatment, with 5-year survival rates of only ~5%. Interestingly, recent evidence suggests that HCV-associated tumours respond differently to therapy, providing a potential opportunity to stratify therapy. In addition, concomitant treatment of both tumours and the underlying virus infection may greatly improve patient quality of life.
We have assessed Oncolytic Reovirus therapy for the treatment of both HBV and HCV-associated HCC. We found that Reovirus displays profound anti-tumour and anti-viral effects *in vitro* and *in vivo* through both direct and indirect mechanisms, namely: potent direct HCC oncolysis, stimulation of anti-viral cytokines from both liver/tumour cells and liver-resident lymphocyte populations, and the direct stimulation of liver-resident natural killer cell tumour killing. The culmination of these effects is demonstrable using *in vivo* HCC models, with Reovirus effectively reducing tumour growth, decreasing viral replication and enhancing the efficacy of current HCC standard of care.

LI16We1624

Offered paper – Interaction of chikungunya virus with human mononuclear phagocyte cells *in vitro* and *in vivo*

Adrian Zagrajek[1], David Hallengärd[2], Pierre Roques[4], Peter Liljeström[2], Finn Grey[1], John Fazakerley[1,3]

[1]The Roslin Institute, University of Edinburgh, Edinburgh, UK, [2]Karolinska Institutet, Stockholm, Sweden, [3]The Pirbright Institute, Pirbright, UK, [4]Institut des Maladies Emergentes et Thérapeutiques Innovantes, CEA, Paris, France

Chikungunya virus (CHIKV) generally causes an acute disease in humans, where symptoms usually resolve within a week. However, some people infected with CHIKV develop chronic arthritis/arthralgia. In this study, a recognition element (RE) for a haematopoietic-specific miRNA was incorporated into the genome of CHIKV to investigate if blocking virus replication in cells of the mononuclear phagocyte system alters virus kinetics *in vitro* and pathogenesis *in vivo*. The replication of the modified viruses was investigated *in vitro* in macrophage/monocyte cell lines (Thp-1, IC-21) and in HEK 293 cells, which were modified to express the miRNA of interest under the control of an inducible tetracycline promoter. Incorporation of the miRNA RE into the genome of CHIKV was successful. Virus replication in all cells expressing the miRNA was reduced by at least 90% when compared to control viruses. Virus replication was not affected in cell lines not expressing the miRNA. The virus was found to be genetically stable *in vitro* following multiple passages on BHK-21 cells. The long-term goal of this study is to determine whether restriction of virus replication in haematopoietic cells alters the pathogenic outcome of disease in mouse and non-human primate models of this disease.

LI16We1636

Offered paper – The localisation and function of the non-structural 5A (NS5A) protein of hepatitis C virus (HCV) is dependent upon phosphorylation and is modulated by the inhibitor Daclatasvir

Douglas Ross-Thriepland, Mark Harris

The University of Leeds, Leeds, UK

Hepatitis C virus (HCV) currently infects around 180 million people worldwide. One of the viral non-structural proteins, NS5A, is highly phosphorylated, has no intrinsic enzymatic activity but is nevertheless essential for multiple stages of the virus life cycle; including genome replication, virus assembly and virus persistence. Recent work in our lab has shed more light on the complexities of this phosphorylation, and identified numerous phosphorylation events that were shown to be important for virus replication. However, the molecular details of how these phosphorylation events effect NS5A function remain elusive. To address this we have utilised SNAP-tag technology and confocal microscopy to investigate the effects of NS5A phosphorylation on the sub-cellular trafficking and localisation of NS5A. We show here that loss of specific phosphorylation events result in dramatic shifts in the localisation of NS5A, with only a modest cost to replication efficiency. Further applications of the SNAP-tag technology has allowed us to investigate in greater detail the mechanism of the new NS5A inhibitor, Daclatasvir (Bristol-Myers Squibb), which has been shown to rapidly modulate NS5A phosphorylation, while not inhibiting replication until a later time point.

LI16We1648

Offered paper – Carrot internal necrosis disease is associated with infection by *Carrot yellow leaf closterovirus*

Please note: Abstracts are published as received from the authors and are not subject to editing.

Adrian Fox, Anna Skelton, Ian Adams, Rachel Glover

Fera, York, UK

Traditional diagnostic methods in plant virology are targeted towards specific pathogens. With limited options for non-targeted diagnostics for plant pathology applications, rationalising symptoms to pathogenic causes has been challenging. With the advent of deep sequencing technology, novel approaches to non-targeted diagnostics are being pursued.
Internal necrosis of carrot, anecdotally linked to virus infection, has been observed in UK crops for at least the last 10 years. Carrot samples were collected from a crop exhibiting root necrosis. Approximately equal numbers of affected and unaffected carrots were sampled. These samples were tested for the presence of the established carrot viruses: The presence of these viruses did not correlate with symptomatic roots either as single or multiple infections.
A sub-sample of 24 carrots of mixed virus status was subjected to Next-generation sequencing. At least six novel or unexpected plant viruses were found within the sub-sampled carrots. The results from these tests suggested *Carrot yellow leaf closterovirus* (CYLV) was associated with symptomatic roots. From these data necrosis without CYLV is estimated to be rare in the sampled population.
This work was carried out with funding from the HDC under project FV382a.

LI16We1654

Manipulating virus genome dinucleotide frequencies to control pathogenicity

Eleanor Gaunt[1], Inga Dry[1], Rennos Fragkoudis[2], Peter Simmonds[1]

1University of Edinburgh, Edinburgh, UK, 2The Pirbright Institute, Pirbright, UK

Almost without exception, RNA viruses infecting vertebrates show marked suppression of CpG and UpA dinucleotide frequencies even though processes directing dinucleotide frequencies in vertebrate genomes (such as methylation-induced mutation that removes CpG) cannot operate on viral RNA.
A panel of Theiler's murine encephalomyelitis virus (TMEV) mutants in which the amino acid code was preserved but dinucleotide frequencies were altered was constructed. In murine macrophages (RAWs), viruses with more CpG and UpA dinucleotides (CpGH, UpAH) were attenuated compared with wildtype and control virus (3.4-22.4 fold decrease in titre; RNA replication was halved). Viruses with decreased CpG and UpA (CpGL, UpAL) were slightly fitter (1.7-2.4 fold increase in titre; RNA replication was nearly four-fold increased). Findings were similar in Huh7 cells.
Preliminary *in vivo* work found that mice infected with CpGH and UpAH strains lost less weight than mice infected with wildtype and control viruses; mice infected with CpGL and UpAL viruses did not. *In vitro* findings have been recapitulated using other viruses including another picornavirus, echovirus 7. Future work includes competition experiments, and characterisation of replication of these strains in RAW cells with components of the innate immune system knocked out. This approach is purported as a novel vaccine development strategy.

LI16We1706

Offered paper – The Impact of GBV-B Sequence Variation on Infection Outcome

Ori Bowen[1], Robert Goldin[2], Ed Mee[1], Peter Karayiannis[3], Nicola Rose[1]

[1]Division of Virology, NIBSC, Potters Bar, Hertfordshire, UK, [2]Department of Pathology, Division of Medicine, Imperial College London, London, UK, [3]St George's, University of London Medical School at University of Nicosia, Nicosia, Cyprus

GBV-B is a flavivirus distinct from but phylogenetically closely related to Hepatitis C virus (HCV). The viruses have similar genome length and organisation, with functional homology in defined regions. There is limited treatment efficacy and no licensed vaccine for HCV. Using the surrogate GBV-B/tamarin model of HCV of acute infection we find that some animals display a typical acute viraemia and others have prolonged infections. Understanding the impact of viral variation on viral persistence could inform antiviral design. Whole-genome deep sequencing of virus recovered from tamarins with typical viraemia profiles revealed contrasting structural protein conservation and non-structural protein diversity, primarily in the NS5A protein C-terminus. Mapping the position of GBV-B NS5A residue changes reveals an analogous pattern to those reported for HCV NS5A. Modelling their effects on predicted secondary structures highlights changes in key regions, indicative of their functional effects. In tamarins with prolonged infection, major variants in the envelope protein have been observed alongside non-structural protein changes. Continuing investigations aim to assess viral variants in the liver and the role of sequence variation on success of clearance of virus from the periphery. Such detailed sequence variation has not previously been reported for GBV-B and may inform immunotherapeutic development.

LI16We1718

Offered paper – Highly pathogenic recombinant form of a picorna-like Deformed Wing Virus of the honeybee is selected by *Varroa* mite transmission: insights from high throughput sequencing

Eugene Ryabov, Jessica Fannon, Graham Wood, Jonathan Moore, Nigel Burroughs, David Evans

University of Warwick, Coventry, UK

Honeybees are threatened by the global spread of the mite *Varroa destructor* which vectors viral pathogens, the most important of which is Deformed Wing Virus (DWV). In the absence of *Varroa DWV* accumulates to low levels and is asymptomatic. Conversely, mite-infested colonies show markedly elevated DWV levels, with overt symptoms including impaired development and enhanced overwintering colony losses.
To elucidate the effect of Varroa on DWV diversity and levels, we exposed *Varroa*-naïve larvae to orally and mite-transmitted DWV, using mite feeding or virus injection to the haemolymph. We analysed virus populations by NGS of total RNA, small RNAs, or DWV cDNA fragments. We monitored viral levels and diversity in individual honeybees and associated mites, and the host RNAi responses. We observed dramatic reduction of DWV diversity in the mite-exposed and virus-injected honeybees which showed a striking increase of near-clonal pathogenic DWV variant.
We suggest that the differences in DWV levels and diversity in *Varroa*-free and *Varroa*-exposed honeybees were due to preferential amplification of highly pathogenic DWV strain after transmission

by the mite. The identification of a virulent virus variant improves understanding of the mechanistic role of viral vectors and transmission routes in virus evolution and defines potential targets for therapy.

LI17
Virology workshop: negative strand RNA viruses

LI17We0900
Offered paper – Quantitation of protein composition reveals both conserved and host-specific features of influenza virion architecture

Edward Hutchinson[1], Philip Charles[1], Svenja Hester[1], Benjamin Thomas[1], David Trudgian[1,2], Ervin Fodor[1]

[1]Sir William Dunn School of Pathology, University of Oxford, Oxford, UK, [2]UT Southwestern Medical Center, Dallas, TX, USA

Influenza virions incorporate proteins encoded by both the virus and its host, but their pleomorphic structure has prevented detailed analysis of their composition. We developed a method for determining protein ratios based on label-free absolute quantitation of mass spectra, allowing highly sensitive quantitation of the protein composition of influenza virions. Using this, we demonstrated that 'spherical' influenza viruses have a consistent overall virion architecture, with viral proteins and certain host proteins incorporated in a fixed ratio regardless of the host species and for a range of influenza A and B viruses. To our surprise, the increased sensitivity of detection afforded by this method revealed routine incorporation of the NS1 protein, previously thought to be non-structural; this finding was confirmed by Western blotting. The host proteins in virions resemble those in host-derived microvesicles, suggesting similarities in the biogenesis and transport of these enveloped structures. In addition, aspects of virion composition varied between hosts. For example, we show that the virion membrane is highly enriched with members of the tetraspanin superfamily, but that the tetraspanins selected vary between mammalian and avian hosts. In this way the architecture of an influenza virion is shaped by both the viral genome and the host species.

LI17We0912
Offered paper – The M segment of the 2009 (H1N1) influenza virus confers increased neuraminidase activity, filamentous morphology and contact transmission to A/Puerto Rico/8/1934-based viruses

Patricia J. Campbell, Constantinos S. Kyriakis, Shamika Danzy, Martin J. Deymier, Anice C. Lowen, John Steel

Emory University, Atlanta, GA, USA

The 2009 H1N1 lineage emerged following reassortment between two poorly transmissible (in human) swine influenza virus lineages. Toward identifying determinants of transmission, we generated reassortants between the pandemic isolate A/Netherlands/602/2009 [H1N1] (NL602), and A/Puerto Rico/8/1934 [H1N1] (PR8) and evaluated the resultant viruses in a guinea pig transmission model. NL602 virus spread efficiently, while PR8 virus did not transmit. Inclusion of of the HA, NA and M segments of NL602 in the PR8 background yielded a virus with indistinguishable contact transmissibility to the pandemic strain. The pandemic M segment alone accounted for much of the improvement in transmission. Toward understanding the underlying mechanism, we evaluated neuraminidase activity and virion morphology of the reassortant viruses. Transmission was found to correlate with higher neuraminidase activity and a more filamentous morphology. We found that introduction of the pandemic M segment alone resulted in an increase in the neuraminidase activity of two pairs of otherwise isogenic PR8-based viruses. Thus, our data demonstrate that the influenza virus M segment can impact neuraminidase activity and, perhaps through this mechanism, have a potent effect on transmissibility.

LI17We0924
Offered paper – The role of influenza virus protein neuraminidase's transmembrane domain on influenza virus budding, and its effect on influenza virus morphology

Scott Roddy, Chris Wilson, Jeremy Rossman

University of Kent, Canterbury, UK

Influenza virus neuraminidase (NA), a type II transmembrane glycoprotein protein has previously been shown to play a role in virion formation during influenza A virus budding. Although the exact mechanism by which NA contributes to virion formation and morphology is currently unknown. Previous research has shown that mutations of the transmembrane domain (TMD) can result in alteration of virion morphology, particularly in production of filament like virus particles.
In this research we used both partial and full mutations of the A/WSN/33 TMD, a primarily spherical strain with the TMD of A/Swine/09 strain to evaluate the effects on virion morphology.
This study uses a transfection based virus like particle (VLP) system, along with viral infection of 293T cells, with virion and VLP morphology analysed by immunofluorescence confocal microscopy. Our preliminary results show the production of virion's and VLP's with an elongated filament like morphology, suggesting that NA's TMD may play a role on in influenza virus morphology.

LI17We0936
Offered paper – The Role of the Avian Allele of the Influenza A Virus NS1 Protein in Setting Host Range and Pathogenicity

Matthew Turnbull[1], Helen Wise[1], Nikki Smith[1], Marlynne Quigg-Nicol[1], Yvonne Ligertwood[1], Gareth Hardisty[1], Rebecca Dunfee[2], Pip Beard[1], Jeffery Taubenberger[2], Bernadette Dutia[1], Paul Digard[1]

[1]The Roslin Institute, The University of Edinburgh, Edinburgh, UK, [2]The National Institute of Allergy and Infectious Diseases, Bathesda, Maryland, USA

Two 'alleles' of the NS segment circulate in non-chiropteran influenza A viruses. The 'A-allele' is found in avian- and mammalian-adapted viruses, but the 'B allele' is almost exclusively avian. A plausible hypothesis for the restricted host range of the B allele is that its NS1 protein is maladapted for controlling the mammalian immune response. To test this, a variety of avian influenza virus NS genes were reassorted into the mouse-adapted A/Puerto

Please note: Abstracts are published as received from the authors and are not subject to editing.

Rico/8/1934 (PR8) virus. All viruses replicated to similar titres in a variety of human and other mammalian cell lines, and also showed similar sensitivities to IFN-ß treatment. *In vitro* co-infection ("competition") studies revealed subtle fitness differences between WT PR8 and exemplar A and B allele reassortants, but surprisingly the order was PR8 > B > A. These viruses replicated to similar titres in the lungs of infected mice, and provoked indistinguishable lung pathology. However, both viruses with an avian segment 8 caused less weight-loss than parental PR8, with the A-allele virus showing the greatest attenuation. Both reassortant viruses elicited lower amounts of pro-inflammatory cytokines in the lung than WT PR8. Thus the B-allele of NS1 does not necessarily attenuate virus replication in a mammalian host but does attenuate disease.

LI17We0948

Offered paper – Within host evolution and cross-species transmission of equine influenza virus non-structural protein NS1

Adam Rash, Alana Woodward, Liz Medcalf, Debra Elton

Animal Health Trust, Newmarket, Suffolk, UK

Equine H3N8 influenza virus (EIV) emerged in 1963, causing a pandemic in horses. The virus is thought to be of avian origin, continues to circulate in horses and has undergone further cross-species transmission into dogs.

Sequence analysis of the NS1 gene from a panel of virus isolates revealed variation between the avian-like prototype of H3N8 EIV, isolates from the 1980s-90s, the current Florida sublineage and canine H3N8 viruses, suggesting host-related adaptation. In particular, variation was observed at the C-terminus of NS1 with the terminal four amino acids changing from 1963 through to the 1990s, whilst NS1 of the Florida sublineage has been truncated by 11 amino acids. Interestingly, the NS1 of canine H3N8 was also truncated in early isolates, but more recent strains have full length NS1 with a novel C-terminus.

The C-terminus of influenza NS1 has previously been shown to include a PDZ-binding domain that differs between avian and human isolates. Here we demonstrate the effect of altering the C-terminal sequences of EIV NS1 on virus replication in cell and tissue explant cultures from different host species. We also show striking differences in nuclear localisation properties of the NS1s in cells of avian, equine, canine and primate origin.

LI17We1000

Offered paper – Evolution of highly pathogenic avian influenza H7N7 *in ovo*

Amanda Hanna[1,2], Wendy A Howard[1], Alejandro Núñez[1], Daniel Hicks[1], Wendy S Barclay[2], Jill Banks[1]

[1]Animal Health and Veterinary Laboratories Agency (AHVLA), Surrey, UK, [2]Imperial College, London, UK

Outbreaks of Highly pathogenic avian influenza (HPAI) may infect millions of poultry, causing devastating disease, and the viruses may also have the potential to cause severe human infections. Observations from natural outbreaks and laboratory experiments support the established dogma that HPAI viruses can emerge from low pathogenicity avian influenza (LPAI) precursors. HPAI has only been seen in some H5 and H7 subtypes of avian influenza viruses and the multi-basic cleavage site (MBCS) in the haemagglutinin (HA) gene has been described as the main pathogenic determinant of HPAI in chickens.

We aimed to develop an *in ovo* model that could support LPAI evolution to HPAI. Reverse genetics viruses were made based on A/chicken/England/11406/08 (H7N7) HPAI. This virus contains a MBCS and emerged in the field from a LPAI precursor containing a rare di-basic CS (H7N7DBCS). Three isogenic viruses were rescued containing either the wild-type MBCS, the DBCS or a typical LPAI single-basic CS. Following inoculation and passage into 14-day-old embryonated chicken eggs, H7N7DBCS showed evidence of spontaneous evolution to a HP genotype and phenotype by immunohistochemistry and DNA sequencing. Our findings present a model that can be used to understand the mechanisms behind the emergence of HPAI.

LI17We1012

Offered paper – Genotypic variation of contemporaneous influenza viruses circulating within European swine

Simon Watson[1], Pinky Langat[1], Scott Reid[3], Stephanie Franz[1], Mike Kelly[3], Tommy Tsan-Yuk Lam[2], Oliver Pybus[2], Paul Kellam[1], Ian Brown[3]

[1]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, UK, [2]Department of Zoology, University of Oxford, Oxford, Oxfordshire, UK, [3]Animal Health and Veterinary Laboratories Agency, Weybridge, Surrey, UK

The 2009 H1N1 influenza pandemic emerged from swine containing human-derived segments that had evolved within swine to become genetically- and antigenically-distinct from human influenza viruses. This zoonosis brought about the realisation of a public-health need to monitor the evolution and molecular epidemiology of influenza in swine: the so-called "One Health" approach. To this end, the European Surveillance Network of Influenza in Pigs (ESNIP) consortium was set up for the genetic and serological surveillance of European swine influenza viruses (SIV). As part of this consortium, we have used high-throughput sequencing on SIV-positive samples from local farms of contributing partners, generating 452 whole-genome sequences. Our results show that the H1N1 pandemic genotype is present in swine across Europe, with evidence of frequent reassortment events between this genotype, the established Eurasian avian-like (EA) swine lineage, and human-origin haemagglutinin and neuraminidase segments. Importantly, we observe a triple-reassortant involving an EA internal gene cassette with human seasonal H3N2 surface glycoproteins and a pandemic matrix protein. This constellation has been implicated in human cases at agricultural fairs in the United States, and is therefore of significant public-heath interest.

LI17We1100

Offered paper – High resolution structural analysis of the Nairovirus nucleocapsid protein, and its association with HSP70.

Rebecca Surtees[1], Antony Ariza[1], Stuart Dowall[2], Roger Hewson[2], Chi Trinh[1], Thomas Edwards[1], John Barr[1]

[1]University of Leeds, Leeds, UK, [2]Public Health England, Porton Down, Wiltshire, UK

Crimean Congo haemorrhagic fever virus (CCHFV) and Hazara virus

(HAZV) are tick-borne arboviruses of the *Bunyaviridae* family, genus *Nairovirus.* CCHFV causes severe haemorrhagic disease and death in humans, whereas HAZV has not been associated with human disease, although both viruses cause similar disease in interferon receptor knock out mice. In order to investigate further similarities between HAZV and CCHFV, to determine whether HAZV could act as a valid CCHFV model, various structural and cell biological aspects of the CCHFV and HAZV replication cycle were investigated. We solved the crystal structure of the nucleocapsid protein (N) of HAZV to a resolution of 2.7 Å. HAZV N was shown to have a highly similar overall structure to CCHFV N, consisting of a globular domain containing amino acid residues from both the N and the C-terminus, and an extending arm domain, with a potential caspase cleavage site at its apex. Cellular heat shock protein 70 (HSP70) was found to be an interacting partner of the N protein of CCHFV and HAZV, both in cells and in infectious virus particles. The significance of this interacting partner to the replication cycle of HAZV was investigated using siRNA knock down of endogenous HSP70.

LI17We1112

Offered paper – Comparison of the cellular effects of the non-structural NSs proteins of Rift Valley fever virus and a newly emerged phlebovirus causing haemorrhagic fever in China

Benjamin Brennan, Richard Elliott

MRC University of Glasgow Centre for Virus Research, Glasgow, UK

Rift Valley fever virus (RVFV, family *Bunyaviridae*) is a mosquito-borne pathogen of both livestock and humans. In humans, the disease can progress to encephalitis, retinal vasculitis or haemorrhagic fever, with a case fatality rate of up to 1%. Recently a new member of the *Phlebovirus* genus, called severe fever with thrombocytopenia syndrome virus (SFTSV), was discovered in China. SFTSV is spread by *Haemaphysalis longicornis* ticks. Disease symptoms include high fever, thrombocytopenia, and multi-organ dysfunction, and a case fatality rate of 12 - 30% has been reported. The non-structural NSs protein of RVFV is the major virulence factor of the virus that acts via a number of well-characterized mechanisms. To investigate the function of SFTSV NSs we generated a recombinant RVFV expressing the SFTSV NSs protein in place of its own NSs. The growth characteristics of the recombinant virus, including protein expression, NSs cellular localisation, RNA synthesis and the effect of SFTSV NSs on host cell antiviral responses such as Protein Kinase R have been analysed. These studies have revealed significant mechanistic differences between the two NSs proteins, and provide us with a preliminary understanding of the cellular action of a virulence factor of a new and truly emerging arboviral pathogen.

LI17We1124

Offered paper – An interaction between Clade B New World Arenavirus Nucleoproteins and Apoptosis-Inducing Factor 1 (AIF1) is required for efficient viral replication

Bjoern Meyer, David Jackson

University of St Andrews, St Andrews, UK

A number of arenaviruses cause haemorrhagic fever in humans with mortality rates of up to 30%, however no Arenavirus-specific antiviral therapies currently exist. The highly pathogenic New World Arenaviruses (NWAs), such as Junin virus, are all located in the same phylogenetic clade (Clade B). However, clade B also contains viruses that do not cause human disease, such as Tacaribe virus (TCRV), and these therefore represent model systems for studying arenavirus molecular biology under low containment conditions. The aim of our research, using TCRV as a model system, is to gain a better understanding of Arenavirus replication at the molecular level by identifying and characterising essential arenavirus-host interactions, thereby uncovering potential antiviral drug targets. The multifunctional nucleoprotein (NP) has roles in many stages of the viral replication cycle making it an ideal potential drug target. We have identified a conserved interaction between multiple Clade B Arenavirus NPs and Apoptosis-Inducing Factor 1 (AIF1). TCRV infection of an AIF1 knockdown cell-line resulted in a significant reduction in viral yield. This indicates the importance of the NP-AIF1 interaction during viral replication and highlights the prevention of this interaction as a potential target for the design of antiviral drugs active against all pathogenic NWAs.

LI17We1136

Offered paper – Elucidation of the interactome of ebola virus VP24

Isabel Garcia Dorival[1], Stuart Dowall[2], Weining Wu[1], Stuart Amstrong[1], John Barr[3], Miles Carroll[2,4], Roger Hewson[2,4], Julian Hiscox[1,4]

[1]Department of Infection Biology, Institute of Infection and Global Health; University of Liverpool, Liverpool, UK, [2]Public Health England, Porton Down, UK, [3]School of Molecular and Cellular Biology, University of Leeds, Leeds, UK, 4Health Protection Research Unit in Emerging Infection (including zoonoses) and Biological Threats, Liverpool, UK

Ebola virus causes severe hemorrhagic fever; due to the requirement of high containment facilities this virus is difficult to study. The Ebola virus genome encodes eight proteins, which may have multiple functions in the host cell. Elucidation of the interactome of Ebola virus in the host cell will help in understanding why this virus is so pathogenic. VP24 of Ebola virus has a critical role in the evasion of the immune response; VP24 acts as an interferon antagonist and is responsible for the inhibition of the interferon type I response; which is a key feature of filovirus pathogenesis. To better understand the functions of VP24 and its potential mechanism of action in the cell, proteomics was used to map which cellular proteins could interact with VP24. To do this VP24 was fused to EGFP and a highly specific EGFP trap used in conjunction with label free quantitative proteomics to discriminate potential real from background interactions. Sixty proteins were identified that had a high probability of interacting with VP24, and included proteins identified in previous studies. Some of the novel proteins identified in this study suggested that VP24 may interact with cellular ion channels and nuclear import/export pathways.

LI17We1148

Offered paper – Wild type measles virus induces the virus receptor PVRL4 in human brain endothelial cells

Haniah Abdullah, Adam Jeffers, Jake Pulford, Louise

Please note: Abstracts are published as received from the authors and are not subject to editing.

Cosby

Queen's University Belfast, Belfast, UK

In the long term measles virus (MV) complications subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis brain endothelial cell (BEC) infection is likely to play a major role in virus spread into surrounding brain tissue facilitated by damage to the blood brain barrier. We previously found in SSPE brain that BEC in some blood vessels are infected but the entry receptor used is unknown. Wild type (WT) viruses do not use CD46 and BEC are SLAM negative. We found that the MV epithelial receptor PVRL4 (Nectin 4) was induced by virus infection of human BEC (HBEC). Extensive monolayer destruction associated with activated caspase 3 staining was observed in infected HBEC and in a mouse model. Tumour necrosis factor related apoptosis inducing ligand (TRAIL), but not FAS ligand, was induced by MV infection. Extensive monolayer destruction associated with activated caspase 3 staining was observed (Abdullah et al. 2013, J. Neuropath. Expt. Neurobiol., 72, 681-696.). We have subsequently determined that PVRl-4 is up-regulated early in infection and we will report the mechanism involved. This is the first demonstration that WT MV infection of BEC induces TRAIL in vitro and in vivo with subsequent apoptosis and up-regulation of PVRL4.

LI17We1400

Offered paper – "PPRV, The role of its accessory proteins in the inhibition of the induction of IFNβ"

Beatriz Sanz Bernardo[1,2,] Steve Goodbourn[2], Michael Baron[1]

1The Pirbright Institute, Pirbright, Surrey, UK, 2SGUL, London, UK

Peste des petits ruminants virus (PPRV) is a morbillivirus that causes clinical disease in sheep and goats. We show how PPRV interferes with the induction of IFNβ, an immediate cell response to virus infection. Infection with PPRV itself does not induce the activation of the IFNβ promoter in Vero cells during the first 24 hours. This appears to be only in part due to active suppression of IFN induction; while the induction of IFNβ was lower in PPRV-infected cells, whether this induction was through the MDA-5 pathway (transfection with poly(I:C)) or the RIG-I pathway (infected with DI-containing Sendai virus), the effect of PPRV infection was minor. This reduction is greater in goat fibroblasts, suggesting a species specificity. The inhibition of poly(I:C)-induced IFNβ can be reproduced when the viral accessory V protein is expressed in transfected cells, and immunoprecipitation assays showed that V directly binds to MDA-5. The mechanism by which PPRV affects the RIG-I pathway is unclear: neither of the viral accessory proteins appear to bind RIG-I but each protein, like the virus, has a small effect on the induction of IFNβ through RIG-I.

LI17We1412

Offered paper – Activation of the interferon induction cascade by influenza A viruses requires viral RNA synthesis and nuclear export

Marian Killip, Matt Smith, David Jackson, Richard Randall

University of St Andrews, St Andrews, Fife, UK

We have examined the requirements for virus transcription and replication, and thus the roles of input and progeny genomes, in the induction of interferon (IFN) by influenza A viruses using inhibitors of these processes. In the presence of cycloheximide or NP siRNA, which inhibit viral protein synthesis and thus cRNP and progeny vRNP synthesis, strong activation of the IFN induction cascade occured in A549 cells infected with a range of influenza A viruses. In contrast, activation of the IFN induction cascade was very effectively abrogated by treatment with actinomycin D and other transcription inhibitors, which correlated with the inhibition of the synthesis of all viral RNA species. Furthermore, 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole, an inhibitor that prevents viral RNA export from the nucleus, was also a potent inhibitor of IRF3 activation; thus, both viral RNA synthesis and nuclear export are required for IFN induction by influenza A viruses. Whilst the exact nature of the viral PAMPs remains to be determined, our data suggests that in this experimental system, the major influenza A virus PAMPs are distinct from incoming genomes or progeny vRNPs.

LI17We1424

Offered paper – Inhibitors of the interferon response enhance virus replication *in vitro*

Claire Stewart, Richard Randall, Catherine Adamson

School of Biology, Biomedical Sciences Research Complex, University of St Andrews, St Andrews, UK

Virus infection triggers the cellular interferon (IFN) response to elicit a powerful anti-viral state. However, viruses encode viral IFN antagonists to circumvent the IFN response. Knockout of IFN antagonist function facilitates basic research and various clinical applications e.g. designed live-attenuated vaccines. However IFN-sensitive viruses can be difficult to grow to high-titer in tissue-culture. The default option is a limited selection of cell-lines (e.g. Vero cells) that have lost their ability to produce IFN. Yet many viruses do not grow efficiently in Vero's. We demonstrate that supplementing tissue-culture media with IFN inhibitors provides a simple, effective and flexible alternative to enhance virus growth in cell-lines of choice. IFN inhibitors targeting different components of the IFN response (TBK1, IKK2 and Jak1) significantly increased replication of a variety of viruses; including vaccine candidates with disabled IFN antagonists (Respiratory Syncytial Virus, Influenza, Bunyamwera), traditionally-attenuated vaccine strains (Measles, Mumps) and wildtype viruses (Respiratory Syncytial Virus, Bunyamwera) in human cell-lines or a selection of cell-lines derived from different mammalian species. The method affords a number of benefits, including for viruses that replicate inefficiently *in vitro* due to IFN sensitivity, species constraints of the viral IFN antagonist, other attenuating mutations or intrinstic properties of the wild-type virus.

LI17We1436

Offered paper – Activation of MDA-5 and RIG-I by Paramyxovirus Defective Interfering (DI) Particles

Craig Ross[1], John Short[2], Marian Killip[2], Dan Young[2], Kay Childs[1], Derek Gatherer[3], Andrew Davison[3], John McCauley[4], Rick Randall[2], Steve Goodbourn[1]

[1]St. George's, University of London, London, UK, [2]University of St. Andrews, St. Andrews, UK, [3]University of Glasgow, Glasgow, UK, [4]National Institute for Medical Research, London, UK

Paramyxoviruses are efficient inducers of type I interferon only

when preparations are rich in defective interfering (DI) particles. Cell sorting experiments demonstrate that IFN induction at the single cell level associates with the presence of DI particles. We have prepared nucleocapsid RNA from DI-rich preparations of parainfluenzavirus 5 (PIV5) and Sendai virus and subjected these to deep sequencing. Preparations of both viruses were characterised in which the predominant DI is of trailer copyback type; however, a distinct preparation of Sendai virus was enriched for internal deletion DIs with only a low abundance of copyback structures. The DI structures found in RNPs were also found in infected cells. Further analysis of specific DI particles showed that they are sufficient to act as pattern associated molecular patterns (PAMPs) and demonstrated differing abilities to activate the pattern recognition receptors (PRR) RIG-I and MDA-5: this was dependent on the size and structure of the DI molecule.

LI17We1448

Offered paper – The influence of CpG and UpA dinucleotides on influenza A virus replication *in vitro* and *in vivo*

Huayu Zhang[1], Helen Wise[1], Nicola Atkinson[1], Pip Beard[1], David Evens[2], Paul Digard[1], Peter Simmonds[1]

[1]University of Edinburgh, Edinburgh, UK, [2]University of Warwick, Coventry, UK

Most ssRNA viruses infecting mammals show profound suppression of CpG and UpA dinucleotide frequencies. We previously demonstrated that mutants of echovirus 7 (E7; a picornavirus) with increased frequencies of CpG and UpA showed impaired replication, while remarkably removal of CpGs and UpAs showed enhanced replication and rapidly outcompeted wildtype virus.
To investigate the *in vivo* significance of these observations, we generated mutants of influenza A virus with altered dinucleotide frequencies in segment 5. *In vitro*, CpG- and UpA-high mutants showed substantially slower replication kinetics, lower infectivity/RNA ratios (0.09-0.13) and small plaque phenotypes (3-5 fold smaller). Conversely, the CpG/UpA-low segment 5 rapidly outcompeted wildtype, recapitulating findings in E7.
Mice inoculated with the replication-impaired CpG-high and UpA-high mutants showed lower weight loss (6-9% compared to 20%) and 4.5x and 13x lower viral titre than wildtype. Conversely, CpG/UpA-low mutants showed similar weight loss but 1.4-fold greater viral titre in lung compared with wildtype.
Wildtype induced pathology in lung- more extensive than the CpG/UpA-low mutant despite the latter's greater replication. CpG- and UpA-high mutants induced infiltration and inflammatory changes despite impaired replication. Dinucleotide composition of many RNA viral genomes is subject to selection pressures independently of coding capacity and profoundly influences host/pathogen interactions.

LI17We1500

Offered paper – Species-specific host factors for Influenza A Virus replication

Laura Martin-Sancho, Alexander Karlas, Aki Imai and Thomas F. Meyer

Max Planck Institute for Infection Biology, Department of Molecular Biology, Berlin, Germany.

Many influenza A virus (IAV) strains show limited replication in species other than their natural host, aquatic birds. This host tropism could be due to differences in host cell factor requirements. Five RNAi-based screens identified multiple host factors essential for IAV replication in human cells, but requirements in other host species are unknown. To address this, we carried out a targeted siRNA screen in avian and porcine cells. Using data from published screens, we identified genes that have avian and porcine orthologs and confirmed expression in our cell lines using microarrays. Customized siRNAs were designed for the resultant 84 genes.
Our screen assessed viral replication, cell viability and interferon induction. After excluding toxic and immunostimulatory siRNAs, cell HTS analysis revealed thirty genes required for influenza virus replication in human, avian and porcine cells. Many of these were associated with mRNA splicing, nuclear transport and cellular trafficking. In addition we identified thirteen genes required by the virus in human or porcine but not in avian cells. These included genes involved in secretion, interferon and anti-apoptotic response. It is anticipated that this siRNA cross-species screen will elucidate the role of specific host factors in IAV tropism, providing novel target candidates for host-directed therapy.

LI17We1512

Offered paper – Global reprogramming of the host SUMO proteome during influenza virus infection

Filip Golebiowski[1], Patricia Domingues[1], Michael Tatham[2], Ron Hay[2], Benjamin Hale[1]

[1]University of Glasgow, Glasgow, UK, [2]University of Dundee, Dundee, UK

Influenza viruses are major seasonal human and animal pathogens, and can cause devastating pandemics. Peculiarly among RNA viruses, influenza viruses replicate in the host-cell nucleus, and we observe that virus infection triggers a massive rearrangement of host SUMOylation, a key posttranslational regulatory process in the cell. By employing high-throughput quantitative proteomic and bioinformatic technologies, we have identified >1000 putative SUMO substrates in human lung cells, and quantified the specific changes to SUMOylation caused by influenza virus infection. While for the majority of substrates, SUMO modification remains unchanged or slightly decreased, a group of ~200 target proteins show significantly enhanced SUMO conjugation, which can be as great as 100-fold. Furthermore, we identified several novel putative SUMOylation substrates among viral proteins. Strikingly, bioinformatic and functional analyses revealed that virus triggered SUMOylation is qualitatively distinct from the SUMO response mediated by some other stimuli, such as heat shock, ionizing radiation or bacterial infection, thereby potentially identifying several SUMO modified human proteins that may play unique roles during the interplay between influenza viruses and their hosts. Overall, our global analysis of SUMO posttranslational modifications establishes a new framework for understanding the unique way in which the host-cell nucleus is 're-wired' during influenza virus infection.

LI18

Virology workshop: clinical virology

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI18We0900

Offered paper – Enhanced diagnostic and management strategies to improve the identification and outcome of individuals with encephalitis

Chandanjit Nijjar[1,2], Julia Granerod[2], Melvyn Smith[1], Mark Zuckerman[1,2], Kevin Brown[2], David Brown[2]

1Kings College Hospital, London, UK, 2Public Health England, London, UK

A 2005-2008 study of the aetiology of encephalitis in England reported 37% were of unknown cause. The aims of this current study included developing a diagnostic algorithm for investigating encephalitis; auditing current practice in laboratory investigations of encephalitis in the UK Clinical Virology Network; and develop a 14 target multiplex real-time PCR and intrathecal antibody tests for analysing CSF samples as a reference diagnostic service.
18 audit questionnaires were completed, findings included HSV DNA was not tested in all laboratories; 4 did not use internal controls and 7 did not request intrathecal antibody tests. From March 2013, 106 patients with encephalitis were admitted to King's College Hospital and CSF and serum samples collected and stored. A cause was identified in 8.5% (4.7% VZV and 3.8% CMV DNA positive). A 14 target multiplex real-PCR assay has been optimised and validated on the Rotor-gene 6000, together with a 16s rRNA PCR. Additionally, intrathecal antibody detection methods were optimised, targeting HSV, JCV, VZV, measles and rubella viruses. Results will be used to identify samples for virus discovery by next generation sequencing methods.
The audit demonstrated that guidance using a diagnostic algorithm would be helpful and further test results for the clinical samples will be presented.

LI18We0912

Offered paper – Do corticosteroids reduce swelling and improve outcome in herpes simplex virus encephalitis? A pilot study generating preliminary data for the phase III randomised controlled COHESIVE (COrticosteroids in HErpes SImplex Virus Encephalitis) study

Sylviane Defres[1], Simon Keller[2], Rishma Vidyasagar[3], Kumar Das[5], Neil Roberts[4], Laura Parkes[3], Tom Solomon[1,5]

[1]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK, [3]Centre for Imaging Sciences, University of Manchester, Manchester, UK, [4]Division of Medical and Radiological Sciences, University of Edinburgh, Edinburgh, UK, 5Walton Centre NHS Foundation Trust, Liverpool, UK

Background: Herpes simplex virus (HSV) encephalitis is a devastating brain infection. Outcomes remain poor despite aciclovir treatment. The role of corticosteroids remains uncertain: they may improve outcome by reducing swelling and inflammation, or cause deterioration by allowing uncontrolled viral replication.
Methods: To provide preliminary data for a randomised controlled trial of dexamethasone in HSV encephalitis, we examined change in temporal lobe volume and oedema (between admission and 4 weeks) on MRI brain scans of patients with paired scans.
Results: Temporal lobe volume (estimated using the Cavalieri method of design-based stereology) increased on average by 1.6% (standard deviation 0.11%) between first and second scans for five patients who had no steroid treatment, and decreased by 8.5% in two patients who had corticosteroid treatment. Cerebral oedema, as determined by FLAIR, increased by 9% in patients who did not receive corticosteroids, and decrease by 29% in the two patients who did.
Interpretation: In this preliminary study, brain swelling increased in patients not treated with corticosteroids, but decreased in those given corticosteroids. A phase III randomised controlled trial, the COHESIVE (COrticosteroids in HErpes SImplex Virus Encephalitis) study, starting 2014, will provide a definitive answer to whether corticosteroids improve the outcome.

LI18We0924

Offered paper – Construction of chimeric Arctic-like rabies virus glycoproteins rescues pseudovirus titre to permit use in serological studies

Emma Bentley[1,2], Ruqiyo Ali[1], Daniel Horton[3], Ashley C. Banyard[3], Edward Wright[1,2]

[1]Faculty of Science and Technology, University of Westminster, London, UK, [2]Viral Pseudotype Unit (Fitzrovia), University of Westminster, London, UK, [3]Wildlife Zoonoses and Vector Borne Diseases, Animal Health and Veterinary Laboratories Agency, Surrey, UK

Arctic-like rabies (AL RABV) viruses, a lineage of RABV circulating widely in the Middle East and Asia, have distinct antigenic and genetic characteristics. Similar to other members of the RABV species, they cause a zoonotic disease ultimately leading to death of infected patients. RABV glycoprotein (G) lentiviral pseudovirus (PV) has shown to be a highly sensitive and specific surrogate to live virus in neutralisation assays. However, using wildtype AL RABV G failed to generate infectious PV. Thus, we investigated the generation of chimeric AL RABV G to increase the PV titre. Chimeras were constructed by splicing the ecto- and transmembrane domains of four AL RABV G strains with the cytoplasmic domains of vesicular stomatitis virus (VSV) or RABV challenge virus standard-11 (CVS-11) G. PV produced expressing wildtype or chimeric G revealed chimeric AL RABV with VSV G but not CVS-11 significantly increased PV titres. Hence we have sought to undertake neutralisation studies with the AL RABV VSV G chimeras, testing the efficacy of current vaccines and antivirals against this RABV subset.

LI18We0936

Offered paper – Trends in rabies post-exposure treatment in England and Wales, 2001-2012

S Winchester [1], H Kirkbride[2], D Brown[1], K Brown [1]

[1]Virus Reference Department, Microbiology Services, PHE Colindale, 61 Colindale Avenue, London NW9 5EQ

[2] Gastrointestinal, Emerging and Zoonotic Infections Department, Microbiology Services, PHE Colindale, 61 Colindale Avenue, London NW9 5EQ

Background Rabies vaccine and human rabies immune globulin (HRIG) issues were reviewed to inform future recommendations.

Methods
Post-exposure treatment (PET) rabies enquiry forms at PHE Colindale for England and Wales 2001-2012 were retrospectively analysed.
ResultsPET vaccine issues increased from <400 in 2001 to >1100 in 2012 and HRIG issues increased from <200 to >400. Bat exposures increased from <10 to >100 and the majority of these were in the UK. 24% (1951/8131), 17% (1357/8131) and 16% (1269/8131) of rabies PET issues were due to exposures in South East Asia, South Asia and Western Europe, respectively. In countries with a high number of exposures, PET issues per 100,000 visits increased from 1 to 11, 11 to 12, 4 to 14, 17 to 25, 24 to 44 and 5 to 8 in China, India, Morocco, Sri Lanka, Thailand and Turkey, respectively.
Conclusions There was a more than three-fold increase in rabies PET issues due to increases in bat and travel related exposures. The increase in bat exposures follows the death of a bat handler in Scotland in 2002. The increase in travel related PET was not accounted for by increased travel alone as increases in issues per visits were observed.

LI18We0948

The Prevalence of Mixed Genotype Hepatitis C Virus Infections

Anna McNaughton[1], Rory Gunson[2], Kate Templeton[3], Carol Leitch[1]

[1]MRC-University of Glasgow Centre for Virus Research, Glasgow, UK, [2]West of Scotland Specialist Virology Centre, Glasgow, UK, [3]Edinburgh Specialist Virology Centre, Edinburgh, UK

Mixed genotype (gt) hepatitis C virus (HCV) infections, occurring when individuals are infected with multiple HCV genotypes, have been documented in the literature at rates of 5-25.3%. Currently, the prevalence of mixed HCV infection within the UK is relatively unknown.
To assess the prevalence of mixed infections in UK patients, a highly sensitive gt-specific PCR assay was developed to detect gt1a/gt3a mixed HCV infections. Assay sensitivities were demonstrated using known dilutions of synthetic RNA transcripts. Minor gt1a variants were detected in 8.7% (11/126) of gt3a-diagnosed patients. Conversely, no gt3a co-infecting strains were observed in gt1a-diagnosed individuals (n=48). A real-time PCR assay was developed to quantify the major and minor genotypes present in mixed infections. Proportions of hidden gt1a in gt3a-diagnosed individuals were found to range from 0.01-21% of the total viral load.
Mixed HCV infections, comprising predominant gt3a strains with co-infecting minor gt1a strains, were detected in 8.5% of patients. Further work is vital to improve our understanding of mixed infections and their impact on clinical progression. As the newly-licensed direct-acting anti-virals targeting HCV have gt-specific activity, studies are required to assess whether mixed infections may contribute to the partial- and non-response outcomes observed during HCV treatment.

LI18We1000

Offered paper – Infection of liver-derived progenitor-like cell populations with hepatitis C virus

Matthew Bentham, Anjana Patel, Adel Jebar, Rajiv Dave, Alan Melcher, Heiko Wurdak, Stephen Griffin

University of Leeds, West Yorkshire, UK

Hepatitis C virus (HCV) infection is responsible for over 30% of hepatocellular carcinomas (HCC) worldwide, although the mechanism by which the virus predisposes to malignancy is controversial. Dogma states that tumours arise as a consequence of increased inflammation-associated cell turnover within cirrhotic livers, yet growing evidence supports a direct HCV oncogenic effect. In particular, several studies have demonstrated the ability of HCV to drive cells towards a de-differentiated state, which may predispose cells to oncogenic change and the formation of cancer initiating cells (CICs). However, another source of CIC is adult progenitor cell populations – cells that naturally share many characteristics of CICs, leading to the intriguing possibility that HCV may promote the transformation of such cells.
We have optimised the cultivation of primary cell populations with self-renewal capacity sourced from adult human liver tissue. These diverse and heterogeneous cultures can be propagated under “stem-cell” like conditions for multiple passages and express markers consistent with undifferentiated cells that are not yet committed towards the hepatocyte/cholangiocyte lineage. We demonstrate the ability of cell culture-derived HCV to infect these cells at low frequency, providing a novel event that could conceivably result in liver cell transformation *in vivo*.

LI18We1012

Offered paper – Genetic Diversity of Porcine Group A Rotavirus Strains in the UK

Rebecca Chandler-Bostock[1], Laura Hancox[1], Sameena Nawaz[2], Oliver Watts[1], Miren Iturriza-Gomara[3], Kenneth Mellits[1]

[1]University of Nottingham, Nottingham, UK, [2]Virus Reference Department, Public Health England, Colindale, UK, [3]University of Liverpool, Liverpool, UK

Rotavirus is a double-stranded RNA virus which causes diarrhoeal disease in young mammals. In pigs, an outbreak of rotavirus causes significant losses in production to farmers. A wide range of Group A rotavirus (GARV) genotypes were found on UK farms between 2010 and 2012. These were VP7; G2, G3, G4, G5, G9 and G11 and VP4; P[6], P[7], P[8], P[13], P[23], and P[32].
G9 sequences from this study were distinct from human G9 sequences found in the UK; suggesting that the origin of human G9 strains in the UK is unlikely to be zoonotic transmission from pigs with in the UK. Multiple lineages of P[6] sequences were found, many sequences clustered with lineages associated primarily with human rotavirus, this suggests multiple interspecies transmission events between pigs and humans. Interestingly, although P[8] rotavirus is almost exclusively found in humans, one P[8] sequence was identified in a pig, closely related to UK human rotavirus strains with P[8]-3 lineage, so is likely to be the result of an interspecies transmission between human and pig.
An understanding of prevalent genotypes may rationalize vaccine development for pigs specifically, as other vaccines target different GARV genogroups and it remains to be tested if they offer cross-protection.

LI18We1100

Offered paper – Is a comparative serology approach a requisite for the evaluation of pre-

Please note: Abstracts are published as received from the authors and are not subject to editing.

existing serological cross-reactivity in human populations?

Eleonora Molesti[1], Francesca Ferrara[1], Giulia Lapini[2], Emanuele Montomoli[2], Nigel Temperton[1]

[1]University of Kent, Chatham Maritime, UK, [2]University of Siena, Siena, Italy

The human population is constantly exposed to multiple influenza A subtypes due to zoonotic spillover and rapid viral evolution driven by intrinsic error-prone replication and immunological pressure. In this context, antibody responses directed against HA and NA proteins, are of importance since they have been shown to correlate with protective immunity (Gerhard, 2001).
Serological techniques, detecting these responses, play a critical role for influenza surveillance, vaccine development and evaluation (Katz et al., 2011). As the recent pandemics and avian influenza outbreaks demonstrated, there is a need to be better prepared to assess the contribution of the antibody responses against newly emerged viruses and to evaluate the extent of pre-existing serological cross-reactivity in populations (Liu et al., 2013).
In this study, 68 serum samples collected from the Italian population between 1992 and 2007 were found to be positive for antibodies against H5N1 as determined by Single Radial Hemolysis (Gentile et al., 2007) but negative when evaluated using Haemagglutination Inhibition and Microneutralisation assays. As a result of these discordant serological findings, the increased sensitivity of lentiviral pseudotypes was exploited in pseudotype-based neutralisation assays (pp-NT) and results obtained provide further insight into the complex nature of humoral immunity against influenza A viruses.

LI18We1112

Offered paper – Clinical Assessment of a Novel Recombinant Simian Adenovirus ChAdOx1 as a Vectored Vaccine Expressing Conserved Influenza A Antigens

Lynda Coughlan[1], Richard Antrobus[1], Tamara Berthoud[1], Matthew Dicks[2], Adrian Hill[1], Teresa Lambe[1], Sarah Gilbert[1]

[1]The Jenner Institute, University of Oxford, Oxford, UK, [2]King's College London School of Medicine, London, UK

Human Adenovirus Type-5 (Ad5) vectors are used most commonly in clinical trials. Adenoviruses (Ads) are promising vaccine vectors for infectious diseases due to their ability to induce and boost cellular immunity to transgene antigens. However, high level pre-existing immunity in humans has the potential to limit the efficacy of Ad5-based vaccines. In recent years this issue has prompted the investigation of rare species or non-human Ad vectors.
We report the first dose-escalation Phase I clinical trial to determine safety and immunogenicity of replication-defective chimpanzee Ad, ChAdOx1, expressing conserved antigens from Influenza A, nucleoprotein (NP) and matrix protein-1 (M1). There were no serious adverse events at any dose and the vaccine was well tolerated up to $2.5x10^{10}$vp. At the highest dose ($5x10^{10}$vp), 3/6 volunteers experienced adverse reactions including fever, fatigue, malaise and headache. Peak T-cell responses (ELISpot) to NP+M1 peptide pools were observed D14 post-vaccination and were increased ~3-fold ($5x10^{8}$vp), 6-fold ($5x10^{9}$vp), 7-fold ($2.5x10^{10}$vp) and 4-fold (5x1010vp) over baseline (D0). A small number of volunteers received a boost vaccination with poxviral vector MVA-NP+M1 ~8 weeks post-prime with ChAdOx1-NP+M1. MVA-NP+M1 boosted T-cell responses to levels comparable to the initial ChAdOx1-NP+M1 prime vaccination. These data highlight the potential for clinical use of ChAdOx1.

LI18We1124

Offered paper – Development of aptamer-based ELISA for the diagnosis of avian influenza viruses

Beata Adamiak[1], Thao Le[2], Sanjiv Sharma[2], Rob Fenton[2], John W McCauley[3], Anthony EG Cass[2], Munir Iqbal[1]

[1]The Pirbright Institute, Compton Laboratory, Compton, Newbury, Berkshire, UK, [2]Imperial College, Department of Chemistry and Institute of Biomedical Engineering, London, UK, [3]MRC National Institute for Medical Research, Division of Virology, Mill Hill, London, UK

Avian influenza viruses (AIV) cause heavy losses for poultry production world-wide. The diagnosis of AIV infection in poultry is complex requiring rapid, easy-to-use, and highly sensitive tests that can detect and differentiate poultry viruses at the pen-side. Considering these needs, we are developing new AIV tools based on single stranded (ss) nucleic acid molecules, "aptamers" that specifically bind to AIV antigens.
Here we demonstrate the use of ss DNA aptamers for enzyme linked immunosorbent assay (ELISA) based diagnostics. Selection of aptamers was against H5N1 and H9N2 antigens using SELEX. The selected aptamer showed specific affinity for the AIV antigens of H5, H7 and H9 subtypes but not NDV. Direct and indirect ELISA methods were tested using virus-, specific antiserum-, chicken IgG-, and streptavidin-coated plates. All above approaches exhibited promising potential for development of highly sensitive aptamer-based assays that will diagnose causative agents during outbreak and will have implications for the implementation of appropriate disease control measures.

LI18We1136

Offered paper – Burden of Influenza B virus infections in Scotland - Epidemiological investigations from 2000 - 2013

Heli Harvala [1], Donald Smith[2], Karina Salvatierra [2], Rory Gunson [3], Beatrix von Wissmann[4], Arlene Reynolds[4], Catherine Frew[3], Alastair MacLean[3], Alison Hunt[4], David Yirrell[5], Peter Simmonds[2], Jim McMenamin[4] and Kate Templeton[1]

[1] Specialist Virology Centre, Department of Laboratory Medicine, Royal Infirmary Edinburgh; [2] Centre for Immunology, Infection and Evolution, University of Edinburgh [3] West of Scotland Specialist Virology Centre, Glasgow, UK; [4] Health Protection Scotland, Glasgow; [5] Department of Medical Microbiology, Aberdeen Royal Infirmary; [6] Department of Medical Microbiology, Ninewells Hospital and Medical School, Dundee, UK

The burden of influenza B infection in Scotland was investigated over 13 years. Influenza A and B viruses co-circulated throughout the study period with numbers of influenza B infections exceeding those of influenza A during six seasons. Both Victoria and Yamagata lineage influenza B viruses were detected most seasons. In 2012-2013, influenza B virus infections accounted for 45% of all influenza infections with highest incidences in those <5 years. Over this period, influenza A infections led to a large number of ICU admissions (81

patients) with a significant mortality rate of 36% (29 deaths). Rates for influenza B were substantially lower (37 admissions, 3 deaths). However, most of the latter admissions had not been immunized (25%) or treated with antivirals (60%). This highlights the need for more vigilant influenza vaccination and the prompt usage of antivirals for those within identified risk groups. In the light of the higher disease burden of influenza B among children compared with older age groups, and the co-circulation of influenza B viruses of both lineages, combining the newly introduced vaccination programme for children with the use of a quadrivalent vaccine in future may provide the opportunity to improve the control of influenza B.

LI18We1148

Offered paper – Replication of human influenza viruses in dog tracheas: implications for viral emergence

Gaelle Gonzalez[1], John.F Marshall[2], Joanna Morrell[1], David Robb[3], Pablo.R Murcia[1]

[1]MRC-University of Glasgow Centre for Virus Research , Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, [2]Weipers Centre Equine Hospital, School of Veterinary Medicine, University of Glasgow, Glasgow, UK, [3]Charles River Laboratories, Preclinical Services, Tranent, Edinburgh, UK

Influenza viruses (IAVs) represent a significant risk to human and animal health. Cross species jumps by IAVs between mammals are uncommon but do occur and have generated diseases outbreaks in new host species. Determining the mechanisms of cross-species transmission is critical to improving our understanding of viral emergence. As previous reports have demonstrated infection of the domestic dog by human, avian and equine influenza viruses, we wanted to determine if dogs could act as a "mixing vessel" for the generation of influenza viruses with pandemic potential.

Dog tracheal explants were infected with a variety of human IAVs (laboratory strains, clinical isolates, reverse genetics generated viruses) and reassortants between canine and human IAVs. Viral replication was quantified and changes in the histology and ciliary activity of infected explants were assessed over a 7-day period to compare the phenotype of infection of each virus.

We observed that human IAVs exhibited differential abilities to infect dog tracheas and reassortment between canine and human influenza viruses lead to the generation of viable viruses. Our results indicate that the dog constitutes a natural host where novel IAVs with pandemic potential can be generated.

LI19

Virology workshop: retroviruses

LI19We1600

Offered paper – Host-Parasite Genetic Conflict at the Nuclear Pore Complex

Paul Rowley[1], Kurt Patterson[2], Suzanne Sandmeyer[2], Sara Sawyer[1]

[1]The University of Texas at Austin, Austin, Texas, USA, [2]The University of California at Irvine, Irvine, California, USA

Many viruses must enter the nucleus of a host cell before they can replicate. In these cases, the virus must find a way to transport itself through the nuclear pore complex (NPC). We have found that, in the *Saccharomyces* species of yeasts, proteins of the NPC (nucleoporins) are evolving rapidly, consistent with Darwinian positive selection. Ty elements are retrotransposons that have an analogous lifecycle to retroviruses, and are the only yeast parasite that transits through the NPC. We tested the hypothesis that the rapid evolution of certain nucleoporins has been driven by Ty elements, where the host NPC has been selected for variants that exclude Ty from accessing the nucleus. Despite ~20 million years of evolution, we were able to precisely replace the rapidly evolving nucleoporins *NUP82* and *NUP84* from *S. cerevisiae* with the orthologous genes from different yeast species. Replication of Ty1 elements was significantly affected by orthologous gene replacement of these nucleoporins; however, *NUP84* replacement had no effect on Ty3 replication. This work demonstrates that nucleoporin evolution can alter the replication of specific Ty elements, and that the NPC may defend against nuclear entry of parasites and influence cross-species transmission.

LI19We1612

Offered paper – Close relatives of fish retroviruses identified in primates

Katherine Brown[1], Ed Louis[2], Richard Emes[1], Rachael Tarlinton[1]

[1]University of Nottingham, Nottingham, UK, [2]University of Leicester, Leicester, UK

Several types of cancer in fish are caused by retroviruses, including those responsible for major outbreaks of disease, such as walleye dermal sarcoma virus and salmon swim bladder sarcoma virus. These are all members of the epsilonretrovirus genus. Epsilonretroviruses have become endogenous on several occasions, integrating into germline cells to become part of the host genome, and sections of fish and amphibian genomes are derived from epsilonretroviruses. However, epsilonretroviruses in mammals are almost unknown.

We have developed a novel pipeline to screen full genomes for endogenous retroviruses, with an improved ability to identify epsilonretroviruses. Using this pipeline, we have located over 500 endogenous epsilonretroviruses in primate genomes. Genomes from 32 species of mammals and birds were screened and these viruses were found in all primate genomes but no others. The majority appear to have entered the genome of a common ancestor of old and new world monkeys at least 40 million years ago.

Based on these results, there is an ancient evolutionary relationship between epsilonretroviruses and primates. Clearly, these viruses have the potential to infect primates, including humans, and were at some point a common primate pathogen. This result raises questions about the evolutionary history of other groups of retroviruses.

LI19We1624

Offered paper – Modelling the Neuropathological Consequences of HIV Vaccines that Confer Partial Protection

Debbie Ferguson[1], Claire Ham[1], Andrea Meiser[2], Steve Patterson[2], Neil Berry[1], Neil Almond[1]

[1]National Institute of Biological Standards and Control, Potters Bar,

Please note: Abstracts are published as received from the authors and are not subject to editing.

Herts, UK, [2]Imperial College, London, UK

Antiretroviral therapy (ART) has effectively turned HIV into a chronic disease, with some entering a 3rd decade of suppressed viral loads. However, treated patients are developing co-morbidities including HIV-associated neurocognitive impairment (HIV-NCI). Using a non-accelerated ART-free SIV animal model with viral replication kinetics similar to human HIV infection, we have shown long term loss of viral control is not required for neuropathology to develop and the process begins within days of infection.

As part of a vaccine study macaques were immunised with an SIV*gag* based vaccine regimen. Although vaccination did not prevent virus acquisition or reduce set point viral loads, it did result in a significant blunting of the primary viremia compared with naïve challenge controls.

Immunohistochemical examination of brain sections from macaques at termination revealed neuroinflammatory responses present in both vaccinated and naïve infection control animals. However the degree of astrogliosis, microgliosis, perivascular macrophage, T cell influx and expression of CNPase1 (olidodendrocytes) was significantly reduced following vaccination.

If blunting of primary viremia alone modulates HIV induced neuropathology then new treatments may need to be developed that prevent or reverse the pathology, as identifying HIV acquisition before or at seroconversion is rarely achieved in clinical practice.

LI19We1636

Offered paper – Deep sequence analysis of transmitted founder viruses in a low dose mucosal SIV challenge study delivering partial vaccine protection

Claire Ham[1], Ed Mee[1], Christiane Stahl-Hennig[2], Steve Patterson[3], Neil Almond[1], Neil Berry[1]

[1]Division of Virology, NIBSC, Potters Bar, EN6 3QG, UK, [2]German Primate Centre, Kellnerweg 4, Gottingen, Germany, [3]Chelsea and Westminster Hospital, Imperial College, London, UK

Deep sequence analysis of the viral populations which represent transmitted founder viruses of HIV/SIV vaccine trials is beginning to provide insights into the potential protective mechanisms involved, particularly where partial vaccine protection has been observed. We sought to determine the impact of immunisation with a recombinant SIVmac239 *gag* DNA/adenoviral boost vaccine regimen on the sequence of those viruess that established infection in groups of Mauritian derived cynomolgus macaques. As plasma SIV RNA levels in challenge controls and vaccinates differed significantly in the immediate post-challenge period, we sought to define the founder virus population in each group by deep sequencing and compare it with the challenge virus stock SIVmac251/CSH. SNP frequencies across *gag* as detected by either 454 or MiSeq sequencing technologies were compared with the CSH consensus. As macaques were MHC typed we will present an analysis of the sequence populations perturbed following vaccination, stratified by viral load and/or MHC type, to identify signature sequences of vaccine efficacy. These data will inform our ability to identify key signature motifs of vaccine mediated virus control informing improved HIV vaccine design.

LI19We1648

Offered paper – HIV patients with protease inhibitor mutations: Identification of novel Gag mutations and potential changes in UTR RNA structure using ultra-deep sequencing

Leonardo Chanqueo [1,3], Howard Martin [2], Kim Brugger [2], Andrew Lever [1], Jane Greatorex [3]

[1]Department of Medicine, University of Cambridge, UK

[2]Department of Molecular Genetics, Addenbrookes Hospital, Cambridge, UK [3]Public Health England, Cambridge, UK

The HIV Gag polyprotein has been demonstrated to modulate HIV resistance. The aim of our project was use Ultra Deep Sequencing (UDS) to identify novel Gag mutations and changes in the 5' UTR sequences when protease inhibitor resistance occurs. Samples were chosen from patients on protease inhibitor (PI) treatment and with PI mutations who had had an HIV genotyping test in the past 12 months.

Eight sequences were obtained from twelve patient samples using primers for the UTR. The potential RNA secondary structures of each were obtained and folded using Mfold version 3.5. None of the associated RNA structures deviated significantly from the wild type virus. However, in 4/8 RNA sequences it was possible to demonstrate that multiple RNA structures could potentially exist simultaneously. This might indicate evolution of RNA structure over time.

Ten Gag sequences were generated and in the protease cleavage sites the following mutations were found: MA/CA = I138L, CA/p2 = N372 K/G/A. In addition, 65 mutations were found in Matrix, 36 mutations in Capsid, 20 in Nucleocapsid, 3 in p1 and 46 in p6.

We successfully demonstrated the use of UDS for the discovery of both novel resistance mutations and RNA secondary structures in HIV sequences.

LI19We1700

Offered paper – Functional analysis of the CA-SP1 junction in HIV-1 Gag: A critical assembly domain and molecular target of HIV-1 maturation inhibitors.

Christopher Murgatroyd, Catherine Adamson

University of St Andrews, St Andrews, Fife, UK

The CA-SP1 junction in HIV-1 Gag plays a critical role in virus assembly and acts as the target of HIV-1 maturation inhibitors, which specifically inhibit CA-SP1 cleavage during viral proteolytic maturation. We previously identified two mutations, SP1-A3V and SP1-A3T that confer resistance to the maturation inhibitor bevirimat and impose a replication defect on HIV-1. Interestingly the SP1-A3V replication defect is reversed by a second-site compensatory mutation CA-G225S and partially rescued by inhibitory concentrations of bevirimat. Further selection experiments identified a panel of second-site mutations that compensate for SP1-A3V. Characterization of these mutants in Jurkat T cells demonstrated that, like the CA-G225S mutation, SP1-V7I and SP1-P10Q mutations reverse the SP1-A3V but not the more severe SP1-A3T replication defect. However the SP1-T8I mutation compensates for both the SP1-A3V and SP1-A3T mutations. The SP1-A3V, -A3T and -T8I mutations impose virus assembly defects, which are reversed by the SP1-residue-3/SP1-T81 combinations but not the other compensatory mutations. We are currently examining the effect of these mutations and drug binding on Gag-Gag multimerization, virus

morphology and viral genome packaging. These studies will provide further understanding of the role of this region of Gag in HIV-1 assembly and aid the development and characterization of second-generation maturation inhibitors.

LI19We1712

Offered paper – Investigation of a 3-helix motif in the HIV-15'UTR

Sarah K Mercier[1], Julia C Kenyon[1], Liam J Prestwood[1], James D Stephenson[2], Andrew ML Lever[0]

[1]Department of Medicine, University of Cambridge, Box 157, Level 5 Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ, UK, [2]University of Hawaii, NASA Astrobiology Institute, 2680 Woodlawn Drive, Honolulu, Hawaii 96822, USA

Recently published 3D model of a 240nt region of the 5'UTR HIV-1 sequence identified a 3 helix junction. Using single-molecule FRET with geometric restraints, a putative kink turn (k-turn) located at the junction of the PBS, the polyA stem loop and the packaging signal stem loops 1-3 was suggested.
K-turns are an important structural RNA motif that introduces a tight kink (~60°) into the axis of helical RNA such that they play a role in many aspects of RNA function and serve as binding sites for a number of proteins. The classical k-turn comprises a Watson-Crick paired canonical helix (C-helix), a three nucleotide bulge followed on the 3 side by a G:A pair, an A:G pair motif and a further non-canonical helix (NC helix).
There are many variations to the classic k-turn structure. To investigate this putative k-turn further we introduced mutations into the GA:AG motif, the C and NC-helix. Mutagenesis confirms the importance of the 3-helix junction motif and highlights the flexibility of the region in accommodating some sequence variation if key helix features are maintained. Further work is ongoing to establish the exact tertiary structure and to investigate the role of this region in the virus life cycle.

LI20

Virology workshop: respiratory viruses

LI20We1400

Offered paper – A high resolution electron microscopy study of within-host dynamics of canine influenza virus infection

J. Morrell, S. Vijayakrishnan, G. Gonzalez, D. Bhella, P. Murcia, Joanna Morrell, Swetha Vijayakrishnan, Gaelle Gonzalez, David Bhella, Pablo Murcia

MRC, Centre for Virus Research, Glasgow, UK

Influenza A viruses (IAVs) are significant pathogens of humans and animals whose natural host is wild waterfowl (and possibly bats), but have evolved to gain the ability to jump host barriers to infect other animals. Equine influenza virus (H3N8) has been a pathogen of the horse since 1963, but in the early 2000's it jumped the species barrier to establish in dogs as a novel respiratory virus (canine influenza virus [CIV]). To gain insight of the *in situ* pathogenesis of CIV along the course of infection in the target tissues of the natural host, we performed an electron microscopy (EM) study on dog tracheal explants infected with CIV. Time course experiments allowed us to characterise dynamics of the ultrastructural changes associated with CIV infection.
By combining tracheal explants with EM we obtained a high-resolution description of the dynamics of influenza infection within the host. Our results show that the ultrastructural changes that occur within the tracheal epithelium due to infection are consistent with light microscopy findings. Our next step will be to utilise EM to directly visualize influenza virus particles at different stages of the infection process.

LI20We1412

Offered paper – Relative respiratory syncytial virus cytopathogenesis in well-differentiated primary paediatric airway epithelial cell cultures from the upper and lower respiratory tract

Hong Guo-Parke[1], Paul Canning[1], Isobel Douglas[2], Remi Villenave[1], Liam Heaney[1], Peter Coyle[3], Jeremy Lyons[2], Michael Shields[1,2], Ultan Power[1]

[1]Queen's University Belfast, Belfast, UK, [2]The Royal Belfast Hospital for Sick Children, Belfast, UK, [3]Regional Virus Laboratory, Royal Victoria Hospital, Belfast, UK

Respiratory syncytial virus (RSV) is a major pathogen that primarily infects airway epithelium. Most infants suffer mild upper respiratory tract (URT) symptoms, while approximately one third progress to lower respiratory tract (LRT) involvement. However, little is known about relative RSV cytopathogenesis in infant URT versus LRT.
To address this, we generated well-differentiated nasal (WD-PNEC) and bronchial (WD-PNEC) epithelial cell cultures from the same individuals and infected them with a recent RSV clinical isolate. RSV tropism, infectivity, cytopathology, growth kinetics, cell sloughing, apoptosis, and cytokine/chemokine responses were determined.
RSV infection in both cultures was restricted to apical ciliated cells and occasional non-ciliated cells, but not goblet cells, without causing gross cytopathology. Infection resulted in apical release of progeny virus, increased apical cell sloughing, apoptosis and occasional syncytia. RSV growth kinetics and peak titers were higher in WD-PBECs, coincident with higher ciliated cell contents, cell sloughing and slightly compromised tight junctions. However, pro-inflammatory chemokine and lambda interferon responses were similar for both cultures.
RSV induced remarkably similar cytopathogenesis and pro-inflammatory responses in WD-PNECs and WD-PBECs that reproduce many hallmarks of RSV pathogenesis in infants. WD-PNECs may provide an authentic surrogate model with which to study RSV cytopathogenesis in infant airway epithelium.

LI20We1424

Offered paper – Human RSV M protein interactions with host proteins reveal new cellular ligands involved in viral nuclear localization and innate defense

Sarit Kipper[1], Leon Caly[2], Teofano Panayiotou[3], Eran Bacharach[4], David Jans[2], Doron Gerber[1], Monika Bajorek[3]

[1]Bar Ilan University, Ramat Gan, Israel, [2]Monash, Melbourne, Australia, [3]Imperial College London, London, UK, [4]Tel Aviv University, Tel Aviv, Israel

Please note: Abstracts are published as received from the authors and are not subject to editing.

Respiratory syncytial virus (RSV) is the commonest cause of bronchiolitis in infants, but also kills many elderly people. There is no vaccine or specific treatment. The RSV Matrix (M) protein is found in the nucleus early in infection, localizes into viral Inclusion Bodies (IBs) and is crucial to viral assembly and budding at plasma membrane.
Identifying the RSV M interactions with the host may assist in understanding possible effects on transcription inhibition in the nucleus, innate immunity down-regulation and the formation of infectious RSV particles. We therefore screened for RSV M-host protein interactions using a high throughput microfluidics platform, using a custom human proteome library as molecular bait.
We identified 23 novel binding partners involved in a range of cellular pathways; signalling, transcription regulation, innate immunity, membrane remodelling and trafficking. One of the identified proteins involved in innate immunity localized within large RSV IBs. This finding joins previously published reports suggesting that IBs may have a role in modulating the innate immune response during infection by sequestering specific host factors. Further, depletion of a nuclear factor ZNF502 interacting with M resulted in decreased viral titre, implying that this interaction is involved in RSV life cycle.

LI20We1436

Offered paper – The role of the HRSV polymerase cellular interactome in viral transcription and replication and L protein function

Weining Wu[1], Diane Munday[1], Nikki Smith[2], Jenna Fix[3], Paul Digard[2], John N. Barr[4], Andrew Easton[5], Jean-François Eléouët[3], Julian A. Hiscox[1]

[1]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]The Roslin Institute, University of Edinburgh, Edinburgh, UK, [3]Unité de Virologie et Immunologie Moléculaires, INRA, Paris, France, [4]School of Molecular and Cellular Biology, University of Leeds, Leeds, UK, [5]School of Life Sciences, University of Warwick, Warwick, UK

The human respiratory syncytial virus (HRSV) polymerase complex is critical for virus biology, and a target for anti-virals. We have focused on two of the polymerase proteins: the L protein (the RdRp) and its cofactor the P protein. Using biologically active L and P proteins that have been fused to EGFP, we have developed label free quantitative proteomics coupled to highly specific EGFP traps to define their cellular interactomes. Significant interacting proteins have been identified including heat shock proteins and their co-chaperones. Ablation of chaperone function using small molecule inhibitors demonstrated that chaperone activity is required for L protein stability, and treatment of infected cells demonstrated that progeny production could be reduced by up to five logs. Deep sequencing and continuous passage of virus in the inhibitors demonstrated that no resistant viruses emerged.

LI20We1448

Offered paper – Non-canonical interplay between influenza A virus (FLUAV) and the host SUMO system

Patricia Domingues, Ben Hale

MRC - University of Glasgow Centre for Virus Research, Glasgow, UK

Protein modification by SUMO (SUMOylation) contributes to almost all aspects of nucleus biology, the key organelle for FLUAV genome replication and transcription. Here, we observe that FLUAV infection causes a dramatic redistribution of intra-nuclear SUMO, which parallels a global increase in SUMOylation. FLUAV-induced SUMOylation is characterized by the initial appearance of several specific, low MW SUMO-reactive conjugates, followed by the subsequent development of additional higher MW conjugates. By chemically dissecting FLUAV replication, we show that the increase in SUMOylation is dependent on virus genome replication and protein synthesis, but not vRNP export. Analysis of classical virus-activated stress pathways using genetically-engineered cells and chemical agonists revealed that FLUAV-induced SUMOylation does not correlate with activation of IFN, unfolded protein, apoptotic or DNA damage response pathways.
Strikingly, FLUAV-triggered SUMOylation is phenotypically distinct from canonical SUMO conjugate inducers, such as thermal or proteotoxic stresses. Furthermore, siRNA depletion studies suggest that the SUMO conjugation pathway is required for efficient FLUAV replication, an observation that contrasts with the antiviral role of SUMO pathways against nuclear-replicating DNA viruses. Overall, our data describe a complex non-canonical interplay between FLUAV and the host nuclear SUMO system, and highlight that FLUAV may be hijacking aspects of SUMOylation to facilitate replication.

LI20We1500

Offered paper – Swine IFITM proteins protect against influenza A virus infection *in vitro*

Caroline Lanz, Silke Stertz

Institute of Medical Virology, Zurich, Switzerland

The zoonotic potential of swine influenza viruses is a major threat to human health and causes huge economical losses, as could be seen during the swine-origin influenza pandemic in 2009.
The antiviral potential of interferon-induced transmembrane proteins (IFITMs) in human and chicken against a wide range of viruses, including influenza A virus, has been described. However, little is known about the expression and the antiviral activity of IFITM proteins present in swine. To close this gap, we cloned the swine homologs of IFITM1, IFITM2, IFITM3 and IFITM5 and stably introduced them into newborn pig trachea epithelial cells (NPTr). When infecting these cell lines with A/WSN/1933 we observed a significant reduction in viral titers compared to control cells. Furthermore, we tested the antiviral restriction capacity of swine IFITM proteins via a mini-genome reporter assay in NPTr and HEK293T cells and could confirm the results obtained in the viral titer experiments. Localization studies revealed distinct patterns for the different IFITM proteins with either prominent plasma-membrane accumulation or an endosome-type distribution. In sum, we cloned and characterized the swine IFITM proteins regarding their localization and antiviral activity.

LI20We1512

Offered paper – Inhibiting the Beta 6 Integrin Protects Mice from Diverse Respiratory Viral and Bacterial Infections by Reducing Acute Lung

Injury and Inflammation

Stacey Schultz-Cherry[1], Victoria Meliopoulos[1], Michael Johnson[1], Nicholas van de Velde[1], Carlos Huerta[1], Jason Rosch[1], Paul Thomas[1], Dean Sheppard[2], Peter Murray[1]

[1]St Jude Children's Research Hospital, Memphis, TN, USA, [2]University of California San Francisco, San Francisco, CA, USA

Integrins modulate cellular function and influence innate immune responses. The beta 6 integrin is only expressed in epithelial cells during development or injury then plays a critical role in controlling acute lung injury. Given that beta 6 levels are increased in the lungs of influenza virus-infected mice we investigated its role in pathogenesis. A/California/04/09 (H1N1) influenza virus infected C57Bl/6 (WT) or β6-/- KO mice were monitored for morbidity, pathology, immune responses and lung function at different times post-infection. KO mice had increased survival (MLD50 105.3) as compared to WT mice (MLD50 102.5) associated with improved lung function, decreased pathology and inflammation. Although the overall viral titers did not differ, KO mice better controlled viral spread within the lungs. We hypothesize that the increased production of surfactant protein D and elevated numbers of activated lung (CD11c+/b+ double positive) macrophage are important for infection. Importantly, our findings are not influenza virus specific. KO were also protected from Sendai virus, *Streptococcus pneumonia* and secondary *Streptococcus pneumonia* following influenza infection. Our results suggest that the β6 integrin plays a previously unrecognized role in influenza pathogenesis regulating inflammation and the resulting lung damage by modulating communication between the infected epithelium and the innate immune response.

LI20We1600

Offered paper – The role of SPLUNC1 in the pulmonary innate immune response to Influenza A virus

Nathifa Moyo[1], Tessa Walsh[1], Gail Leeming[1], Mark Tompkins[2], Ralph Tripp[2], James Stewart[1]

[1]University of Liverpool, Liverpool, UK, [2]University of Georgia, Athens, USA

SPLINC1 is an epithelial host defense protein produced in the mammalian upper respiratory tract and is implicated in anti-inflammatory and immunomodulatory roles in response to infection. The function of this protein is yet unresolved in many species and by elucidating this will aid to clarify its physiological role within the lungs. The objective of this study was to quantify the expression of SPLUNC1 in response to Influenza A virus. Mice were infected with different strains of varying pathogenicities of Influenza A virus and analysed by immunohistochemical methods at specific time points during the infection period. Imaging software was then used to quantify the level of protein expression within the respiratory epithelium. It was found that the level of SPLUNC1 decreased in response to H1N1 and H5N1 infections. This alteration in expression was associated with an inflammatory response within the respiratory tissue surrounding the epithelium. Differences were also observed in SPLUNC1 expression in response to the pathogenicity of the virus. There was a significantly reduced SPLUNC1 expression in the tissues infected with the high pathogenic strains of both H1N1 and H5N1. Our data indicate that SPLUNC1 is a crucial component of the mucosal innate immune defense against Influenza A virus.

LI20We1612

Offered paper – Transient potential channels as potential therapeutic targets for virus induced asthma exacerbations: Studies in a human neuronal cell model

Hani'ah Abdullah, Hanagh Winter, John Corry, Liam Heaney, Lorcan McGarvey, Louise Cosby

Queen's University Belfast, Belfast, UK

Airway nerves control crucial reflexes such as cough and bronchoconstriction. In asthma and other respiratory conditions these reflexes become hyperactive, typically triggered by exposure to environmental irritants and are exacerbated during viral infections. The precise mechanism of how virus infection induces hypersensitisation, causing bouts of cough and wheezing, is unknown. The largest group of receptors with the capacity to detect noxious stimuli is the transient receptor potential (TRP) channel family, present on both neuronal and non-neuronal airway cells. TRPA1 has been implicated in the pathophysiology of acute and chronic cough. Our previous study demonstrated that soluble factors induced by human rhinovirus (HRV)infection up-regulate TRPA1 in a neuronal cell model .Factors such as IL-8 and NGF were implicated and may play an important role (Abdullah et al., *Thorax* 69:46-54, 2013). Based on this finding we explored the potential inflammatory factors which are responsible for up-regulating TRPA1 at the protein and mRNA levels. Our preliminary results suggest that treatment of virus induced supernatants with neutralizing IL-8 or NGF antibodies inhibits the up-regulation of TRPA1 by HRV infection. Understanding the mechanism of virus induced hyper- expression of TRP channels may indicate novel potential therapeutic targets for treatment of asthma and other respiratory conditions.

LI20We1624

Offered paper – Production of Influenza B haemagglutinin lentiviral pseudotype particles and their use in neutralization assays

Francesca Ferrara[1], Eva Böttcher-Friebertshäuser[2], Davide Corti[3], Simon Scott[1], Paul Kellam[4], Sarah C Gilbert[5], Nigel Temperton[1]

[1]Viral Pseudotype Unit, School of Pharmacy, University of Kent, Chatham Maritime, Kent, UK, [2]Institute of Virology, Philipps University Marburg, Marburg, Germany, [3]Institute for Research in Biomedicine, Bellinzona, Switzerland, [4]Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK, [5]The Jenner Institute, University of Oxford, Oxford, UK

Influenza B (FluB) viruses cause respiratory disease epidemics in human populations. Furthermore an Influenza B strain is routinely included in seasonal vaccination and recently a quadrivalent vaccine containing two FluB strains was developed.
Serological methods permit the evaluation of Influenza epidemiological distribution and are additionally used to evaluate vaccine efficacy. However the haemagglutination inhibition assay has been shown to be relatively insensitive for the detection of antibodies against FluB viruses. The use of replication-defective viruses, such as pseudotypes, in microneutralization assays is an accepted and safe alternative approach to study antibody responses elicited by natural infection or vaccination. We have produced a panel of Influenza B haemagglutinin (HA) pseudotypes using a standard

Please note: Abstracts are published as received from the authors and are not subject to editing.

pseudotype-producing transfection protocol. To activate FluB HA we have explored the use of two proteases, the transmembrane protease serine 2 and the human airway trypsin-like protease, by adding the relevant encoding plasmid to the transfection mixture. When tested for their ability to transduce target cells, the influenza B pseudotypes exhibit high transduction titers. With these newly developed reagents, which have hitherto not been reported in the literature, we are currently investigating the feasibility of using Influenza B pseudotypes as surrogate antigens in neutralization assays.

LI20We1636

Offered paper – Direct Measurement of Influenza A Haemagglutinin and Neuraminidase Balance

Donald Benton, Stephen Martin, Stephen Wharton, John McCauley

MRC National Institute for Medical Research, Mill Hill, London, UK

A major factor underlying Influenza A virus transmissibility is the interaction of the two surface glycoproteins, Haemagglutinin (HA) and Neuraminidase (NA), with the cell surface.
It has been hypothesised that there is a fine balance between the receptor binding properties of the HA and the receptor destroying activities of the NA. The HA receptor binding needs to be strong enough to ensure efficient binding to the cell but not so strong as to prevent virus release. The NA activity needs to be strong enough to allow release of the virus from the cell surface after budding but not so strong as to impede receptor binding. This HA and NA balance is determined by a number of viral properties: the kinetic parameters of HA binding and NA enzymic activity, virus shape and the proportions and distribution of the two glycoproteins on the virion. Biophysical assays have been carried out to measure the contributions of HA and NA to virus binding to human and avian receptor analogues. These assays use bio-layer interferometry and show the binding and release of viruses from a biosensor in real-time. These data can be used to infer characteristics of virus interactions with the cell surface.

LI20We1648

Offered paper – A promoter mutation in the HA segment of influenza A virus generates an effective candidate live attenuated vaccine.

Ruth Harvey, Rachel Johnson, Kirsty MacLellan-Gibson, James Robertson, Othmar Engelhardt

National Institute for Biological Standards and Control, Potters Bar, UK

Haemagglutinin (HA) is the major immunogenic component of influenza vaccines. It has previously been shown that mutations can be introduced in the non-coding region of influenza segments which upregulate protein expression. Here we describe the development of a new candidate vaccine virus (NIBRG-93) based on the widely used laboratory strain A/PR/8/34 (PR8) with a modified 3' non-coding region of segment 4 (haemagglutinin). We show that this virus has enhanced haemagglutinin production, but that it is also significantly attenuated. Further analysis of the virus by EM shows that the modified virus produces 'virus-like particles' which consist of mostly budded cell membrane covered in HA but lacking M protein. We demonstrate that this candidate vaccine virus is an effective live attenuated vaccine virus protecting against lethal challenge, and reducing shedding of virus in infected mice.

LI20We1700

Offered paper – Mitigating risk: A strategy for working with HPAI influenza

Wendy Barclay[2], Konrad Bradley[2], Jason Long[2] , Joe James[1,2], Munir Iqbal[1], Kolli Reddy[1], Karen Staines[1], Colin Butter[1], Holly Shelton[1]

[1]The Pirbright Institute, Compton, UK, [2]Imperial College London, London, UK

To study influenza virus pathogenesis the contribution of specific viral proteins and RNA structures should be investigated in the context of infectious virus. For researchers working with highly pathogenic avian influenza (HPAI) this can pose a real problem since the biosafety containment requirements can be highly prohibitive. We propose a universal risk mitigation strategy for influenza viruses, including HPAI strains, that allows the internal gene constellations to be probed under low containment conditions by pseudotyping with the surface glycoproteins, (HA and NA), from the well characterised vaccine strain A/PR/8/34 (PR8). Using reverse genetics we generated a series of recombinant viruses that possessed the internal genes from HPAI H5N1, H9N2 and human pH1N1 reassorted with the external surface genes, HA and NA from PR8. We assessed their characteristics *in vivo* in poultry and *in vitro* in relevant cell types including chicken tracheal organ and primary human airway cultures. The pseudotyped viruses were attenuated for viral dissemination when compared to viruses bearing homologous surface antigens. These viruses were also neutralised efficiently by pH1N1 post vaccination sera. Such a strategy could be adopted when novel influenza isolates are identified such as the recent H7N9 infections in China.

LI20We1712

Offered paper – Novel inhibitors targeting the drug-resistant proton channel of Influenza A Virus

Claire Scott[1], Jayakanth Kankanala[1], Ian Tietjen[2], David Fedida[2], Wendy Barclay[3], Richard Foster[1], Stephen Griffin[1]

[1]University of Leeds, Leeds, UK, [2]University of British Columbia, Vancouver, Canada, [3]Imperial College London, London, UK

As a consequence of the rapid onset of influenza pandemics, there is often insufficient time to produce vaccines prior to the virus becoming established; therefore antiviral prophylaxis represents the only effective mechanism to control the infection. Two classes of antiviral drugs are currently licensed for influenza A virus treatment, these target either the viral neuraminidase protein or the M2 proton channel. Widespread resistance in circulating strains has meant that existing adamantane drugs targeting M2 are no longer recommended for treatment. However, M2 remains an ideal target for antiviral development, making novel compounds targeting drug resistant variants highly desirable.
Despite abundant atomic structural information, controversy surrounds the mechanism by which adamantane inhibitors block M2 channel activity, with both lumenal and peripheral binding sites

proposed. We have embarked upon a structure-guided rational drug design programme targeting M2 from the adamantane resistant pandemic H1N1 "swine" influenza A virus. Critically, we have not only generated the first M2-targeted swine 'flu inhibitors effective both *in vitro* and in culture that are structurally entirely distinct to adamantanes, but our findings also support the presence of two druggable sites on the M2 channel complex. These results could pave the way to novel inhibitors of pandemic 'flu strains.

POSTERS

General Microbiology

GM/01

Development of a Worldwide Vaccine for Porcine Rotavirus Using Interferon Deficient Cell Lines.

Nathan Meade[1], Rebecca Chandler-Bostock[1], Marian Killip[2], Richard Randall[2], Kenneth Mellits[1]

[1]The University of Nottingham, Nottingham, UK, [2]The University of St Andrews, Fife, UK

Rotavirus is an infectious disease causing gastroenteritis and stunted growth in young mammals including livestock such as pigs. Outbreaks in pig herds cause poor growth performance, an increase in morbidity and higher mortality rates. This leads to a reduction in earnings for farmers, reduced sustainability and a lower quality of pork worldwide. Viruses can be characterised by their ability to circumvent immune responses. Rotavirus contains a number of properties enabling it to circumvent the innate immune response known as the Interferon system. Interferon (IFN) deficient modified MA104 cell lines utilising two viral proteins; bovine viral diarrhoea virus (BVDV) Npro protein and parainfluenza virus 5 (PIV5) V protein, have successfully been produced and characterised. These cell lines render IFN antagonism properties redundant during rotavirus infection and replication. A proposed technique of serial infection of modified MA104 cell lines is being investigated, using the porcine OSU lab strain of rotavirus. At different time points the serial infection will be monitored using a rotavirus plaque assay. We hope to determine whether this potential method can be used to produce attenuated viral vaccine candidates for the porcine model.

GM/05

Effect of dehulling and fermentation with r. Species on vitamins, minerals and antinutritional content of velvet (mucuna utilis) flour

Olanrewaju Ifejesu Balogun[1], Olawale Peter Olatidoye[1], Ezekiel Olateju Otunola[2]

[1]Yaba College of Technology, Yaba, Lagos, Nigeria, [2]Ladoke Akintola University of Technology, Ogbomoso, Oyo, Nigeria

Changes in vitamins, mineral and antinutritional factors of velvet bean flours as a result of dehulling and fermentation with *R. oligosporus* and *R. stolonifer* were investigated. The samples were subjected to solid state fermentation at 30^{0}C for 72hr. Samples were analyzed using established procedures at twelve hourly intervals during fermentation. After fermentation, samples were dried in hot air oven at 55°C for 24hr, milled and sieved appropriately. Vitamins A, B^2 and niacin were found to have increased in undehulled samples. Vitamins and niacin content of dehulled samples were observed to have reduced when compared with corresponding values in undehulled samples. General increase, significant at P<0.05, was observed in the quantities of minerals in the flour samples (calcium, iron, potassium, magnesium and phosphorous) in both undehulled and dehulled samples. The levels of anti nutritional factors trypsin inhibitor, phytic acid and oligosaccharides, decreased significantly in all the samples. The result of this study suggests that the fermentation process could be a viable option in the detoxification of velvet beans and also effective in improving its nutritional status and can also be explored as a good diet-enrichment method especially among economically weaker sections of peoples in developing countries.

Keywords: Fermentation, vitamin, mineral, antinutrient, velvet bean, Rhizopus species

GM/06

Optimization of fermentation process for Korean blueberry by various strains of probiotic lactic acid bacteria

Hyo-Nam Song[1]*, Myung-Soo Park[1], Ho-Sik Youn[2], Jae-Jung Park[2] and Christer Hogstrand[3]

[1]Department of Oriental Medical Food and Nutrition, Semyung University, Jecheon, 390-711, Republic of Korea

[2]WellRun B&F, Cheonan, 330-872, Republic of Korea

[3]Diabetes and Nutritional Sciences, King's College London, SE1 9NH, United Kingdom

Blueberries contain bioactive compounds with potential health benefits. The objectives of this study were to evaluate the antidiabetic characteristics of blueberries cultivated in South Korea and to optimise the fermentation process by different strains of lactic acid bacteria to maximise antidiabetic properties. Approximate composition, mineral and free sugar contents, free amino acid and free fatty acid compositions of raw Korean blueberries were determined. Juices from the blueberry homogenates were fermented by inoculation of seven strains of *Lactobacillus spp.; L. brevis, L. fermentum, L. plantarum, L. salivarius salicinius, L. rhamnosu, L. paracasei, L. acidophilus* and another two of *Leuconostoc mesenteroides* and *Streptococcus thermophiles.* Various fermentation conditions were investigated with 0.5, 1, 3, 5, 10% of inoculum for 1, 3, 5, 7, 10, 15 days at 37°C. Growth rates, pH and total polyphenol contents were measured. Antioxidant activity was determined by the free radical scavenging activity with 2,2-diphenyl-1-picrylhydrazyl and the changes of trans-resveratrol contents were analyzed by HPLC. *L. plantarum* produced the highest level of *trans*-resveratrol and revealed the highest DPPH radical scavenging activity through fermentation of 3%, 3 days at 37^{0}C. Fermented blueberry is expected to be a good natural source of *trans*-resveratrol and other antidiabetic compounds.

GM/07

Bacterial microcompartments in heterotrophic bacteria are non-uniform polyhedra resembling Johnson solids

Mingzhi Liang[1], Martin Warren[2], Alasdair McDowall[3], Grant Jensen[3], Sukanta Bag[4], Kingshuk Choudhury[4], Mike Prentice[1]

[1]University College Cork, Cork, Ireland, [2]University of Kent, Canterbury, UK, 3California Institute of Technology, Pasadena, USA,

Please note: Abstracts are published as received from the authors and are not subject to editing.

4Duke University, Durham, USA

Bacterial microcompartments (BMC) are intracellular polyhedral structures specified by a chromosomal operon. Carboxysomes, the best characterised BMC, are found in cyanobacteria and chemoautotrophs, facilitating carbon fixation by concentrating RuBisCo. BMC of similar appearance are also present in heterotrophic bacteria, where they contain a variety of enzymes. Carboxysomes extracted and purified by gradient density centrifugation are thin-shelled structures about 100 nm in diameter thought to be icosahedral on electron cryotomography (ECT). This may be an extraction artefact as intracellular carboxysomes are not icosahedral on ECT. ECT of bacterial cytoplasm is subject to technical limitations based on cell size. By using cell wall-directed antibiotic treatment we obtained small and fragile *E.coli* cells expressing recombinant functional microcompartments imaged with satisfactory resolution by ECT. Tomograms showed both intracellular and free microcompartments are of variable size and shape, and did not show icosahedral symmetry. Most resembled deformed Johnson solids, non-uniform polyhedra with a larger surface area:volume ratio than icosahedra. Although having more structural components and constituent enzymes than carboxysomes, the BMC show similar tight packing of contents and regular interior organization. Cytoplasmic filaments were found located close to the cell membrane, suggesting that there is an apparent relationship between BMC and the bacterial cytoskeleton.

GM/08

The role of host cell ion channel function during the human respiratory syncytial virus lifecycle

Hasa Taqi[1], Julian Hiscox[2], John Barr[1], Jamel Mankouri[1]

[1]University of Leeds, Leeds, UK, [2]University of Liverpool, Liverpool, UK

Infection with human respiratory syncytial virus (HRSV) causes both acute and chronic respiratory problems in children and the immunosuppressed. There is currently no HRSV vaccine, although prophylactic immunotherapy offers protection to at risk individuals. However, treatment is expensive and incompletely protective.
Ion channels are pore-forming proteins that regulate ion homeostasis across membranes in cells, acting as signaling proteins that regulate many aspects of cell physiology from cell cycle progression to apoptosis and gene transcription. Since ion channels play a key role in many aspects of lung cell physiology, we investigated their involvement during HRSV infection.
Using ion channel modulating drugs we showed that K^+ channel blockers reduced both HRSV growth and viral protein expression, suggesting K^+ activity is required during HRSV infection. Conversely, the inhibition of voltage gated Na+ channels with tetrodotoxin and Lidocaine led to enhanced levels of infection, and increased HRSV protein expression, suggesting Na^+ channel inhibition acted to promote HRSV infectivity.
This study demonstrates a dynamic role of specific ion channel families in the HRSV lifecycle. This may pave the way for future studies highlighting ion channels as druggable targets for a repertoire of lung-associated virus infections.

GM/09

Investigating the role of rhomboid proteases in *Dictyostelium discoideum*

Mehak Rafiq[1], David Traynor[2], Elinor Thompson[1]

[1]University of Greenwich, Kent, UK, [2]MRC LMB, Cambridge, UK

Rhomboid intramembrane proteases are conserved across evolution. Although they cleave disparate substrates, their location does mean that their proteolysis of membrane-tethered preproteins produces a common role in signalling or activation by cleavage. In the apicomplexa, rhomboid proteases facilitate infection: the bacterium *Providencia's* rhomboid activates a transport channel protein, facilitating quorum sensing. The rhomboids of the social amoeba *Dictyostelium discoideum* have not been explored. This biomedical model organism is useful in the study of eukaryotic regulatory pathways, having many common genetic networks with higher eukaryote cells, uni- and multicellular growth phases, phagocytosing bacterial prey, and being motile and chemotactic. A small group including four apparently enzymatically-active rhomboids was identified in *Dictyostelium*. We found that development was unaltered following deletion of *rhmC* whereas attempts to knockout the putative mitochondrial *rhmD* proved lethal. *rhmA*- and *rhmB*-null mutants give rise to changes in development, *rhmA*- having an altered response to chemoattractants and demonstrating decreased motility. rhmB-null cells have lower viability, smaller spore size and a decreased response to cAMP. These results correspond with RTPCR analysis, in which *rhmA* and *B* transcript levels are highest during chemotaxis-led aggregation of cells to the multicellular growth phase. Preliminary TEM intriguingly suggests a possible mitochondrial role for RhmA.

GM/11

Diversity of Indigenous Yeast in a Traditional Irish Cider Fermentation

Sinead O' Loughlin[1], Aedin O'Brien[1], Eoin Moynihan[1], Erinn Quinn[1], Marine Börlin[2], Isabelle Masneuf-Pomarede[2], John Morrissey[1]

[1]University College Cork, Cork, Ireland, [2]USC Oenologie-INRA, Université Bordeaux Segalen, Villenave d'Ornon, France

Natural yeast diversity contributes to the complexity of flavours found in traditional and craft cider. Identification and study of these yeasts could lead to discovery of new strains with biotechnological relevance. Flavours conventionally differ between commercial and traditional craft cider. Traditional fermentation relies on natural microbiota to drive the spontaneous fermentation of apple juice, whereas commercial companies replace/out-compete this diversity by introducing strains of *Saccharomyces bayanus/uvarum/cerevisae*. This investigation was undertaken to identify the yeast diversity associated with a traditional Irish fermentation using culture based methods and molecular identification. Yeasts were cultured over the course of fermentation and a number of yeasts were identified at each time point by sequencing the D1/D2 domain of the 26S rRNA gene. Results revealed a range of non-*Saccharomyces* species present at the early stages. Further study of early stage diversity should be undertaken, as some species may play a role in the fermentation process/contribute traits to the final product. From Day 6 onwards, an isolate of *S. uvarum* dominates. Using microsatellite markers to compare this strain to other *S.uvarum*, it was established this is a novel strain related to others previously recovered from apple and pear fermentation. Future work will further characterize this strain.

GM/12

Poly-gamma-glutamic acid (γ-PGA) - a promising biosorbent for removal of heavy metals

Adetoro Ogunleye, Iza Radecka, David Hill, Craig Williams

University of Wolverhampton, Wolverhampton, West-midlands, UK

Poly-gamma-glutamic acid (γ-PGA), an unusual natural anionic biopolymer composed of D- and/or L-glutamic acid units polymerized through amide linkages between α-amino acid and γ-carboxylic acid groups, was synthesized by three bacterial strains - Bacillus subtilis (natto), Bacillus licheniformis 9945a and Bacillus licheniformis 9945. Three culture media - one containing glycerol, citric acid and L-glutamic acid as carbon sources, another, having citric acid, sucrose and L-glutamic acid as carbon sources and the third one with sucrose and L-glutamic acid as its sources of carbon were used in this study. Each strain produced γ-PGA extracellularly when grown aerobically in one or all three media. The biopolymers produced were identified as γ-PGA by Fourier transform infrared spectroscopy (FTIR). The effects of different fermentation temperatures (37°C and 50°C) and media on bacterial growth, production and molecular weight of γ-PGA were investigated. The metal binding affinity of γ-PGA was also studied and it was found that it binds heavy metals. The optimal γ-PGA yield of 11.45g/l as well as the highest molecular weight of 1,650 kDa was obtained when *Bacillus subtilis* (natto) was grown aerobically at 37°C for 96 hours in a culture medium having citric acid, sucrose and L-glutamic acid as carbon sources.

GM/13

Recombinant protein production using bacterial autotransporter technology for the development of vaccines

Irene Beriotto[1,2], Yanina Sevastsyanovich[1], Ian Cadby[1], Silvana Savino[3], Mikkel Nissum[3], Giacomo Romagnoli[3], Adam Cunningham[1], Calman MacLennan[2], Ian Henderson[1]

[1]University of Birmingham, Birmingham, UK, [2]Novartis Vaccines for Global Health, Siena, Italy, [3]Novartis Vaccines and Diagnostics, Siena, Italy

Recombinant protein production (RPP) is a crucial step in the creation of biologics, nowadays used for the treatment of a wide range of diseases. *E. coli* is the preferred organism for RPP in industrial settings for its ease of manipulation, however as the protein is expressed in the cytoplasm an expensive process of purification is needed to obtain the quality required. Thus, a need for cheaper and more versatile systems has arisen.
Our laboratory has developed a method for the selective extracellular accumulation of recombinant proteins using the autotransporter secretion pathway. This technology involves the engineering of the autotransporter protein, Pet for the secretion of the protein of interest into the culture medium. Compared to other recombinant protein production systems this technology allows reduction of the quantity and diversity of process-related impurities, the number of downstream processing steps and the process development time, resulting in less expensive protein production. Here, we report the effect that different host strains and recombinant versions of an autotransporter protein have on the production of “difficult” proteins that have potential relevance to the pharmaceutical industry.

GM/14

Proteomic Analysis of *Cellvibrio japonicus*

Andrew Porter[1], Paddy Lavery[2], Lynn Dover[1], Meng Zhang[1], Gary Black[1]

[1]Northumbria University, Newcastle Upon Tyne, UK, [2]Nonlinear Dynamics, Newcastle Upon Tyne, UK

Plants and crops contain a large proportion of hemicellulose. Hemicellulosic biomass represents a huge and relatively untapped source of organic carbon. Current processing methods for this crystalline substrate involve extreme conditions for hydrolysis; however the use of carbohydrate degrading enzymes could increase the viability and reduce the cost of the process. The analysis of the proteins associated with carbohydrate degradation and metabolism are of significance to many industries such as, food, textile and especially biorefineries. The aim of this study is to target the proteome of *Cellvibrio japonicus* UEDA 107 which has over 130 enzymes associated with carbohydrate degradation. Three methods of LC-MS Gas Phase Fractionation (GPF), 2-D Gel Electrophoresis (2DE) and 1D SDS PAGE gel slicing were employed in order to validate any potential up or down regulated candidates. Data analysis carried out with Nonlinear Dynamics Progenesis LC-MS and SameSpots software. This work has provided an insight into the growth and cellular adaptation of the *C. japonicus* to a range of biomass sources, in addition to highlighting candidates which have downstream application in the saccharification of hemicellulosic biomass.

GM/15

Mutagenesis Of *Corynebacterium Glutamicum* Through Ethyl Methanesulfonate For Enhanced Production of Glutamic Acid

Misbah Jamil

Gcu Lahore, Lahore, Pakistan

The present cogitation adduce to the melioration of *Corynebacterium glutamicum* strain WA4 for embellish glutamic acid production in 250 ml Erlenmeyer flasks. *Corynebacterium glutamicum* isolated from soil samples collected from sugar factories area in pattoki and Lahore. Bacterial strain grown on different fermentation media based on glucose urea and molasses to obtain maximum yield of glutamic acid . Chemical mutation using ethyl methane sulfonate (EMS 50-300 µ/ml) was commence for 10-60 min.The amino acids were observed both quantitatively and qualitatively. Paper chromatography was employed for qualitative analysis of amino acids. While acidic ninhydrin method (colorimetric method) was employed for quantitative analysis. The amount of glutamic acid produced by wild strain WA4 in molasses based medium was 0.8g/litre, 2.5g/litre, 3.3g/litre, 2.7g/litre at 24 ,48 ,72 and 96 hours incubation under optimum condition respectively in media .Which is enhanced by EMS mutant in the same medium was 8.2g/litre, 8.4g/litre, 8.7g/litre, 9.7g/litre . The objective of this work is to explicate a cogent high yielding , feedback insensitive mutant strain and optimization of its medium pH for maximum production of glutamic acid .

Please note: Abstracts are published as received from the authors and are not subject to editing.

GM/16

Mechanisms of MRSA and MSSA interaction with Acanthamoeba and application of photodynamic therapy

Saleh Alghamdi

University of Essex, UK

Acanthamoeba feed on Gram positive bacteria such as *Staphylococcus aureus* (MSSA) and the multi-drug resistant MRSA which facilitates their spread and disease. This suggests that amoebae and bacteria are involved in complex interactions, which plays important roles in the environment and human health.The first aim of this investigation is to study the molecular mechanisms of this interaction and the second is to find alternative methods to target MRSA using photodynamic therapy (PDT).Clinical isolates of *Acanthamoeba castellanii,* MRSA and MSSA were used here. *Acanthamoeba* was grown in 24-well plates and live bacteria were added for one hr. This assay is based on utilizing the molecular oxygen in the biological system with photosensitising drug (m-Tetra hydroxyphenyl chlorine), and a suitable light, producing reactive oxygen species (ROS). To elucidate the mechanisms of *Acanthamoeba* phagocytosis, several inhibitors including, LY294002, Genistein, and sodium orthovanadate were used. Our findings revealed that all inhibitors significantly reduced *Acanthamoeba* phagocytosis. The PDT had significant positive effect on the survival of bacteria and reduces the MIC to less than 1µg/ml. Additionally, PDT demonstrated high efficacy on *Acanthamoeba* survival. In conclusion, the PDT is a promising methodology not only for tumour treatment but could be also for infections.

GM/17

The Protective effect of bacterial derived polymer Gamma polyglutamic acid (γ-PGA) on bacteriophages

Ibrahim Khalil

University of Wolverhamptom, UK

The emergence of resistant bacteria to many available antibiotics and disinfectants, has made the development of alternatives, or supplementary treatments to antibiotics and disinfectants one of the important priorities that concerns modern medicine. The ability of bacteriophages to persist in harsh environmental conditions for extended periods is limited by various factors. Formulations that increase the longevity of phages in such environments are required to improve their viability. The aim of our research was to develop a protective formulation that increases the life span of bacteriophages under adverse environmental conditions. Bacteriophages formulated with 1% of the bacterial derived polymer poly-gamma-glutamic acid (γ-PGA) in this study showed significant increase in their survival rate, as compared to non-treated bacteriophages after the exposure to extreme environmental conditions over period of time, such as heat at 50, 60 and 70° C. Also the viability of treated bacteriophages and non-treated bacteriophages showed significant difference, when evaluated at different pH such as, 11, 7, 4 and ~ 3. Furthermore, UV light known to have a dramatically affect on phages yet the treated phage can survive longer after the exposure to 245 nm UV radiation, where as the non-treated bacteriophages were clearly affected. γ-PGA is a biodegradable, non toxic and non immunogenic polymer. The obtained results and the unique characteristics of the used polymer could open the door for a new gene therapy or anticancer therapy. This smart polymer might also capable to protect other useful viruses such as those used in experimental cancer therapies, for instance, adenoviruses, in the same manner of protecting bacteriophages.

GM/29

The Effect of Biotic and Abiotic Factors on Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Bacteria in Soil

Zohreh Khorasanizadeh

University of Hertfordshire, UK

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental contaminants with two or more aromatic rings and originating from different emission sources. They are extremely toxic to human, animals and plants. Consequently, the need to expand economical and practical remediation technologies for PAH contaminated sites is evident. The degradation was studied on the key model PAH (phenanthrene, anthracene, fluoranthene and pyrene) in J. Arthur Bower's top soil. The hypothesis for this study was that roadside soil would contain PAH degrading bacteria; pH would influence the microbial degradation of PAH, chemical oxidation of PAH would be as efficient as microbial breakdown of PAH and mobilising agents, would move PAH throughout soil, potentially making the PAH more available for biodegradation. The greatest degradations were found for the lowest molecular weight PAH, phenanthrene and anthracene; whilst lowest degradation was observed for higher molecular weight PAH, fluoranthene and pyrene. This study indicated microbial biodegradation was the most effective technique for removing of the PAH from contaminated soil, which was cost effective and easier to perform in comparison to the other two techniques. Microbial biodegradation could be improved by adjusting pH through liming if soil was acid.

LI02

Pseudomonas signalling, secretion and social interactions

LI02/01

Identifying novel effectors of the key second messenger cyclic di-GMP in *Pseudomonas fluorescens* SBW25

Eleftheria Trampari[1], Richard Little[1], Jacob Malone[1,2]

[1]John Innes Centre, Norwich, Norfolk, UK, [2]University of East Anglia (UEA), Norwich, Norfolk, UK

Cyclic di-GMP (c-di-GMP) is a second messenger ubiquitous in bacteria. It regulates transcription and post-transcriptional procedures as well as allosterically controlling protein function. Macroscopically, c-di-GMP action leads to biofilm formation, secondary metabolite production and the switch between motile and sessile states of living. While the mechanisms of c-di-GMP metabolism are well understood, the downstream targets of this signalling molecule are currently poorly characterised.
The aim of this project is to identify and characterise potential c-di-GMP targets in the commensal soil bacterium Pseudomonas fluorescens SBW25. To accomplish this goal, c-di-GMP capturing

experiments were conducted that led to a list of potential effectors of c-di-GMP. The most interesting targets were selected, and tested for binding using alternative techniques including capillary based radiolabelled assays, differential scanning fluorimetry and gel-shift assays. We are currently examining the biological function and regulation of several newly identified c-di-GMP binding proteins.

LI02/02

Elucidating the role of the transcriptional regulator RccR from *Pseudomonas fluorescens* in rhizosphere colonisation

Rowena Fung[1,2], **Richard Little**[1], **Jacob Malone**[1,2]

[1]John Innes Centre, Norwich, UK, [2]University of East Anglia, Norwich, UK

Pseudomonas fluorescens SBW25 is a soil dwelling bacterium that suppresses pathogens whilst promoting plant growth. SBW25 colonises plant roots, a phenotype directly related to the effectiveness of plant-growth promotion and biocontrol. The second messenger cyclic-di-GMP is a signalling molecule that affects bacterial processes including the switch between sessile and motile lifestyles. Our lab is interested in the role cyclic-di-GMP has in controlling rhizosphere colonisation.
RccA, a predicted cyclic-di-GMP signalling protein, has previously been identified as being up-regulated in the rhizosphere. Immediately downstream of *rccA* is *rccR*, a transcriptional regulator consisting of sugar isomerase and DNA binding domains. We have demonstrated that *ΔrccA* and *ΔrccR* have root colonisation deficiencies compared to SBW25. To further characterise RccR, we purified the His-tagged protein by FPLC. Subsequent EMSA analysis revealed that *RccR* binds to its own promoter, confirming transcriptional fusion assays that show negative autoregulation by RccR.
C-di-GMP is known to affect bacterial motility, prompting the investigation of whether swarming motility of *rccA* or *rccR* mutants and over-expression strains was affected. On KB swarm agar, the *rccA* mutant has a markedly different phenotype to SBW25; however, wild-type swarming is restored in an *rccAR* mutant, suggesting that *rccA* and *rccR* are genetically linked.

LI02/03

Characterization of class 1 integrons in multidrug resistant *Pseudomonas aeruginosa* isolated from diarrhoeic cattle in Egypt

Helmy Torky, **Adel M. Khadr**

Faculty Of Vet. Medicine, Alexandria, Egypt

Pseudomonas aeruginosa infections are normally difficult to eraddicate due to acquired resistance to many antibiotics. Although *P.aeruginosa* is not generally considered a common cause of infectious diarrhea in animals,it was the predominant organism isolated from the faeces of diarrhoeic cattle in a farm in Egypt.Integrons are potentially mobile DNA elemants which comprise a site-specific recombination system capable of capturing and expressing antibiotic resistance genes. A total of 14 clonally unrelated multidrug-resistant *P. aeruginosa* strains were isolated and identified. PCR and DNA sequencing were used for screening and characterization of class 1 integrons and their antibiotic resistance genes. Six P. aeruginosa isolates were positive for class 1 integrons,of which 3 isolates harbored antibiotic resistance gene cassettes.
The identified resistance genes within class 1 integrons were aminoglycosides adenyltransferase type A, *aadA1,* which confers resistance to streptomycin and
spectinomycin and dihydrofolate redutase type A, *dfrA1*, *dfrA5* and *dfrA17,* which confer resistance to trimethoprim. To the best of our knowledge, this is the first report of class
1 integrons in p .aeruginosa isolated from diarrhea in animals

LI02/04

Pseudomonas aeruginosa quorum sensing communication with macrophages - the role of aquaporins

Angelika Holm, Joanna Gradin, Karl-Eric Magnusson, Elena Vikström

Division of Medical Microbiology, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

Existing in biofilm communities, *Pseudomonas aeruginosa* regulate multiple virulence factors and biofilm maturation by the quorum sensing (QS) system and cause pulmonary, urinary tract, burn and wound infections. During infection and inflammation, water homeostasis is important as water channels, or aquaporins (AQP) regulate human cell shape, volume, motility and migration in tissues. We aimed to investigate how *P.aeruginosa* QS communication affects phagocytic activity of human primary macrophages, its cell volume, AQP9 expression and distribution. Macrophages were infected with *P.aeruginosa* PAO1 wild-type strain and its lasI-/ *rhlI*- mutant (lacking production of QS molecules 3O-C12-HSL and C4-HSL) at multiplicities of infection 1, 10 and 100. We observed a significant increase in phagocytic capacity of macrophages infected with wild-type bacteria compared to those infected with the mutant. Using live cell imaging, we found that treatment of macrophages with 50 µM 3O-C12-HSL resulted in a rapid and prolonged cell-volume increase. Furthermore, with quantitative PCR, immunoblot, classical confocal and advanced high-resolution fluorescence microscopy we demonstrated, that AQP9 mRNA expression and protein distribution were affected in 3O-C12-HSL-treated macrophages. Thus, we recognize the importance of water homeostasis and AQPs during bacterial - human cell communication, but the details of the cellular mechanisms remain to be further elucidated.

LI02/05

Transcriptional regulation by molecular interaction of two different DNA-bound regulators

Abdelali Daddaoua[1], **Tino Krell**[1], **Juan Luis Ramos**[2]

[1]Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas, Granada, Spain, [2]Department of Biotechnology, Abengoa Research, Sevilla, Spain

The transcriptional control of glucose metabolism and synthesis of exotoxine A, the major virulence factor of *Pseudomonas aeruginosa,* is tightly interwoven. Central to this concerted regulation is a pair of transcriptional regulators that belong to different families, namely PtxR (LysR family) and PtxS (LacI family). We have now deciphered the molecular mechanism of the concerted PtxR/PtxS action (1, 2). In the case of the glucose dehydrogenase promoter Pg*ad* PtxR

Please note: Abstracts are published as received from the authors and are not subject to editing.

and PtxS bind to their respective operator sites that are some 50 bp apart. An interaction of both DNA-bound regulators creates a pronounced DNA loop that prevents promoter binding of the RNA polymerase. Using site-directed mutagenesis key residues in this protein-protein interaction were identified. The dissociation of this protein complex and consequently the reversal of DNA loop formation is induced by the specific binding of 2-ketogluconate to PtxS, which is an intermediate of the glucose metabolism. This study reveals part of the molecular detail that connects the regulation of bacterial virulence and glucose metabolism. The molecular interaction between two different, DNA bound regulator proteins corresponds to a novel mechanism in transcriptional regulation.

1. Daddaoua *et al*. (2014) N*ucleic Acids Res*; 41(22):10150-6
2. Daddaoua *et al*. (2012) *PLOS One;* e39390

LI02/06

Linking fatty acid-mediated signalling with genetic regulatory networks in *Pseudomonas aeruginosa*

Diana Palenzuela, Ye Chen, Paul Williams, Stephan Heeb, Miguel Cámara

School of Life Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham, UK

*Pseudomonas aeruginosa i*s an ubiquitous organism which can adapt to a wide range of environments using an intricate network of regulatory circuits. These include quorum sensing and two-component systems which exploit the production and sensing of a variety of small signalling molecules. One of these is cis-2-decenoic acid (CDA), belonging to the family of Diffusible Signal Factors (DSF) produced by many Gram-negative organisms. This molecule has an important role in biofilm dispersal in *P. aeruginosa* and can also disperse biofilms of other species such as *Staphylococcus aureus,* although the overall role of CDA in the biology of *P. aeruginosa* remains unclear. To further investigate this, we performed RNA sequencing analysis of *P. aeruginosa* grown in the presence and absence of CDA and we found that the mRNA levels of genes responsible for multiple traits were altered. Additionally, CDA repressed the transcription of the two small regulatory RNAs, RsmZ and RsmY, and it also impacted on different quorum sensing systems. We are currently investigating the mechanisms by which CDA regulates some of the RNAs identified with a view to gain a better understanding of the role played by this signal molecule in the adaptation of *P. aeruginosa* to different environments.

LI02/07

Genes encoding CheR-TPR fusion proteins are predominantly found in gene clusters encoding chemosensory pathways with alternative cellular functions

Míriam Rico Jiménez[1], Francisco Muñoz Martínez[1], Cristina García Fontana[1], Carlos Alfonso[2], Tino Krell[1]

[1]Estacion Experimental del Zaidin, CSIC, Granada, Spain, [2]Centro de Investigaciones Biológicas, CSIC, Madrid, Spain

Chemosensory pathways are major signal transduction mechanisms that either mediate motility or possess alternative cellular functions (ACF). CheR methyltransferases are core enzymes of chemosensory pathways. Some CheRs are fused to tetratricopeptide repeat (TPR) domains, which are typically involved in ligand binding. To cast light into the relevance of these fusion proteins, we analyzed this uncharacterized family. CheR-TPRs are widely distributed in GRAM-negative bacteria. Most strains contain a single CheR-TPR and its abundance does not correlate with the number of chemoreceptors. The majority of CheR-TPR genes were found in gene clusters that harbor multidomain response regulators in which the REC domain is fused to different output domains. Since the presence of multidomain response regulators is a distinctive feature of the ACF class of pathways, we conclude that CheR-TPR proteins are another characteristic feature of this pathway class. We characterized WspC of *Pseudomonas putida* as a representative example of a CheR-TPR fusion. The affinities of WspC-Pp for S-adenosylmethionine and S-adenosylhomocysteine were comparable to those of prototypal CheR. The removal of the TPR domain did not impact significantly on the binding constants. WspC-Pp was found to be monomeric, ruling out a role of the TPR domain in self association.

LI02/08

The effect of manuka honey treatment on iron acquisition and quorum sensing in *Pseudomonas aeruginosa*

Michael Pascoe, Sarah Maddocks

Cardiff Metropolitan University, Cardiff, UK

Pseudomonas aeruginosa infects burn wounds where it grows as a biofilm, being very difficult to treat. Within the host environment microorganisms must acquire vital nutrients, such as iron, for survival and co-ordinates this via the process of quorum sensing.   In *P. aeruginosa* iron acquisition via the production of siderophores and quorum sensing is intrinsically linked and both play a fundamental role in virulence.

Manuka honey is a broad spectrum, topical antimicrobial. It is bactericidal against *P. aeruginosa* and affects the expression of multiple genes, including a number of virulence factors. This study aimed to ascertain whether manuka honey effected the expression of iron acquisition and quorum sensing when applied to *P. aeruginosa* at sub-lethal concentrations.

The chrome azurol assay was used to show that manuka honey treatment mediated a reduction in siderophore production. However, RT-PCR showed that this was not correlated with a reduction in the expression of *pvdQ,* which is involved in biosynthesis of the siderophore, pyoverdin. Two quorum sensing genes, *mvfR* and *rhlR* were also investigated by RT-PCR but manuka honey treatment did not alter their expression either, despite evidence that has previously shown that honeys can inhibit quorum sensing; it is possible that other targets are involved.

LI02/09

Interactions of microbes during chronic lung infection in cystic fibrosis patients

Joanne Fothergill[1], Raffaele de Leon[2], Jake Bundy[2], Martin Walshaw[3], Craig Winstanley[1]

[1]Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [2]Faculty of Medicine, Department of Surgery & Cancer, Imperial College, London, UK, [3]Liverpool Heart and Chest Hospital, Liverpool, UK

Bacteria in chronic infections can form complex, interacting

communities. These communities diversify due to interactions with both the environment and each other. The Cystic Fibrosis (CF) lung facilitates the cohabitation of microbes and we are just beginning to understand the extent of this diversity. We aimed to study the community structure and interspecies interactions by using both an *in vitro* model and deep sequencing of patient samples.
We have developed co-culture and multispecies biofilm models in which microbial interactions can be investigated. Using an artificial sputum medium, pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia sp* have been studied to determine whether interspecies interactions facilitate the virulence of *P. aeruginosa*. In addition, metabolic footprinting of these cultures was used to determine changes in small-molecule metabolites. To further characterise the CF lung microbiome, we have collected longitudinal sputum samples from adult CF patients and sequenced 16S rRNA sequences. This enables the microbial community in each sample to be identified and compared to determine whether additional factors influence the microbial composition of the lung. Considerable differences in the microbial population could be found between samples.
Understanding complex interactions may uncover novel therapeutic targets and ultimately lead to altered CF patient management.

LI02/10

Differential and temperature-dependent riboregulation by RsmA and RsmN in *Pseudomonas aeruginosa*

Hazel Silistre, Laura Lovelock, Marco Messina, Jonas Emsley, Mark Searle, Miguel Cámara, Stephan Heeb, Paul Williams

University of Nottingham, Nottingham, UK

Gene regulation in bacteria occurs transcriptionally, e.g. through quorum sensing and two-component systems, and post-transcriptionally via riboregulators. The latter includes the Rsm (regulator of secondary metabolism) system that constitutes a family of RNA-binding proteins whose action is modulated by small RNAs. In previous work, complementation of swarming deficiency in a *P. aeruginosa* rsmA mutant resulted in the discovery of RsmN, a protein similar to RsmA. Structural analysis of purified RsmN revealed conserved β-sheets, and a similar RNA-binding pocket, but also a swapped α-helix which confers it a distinct fold compared with RsmA. The highly conserved residue R62 was found to be essential for RNA binding and biological function. In this study, we further investigated the functional role of RsmN, the conditions under which it operates and the role of R62.
Production of protease, elastase and pyocyanin can be restored in a double *rsmArsmN* mutant in a temperature-dependent manner by complementation with *rsmN*. Overexpression of *rsmN* also complements several *rsmA* phenotypes such as swarming motility, protease and elastase production, whereas this is abolished with an R62A substitution.
These results show that RsmA and RsmN carry out similar functions but under different conditions, and that R62 is essential for biological function.

LI02/11

The implications of synergistic Quorum Senising in *Pseudomonas aeruginosa*

James Gurney[1], Dan Cornforth[2], Roman Popat[2], Luke McNally[2], Thom-Scott Philips[3], Sam Brown[2], Steve Diggle[1]

[1]University of Nottingham, Nottingham, UK, [2]University of Edinburgh, Edinburgh, UK, [3]Durham University, Durham, UK

Quorum Sensing (QS) research has traditionally focused on understanding mechanism, with less attention paid to evolutionary considerations of QS. Many bacterial species produce more than one QS signal. A key question evolutionary is why make multiple QS signal molecules? Do molecules have overlapping roles in gene regulation in a non-additive manner and do bacteria have a fitness benefit by using two or more signal molecules? We test these questions using the opportunistic pathogen P*seudomonas aeruginosa*, which produces two major N-acylhomoserine lactone (AHL) signals.
We show that interactions of two signal molecules at the level of gene transcription can lead to synergistic interactions, resulting in (1) drastic regulation of QS- controlled genes and (2) 14 distinct QS regulons (3) a fitness benefit in using two or more molecules. These regulons are controlled depending on the ratios of signals that bacteria encounter. We have shown that the expression of these regulons matches the expected profiles of microbial densities in different environments. Lending support to the notion that bacterium can use production of signals molecules to sense population density and the environment factors such as mass transfer. We also show that in certain environments there is a fitness benefit to having both signals.

LI02/12

A novel virulence strategy for *Pseudomonas aeruginosa* mediated by an autotransporter with arginine-specific aminopeptidase activity

Esteban Paredes-Osses[1], Jeni Luckett[1], Owen Darch[1], Chase Watters[2], Manal AbuOun[3], Victoria Wright[1], Stephan Heeb[1], Stephanie Pommier[1], Kendra Rumbaugh[2], Miguel Camara[1], Charles Laughton[1], Kim Hardie[1]

[1]University of Nottingham, Nottingham, UK, [2]Texas Tech University Health Sciences Center, Lubbock, Texas, USA, [3]Animal Health and Veterinary Laboratories Agency, Surrey, UK

Pseudomonas aeruginosa is a major cause of nosocomial infections, particularly in patients with burns and cystic fibrosis. The *P. aeruginosa* genome encodes at least four proteins displaying the characteristic three domain structure of autotransporters. Autotransporters are the principal family of secreted proteins in Gram-negative bacteria, and those characterised are virulence factors. Now, we reveal that the PA0328 autotransporter is a cell-surface tethered and arginine-specific aminopeptidase. Therefore, we have named AaaA. We demonstrate that AaaA offers a fitness advantage in environments where the sole source of nitrogen is peptides with an amino terminal arginine. This could be vital for establishing an infection as the lack of AaaA led to attenuation in a murine chronic wound infection which correlated with lower levels of some cytokines. Moreover, we show evidence that aaaA might be regulated by genes related with quorum sensing, metabolism and with the alternative sigma factor of RNA polymerase. Structural modelling has identified the putative active site of AaaA, and mutants of AaaA with single amino acid changes were created. We

Please note: Abstracts are published as received from the authors and are not subject to editing.

will show how these mutants are enabling us to define the active site of AaaA thereby facilitating the screening for inhibitors that could be exploited as potential therapeutic agents.

LI02/13

An LmbE-like putative deacetylase contributes to fluoroquinolone tolerance in *Pseudomonas aeruginosa*

Veerle Liebens[1], Valerie Defraine[1], Annelies Van der Leyden[1], Valerie De Groote[1], Carolina Fierro[1,2], Serge Beullens[1], Natalie Verstraeten[1], Cyrielle Kint[1], Ann Jans[1], Kathleen Marchal[1,2], Wim Versées[3], Maarten Fauvart[1], Jan Michiels[1]

[1]KU Leuven, Leuven, Belgium, [2]Ghent University, Ghent, Belgium, [3]Vrije Universiteit Brussel, Brussel, Belgium

A major cause of treatment failure of infections caused by *Pseudomonas aeruginosa* is the presence of persister cells. The mechanism of persister formation in *P. aeruginosa i*s largely unknown and only few genetic determinants have been linked to *P. aeruginosa* persistence. Building on a previously published high-throughput screening, we here present ndpA (N-deacetylase involved in persistence) as a new gene involved in non-inherited fluoroquinolone tolerance in *P. aeruginosa.* Fluoroquinolone tolerance of an *ndpA* mutant is strongly reduced both in planktonic culture and in a biofilm model whereas *ndpA* overexpression causes an increase of the persister fraction. In addition, susceptibility of the *ndpA* mutant to different classes of antibiotics is not affected. *ndpA* is part of the conserved LPS core oligosaccharide biosynthesis gene cluster but no difference in LPS profile could be detected upon inactivation or overexpression of ndpA. Primary sequence analysis suggests that *NdpA* acts as an LmbE-like *N*-deacetylase. Localization studies indicate that *NdpA* is an integral inner membrane protein, with the catalytic domain residing in the cytoplasm. Experiments are under way to determine whether enzymatic activity and subcellular localization are important for NdpA's role in persistence, possibly providing targets for the rational design of anti-persister therapies.

LI02/14

Cyclic-di-GMP signalling and regulation of *Pseudomonas aeruginosa* lifestyles

Martina Valentini, Joana Moscoso, Kailyn Hui, Alain Filloux

MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College London, London, UK

Bacterial physiologic behaviour can switch between a single-cell planktonic motile lifestyle and a sessile multicellular existence, i.e. biofilm. The two different lifestyles correlate in *Pseudomonas aeruginosa*, being also a pathogen, with an acute or chronic infection strategy, respectively.
At the heart of this decision making process are two main regulatory networks: the cyclic-di-GMP (c-di-GMP) signalling and the Gac/Rsm pathways.
The c-di-GMP molecule, an universal second messenger in eubacteria, regulates developmental transitions, adhesion, biofilm formation, motility and virulence. Cellular c-di-GMP levels are modulated by two classes of proteins: diguanylate cyclases and phosphodiesterases.
The Gac/Rsm pathway converges into the translational repressor RsmA and regulates *Pseudomonas* virulence, motility and quorum sensing.
We have previously shown that c-di-GMP and the Gac/Rsm pathways intersect and that both are instrumental to the switch between the two lifestyles. Particularly, we observed that c-di-GMP level increased in strains with mutations relieving from RsmA repression. By mutating genes belonging to both networks and testing the diverse phenotypes, we identified at least two diguanylate cyclases whose activity is directly linked to the Gac/Rsm pathway.
P. aeruginosa has several genes encoding diguanylate cyclases, demonstrating that some are linked to the Gac/Rsm pathway corroborates the idea of a high specificity in the c-di-GMP signalling.

LI02/15

Regulation of the turnover of ciyclic diguanylate (c-di-GMP) in *Pseudomonas putida* KT2440 via repressors of stationary phase metabolites (Rsm) proteins

Óscar Huertas-Rosales, Manuel Espinosa-Urgel, María Isabel Ramos-González

Department of Environmental Protection, Estación Experimental del Zaidín - CSIC, Granada, Spain

The second messenger c-di-GMP modulates the transition between sessile and motile lifestyles in bacteria. It is synthesized by the diguanylate cyclase activity of GGDEF domains and hydrolysed by specific phosphodiesterase activity of EAL/HD-GYP domains. In previous studies, the GGDEF/EAL domain-containing response regulator Rup4959 of *Pseudomonas putida* KT2440 was shown to increase the levels of intracellular c-di-GMP when expressed from its own promoter in multicopy, causing a pleiotropic phenotype that includes flocculation. Expression of rup4959 is RpoS-dependent and modulated by root exudates and O^2 tension.
Sequences resembling the consensus recognized by the regulators CsrA/RsmA were identified in the leader sequence of *rup*4959 mRNA and overlapping the ATG. In *P. putida,* three loci (PP1746, PP3832, PP4472) show 56%, 67% and 81% identity with Pseudomonas *aeruginosa rsmA*, respectively. We have therefore renamed them *rsm*A (PP4472), *rsm*E (PP3832; based on *Pseudomonas* fluorescens nomenclature) and rsmI (PP1746).
To analyze the implication of these rsmA homologues in the regulation of rup4959 expression, null mutants in each gene, double mutants and a triple mutant were constructed. Expression of a $P^{rup4959}$::´lacZ fusion was significantly higher in the mutants than in the wild type at the beginning of stationary phase. Consistently, flocculation was accelerated and enhanced in the mutants

LI02/16

The lipopeptide biosurfactant viscosin plays important roles for both attachment and detachment of *Pseudomonas fluorescens* SBW25 biofilms

Lise Bonnichsen[1], Nanna Svenningsen[1], Tim Tolker-Nielsen[2], Ole Nybroe[1]

[1]Section of Genetics and Microbiology, Faculty of Science, University of Copenhagen, Copenhagen, Denmark, [2]Department of International

Health, Immunology and Microbiology, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark

Pseudomonas lipopeptide biosurfactants play important roles for biofilm formation. For example, mutant strains of *P. fluorescens* SBW25 lacking viscosin biosurfactant production are reported to be impaired in biofilm formation. However, the detailed role of viscosin for biofilm development and architecture has not been clarified, and the aim of this study was to provide detailed information on the role of viscosin for SBW25 biofilm formation and detachment. The SBW25 wild type (wt) initially formed more biofilm in microtiter wells than a viscosin-negative *visc*A mutant strain, while the mutant formed substantially more biofilm after extended incubation. The dynamics in biofilm architecture was studied for *gfp*-tagged wt and *visc*A mutant strains in flow chamber systems. A quantitative analysis showed that the wt formed more developed microcolonies than the mutant in the initial state of biofilm formation (day 1). However, in mature biofilms (day 2-5), the mutant maintained a thick biofilm whereas the wt biofilms started to detach on day 3. We speculate that viscosin initially play a role in adhesion of planktonic cells and microcolony formation, and later is involved in detachment of cells from mature biofilms. We are currently exploring the dynamics of viscosin production by a mCherry based viscA bioreporter strain.

LI02/17

Defining the impact of clinically-relevant PhoQ and PmrB mutations on the polymyxin resistance, virulence and quorum sensing activity of *Pseudomonas aeruginosa*

Matthew T. Robinson[1], Matthew Fletcher[2], Paul Williams[2], Steve L. Porter[1], Alan R. Brown[1]

[1]Biosciences, University of Exeter, Exeter, UK, [2]Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK

Resistance to polymyxin antibiotics within clinical isolates of *Pseudomonas aeruginosa* is commonly associated with mutations within PhoPQ and/or PmrAB two-component systems (TCSs) which regulate the expression of the aminoarabinose biosynthetic pathway. Using isogenic strains differing only by the presence of clinically-relevant mutations in *phoQ* (*phoQ*[V260G]) and *pmrB* (*pmr*B[M292T]), we assessed the extent to which these alleles impact on virulence and polymyxin resistance. Both mutant alleles individually conferred intermediate- to low-level polymyxin resistance, consistent with reports that second-step mutations are required for high-level resistance. Both mutant alleles resulted in reduced cytotoxicity and attenuated virulence in the *Galleria mellonella* infection model, whilst the *pho*Q[V260G] allele was associated with reduced biofilm formation. These phenotypes are comparable to those reported in late-stage *P. aeruginosa* isolates from the cystic fibrosis lung, suggesting that the phenotypic adaptations observed in chronic isolates may in part reflect emergence of phoQ and pmrB mutant alleles. Both mutant alleles were also associated with altered activity of the alkyl quinolone (AQ)-based quorum sensing system. The nature of the interaction between the PhoPQ/PmrAB TCSs and the AQ system is the subject of ongoing studies, as is the extent to which altered AQ activity contributes to the observed phenotypes.

LI02/18

The impact of *Pseudomonas aeruginosa* lectin LecB on signaling processes during bacterial cell internalization

Katja Kühn, Winfried Römer

BIOSS -Center for Biological Signaling Studies, Freiburg, Germany

P. aeruginosa is a major opportunistic and nosocomial pathogen causing severe infections of the pulmonary tract with a particular risk for cystic fibrosis patients.

As well, antiobiotic resistance becomes an increasing problem; hence, it is of major interest to identify signaling processes as well as proteins and lipids, which are crucial for the infection and represent potential drug targets for therapy.

Even if *P.aeruginosa* generally is an extracellular bacterium, the endocytic uptake and the attachment to host cells are important pathogenicity factors to evade the immune response and to transmigrate cells reaching other target organs and the blood stream. However, the distinct signaling mechanisms and molecules controlling these processes are not yet described.

Previously described virulence factors of *P. aeruginosa* are lectins, carbohydrate-binding proteins, which are supposed to contribute to attachment during infection and to biofilm formation (Tielker et al. 2005). It is of special interest to investigate if the lectins also have an impact on host signaling pathways acting not only as attachment factor but also in the induction of cellular signaling cascades.

LI02/19

A Drug Repositioning Screen Identified Pentetic Acid as A Potential Therapeutic Agent to Suppress Elastase-mediated Virulence of *Pseudomonas aeruginosa*

Mia Gi[1], JaeYoung Choi[1], Junhui Jeong[1], Kang-Mu Lee[2], Yongjin ParK[2], Keehoon Lee[2], Sang Sun Yoon[2]

[1]Department of Otorhinolaryngology Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Republic of Korea, [2]Department of Microbiology and Immunology, Brain Korea 21 PLUS Project for Medical Science, Yonsei University, Seoul, Republic of Korea

Pseudomonas aeruginosa, a Gram-negative bacterium of clinical significance, produces elastase as a predominant exotoxin. Here, we screened a library of chemical compounds currently used for human medication and identified Diethylene Triamine Pentaacetic Acid (DTPA, Pentetic acid) as an agent to suppress the production of elastase. Elastase activity found in the prototype P. aeruginosa strain PAO1 was significantly decreased, when grown with as low as 20 μM DTPA. Supplementation with Zn^{2+} or Ca^{2+} ions restored the suppressive effect of DTPA suggesting that DTPA-mediated decrease in elastase activity is associated with its ion-chelating activity. In DTPA-treated PAO1, transcription of elastase-encoding lasB gene and the level of pseudomonas quinolone signal (PQS), a molecule that mediates *P. aeruginosa* quorum sensing (QS), were significantly downregulated, proposing a potential involvement of PQS QS system in the DTPA-mediated elastase suppression. Biofilm formation was also decreased by DTPA treatment. When A549 airway epithelial cells were infected with PAO1 in the presence of DTPA, viability of A549 cells was substantially increased. Furthermore, intranasal delivery of DTPA to the PAO1-infected mice alleviated pathogenic

Please note: Abstracts are published as received from the authors and are not subject to editing.

effects of PAO1 in animals. Together, our results uncovered a novel function of an old molecule, application of which may help treat *P. aeruginosa* airway infection.

LI02/20

Pseudomonas alkylquinolone signal molecules: an interkingdom dimension

F. Jerry Reen, John Phelan, Lorna Gallagher, Claire Adams, Fergal O'Gara

University College Cork, Cork, Ireland

Pseudomonas aeruginosa is a highly adaptable organism, capable of colonising a wide variety of niches including burn wounds and immunocompromised patients and it is the main pathogen associated with morbidity and mortality in Cystic Fibrosis patients. *P. aeruginosa* produces >50 alkylquinolones (AQs) that differ structurally on the basis of substitution at the C3 position, N-oxide substitution of the quinolone nitrogen and modification of the alkyl side chain. While several alkylquinolones have been characterised as antibiotics, a role as QS signal molecules in cell-cell communication has been revealed for 2-heptyl-3-hydroxy-4-quinolone [*Pseudomonas* quinolone signal (PQS)] and its immediate precursor 2-heptyl-4-quinolone (HHQ). Research in the BIOMERIT Research Centre has revealed an interkingdom dimension to the bioactivity of HHQ/PQS, modulating the behaviour of other bacterial and fungal species. Phenotypes such as motility, biofilm formation and growth were affected in a broad spectrum of bacterial species, while HHQ was found to suppress biofilm formation of the co-colonising yeast pathogen *Candida albicans*. As the structural properties underlying this AQ interkingdom phenomenon remain poorly understood, a suite of structural analogues have been generated. These will enable us to delineate and assign phenotypes to key functional groups present on the basic quinolone framework.

LI02/21

Sticky but not so sweet: The Interaction of *Pseudomonas aeruginosa* with Mucus, Mucin and Glycans

Patrick Moore[1], Julie Naughton[1], Valerie Urbach[2], Paul McNally[2], Michelle Kilcoyne[3], Lokesh Joshi[3], Stephen Carrington[4], Marguerite Clyne[1]

[1]The School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, [2]The National Children's Research Centre, Crumlin, Dublin 12, Ireland, [3]Glycoscience Group, National University of Ireland, Galway, Ireland, [4]Veterinary Science Centre, University College Dublin, Dublin, Ireland

Pseudomonas aeruginosa colonises mucus in the lungs of individuals with cystic fibrosis (CF) and interacts with glycans present on mucins and on the host cell surface. Neo-glycoconjugate arrays were probed with *P. aeruginosa* strain PAO1 and strains isolated from CF patients in order to identify potential inhibitors of initial colonization. The interaction of the organism with mucus secreting cells and with purified mucins from humans and animals using a novel mucin microarray platform was also assessed. Strong binding of CF *P. aeruginosa i*solates and strain PAO1 to mannose, glucose, galactose, fucose and to the blood group antigens Lewisx, Lewisb, and H2 was detected. Differences in binding to sialyl Lewisx, Lewisy and 3' and 6' sialyl lactose was observed between strains. Similar binding profiles were seen for strains grown to log and stationary phase. The strength of the interaction of *P. aeruginosa* with mucins was not as strong as that seen with individual glycans. Infection assays suggest that *P. aeruginosa* displays a predilection for mucus on the surface of cells and aggregates within the mucus. These results suggest that glycans present on the host cell surface and mucins are potential inhibitors of initial colonization by *P. aeruginosa* in the CF lung.

LI02/22

Unravelling the role of the small RNA-binding regulatory protein RsmA in the post-transcriptional regulation of the blue copper redox protein Azurin production in *Pseudomonas aeruginosa*

Jean Dubern[1], Birgit Koch[1,2], Natalio Krasnogor[2], Tuval Ben-Yehezkel[3], Ehud Shapiro[3], Miguel Cámara[1], Stephan Heeb[1]

[1]Centre for Biomolecular Sciences, School of Life Sciences, University of Nottingham, Nottingham, UK, [2]School of Computer sciences, University of Newcastle, Newcastle, UK, [3]Department of Biological Chemistry and Department of Computer Science and Applied Mathematics, The Weizmann Institute of Science, Rehovot, Israel

Azurin is a small copper redox protein synthetised by the opportunistic human pathogen *Pseudomonas aeruginosa* and some other bacteria as a secreted factor able to induce macrophage apoptosis, although its precise function remains unknown. Azurin production is positively controlled at the post-transcriptional level by the small RNA-binding protein RsmA. This regulation is not exerted upstream of the azu start codon and cannot be exerted downstream of its rho-independent transcriptional terminator. We hypothesised that RsmA enhanced the stability of the *azu* mRNA by binding to it, increasing translation yields. Computer analysis suggests that the *azu* mRNA contains three potential RsmA binding sites. To investigate this, we introduced a combination of silent mutations within the *azu* gene which change the predicted RsmA binding sites without altering the encoded protein sequence. This was achieved with a synthetic biology approach based on DNA fragment recycling by which a library of 27 combinatorial variants has been constructed. This library has been introduced into an rsmA conditional mutant having an Δazu deletion. The levels of azurin produced by the different clones of this library have been monitored following the induction of rsmA and nucleotides required for the control of its production have been identified.

LI02/23

Quorum sensing regulation preserves co-operation

Richard C Allen, Luke McNally, Roman Popat, Sam P Brown

Centre for Immunity, Infection and Evolution, University of Edinburgh, Edinburgh, UK

Signal blind mutants (ΔLasR) are naturally occurring *Pseudomonas aeruginosa* strains that are defective for cell to cell signalling (quorum sensing). These mutants can produce signal but do

not respond to it, having reduced expression of quorum sensing regulated genes. Public goods, like secreted protease, are often regulated by quorum sensing and so signal blind mutants act as cheats, exploiting wild-type co-operators. Critically, signal blind strains are unable to increase their signal output in line with wild-type strains, because signal production in wild-type cells is increased in response to signal via a positive feedback loop. We compete wild-type co-operators against signal blind cheats at a range of frequencies and demonstrate that signal in the environment decreases as wild-type cells become rare. We show that the change in signal concentration causes wild-type cells to behave as phenotypic cheats when rare, secreting low levels of protease. Using competition experiments we then demonstrate that reducing co-operation when in the presence of a high number of cheats can lead to negative frequency dependent wild-type fitness. We model competition in a meta-population and show that repressing co-operative phenotypes when rare allows quorum sensing controlled co-operation to persist in the face of exploitation by signal blind mutants.

LI02/24

PQSIS - a bioreporter for screening inhibitors of 2-alkyl-4(1H)-quinolone (AQ)-dependent quorum sensing in *Pseudomonas aeruginosa*

Eliza Ye-Chen Soh, Siri Ram Chhabra, Miguel Cámara, Stephan Heeb, Paul Williams

University of Nottingham, Nottingham, UK

Pseudomonas aeruginosa produces 2-alkyl-4(1H)-quinolones (AQs) including the "*Pseudomonas* quinolone signal" (PQS) and its precursor HHQ that are quorum sensing (QS) signalling molecules. Their biosynthesis rely on PqsR-dependent transcription of the *pqsABCDE* operon. AQ signalling is pivotal in biofilm development and the expression of various virulence factors, making it a promising target for antimicrobials. A bioreporter (PQSIS) for detecting activators or inhibitors of the AQ QS system was developed. We constructed an AQ-negative *P. aeruginosa* PA01 strain by incorporating a *ΔpqsAΔpyrF* double knockout and an IPTG-inducible pqsR gene, into which a plasmid with the pqsA promoter fused to *pyrF* was established. PyrF encodes orotidine-5'-phophate decarboxylase for uracil prototrophy and mediates fluoroorotic acid (FOA) sensitivity. In the presence of PQS (or any agonist) and FOA, the pqsA-pyrF fusion is induced resulting in growth inhibition, whereas a PqsR inhibitor will 'rescue' the bioreporter cells. PQSIS was activated most sensitively by a PQS analogue, 7Cl-PQS, whereby only 24 nM was required for complete growth inhibition compared with 190 nM and 25 µM for PQS and HHQ respectively. This response can be quenched with quinazolinone antagonists of PqsR. PQSIS can be used for screening novel AQ agonists or antagonists among natural and synthetic compound libraries.

LI02/25

Co-evolution of the ATPase ClpV and the TssB/C sheath distinguishes two type VI secretion subclasses

Sara Planamente, Alain Filloux

MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College, London, UK

Pseudomonas aeruginosa uses the type VI secretion system (T6SS) for delivering toxins in target cells. The T6SS is a weapon for bacterial warfare whose assembly involves ~13 core proteins and shares similarities with the tail of the bacteriophage T4. Among the T6SS components, two proteins, TssB and TssC, form contractile tubules resembling the tail sheath of bacteriophages. Contraction of the TssB/TssC sheath triggers the T6SS dynamics and injection of toxins into neighbouring bacteria. The sheath is subsequently degraded by the AAA$^+$ ATPase ClpV, which binds TssC, allowing a new round of T6SS assembly.
In this study, the structure of the *P. aeruginosa* accessory protein TagJ/HsiE1 was solved in complex with the N terminus of HsiB1 (TssB homologue). It was found that HsiE1 interacts with ClpV1 suggesting it helps targeting the ATPase to the sheath. We solved the structure of the *P. aeruginosa* ClpV1 N-terminal domain, and observed key differences compared to that of the well characterized *Vibrio cholerae* homologue. Phylogenetic analyses show that these alterations are the hallmark of ClpVs associated with TagJ/HsiE1 and distinguish two T6SS subclasses. The N termini of TssCs and TssBs also vary depending on the subclass, indicating a co-evolution between TssC, TssB, TagJ/HsiE and ClpV.

LI02/26

Pseudomonas aeruginosa can be detected in a polymicrobial competition model using impedance spectroscopy with a novel biosensor

Andrew Ward[1], Patricia Connolly[1], Nicholas Tucker[2]

[1]University of Strathclyde, Department of Biomedical Engineering, Glasgow, UK, [2]University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Science, Glasgow, UK

Electrochemical Impedance Spectroscopy (EIS) is a powerful technique that can be used to elicit information about an electrode interface. Here, we highlight six principal processes by which the presence of microorganisms can affect impedance and show how one of these - the production of electroactive metabolites - changes the impedance signature of culture media containing *Pseudomonas aeruginosa*. EIS, was used in conjunction with a low cost screen printed carbon sensor to detect the presence of *P. aeruginosa* when grown in isolation or as part of a polymicrobial infection with *Staphylococcus aureus.* By comparing the electrode to a starting measurement, we were able to identify an impedance signature characteristic of *P. aeruginosa.* Using mutant strains we are able to show that one of the changes in the impedance signature is due to pyocyanin and associated phenazine compounds. The findings of this study indicate that it might be possible to develop a low cost sensor for the detection of *P. aeruginosa* in important point of care diagnostic applications. In particular, we suggest that a development of the device described here could be used in a polymicrobial clinical sample such as sputum from a CF patient to detect *P. aeruginosa*.

LI02/27

Pseudomonas aeruginosa PA01 strain variability

Annapaula Correia[1], Josh Quick[2], Szymon Calus[2], David Livermore[1], Jacob Malone[3], Nick Loman[2], John Wain[1]

[1]Medical Microbiology, University of East Anglia, Norwich, Norfolk, UK, [2]Institute of Microbiology and Infection, University of Birmingham, Birmingham, UK, [3]Molecular Microbiology,The John Innes Centre, Norwich, Norfolk, UK

Please note: Abstracts are published as received from the authors and are not subject to editing.

Pseudomonas aeruginosa PA01 is a commonly used reference strain, providing the foundation for much *Pseudomonas r*esearch. Variation caused by the accumulation of SNPs is well known in PA01, but its effects on phenotype are not well described. Here, we present a comprehensive study of four representative strains of PA01: the first from the Leibniz Institut DSMZ German Collection of Microorganisms and Cell Cultures, the second, a descendant from the *American Type Culture* Collection and the last two, colonial variants of PA01 from the UK National Collection of Type Cultures (NCTC). Whole genome sequencing (WGS), microscopy, growth curves and biofilm assays were performed for all four organisms. WGS revealed mutations in genes associated with biofilm phenotypes; microscopy showed differences in colony morphology; growth curve analysis revealed different growth rates among the four organisms. Variation in ability to form biofilms was seen between the large and small NCTC colonial variants. Such variations, accumulating over time and sub-culturing, are a cause for concern. We recommend researchers should routinely perform WGS of their standard strains such as PA01, publishing these data alongside their experimental results, to allow other researchers to assess the likely impact of any genetic diversity on reproducibility.

LI02/28

Development of an *ex vivo* porcine lung model for studying growth, virulence and signalling of *Pseudomonas aeruginosa*

Freya Harrison, Aneesha Muruli, Steven Higgins and Stephen P. Diggle

School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, UK

The evolution of bacterial public goods such as quorum sensing (QS) signal molecules and exoenzymes is subject to socially-imposed selection, which is a product of bacterial population and community structure. Key questions about social evolution and virulence have been addressed in *in vitro* experiments and acute mouse infections, but it is difficult to extrapolate from these to the common *in vivo* situation: chronic multi-strain and multi-species infections of spatially-structured organs, such as the cystic fibrosis (CF) lung. This means that it is hard to evaluate the therapeutic potential of novel treatments for chronic infection that are based on disrupting social behaviours like QS.

We have developed an infection model that mimics chronically-infected human lung: *ex vivo* pig lung (EVPL). We can quantify *P. aeruginosa* growth, QS, virulence factor production and tissue damage in this model. We report that *lasR* mutants, which do not respond to 3-oxo-C12-HSL-mediated QS, exhibit reduced virulence in EVPL. However, lasR mutants grow as well as, or better than, a corresponding wild type strain. Our data are not consistent with the hypothesis that *lasR* mutants act as social 'cheats' in the lung and have implications for the likely therapeutic success of QS inhibitors.

LI02/29

A novel subclass of MMBL-organized proteins in *Pseudomonas*

Maarten Ghequire, René De Mot

Centre of Microbial and Plant Genetics, University of Leuven, Kasteelpark Arenberg 20 bus 2460, 3001 Heverlee, Belgium

The LlpA protein from *Pseudomonas putida* represents the prototype of a novel family of antimicrobial agents with bacteriocin-like properties. This protein is constituted of two structurally similar monocot mannose-binding lectin (MMBL) domains, and followed by a short C-terminal extension. Previously it was demonstrated that only one of the MMBL domains is involved in the binding of carbohydrates, while the other domain is the primary determinant of target strain specificity. The presence of both modules is mandatory to obtain a fully active molecule.

We identified a new putative lectin-like bacteriocin, termed LlpB, consisting of only a single MMBL domain. So far this protein could only be retrieved in genomes of pseudomonads, whereas LlpAs are more widespread and occur in other genera as well. Structural predictions indicate that LlpB adopts a fold that is similar to LlpA. Preliminary tests with recombinant His-tagged protein suggest that LlpB is a functional antibacterial protein, acting with genus-specific activity. The latter is somehow surprising as this would imply that target strain specificity and the carbohydrate-binding function of this protein are essentially united in one functional module, as compared to LlpAs.

LI02/30

Characterizing the role of MifSR two-component system proteins in regulating *Pseudomonas aeruginosa* metabolism

Gorakh D. Tatke[1], Kumari H[2], Eugenia Silva-Herzog[2], Lourdes Ramirez[1] and Kalai Matheea[2]

[1]Department of Biological Sciences, College of Arts and Sciences, and [2]Department of Molecular Microbiology and Infectious Diseases, Herbert Wertheim College of Medicine, Florida International University, Miami, FL.

Pseudomonas aeruginosa is a Gram-negative, metabolically versatile opportunistic pathogen. It has a propensity for causing incapacitating infections in individuals with compromised immunity. *P. aeruginosa* infections are difficult to treat due to the expression of a multitude of virulence factors and its extraordinary intrinsic and acquired resistance to a gamut of clinically significant antibiotics. This ability, in part, is mediated by several two-component regulatory systems (TCS) that play a crucial role in regulating virulence mechanisms and metabolism. MifSR is one such TCS known to regulate biofilm formation. In addition, our *in silico* analysis revealed the presence of a two gene poxAB operon, 81 bp downstream of *mifSR g*enes. The function of poxA is still unclear and *poxB* encodes for a β-lactamase. The close proximity of *mifSR* genes to *poxB* hinted the role of MifSR TCS in regulating antibiotic resistance. However, no difference was observed in the antibiotic resistance profile of *mifSR* mutants compared to *P. aeruginosa* prototypic strain (PAO1). Subsequently, our phenotypic microarray data (BioLOG) and growth profiling studies indicates the inability of *mifSR* mutants in utilizing α-ketoglutarate (α-KG), a key tricarboxylic acid (TCA) cycle intermediate as a sole carbon source. This indicates the role of *mifSR* TCS in regulating metabolism by modulating the TCA cycle. Furthermore, growth curve analysis of mifSR mutants compared to *P. aeruginosa* PAO1 demonstrates no difference in the growth pattern in presence of sugars and other TCA cycle intermediates (except α-KG) as the sole carbon source. In addition, complementation of *mifSR* mutants with *mifSR* genes restores their growth in media supplemented with α-KG. Thus, growth profile analysis and complementation studies together, clearly indicates

the role of MifSR TCS in regulating *P. aeruginosa* metabolism, specifically TCA cycle, by modulating α-KG utilization

LI03
Cell Cycle

LI03/01
The role of dynamin-like proteins in the developmental control of cell division in *Streptomyces*

Susan Schlimpert[1,2], Sebastian Wasserstrom[2], Elisabeth Barane[2], Maureen J. Bibb[1], Mark J. Buttner[1], Klas Flärdh[2]
[1]Department of Molecular Microbiology, John Innes Centre, Norwich, UK, [2]Department of Biology, Lund University, Lund, Sweden

The *Streptomyces* developmental program involves mycelial growth and differentiation into dormant uni-genomic spores. During the reproductive phase, a massive and synchronous cell division event leads to the deposition of 50 or more regularly spaced FtsZ-rings along the sporogenic hyphae within a short time. Among the regulatory genes identified to be central to developmentally-controlled cell division is the transcription factor *whiH*. Exploiting the ability of *S. venezuelae* to sporulate in liquid culture, we have characterized the *WhiH* regulon. Through this route, a WhiH target promoter was identified that controls an operon of two genes encoding dynamin-like proteins (DynAB).
Dynamin-like proteins are large GTPases that play critical roles in diverse cellular processes in eukaryotes that require membrane fusion or fission. Although bacterial dynamin-like proteins have been partially characterized, their precise function in bacteria has remained poorly understood. Interestingly, in *Streptomyces*, the disruption of DynAB leads to the creation of long compartments containing more than one copy of the chromosome, indicating that the dynamins are required for normal sporulation septation, and for the regular assembly of cytokinetic FtsZ-rings. The dynamin mutant phenotype very closely mimics the *whiH* phenotype, suggesting that the dynamins largely mediate the effect of WhiH on developmentally-controlled cell division.

LI03/02
Coming close to the edge: subpolar organization by bactofilins in *Myxococcus xanthus*

Lin Lin[1,2,] Andrea Harms[3], Anke Treuner-Lange[3], Jörg Kahnt[3], Lotte Søgaard-Andersen[3], Martin Thanbichler[1,2]
[1]Max Planck Research Group "Prokaryotic Cell Biology", Max Planck Institute for Terrestrial Microbiology, Marburg, Germany, [2]Falculty of Biology, Philipps University, Marburg, Germany, [3]Department of Ecophysiology, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Bacterial cells show surprisingly highly ordered temporal and spatial organization. This requires the precise subcellular positioning of many proteins. Several mechanisms have been identified that contribute to protein subcellular positioning. These include the establishment of landmark structures at certain regions within the cell, which further recruit other proteins. So far, most of these cellular landmarks have been found at either mid cell or cell poles. Here, however, we describe an unusual mechanism where landmark structures built by three bactofilin homologs (BacN-P) fill the subpolar regions, thereby tethering proteins at a defined distance from the cell poles in *Myxococcus xanthus*. Bactofilins are a class of cytoskeletal elements that are conserved and widespread among bacteria, with no similarity to other known cytoskeletal proteins. In *M. xanthus,* BacN-P are essential for the precise subpolar localization of multiple proteins, including the key chromosome segregation components ParA and ParB. Moreover, we have identified a ParB-like protein, BadA, which associates with BacN-P and has a role in this mechanism. Our data suggest that the bipolar BacN-P structures anchor ParAB and BadA to their proper positions close to the cell poles, thus ensuring the robustness of chromosome segregation.

LI03/03
The Indole Pulse: A new perspective on indole signalling in *Escherichia coli*

Hannah Gaimster[1], Jehangir Cama[2], Silvia Hernández-Ainsa[2], Ulrich Keyser[2], David Summers[1]
[1]Department of Genetics, Cambridge, UK, [2]Cavendish Laboratory, Cambridge, UK

Indole inhibits *E. coli* division at concentrations of 3-5 mM. However, the biological relevance of such high concentrations has been questioned. The phenotypes of indole producing and indole non-producing strains were monitored. In the short term indole producers suffered a growth disadvantage, across longer period they were significantly more viable than there non indole producing counterparts. Addition of 1 mM indole to the non indole producing culture was unable to complement the phenotype. This suggested that production of indole was key, rather than just the presence of indole. Indole accumulation in a culture supernatant was assayed, this showed that indole is made rapidly over a short period during the transition from exponential to stationary phase. We propose that here, indole concentrations inside the cell can be substantially higher that outside. We call this transient high level of indole as 'pulse' signalling. We have also shown that indole has a 90-fold higher affinity for membranes than the cytoplasm. Direct measurements of cell-associated indole during the exponential-stationary phase transition have shown that up to 60 mM indole is associated with cells. We suggest that indole accumulated within the cell is likely to be involved in a controlled arrest of the cell cycle.

LI04
Metabolic engineering for biotechnology: fundamental knowledge to societal benefit

LI04/01
Cyclodextrin glucanotransferase immobilisation onto functionalized magnetic double mesoporous core-shell silica nanospheres

Abdelnasser Ibrahim[1,2], Ali Al-Salamah[1], Ahmed El-Toni[3], Mohamed El-Tayeb[1], Yahya Elbadawi[1]
[1]King Saud University, Riyadh, Saudi Arabia, [2]National Research Center, Cairo, Egypt, [3]King Abdullah Institute for Nanotechnology, King Saud University, Riyadh, Saudi Arabia

Cyclodextrin glucanotransferase (CGTase) from *Amphibacillus sp.*

Please note: Abstracts are published as received from the authors and are not subject to editing.

NPST-10 was immobilised onto amino-functionalised magnetic double mesoporous core-shell silica nanospheres (mag@d-SiO2@m-SiO2–NH2), and the properties of immobilised enzyme were investigated. The synthesis process of the nanospheres included preparing core magnetic magnetite (Fe3O4) nanoparticles, coating the Fe3O4 with a dense silica layer, followed by further coating with functionalised or non-functionalised mesoporous silica shell. The structure of the synthesised nanospheres was characterised using TEM, XRD, and FT-IR analyses. Comparison of CGTase immobilisation by physical adsorption and covalent attachment indicated that enzyme immobilisation using covalent attachment onto the activated mag@d-SiO2@m-SiO2-NH2 was the most effective, showing one of the highest immobilisation yield (98.1%) and loading efficiency (96.2%) reported so far for CGTase. In addition, the immobilised CGTase can be easily recovered using an external magnetic field. Immobilised CGTase exhibited significant improvement of thermal and pH of stability upon immobilisation. Furthermore, the immobilised enzyme exhibited good operational stability, retaining 56.3% of the initial activity after reutilizations for ten successive reactions, respectively. The applied nanomaterials and immobilisation protocol are promising approach for industrial production of cyclodextrins using immobilised CGTase. To best of our knowledge, this is the first report about CGTase immobilization on magnetic double mesoporous core-shell silica nanospheres.

LI04/02

Isolation, identification and cultivation of isosaccharinic acid degrading alkaliphilic microbes from a hyperalkaline environment

Christopher Charles, Simon Rout, Paul Humphreys

Huddersfield University, Huddersfield, West Yorkshire, UK

The UK intermediate level waste (ILW) inventory contains a range of cellulosic materials originating from nuclear operations. These materials are expected to undergo alkaline hydrolysis under the alkaline conditions of a deep geological repository. This hydrolysis generates a range of cellulose degradation products (CDP) including α and β forms of isosaccharinic acid (ISA) which may enhance the mobility of radionuclides through complexation reactions. The microbial degradation of ISA may therefore have an impact on radionuclide migration by the removal of these complexants. Historical disposals of lime kiln wastes in Derbyshire, UK have generated a hyperalkaline environment which potentially represents an analogue for cementitious radioactive waste disposal. Analysis of sediments and pore waters from the site indicate the in-situ generation of ISA. Sacrificial samples of cotton placed within the site also showed evidence of ISA generation following 3 months emplacement. Microbial consortia recovered from these samples and cultured at pH 11 under fermentative and sulphate-reducing conditions are capable of ISA degradation with the subsequent production of fermentation end products. Phylogenetic analysis of microbial communities present on recovered cotton samples and associated microcosms has been performed via the construction of clone libraries and the sequencing of associated 16S ribosomal DNA.

LI04/03

Fusion of Pyruvate decarboxylase (Pdc) and Alcohol dehydrogenase B (AdhB) increases ethanol production in *Escherichia coli*

Jan Lyczakowski, Aleksandra Lewicka, Christopher French

The University of Edinburgh, Edinburgh, Midlothian, UK

Ethanol is most widely used biofuel, and improvements in ethanol production have the potential for significant impact on commercialisation of biomass derived liquid fuel replacements. Microbial ethanol production is catalysed by two enzymes, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) which convert pyruvate to ethanol via acetaldehyde. Enzymatic fusions have been reported to increase product yields in various processes, this beneficial effect being attributed to enhanced substrate channelling. In case of PDC and ADH, an additional beneficial factor might be decreased exposure of the cell to toxic acetaldehyde. A fusion of PDC and ADH was generated and expressed in *Escherichia coli.* The fusion enzyme was demonstrated to possess both activities, albeit at reduced levels compared to co-expression of the two separate proteins. However, cells expressing the fusion protein generated ethanol more rapidly and to higher levels than cells co-expressing PDC and ADH, suggesting a specific rate enhancement due to the fusion of the two enzymes. This may result in improved process economics for bioethanol production using recombinant bacteria.

LI04/04

Analysis of rhamnolipid production in *Burkholderia thailandensis* E264, a potential non-pathogenic alternative to *Pseudomonas aeruginosa*

Scott Funston, Michelle Rudden, Mohamed Elshikh, Roger Marchant, Ibrahim Banat

University of Ulster, Coleraine, UK

P. aeruginosa is the best known rhamnolipid producing bacteria and therefore, much of the research in the field of rhamnolipids has been carried out using this organism. There is, however, one major issue with regards to *P. aeruginosa* and its classification as a Group II microorganism i.e. an opportunistic human pathogen. This would lead to increased production cost and risk, making rhamnolipids less commercially viable when compared to chemical surfactants. One way this problem can be overcome is to use or develop a non-pathogenic strain of bacteria that is able to produce rhamnolipids at a rate equal to (or greater than) that of *P. aeruginosa*. *Burkholderia thailandensis* is a gram negative bacterium commonly found in the soils and stagnant waters of central and north-eastern Thailand. It has a high physiological and genetic similarity to *Burkholderia pseudomallei* (known to cause meliodosis in both humans and animals) however, *B. thailandensis* E264 was reported to be non-pathogenic (Brett, et. al., 1998., Koh, *et. al.*, 2012). This study has shown that *B. thailandensis* E264 is capable of rhamnolipid production and mainly produces the di-rhamnolipid Rha-Rha-C14-C14. *B. thailandensis* E264 was grown under different conditions to see if the rate of rhamnolipid production could be increased.

ABSTRACTS

LI04/05

The parologous pyruvate kinases in *Streptomyces coelicolor* have distinct roles in growth and antibiotic production

Jana K Hiltner[1], Pablo Cruz-Morales[2], Lorena T Fernandez-Martinez[3], Hrvoje Petkovic[4], Iain A Hunter[1], Juan F Barona-Gomez[2], Paul A Hoskisson[1]

[1]Strathclyde Institute of Pharmaceutical and Biomedical Sciences, University of Strathclyde, Glasgow, UK, [2]Langebio Cinvestav, Irapuato, Mexico, [3]John Innes Centre, Norwich, UK, 4Acies Bio Ltd, Llubljana, Slovenia

Streptomyces species are prolific producer of antibiotics, nevertheless analysis of complete genomes still shows that there are many biosynthetic clusters present that are silent under normal cultivation conditions. The current increase in clinical antibiotic resistance requires the discovery of new antibiotics, but also a greater understanding of antibiotic production for industrial exploitation. Our interest is in studying the transition of primary metabolites into secondary metabolism. We focus on the PEP-PYR-OAA node of central carbon metabolism using *Streptomyces coelicolor* as a model and have identified pyruvate kinase influences antibiotic production. The genome encodes two parologous pyruvate kinase genes - SCO2014 (pyk1) and SCO5423 (pyk2). Phenotypic analysis of the mutants revealed differences in their physiological role, pyk2 exhibits altered growth on glucose, whereas pyk1 mutants show a difference in antibiotic production. We used cross-species complementation experiments with E.coli ΔpykF, ΔpykA and ΔpykA/pykF double mutant and complemented these with *pyk1* and pyk2 from *S.coelicolor* on different media to clarify the physiological role. Furthermore pyk1 and pyk2 were overexpressed in E.coli for a detailed characterisation of their biochemical properties. Our data show that parologous genes in primary metabolism have distinct physiological roles in *Streptomyces* that impact significantly on growth and the production of antibiotics.

LI04/06

Tailoring *Streptomyces* antibiotic biosynthesis: Producing novel minor groove binder antibiotics

Emilio Cortés-Sánchez[2], Sara de Ornellas[1], Glenn Burley[1], Paul A Hoskisson[2]

[1]Strathclyde University , Organic Chemistry, Glasgow, UK, [2]Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, UK

The rise of antibiotic resistant strains and the decline of antibiotic discovery have resulted in an urgent need to develop and discover novel antimicrobials. Minor groove binders (MGB's) are polyamide-derived compounds that show antibiotic, anticancer and antiviral activity by binding to the minor groove of the DNA helix.
MGB's are naturally produced by a range of Streptomyces species. In order to produce novel drugs, we are using synthetic biology and combinatorial biosynthesis to explore pyrrole amide pathway modification in an attempt to assemble novel derivatives of natural antibiotics.
We transformed several *Streptomyces* strains with an MGB producing biosynthetic cluster on an integrating cosmid clone containing four independent non-ribosomal peptide synthetases. These strains were cultured and sampled to look for the production of novel compounds. Culture supernatants were subjected to organic extraction and analysis by bioassay, HPLC , LCMS and HRMS. Our data showed that some of these strains produced a novel compound, not present in the fermentation broths of the parental strain or from the transformed cosmid, HPLC and MS analysis suggest the assembly of a novel compound that also exhibits antibiotic activity according to bioassays. Future work includes characterisation of the molecule and identification of its biosynthetic pathway.

LI04/07

Expanding the potential of mutasynthetic approaches for Pseudomonic acids

Yusra Alsammarraie, Anthony Haines, Christopher Thomas

University of Birmingham, Birmingham, UK

Background: Polyketides are bioactive natural products and some of the most important medicines; they are synthesized by bacteria, fungi and some plants and represent an important group of clinically useful compounds which include antibiotics like erythromycin and tetracycline. Re-engineering polyketide biosynthetic pathways has been a major objective but a parallel approach is to join the products of different pathways .
Objective: Thioamarinol is a hybrid between marinolic acid (a pseudomonic acid) and a pyrrothine, joined via an amide bond. Our aim is to explore ways to create other amines using both *P.fluorescens* NCIMB10586 the (Mupirocin producer) and *P.alteromonas* SANK73390 that makes thiomarinol.
Methods: Gene cloning and mutagenesis were used to manipulate the biosynthetic genes of different polyketide systems to explore mutasynthesis.
Results: Our results shows that SimL (an amide ligase in an Aminocoumarin biosynthetic pathway) has no negative effect on Mupirocin or Thiomarinol production, which makes it a strong candidate for creating hybrid compounds based on Mupirocin.
Conclusion: The outcome of the current experiments will determine to what extent Aminocoumarins can be used to combine independent antibiotic biosynthetic pathways and consequently aid in developing a new family of hybrid derivatives that may extend the effective use of mupirocin against MRSA.

LI04/08

Mariner-Transposon Mediated Random Mutagenesis in *Clostridium* species

Alexander Grosse-Honebrink, Ying Zhang, Nigel P. Minton

University of Nottingham, Nottingham, UK

Random transposon mutagens are an essential tool in the armoury of the molecular biologist and facilitate the application of forward genetics. Through their deployment one can screen for a desired phenotype, and then very simply determine the genotype of the mutant isolated. Through forward genetics, factors are identified that were previously not known to be involved in the process under investigation.
Here we describe the adaptation of a *mariner* transposon system previously established for *Clostridium difficile* (Cartman and Minton, 2010). The technology consists of a conditional replicon, the Himar9 transposase and its transposable element. The methodology is

Please note: Abstracts are published as received from the authors and are not subject to editing.

universally applicable to any *Clostridium* species and has been exemplified in the biotechnologically relevant species *Clostridium acetobutylicum* and *Clostridium sporogenes.*

LI04/09

Engineering amino acid producing *Corynebacterium glutamicum* for access to alternative carbon sources

Christian Matano[1], Jung-Won Youn[1], Tobias Meiswinkel[1], Andreas Uhde[2], Gerd Seibold[2], Volker Wendisch[1]

[1]Bielefeld University, Bielefeld, Germany, [2]University of Cologne, Cologne, Germany

The Gram-positive soil bacterium *Corynebacterium glutamicum* is a versatile workhorse for biotechnology. Via metabolic engineering has been possible to establish large-scale amino acid production processes and to broaden the spectrum of products obtainable, to organic acids, alcohols, terpenoids and diamines.
The natural ability of *C. glutamicum* of utilizing a variety of carbohydrates, alcohols, and organic acids as single or combined carbon and energy sources and the natural resistance to various growth inhibitors such as furans, phenols, and acids, formed during lignocellulose pretreatment make this organism ideal for the prospect of production processes from agro-wastes.
Moreover, *C. glutamicum* can be engineered for the utilization of alternative carbon sources that do not have competing applications in the food industry, since the intensive utilization of media based on starch and molasses (main industrial sources of glucose and sucrose) is an emerging social and economic issue.
Examples of this trend is the development of strains able to utilize hitherto inaccessible carbon sources, through the characterization of already existing pathways as well as the engineering of heterologous pathways, in order to gain access to the sugar fraction of lignocellulosic hydrolysates and the amino sugars glucosamine, and N-acetyl glucosamine constituting the chitin fraction of shellfish waste.

LI04/10

Development of a pyruvate decarboxylase for use in thermophilic bacteria

Lisa Buddrus, Michael J. Danson, David J. Leak

University of Bath, Bath, UK

Bioethanol produced from organic waste, as a second generation biofuel, is an important renewable energy source. Yeast and a very limited range of bacteria use the homoethanol fermentation pathway which employs pyruvate decarboxylase (PDC), in conjunction with alcohol dehydrogenase (ADH), to convert pyruvate to ethanol. Of the known bacterial PDCs, that from *Zymomonas mobilis* is the most extensively characterized. The *pdc* and *adh* genes of *Z. mobilis* have been successfully cloned and expressed in *E. coli* and a number of other mesophiles, thus creating very effective ethanologenic strains. Despite extensive screening no PDC has been identified in a thermophilic organism yet. The most thermostable PDC, from the mesophile *Zymobacter palmae*, is active at 65°C (when sourced from its native host or a recombinant mesophile), but not expressed in its active form at temperatures above 55°C. We believe that this is not simply a problem of protein thermostability, but also one of protein folding at high temperatures.
Using *Geobacillus thermoglucosidasius* as a host platform, we are endeavoring to develop a PDC which both folds and functions at high temperatures in order to create a thermophilic version of the homoethanol pathway for use in both this host and others.

LI04/11

Construction of a signal peptide library to screen for optimal secretion in the industrially relevant thermophile *Geobacillus thermoglucosidasius*

Ali H. Hussein, David J. Leak

University of Bath, Bath, UK

Certain industrial microbial processes can be enhanced when the organism being exploited is able to efficiently secrete relevant enzymes into the extracellular milieu. This potentially rate limiting step is, in part, mediated by N-terminal signal peptides of varying length and amino acid sequence.
The facultatively-anaerobic, gram-positive thermophile *Geobacillus thermoglucosidasius* can ferment a range of pentose and hexose sugars and has been metabolically engineered to produce bioethanol at a high yield. However, its substrate range could be expanded by secreting enzymes for the breakdown of oligosaccharides. At present, *G. thermoglucosidasius* has not had its secretion machinery or secretome characterised, although, genome sequence analysis has identified proteins containing signal peptides.
Evidence from the *Bacillus* genus suggests that no one-size-fits-all signal peptide exists for the optimal secretion of various proteins. Therefore, we developed a simple system for the identification of the best native signal peptides predicted in *G. thermoglucosidasius.* Secretion signals have been introduced upstream of cellulolytic reporter genes, which can be quantitatively assessed with simple metachromatic colony agar and spectrophotomeric assays.
The construction of a *G. thermoglucosidasius* signal peptide library paves the way for several industrial applications, and will serve as a powerful tool for a comprehensive study of secretion in *Geobacillus.*

LI04/12

Construction of the genome-scale metabolic model for *Geobacillus thermoglucosidasius*

Beata Lisowska, David Leak, John Pinney

Bath University, Bath, UK

The threat of global warming and the finite nature of fossil fuels necessitate the development of sustainable sources of fuels and chemicals from renewables.
Lignocellulosic material (LCM) from plants is an abundant renewable source of carbohydrate that can serve as a feedstock for biofuel production. Due to the recalcitrant and complex nature of LCM, it is necessary to utilize microbes that can efficiently ferment a broad range of complex polysaccharides into useful products.
Geobacillus thermoglucosidasius is Gram-positive thermophilic eubacterium (45-70 ^{0}C) that has the ability to convert pre-treated LCM into ethanol. This organism has been genetically engineered such that its yield of ethanol production is in excess of 90% of the theoretical maximum (Cripps *et al.,* 2009). There remains considerable scope to develop *G. thermoglucosidasius* to produce alternative fuels and chemicals of industrial importance.
For such a useful bacterium the understanding of the global metabolism remains poorly characterized. To gain a better

insight into the metabolic pathways and capabilities of *G. thermoglucosidasius* we have applied a bottom-up approach to construct a comprehensive metabolic model of the organism. Our model is build from manually annotated genome and incorporates data from wet lab experiments for accurate *in silico* analyses.

LI04/13

Rewiring antibiotic biosynthesis clusters using synthetic biology

Ashley Chessher, Matthew Cummings, Eriko Takano

University of Manchester, Manchester, UK

The emerging field of synthetic biology is envisaged as a revolutionary approach to manipulate biological systems. Unlike traditional genetic engineering strategies, synthetic biology incorporates an engineer's perspective and seeks to rationally design and construct standardised "parts" (genetic elements such as promoters). These individual parts can then be assembled to form functional modules, which in combination could generate entirely *de novo* metabolic pathway. Antibiotics are particularly amenable to a synthetic biology approach due to the inherent modular nature of polyketides (PKS) and non-ribosomal peptides (NRPS). Evidence suggests that many secondary metabolites are encoded within cryptic biosynthetic gene clusters and, hence, not readily expressed. In the face of increasing antibiotic resistance, the requirement for novel antibiotic scaffolds is becoming an urgent priority. Using a synthetic biology approach, we aim to refactor cryptic biosynthetic gene clusters from *Streptomyces* and develop novel expression systems with the aim of synthesising new antibiotics.

LI04/14

Enhancing bioproduction of styrene using *in situ* product recovery

Luca Rossoni, Patricio Zapata Henriquez, Stephen J Hall, David Archer, Gill Stephens

University of Nottingham, Nottingham, UK

Styrene is a large volume commodity chemical which is used as precursor for a range of useful polymers and co-polymers. The conventional styrene synthesis is performed through dehydrogenation of petroleum-derived ethylbenzene. Recently, styrene synthesis from renewable resources has been reported, involving the biocatalytic conversion of endogenous L-phenylalanine to styrene by expressing *Arabidopsis thaliana* phenylalanine ammonia lyase (PAL2) and *Saccharomyces cerevisiae* trans-cinnamate decarboxylase (FDC1) in engineered *Escherichia* coli (McKenna & Nielsen, 2011). However, production titre (260 mg/L) was far from being industrially viable and, to the best of our knowledge, no significant improvements have been reported to date. The main limiting factor for such a low final product concentration is the toxicity of styrene (around 3 mM). To increase styrene production, we tested an engineered *E. coli s*train in a process that involved in situ product recovery into biocompatible ionic liquids in a biphasic system, overcoming styrene toxicity issues. Moreover, a hypothetical trans-cinnamate decarboxylase (OHBA1) from *Aspergillus niger* was tested as an alternative enzyme for styrene biosynthesis. Its potential in catalysing the conversion of *trans-cinnamic* acid to styrene was compared to the already reported FDC1.

McKenna, R. & Nielsen, D. R. (2011). Metabolic Engineering 13, 544-554.

LI04/15

Engineering the bacterial flagellum: conversion into a high efficiency protein secretion machine

C.A. Green[1], M. Hicks[1], F. Ying[1], P.C. Wright[2] and G.P. Stafford[1]

[1] *Integrated BioSciences, School of Clinical Dentistry, The University of Sheffield, Sheffield, S10 2TA, UK*

[2] ChELSI, Chemical and Biological Engineering, The University of Sheffield, Sheffield, S1 3JD, UK

When biomanufacturing proteins within bacteria, secretion directly into the culture media is desirable, as product will be free of cytoplasmic contaminants, proteolysis reduced, and downstream processing simple. While bacteria contain several protein secretion systems, we have focussed on re-engineering a naturally occurring one-step high capacity secretion machine, namely the flagellar type III secretion system (FT3SS) whose normal function is to assemble the flagellum. Flagellar structural proteins –including several thousand flagellin monomers- are extruded through the flagella lumen before assembly at the distal growing tip. Flagella biogenesis is extensively characterised and indicates that the flagellum represents a structure that is amenable to engineering into an efficient protein secretion device.

We have constructed *E. coli* strains with truncated flagella and demonstrated directed secretion of a range of eukaryotic and prokaryotic proteins in a modular secretion construct, which harbours a secretion signal peptide, purification tag and cleavage sites for simplified downstream processing.

We are currently working to improve secretion by re-engineering flagellar regulatory circuits to increase the secretion capacity of the FT3SS in *E. coli.* We will present our data showing secretion of a number of exemplar proteins and will also highlight metabolic network manipulation to increase production of proline rich eukaryotic proteins.

LI05

Sexually transmitted and reproductive diseases in humans and animals

LI05/01

HPV genes regulate autophagy in epithelial cells

Chara Charsou[1], Ramya Gundurao[2], Juergen Haas[2], Kate Cuschieri[3], Sarah Howie[1]

[1]*University of Edinburgh, MRC Centre for Inflammation Research, Queen's Medical Research Institute, Edinburgh, UK,* [2]*University of Edinburgh, Division of Pathway Medicine, Medical School, Edinburgh, UK,* [3]*Specialist Virology Centre, Royal Infirmary of Edinburgh, Edinburgh, UK*

Human papilloma virus (HPV) is one of the commonest infections of keratinocytes in skin and mucosal tissues. Persistent infection with High-Risk HPV types can lead to cellular transformation and cancer. HPV-16 and 18 high- risk (HR) HPV types are the most abundant and are responsible for almost 70% of cervical cancers. Autophagy, a process of intracellular compartment degradation is a physiological response of the cell to stress, maintaining the balance between cell death and cell survival. The role of autophagy in HR-HPV related tumour development and progression is unknown although proteins associated with the pathway have been suggested as markers of

Please note: Abstracts are published as received from the authors and are not subject to editing.

disease progression. Here, we are investigating *in vitro* the potential role of autophagy in different epithelial cell lines infected with HPV types 16 and 18 as well as the role of early and late HPV16 genes in autophagy regulation in keratinocytes. We are also assessing the role of the mitochondrial apoptotic pathway in correlation with the autophagic process. Our results together with the *ex vivo* data on differential regulation of autophagic markers in patient samples will hopefully be useful as markers of disease progression for patients with HPV related disease and indicate novel treatment pathways.

LI05/02

Development of a loop mediated isothermal amplification (LAMP) assay for the rapid detection of *Mycoplasma genitalium*

Thomas Edwards[1], Patricia Burke[1], Helen Smalley[1], Liz Gillies[2], Glyn Hobbs[1]

1Liverpool John Moores University, Liverpool, UK, 2Mast Group Ltd, Liverpool, UK

Mycoplasma genitalium is a sexually transmissible, pathogenic bacterium, and a significant cause of non-gonococcal urethritis (NGU) in both men and women. Due to the difficulty of the culture of *M. genitalium* from clinical samples, laboratory diagnosis is almost exclusively carried out using nucleic acid amplification tests (NAATs). So far, the application of rapid isothermal amplification methods to *M. genitalium* detection has been limited, and the majority of testing is carried out using either commercially available or "in house" PCR assays. Loop mediated isothermal amplification (LAMP) is a novel nucleic acid amplification technology, utilising a set of four primers specific to six distinct regions of the target DNA sequence, in order to amplify target DNA in a highly specific and rapid manner. A LAMP assay was designed to the pdhD gene of *M. genitalium*, and the limit of detection of the assay was compared to two widely used M. genitalium PCR assays from the literature; a 16S rRNA assay, and an MgPa assay. The pdhD LAMP assay was found to be equally sensitive as the PCR assays, and enabled more rapid testing.

LI08

Evolution of microbial populations within the host

LI08/01

Characterisation of the *yehUT* two-component regulatory system of *Salmonella enterica* serovar Typhi and Typhimurium

Vanessa Wong[1], Derek Pickard[1], Lars Barquist[1,3], Karthikeyan Sivaraman[3,4], Andrew Page[1], Peter Hart[5], Mark Arends[2,6], Kathryn Holt[7], Leanne Kane[1], Lynda Mottram[1,8], Louise Ellison[1], Ruben Bautista[1], Chris Mc Gee[1], Sally Kay[1], Thomas Wileman[1], Linda Kenney[9,10], Calman MacLennan[5,8], Robert Kingsley[1], Gordon Dougan[1]

[1]Wellcome Trust Sanger Institute, Cambridge, UK, [2]University of Cambridge Department of Pathology, Cambridge, UK, [3]European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Cambridge, UK, [4]Positive Bioscience Ltd, Mumbai, India, [5]Medical Research Council Centre for Immune Regulation and Clinical Immunology Service, Birmingham, UK, [6]University of Edinburgh Division of Pathology, Edinburgh, UK, [7]The Department of Biochemistry and Molecular Biology and Bio21 Molecular Science and Biotechnology Institute, Melbourne, Australia, 8Novartis Vaccines Institute for Global Health, Siena, Italy, 9Mechanobiology Institute, Queenstown, Singapore, 10Department of Microbiology and Immunology, Chicago, USA

INTRODUCTION: Proteins exhibiting hyper-variable sequences within a bacterial pathogen may be associated with host adaptation. Several lineages of the monophyletic pathogen *Salmonella enterica* serovar *Typhi* (S. Typhi) have accumulated non-synonymous mutations in the putative two-component regulatory system *yehUT.* There is very little known about *yehUT,* particularly in Salmonella. In this study we provide new data that sheds light on *yehUT* at the genetic and proteomic level. METHODS: We evaluated the function of yehUT in S. Typhi BRD948 and S. Typhimurium ST4/74 using a range of experiments, including DNA microarrays, qRT-PCR, tissue invasion assays, serum bactericidal assays, phenotypic (BIOLOG) assays, antimicrobial-susceptibility testing, Vi phage testing (S. Typhi only) and different murine models (S. Typhimurium only). RESULTS: Transcriptome analysis identified the cstA gene, encoding a carbon starvation protein as the predominantly yehUT-regulated gene in both these serovars. Furthermore, virulence genes SPI1 are differentially regulated by *yehUT* in S. Typhi. Deletion of *yehUT* had no effect on the ability of these mutant *Salmonella* to invade epithelial cells or induce colitis in a murine model. Growth, metabolic and antimicrobial-susceptibility tests identified no obvious influences of *yehUT* on these phenotypes. CONCLUSION: This is the first in-depth investigation of the function of *yehUT* in *Salmonella.*

LI08/02

Stx-phage vB_EcoP 24B sculpting host bacterial function

Giles Holt[1], Alan McCarthy[2], Heather Alison[2], Darren Smith[1]

[1]Northumbria University, Newcastle Upon Tyne, UK, [2]University of Liverpool, Liverpool, UK

Lambdoid-like Stx-bacteriophages are the viral entities responsible for the dissemination of Shigatoxin (Stx) and conversion to Shigatoxigenic *Escherichia* coli (STEC). Stx-phages have an increased genome size compared to their Lambda ancestor and can carry > 33% extra DNA. A high proportion of these extra/accessory genes are located in late gene region of the phage with no determinable function using bioinformatic analyses. Importantly these genes are conserved between globally diverse phage isolates and thus may encode for an important function. This study uses the Biolog microbial phenotypic array to compare the alteration in respiration of the *Escherichia* coli host (MC1061) both prior (naïve) and subsequent to infection (lysogen) with sequenced Stx-phage vB_Ecop 24B. Differences in the utilisation of carbon, nitrogen and phosphate sources were identified as the lysogen was able to respire utilising Uridine-2-monophosphate and lost the ability to utilise b-D-Allose through subversion and genome insertion respectively. The lysogen also gained increased resistance to -Lactam antibiotics and other surface acting chemicals that may offer positive selection to the host. Metabolomic analyses of both the host and lysogen were characterised from early exponential to stationary growth phase in standard culture conditions where phage related difference could be identified.

LI08/03

Changes in phase variable genes of *Campylobacter jejuni* strain 11168 during long-term colonisation of chickens

Lea Lango-Scholey[1], Michael A. Jones[1], Alexandra Woodacre[2], Christopher D. Bayliss[2]

[1]University of Nottingham, Sutton Bonington, UK, [2]University of Leicester, Leicester, UK

Campylobacter jejuni colonises gastrointestinal tract of poultry asymptomatically to very high levels. In humans, *Campylobacter* is the main cause of bacterial gastroenteritis, with estimated 460,000 cases annually in the UK alone. The genome of C. *jejuni* NCTC11168 contains 28 loci that are subject to phase variation (PV) due to changes in polyG/polyC repeat tracts. Many of these loci are predicted to play a role in modification of surface structures - capsule, lipo-oligosaccharide and flagella - which are often targeted by host adaptive immunity. However, the role of PV during persistence in chickens has so far received limited attention. Here we report preliminary results of an *in vivo* experiment to investigate the role of PV during host colonisation and immune avoidance. Chickens were inoculated with strain NCTC11168 and samples were collected two, four and nine weeks after colonisation. A method was developed for the simultaneous analysis of all 28 polyG/polyC tracts in multiple isolates from input and output populations. A trend was detected wherein heightened levels of variation occurred as a function of the length of host colonisation. These findings suggest PV has a major role in increasing genetic and phenotypic diversity during host persistence and may contribute to immune escape.

LI08/04

The influence of temperate phage on *Pseudomonas aeruginosa* and the effect on Chronic Respiratory Disease Sufferer's

Francesca Everest[1], Mohammad Tariq[1], Anthony de Soyza[1,4], Audrey Perry[3], John Perry[3], Stephen Bourke[4], Stephen Cummings[1], Clare Lanyon[1], Darren Smith[1]

[1]Northumbria University, Newcastle Upon Tyne, UK, [2]Newcastle University, Newcastle Upon Tyne, UK, [3]Freeman Hospital, Newcastle Upon Tyne, UK, [4]Royal Victoria Infirmary, Newcastle Upon Tyne, UK

Bronchiectasis arising from either congenital (Cystic Fibrosis/CF) or acquired (non-Cystic Fibrosis Bronchiectasis/nCFBR) chronic respiratory disorders are partially characterised through sequential inflammation events in the lung. Both diseases elicit increased mucus production due to their pathophysiology and diminished mucocilliary clearance offers an environment for opportunistic pathogens e.g. *Pseudomonas aeruginosa* (PA) to colonise. Adaptation by PA to the chronic lung occurs progressively alongside the onset of disease. It is also possible to clinically attenuate/negate bacterial colonisation in nCFBR whereas in CF, we use this to discriminate between induced phages when modelling their adaptation over time. This study models phage induction, cross infection, phage genome comparison and twitching studies linking the results to clinical data. This allows identification/stratification of markers of adaptation between the phages/host and how this relates to the deriving disease state. The data describes how phages derived from PA in older CF patients are: (i) highly adapted to infect PA that can colonise the lung, (ii) have higher rates of self-infection, (iii) have distinct infection profiles that prescribe the bacterial origin of the phage and (iv) how the addition of phage encoded genetic traits offer positive selection for their bacterial host.

LI08/05

The composition of polymicrobial populations of wound associated bacteria changes when exposed to different human protein ligands and antimicrobials

Patricia Alves[1], Edurne Urrutia[1], Kevin Purdy[2], Sarah Maddocks[1]

[1]Cardiff Metropolitan University, Cardiff, UK, [2]University of Warwick, Coventry, UK

Wound infections can be comprised mixed populations of bacteria which commonly grow as a biofilm, impairing the wound healing process. Biofilms present a problem for diagnosis and antimicrobial treatment, which is compounded when multiple species are present. This study demonstrates how a three species biofilm *(Pseudomonas aeruginosa, Streptococcus pyogenes and Staphylococcus aureus),* changes over time, and the impact that human ligand proteins, have on the species composition. Additionally it establishes how the microbial population fluctuates during treatment with antimicrobial manuka honey.

In a three species biofilm *P. aeruginosa* predominates at 24h, with *S. pyogenes* comprising the smallest proportion of the population. After 48 and 72 h numbers of *P. aeruginosa* decline and those of *S. pyogenes* increase; the proportion of the biofilm comprised of S. aureus remains relatively constant. When fibronectin, collagen or fibrinogen are used as a ligand for biofilm growth the population composition is distinct and changes considerably depending upon which ligand is present. Introducing manuka honey to the developing polymicrobial biofilm shifts the population composition from *P. aeruginosa* > *S. aureus* > *S. pyogenes* to one in which each species is present in almost equal number, the principal change being fewer *P. aeruginosa* and more *S. pyogenes.*

LI08/06

Characterising temperate bacteriophage induced from *Burkholderia Cepacia Complex* isolates, originating from the lungs of Cystic Fibrosis patients

Mohammad Tariq[1], Francesca Everest[1], Giles Holt[1], Anthony de Soyza[2], Audrey Perry[2], John Perry[2], Stephen Cummings[1], Clare Lanyon[1], Darren Smith[1]

[1]Northumbria University, Newcastle upon Tyne, UK, [2]Freeman Hospital, Newcastle upon Tyne, UK

Cystic Fibrosis (CF) is the most common autosomal recessive disorder in the UK, currently affecting over 10,000 people.
The causative mutation in the Cystic Fibrosis Transmembrane Conductance Regulator gene alters the chloride channel, giving rise to a thick mucus layer. Through continuing inflammatory responses at the epithelial surface, scar tissue decreases the mucocilliary movement, negating clearance of mucus from the lung. This mucus provides a nutrient rich environment ideal for opportunistic bacteria such as *Burkholderia Cepacia Complex* (BCC) to colonise. *Burkholderia* sp. comprises of 17 sub species of which 5 are commonly found in the lungs of CF patients. BCC is

Please note: Abstracts are published as received from the authors and are not subject to editing.

more problematic than *Pseudomonas aeruginosa* (PA), the most common bacteria found in the lungs of CF patients, due to increased antimicrobial resistance. Temperate bacteriophages are viruses that infect and subvert their bacterial hosts through integration into the bacterial chromosome (prophage) and adapt alongside their host. We elucidate that there are less inducible bacteriophages present in this bacterial background when compared to PA from the CF lung. This study presents the induction of temperate phages from 47 CF, BCC isolates comparing (i) cross infection data (ii) incidence (iii) genome comparison and (iv) these data prescriptively modeled against clinical and microbiological data.

LI08/07

Sub-inhibitory concentration of antimicrobials increases resistance gene transfer between MRSA strains

Kinga Stanczak-Mrozek, Jason Hinds, Jodi A Lindsay

St George''s, University of London, London, UK

Methicillin-resistant Staphylococcus aureus (MRSA) is a major human antimicrobial-resistant (AMR) pathogen, and host colonization is the major risk for infection. Resistance genes to all antimicrobials have been found in MRSA clinical strains but no strains have accumulated resistance to all. Transduction via bacteriophage is the main mechanism of gene transfer in *S. aureus*, and all clinical strains harbour at least one prophage. Half of MRSA positive nasal swabs from patient's admitted to St Georges' Hospital contained free phage particles, and patients are frequently exposed to antimicrobials. The aim of this study was to investigate whether exposure of clinical MRSA strains to different antimicrobials could trigger phage induction and increase the efficiency of transfer of resistance genes. Nine antimicrobials at sub-inhibitory concentrations were tested, and all induced more infectious bacteriophage particles than without antimicrobials. Compared to control conditions, all antimicrobials induced more transducing particles, however the ratio of each type of particles differed for each antimicrobial. These findings suggest antimicrobial exposure during colonization could contribute to the enhanced spread of resistance genes between MRSA, potentially leading to fully resistant MRSA.

LI08/08

Deletions comprising TonB-dependent receptor genes frequently occur during adpatation of *Pseudomonas aeruginosa* to the CF lung environment

Jozef Dingemans[1,4], Lumeng Ye[1], Falk Hildebrand[1,6], Francesca Tontodonati[1], Michael Craggs[1], Florence Bilocq[2], Daniel De Vos[2,1], Aurélie Crabbé[3], Rob Van Houdt[4], Anne Malfroot[5], Pierre Cornelis[1]

[1]Department of Bioengineering Sciences, Research Group Microbiology, Vrije Universiteit Brussel, Brussels, Belgium, [2]Laboratory for Molecular and Cellular Technology, Queen Astrid Military Hospital, Brussels, Belgium, [3]The Biodesign Institute, Center for Infectious Diseases and Vaccinology, Arizona State University, Tempe, Arizona, USA, [4]Unit of Microbiology, Expert Group Molecular and Cellular Biology, Institute for Environment, Health and Safety, Belgian Nuclear Research Centre (SCK CEN), Mol, Belgium, [5]Department of Pediatric Pulmonology, Cystic Fibrosis Clinic and Pediatric Infectious Diseases, Brussels, Belgium, 6European Molecular Biology Laboratory, Heidelberg, Germany

Pseudomonas aeruginosa chronically infects the lungs of cystic fibrosis (CF) patients, leading to persistent lung inflammation, and ultimately, death of the patient. In a previous study, it has been shown that events leading to genome reduction frequently occur during adaptation of this opportunistic pathogen to the CF lung (Rau et al., 2012). In this study, we have determined the genetic relatedness of 54 *P. aeruginosa* isolates, collected from 22 CF patients using a combination of rep-PCR and multiplex PCR targeting ferripyoverdine and S-pyocin genes. Interestingly, we found that in a number of *P. aeruginosa* isolates, the fpvB TonB-dependent ferripyoverdine receptor gene was deleted. Furthermore, we found that several other TonB-dependent receptor genes had been deleted during colonization of *P. aeruginosa* in the CF lung in several patients attending different CF reference centers. Whole-genome sequencing of a potentially transmissible *P. aeruginosa* clone lacking the *fpvB* gene revealed that a large number of potentially deleted genes were shared with isolates of the DK2 clone that dominates the CF population at the Copenhagen CF center. Conclusively, it was shown that parallel pathways comprising genome rearrangements are followed by P. aeruginosa leading to highly adapted CF clones.

LI08/09

Whole genome multi-locus typing of six *Bordetella* species

Sofia Hauck

University of Oxford, Oxford, UK

Bordetella pertussis, an obligate human pathogen with low genetic diversity, remains endemic globally despite widespread vaccination against it. Recent pertussis outbreaks have included other *Bordetella* species, either as co-infections or as co-occurring outbreaks. A whole genome analysis of six species in the *Bordetella* genus was conducted to determine genetic similarities and phylogeny, and aid in the search for better genetic markers for diagnosis and surveillance. Finished *Bordetella* genomes added to the Bacterial Isolate Genome Sequence Database (BIGSdb) were compared to determine the core genome. They and 96 newly sequenced draft genomes were analysed with multi-locus sequence typing (MLST) techniques, using both the *Bordetella* core genome scheme and a ribosomal gene scheme applicable to all bacteria (rMLST). The two schemes' results generated phylogenetic trees with similar topology, supporting rMLST as representative of the whole genome and as a scalable, backwards compatible, phylogenetically consistent identification system. B. bronchiseptica was found to be polyphyletic and form three groups that differ in virulence-associated genes. *B. holmesii*, a recently identified species, was found to be most closely related to *B. avium*, a species found only in birds, and is the third independent case of *Bordetella* evolving to infect human hosts.

LI08/10

Determination of pneumococcal density in experimental human carriage

Jenna F. Gritzfeld[1], Amelieke J. H. Cremers[2], Gerben Ferwerda[2], Daniela M. Ferreira[1], Aras Kadioglu[3], Peter W. M. Hermans[2], Stephen B. Gordon[1]

[1]Respiratory Infection Group, Liverpool School of Tropical Medicine,

Liverpool, UK, [2]Department of Pediatrics, Radboud UMC, Nijmegen, The Netherlands, [3]Department of Clinical Infection, Microbiology and Immunology, Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

Introduction: Density and duration of pneumococcal carriage may affect the likelihood of transmission and invasive disease. Because of its importance in both spreading and causing disease, carriage has been suggested as an endpoint in future vaccine trials. Culture, the current gold standard for detection, may not be sensitive enough to detect changes at low density.

Method: Healthy adult volunteers received an intranasal inoculation with *Streptococcus pneumoniae* serotype 6B. Pneumococcal density in nasal washes collected at six time points post-inoculation was determined by culture and qPCR. Natural pneumococcal carriers detected at an initial screen were followed in parallel.

Results: In 332 nasal washes from 79 volunteers, the sensitivity and specificity of detection by qPCR, compared with culture, were 92.3% and 75.9%. The estimation of pneumococcal density by culture and qPCR was highly correlated (rs=0.77, P<0.0001), although qPCR had a lower detection limit. Pneumococcal density fluctuated within a carriage episode and occasionally fell under the detection limit of culture and/or qPCR. Similar fluctuations in density were observed in natural carriers. The duration of a carriage episode was underestimated when using only one of the methods.

Conclusion: Pneumococcal carriage is a dynamic event. Culture and qPCR are complementary in surveying carriage episodes.

LI08/12

Comparative genomics and transcriptomics of *Sodalis glossinidius,* a secondary endosymbiote of the Tsetse Fly

Ian Goodhead, Alistair Darby

University of Liverpool, Liverpool, UK

Tsetse flies, *(genus Glossinidae),* are the insect vector of protozoan African Trypanosomes: bites of which can lead to infections in man (Sleeping Sickness), and similar infections in non-human animals (Nagana). Tsetse harbours three bacterial endosymbionts: W*olbachia pipientis; Wigglesworthia glossinidiae* and *Sodalis glossinidius,* the latter of which is a commensal secondary symbiont that increases the susceptibility of teneral flies to trypanosome infection.

At present, the mechanisms underlying this interaction between bacteria, trypanosome and tsetse fly remain unresolved. Fortunately, *Sodalis* forms an experimental symbiosis with the *Drosophila* S2 cell line meaning that symbiont/host interaction can be studied using classical molecular genetics.

Sodalis is undergoing genome reduction and loss of function; 51% of its ~4Mbp genome encodes for 'functional' proteins. We have sequenced six further species of *Sodalis* and are combining these data with transcriptomic and proteomic datasets to create a highly-annotated functional gene set for *S. glossinidius.* Furthermore, we have performed Illumina HiSeq directional RNAseq on cDNA isolated from *Drosophila* S2 cultures infected with S. *glossinidius* to elucidate insect genes important in the establishment of infection/symbiosis. Our study will identify target genes at the bacterium/parasite/host interface that can be tested for potential intervention strategies to counteract trypanosome infection in sub-Saharan Africa

LI08/13

Discovery of antimicrobial peptides active against antibiotic resistant bacterial pathogens

Arif Felek[1], Catherine O'Neil[1], Mathew Upton[2]

[1]Microbiology and Virology Unit, School of Medicine, University of Manchester, Manchester, M13 9PL. UK

[2]School of Biomedical & Healthcare Sciences, Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth University, Plymouth, PL4 8AA. UK

Spread of antibiotic resistance (ABR) among bacteria, combined with diminished new antibiotic discovery, is an increasing threat to human health. Bacterially derived antimicrobial peptides (AMP) can be the solution. This study embraces natural antimicrobial product screening, combined with HPLC, mass spectrophotometry and bioinformatic techniques to identify novel bacteriocins active against ABR bacteria.

During the study two promising lead AMPs have been identified, Peptide NI04, from *Bacillus pumilus,* shown to inhibit Gram positive pathogens (Meticilin resistant *S. aureus* and vancomycin resistant Enterococcus) and the anti-Gram negative agent NI05 (ESBL *E.coli*, KPC K. *pneumonia*, *P. aeruginosa*), isolated from *Klebsiella pneumoniae.*

Further study of NI04 and NI05 demonstrated high stability against digestive enzymes and heat. NI04 was also observed to be very potent with MIC values as low as 320au/ml for MRSA and VRE isolates and was non haemolytic in blood and non-toxic to keratinocyte cell lines (up to10xMIC). The mass data (NI04: 6650 Da and NI05: 1796.253 Da) obtained from MALDI-TOF MS and physiochemical studies performed indicate novelty of these agents. The genome sequence of the producer isolates have been determined in order to facilitate identification of the genetic loci required for production of these AMPs following *de novo* peptide sequencing.

LI08/14

Differential complement sensitivity of *S. pneumoniae* and *S. mitis*

Helina E. Marshall[1], Robert J. Wilson[1], Håkon V. Rukke[2], Fernanda C. Petersen[2], Jeremy S. Brown[1].

[1]Centre for Inflammation and Tissue Research, Department of Medicine, Royal Free and University College Medical School, Rayne Institute, United Kingdom [2]Department of Oral Biology, Faculty of Dentistry, University of Oslo, Norway

Streptococcus pneumoniae and *Streptococcus mitis* are naso-oropharyngeal commensals that are genetically similar. However, *S. pneumoniae* is highly pathogenic and a common cause of pneumonia and septicaemia, whereas *S. mitis* rarely causes disease. We hypothesise that differences in sensitivity to innate immunity may underlie these differences in virulence phenotype. We compared sensitivity of *S. pneumoniae* and *S. mitis* to neutrophil killing. After opsonisation with serum but not with heat-treated serum or PBS, *S. mitis* was markedly more sensitive to neutrophil killing compared to *S. pneumoniae.* These differences suggested *S. mitis* was relatively complement sensitive, and flow cytometry assays of C3b/iC3b deposition confirmed there was increased complement opsonisation of *S. mitis* compared to *S. pneumoniae.* *S. pneumoniae* resistance to complement is partially dependent on binding of the immune regulator Factor H by the surface protein,

Please note: Abstracts are published as received from the authors and are not subject to editing.

PspC. We therefore investigated Factor H binding to *S. mitis* using flow cytometry. The results demonstrated that there was no significant factor H binding to *S. mitis*. These data suggest that an inability to bind factor H might underpin *S. mitis* sensitivity to opsonisation with complement and neutrophil killing compared to *S. pneumoniae*, and therefore contribute to the differences in virulence between these two commensal species.

LI08/15

A virulent keratitis-associated strain of *Pseudomonas aeruginosa* exhibits prolonged survival time in contact lens fluid

Amanda Hall[1], Joanne Fothergill[1], Stephen Kaye[1,2], Timothy Neal[1,2], Craig Winstanley[1]

[1]University of Liverpool, Liverpool, UK, [2]Royal Liverpool and Broadgreen NHS Trust, Liverpool, UK

Bacterial keratitis is a major cause of visual loss with approximately 6000 cases per year in the UK. *Pseudomonas aeruginosa* accounts for 25% of cases with an increased prevalence among contact lens wearers. Previous work, using the Array Tube (Alere) genotyping method has identified a core cluster of *P. aeruginosa* strains associated with keratitis.

Contact lens fluid survival rates were compared between nine keratitis-associated *P. aeruginosa* strains, representing strains from both within the core cluster and outside the cluster, together with two control strains. Opti-Free Replenish contact lens solution was challenged with approximately 106 cfu/ml and samples were taken at five time points ranging from one minute to 24 hours.

For most of the isolates 99.9% kill was achieved after ten minutes and for the control strains after one minute. However, one isolate, which was associated with prolonged healing time, and a more severe case of keratitis (39016) did not achieve 99.9% kill, even after 4 hours.

There was no significant difference between survival times for those strains within the core cluster and those outside. This study highlights that some clinical strains may be highly resistant to contact lens fluid, and that current control strains may not be sufficiently representative.

LI08/16

Bacteriophages from a transmissible strain of *Pseudomonas aeruginosa* and their role in bacterial competitiveness

Emily Jones[1], Michael Brockhurst[3], Chloe James[2], Craig Winstanley[1]

[1]University of Liverpool, Liverpool, UK, [2]University of Salford, Manchester, UK, [3]University of York, York, UK

The Liverpool epidemic strain (LES) is a transmissible strain of *Pseudomonas aeruginosa* that causes chronic lung infections in individuals with cystic fibrosis (CF). The LES is associated with a worsened prognosis relative to other *P. aeruginosa* strains. An *in vivo* study using a rat model of chronic lung infection revealed that strain LESB58 has increased competitiveness, due in part to three of the six prophages it harbours. Five of the prophages are active, and high levels of free phages can be detected in CF sputum. We proposed that the LES could supersede other resident *P. aeruginosa* strains by prophage-induced lysis of susceptible competitor strains. We competed fluorescently labelled, isogenic PAO1 strains that differ only by the presence or absence of a LES prophage. We tested three LES phages that are able to form stable lysogens in PAO1, individually and in combination. Lysogens were successfully able to invade an established non-lysogen population, as well as resist invasion by non-lysogens. There were marked differences in the invasion potential of the different phages. We conclude that the LES uses its resident prophages as a weapon to obtain and uphold its stance as the dominant *P. aeruginosa* clone in many CF individuals.

LI09

RNA and riboswitches in bacterial regulation

LI09/01

Differential gene expression of two closely-related *Salmonella* Typhimurium strains under infection-relevant conditions

Rocío Canals[1], Disa Hammarlöf1, Carsten Kröger[1], Sathesh Sivasankaran[2], Karsten Hokamp[2], Roy Chaudhuri[3], Siân Owen[1], Jay Hinton[1]

[1]Institute of Integrative Biology, University of Liverpool, Liverpool, UK, [2]Department of Microbiology, Moyne Institute of Preventive Medicine, Trinity College Dublin, Dublin, Ireland, [3]Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK

Salmonella Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. However, some variants of this serovar have been associated with an emergent invasive *Salmonella* disease in sub-Saharan Africa which usually affects susceptible HIV+ or malarial individuals. These *S. Typhimurium* variants constitute the Sequence Type ST313. A genomic comparison between an ST313 isolate, D23580, and the well-characterised European 4/74 strain (ST19) shows that both strains share 94% of coding genes. Genetic differences include 1000 SNPs, D23580-specific prophages and the presence of pseudogenes. The primary transcriptome of 4/74 has been published [1]. To determine if different gene expression patterns reflect altered pathogenic mechanisms between the two strains, RNA-seq-based transcriptomic data were obtained for strains 4/74 [2] and D23580 grown under sixteen infection-relevant *in vitro* conditions. The comparative transcriptomic analyses revealed that the proportion of differentially expressed genes varied between different stress conditions, from 1.5% of all genes after oxygen shock, to 15% of all genes following low-iron shock. We are investigating whether these patterns of differential gene expression reflect an alteration in regulatory mechanisms between these strains.

1. Kröger *et al* (2012) PNAS 109: E1277-86.
2. Kröger *et al* (2013) *Cell Host Microbe* 14: 683-95.

LI09/02

Detection of small RNAs in *Clostridium difficile* strain 630

Nicola Moore, Geoff McMullan, Chris Gill, Nigel G. Ternan

University of Ulster, Coleraine, UK

Clostridium difficile is a nosocomial pathogen that is the most common cause of antibiotic associated diarrhoea worldwide. Small RNAs (sRNAs) are short sequences of RNA between 50 and 500

nucleotides in length, located in intergenic regions of the bacterial genome that can act to modulate gene expression. Here, we aimed to experimentally verify a subset of sRNAs in *C. difficile* 630 and analyse their expression during growth. RNA with high RIN was isolated at different stages of growth, reverse transcribed and the resultant cDNA used in RT-qPCR to demonstrate sRNA expression. DNA sequencing further validated the identity of the amplified PCR product. Primers for all 10 sRNAs yielded an amplicon of the expected size with both gDNA and cDNA template, confirming the presence and expression of these 10 sRNAs within *C. difficile* 630. Expression of two sRNAs (*scdf*1811.1 and scdf21.1) was analysed at various stages of growth. *scdf*1811.1 showed highest expression levels during mid log whereas *scdf*21.1 showed highest expression during stationary phase. We have experimentally identified expression, and expressional changes in several *C. difficile* 630 sRNAs. This work paves the way for further sRNA expression studies and clarification of their role in gene regulation within this pathogen.

LI09/03

The sRNA landscape of intra-macrophage Salmonella Typhimurium

Shabarinath Srikumar[1,2], Carsten Kröger[2], Magali Hébrard[2], Aoife Colgan[2], Michael Beckett[2], Lu Wang[3], Sathesh K Sivasankaran[2], Katie Breen[2], Karsten Hokamp[4], Daoguo Zhou[3], Jay C. D. Hinton[1,2]

[1]Department of Functional and Comparative Genomics, Institute of Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK, [2]Department of Microbiology, School of Genetics & Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin, Ireland, [3]Department of Biological Sciences, Purdue University, West Lafayette, USA, 4Institute of Genetics, Trinity College, Dublin, Ireland

Salmonella enterica serovar Typhimurium is an adaptable micro-organism that thrives in a variety of environmental niches, including the GI tract of many animals including humans. The bacteria invade and replicate within eukaryotic cells and *Salmonella* gene expression is modulated in response to the hostile environments encountered during infection. The identification of large numbers of small regulatory RNAs (sRNA) in *Salmonella* is bringing new insight into the post-transcriptional control of gene expression. To elucidate new aspects of *Salmonella* gene regulation that are critical for infection, we used RNA-seq to identify the expression profile of S. *Typhimurium* sRNAs during the infection of mammalian cells. Of the 282 sRNAs identified in *S. Typhimurium* to date, 36 were up-regulated and 120 were down regulated more than 2-fold within macrophages. RyhB-1 is the most highly up-regulated sRNA in intra-macrophage *Salmonella* and is known to respond to iron-limiting conditions. InvR, a *Salmonella* Pathogenicity Island 1-associated sRNA is the most down-regulated sRNA. The STnc1850 sRNA is also down-regulated in macrophages, and analysis of an STnc1850 deletion mutant showed that it is required for virulence in macrophages and mice. We recently discovered that STnc1850 controls acid resistance *in Salmonella*, and these experiments will be presented.

LI09/04

RfaH suppresses sRNA MicA inhibition of *fimB* expression in *Escherichia coli* K-12

Alexandra Moores, Ian C Blomfield

University of Kent, Canterbury, UK

The phase variation (reversible on-off switching) of the type 1 fimbrial adhesin of *E. coli* involves a DNA inversion catalyzed by FimB (switching in either direction) or FimE (on-to-off switching). Here we demonstrate that RfaH activates expression of a FimB-LacZ protein fusion, while having a modest inhibitory effect on a comparable *fimB-lacZ* operon construct and on a FimE-LacZ protein fusion, indicating that RfaH selectively controls *fimB* expression at the post transcriptional level. Further work demonstrates that loss of RfaH enables sRNA *MicA* inhibition of *fimB* expression even in the absence of exogenious inducing stress. This effect is explained by induction of σE, and hence MicA, in the absence of RfaH. Further work shows that *FimB* recombination is diminished at 28°C in comparison to 37°C and that this effect reflects enhanced fimB translation at the higher temperature. Thermoregulation is shown to result from increased *MicA* inhibition of *fimB* expression at the lower temperature. This effect reflects increased expression of *micA*, despite a counteractive decrease in MicA activity at the lower temperature.

LI11

Prokaryotic microbial infection forum

LI11/01

Phenotypic characterization of multidrug resistance patterns (MDR) among methicillin resistant *Staphylococcus aureus* (MRSA) from Libya

Mohamed O Ahmed

Tripoli University, Tripoli, Libya

The emergence of multi-drug resistant (MDR) among methicillin resistant *Staphylococcus aureus* (MDR-MRSA), is alarming worldwide problem. This study was performed to reanalyze a collection of methicillin-resistant *Staphylococcus aureus* (MRSA) previously reported from Libyan hospitals for the extent of MDR phenotype patterns. A total of 85 MRSA isolates previously identified using standardized and definite laboratory techniques were further analyzed. Based on the pattern of antibiotic susceptibility testing, previously reported for 8 different classes of antibiotics, MDR was identified based on the expression of resistance to 3 or more classes of antibiotics. MDR- MRSA phenotype was identified in 46/85 (54%) and specific phenotypic resistance (e.g. MLSB) were also within the MDR-MRSA. Analysis and classification of MRSA from Libyan sources is limited and antibiotic susceptibility pattern is extremely important. This limited study shows the large presence of MDR phenotypes and emphasize the important to characterize as well as classify MRSA pattern-phenotypes according to international guidelines.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI11/02

The relation between polymerase chain reaction (PCR) and other diagnostic tests of TB- patients with reference to blood groups.

Mohemid Al-Jebouri, Nuha Wahid

College of Medicine,University of Tikrit, Tikrit, Iraq

Tuberculosis is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites i.e. extra-pulmonary TB. Polymerase chain reaction (PCR), such as nucleic acid amplification (NAA) tests are used to amplify DNA for rapid identification of acid-fast bacilli. In the current study there were 150 patients (13 pulmonary TB, 5 extra-pulmonary TB and 132 suspected TB patients) and 150 healthy control. The patient were examined for the presence of TB utilizing QuantiFERON-TB Gold In-Tube assay, polymerase chain reaction (PCR), AFB smear, OnSite TB rapid test, erythrocyte sedimentation rate(ESR) and chest X-ray. PCR and QFT-GIT were found positive in almost 45% of the patients tested.The simultaneous positivity of PCR test and AFB smear, OnSite TB rapid test, erythrocyte sedimentation rate(ESR) and chest X-ray was variable.It was concluded that blood group (ABO) and rhesus typing were found to be the most prevalent in pulmonary TB patients with blood group ORh +ve and the tested carried out were blood group related at some times. Real-time polymerase chain reaction (PCR) is a specific tool for early diagnosis of TB in a variety of clinical samples particularly with sputum.

LI11/03

Correlation of interleukin-31 serum levels with staphylococcal colonization in pediatric atopic dermatitis

Hala Ibrahim[1], Hadir Okasha[2], Carmen Farid[1]

[1]Dermatology, venereology and andrology Faculty of medicine Alexandria university, Alexandria, Egypt, [2]Medical microbiology and immunology department faculty of medicine Alexandria university, Alexandria, Egypt

Pruritus is a distressing symptom in atopic dermatitis (AD). IL-31 has been a focus of research for its relation to severity of dermatitis and pruritus as well. Staphylococcus aureus enterotoxins were suggested to induce its expression and hence augment pruritus in AD patients.
The objective of this study was to verify the relationship between clinical severity of AD, serum IL-31 levels and density of Staphylococal aureus colonization in AD children.
Thirty AD children of different severities were included and 30 healthy subjects served as controls. SCORAD score was calculated for all patients, serum IL-31 as well as Staphylococal aureus colonization density were determined for patients before and after receiving treatment for their condition as well as for controls. Statistical analysis of the data was then carried out.
A significant association was found between IL-31 serum levels and occurrence of AD but it did not correlate with disease severity nor with pruritus intensity. Staph colonization density correlated positively with disease severity, pruritus intensity both before and after treatment, but it did not correlate with serum levels of IL-31.

LI11/04

The importance of prudent antibiotic use in medicine: A review of previous campaigns designed to increase public awareness.

David Stockell

University of Leeds, West Yorkshire, UK

This report aimed to review previous attempts to increase public awareness of the importance of prudent antibiotic use. The level of success was determined according to what percentage of the general public recalled the campaign (penetration), whether the campaign led to changes in attitude towards antibiotics, and whether this in turn led to a decrease in antibiotic consumption.
The 1999 'Andybiotic' campaign was analysed using data from a Department of Health survey. A 2008 campaign run in association with European Antibiotic Awareness Day was analysed using data from a large 'before and after' household survey carried out by McNulty et al and published in the Journal of Antimicrobial Chemotherapy.
In addition to investigating how successful these two campaigns were, the report also includes data on current public opinions towards antibiotics in order to try to guide future antibiotic campaigns in the UK. This data, along with analysis of the two previous campaigns, shows that future campaigns should be directed at young females and individuals with a lesser education. Additionally, they should make use of television and radio advertising in order to achieve the levels of success shown by campaigns in other countries such as Australia and France.

LI11/05

Hetero-resistance to vancomycin in Coagulase-negative Staphylococci (CoNS) isolated from neonatal blood cultures

Issam Alshami[1,2], Kawthar I Mohammed[1], Nada A M Abdel aziz[1], Rehab Eltahlawi[1], Ahmed E Alharbi[1], Sawsan Awad[2]

[1]Taibah University, Almadinah, Saudi Arabia, [2]University of Manchester, Manchester, UK

Coagulase-negative staphylococcus (CoNS) is the most common isolate from neonatal blood cultures and significantly impacts patient mortality and morbidity. Decreasing glycopeptide susceptibility has been observed among these isolates and prompted the use of aggressive surveillance measures to recognise strains demonstrating hetero-resistance.
In this particular study, 45 clinical isolates from The Maternity and Children Hospital were studied. Different methods were applied on all isolates in order to assess their tolerance to vancomycin including MIC and MBC testing, Time-Kill kinetics and Population Analysis Profiling.
None of the samples were found to be totally resistant to vancomycin even though different degrees of susceptibility pattern existed. Out of all the 45 isolates, 4 strains of CoNS demonstrated the phenomenon of heterogeneous resistance producing subpopulations.
Screening CoNS isolates for the detection of heterogeneous strains can currently broaden our ability to understand the role of heterogeneous resistance in clinical treatment failures, to predict them and to prevent them. Further research need to be conducted

in order to find ways to standardize and improve methods for screening various strains. This can ultimately lead to a more reliable and definitive means of identifying resistant strains and therefore help us prevent any future clinical treatment failures.

LI11/06

Antibacterial activity of extracts from plants used in Sudanese traditional medicine

Ahmed Abdulhag[1], Nezar Alhebshi[1], Nader Whaba[1], Najiha Hashim[2], Assad KHalid[1]

[1]Jazan University, Jazan, Saudi Arabia, [2]University Malayia, Kuala Lumpur, Malaysia

Background: Infections with multi-drug resistant bacteria are difficult and expensive to treat and result in significant mortality, morbidity and economic burden. Among strategies proposed to control this phenomenon is to search for alternative, novel antibacterial agents from natural sources. Objective:, the objective of this study was to assess the antibacterial activity of extracts of plants and herbs used traditionally in Sudan for treatment of infections. Materials and methods: samples of 33 plants were obtained and authenticated. Samples were extracted in 80% ethanol. The extracts were reconstituted in DMSO at 100 mg/ml and tested in agar diffusion assays against a panel of reference and clinical strains of major human pathogens. MIC and MBC were then determined for active extracts using broth-microdilution protocol. Results: 11 extracts exhibited activity against one or more of the tested bacteria; twenty-two did not show any activity against any of the tested pathogens. Only 2 plants, Peganum harmala and Acaia arabica, showed strong activity against both gram positive and gram negative tested bacteria (MIC and MBC= 0.78-3.125 mg/ml), including Acintobacter and *Pseudomonas*. Significance: this screening resulted in a short list of plants that can be considered for further testing and phytochemical analysis to identify active components.

LI11/07

Studying *Shigella sonnei* Virulence using the *Galleria mellonella* Model

Rasha Y. Mahmoud[1,2], Wenqin Li[1], Mohamed Emara[2], R A Eldomany[2], Jun Yu[1]

[1]Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, Scotland, UK, [2]Department of Microbiology and Immunology, Faculty of Pharmacy, Helwan University, Cairo, Egypt

Invertebrate models have become increasingly popular in studying host-microbe interaction because they are cost effective and have less ethical concerns. Larvae of wax moth *Galleria mellonella* show a great value in such studies as; the larvae innate immune system shares remarkable similarities to that of mammals, larvae are able to live well at the human body temperature and larvae are large in size to receive defined dosages of pathogens. *Galleria mellonella*, have been experimentally exploited as an alternative to the animal models in studying the pathogenesis of different bacteria like *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. Here we test the ability of *Galleria mellonella* using in studying the *Shigella sonnei* pathogenesis.
To establish molecular mechanisms of *Shigella pathogenesis*; we constructed a numbers of deletion mutations in genes known to be necessary for *Shigella virulence* including; *dsbA, icsA, mxiD, ipaB*, and *mam* genes. In comparison with wild type *Shigella sonnei* strain 86, all mutants showed significant increase of LD50. The results suggest that the model is invaluable in assessing the roles of virulence factors.

LI11/08

Characterization of *Porphyromonas gingivalis* mutants and identification of specific heme-binding proteins

Geoffrey Tompkins[1], Bikiran Pardesi[1], Alan Carne[2]

[1]Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, Otago, New Zealand, [2]Department of Biochemistry, University of Otago, Dunedin, Otago, New Zealand

The gram-negative heme-requiring anaerobic bacterium *Porphyromonas gingivalis* is implicated in adult periodontitis. As the bacterium lacks porphyrin-biosynthetic enzymes, it must acquire heme from exogenous sources. Several heme-binding outer-membrane proteins (OMP) have been reported but none identified as a high-affinity site. In this study, six reported heme-binding proteins expressed by *P. gingivalis* (HPB35, IhtB HmuY, HmuR,Tlr and HusA) were individually deleted by allelic exchange using an ampicillin-resistance element and the mutant colonies were screened by BSA-enriched PCR. The growth rates, pigmentation and SDS-PAGE OMP profiles of the mutants were indistiguishable from those of the wild-type. Porphyrin binding measured using the fluorescent heme analogue zinc-protoporphyrin IX under stringent conditions was not diminished by more than 15% in any of the mutants. OFFGEL analysis of *P. gingivalis* OMPs extracted using mild detergent followed by heme detection by tetramethyl benzidine (TMBZ) and identification by MALDI-TOF/TOF, revealed a low molecular weight protein (≈ 20 kDa, unidentified), RagA (110kDa), RagB (55kDa) and proteases in the TMBZ-positive fractions. In conclusion, our study suggests that none of the previously reported heme-binding proteins is responsible for specific heme binding but provides several alterntive proteins as candidates for the high-affinity heme-binding site.

LI11/09

Pharmacokinetic, pharmacodynamic and combination therapy considerations to reduce antimicrobial resistance

Germander Soothill[1], Yanmin Hu[2], Anthony Coates[2]

[1]Barts and The London School of Medicine and Dentistry, London, UK, [2]Medical Microbiology, Centre for Infection, Division of Clinical Studies, St George's, University of London, London, UK

Worldwide the crucial role of antimicrobials in healthcare is being put in jeopardy by the emergence of widespread antimicrobial resistance. In the past, development of new antimicrobials kept medicine just ahead of that problem, but only three new classes of antimicrobials have reached the market in the last thirty years. Soon there may not be effective treatments against many bacterial infections, particularly those caused by Gram-negative organisms. In this work we examined two strategies to prevent resistance by changing the way current antimicrobials are used: A) Consideration of pharmacokinetic and pharmacodynamic antimicrobial properties.

Please note: Abstracts are published as received from the authors and are not subject to editing.

Firstly, how the dose of antimicrobials can affect resistance. Secondly, how different distribution and excretion properties of antimicrobials can have variable affect on the microbiota and so cause differing amounts of resistance. B) Combination therapy to prevent resistance development in the treatment of Mycobacterium tuberculosis is well established, however this organism has unique properties. Use of combination therapy as a strategy for prevention of resistance in the treatment of other bacterial infections, such as Pseudomonas aeruginosa and Neisseria gonorrhoeae, has been explored. This work reviewed the evidence for use of both the above strategies.
Can We Prevent Antimicrobial Resistance by Using Antimicrobials Better? Pathogens, 2013, 2(2), 422-435.

LI11/10

Biofilm forming *Staphylococcus aureus*: threat to dental carries disease chemotherapy

Elizabeth Mitaki, Judith Okoth

Jomo Kenyatta University Of Agricuture & Technology, NAIROBI, Kenya

Microorganisms that have ability to form biofilms have remarkable tolerance to killing by antimicrobial agents. They consume the agent decreasing the concentration of the drug to a level that would be ineffective in the deeper region of the biofilm thus providing a 'protection' to the microorganism beneath. Estimation of biofilm formation was done by first culturing S. aureus overnight in Tryptone Soy Broth (TSB) supplemented with 0.25% glucose. 200ul cell suspension was innoculated per well to 16 well polysterone micro titlre plates then incubated for 18 hours at 37 degrees celcius afterwards washed, dried and stained with 0.1% saffranin, rinsed and absorbance was determined at a wavelength of 490nm. Using neem leaves exracts as a possible antibacterial agent at different concentrations gave the following readings; water extract 2.001 on 25ul addition,1.954 on 50ul and 1.369 on 75ul.Chloroform extract showed 2.008 on 25ul, 1.890 on 50ul and 1.256 on 75ul. Finaly methanol had 2.007 on 25ul, 1.733 on 50ul and 1.134 on 75ul addition. There was notable formed biofilms demonstrated by the readings at 490nm wavelength. we conclude that the ability of Staphylococcus aureus to form biofilms affects the reaction of any antibacterial compounds. Key Words;Biofilm, Antibacterial, Dental Carries

LI11/11

Isolation of uncommon pasteurella multocida strains from cattle in north central nigeria

Manasa Sugun

National Veterinary Research Institute Vom, Nigeria, Jos, Plateau state, Nigeria

The prevalence of P. multocida, an aetiologic agent of bovine haemorrhagic septicaemia was studied using a purposive sampling technique in north central Nigeria. A total of 18 positive isolates of P. multocida were obtained from 175 lungs, liver, and spleen samples examined, giving an isolation rate of 10.3 %. T The eighteen isolates were confirmed as P. multocida by Microbact GNB 24E supplied software version Microbact TM 200 identification package V2.03 (Windows TM). By the software interpretations package the percentage probabilities of 12 isolates were above 75% and 6 others were below 75%. The study confirmed the presence of the African capsular strain E (511 bp) and a unique capsular F (851 bp). The P. multocida strains were somatically typed as: P. multocida E: 3, 4 and P. multocida E: 2, 5. Also of interest is capsular group F somatically untypeable that has been identified for the first time from calves in Nigeria. These strains have not previously been reported in Nigeria or within the West African sub-region. These could redefine the vaccine strategy as the current vaccine used in Nigeria contain P multocida B: 3,4 and E: 2.

LI11/12

Molecular epidemiology of *Staphylococcus aureus* with ERIC-PCR method

Shadieh Abdollahi

Cellular & Molecular Research Center and Microbiology Department, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj- Iran, Iran

Background and Objectives: *S. aureus* is one of the most significant etiological agents responsible for healthcare-associated infections. The aim of this study was to show the genetic relationship in S. *aureus* isolates and their transmission pattern between hospitals.
Materials and Methods: 90 *S.aureus* strains, isolated from hospitalized patients in the intensive care unit and infectious wards of Besat and Toohid hospitals, Sanandaj. Antimicrobial susceptibilities were determined by the disc diffusion method, Methicillin resistance was done by agar screen test and the resistance inducible by the D-Test. By ERIC-PCR technique relationship of strains was determined based on the similarities between DNA fingerprints by using Jaccards coefficient in the SAHN program of the NTSYS-pc software.
Results: Fourteen different antimicrobial patterns were observed. 46.7% of the strains were susceptible to all antimicrobials tested. The ERIC-PCR profiles allowed typing of the 90 isolates into 75 ERIC-types which were grouped into eleven main clusters (C1-C11). The Fourth group with the largest number was formed 17 strains. Agreement between antibiotic patterns and rep-profiles was not observed for most isolates.
Conclusion: The results of our study also showed that most of *Staphylococcus* isolated produced different genomic fingerprint patterns, therefore, dissemination source of infection is different.

LI11/13

Lemierre's syndrome: The forgotten disease? A case report

Amera Elzubeir[1], Lee Elzubeir[2]

[1]Heart of England NHS Trust, Birmingham, UK, [2]University of Leicester, Birmingham, UK

Lemierre's syndrome is characterised by primary oropharyngeal infection, leading to secondary development of septic thrombophlebitis of the internal jugular vein with subsequent septicaemia and metastatic emboli, to which the lungs is one of the most commonly affected organs 1,2. Lemierre's syndrome is caused by super infection with Fusobacterium Necrophorum. Fusobacterium Necrophorum is a non-motile anaerobic gram negative bacilli. In the pre-antibiotic era this syndrome was far more common and was often fulminant and fatal in 7-15 days, with a 90% mortality rate1,2,3. However with the advent of antibiotics in the late 1940's there was a rapid decline in the number of reported cases of Lemierre's syndrome and it soon became the "forgotten

disease"3,4, 5. However in the last decade there appears to have been a resurgence of this syndrome. Emergence of antibiotic resistance may explain the recent rise in the number of reported cases. Treatment involves a prolonged course of intravenous antibiotics, the role of concomitant anticoagulation however still remains controversial. We present a case of an 18 year old Caucasian male diagnosed with Lemierre's syndrome to illustrate its classical presentation, common pitfalls in diagnosis and optimal management.

LI11/14

Factors that enhance the ability of *Pseudomonas aeruginosa* to resist the action of antibiotics

Wafa Almatrood, Glyn Hobbs, Helen Smalley, Katie Evans

Liverpool John Moores University, Liverpool, UK

P. aeruginosa is an opportunistic pathogenic bacterium, which has clinically become the most prevalent bacterial pathogen in nosocomial infections, due to the fact that it is capable of resisting the effect of a wide range of antimicrobial agents. Several factors have been identified as having a significant influence on the capacity of *P. aeruginosa* to resist the antibacterial action. This study has aimed to evaluate the consequence of different temperature and inoculums density on the susceptibility of *P. aeruginosa* to the minimum inhibitory concentrations (MIC) of the antimicrobial agents. In the present study, *P. aeruginosa* strain PA01 was subjected to the action of aminoglycoside and polymyxins E agents at different concentrations. Experiments were carried out to establish the organism's MIC to the antibiotics. It has been observed that temperature and inoculum density clearly affected the outcome of the susceptibility of *P. aeruginosa* to the antibiotics. The results of the tests conducted during this study revealed that both factors, incubation at low temperature and high inoculation density increased the measured MICs values of the antimicrobial agents against *P. aeruginosa*. These observations indicated that a number of factors employed in susceptibility tests can contribute to change the MIC values.

LI11/15

Molecular analysis of class I integron genes in clinical Staphylococcus isolates

Rashid Ramazanzadeh

1Cellular & Molecular Research Center and Microbiology Department, & Student Research Committee Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran, sanandaj, Kurdistan, Iran

Introduction and Objectives: Staphylococcus is an important Nosocomial infectious agent which is notorious for rapidly gaining antimicrobial resistance genes. Integrons are a series of mobile genetic elements that are able to express gene cassettes encoding various antibiotic resistances. This study aimed to identify integron class I gene cassettes in clinical Staphylococcus isolates recovered from patients in Sanandaj, Iran hospitals.

Materials and Methods: A total of 200 Staphylococci spp. was recovered from nose and throat swabs of patients (ICU and infection wards) in Toohid and Beasat hospitals in Sanandaj, Iran. Following bacterial DNA extraction, Class I Integron gene was detected by PCR.

Results: Out of the 200 Staphylococci spp. , 81 (40.5%) isolates were carriers of class I integron . The integron expressing isolates included 35 cases (23.5%) of Staphylococcus epidermidis, 37 cases (40.1%) of Staphylococcus aureus, and 9 cases (36%) of Staphylococcus saprophyticus.

Conclusion: Results indicated that frequency of class I integron gene is quite high among clinical Staphylococcus isolates in Sanandaj area. For control of antibiotic resistance spread, screening of clinical samples for these genes and elucidation of their genetic diversity is crucial

LI11/16

Investigating pathogenesis and virulence of the human pathogen, *Vibrio vulnificus*

Selina Church[1], Craig Baker-Austin[2], Stephen Michell[1]

[1]University of Exeter, Exeter, UK, [2]CEFAS, Weymouth, UK

The aim of this project is to use both a bioinformatic and wet lab approach to identify novel virulence genes in V. vulnificus, a Gram negative opportunistic pathogen found predominantly in estuarine environments. Using these approaches we identified a novel putative type VI secretion system (T6SS) in V. vulnificus.

The T6SS is the most recently identified secretion system in Gram negative bacteria. It is hypothesised to form an inverted bacteriophage like puncturing device and has been shown to possess both anti-eukaryotic as well as anti-prokaryotic properties. Investigation into the T6SS of V. vulnificus has demonstrated that V. vulnificus contains a functional T6SS. Additional mutagenesis studies have demonstrated that the functional T6SS of V. vulnificus produces anti-prokaryotic effectors.

LI11/17

Redeployment of an old antibiotic: The potential therapeutic benefit of treating community associated-methicillin resistant *Staphylococcus aureus* with oxacillin

Justine Rudkin[1,2], Maisem Laabei[2], Andrew Edwards[3], Hwang-Soo Joo[4], Michael Otto[4], Katrina Lennon[2], James O'Gara[1], Nicholas Waterfield[2], Ruth Massey[2]

[1]National University of Ireland, Galway, Ireland, [2]University of Bath, Bath, UK, [3]Imperial College London, London, UK, 4NIAID, NIH, MD, USA

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections in predisposed patients. Healthcare-associated MRSA (HA-MRSA) has largely been confined to clinical environments due to high fitness cost, reduced toxicity and attenuated virulence associated with methicillin resistance. Recently emerged hyper-virulent community-associated MRSA (CA-MRSA) can cause severe disease in otherwise healthy individuals. Its success has been attributed to expression of low fitness cost antibiotic resistance and high toxicity. We recently reported that elevated expression of the methicillin resistance gene, *mec*A, in HA-MRSA was accompanied by reduced toxicity and disease severity. Attenuated toxicity is not a characteristic of hyper-virulent CA-MRSA, which are generally less resistant to methicillin. We therefore sought to reduce the toxicity and virulence of a major epidemic CA-MRSA clone by increasing its level of *mec*A expression. To achieve this we grew MRSA in the presence of oxacillin to activate the mecA gene and demonstrated that we could modulate the virulence

Please note: Abstracts are published as received from the authors and are not subject to editing.

factor expression profile of CA-MRSA, reduce expression of many key toxins and block the accessory gene regulator quorum sensing system. These findings reveal a potential therapeutic benefit in the treatment of CA-MRSA with oxacillin or related b-lactams. We are currently testing this hypothesis in an animal model.

LI11/18

Phenotypic Patterns of Broad Spectrum β-lactam Resistance in Faecal *E. coli* Isolated from Severely Malnourished and Nourished Children Attending Mbagathi District Hospital, Nairobi: A Case-Control Study

Samuel Njoroge[1,2], John Kiiru[2], Gideon Kikuvi[2], James Berkley[3]

[1]Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya, [2]Center for Microbiology Reseach, Kenya Medical Research Institute, Nairobi, Kenya, [3]Centre for Geographic Medicine Research, Wellcome Trust Research Programme, Kilifi, Kenya

Background: Severely malnourished children have increased risk of being put on antibiotics because of co-morbidities. The study's objective was to characterize the *Escherichia coli* β-lactamase mediated resistance to broad spectrum β-lactam antimicrobials among this population and compare them with nourished children as Controls. Settings and Design: In this cross-sectional, hospital based setup, 109 E. coli isolates were obtained from each group, one isolate per subject. Methods and Material: Stool or anal swabs were collected, enriched in buffered peptone water and cultured on MacConkey and Eosin Methylene Blue agars. Biochemical test were used to identify E. coli. Antibiograms to determine phenotypic resistance were determined using a panel of 14 drugs. Only the isolates showing synergy between Ampicillin-Calvulanic acid and one or more third generation cephalosporins were picked as Extended Spectrum β-Lactamase (ESBL) producers. Statistical Analysis: Differences in ESBL rates and susceptibility percentages between Cases and Controls were evaluated for significance using 2-tailed Fisher's exact test. Results: Prevalence of ESBL phenotype was higher in severely malnourished children (39%) as compared to the controls (7%). The pAmpC's-like phenotype was observed in 11% isolates. Conclusions: Isolation of ESBL-E. coli among severely malnourished children is high.

LI11/19

JHP0940 expressed from *Helicobacter pylori* induces a proinflammatory response from gastric epithelial cells *in vitro*

Maher M. Alandiyjany, Jane I. Grove, Robin M. Delahay

Centre for Biomolecular Sciences and Nottingham Digestive Diseases Centre, University of Nottingham, Nottingham, UK

Many H. pylori strain-specific genes are encoded within highly variable chromosomal plasticity zones (PZs). Most PZ genes are of unknown function but the encoded products of some are recognised to contribute to the virulence potential of infecting strains. The JHP0940 protein encoded within the PZ of strain J99 is one such example. Studies have shown it to be highly expressed in response to the interaction of H. pylori with the gerbil gastric mucosa and addition of purified recombinant JHP0940 to cultured macrophages has been demonstrated to activate NF-kB leading to upregulation of proinflammatory cytokines such as TNFα and IL-8.

We aimed to investigate the ability of native JHP0940 expressed from *H. pylori* to interact with cultured epithelial cells *in vitro*. A module comprising a kanamycin resistance cassette and 5'-FLAG-tagged jhpo940 under transcriptional control of the constitutive flaA promotor was inserted into the coding sequence of cagE in several clinical strain backgrounds. Then strains were assessed for JHP0940 expression, secretion and host cell interaction. Our data indicates that natively expressed JHP0940 is secreted from certain strains and induces expression of IL-8 from gastric epithelial cells in vitro. The C-terminus of the protein is determined to be important in these respects.

LI11/21

Comparison of probiotics for prevention of dental cariology

SungHoon Lee[1], YoungJae Kim[2]

[1]College of Dentistry, Dankook University, Cheonan, Republic of Korea, [2]School of Dentistry, Seoul National University, Seoul, Republic of Korea

Objectives: Dental caries is induced by oral biofilm containing Streptococcus mutans. Probiotic bacteria have been known to promote human health. Thus, the information of probiotics for oral health has been lack yet. In this study, we investigated influence of various probiotics on oral bacteria or cariogenic biofilm and evaluated candidate probiotics for dental caries among them. Methods: The antimicrobial activity of the spent culture medium of probiotics for oral streptococci was performed. Probiotics were added during the biofilm formation with salivary bacteria including S. mutans. The oral biofilms were stained with a fluorescent dye and observed using the confocal laser scanning microscope. To count bacteria in the biofilm, the bacteria were plated on MSB and BHI agar plates after disrupting the biofilm and cultivated. Results: Among probiotics, Lactobacillus species strongly inhibited growth of oral streptococci. Moreover, Lactobacillus species strongly inhibited formation of cariogenic biofilm model. The expression of gtfs was significantly reduced by L. rhamnosus. The integration of L. rhamnosus into the biofilm model did not exhibit. Conclusion: L. rhamnosus may inhibit oral biofilm formation by decreasing glucan production of S. mutans and antibacterial activity, which can be a candidate for caries prevention strategy.

LI11/22

Characterisation of lipoproteins in *Staphylococcus aureus*

Ashraf Elgallali, Howard Foster

University Of Salford, Manchester, UK

Staphylococcus aureus is an extremely successful opportunistic bacterium capable of causing a wide range of infections, and is becoming increasingly virulent and resistant to antibiotics. Various methods have been used to analyse the pathogenic behaviour of *S. aureus* including genomics, transcriptomics, and proteomics. Due to the broad ranging functionality of cell wall lipoproteins in S. aureus, which comprise a large family of membrane-anchored proteins, were shown to perform various roles in bacterial activity and attract a particular interest to investigate their virulence and survival influences. The initial part of this study was to find out whether the lipoproteins that been identified are carry the same molecular

characterisation among all *S. aureus* strains, and examined their expressions under different growth conditions. The PCR results show high similarity in Lpp among the examined strains, whereas qPCR outcomes were varied in Lpp genes expression within different growth phases and between the examined strains. Phylogenetic trees were generated in two different ways, in one, genes sequences were concatenated into a one alignment, then analyzed to generate the strains tree, while in the second, phylogeny trees were inferred individually from each gene, a consensus of these genes phylogeny was used to represent the Lpp distribution.

LI11/23

Fcγ receptor mediated uptake of C-Reactive Protein-opsonised *Neisseria meningitidis* into human phagocytic cells

Ashanthie Tudugalle, **Rosalyn Casey, Jane Newcomb, Johnjoe McFadden, Kikki Bodman-Smith**

University of Surrey, Guildford, Surrey, UK

The acute phase protein C-reactive protein (CRP) is a powerful opsonin binding to pathogens such as Neisseria meningitidis (Nm); aiding their uptake by human phagocytes (macrophages). The major receptors for CRP are the immunoglobulin Fcγ receptors (FcγR) I and II; what is less clear is whether CRP-opsonised Nm is also taken up by FcγRs on these cells and by other antigen presenting cells such as dendritic cells (DCs).
Peripheral blood monocyte derived macrophages and dendritic cells were incubated with green fluorescent protein (GFP) expressing Nm with and without CRP to investigate (using fluorescent confocal microscopy) CRP-opsonised Nm association with these phagocytes. Purified human IgG and antibodies specific to Fcγ receptors I (CD64) and II (CD32) were pre incubated with both cell types to block the Fcγ receptors.
Association of Nm with phagocytic cells was significantly enhanced when the meningococci were opsonised with CRP. Cells pre-incubated with human IgG significantly reduced the uptake of CRP-opsonised Nm as did pre-blocking of the cells with FcγRI (CD64) and II (CD32) specific antibodies, confirming the involvement of Fcγ receptors in CRP-mediated uptake of the meningococcus into these cells. Whether this enhanced CRP mediated uptake is helping or hindering the meningococci is yet to be elucidated.

LI11/24

Mechanisms of mecillinam resistance *in vitro* and in clinical isolates of *Escherichia coli*

Elisabeth Thulin, **Dan I Andersson**

Uppsala University, Uppsala, Sweden

Due to antibiotic resistance development there is a shift in what antibiotics are used for treatments of different infectious diseases. One example of this is uncomplicated urinary tract infection, where the first hand choice of antibiotic in general practice in Sweden has changed from trimethoprim to mecillinam. The frequency of mecillinam resistance in clinical isolates is low, which is somewhat of a paradox since numerous genes that can confer high resistance to mecillinam when mutated have been found *in vitro*.
We have isolated *in vitro* mecillinam resistant mutants, identified their resistance mutations, characterized them in terms of resistance level and fitness and compared them to mecillinam resistant clinical isolates. Most of the *in vitro* isolated mutants had previously known mecillinam resistance mutations but we also found several novel mecillinam resistance genes.
The clinical and the *in vitro* mecillinam resistant isolates differed in a number of ways, for example in fitness levels and which mecillinam resistance mutations they acquired. Importantly: all clinical isolates had a mutation in the known mecillinam resistance gene *cysB*. A *cysB* mutation only cause intermediate resistance, but we believe that the acquisition of it is an important stepping stone for obtaining higher resistance in the clinical setting.

LI11/25

Bacterial Biofilm Formation and Exopolysaccharide Characterization on Mineral Media with Different Carbon Source

Sirwan Akbar, **Paul Humphreys, Andrew Laws**

The university of Huddersfield, Huddersfield, UK

The formation of biofilms and associated extracellular polymeric material (EPS) are important factors in the environmental survival and virulence of a range of pathogens. The biofilm forming capacity of a number of clinical isolates of both Stenotrophomonas maltophilia and Pseudomonas aeruginosa was assessed using a mineral media enriched with a range of carbon sources; Glucose, Glycerol and Ethanol. Significant variations in biofilm forming capacity were seen between isolates when assessed against hydrophobic plastic surfaces. In some cases, isolates were able to form biofilms when suspended in sterile ultrapure water. EPS extracted from strong biofilm forming strains were shown to be carbohydrate based via 1H NMR. Monomer analysis of the extracted EPS via MALLS and HPAEC indicated that the EPS was composed of several sugars, which varied depending on culture media and species. The EPS was primarily composed of glucose, rhamnose, glucosamine, mannose and glucuronic acid for Pseudomonas aeruginosa while for Stenotrophomonas maltophilia were composed of glucose, rhamnose, 2-deoxy-D-glucose, glucoseamine, Galactose, fucose and mannose.

LI11/26

Localization of Autotransporter EspC Protein Secretion

Mahmoud Ashawesh, **Christopher Penfold, Kim Hardie**

The University of Nottingham, Nottingham, UK

Autotransporters (ATs) are the largest family of virulence factors secreted from Gram negative bacteria and share a unique unifying structure comprising three domains; the N-terminal signal sequence, the central functional passenger domain, and the pore forming C-terminal outer membrane transporter domain (the -barrel domain). The serine protease AT EspC is secreted by Enteropathogenic Escherichia coli (EPEC), but the precise steps by which EspC is secreted are still unknown. Here we show that EspC tagged with fluorescent mCherry protein locates to a structure that resembles the spiral cytoskeleton of E. coli when it has its -barrel domain attached. A mutant version of EspC-mcherry lacking the C-terminal -barrel domain has a diffuse localization. To investigate whether the helical bacterial cytoskeleton is required for EspC secretion, the structure of the actin homologue MreB (the main helical cytoskeletal component) was perturbed with A22. A22 altered the localisation of both forms of

Please note: Abstracts are published as received from the authors and are not subject to editing.

EspC-mCherry. A22 also interfered with the processing of the EspC-mCherry mutant lacking the -barrel domain in a dose-dependent manner. Further investigations are required to understand the molecular interactions that are involved in the localisation of EspC and discount the possibility of artifacts that can arise as a consequence of using florescent proteins.

LI11/27

A comparison study of different methods used in the detection of giardia lamblia on fecal specimen of children

Farooq Baig[1], Saleem Khanzada[2], Javaid Budvi[3]

[1]Muhammd Medical College, Mirpurkhas, Pakistan, [2]LUMHS, HYDERABAD, Pakistan, [3]GMMC, SUKKHAR, Pakistan

OBJECTIVE: The purpose of this study was to compare results obtained, between direct immunofluorescent assay (DFA), and two conventional staining methods.DESIGN: Hundered and fifty children fecal specimens were collected and examined by each method. The O&P and the DFA were used as the reference method.SETTING: The study was performed at the laboratory in the Basic Medical Science Institute JPMC Karachi.MAIN OUTCOME: The amount of agreement and disagreement between methods.1. Presence of giardiasis in our population.2.The sensitivity and specificity of each method. RESULTS: There was 45(30%) positive 105(70%) negative on DFA, 41(27.4%) positive 109(72.6%) negative on iodine and 34(22.6%) positive 116(77.4%) on saline method. The sensitivity and specificity of DFA in comparision to iodine were 92.2%, 92.7% respectively. The sensitivity and specificity of DFA in comparisoin to saline method were 91.2%, 87.9% respectively. The sensitivity of iodine method and saline method in compariosn to DFA were 82.2%, 68.8% respectively. There is mark diffrence in sensitivity of DFA to conventional method. CONCLUSION: The study supported findings of other investigators who concluded that DFA method have the greater sensitivity. The immunologic methods were more efficient and quicker than the conventional O&P method.ABBREVIATIONS: DFA = direct immunofluorescent assay; O&P = ova and parasite;

LI11/28

PhoP regulator of pathogenic *Neisseria meningitidis* is linked with infectivity of epithelial cells

Hajah Mohd Afsar, Tom Mendum, Ricardo Balhana, Jane Newcombe Newcombe, Johnjoe McFadden, Kikki Bodman-Smith

University of Surrey, Guildford, Surrey, UK

Neisseria meningitidis is classically an asymptomatic colonizing bacterium living on mucosa of nasopharyngeal tissue in approximately 10-30% of the human population. This colonization, however, can act as an entry point to the blood, leading to life threating septicaemia and meningitis in immunosuppressed hosts. Early studies identified human epithelial and endothelial cells as targets for N. meningitidis, and a central role in this response is played by two-component systems (TCSs), which sense the environment and drive the cellular responses. Recent evidence indicated that TCS (PhoP/PhoQ) is linked with the virulence of N. meningitidis. We set out to investigate whether PhoP regulator is involved in the invasion process of the epithelial cells to underpin the complex network of virulence regulation and to discover the potential targets for novel antimicrobial drugs and vaccines. Initial investigations formulated a model based on bacterial invasion of A549 epithelial cells. Results showed a significant reduction in the phoP mutant bacteria count when compared with the wild-type, and these findings were consistent with previous studies. The results indicated that PhoP regulator is a genetic switch which regulates the expression of outer membrane proteins and capsular genes in response to the changing conditions within the host cells.

LI11/29

An evaluation of factors contributing to a VTEC risk assessment for fresh produce

Bernhard Merget[1], Norval Strachan[2], Ken Forbes[2], Fiona Brennan[3], Nicola Holden[1]

[1]The James Hutton Institute, Dundee, UK, [2]The University of Aberdeen, Aberdeen, UK, [3]The James Hutton Institute, Aberdeen, UK

Fresh produce is recognised as an important source of food-borne disease: 25% of all food-borne outbreaks from well-known enteric bacteria, such as verocytotoxigenic *Escherichia* coli (VTEC) and non-typhoidal *Salmonella enterica*, arise from fruit and vegetables. There is evidence that bacteria are able to actively interact with growing plants and use them as alternative hosts. However, many questions surrounding the relative risk factors remain: the 'fitness' of different bacterial isolates; the physiological status of the bacteria; variability between plant species and/or cultivars; different plant tissue types; and external and internal locations on the plant.
The aim of the project is to assess the risk to humans by testing the hypothesis that plants represent a pathway of transmission from animal sources, underpinned by molecular interactions between pathogens and plant hosts. Bacteria-plant infection assays were routinely set up on leafy greens or sprouted seeds with VTEC O157:H7. Biofilm formation on plants was determined, as well as growth and survival curves. Plant-associated bacteria were enumerated, which will provide extrapolated data for the point of harvest. All factors (laboratory and literature based) will then be included in a quantitative risk assessment for fresh produce in Scotland, which can be used by producer and distributer.

LI11/30

The role of fructose-1, 6-bisphosphate aldolase (FBA) in the pathogenesis of *Neisseria meningitidis*

Fariza Shams[1], Neil J. Oldfield[1], SiKei Lai[1], Akhmed Aslam[1], Alan Berry[2], Karl G. Wooldridge[1], David P. J. Turner[1]

[1]Molecular Bacteriology and Immunology Group, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK, [2]Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, UK

Neisseria meningitidis resides harmlessly in the human nasopharynx, but may sometimes cause fatal infections. Fructose 1,6 bisphosphate aldolase (FBA), a glycolytic pathway enzyme, has been described as having moonlighting functions in N. meningitidis. This study aimed to elucidate the putative role of FBA in the pathogenesis of N. meningitidis. SDS-PAGE and Flow cytometry showed FBA is a ubiquitously expressed and surface

exposed protein of both pathogenic and commensal Neisseria spp. At present, N. meningitidis (and other species of Neisseria) are the only Gram-negative species in which FBA has been found on the bacterial surface. Binding of plasminogen by FBA was also investigated. Plasminogen was found to bind to intact Neisseriae and to purified wild type rFBA and a mutated FBA lacking aldolase activity. 6-aminocaproic acid, a lysine analogue inhibited plasminogen binding by FBA suggesting involvement of lysine residues. Interestingly, a truncated FBA spanning the C-terminal 134 amino acids of FBA and containing most of its lysine residues was also able to bind plasminogen. Moreover anti-FBA antibodies were able to inhibit binding in a dose dependant manner. In conclusion, FBA appears to contribute to plasminogen binding on the surface of Neisseria spp. via its C-terminal lysine residues, independent of aldolase activity.

LI11/32

Culture positive confirmed infections in cancer patients –Role of CRP, PCT and ANC

Vivek Bhat, Preeti Chavan, Prafulla Thakkar, Manikchandra Tiwari

ACTREC, Navi Mumbai, India

Introduction:
Infections are an important cause of morbidity and mortality in immunocompromised cancer patients. This study was performed to study the sensitivity of C- Reactive protein (CRP), Procalcitonin (PCT) and absolute neutrophil count (ANC) in culture positive confirmed infections in cancer patients.
Methods:
A total of 38 culture positive confirmed infections in cancer were tested for CRP, ANC and PCT to determine their role in detecting bacterial infections. Aerobic bacterial cultures and susceptibility testing was performed as per standard microbiology laboratory procedures. ANC was performed in HMX by Beckman coulter, CRP in the Siemens Dimension RXL analyzer and Procalcotonin by the Brahms PCT-Q (Thermo –Scientific) test.
Results:
Common organisms isolated included E.coli (14), Klebsiella pneumoniae(7), Pseudomonas aeruginosa(4), Acinetobacter (3) and staphylococci (4). CRP was high in 100% of the cases. ANC was high in 87% cases without neutropenia. PCT was high in only 45% of the cases. However the PCT was high among all the five non neutropenic septicemia cases.
Conclusion:
CRP was high in all and ANC in most of the culture confirmed infections. PCT was high in all non neutropenic sepsis cases but the sensitivity was less than 50% for other infections

LI11/36

Discovery of inhibitors of the *Staphylococcus aureus* global regulator, agr

Yanin Jaiyen[1], Fabio Rui[2], Victoria Steele[1], Ewan Murray[1], Weng Chan[2], Paul Williams[1]

[1]School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, Nottingham, UK, [2]School of Pharmacy, University of Nottingham, Nottingham, UK

Staphylococcus aureus is one of the most important pathogenic bacteria. Developing new drug therapies is an urgent issue as many clinical cases report drug resistance against almost every antibiotic currently available. S. aureus infection is enabled by a highly co-ordinated change in the bacterial genome expression that is ultimately controlled by a few key regulators such as the S. aureus quorum sensing (QS) system agr. Here we have screened a small library of related novel compounds for their ability to reduce S. aureus exotoxin production in a variety of S. aureus strains, including the highly virulent MRSA USA300. Data indicate that one of our compounds (FR44) reduced the production of exotoxins -haemolysin and TSST but also increased the expression of protein A circumstantially implying that FR44 maybe targeting the S. aureus agr system. Significantly, this compound did not inhibit bacterial growth at the concentrations that inhibited toxin production therefore there would be less selective pressure for mutants resistant to this novel compound to arise. In conclusion, our data imply that FR44 targets and inhibits the S. aureus QS system agr and we are currently working to decipher the mechanism by which it achieves this.

LI11/37

Characterization of lactate utilization and its implication on the physiology of *Haemophilus influenzae*

Sabine Lichtenegger[1], Isabelle Bina[1], Sandro Roier[1], Stilla Bauernfeind[2], Kristina Keidel[2], Stefan Schild[1], Mark Anthony[3], Joachim Reidl[1]

[1]University of Graz, Graz, Austria, [2]University of Wuerzburg, Wuerzburg, Germany, [3]Oxford University Hospitals, Oxford, UK

Earlier work demonstrated, that L-lactate metabolism is associated with serum resistance and survival of Haemophilus influenzae type b *in vivo*. To gain insight into lactate utilization of non-typeable strains deletion mutants of the L-lactate dehydrogenase (lctD) and permease (lctP) of NTHi 2019 and laboratory strain Rd KW20 were generated and characterized. Comparison of COPD isolate 2019 with the corresponding lctP mutant did not reveal a lactate dependent alteration in serum resistance. Interestingly, we observed a 4-fold attenuation of the mutant strain in a murine model of nasopharyngeal colonization, highlighting a potential role of L-Lactate to persist in this niche. Characterization of lctP transcriptional control shows a negative feedback regulation in the presence of L-lactate, depending on the ArcAB two component system. Additionally, for 2019 it was found, that available but not metabolized lactate may have an ArcAB independent signalling function leading to increased cell growth in late log phase. We conclude that L-lactate is not only an important carbon-source but may also act as signal substrate, fine tuning the globally acting ArcAB regulon and potentially a yet unidentified signalling system. Additionally, our data suggest that the ability to take up lactate provides a selective advantage during colonization of the nasopharynx.

LI11/38

Footrot and interdigital dermatitis in sheep: histopathology and *D. nodosus* colonisation

Grazieli Maboni, Melissa Bexon, Catrin Rutland, Kerstin Baiker, Sabine Tötemeyer

Please note: Abstracts are published as received from the authors and are not subject to editing.

University of Nottingham, Loughborough, UK

Footrot is an infection of the ovine interdigital skin caused by Dichelobacter nodosus co-infecting with Fusobacterium necrophorum, the latter is essential for an intermediate disease state, interdigital dermatitis (ID). A previous study identified D. nodosus in sheep with different clinical condition. The highest amount of D. nodosus was found in sheep with ID; however, this study sampled a small number of animals1. The aim of this research was to identify correlations between D. nodosus, total eubacteria and clinical condition. Post slaughter biopsies were collected (n= 200) from the skin-hoof interface scored for conformation, ID and footrot. Tissue sections were fixed for histology and bacterial DNA was extracted. D. nodosus and total eubacterial DNA was enumerated by quantitative PCR. ID and footrot lead to progressive chronic-active pododermatitis with a mixed lymphocytic and neutrophilic infiltration with a significant correlation between clinical score and histopathological score (p=0.0009). Data will also be presented comparing total eubacterial and D. nodosus load in relation to clinical lesion scores. This study enables us to profile the bacterial load in the different clinical conditions. This information contributes to a more targeted development of vaccines used to control footrot.
1Calvo-Bado, L.A., et al. (2011). ISME Journal, 5, 1426.

LI11/39

Role of Fibroblast Growth Factor1 in interaction of *Neisseria meningitidis* with Human Brain Microvascular Endothelial Cells

Sheyda Azimi, Lee. M Wheldon, Neil. J Oldfield, Karl. G Wooldridge

University of Nottingham, Nottingham, UK

Neisseria meningitidis (meningococcus) is an obligate human commensal bacterium that can cause meningitis and sepsis. Crossing the Blood-Brain Barrier (BBB) is a crucial step in the development of meningitis, but the mechanisms used by the meningococcus to achieve this are not fully understood. The aim of this study was to investigate the role of the Fibroblast Growth Factor1-IIIc isoform (FGFR1-IIIc) in the attachment to, and invasion of, Human Brain Microvascular endothelial cells (HBMECs) by N. meningitidis. Confocal microscopy showed that micro-colonies of adhered N. meningitidis recruit activated FGFR1. Direct interaction between meningococci and the extracellular domain of FGFR1-IIIc was demonstrated by ELISA confirming the ability of this bacterium to bind FGFR1-IIIc. Other bacterial meningeal pathogens, including Streptococcus pneumoniae and Haemophilus influenzae, were unable to bind to this receptor confirmed specificity. This study identified a novel receptor for meningococci, FGFR1, which may play an important role in the pathogenesis of this pathogen, and may constitute a new therapeutic and prevention target for disease caused by these bacteria.

LI11/40

The mechanism of natural compounds controls intracellular bacterial pathogens

Zainulabedeen Mirza, Jun Yu

Strathclyde university, glasgow, UK

Dysentery (shigellosis) predominantly occurs in children and older people with high levels of morbidity and mortality. It is estimated that about 165 million Shigellosis episodes occurring word wide mostly in developing countries. Most of the strains can be killed by antibiotics. However, some of the strains of Shigella became resistant to antibiotics. These strains became problems in global because of it replace with more diverse Shigella strains that there are no vaccine targeting Shigella. Natural compound such as propolin D essentially inhibited bacterial intracellular growth by unknown mechanisms. Methods such as cell invasion killing, in vivo and in vitro assays, RT-PCR are used to test propolin D for its anti-S. sonnei activity and its mechanisms. Propolin D penetrated into cell, while all others compounds were stuck on host cell membrane. Propolin D shows in vitro activities by reduced Shigella growth with glutathione concentration of 5 to 10mM. Growth curve of Shigella 86 and DsbA mutant were determined in m9 medium in different stress responses. Propolin D treatment causes expression of dsbA gene of Shigella 86 which suggest Propolin D interferes with the function of Shigella DsbA.

LI11/41

Phenotypic and Molecular Characterisation of *BlaCTX*gene and *GyrA* Genes in *Shigella sp.* from Diarrhoeal Patients' Stool Samples

Anthonia Oluduro, Biola Isawumi

Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

The study reported the antibiotic susceptibility profiles and isolation of plasmid factors and resistant genes in Shigella sp. recovered from stool samples of diarrhoeal patients.

One hundred and forty-eight diarrhoeal patients' stool samples collected from various health institutions in Ile-Ife, Osun State, Nigeria were examined for the presence of Shigella sp. Isolation was done on Salmonella-Shigella agar plates at 37 0C and isolates identity confirmed by biochemical characterization. Antibiotic susceptibility of isolates was by Kirby-Bauer's disc diffusion technique and isolation of plasmid DNA in the multiple antibiotic resistant (MAR) isolates was also done appropriately. Molecular detection of resistance (BlaCTX and gyrA) genes was by Polymarse chain reactions.A total of 58 Shigella species were recovered. Susceptibility to antibiotics was in varying proportions. All isolates were resistant to ceftriazone and resistance was mostly to nitrofurantoin (96.5%), augmentine (94.8%) and amoxicillin 82.2%). Generally, 80% of the Shigella sp was multiple antibiotic resistant type, displaying 22 various MAR patterns. Plasmid DNA bands of varying sizes (1.17-23.13 kb), GyrA and BlaCTX genes were isolated from the representatives MAR isolates. The recovery of multiple antibiotic resistance and resistance genes in Shigella sp. in the study is of great concern considering its therapeutic cost implications.

LI11/42

Surface conditioning allows efficient attachment of *Campylobacter jejuni* biofilms to abiotic surfaces

Helen Brown[1], Mark Reuter[1], Roy Betts[2], Arnoud van Vliet[1]

[1]Institute of Food Research, Norfolk, UK, [2]Campden BRI, Gloucestershire, UK

Introduction: *Campylobacter jejuni* is one of the leading causes of infectious intestinal disease in the developed world, and its

public health and economic burden makes reducing its prevalence a priority. Biofilms are a well-known method for environmental survival and transmission, and we have previously shown that in laboratory conditions, *C. jejuni* biofilm formation is increased in aerobic conditions. Here we have investigated biofilm formation of *C. jejun*i on abiotic surfaces in the presence of meat juices, representing a food-chain relevant model.
Results: Meat juices are known to form a conditioning film on food-chain relevant abiotic surfaces, such as glass, plastic and stainless steel. Using a metabolic dye (TTC)-based staining method and microscopy, we have shown that surface conditioning both supports *C. jejuni* growth, and enhances biofilm formation. This effect was observed in several *C. jejuni* and *C. coli* isolates, and surprisingly also in a biofilm-deficient flaAB mutant of *C. jejuni*.
Discussion: Meat juices are present throughout the food chain, and offer foodborne bacteria a means of increased survival via biofilms. A greater understanding of the mechanisms underlying biofilm formation in food matrices will undoubtedly aid in reducing the burden of bacterial contamination of the food chain, and ultimately safer food.

LI11/43

Galleria mellonella: an *in vivo* model for assessing the synergistic effects of macrolides with colistin or polymixin B against *Acinetobacter baumanii*

Alice Gillett, Nicola Crewe, Ronald Dixon

University of Lincoln, Lincoln, UK

Opportunistic multi-resistant (MDR) Acinetobacter baumannii, a common cause of infections in critically ill patients with a compromised immune system, continues to be a great threat in hospital environments. A large percentage of A. baumannii are resistant to most broad spectrum antibiotics resulting in infections that are only susceptible to agents of 'last resort' such as the polymyxins or tigecycline. However with increasing resistance reported to these and concerns about their tolerance, new methods of treatment are needed. This study uses Galleria mellonella, the greater wax moth larvae, as a model host to examine the efficacy of combinations of existing licensed antibiotics for treatment of A. baumannii infections. G. mellonella larvae were infected with lethal levels of A. baumannii and treated with macrolides, colistin or polymyxin B, both alone and in combination. Combining colistin or polymyxin B with macrolides increased the survival rates of A. baumanii infected G. mellonella when compared to treatment with a single drug, providing evidence of a synergistic effect that could be used to treat serious infections in humans. Based upon this data we are expanding our model to investigate the treatment of biofilms, a common source of nosocomial infections.

LI11/44

An Audit of Antimicrobial Choice and Administration for Neutropenic Sepsis at Gloucestershire Royal Hospital and Cheltenham General Hospital

Hannah Giles[1,2], John Boyes[3]

[1]University of Birmingham, Birmingham, UK, [2]City Hospital, Birmingham, UK, [3]Gloucestershire Hospitals NHS Trust, Gloucestershire, UK

Neutropenic sepsis is a medical emergency with mortality rates ranging from 2% to 21%. In light of the findings of the NCEPOD report concerning patients who died within 30 days of receiving systemic anticancer therapy, an audit of the management of patients with neutropenic sepsis was carried out at Gloucestershire Royal and Cheltenham General Hospitals between May 2013 and July 2013.
24 patients were treated for suspected neutropenic sepsis; 18 new admissions and six inpatients. 100% had blood cultures sent and 81% of these were sent prior to the first antibiotic dose, but only 4.8% had the correct number of blood cultures sent. 85.7% patients received their first dose of empirical antimicrobial therapy within 60 minutes. However, prescribing of additional agents to cover atypical respiratory infections and suspected C. difficile diarrhoea was poor: 40% received antibiotic cover for atypical pneumonia and 14.3% received appropriate antibiotics to treat suspected C. difficile infection. Eight patients had antifungal therapy initiated; this was appropriate in all cases and an appropriate antifungal agent was used.
Further education is needed to emphasise the importance of timely investigations and the prescribing of additional agents to cover pathogens that may not be covered by empirical therapy.

LI11/45

Staphylococcus aureus biofilm prevention stratagies after orthopedic prosthetic surgery

Laurence Stipetic[1], Matthew J. Dalby[1], Nikolaj Gadegaard[1], Karl E.V. Burgess[1,2]

[1]The University of Glasgow, Glasgow, UK, [2]Glasgow Polyomics, Glasgow, UK

Bacterial biofilm formation on orthopaedic implants is a major cause of implant failure. Strategies to prevent biofilms are needed to reduce implant failure. Two such strategies to inhibit biofilm formation are through physical or chemical interventions. One such physical method is through altering surface topography at a nano-scale, inducing mechanotransduction, subsequently changing cellular behaviour. One chemical intervention is through the use of an anti-microbial agent, such as triclosan.
Here the effect of changing surface topography and the effects of triclosan on biofilm formation are studied using cell culture, microscopy and a molecular approach, metabolomics, against an isolate of *Staphylococcus aureus* from an infected prosthesis.
Results show that certain surface topographies alter the adherence of bacterial cells compared to a planar surface. These topographies could represent novel anti-microbial surfaces and could be used as a future development of orthopaedic implants. Triclosan mode of action against bacterial cells was also tested for anti-biofilm properties. Through applying metabolomics a molecular 'snap shot' of cell metabolism in response to triclosan was obtained, allowing for greater understanding of drug mode of action. Changes in cellular metabolism are shown in cells having been treated with increased triclosan concentrations, thus advocating triclosan as an anti-biofilm agent.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI11/46

Dientamoeba fragilis as a cause of diarrhoea: fast tracking development of new diagnostics

Joel Barratt, Damien Stark, John Ellis

University of Technology Sydney, Sydney, NSW, Australia

Dientamoeba fragilis is a parasite found in the human gastrointestinal tract and it is emerging as a cause of diarrhoeal disease. Diagnosis of infection is typically performed by light microscopy using fecal specimens, although real time PCR is increasingly being used by diagnostic labs. In order to provide reagents for development of rapid diagnostic tests and to investigate this parasites ability to cause disease, a transcriptome has been generated from cultured trophozoites using Illumina next generation sequencing. Functional annotation of the RNA sequence data has shown that D. fragilis express a wide range of cathepsins that are likely to be virulence factors in this species. These types of proteins are well known in parasitology to play a central role in disease-related processes, and so these studies are now providing very strong support for the role of D. fragilis as a human disease-causing pathogen. In addition, the sequence data is allowing fast track development of a new range of diagnostic tests for dientamoebiasis based on antibodies generated to peptide sequences.

LI11/47

Using next generation sequencing to understand the trade-off between phage resistance and in planta virulence in *Pseudomonas syringae*

Sean Meaden, Britt Koskella

University of Exeter, Cornwall, UK

Phage-imposed selection on bacterial populations should strongly favour the evolution of resistance mechanisms, but bacteria may face a trade-off between investment in reproductive fitness, such as growth rate, and investment in defense against phages. The magnitude of such a trade-off is likely to be context-dependent, and accordingly costs that are key in shaping microbial evolution in nature may not be easily observable in an artificial laboratory environment. We assess the costs of phage resistance for a plant pathogenic bacterium in its natural plant host (Tomato) versus in a nutrient-rich, artificial medium. We demonstrate that mutants of *Pseudomonas syringae* that evolved resistance via a single mutational step pay a substantial cost for this resistance when grown on their tomato plant hosts, but do not pay any measurable growth costs in nutrient-rich media in the absence of phages. Next generation sequencing and comparative genomics identified the mutational basis of these phenotypic differences. The combined phenotypic and genotypic data underscores the importance of phage-mediated selection on bacterial host life history traits.

LI11/48

Antimicrobial Sensitivity of Methicillin-Resistant *Staphylococcus aureus* (MRSA) isolates collected from healthcare and community facilities in Libya

Said Wareg[1,2], Mohamed Daw[1], Howard Foster[2]

[1]University of Tripoli, Tripoli, Libya, [2]University of Salford, Salford, UK

The prevalence of MRSA in healthcare and community settings in Libya was studied in clinical specimens submitted to the microbiology laboratories at Tripoli Central Hospital, Tripoli Trauma / Accident Hospital, Tripoli Medical Centre and Tripoli Burn Hospital. Antibiotic susceptibility patterns were determined using the Kirby and Bauer disc diffusion susceptibility testing method. The prevalence of Inpatient Healthcare Associated MRSA (IP-MRSA), outpatient-Healthcare Associated MRSA (OP-MRSA) and community carried MRSA (CC-MRSA) was 43%, 37% and 38% respectively. All the isolated strains of MRSA displayed resistance to fusidic acid and strains had multiple drug resistance (MDR) to 2- 9 antibiotics for IP-MRSA, 2-7 antibiotics for OP-MRSA and 2-6 antibiotics for CC-MRSA. The most frequent MDR was resistance to fusidic acid, ciprofloxacin, streptomycin and clindamycin. This study has shown that MRSA is equally prevalent in inpatient, outpatient and community settings. Analysis of a survey of the availability of antibiotics showed a lack of restrictions on the supply of antibiotics and widespread use by the general public which may be responsible for the fusidic acid resistance. Zone size criteria for fusidic acid also need to be reviewed.

LI11/49

Analysis of antimicrobial resistance and virulence - associated genes of e. Coli from dairy cattle of zambia

Geoffrey Mainda, Mark Stevens, Mark Broonsvoort, David Gally

University of Edinburgh, Roslin Institute, Edinburgh, UK

Some studies have suggested that both livestock and wildlife could serve as reservoirs of diverse antimicrobial resistant E. coli and pathotypes in Zambia.

In a pilot study, E. coli was isolated from faecal samples from 14 dairy herds. Multiplex PCR was used for phylo-grouping and detection of virulence- associated genes. Further, a subset of 40 strains was selected with a view to conducting whole genome sequencing and in-depth analysis.

From the 71 animals investigated, 19.7% (95% CI: 9.8 - 29.6) were resistant to one or more antimicrobial agents, Oxytetracycline being the most prevalent (14.1%; 95%CI: 5.5 - 22.7), Sulfamethoxazole (7.0% 95%CI 0.1 - 14.0), Ciprofloxacin (5.6% 95% CI: -3.4 - 14.7) and Ampicillin (1.4% 95%CI: -3.3 - 6.1). The phylo-grouping assigned group A (11%), group B1 (78%), group B2 (1%), group D (4%) and 5% un assigned. PCR revealed 4% possessed the gene for Shiga toxin 2, 1% Shiga toxin 1, 4% had intimin but none possessed *rfbo157*, indicating that non-O157 Shiga toxin-positive strains are present that may have zoonotic potential. Ongoing studies seek associations between antibiotic resistance and prescribing trends and will examine the risks posed to human health by E. coli found in Zambian cattle.

LI11/50

Phylogenetic Fingerprinting Using Short Insertion Sequence Elements

Athanasios Mourikis[1], Robert Powell[1], Lisa Crossman[1]

[1]University of East Anglia, Norwich, Norfolk, UK, [2]SequenceAnalysis.co.uk, Norwich, Norfolk, UK

Escherichia coli O104:H4 was the causal agent of a serious foodborne outbreak of beansprouts centred on Germany in

2011. The disease took a serious acute form with an increased probability of contracting potentially fatal haemolytic-uraemic-syndrome (HUS). Due to the swift and acute response to the pathogen, we hypothesised that the genome holds within it an example of a recent evolutionary event, *e.g.* a recent horizontally transferred region or gain of a plasmid.
A large number of insertion sequences are present in specific regions of the chromosome and of the plasmids from this organism. The insertion sequences (IS elements) in the genome were of different families on the plasmids compared to the chromosome. Using manually curated data on the IS element families, Crossman (2011) described the plasmids and chromosome as originating from different genetic backgrounds.
In the current study, we create an automated Perl pipeline "MEC" capable of typing *E. coli* strains involving variable region identification, IS element detection and characterisation. IS element families were identified across example genomes of *E. coli* to create a phylogenetic fingerprint. We show that the mobile element phylogenetic fingerprinting can be used to produce a robust phylogenetic tree. The MEC pipeline is available from github.

LI11/51

A role for *Staphylococcus aureus* Elastin Binding Protein in host cell invasion

Christina Merakou, Charalampia-Georgia Korea, Fabio Bagnoli, Robert Janulczyk, Meera Unnikrishnan

Novartis Vaccines & Diagnostics, Siena, Italy

Staphylococcus aureus is responsible for disorders ranging from skin infections to septic shock. Although it is considered to be an extracellular pathogen, it can also have an intracellular lifestyle. The molecular mechanisms involved in S. aureus-mediated host cell invasion are unclear. While the fibronectin binding protein has been reported to be a major invasin, there is little known about other cell surface proteins in host cell invasion.
In order to characterize invasins of S. aureus, we screened a selection of S. aureus Newman mutants of cell wall and extracellular proteins for invasion efficiency into human cells. We found that a mutant of EbpS, a previously protein, demonstrated a significant reduction in invasion of human epithelial cells. Phenotype was reversed by genetic complementation of EbpS. Furthermore, we observed that internalization of wild type S. aureus is serum dependent, and the reduction of invasion of the EbpS mutant was maximal in presence of optimal amounts of serum. These data indicate a role for a soluble serum ligand in Ebp-mediated host cell invasion. Furthermore, we are currently studying downstream host cell signalling pathways in response to wildtype and EbpS mutant. Our data suggest that the staphylococcal cell surface proteins EbpS mediates staphylococcal invasion.

LI11/52

T-helper 17 (Th17) cell responses predominate in the *Helicobacter pylori*-infected gastric mucosa, and correlate with the severity of inflammation

Emily Staples, Richard Ingram, Abed Zaitoun, John Atherton, Karen Robinson

University of Nottingham, Nottingham, UK

Helicobacter pylori is the leading cause of peptic ulcer disease and gastric cancer, both of which are driven by inflammation. Th1 and Th17 cells contribute to inflammation and pathology in infected animals, but their role in humans is unclear. We therefore aimed to quantify these responses in human gastric tissue.
Gastric biopsies were collected under ethics approval from 166 consenting patients undergoing an upper GI tract endoscopy (115 infected, 51 uninfected). Biopsies were lysed and cytokines assayed using Luminex and RT-qPCR. Flow cytometry was used to determine cellular sources of interleukin-17 (IL-17). Severity of inflammation and neutrophil infiltration was assessed by histopathological scoring.
Significantly higher concentrations of IL-17 (signature Th17 cytokine) were present in biopsies from infected compared to uninfected donors (4.3-fold; p=0.0001). Higher concentrations of other Th17-associated cytokines were also present. There were no differences in interferon-gamma (signature Th1 cytokine), with concentrations approximately 100-fold lower than IL-17. IL-23 (Th17-differentiating factor) concentrations were 500-fold higher than IL-12p70 (Th1-differentiating factor). The majority of IL-17-secreting cells were CD4+ lymphocytes, and IL-17 levels correlated with inflammation (Rs=0.262, p=0.039) and neutrophil infiltration (Rs=0.370, p=0.003).
Mucosal Th17 responses to *H. pylori* therefore predominate over Th1 in humans, and play a major role in disease processes.

LI11/53

Experimental coevolution can increase the phage infectiveness to *Pseudomonas aeruginosa* cystic fibrosis bacterial isolates

Ville-Petri Friman[2,1], Daniel Soanes-Brown[1], Pawel Sierocinski[1], Søren Molin[3], Helle Krogh Johansen[4], Angus Buckling[1]

[1]University of Exeter, Penryn, UK, [2]Imperial College London, Ascot, UK, [3]Technical University of Denmark, Copenhagen, Denmark, [4]Department of Clinical Microbiology Rigshospitalet, Copenhagen, Denmark

Phage therapy – The use of viruses to specifically kill the disease-causing bacteria – could become evolutionary sustainable alternative for antibiotics because phages can overcome bacterial resistance by coevolving more infective. Here we studied *in vitro* ﬁf experimental coevolution can increase the efficiency of model phage therapy with *Pseudomonas aeruginosa* bacteria isolated from the lungs of relatively young, intermittent (1-16 months), and relatively old, chronically (1-21 years) colonized cystic fibrosis (CF) patients. We found that coevolved phages were more efficient in reducing bacterial densities because of weaker resistance evolution; only 20% of bacteria evolved resistance to coevolved phages, while 40% of bacteria evolved resistance to ancestral phages. While resistance evolution did not differ between intermittent and chronic isolates, it incurred higher growth cost for chronic isolates. As a result, selection by coevolved phages led higher bacterial density reduction with chronically colonised bacteria. Moreover, while the ecological and evolutionary effects of phage therapy varied clearly less with chronic versus intermittent isolates, some of the bacteria in both classes were unable to evolve resistance to coevolved phages. Together these results show that phage therapy could be especially useful against chronic *P. aeruginosa* lung infections that are often characterized by multidrug resistance.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI11/54

Viral parasites constrain protist predation-driven attenuation of *Pseudomonas aeruginosa* PAO1 virulence in multi-enemy communities

Ville-Petri Friman[1,2], Angus Buckling[2]

[1]*Imperial College London, Ascot, UK,* [2]*University of Exeter, Penryn, UK*

The coincidental theory of virulence predicts that bacterial pathogenicity could be a by-product of selection by natural enemies in environmental reservoirs. However, current results are ambiguous and the simultaneous impact of multiple ubiquitous enemies, protists and phages, on virulence evolution has not been previously investigated. Here we tested experimentally how *Tetrahymena thermophila* protist predation and PNM phage parasitism (bacteria-specific virus) alone and together affect the evolution of *Pseudomonas aeruginosa* PAO1 virulence (measured in wax moth larvae). Protist predation selected for highly defensive small colony types, both in the absence and presence of phage, which showed attenuated virulence and growth. While, phage selection alone did not affect the bacterial phenotype, it weakened protist driven anti-predatory defence (biofilm formation), its associated pleiotropic growth cost and the correlated reduction in virulence. These results suggest that protist selection is a strong coincidental driver of attenuated bacterial virulence, while phages can constrain this effect due to effects on population dynamics and conflicting selection. Establishing these kind of causal links could allow us to use microbial community composition as a predictor for environments that might act as hot spots for coincidental selection of bacterial virulence.

LI11/55

Adjuvant therapy to treat fluoroquinolone resistance in clinical isolates of *Klebsiella pneumoniae*

João Anes, Matthew P. McCusker, Seamus Fanning, Marta Martins

UCD centre for Food Safety, School of Public Health, Physiotherapy and Population Science, University College Dublin, Dublin, Ireland

Failure to treat Multidrug resistant (MDR) bacteria has become a major public health problem. New approaches have been developed using adjuvants combined with antibiotics to create a synergistic effect increasing the susceptibility of bacteria.

A collection of clinical isolates of Klebsiella pneumoniae was screened for resistance to several antibiotic classes. In order to reverse/reduce the antibiotic resistance adjuvant compounds were tested in combination with antibiotics.

Klebsiella pneumoniae isolates (n=12) from St. Vincent's University Hospital (Dublin, Ireland) were screened for their MDR phenotype. Isolates resistant to fluoroquinolones (namely, ciprofloxacin-CIP, nalidixic acid), tetracycline (TET) and chloramphenicol (CHL) were selected for further studies. The MIC for antibiotics and the adjuvants: chlorpromazine (CPZ), 1-(1-naphthylmethyl)-piperazine (NMP), thioridazine (TZ), phenylalanine arginine -naphthylamide (PAN) was determined (broth microdilution). The EtBr-agar cartwheel method was performed to confirm the possible contribution of efflux to the multidrug resistance of the strains. The resistance to fluoroquinolones was also confirmed by sequencing the related-genes, gyrA, gyrB, parA and parC.

Resistance to CIP and TET decrease in the presence of CPZ and NMP. The majority of the strains (n=10) in the presence of NMP reverted to a susceptibility profile.

This study highlights the relevance of alternative therapies to treat MDR bacteria.

LI11/56

Uncovering the virulence potential of *Salmonella* Agona PFGE-type SAGOXB.0066

Marta Martins, Matthew McCusker, Seamus Fanning

School of Public Health, Physiotherapy and Population Science; UCD Centre for Food Safety; Centre for Molecular Innovation and Drug Discovery; University College Dublin, Belfield., Dublin, Ireland

We studied the virulence potential of *Salmonella enterica* serovar Agona PFGE-type SAGOXB.0066 strain, directly linked to a pan European outbreak in 2008.

Ex-vivo studies were conducted with Caco-2 cells and human THP-1 macrophages. *Salmonella* Typhimurium SL1344 was used as a reference. The invasiveness and intracellular growth was assessed using standard methods. Survival assays and microscopy were conducted in infected macrophages (up to 7 days). Supernatants of the cultures were kept for cytokines analysis and macrophage activation (Griess test). Cytokines were analysed using the V-PLEX proinflammatory panel (Meso Scale Delivery, Rockville, MD) and readings performed in a SECTOR® Imager 6000.

S. Agona SAGOXB.0066 showed an increased ability to adhere (10-fold) and invade (20-fold) Caco-2 cells when compared with SL1344. After 7 days, S. Agona exhibited an ability to survive and replicate inside macrophages (10-fold), while SL1344 was cleared. Microscopy studies of S. Agona-infected macrophages showed a significant increase in the number and size of vesicles. Significant differences were obtained in macrophage activation and cytokine production.

In this study, we analysed the virulence of *Salmonella* Agona PFGE-type SAGOXB.0066. These findings may explain why this particular S. Agona pulse-field type has caused numerous outbreaks including the pan European occurrence in 2008.

LI11/57

Death By Worm Star: Leucobacter are diverse natural pathogens of Caenorhabditis

Laura Clark[1], Marie-Anne Felix[2], Dave Stroud[1], Maria Gravato-Nobre[1], Jonathan Hodgkin[1]

[1]*Department of Biochemistry, University of Oxford, Oxford, UK,* [2]*Institute of Biology, Ecole Normale Superieure, Paris, France*

We have isolated two novel strains of Leucobacter that are natural pathogens of the nematode Caenorhabditis. The bacteria were discovered as a co-infection of a wild Caenorhabditis found on rotting banana trunks on Cape Verde, and successfully transferred to the laboratory nematode Caenorhabditis elegans.

One bacterium, Verde1, attaches to the nematode cuticle in a dense fur-like covering and impairs the movement of worms on solid medium. In liquid culture, Verde1 causes the worms to aggregate by their tails into "worm stars" from which the nematodes are unable to escape [1].

The second bacterium, Verde2, causes rectal infection and tail swelling similar to that of the nematode pathogen Microbacterium nematophilum, and is rapidly lethal to worms.

We present progress on this novel strategy of nematode-trapping

and characterisation of the host-pathogen interactions.

1. Hodgkin et al 2013 Two Leucobacter strains exert complementary virulence on Caenorhabditis including death by worm-star formation. Current Biology 23, 2157-2161.

LI11/58

Isolation and characterisation of listeria monocytogenes in ready-to-eat foods

Laura Luque[1], **Edward Fox**[2], **Séamus Fanning**[1]

[1]UCD School of Public Health, Physiotherapy and Population Science, UCD Centre for Food Safety, University College Dublin, Dublin, Ireland, [2]CSIRO Animal, Food and Health Sciences, Werribee,Victoria, Australia

Listeria monocytogenes is a difficult organism to control in food-processing facilities, as it is resistant to stresses and form biofilms, thus impeding the effectiveness of sanitation procedures. Outbreaks are usually associated with Ready-To-Eat (RTE) foods, causing large economic losses and recalls.
This study focuses on the identification of Listeria monocytogenes in food-processing facilities using molecular methods, sub-typing of the isolates to understand the ecology, epidemiology and understanding reasons for persistence of the strains in the food chain.
Strains were obtained from environment and food every 2 months during 6 months, from 12 Food Business Operators around Ireland. The samples were analysed following ISO 11290-1. The presumptive positives isolates were confirmed by RT-PCR and the confirmed positives isolates serotyped by multiplex PCR and Antisera testing. The presence of Listeriolysin S was tested by PCR and PFGE to identify persistent strains.
6% of the samples were positive for L. monocytogenes with the most prevalent serotype being 1/2a. Additionally, genotyping showed the contamination route in different cases.
This study shows the relevance of positively identify L. monocytogenes in food samples. L. monocytogenes must be controlled in food-processing facilities, ingredients, finished and unfinished products to determine critical control points and implement safety corrective actions.

LI11/59

The human B cell response to factor H binding protein of Neisseria Meningitidis

Prince Yalley[1], **Hedda Wardemann**[1], **Christoph Tang**[2]

[1]Max Planck Institute for Infection Biology, Berlin, Germany, [2]Sir William Dunn School of Pathology, Oxford University, Oxford, UK

Neisseria meningitidis are commensals of the human nasopharynx residing asymptomatically in 10-40% of the population. In a substantial number of infections, the encapsulated bacteria enter the blood across the nasopharyngeal epithelial barrier, often with fatal outcomes including meningitis and septicemia.
Immunity to N. meningitidis is associated with the development of protective serum bactericidal activity that involves complement activation. Antibody-mediated opsonisation may also contribute significantly to bacteria killing. Naturally acquired serum antibodies are predominantly directed against capsular polysaccharide (PS) antigens. However, PS antigens do not elicit long-lasting memory responses and fail to elicit universal humoral protection due to strain variation. Protein antigens which are able to elicit long-lasting immunity may induce more potent protective antibodies against N. meningitidis. A prime candidate is factor H binding protein (fHbp).

fHbp, expressed on almost all strains of N. meningitidis, contributes to evasion of complement mediated killing. It has been shown to induce broad protection (anti-fHbp antibodies) against a diverse collection of N. meningitidis strains in mouse models, thus making it an important vaccine candidate antigen. However, humoral immunity to fHbp in humans has not been characterized yet. The aim of the project is to characterize the B-cell response to fHbp of N. meningitidis.

LI11/60

Isolation and characterization of membrane vesicles from Streptococcus pneumoniae

Mario Codemo, **Sandra Muschiol, Laura Plant, Murat Balaban, Birgitta Henriques Normark**

Karolinska Institutet, Stockholm, Sweden

Microbes have evolved over millennia to become adapted to the environments that they occupy. For this purpose, they have evolved specific tools to mediate interactions with the environment. One such tool that prokaryotes have evolved includes the production of membrane vesicles (MVs). MVs in Gram negative bacteria are 10-300 nm spherical blebs derived from the outer membrane and have been shown in Gram negatives to affect protein secretion, immune activation and suppression, stress response, attachment, internalization and virulence.
In Gram negative bacteria MVs are distinct from membranous blebs produced during cell lysis as they are produced as a regulated secretion event. The MV secretion functions to disseminate virulence factors including toxins and degradative enzymes into the extracellular milieu. Once released, MVs have been demonstrated to function offensively as a virulence factor delivery mechanism, as well as defensively, to aid in the colonization of a host.
So far nothing is known about membrane vesicles in pneumococci. We have purified membrane vesicles from pneumococcal cultures and are currently characterizing these vesicles by various biochemical and molecular methods, including SDS-PAGE, western blotting and electron microscopy. In particular, we are looking into the size distribution and protein content of membrane derived pneumococcal vesicles.

LI11/61

The effect of *Bacillus subtilis* on *Campylobacter* and the caecal microbiome of broiler chickens

Adrian Horton[1], **Toby Wilkonson**[1], **Dave Leemans**[1], **Vince Theobald**[1], **Szymon Calus**[1], **Sarah Gaunt**[2], **Rebecca McDowell**[2], **Michael Lee**[1], **Jamie Newbold**[1], **Nigel Scollan**[1], **Justin Pachebat**[1]

[1]IBERS, Aberystwyth University, Aberystwyth, UK, [2]Eminate, Sutton Bonington, UK

Campylobacter is the leading cause of bacterial gastro-enteritis in the industrialised world. Campylobacter thrive in the avian gut and the primary source of human infection is through the food chain with poultry meet being contaminated during the slaughter process. Here we report on the use of the GRAS registered probiotic *Bacillus subtilis*, incorporated into commercial feed, as an alternative method

Please note: Abstracts are published as received from the authors and are not subject to editing.

to reduce the load of thermotolerant *Campylobacter* spp. in Ross 308 broiler chickens. We also report on the use of 16S Ion Torrent sequencing to profile the effects of B. subtilis on the broiler chicken caecal microbiome.
The caecal contents of commercial chickens fed a normal diet, supplemented with or without *B.subtilis* spores, were extracted on days 14, 21, 28 and 36 of the trial and and variable regions 1 and 2 of the 16s rRNA gene amplified by PCR, and sequenced on an Ion Torrent.
Results indicate that *B. subtilis,* when used as a food supplement, has the potential to reduce levels of *Campylobacter* present in chicken caeca. This study also provides a snap-shot of the chicken cecum microbial community, and the modulations of this community in response to *B. subtilis* in poultry feed.

LI11/62

Investigating the role of the non-integrin laminin receptor in the pathogenesis of meningococcal meningitis

Sozan M. Qarani, Shaun S. Morroll, Akhmed Aslam, Neil J. Oldfield, Karl G. Wooldridge and Dlawer A. A. Ala'Aldeen

Molecular Bacteriology and Immunology Group, School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, NG7 2RD

Neisseria meningitidis, Haemophilus influenzae b (Hib) and *Streptococcus pneumoniae* are the main causes of bacterial meningitis. These pathogens target laminin receptor (LamR) as a receptor on the surface of human brain microvascular endothelial cells to invade central nervous system. In this study we aimed to investigate the regions of LamR that are responsible for binding to the *N. meningitidis* ligands, PorA and PilQ. Several targeted mutations were introduced into recombinant LamR and enzyme-linked immuno-sorbent (ELISA) assays used to test the interaction of the purified mutant LamR proteins with meningococcal outer membrane protein PorA, PilQ and *N. meningitidis* bacteria. We identified specific residues which are required for optimal LamR-PorA binding. We also demonstrated that meningococcal PorA and PilQ act synergistically as ligands for LamR.

LI11/63

Persistent Salmonella infection is controlled by PPARδ, a host regulator of fatty acid metabolism

Nicholas Eisele[1], Thomas Ruby[1], Amanda Jacobson1, Paolo Manzanillo[2], Jeffery Cox[2], Lilian Lam[1], Lata Mukundan[2], Ajay Chawla[2], Denise Monack[1]

[1]Stanford University, Stanford, CA, USA, [2]University of California, San Francisco, San Francisco, CA, USA

Upon the resolution of acute salmonellosis, some mammalian hosts develop an asymptomatic chronic infection where bacteria colonize macrophages residing in systemic tissues. However, the host cell physiology and metabolic requirements supporting bacterial persistence are poorly understood. Macrophages are a dynamic cell type that exhibit diverse activation states depending on the local concentration of cytokines. Using *ex vivo* and murine models of infection, we observed an elevated level of Th2 cytokines in persistently infected tissues and that bacteria preferentially associates with alternatively activated macrophages. We subsequently found that PPARδ, a eukaryotic transcription factor involved in sustaining a program of fatty acid metabolism and the alternatively activated phenotype, is up-regulated and directly activated in *Salmonella*-infected macrophages. PPARδ deficiency dramatically inhibits Salmonella replication, which is linked to the metabolic state of macrophages and the level of intracellular glucose available to bacteria. Pharmacological activation of PPARδ increases glucose availability and enhances bacterial replication in macrophages and mice, while *Salmonella* fail to persist in *Ppar* null mice. Taken together, these data suggest that alternatively activated macrophages represent a unique niche for long-term intracellular bacterial survival and link the PPARδ-regulated metabolic state of the host cell to persistent bacterial infection.

LI11/64

Highly multiplexed and quantitative pathogen detection reveals importance of multiple infections

Keith Stanley[1], Richard Hale[2]

[1]AusDiagnostics PTY, Sydney, Australia, [2]AusDiagnostics UK Ltd, Chesham, UK

Multiplex PCR is widely used in hospital microbiology departments to identify pathogens in clinical samples. However, development of multiplex reactions is not straightforward and in particular, if there is more than one pathogen present, signal from those at lower levels can be lost due to competition for reactants. Multiplex Tandem PCR (MT PCR) avoids this problem by splitting the reaction into two steps. The first step uses only fifteen cycles of multiplexed pre-amplification. Consequently, less than 1% of reactants are consumed and quantification of the less abundant pathogens is preserved. Quantitative reactions of fifty-plex are possible using this method. Quantification is established in the second step and is expressed as relative gene expression normalised to an internal control. This method reveals common occurrence of dual pathogen infection - especially respiratory and enteric samples. This information may help clinicians make better decisions in treatment strategy.
From a technical perspective, independence of targets in MT-PCR also simplifies the validation process and enables new multiplex panels to be developed rapidly in response to new guidelines or changing clinical needs.

LI12

Environmental microbiology forum

LI12/01

Studies on the Effect of Silicon Compounds on Microbial Growth

Bassam Al johny

King Abdulaziz University, Faculty of Science, Biological Science Department, Jeddah, Saudi Arabia

Silicon is the second most abundant element (after oxygen) on Earth, making up 27.7 % of the crust by mass, and is the eighth most abundant element in the universe. Silicon compounds, which are efficient at adsorbing gases and volatiles, remove combined

carbon and nitrogen from the atmosphere, and these may then act as nutrient source for bacterial growth (Soomor, 2000). With the exception of diatoms, which contain silicon as a constituent part of their cell walls, a few studies have shown that fungi and bacteria can solubilise insoluble silicon compounds, it is likely that there exist important interactions between this element, bacteria and fungi (Wainwright .,et al 1997).The present study with various bacteria species has indicated that bacteria dissolves insoluble inorganic silicon, which causes the release and accumulation of silicon, while several types of silicon compounds were examined to assess the effect of these compounds on Aurobasidium pulluans growth under normal and Oligotrophic conditions. This proposes that silicic acid stimulates the growth of A.pulluans under normal conditions, whereas other compounds had an inhibitory effect on fungal growth under Oligotrophically. In addition, the effect of silicic acid on the respiration rates of B.subtilis with HgCl2 have been studied.

LI12/03

Microbiota and microbiological Quality and Safety of Food Related Spices and Herbs marketed in Saudi Arabia

Rashad Al-Hindi

King Abdulaziz University, Jeddah, Saudi Arabia

Eleven kinds of food related spices and herbs were chosen for study, namely, black pepper, cardamom, cinnamon, clove, coriander, cumin, curry, galangal, ginger, juniper and turmeric. Microbial counts were monitored as mean log10 cfu/g sample in three available different commercial presentations, whole non-packaged (WN), ground non-packaged (GN) and ground polyethylene packaged (GP). Most spices and herbs samples, especially black pepper, were highly contaminated conerning total aerobic mesophilic bacteria (TAMB) ranging >log 4 cfu/g. 100% of the samples examined harboured mesophilic aerobic sporeformer bacteria (MASB). Lactic acid bacteria (LAB) were demonstrated in four kinds of spices and herbs, namely, black pepper, curry, juniper and turmeric. Black pepper was heavily contaminated while cumin was moderately contaminated and ginger was less contaminated based on their yeast and mold (Y&M) counts. Coliform bacteria (CFB) were found to occur in only two kinds of spices, black pepper and coriander of the different commercial presentations. Of the (WN) samples only black pepper and clove showed low counts of coagulase (+) staphylococci. On the other hand, (GP) samples were free from coagulase (+) staphylococci. A significant decrease in (TAMB) analysis item counts was noticed in (GP) samples ($p<0.05$) when compared with the other two commercial presentations samples.

LI12/04

Isolation and characterization Streptomyces from soils that exhibit resistance to penicillin G

Wedad Alkut, Glyn Hobbs, Katie Evans, Joanne Foulkes

Liverpool John Moores University, Liverpool, UK

Streptomycetes are gram-positive bacteria, commonly found in soil and are known antibiotic-producers. Ten strains of Streptomycetes were isolated and characterized morphologically and from 16s rDNA sequences. The morphology study and the16s rDNA sequences of these strains indicated that they belong to the species Streptomyces. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of penicillin G for Streptomyces strains were measured by plate culture using starch casein agar. Four strains (W1, W3, W6 and W7) showed growth up to 400 µg/ml, which indicate that the strains were highly resistant against penicillin G. Strains W4 and W10 were un-able to grow at concentration above 200 µg/ml. Strain W9 was un-able to grow at concentration above 50 µg/ml, while strains W2, W5 and W8 were sensitive to penicillin G. Also, The MICs of penicillin G were measured using OxoPlates® in 96-well culture plates employing Mueller-Hinton broth. The MICs of all strains ranged from 0.625-10 µg/ml. The highest MICs (10 µg/ml) was with strains W1, W3, W6 and W7 and the lowest MIC was undetected in strain W2. The highest MBCs (20 µg/ml) was detected in strains W1, W3, W6 and W7 and the lowest MBC was 0.3125 µg/ml in strain W2.

LI12/05

Isolation and characterisation of imipenem-resistant bacteria in river water and farm soils

Fahd Alkhaleefah[1], Christine Murphy[1], Mark Osborn[2]

[1]University of Hull, Hull, UK, [2]RMIT University, Melbourne, Australia

Environmental bacteria are now being incriminated as sources of imipenem resistance genes with the potential for transfer to clinical isolates. Thus, the current study aims to investigate and characterise imipenem-resistant (IMR) bacteria within environmental samples. Water samples from Beverley Beck and soil samples from Riseholme and Lodge Farms in the U.K. were cultured onto imipenem-containing media. Phenotypic resistance to imipenem was observed in 4.5% and 13.5% of water and soil isolates, respectively, with MIC ranging from 4-32 mgl-1. 16S rRNA gene sequencing of 126 IMR isolates identified 13 different water and 25 soil species including known opportunistic pathogens, e.g. *Stenotrophomonas* spp. B-lactamase activity was detected in 53% of resistant isolates including detection of metallo-beta-lactamase (MBL), *Klebsiella pneumoniae* carbapenemase (KPC), and AmpC beta-lactamase in 74.6%, 14.9%, and 35.8% of isolates, respectively. Multiple antimicrobial resistance was observed in 56.4% of isolates. PCR and sequencing analysis using 12 different primer sets targeting IMR genes showed that resistant genes within these river water and farm soil isolates were not related to IMR genes within antibiotic resistant clinically important species. This indicates a potentially large and divergent gene pool for imipenem resistance within natural environments.

LI12/06

Effect of pH on the microbial degradation of anaerobic alkaline cellulose degradation products relevant to geological disposal.

Simon Rout[1], Christopher Charles[1], Charalampos Doulgeris[1], Alan McCarthy[2], David Rooks[2], Paul Loughnane[2], Andy Laws[1], Paul Humphreys[1]

[1]University of Huddersfield, Huddersfield, UK, [2]University of Liverpool, Liverpool, UK

Under anaerobic, alkaline conditions cellulosic materials are hydrolysed to a range of cellulose degradation products (CDP)

Please note: Abstracts are published as received from the authors and are not subject to editing.

capable of supporting microbial metabolism. The α and β forms of isosaccharinic acid are major components of CDP and are of importance to radioactive waste disposal since they are capable of complexing some radioelements and may increase their migration. Through the development of stable batch fed microcosms at three pH values (7.5, 9.5, 10.0), this study demonstrates that pH plays a major role in the degradation profiles of CDP and associated microbial communities. At pH 7.5, the data indicate a two stage degradation of ISAs to common fermentation end products, followed by methanogenesis through acetotrophic and hydrogenotrophic pathways. As pH increases, ISAs remain in the reactor fluid beyond the sampling period as a result of diminished rates of fermentation. Changes in community structures suggest that methanogenesis is impaired causing an accumulation fermentation products alongside other recalcitrant organic compounds at elevated pH. These may remain a source of complexants within a disposal facility.
This work was partially funded by the RCEP/NDA-RWMD Geological Disposal of Nuclear Waste programme.

LI12/07

Characterisation of Novel Iso-Saccharinic Acid Degrading Bacteria

Isaac Ampaabeng Kyeremeh, Chris J. Charlse, Simon P. Rout, Paul N. Humphreys, Andy Laws

University of Huddersfield, Huddersfield, UK

UK intermediate level radioactive waste contains a range of cellulosic materials that are susceptible to alkaline hydrolysis under the high pH conditions expected to develop within a deep disposal site. This alkaline hydrolysis generates cellulose degradation products (CDP) which are composed of a range of small MW organic compounds. The most important CDP being Iso-saccharinic acid (ISA), due to its ability to complex and mobilise some radionuclides. Consequently, the biodegradation of ISA under relevant environmental conditions is of interest. An alkali tolerant ISA degrading *Clostridium* species has been isolated from a CDP fed, pH 10 microcosms developed from anaerobic freshwater sediments and characterised by 16S rRNA gene sequencing (Accession Number KF514652). The isolate is able to germinate and metabolise ISA across a broad pH range (up to pH 12.0), however the generation of fermentation end products (primarily lactate) results in rapid reductions in pH, suggesting that the bacteria is able to modify its immediate environment to a more metabolically favourable pH.

LI12/08

Predicting the minimum liquid surface tension (γ) activity of pseudomonads expressing surfactants

Ibrahim Mohammed, Yusuf Deeni, Simona Hapca, Andrew Spiers

Abertay University, Dundee, UK

Bacteria produce a variety of chemically-diverse surfactants capable of significantly reducing liquid (aqueous) surface tension (γ, mN.m-1) and having a range of biological roles and biotechnological uses. In order to determine the strongest surfactant activity likely to found by bacterial surveys corresponding to the lowest achievable γ (γMin), we recovered a diverse collection of 355 isolates from contaminated soil and activated sludge using *Pseudomonas*-selective medium and identified 71 (20%) likely to express surfactants *in vitro* by drop--collapse assay. Liquid surface tension reducing ability (LSTRA) was quantitatively determined by tensiometry of culture supernatants, with 57 demonstrating significant LSTRA, lowering g to between 24.5 – 49.1 mN.m-1 (p = 0.05). Individual distribution identification (IDI) analysis was used to identify the theoretical probability distribution that best fitted the γ data (AD = 0.497), and the threshold parameter used to predict a γMin of 24.24 mN.m-1. This was in agreement with predictions based on published mixed–bacterial spp. LSTRA data and earlier work, suggesting a fundamental limit to the ability of bacterial surfactants to reduce g in aqueous systems. This implies a biological restriction on the synthesis and export of these agents or a physical-chemical restriction on their functioning once produced.

LI12/09

Does the hanging of traditional farm fresh turkeys for 21 days after slaughter affect microbiological status?

Sarah Gardner, Grace Burnham

Writtle College, Chelmsford, UK

Current regulations state the maximun time for hanging traditional farm fresh turkeys after slaughter is 15 days. The microbiological status of ten turkeys was investigated over a 21 day period following slaughter. Swabs of refrigerated turkeys were taken from the necks of five hens and five stags on days 0, 7, 14 and 21 after slaughter and dilutions tested for total viable count on nutrient agar. Samples were also plated on specific *E. coli*/coliform, *Listeria, Pseudomonas, Salmonella* and *Campylobacter* agars to identify the presence of any of these food contaminating bacteria. The colonies on these plates were tested using gram staining, catalase and oxidase testing to establish likely identification of the micro-organisms. *Escherichia coli, Staphylococcus* and *Listeria* were present at all time points, with growth on the nutrient agar and *E. coli*/coliform plates increasing over the first 14 days. These numbers were greatly reduced by day 21. On day 7 *Burkholderia cepacia* were identified on the *Pseudomonas* plates. Preliminary results indicate that hanging the turkeys for a further six days does not have a detrimental effect on the product.

LI12/10

Cellulose degradation in anoxic environments: metatranscriptomes and metagenomes

Anshul Gupta, James Houghton, David Rooks, Heather Allison, Alan McCarthy

University of Liverpool, Liverpool, UK

Anaerobic microbial communities that degrade cellulose are strongly associated with the herbivore gut, but relatively unexplored anoxic environments such as landfill sites can be viewed as a potential reservoir of novel cellulolytic agents. High-throughput sequencing of metagenomes and metatranscriptomes enables the analysis of these diverse microbial communities rich in uncultivable species, whilst overcoming the bias associated with the use of amplicon clone libraries. Gene mining for novel polysaccharide hydrolases, including cellulases, is also a core objective of this research. We are currently investigating the metatranscriptomes and metagenomes of cellulose biofilms recovered from landfill leachate and lake sediments. Fosmid DNA libraries have been generated from parallel samples and are screened for cellulase-encoding genes using primers designed from the metatranscriptomic data alongside

conventional expression screens. Bacteroidetes and Firmicutes are two predominant phyla in the lake sediment metatranscriptome, where ca. 40% of the sequences were complete unknowns and >500 predicted ORFs matched glycosyl hydrolases, of which almost half had no BLASTX matches and are therefore potentially novel. Apart from improving our understanding of cellulose degradation in anoxic environments, novel cellulases have commercial significance in biomass processing including production of second-generation biofuels.

LI12/11

Evaluation of Post-Extraction Clean-Up Protocols for Contaminated Nucleic Acid Preparations

Vince Moroney[1], Heather Callahan[1], Suzanne Kennedy[2], Mark Brolaski[1]

[1]MO BIO Laboratories, Carlsbad, California, USA, [2]Baylor College of Medicine, Houston, Texas, USA

Isolation of nucleic acids from complex samples can result in co-extraction of inhibitory substances that are carried through subsequent purification steps. These substances can impact nucleic acid yields, purities, and enzyme-based downstream applications, such as the polymerase chain reaction. Here we describe an improved method of post-extraction clean-up for nucleic acids that employs the precipitation of PCR inhibitors from solution. Efficacy was initially demonstrated by removing humic acid that co-eluted with purified bacterial DNA or RNA. The resulting yields, purities and PCR data were compared. For both DNA and RNA, the presence of humic acid resulted in inaccurate quantitation, low purity, and prevented complete PCR amplification even after dilution of the template. After clean up, yields were more consistent, purity improved, and amplification was uninhibited. To further validate the clean-up protocols, nucleic acids were extracted from an environmental (soil) and a clinical (stool) sample using established methods. Purified nucleic acids were cleaned-up post-extraction and the yields, purities, and PCR results were again compared. We found that commonly used extraction methods did not adequately remove inhibitors from difficult samples such as soil and stool and that post-extraction clean-up was necessary for not only accurate quantitation but the success of downstream applications.

LI12/12

Occurrence and diversity of two tetracycline resistance genes, *tet(B)* and *tet(C)*, in the water columns of the East Sea

Byung Cho, Hyunwoo Chung

School of Earth and Environmental Sciences, Seoul National University, Seoul, Republic of Korea

Bacterial resistance against tetracycline has not been studied much in the open ocean. Although two tetracycline resistance (TcR) genes, *tet*(B) and *tet*(C), have been detected in marine bacterial isolates, most of them weren't from the open ocean. Here, 118 tetracycline-resistant bacterial strains and 25 natural DNA samples were isolated from the East Sea at various locations and depth to detect and assess the diversity of these two genes. Among the two genes, only tet(C) was detected in seawater samples and ~10% of them were unique. Both genes weren't detected in the 118 strains. Further, new PCR primers were developed from "*tet*(B)" and "*tet*(C)" sequences on marine bacterial genomes found at IMG/ER database. PCR with the new primers failed for all the samples. Phylogenetic and protein-homology analysis of those gene sequences from bacterial genomes indicated that they might be new classes of TcR genes owing to low sequence identities with pre-existing TcR genes. Interestingly, putative-*tet*(B)s were homologous to substrate binding proteins. Overall, our results suggest that *tet*(B)s are rare in the open ocean, *tet*(C)s exist in the uncultured marine bacteria, and novel TcR genes exist in the sea at a low level.

LI12/13

A functional RpoS-mediated stress response is retained in long-term soil-adapted *Escherichia coli*

Yinka Somorin[1], Fiona Brennan[2], Florence Abram[1], Conor O'Byrne[1]

[1]National University of Ireland, Galway, Ireland, [2]The James Hutton Institute, Craigiebuckler, Aberdeen, UK

Recent studies have shown that *Escherichia coli* can persist in the external environment for long periods, contrary to an assumption of their transience in the environment but the mechanism for their survival in the soil unclear. The general stress response regulator, RpoS, is important for adaptation of *E. coli* to different environmental stresses, whereas it has been postulated that RpoS could be lost as a trade-off between stress protection and nutritional competence under certain conditions. Thus, this study was conducted to determine whether RpoS is functional in long-term (>10 years) soil-adapted *E. coli* strains or if it has been altered during the adaptation to soil. RpoS activity was determined indirectly using highly RpoS-dependent phenotype assays. All soil strains were acid tolerant, positive for catalase activity and glycogen synthesis, all of which are RpoS-dependent traits. The *rpo*S loci in soil-adapted strains were sequenced and compared with *E. coli* K-12 MG1655. Mutations occurred in all 5 soil-adapted strains but none of the mutations observed led to a truncation of the RpoS. The genotypic analyses are consistent with the RpoS-dependent phenotypes thus confirming that RpoS is functional in the soil-adapted strains.

LI12/14

Effect of soil structure and zeolite augmentation on bioremedation of petroleum hydrocarbon

Williams Joseph, Clive Roberts, David Hill, Iza Radecka

University of Wolverhampton, Wolverhampton, West Midlands, UK

The effect of soil structure and zeolite (clinoptilolite) augmentation on bioremediation was determined using crude oil artificially contaminated silty–clay and sandy soil composition from Hilton, East Shropshire, UK. These soils composition were formulated in the Laboratory based on their particle size distribution. A mixed culture of *Pseudomonas aeroginosa*, *Rhodococcus sp, Acinetobacter sp,* which were selected based on criteria that they were able to utilize hydrocarbons as the sole source of carbon and energy and were able to show significant growth in crude oil, was utilized for the bioremediation investigation. Soil amendment experiments at 30oC for a period of 30 days showed a more rapid and greater extent of apparent oil removal with the addition of both bacterial consortium and clinoptilolite in the clay soil than the sandy soil. There was 72.7% ± 0.8% oil removal by the bacterial consortium in the clay

Please note: Abstracts are published as received from the authors and are not subject to editing.

soil as compared to 55.6% ± 0.7% in the case of the sandy soil while 79.1% ± 0.4% oil removal by the bacterial consortium in the clay soil amended with clinoptilolite as compared to 67.3% ± 0.8% in the case of the amended sandy soils with clinoptilolite. Gas chromatographic profile showed appreciable reductions in hydrocarbon.

LI12/15

Identification of dominant bacterial species in agriculturally contaminated ground water using Multiple Displacement Amplification based approaches

Omolola Akinbami, Leonid Kulakov, Michael Larkin, Chris Allen

Queen's University Belfast, Belfast, UK

One of the great challenges of microbial analysis in natural environments derives from the fact that a large proportion of microorganisms present is not culturable in standard conditions. To overcome this, molecular approaches are widely used nowadays to successfully study environmental microorganisms. Multiple Displacement Amplification (MDA) is especially useful as it can be applied in conjunction with other techniques to identify genes derived from individual microbial cells.

Ground water samples obtained from an agriculturally contaminated site in Ireland were analysed in order to identify dominant bacterial strains encoding nitrate reducing genetic determinants. Several molecular approaches were applied to achieve this; with the most important being MDA assisted PCR. DNA was amplified from individual cells in three water samples possessing different, bacterial communities and it was shown that strains related to *Pseudomonas fluorescens* and *Polaromonas* sp. encoding narG gene (nitrate reductase) dominate the environment studied. Analysis of the corresponding 16S rRNA gene libraries identified the same bacterial strains amongst major species present on this site. However, a number of dominant 16S rRNA gene species found in the libraries (i.e. Janthinobacterium sp., Rhodoferax ferrireducens T118 were not detected when analysing amplified individual cell genomes.

LI12/16

Functional characterization of osmoprotective ATP-binding cassette (ABC) transporter Opp in Rhizobium etli

Shanjun Gao, Maarten Fauvart, Jan Michiels

Centre of Microbial and Plant Genetics, KU Leuven, Leuven, Belgium

Rhizobium etli is a gram-negative soil bacterium that elicits nitrogen-fixing nodules on its leguminous host plant Phaseolus vulgaris, the common bean plant. In order to thrive inside the nodule, R. etli needs to be adapted to the prevailing conditions, such as microoxic environment and presence of osmotic stress. In search of novel symbiotic genes, a genome-wide screening for R. etli symbiotic mutants was performed. Amongst the identified mutants, one carried an insertion in a transporter, classified as belonging to the Peptide/opine/nickel uptake transporter (PepT) family, better known as the oligopeptide ABC-transporter family. This system was named opp. Synteny analysis indicated that the opp system is linked to a conserved gene coding for a dehydrogenase, conserved amongst both symbiotic and pathogenic bacteria belonging to the α-proteobacteria. Plants nodulated by R. etli strains containing defined mutations in the opp genes showed strong reduction in symbiotic nitrogen fixation activity. Also the bacteroids of opp mutants were morphologically different from wild-type bacteroids. In the free-living state, the opp mutants exhibited aberrant cell morphology and their growth was affected by osmotic upshift. Together, our results indicate essential roles for a novel oligopeptide transporter in symbiosis and stress resistance during free-living growth in R. etli.

LI12/17

ScoCyc: a curated genome-scale network model of Streptomyces coelicolor A3(2) metabolism

Georgia Isom, Vincent Poon, Christophe Corre, Gregory Challis, David A. Hodgson, Jonathan D. Moore

University of Warwick, Coventry, UK

ScoCyc is a pathway/genome database for Streptomyces coelicolor A3(2), the model antibiotic-producing bacterium for Streptomyces. It is the only manually curated metabolic pathway database for Streptomyces and contains details of the specific enzymes, reactions and genes that formulate its biochemical network. ScoCyc is maintained using the Pathway Tools software, which allows the database to be updated based on recent publications and experimental data. The database currently consists of 206 pathways, 1398 enzymatic reactions and 1255 enzymes. This detailed network contributes to the understanding of S. coelicolor's complex metabolism and can be used as an accurate metabolic model for simulation experiments and synthetic biology. Consequently, ScoCyc could aid the productivity of antibiotic production in pharmaceutical industries, and could contribute to the discovery of novel antimicrobials. ScoCyc is available at http://biocyc.org.

LI12/18

LEA proteins are involved on desiccation resistance and other abiotic stresses in Azotobacter vinelandii

Julieta Rodríguez, Soledad Moreno, Guadalupe Espín

Instituto de Biotecnología UNAM, Cuernavaca, Morelos, Mexico

In plants, the expression of Late Embryogenesis Abundant Proteins (LEA) correlates closely with the acquisition of tolerance against drought, freezing and salinity stresses. In A. vinelandii we found two genes with homology sharing identity to putative LEA proteins from Arabidopsis thaliana and Artemia franciscana, the sequence of the genes contains a part of the LEA eukaryotic motif, but differs from the distinctive motive found in other bacteria, so this could be an example for the possibility of horizontal gene transfer between domains of life.

The aim of this work is to analyze if LEA proteins are involved on the resistance to desiccation, heat, frezzing and osmotic stress in both cysts and vegetative cells of A. vinelandii.

We constructed mutants for these genes. Cyst and vegetative cells were treated to desiccation for 3 months; osmotic stress was caused by different concentrations of NaCl and Sorbitol. We tested the tolerance of these mutants to high temperatures (60°C for 15 minutes) and frezzing (-20°C for a month).

Results: LEA proteins confer tolerance to desiccation on cyst cells and are necessary to survive at high concentrations of osmotic agents. LEA proteins also protect cyst and vegetative cells against

high and frezzing temperatures.

LI12/19

Distinctive bacterial communities on litter of different tropical tree species

Woosung Kim, Mincheol Kim, Jonathan Adams

Seoul National University, Seoul, Republic of Korea

We took leaf litter of three different species of tropical trees growth in a forest arboretum at FRIM in Malaysia. We extracted DNA and sequenced the 16S gene using HiSeq. Unlike temperate leaf litter, the bacterial community is dominated (>20% of reads) by many different species of Burkholderia. The bacterial community on the leaf litter of each type of tropical tree clusters separately on an ordination, revealing that each type of litter may have its own distinctive bacterial community. We discuss the implications of dominance of Burkholderia in the tropics, and the degree of substrate specialization revealed by tree-species-specific decomposer communities.

LI12/20

Survival of Campylobacter jejuni in nutrient poor water

Kasem Mustafa, Christina Bronowski, Nicola Williams, Paul Wigley, Tom Humphrey, Craig Winstanley

University of Liverpool, Liverpool, UK

Campylobacter jejuni is a major cause of bacterial diarrhoeal disease in humans, showing seasonal variation, peaking in spring. The transmission pathways for ~50% of human cases are unknown, and it is thought that environmental water reservoirs are important. The aim of this study was to compare the survival of different C. jejuni strains in sterile water at different temperatures.
A panel of C. jejuni strains, isolated from diverse sources (human, wild birds and bank vole), representing different sequence types, were tested for their ability to maintain culturability, and the expression of genes reported previously as important for survival was determined using quantitative PCR assays. We identified differences between the strains with respect to the maintenance of colony forming ability at day 3 (for 4°C) and at day 1 (for 25°C). Gene expression profiles also varied, potentially highlighting pathways important for water survival.
Survival in water clearly varies between different strains of C. jejuni with potential implications for strain transmission. Tests for cell viability and the expression of genes during survival in water can be used to elucidate the mechanisms underlying these variations.

LI12/21

A look into the microbial quality of open market foods in malawi

Rudia Lungu, Benson Chilima, Ambonishe Mwalwimba, Watipaso Kasambara, Abel Phiri

CHSU Publi Health Reference Laboratory, Lilongwe, Malawi

Objective
To determine microbial quality of food consumed in open markets and food handlers hygiene practices.
Introduction
Mishandling of food during food preparation or production is often the root of food contamination. In Malawi, ready-to-eat foods in open market places impact the spread of foodborne illnesses. Consumers of such foods usually need only the convenience of buying low priced foods with no concerns of safety, hygiene and quality.
Methods
26 samples of commonly consumed open market food were sampled and cultured to determine microbial contamination. Information on food handling, hygiene and sanitation practices was also acquired.
Results
7 Escherichia coli, 4 Serratia marcescens, 4 Proteus vulgaris, 2 Citrobacter freundii and 1 Proteus mirabilis was isolated with 78% of these having a significant level of contamination.
90% of markets had pay toilets and all markets except one had piped water within premises.
Conclusion
Common contamination was by *Escherichia coli, Serratia marcescens* and *Proteus vulgaris*. Food safety should also focus on food handling after cooking, utensils used and environmental hygiene.
References
1. Market Food Analysis – 2013, CHSU Microbiology Laboratory
2. WHO GFN – Laboratory Protocol, M.L. Mikoleit, 2010

LI12/23

A Genomic Approach To Applying The Use Of Microbial Enhanced Oil Recovery On Unconventional Oil

Sean Goodman[1,2], Heather Allison[1], Andrew Millar[2], Alan McCarthy[1]

[1]University Of Liverpool, Liverpool, Merseyside, UK, [2]BiSN Laboratory Services, Runcorn, Cheshire, UK

Crude oil is currently the principal source of energy, with an estimated 90 million barrels used each day worldwide. Over half of current oil reserves are comprised of unconventional or heavy resources. Nevertheless great difficulty has been had in recovering this oil, with field trials showing recovery rates of below 40% using current recovery methods. Traditionally, most studies have focused on the use of tertiary EOR (chemical/thermal) to improve oil recovery, but due to the environmental and economical disadvantages of these techniques, microbial enhanced oil recovery (MEOR) has been seen as a more practical approach. With numerous species of bacteria already present within these environments, MEOR uses these microbial populations and their metabolic products to increase oil recovery from low producing reservoirs. This study aims to show that unconventional oil is a feasible target for the use of MEOR, using an indigenous strain of bacteria isolated from a reservoir. Furthermore, using a new technique for Next Generation Sequencing, we aim to accurately identify potential MEOR related bacteria within a population from a number of diverse reservoirs, by eliminating the primer bias associated with conventional PCR based sequencing.

LI12/24

Establishment, survival and plant growth promotion of sulphur and phosphorus mobilising bacterial inocula

Please note: Abstracts are published as received from the authors and are not subject to editing.

Jayne Mullally, Achim Schmalenberger

University of Limerick, Limerick, Ireland

Mobilisation of soil nutrients not directly available to plants is one of several key functions of soil microbes and is of great economic value as ecosystem service. In this study, two Rifampicin resistant sulphur and phosphorus mobilising bacteria *(Burkholderia and Acidovorax)* were established in pot experiments with spring barley using biochar as a carrier. Sulphur and phosphorus mobilising activity by the inoculated bacteria were directly linked to plant growth promotion (PGP). Colony forming units of both bacteria were confirmed via colony PCR. Results showed that survival of bacterial inoculants decreased over the course of the pot trials with the *Burkholderia* strain dropping below the detection limit after 6 weeks. Trends in PGP were observed for both inoculants that were below significance. However, both bacteria had a clear impact on the rhizobacteria community composition as revealed via denaturing gradient gel electrophoresis. Biochar as a carrier had only a minor effect on the rhizobacteria community. Results from this study suggest that indigenous soil bacteria have out-competed the inoculants during the course of the experiment. These findings highlight that not only the metabolic capabilities of potential bacterial inoculants are important but also their competitiveness over indigenous rhizosphere microbes.

LI12/25

Microbial community dynamics during biodegradation of fluoranthene in the presence of plants

Sean Storey, Mardiana Mohd Ashaari, Gráinne McCabe, Mary Harty, Rita Dempsey, Owen Doyle, Nicholas Clipson, Evelyn Doyle

University College Dublin, Dublin, Ireland

Polycyclic aromatic hydrocarbons (PAHs) are toxic chemicals whose release to the environment occurs via natural and anthropogenic routes. They are ubiquitous environmental toxins; some are potent carcinogens and are classified as priority pollutants by both the European Union and the United States Environmental Protection Agency. Conventional approaches to environmental remediation include incineration, landfilling or solvent extraction. However, a more sustainable alternative is to use the metabolic potential of biological systems to clean up contaminated environments by degrading toxic compounds to less toxic forms. In this study, bacterial and fungal community responses were investigated during degradation of varying concentrations (0 - 5000 mg kg-1) of a representative PAH, fluoranthene, in the presence or absence of Tomato plants over 30 days. Fluoranthene degraded faster in the presence of plants at 500 and 5000 mg fluoranthene kg-1 soil ($p<0.001$), while 10 mg fluoranthene kg-1 was completely degraded regardless of the presence of a plant. Fluoranthene had a toxic effect on plant fresh weight, height, and spread. Bacterial and fungal community composition in the rhizosphere was significantly different from that in unplanted and bulk samples. The abundance of key degradative genes was higher in the presence of plants.

LI12/26

Comparison of a quantitative PCR assay for *Escherichia coli* against culture based methods in contaminated environmental samples

Joao Fernandes[1,2], Ian Singleton[1]

[1]School of Biology, Newcastle University, Newcastle Upon Tyne, UK, [2]Laboratorio de Analises, Instituto Superior Tecnico, Lisbon, Portugal

Current European guidelines for microbiological water quality are based on the cultivable detection and quantification of microrganisms such as *Escherichia coli*. The primary objective of present study was to provide data to support the use of a new quantitative PCR assay for *E. coli* detection. Primer sets for the amplification of the uidA single copy gene were tested against 65 *E. coli* strains isolated from different environmental water samples and against bacterial species other than *E. coli*. The PCR test detected all *E. coli* isolates and gave negative results with non *E. coli* isolates. Quantification was carried out by a standard curve based on several dilutions of a reference *E. coli* strain (NCTC, HPA) and compared to culture media plate counting. The detection limit of qPCR was 78 cfu. Preliminary qPCR assays (and comparative culture based methods) were performed with environmental water samples and also water samples inoculated with a mix of E. coli strains. The results reveal higher similarity for qPCR and culture based methods with the inoculated water samples suggesting further optimisation in relation to DNA extraction and purification. However, the work also demonstrates that the assay has potential for commercial application as it is rapid and cost-effective.

LI12/27

Inactivation of bacteria on TiO2 and SiO2 surface coatings with Ag or Cu

Mohamed N Abohtera

Salford University, Manchester, UK

Titanium dioxide is a semiconductor with a band gap of 3.2 eV. The major processes that occur upon absorption of a photon of light with sufficient energy to bridge the bandgap e.g. UVA generates an electron in the conduction band and a hole in the valence band. Electron and holes recombine in the bulk or can then diffuse and migrate to the surface where they can react to give highly reactive oxygen species (ROS) such as •OH and O2•– these are responsible for the destruction of organic pollutants including microorganisms. One potential application is the production of self-sterilizing surfaces. Inclusion of metals with antimicrobial activity such as Ag and Cu enhances the antimicrobial effect and gives activity in the dark as well as in UVA illumination. Coatings prepared with Ag and Cu with SiO2 rather than TiO2 are antimicrobial but do not have photocatalytic activity. The different types of surface will be compared for the inactivation of bacteria and their potential application in production of self-disinfecting surfaces will be discussed.

LI12/28

Bio-fungicidal effect of *azadirachta indica* in abating climate change impact on banana productivity

Oladipo, O. G., Ogunkanbi, D.a., Ayo-Lawal, R.a.

National Centre for Technology Management (NACETEM),Federal Ministry of Science and Technology, Obafemi Awolowo University, Campus, Ile – Ife, Nigeria.

Abstract

Incessant global population poses challenge on food security with over 800 million people deprived access to adequate food while about two billion are faced with hunger and malnutrition. Banana (*Musa* spp.) is the world's fourth most important food crop whose cultivation is threatened by climate change. Climate change and extreme weather conditions (flooding, drought, hurricanes etc.) impacts on agriculture resulting in decreased crop productivity and increased disease transmission. *Fusarium oxysporum*, a soil fungus causes Banana vascular wilts of roots, cortex and stele. Environmentally friendly Neem (*Azadirachta indica*) plant extract have been reported to gain importance over inorganic fungicides. This study investigated the efficacy of *Azadirachta indica* seed extract at 10, 20 and 50 % concentrations against F. oxysporum in Potato Dextrose medium using pour plate and cork boring methods. Results showed inhibited growth of *F. oxysporum* with 50 % having the highest growth inhibition. This indicates that *A. indica* seed extract has significant fungicidal effect on *F. oxysporum.* This paper seeks to encourage the use of environmentally safe bio-fungicides that reduce climate change impacts, control pests and diseases, improve banana yield and productivity and hence food security for the teeming population.

LI12/29

The Effect of Biotic and Abiotic Factors on Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Bacteria in Soil

Zohreh Khorasanizadeh

University of Hertfordshire, UK

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental contaminants with two or more aromatic rings and originating from different emission sources. They are extremely toxic to human, animals and plants. Consequently, the need to expand economical and practical remediation technologies for PAH contaminated sites is evident. The degradation was studied on the key model PAH (phenanthrene, anthracene, fluoranthene and pyrene) in J. Arthur Bower's top soil. The hypothesis for this study was that roadside soil would contain PAH degrading bacteria; pH would influence the microbial degradation of PAH, chemical oxidation of PAH would be as efficient as microbial breakdown of PAH and mobilising agents, would move PAH throughout soil, potentially making the PAH more available for biodegradation. The greatest degradations were found for the lowest molecular weight PAH, phenanthrene and anthracene; whilst lowest degradation was observed for higher molecular weight PAH, fluoranthene and pyrene. This study indicated microbial biodegradation was the most effective technique for removing of the PAH from contaminated soil, which was cost effective and easier to perform in comparison to the other two techniques. Microbial biodegradation could be improved by adjusting pH through liming if soil was acid.

LI13

Prokaryotic cell biology forum

LI13/01

Investigating the requirement of the BAM complex components, BamA-E, for the secretion of types Vb, Vc and Ve autotransporters in Gram-negative bacteria

Jessica Rooke[1,2], Timothy Wells[1], Amanda Rossiter[1], Douglas Browning[1], Irene Beriotto[1], Faye Morris[1], Yanina Sevastsyanovich[1], Ian Henderson[1]

[1]University of Birmingham, Birmingham, UK, [2]Cardiff University, Cardiff, UK

Many pathogenic factors in Gram-negative bacteria get to the outer membrane via Type V secretion. Type V secretion is separated into five different subtypes (Va- Ve) based on domain size and organisation. The best characterised subtype are the classical autotransporters (Va), originally thought to contain all elements required for its own secretion. Other factors are needed for secretion however, including the beta-barrel assembly machinery (BAM) complex which, in Escherichia coli, comprises five proteins, BamA-E. Studies show the secretion of Pet (type Va) requires the presence BamA & D but not BamB, C & E (Rossiter et al., 2011). Intimin (type Ve) and YadA (type Vc) secretion also requires BamA (Bodelon et al., 2009; Lehr et al., 2010). The roles of the other BAM complex components for the secretion of types Vb-Ve autotransporters however are largely unknown.

Here, we investigated the requirement of BamA-E in the secretion of type Vb (EtpB), Vc (SadA) and Ve (Intimin) secreted proteins. Our results indicate that BamA is essential for the secretion of EtpB and SadA and also suggests that the non-essential BAM components may play a more vital role than previously thought in the secretion of EtpB and Intimin.

LI13/02

The Paracoccus denitrificans NarK-type nitrate and nitrite transporters; probing nitrate uptake and nitrate/nitrite exchange mechanisms

Alan Goddard[1,4], Shilpa Bali[1], Victor Luque-Almagro[3], Andrew Gates[2], Despoina Mavridou[1], M. Dolores Roldán[3], David Richardson[2], Stuart Ferguson[1]

[1]University of Oxford, Oxford, UK, [2]University of East Anglia, Norwich, UK, [3]Universidad de Córdoba, Córdoba, Spain, [4]University of Lincoln, Lincoln, UK

Nitrate and nitrite transport across biological membranes is often facilitated by members of the major facilitator superfamily (MFS) of transporters. Paracoccus denitrificans contains an unusual arrangement in which two of these transporters, NarK1 and NarK2, are fused into a single protein, NarK. Complementation studies using a mutant strain lacking the nitrate/proton symporter NasA from the assimilatory nitrate reductase pathway are consistent with the proposed transport mechanisms for NarK1 (nitrate/proton symporter) and NarK2 (nitrate/nitrite antiporter). Unexpectedly, E. coli NarK and NarU which act as nitrate/nitrite antiporters can complement deletions in both narK and nasA, implying that these proteins can also act in the net uptake of nitrate. Although it has been previously demonstrated that two arginine residues are required for activity of P. denitrificans NarK1 and NarK2, little is known of the involvement of other residues in the transport process. In this study we have identified an essential proline residue in NarK1 and three essential proline residues in NarK2. There are no obvious amino acid side chain residues in the transmembrane helices to be candidates for reversible protonation during nitrate/xH+ transport, a feature which might relate to the capability of similar proteins to catalyse either nitrate/nitrite exchange or net nitrate uptake.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI13/03

Towards a better understanding of the bacterial type II secretion pathway

Badreddine Douzi, Sandra Michel, Frédéric Cadoret, Geneviève Ball, Bérengère Ize, Sophie Bleves, Chantal Soscia, Christian Cambillau, Romé Voulhoux

CNRS, Marseille, France

The bacterial type II secretion system (T2SS) is unique in its ability to promote the transport of large folded and sometimes multimeric proteins. In this secretion process, exoproteins are first translocated into the periplasm. The final release into the medium requires a multiprotein complex called the secreton. Although the 12 individual components of the secreton have been identified, its mode of action remains obscure. We set up various dedicated in vitro and in vivo protein-protein interaction experiments to identify the *Pseudomonas aeruginosa* Xcp T2SS periplasmic interactome. BIAcore experiments revealed that three Xcp components, XcpP, the secretin XcpQ, and the pseudopilus tip, directly and specifically interact with secreted exoproteins. Affinity chromatography co-purification indicated that the XcpY periplasmic domain interacts with the secreted substrate and a component of the pseudopilus tip XcpW. Interestingly, the periplasmic domain of another member of the Xcp inner membrane platform, XcpZ co-elutes with the XcpY/substrate and the XcpY/XcpW complexes during affinity chromatography. Finally the direct interaction between the secreted substrate and XcpY was confirmed by in situ photo-crosslinking. All together, our results allowed us to propose the most advanced integrative model of Xcp T2SS assembly and function.

LI13/04

Peptidoglycan Modification by Predatory *Bdellovibrio*

Ana Clark, Carey Lambert, Liz (RE) Sockett

University of Nottingham, Nottingham, UK

Peptidoglycan-modification is a vital early event in the predatory life-cycle of *Bdellovibrio bacteriovorus,* both modifying the predator peptidoglycan to squeeze through a small pore in the prey cell outer membrane and also reshaping its prey into the characteristic rounded bdelloplast structure upon invasion. The *Bdellovibrio* genome contains genes encoding two D-ala D-ala carboxypeptidases (DacB enzymes) and Lerner and co-workers (2012) showed that deletion of both of these prevents the Bdellovibrio from rounding-up prey and this is coupled with a reduced overall predation-rate. During an SGM Summer Bursary project, we have set up a microscopic assay to study what happens to the double deletion mutant *Bdellovibrio,* when it invades prey without expressing these DacB proteins. To do this we used a fluorescent prey-cell assay and studied their invasion by timelapse microscopy. The *Bdellovibrio* do still enter prey cells but they occupy a localised niche within prey periplasms. That the niche is hollowed out in the prey-periplasm by the double *dacB* deletion *Bdellovibrio* is shown by the influx of periplasmic fluorescent protein.
Studying these genes and their products will provide us with greater knowledge about how this fascinating bacterium undertakes its unique periplasmic lifecycle and attacks cell walls without initially breaking them.

LI13/05

Identification of Toxin-Antitoxin Systems of *P. aeruginosa* and Study of their Role in Persistence

Marco Garavaglia[1], Patrick Scheu[2], Urvish Trivedi[3], Stephan Heeb[1], Kendra Rumbaugh[3], Kenn Gerdes[2], Paul Williams[1]

[1]University of Nottingham, Nottingham, UK, [2]Newcastle University, Newcastle upon Tyne, UK, [3]Texas Tech University, Lubbock, USA

Toxin-Antitoxin (TA) protein pairs are encoded by adjacent, co-transcribed genes. Toxins mostly act as mRNases decreasing the global translation rate in bacterial cells. The activity of these proteins is modulated by antitoxins that counteract the inhibitory effect of their cognate toxins by direct interaction. Toxins are far more stable than their cognate antitoxins. Indeed, the latter are rapidly degraded by proteases under unfavourable conditions.
E. coli TA systems, along with the Lon protease, have been shown to be involved in the modulation of persistence upon treatment with antibiotics. Persistence is defined as a multidrug tolerance and it is not related to resistance since it does not involve genetic alterations. The Lon protease has been reported to activate TA loci-encoded mRNases in E. coli by catalizing the degradation of their cognate antitoxins. Consistently, Lon plays a role in the regulation of persister cell formation in this bacterium. Interestingly, TA systems are widely conserved amongst bacteria, including major pathogens.
The aim of this work is to study the yet uncharacterized TA systems of the opportunistic human pathogen Pseudomonas aeruginosa, focusing in particular on the possible involvement of these proteins and of the Lon protease in the regulation of P. aeruginosa persistence.

LI13/06

Identifying novel functions in the *Staphylococcus aureus* exoproteome

Jamal Alorabi, Daniel G. Rigden, Malcolm J. Horsburgh

University of Liverpool, Liverpool, UK

Staphylococcus aureus secretes a diverse array of proteins with roles in virulence, survival and nutrient uptake. Individual roles of the exoproteome have been identified including cell adhesion, immune evasion, iron uptake, and antioxidant defence among others. Given the many functions ascribed to the exoproteome a surprising proportion remains uncharacterised. The aim of this study is to reveal new functions within this protein fraction.
Proteins with a signal peptide were retrieved from the complete proteome of S. aureus strain Newman and those with unassigned function were identified. This revealed 56 (33%) out of 169 secreted proteins with a signal peptide (SP) were not ascribed a function. To look for similarity with distantly similar sequences multiple sequence (MSAs) and homologous structures by using modern HH-suite software locally. Three proteins were identified that matched the database significantly, with more then 99% probability and identity ≥ 27%. Each of these 3 proteins match with enzymes and have active sites for indicating roles in cell-matrix interactions, capsule/PIA modification and iron uptake. The roles of the predicted enzymes are being tested using gene inactivation and overexpression/purification/activity studies.

LI13/07

An investigation of the effects of light on the growth of Listeria monocytogenes in food-related environments

Kerrie NicAogáin, Beth O'Donoghue, Conor O'Byrne

National University of Ireland, Galway, Ireland

Listeria monocytogenes is a gram positive bacterium which has become a major concern within the food processing industry. Its ability to withstand harsh environments within food processing factories can lead to the contamination of food products. L. monocytogenes can cause a severe gastrointestinal infection called listeriosis which is a rare infection but has a high mortality rate among immunocompromised people and pregnant women. Recently it has been found that L. monocytogenes possesses a light sensor encoded by the lmo0799 gene. This protein is thought to be involved in the sensing of blue light and in turn the activation of the general stress response through the regulator B. A method for testing the effect of blue light (475nm) in combination with food-related conditions such as different salt concentrations, pH levels and temperatures in 96 well plates has been developed and preliminary results have shown that this system has an inhibitory effect on the growth of L. monocytogenes. It has been observed that the growth of the L. monocytogenes also differs between solid surfaces and in liquid culture. Therefore future study will investigate these differences.

LI13/08

Protein engineering, expression, and purification of soluble methane monooxygenase

Yasin Y Y AL-Luaibi[1], Tim Nichol[1], Malcolm Lock[1], Colin J Murrell[2], Thomas J Smith[1]

[1]Biomedical Research Centre, Sheffield Hallam University, S1 1WB, Sheffield, UK, [2]Department of Environmental Sciences, University of East Anglia, Norwich Research Par, Norwich, UK

Methanotrophic bacteria utilise methane as their sole source of carbon and energy. Soluble methane monooxygenase (sMMO), which is produced by certain methanotrophs such as Methylosinus trichsporium (OB3b), consists of three components: a hydroxlyase with an $(\alpha\beta\gamma)2$ structure with diiron centre in the active site, a coupling/gating protein and an NAD(P)H-dependent reductase. In addition to methane, sMMO also co-oxidises diverse hydrophobic substrates including naphthalene, biphenyl and trichloroethylene (TCE), but not phenanthrene and anthracene.

A number of mutants have been designed and constructed with the intention of increasing the activity of sMMO toward large substrates by diminishing ionic interactions that may control substrate entry into the enzyme and by altering the hydrophobic environment within the active site. Other mutants have been constructed to alter the coordination environment of the diiron centre. In addition, a new affinity tag system has been constructed toward developing a one-step purification system for mutant and wild-type sMMO hydroxylases. The system is highly effective for purification of the hydroxylase from recombinant M. trichosporium OB3b although currently active protein is not consistently obtained. If active recombinant enzyme can be reliably prepared via this system, it will facilitate purification and analysis of sMMO mutants.

LI13/09

The development of a novel cell based assay for the detection of Sortase

Carmen Tong[1], Bethany Mills[1], Victoria Steele[1], Weng Chan[2], Paul Williams[1] and Phil Hill[1]

[1]School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham, University Park, Nottingham NG7 2RD [2]School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, UK

Drug-resistant strains of pathogenic bacteria are becoming an increasing issue globally, and the need for alternative drug therapies is crucial. Sortase are a group of Gram-Positive transpeptidases responsible for cell wall protein anchoring through the recognition of a highly conserved LPXTG motif on secreted cell wall proteins. The presence of Sortase has been directly linked to virulence and ability to establish infection of many Gram-positive bacteria including *Staphylococcus aureus*, and are therefore of particular interest as a target.

We have developed a novel cell based assay which detects the activity of Sortase. This assay utilizes a *Staphylococcus aureus* that has been engineered to surface-express the photoprotein Gaussia Luciferase (GLuc). The GLuc produced possesses an LPXTG motif, meaning that after secretion, is cleaved by Sortase and anchored to the cell wall. However, when Sortase is inactive, the GLuc is secreted into the supernatant. By separating the cell lysate from the supernatant, the activity of Sortase can be determined by observing the levels of bioluminescence in the cell lysate and the supernatant. This cell-based assay can be utilised as a method of screening for novel Sortase inhibitors.

LI14

Prokaryotic genetics forum

LI14/01

The phylogeny of actinobacteria revisited in the light of complete genomes.

Arnab Sen[1], Vincent Daubin[2], Danis Abrouk[2], Alison Berry[3], Isaac Gifford[3], Philippe Normand[2]

[1]University of North Bengal, Siliguri, India, [2]Université Lyon I, Université Lyon, Villeurbanne, France, [3]University of California, Davis, USA

The reconstructed phylogeny of actinobacteria, that has varied a lot over the years, was reassessed using complete genomes. A concatenate of 54 conserved proteins present in single copy and satisfying the Bidirectional BestHit criterion in 100 actinobacterial genomes representing 35 families and 17 orders, was built, yielding a tree which branches were analyzed by bootstrap. In parallel, a 16S tree, a 16S+23S tree and a MLSA tree (AtpI, GyrA, FtsZ, SecA and DnaK) were constructed. All these trees were then tested with the Prunier method to assess the reliability of nodes quantifying lateral transfers using 500 proteins different from those that had been used to build the concatenate tree. The best tree recovered was the concatenate tree. In that tree, the orders *Bifidobacteriales, Coriobacteriales, Corynebacteriales, Micromonosporales, Propionibacteriales, Pseudonocardiales, Streptomycetales* and *Streptosporangiales* were recovered while the *Frankiales* and *Micrococcales* were not. It is proposed that the order *Frankiales*

Please note: Abstracts are published as received from the authors and are not subject to editing.

be split into *Frankiales* (*Frankiaceae*), *Geodermatophiliales (Geodermatophiliaceae*), *Acidothermiales* (*Acidothermaceae*) and *Nakamurellales* (*Nakamurellaceae*). It is also proposed that the order *Micrococcales* be split into *Micrococcales* (*Kocuria, Rothia, Micrococcus, Arthrobacter, Tropheryma, Microbacterium, Leifsonia and Clavibacter)*, *Cellulomonales* (*Beutenbergia, Cellulomonas, Xylanimonas, Jonesia and Sanguibacter)* and B*rachybacteriales* (*Brachybacterium)*.

LI14/02

Linking genes with outer membrane homeostasis – a TraDIS-ional approach

Ashley Robinson[1], Josh Quick[1], Nick Loman[1], Keith Turner[2], Rocky Cranenburgh[3], Ian Henderson[1]

[1]University of Birmingham, Birmingham, West Mids, UK, [2]Discuva, Cambridge, UK, [3]Cobra Biologics, Keele, UK

Gram negative bacteria are characterised by a cell envelope comprised of two membranes separated by a periplasm. The outer membrane of the cell envelope provides natural resistance to many environmental conditions including, especially of interest to medicine, antibiotics. Therefore, understanding which genes are involved with the formation and maintenance of the outer membrane may lead to targets for antimicrobial agents.
Transposon Directed Insertion site Sequencing (TraDIS) is a technique with which it is possible to simultaneously assay the contributions of every gene towards survival against physiological growth conditions. In our lab, we are working towards the development of our own TraDIS experimental design in *Escherichia coli*, with a view to looking for genes involved with the maintenance of the cell envelope.
So far, we have adopted a PCR based approach to the generation of sequencing libraries and these libraries have been sequenced successfully using the Illumina MiSeq. Currently, we are in the process of validating and finalising the necessary bioinformatic analysis, in order to generate an analytical pipeline which will be used in future experiments to tell us which genes contribute to outer membrane homeostasis.

LI14/03

Observation of global transcriptional alteration in *Staphylococcus aureus* during stringent response by next generation sequencing (RNA-seq)

Sari Alhoufie

Slaford University, Manchester, UK

Stringent response is a physiological state that bacteria exhibit to survive when they encounter extreme conditions, such as nutrient limitation and heat or cold shock. In this circumstance, the bacteria can economise their nutrient consumption by reducing its replication, metabolic and other activities to a minimal rate until the surrounding environment is improved.
Mupirocin is a topical agent that been used to treat S.aureus associated wound infection and to decolonise S.aureus from nasal carriage in hospital. It targets protein synthesis in bacteria by inhibiting the synthesis of the isoleucyl-tRNA synthase IleS resulting in accumulation of uncharged tRNA that consequently triggers stringent response in S.aureus.
Sub-inhibitory concentration of mupirocin influence on the transcriptional profiles throughout 1, 12 and 24h has been observed by RNA-sequencing technique. Results revealed that this inhibitory concentration is adequate to trigger stringent response up to 12 hours of exposure. S.aureus recovered from the stress and displayed at 24h similar growth rate and transcriptional profile to normal growth condition at 12h. Comparison between time points transcriptional profile showed interesting alteration on S.aureus genes regulation.

LI14/04

Structural Studies on the V. cholerae ToxR Regulon

Gabriel E. Wagner, Evelyne Schrank, Vera Fengler, Joachim Reidl, Klaus Zangger

University of Graz, Graz, Austria

Cholera, which is an acute intestinal infection caused by the bacterium Vibrio cholerae, is a major threat for developing countries. Despite the availability of vaccination and treatment, infection with V. cholerae is endemic in rural areas. The bacteria have to persist in two quite different environments, the aquatic system and the human host. Therefore the expression of the major virulence factors, the cholera toxin and the toxin coregulated pilus, is tightly regulated. A key feature of this network is the so-called "ToxR regulon", including the proteins ToxR and ToxS. Amongst others, they regulate the transcription of the master virulence gene regulator ToxT. Furthermore, as transmembrane proteins, they are supposed to be important for the signaling across the membrane.
In this study we characterize the periplasmic sensory domains of ToxR and ToxS and the cytoplasmic DNA-binding domain of ToxR. Emphasis is put on the investigation of their redox state and their oligomerization under varying conditions. Together with the determination of the structure and dynamical behavior of the proteins, this should lead to a better understanding of the regulatory mechanisms of V. cholerae.

LI14/05

Random insertion and gene disruption via transposon mutagenesis of *Ureaplasma parvum* using a mini-transposon plasmid

Ali Aboklaish[1,2], Emilie Dordet-Frisoni[3,4], Christine Citti[3,4], John Glass[5], Owen Brad Spiller[1]

[1]Cardiff University, School of Medicine, Department of Child Health, 5th floor, University Hospital of Wales, Cardiff, CF14 4XN, UK, [2]Sebha University, Faculty of Engineering and Technology, Medical Laboratory Sciences Department, P.O. Box 68, Brack, Libya, [3]INRA,, UMR 1225, IHAP, 31076 Toulouse, France, [4]Université de Toulouse, INP, ENVT,, UMR 1225, IHAP, 31076 Toulouse, France, [5]J. Craig Venter Institute,, Rockville MD, USA

While transposon mutagenesis has been successfully used for *Mycoplasma* spp. to disrupt and determine non-essential genes, previous attempts with *Ureaplasma* spp. have been unsuccessful. Using a polyethylene glycol-transformation enhancing protocol, we were able to transform three separate serovars of *Ureaplasma* parvum with a Tn4001-based mini-transposon plasmid containing a gentamicin resistance selection marker. Despite the large degree of homology between *Ureaplasma parvum* and U*reaplasma*

urealyticum, all attempts to transform the latter in parallel failed, with the exception of a single clinical *U. urealyticum* isolate. PCR probing and sequencing were used to confirm transposon insertion into the bacterial genome and identify disrupted genes. Transformation of prototype SV3 consistently resulted in transfer only of sequence between the mini-transposon inverted repeats, but some strains showed additional sequence transfer. Transposon insertion occurred randomly in the genome resulting in unique disruption of genes UU390, UU450, UU520, UU582 for single clones from a panel of screened clones. An intergenic insertion between genes UU187 and UU188 was also characterised. Two phenotypic alterations were observed in the mutated strains: Disruption of a DEAD-box RNA helicase (UU582) altered growth kinetics, while the *U. urealyticum* strain lost resistance to serum attack coincident with loss of expression of a 41 kDa protein.

LI14/06

Pan-genome analyis of emerging pathogen, *Achromobacter xylosoxidans*

Pisut Pongchaikul[1,3], Craig Winstanley[2], Pitak Santanirand[3], Alistair Darby[1]

[1]Institute of Integrative Biology, University of Liverpool, Liverpool, UK, [2]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK, [3]Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Members of the genus Achromobacterare emerging as important pathogens in the human. The majority of Achromobacter infections are caused by Achromobacter xylosoxidans, a non-motile Gram-negative bacilli able to cause a broad range of infections, including both acute and chronic infections. Although the genome of one strain associated with an infection of a Cystic Fibrosis patient, A. xylosoxidans NH44784-1996, has been annotated and published, information about the genome of A. xylosoxidans is generally lacking. In this study, we carried out whole genome sequencing of genomic DNA from 38 A. xylosoxidans, including clinical isolates from the UK (n=10) and Bangkok (n=14) and type strains (n=2), using the Illumina HiSeq platform. The sequence data obtained, combined with the complete genome of A. xylosoxidans NH44784-1996, were analysed using various bioinformatic tools. The results revealed that the core genome was approximately half of the genome size. Pan-genome analysis suggests that the genome is open. Phylogenetic trees based on core genes were used to compare UK and Thai isolates of A. xylosoxidans, as well as identify recombination events within species.

LI14/07

Whole Genome Amplification from clinical samples: Advances in Cryptosporidium genotyping from limited numbers of oocysts

Jenna Alexander[1,2], Stephen Hadfield[2], Guy Robinson[2], Rachel Chalmers[2], Justin Pachebat[1]

[1]Institute of Biological, Environmental, and Rural Sciences, Aberystwyth University, Aberystwyth, Ceredigion, UK, [2]Cryptosporidium Reference Unit, Public Health Wales Microbiology ABM, Swansea, Abertawe, UK

Cryptosporidium is a parasitic protozoan that causes self-limiting but severe gastroenteritis in immuno-competent human hosts and possible prolonged morbidity and/ or mortality in immuno-compromised hosts. The study of Cryptosporidium is hampered by an insufficient understanding of species sub-types and diversity. Whole genome reads can give a wealth of information about infectivity, phylogeny, and host specificity, but to date only three species have been genome sequenced and published. Sequencing directly from clinical samples is often impossible due to the small number of oocysts that can be present in stool samples. To generate enough gDNA for downstream bioinformatic analysis either clinical isolates must be passed through animals, which is time consuming, expensive, and raises ethical issues regarding animal welfare, or whole genome amplification must be performed. Currently gDNA recovery from Cryptosporidium oocysts, and therefore whole genome amplification efficacy, is limited by the high quantities of DNA lost during all extraction and purification steps. We will discuss the development of an efficient method of genomic DNA extraction, isolation, amplification and sequencing of Cryptosporidium spp. DNA from stool samples containing limited numbers of oocysts and the application of this method to clinical isolates stored at the Cryptosporidium Reference Unit.

LI14/08

Dissection of biophysical properties and biological activities of Bet/RecT family DNA recombination proteins

Wen-yang Chen, Tianfan Cheng, Jian-Dong Huang, Rory Watt

University of Hong Kong, Hong Kong, Hong Kong

Single strand DNA annealing proteins (SSAPs) of viral/phage origin play a variety of roles in homologous recombination-based DNA repair and genetic exchange processes in prokaryotes. Bet from bacteriophage lambda and RecT from *Escherichia coli* are the prototypical SSAPs. Bet/RecT family SSAP proteins putatively share a conserved multimeric organization and arrangement of secondary structural units.

Here, we use a combination of biophysical and biological approaches to dissect structure-activity relationships within a diverse selection of SSAP proteins, including: *E. coli* RecT; Lambda-Bet; s065 from the SXT genetic element of *Vibrio cholerae* (SXT-Bet) and Bet from *Laribacter hongkongensis* (LHK-Bet). Single nucleotide alteration and double strand DNA deletion activities were investigated using *E. coli* reporter systems.

We show that Bet/RecT SSAP proteins that have been rationally-truncated at the N-terminus, but not at the C-terminus, exhibit DNA recombination activities. The removal of specific secondary structural units from Bet/RecT SSAPs profoundly affects protein multimer formation in a complex manner. Binding assays indicate that DNA recombination activities are not directly related to single strand DNA binding abilities. Our results suggest that a complex interplay of protein-protein and protein-nucleotide interactions underlie DNA recombination activities in Bet/RecT family SSAP proteins.

LI14/09

Mutualism breakdown in *Wolbachia - Drosophila* association

Ewa Chrostek, Luis Teixeira

Please note: Abstracts are published as received from the authors and are not subject to editing.

Instituto Gulbenkian de Ciência, Oeiras, Portugal

Wolbachia, endosymbionts of many insect species and probably the most prevalent infectious bacteria on the planet, exhibit a range of interesting phenotypes. *Wolbachia* are famous master manipulators of insects reproduction: to favour their spread they can induce male killing, parthenogenesis or cytoplasmic incompatibility. *Wolbachia* can also protect various insect from pathogens, which makes them a perfect tool for the control of vector-borne diseases - mosquitos with *Wolbachia* have already been released in the wild to eliminate Dengue. However, as *Wolbachia* cannot be cultured or genetically manipulated the mechanisms of their interactions with the hosts remain largely unknown.
Our work aims at understanding the interaction of the endosymbionts of *Drosophila melanogaster,* wMel, with their hosts. Although natural wMel variants are mutualistic, the laboratory variant wMelPop proliferates massively in the hosts, causing degeneration of tissues which culminates in insets early death. Sequencing and assembly of the genomes of wMelPop and very closely related wMelCS allowed us to identify genetic differences between the two. We demonstrate that the identified differences are responsible for the pathogenic phenotype of wMelPop.
Our work is essential to understand the biology of *Wolbachia* and it provides the first link between genes and phenotypes in these organisms.

LI14/10

Genome Sequence Determination and Comparative Genomics of Uropathogenic E. coli ST127

Majed Alghoribi[1], Scott Beatson[2], Mathew Upton[3]

[1]Microbiology and Virology Unit, School of Translational Medicine, Manchester, UK, [2]Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Australia, [3]Medical Microbiology, School of Biomedical & Healthcare Sciences (Plymouth University Peninsula Schools of Medicine and Dentistry), Plymouth, UK

Uropathogenic E. coli (UPEC) is the most frequent cause of UTI, being responsible for up to 85% of community acquired UTI and 25% of nosocomial UTI.   An in vivo study using Galleria mellonella showed that larvae inoculated with ST127 isolates (104 colony-forming units) had significantly higher mortality compared to other UPEC including those from ST73 (P≤ 0.0024) and ST131 (P≤ 0.0004). However, an interesting result to emerge from the data was the observation that one strain of ST127 (EC18) did not show any lethal effects, even for high inoculum doses (up to 2.33×107 cfu/10μl).
Using comparative genomic analyses to identify the genetic basis for UPEC ST127 EC18 non-lethality in the Wax moth model G.mellonella. The genomic analyses revealed a major deletion in the O-antigen cluster, which is likely to explain the lack of virulence in the larvae. Evidence of bacterial insertion sequence IS1 was seen at the position where the O-antigen deletion occurs in EC18.
This study illustrates that G.mellonella can be used to investigate the virulence of UPEC strains. The outcome of this analysis will give considerable insight into the association of different E. coli virulence genes with lethality in the experimental model of G. mellonella larvae infection.

LI14/11

Excision and conjugative transfer of the *H. pylori* ICE-like genomic island, *tfs4*

Amberley Stephens, Rob Delahay

Nottingham Digestive Diseases Centre Biomedical Research Unit, University of Nottingham, Nottingham, UK

Introduction
H. pylori has high genetic diversity. Plasticity zones (PZ) are areas of high variability within the population, individual genes and whole zones can be gained and lost. *H. pylori* PZ 1 contains a type IV secretion system, tfs4, which can be variable. *Tfs4* contains markers for conventional ICE-like self-mobilisation, such as a *virD2* relaxase and *xerD* integrase/recombinase genes as well as their recognition sites.
Aim
Our aim is to show Tfs4 is capable of self-mobilisation regardless of its variants.
Results
Initial mating assays show transfer of *tfs4* and an antibiotic marker between donor and recipient strains regardless of the *tfs4* variant present. During transfer, XerD mediated excision of DNA allows circular intermediates (CI) to form. These have been isolated and sequenced to show distinct insertion/excision sites. Isolations of CI's from strain P12 showed formation of CI's is independent of a functional Tfs4 and does not appear to be induced.
Further work
Ongoing work aims to establish whether these CIs are then transferred via Tfs4 and to investigate the mechanism behind the VirD2 relaxase recognition of motifs.

LI14/12

The determination of the ancestral sequence of *Pseudomonas aeruginosa* PAO

Hardeep Naghra[1], David Turner[1,2], Mathew Diggle[2], Chan Kok Gan[3], Richard James[1], Stephan Heeb[1]

[1]University of Nottingham, Nottingham, UK, [2]Queen's Medical Centre, Nottingham, UK, [3]University of Malaya, Kuala Lumpar, Malaysia

The most commonly used strain in the study of the opportunistic pathogen Pseudomonas aeruginosa is PAO1 - a spontaneous chloramphenicol-resistant mutant of the original PAO which was isolated from a wound in Australia in 1954. This original isolate, from which the PAO1, PAO2 and PAO3 strains were derived, is no longer available. PAO1 exists in laboratories across the world as sublines in which varying genotypes have emerged over time. The current reference genome for Pseudomonas aeruginosa PAO1 is based on a laboratory subline from the University of Washington, which contains genetic differences compared to the other laboratory sublines. The most extreme example of this is a 2.2-Mb inversion between two ribosomal operons, rrnA and rrnB, which has so far been found to be unique to the reference strain. It is for this reason that we have built a more complete repertoire of PAO derivatives, by carrying out whole genome sequencing of various laboratory PAO1 sublines, PAO2 and PAO3, in order to deduce the consensus sequence of the ancestral Pseudomonas aeruginosa PAO. This could be used as a universal reference sequence for PAO1, which would be of particular importance for strand-specific RNA sequencing and for the genetic characterisation of laboratory sublines.

LI14/13

A time course transcriptome study of *Salmonella* Typhimurium during the switch from planktonic to biofilm growth

Van Puyvelde S.[1]*, Fierro A.C.[2], De Maeyer, D.[2], De Coster D.[1], Steenackers H.[1], Marchal K.[2], Vanderleyden J.[1]

[1]Centre of Microbial and Plant Genetics, KU Leuven, Kasteelpark Arenberg 20, Heverlee, Belgium

[2]Dept. of Plant Biotechnology and Bioinformatics, U.Ghent, Technologiepark 927, Gent, Belgium

This study describes *Salmonella* biofilm development during the first 24 hours. Hereto, we grew biofilms in a petri dish assay that enables both planktonic and biofilm sampling. Biofilms were first studied phenotypically by counting the cell distribution between the planktonic and biofilm fraction, and by microscopy. The CFU counts of both the planktonic and biofilm fraction revealed a well-timed phenotypic switch from planktonic to biofilm growth.
To unravel the dynamics in the regulatory cascades during this switch from planktonic to biofilm growth, we subsequently conducted a time course transcriptome experiment using tiling arrays. Ten time points each were studied of both the planktonic and biofilm fraction, giving a global overview of the genetic profiles during the switch. Our dataset identifies differentially expressed genes during the different phases of initial biofilm development, namely during: (i) preparation/determination of planktonic cells to become a biofilm cell; (ii) the switch from planktonic to biofilm growth and (iii) biofilm growth. We analyzed these genes by a time-dependent and condition-dependent clustering to classify all changes and defined the responsible regulators by a network approach. Hereto, we constructed a physical interaction network of *Salmonella* Typhimurium.

References

1 Steenackers, Hermans *et al*. (2012), *Food Res Int* 45: 502-531.

LI15

Virology workshop: DNA viruses

LI15/01

To Elucidate The Assembly Of The Epstein-Barr Virus Replisome

Chrisopher Traylen, Rajaei Almohammad, Alison Sinclair

University Of Sussex, Brighton, UK

Epstein-Barr virus (EBV) is a double stranded DNA virus and is a member of the γ-herpesvirus family. EBV widely infects people causing a variety of diseases, from asymptomatic infection to association with certain tumours including Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma. EBV encodes an immediate-early protein called Zta (also known as BZLF1, EB1, ZEBRA), which is important in disrupting latency and which acts a both a transcription and replication factor. Zta co-associates with a variety of different proteins in order to promote transcription and to assemble the replication components necessary for efficient viral replication at the viral lytic origin recognition site (Ori-Lyt). We have identified mutant versions of Zta that are transcription competent but defective for viral replication. We are using these to probe the mechanisms that differentiate the transcription and replication functions of Zta using biochemical analyses of the sizes and composition of Zta complexes using gel-filtration and a proteomic analysis of Zta complexes using SILAC (Stable Isotope Labelling by Amino acids in Cell culture) combined with mass spectrometry analysis. Similarities and differences between the replication competent and replication defective mutants of Zta in EBV positive and negative cells will be presented.

LI15/02

Investigating cellular pathways involved in the egress of BK Polyomavirus

Gareth Evans, Victoria Foster, Colin Crump

Cambridge University, Cambridge, UK

The recent rise in the number of human polyomaviruses and their continued link to cancer, as well as the increased use of immunosupressive therapies leading to polyomavirus-associated diseases such as nephropathy, haemorrhagic cystitis and progress multifocal leukoencephalopathy, has dramatically increased our need to understand the life cycle of these viruses. Many studies have investigated the mechanisms involved in virus entry, gene expression, genome replication and potential transformation, yet little is known about the egress of polyomaviruses from infected cells. Polyomaviruses are non-enveloped and so it is generally assumed that cell lysis is the main method of viral release, although given these viruses establish life-long persistence this may be unlikely and non-lytic mechanisms may be involved in polyomavirus egress. We have screened a large panel of inhibitors to target potential pathways that may be involved in the egress of BK polyomavirus by comparing cell-associated and released infectious titres. We have identified at least one inhibitor, that specifically and significantly reduce virus release, indicating that BK polyomavirus can undergo egress without lysis. Investigations are ongoing into the cellular pathways involved.

LI15/03

Determining the key residues within ASFV virulence factor, DP71L

Claire Barber[1], Chris Netherton[1], Lynnette Goatley[1], Steve Goodbourn[2], Linda Dixon[1]

[1]The Pirbright Institute, Pirbright, UK, [2]St George's, University of London, London, UK

DP71L is one of several virulence factors encoded by the large double stranded DNA virus, African swine fever virus. DP71L is present in all isolates as either a short (70 to 72 amino acids) or long form (184 amino acids) and shares sequence similarity to both cellular GADD34 (growth arrest and DNA damage inducible protein 34) and the neurovirulence factor ICP34.5 of Herpesvirus. It has previously been shown that, like ICP34.5 and GADD34, DP71L recruits PP1 to dephosphorylate phosphorylated eIF2a which has the effect of restoring global protein synthesis, and the downstream effect of inhibiting CHOP activation via the ATF4 pathway. CHOP is a pro-apoptotic transcription factor responsible for cell death during prolonged ER stress.
It has been proposed that DP71L acts as a bridge between PP1 and eIF2a bringing the two components together. We have established the key residues which are critical for function by assessing 17 DP71L mutants for their ability to inhibit induction of CHOP. We

Please note: Abstracts are published as received from the authors and are not subject to editing.

propose that the previously described PP1 binding motif (residues VRF) and a putative eIF2a binding domain (residues LSAVL) are critical for function. Further studies to establish binding of these mutants to eIF2a and PP1 are ongoing.

LI15/04

Quantitiative proteomic analysis to assess the function of the truncated Merkel cell polyomavirus T antigen

Noor Suhana Adzahar, Adrian Whitehouse

University of Leeds, Leeds, UK

Merkel cell carcinoma (MCC) is a rare but highly metastatic skin cancer. Merkel cell polyomavirus (MCPyV) has been detected in the majority of MCC tumour samples. Detection of monoclonally integrated MCPyV genomes within MCC tumour cells indicate MCPyV infection and integration occur prior to expansion and metastasis of the tumour. Moreover, truncation mutations of the Large Tumour antigen (LT) are observed in the integrated genome rendering the virus replication defective. To elucidate the effects of truncated large T antigen on the host cellular proteome, we developed a stable inducible cell line expressing the truncated LT antigen and performed quantitative proteomics analysis. 282 proteins in nuclear samples and 181 proteins in cytoplasmic fractions were showed to be upregulated at least two-fold in samples expressing truncated large T antigen. Surprisingly however, a large number of downregulated proteins were identified in both nuclear and cytoplasmic samples, 1684 proteins and 731 proteins respectively. Bioinformatic analysis is currently being performed to identify cellular networks and pathways that are affected by the MCPyV truncated large T antigen, which may have implications in MCPyV-mediated cellular transformation.

LI15/05

RAB1A plays a key role in production of *Vaccinia virus* extracellular virions

Tali Pechenick Jowers[1], Rebecca Featherstone[1], Danielle Reynolds[1], Samantha Griffiths[2], Juergen Haas[2], Ismar Haga[1], Pip Beard[1]

[1]The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK, [2]Division of Pathway Medicine and Centre of Infectious Diseases, University of Edinburgh, Edinburgh, UK

Vaccinia virus, a member of the *Poxviridae* family, relies on the cellular ER-Golgi system for production of the three membrane wrapped virion forms (IEV, CEV and EEV). However, the wrapping mechanism is imprecisely understood, in particular the contribution to and regulation of the process by cellular proteins. Cellular Rab GTPases mediate multiple vesicle trafficking processes within the cell, including in the ER-Golgi system. RAB1A is a small RAB GTPase cellular protein which facilitates ER to Golgi and inter-golgi trafficking. It also has less well characterised roles in endosome trafficking, integrin protein expression and regulation of the actin cytoskeleton. We discovered that knock-down of RAB1A results in a highly significant reduction in VACV replication in a multi-step growth curve. Crucially in a one-step growth curve the knockdown of RAB1A does not affect either viral gene expression or the formation intracellular mature virions (IMVs) but does result in a markedly reduced number of extracellular enveloped (wrapped) virions. Overall, these data show that RAB1A is a key enabler of VACV morphogenesis through its role in the formation of extracellular VACV virions.

LI15/06

Interferon induction by African Swine Fever Virus and its application to the development of attenuated live vaccines

Ana Luisa Reis, Fuquan Zhang, Lynnette Goatley, Charles Abrams, Dave Chapman, Christopher Netherton, Haru Takamatsu, Linda Dixon

The Pirbright Institute, Surrey, UK

African swine fever virus (ASFV) is a complex DNA virus and the aetiological agent of a domestic pig disease that can range from an acute haemorrhagic disease to unapparent infections. The complexity of the immune response has impaired the development of an effective vaccine. Previous work has established that immunization of pigs with attenuated ASFV strains can induce good levels of protection against lethal challenge with virulent strains. However in some pigs adverse effects occur following immunization. The innate response is essential to prolong the life of the host until the serological and cellular adaptive immune responses provide protection and the generation of the immunological memory crucial to vaccine development. The type I interferon (IFN) response is the first line of innate immunity against viral infection. We are currently characterizing the induction of type I IFN in primary porcine macrophages infected with strains of different virulence. A better understating of how the IFN response is regulated by these different viruses will provide a rational basis for the development of novel vaccine strategies, for example, further attenuation of the virus by deleting genes involved in the evasion of the IFN host system is in progress.

LI15/07

The nucleolar-shuttling protein nucleophosmin (B23) interacts with KSHV ORF57 via nuclear localisation signals

Anja Berndt[1], Marko Noerenberg[2], Adrian Whitehouse[1]

[1]University of Leeds, Leeds, UK, [2]University of Oxford, Oxford, UK

The KSHV ORF57 protein is essential for lytic viral replication. Its main function is the enhancement of nuclear export of viral intronless mRNA transcript. To achieve this, ORF57 binds directly to RNA and components of the human mRNA-export complex TREX, thereby targeting the export complex onto viral mRNAs. Interestingly, the TREX/ORF57/mRNA complex needs to relocate to the nucleolus for efficient export of mRNAs into the cytoplasm. While it is known that ORF57 is important for this relocation, the cellular factors involved have yet to be identified. Recently, we performed SILAC-based proteomic analysis using an ORF57-inducible cell line and results show an increase of the nucleolar-shuttling protein nucleophosmin (B23) in the nucleolus. While B23 is not directly linked to RNA export, it has been shown to be responsible for the nucleolar localisation of the HIV nuclear export protein REV. Further experiments revealed a direct interaction between B23 and ORF57 dependent on the presence of nuclear localisation signals in ORF57. However, the interaction is independent of the ability of ORF57 to

bind RNA, suggesting additional functional aspects of this interaction beside nucleolar localisation alone.

LI15/08

African swine fever virus C-type lectin protein EP153R undergoes differential processing in infected and uninfected cells

Derah Saward Arav[1,2], David Chapman[1], Michael Skinner[2], Linda Dixon[1]

[1]Pirbright Institute, Pirbright, UK, [2]Imperial College, London, UK

African swine fever virus (ASFV) is a large dsDNA virus which causes fatal haemorrhagic fever in domestic swine. The C-type lectin (CTL) domain of ASFV protein EP153R shows similarities to the NK cell receptor CTL family, and in particular to Clec2B, the ligand of NK cell activating receptor NKp80, and is predicted to be involved in immune evasion.

EP153R shows different patterns of expression in ASFV-infected and uninfected cells. In uninfected cells, the expressed protein has a molecular weight of ~37kDa glycosylated and ~18kDa unmodified, and is localised in the ER with no surface expression detected. In cells infected with ASFV, the expressed protein is 2-3kDa smaller and is detected throughout the cytoplasm and at the cell surface. EP153R contains no predicted sites of processing or cleavage, but has putative di-lysine ER retention motifs at the N-terminus. Constructs with mutated di-lysine motifs are being used to determine their role in the ER localisation of EP153R in uninfected cells, along with pulse-chase radiolabelling to track transcription and processing in ASFV-infected and uninfected cells.

The results suggest that EP153R is processed by a virus-encoded/induced enzyme and that localisation and processing of the protein may be important for its function(s) during virus infection.

LI15/09

The role of Src-family kinases in human cytomegalovirus reactivation

Liane Dupont[1], Verity Kew[2], Matthew Reeves[1]

[1]University College London, London, UK, [2]University of Cambridge, Cambridge, UK

Human cytomegalovirus (HCMV) is a significant cause of disease in immunocompromised individuals via primary infection or reactivation from latency. Latency is established in CD34+ hematopoietic cells with differentiation to a dendritic cell (DC) phenotype concomitant with reactivation. A key event for reactivation is the induction of viral immediate early (IE) gene expression from the major immediate early promoter (MIEP) in an ERK-MAPK dependent manner. Clearly, the question remains how pleiotropic IL-6/ERK-MAPK signalling is integrated for specific MIEP activation. To investigate this further, we exploited the observation that classical immature monocyte derived DCs (MoDCs) reactivate HCMV in response to IL-6 whereas immature monocyte derived Langerhans cells do not. Comparative phospho-proteomics of the two cell types revealed haematopoietic cell kinase (HCK), a src kinase, was differentially phosphorylated in reactivating MoDCs – a protein known to interact with gp130 to augment ERK-MAPK signalling. Our initial studies revealed that src kinase inhibitors impacted on HCMV reactivation in MoDCs (but not on the initial stages of lytic infection) suggesting a role for HCK in reactivation. We hypothesise that src kinase activity may be important for the targeting of ERK to a number of down-stream targets important for HCMV reactivation. Studies are ongoing to address this.

LI15/10

Merkel cell polyomavirus small T antigen mediates microtubule destabilisation to promote cell motility and migration through an interaction with PP4C

Laura Knight[1], Gabriele Stakaityte[1], Hussein Abdul-Sada[1], Rachel Wheat[2], Eric Blair[1], Neil Steven[2], Andrew Macdonald[1], David Blackbourn[3], Adrian Whitehouse[1]

[1]University of Leeds, West Yorkshire, UK, [2]University of Birmingham, West Midlands, UK, [3]University of Surrey, Surrey, UK

Merkel cell carcinoma (MCC) is a highly aggressive skin cancer with a high propensity of recurrence and metastasis. Merkel cell polyomavirus (MCPyV) causes the majority of MCC cases due to the expression of MCPyV ST and LT oncoproteins. Although a number of molecular mechanisms have been attributed to MCPyV T antigen-mediated cellular transformation and replication, to date, no studies have investigated any potential link between MCPyV T antigen expression and the highly metastatic nature of MCC. Through a quantitative proteomic approach we have demonstrated that the microtubule-associated protein stathmin functions in MCPyV ST-mediated microtubule destabilisation and cell motility. The phosphorylation status of stathmin regulates microtubule stability and we have established that stathmin phosphorylation levels are altered upon MCPyV ST expression. MCPyV ST is known to function by interacting with multiple cellular phosphatases, therefore to understand the mechanistic action by which stathmin facilitates cell motility we used MCPyV ST binding mutants which fail to interact with either PP2A Aα and PP4C or PP2A Aβ along with various transdominant mutants. These results indicate that stathmin phosphorylation and MCPyV ST-induced cell motility and migration are through the activity of PP4C, which enhances stathmin-mediated microtubule destabilisation.

LI15/11

Vaccinia virus protein 169 modulates host protein synthesis and contributes to virulence

Pavla Strnadova[1,2], Hongwei Ren[1,2], Robert Valentine[1], Michela Mazzon[1,2], Ian Brierley[2], Geoffrey L. Smith[1,2]

[1]Department of Pathology, University of Cambridge, Cambridge, UK, [2]Department of Virology, Faculty of Medicine, Imperial College London, St Mary's Campus, London, UK

Vaccinia virus (VACV) is a well characterised member of the poxvirus family, and the vaccine used to eradicate smallpox. It is a DNA virus that replicates in the cell cytoplasm and encodes about 200 proteins. VACV is being developed as a vaccine against other diseases and understanding the functions of its proteins is important to enable optimisation of its immunogenicity and safety.

A characterisation of VACV protein 169 is presented. It is a small, cytoplasmic protein expressed early after infection. In cells expressing 169, protein synthesis is compromised, with reduced expression levels of both endogenous and over-expressed proteins, in spite of normal RNA levels. As a consequence, expression of 169 also dampens the cell response to different immune stimuli.

Please note: Abstracts are published as received from the authors and are not subject to editing.

Interestingly, deletion of *169* gene (vΔ169) increases virus virulence in both intranasal and intradermal murine models of infection compared with wild type virus. Future work will determine the molecular mechanism by which 169 targets protein synthesis, and investigate the correlation between this effect and the phenotype observed *in vivo.*

LI15/12

Identification of immuno-reactive capsid proteins of malignant catarrhal fever viruses

Kathryn Bartley[1], David Deane[1], Ann Percival[1], Inga Dry[4], Dawn Grant[1], Neil Inglis[1], Kevin Mclean[1], Erin Manson[1], Lisa Imrie[1], David Haig[2], Felix Lankester[3,5], George Russell[1]

[1]Moredun Research Institute, Edinburgh, UK, [2]School of Veterinary Medicine and Science, Nottingham University, Nottingham, UK, [3]Institute for Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, UK, [4]Roslin Institute, University of Edinburgh, Edinburgh, UK, [5]Paul G. Allen School of Global Animal Health, Washington State University, Washington, USA

Malignant catarrhal fever (MCF) is a fatal disease of cattle and other ungulates caused by certain gamma-herpesviruses including alcelaphine herpesvirus-1 (AlHV-1) and ovine herpesvirus-2 (OvHV-2). An attenuated virus vaccine based on AlHV-1 has been shown to induce virus-neutralising antibodies in plasma and nasal secretions of protected cattle. Proteomic analysis and western blotting of virus extracts allowed the identification of seven candidate AlHV-1 virion antigens. Recombinant expression of these candidates and their OvHV-2 orthologues allowed two polypeptides, products of the ORF17.5 and ORF65 genes, to be clearly identified as antigens recognised by antibodies from natural MCF cases or from AlHV-1 vaccinated cattle. These proteins have potential as diagnostic and vaccine antigens.

LI15/13

Inhibiting herpesvirus lytic replication by targeting a cellular interaction partner

Sophie Schumann, Richard Foster, Adrian Whitehouse

University of Leeds, Leeds, UK

Herpesviruses replicate in the nucleus of the host cell and require cellular factors to export viral mRNAs from the nucleus, in order to allow efficient translation of viral proteins in the cytoplasm. However, while mammalian mRNA export is coupled to splicing, many herpesviral mRNAs are intronless. To circumvent this step, all herpesviruses express a highly conserved SR protein, which interacts with the human transcription/export (hTREX) complex to form an export competent ribonucleoprotein particle. This allows for nuclear export of viral mRNAs and subsequent translation of viral proteins. Studying the conserved herpesviral SR protein, ORF57, encoded by the oncogenic gamma-2 herpesviruses Kaposi's sarcoma-associated herpesvirus (KSHV), we present a novel mechanism for specific disruption of the ORF57/hTREX interaction. Targeting a cellular ATPase, which is an essential hTREX component, using a small molecule inhibitor we prevent ATP-cycle dependent remodelling and formation of the viral ribonucleoprotein particle, while allowing endogenous hTREX formation. Bearing in mind the conserved mechanism for herpesvirus intronless mRNA export, we now present a series of compounds that are able to prevent KSHV late protein expression and disrupt virus lytic replication, with limited cytotoxicity.

LI15/14

The effect of cell stress on adenovirus late gene expression

Zeenah Atwan, Keith Leppard

University of Warwick, Coventry, UK

Cellular responses to virus infection and to physiological stresses include many common features, among which are p53 activation and the mobilization and disruption of PML nuclear bodies. We therefore investigated how physiological stress might impact on subsequent virus infection. As a model system, infection of cells that had been stressed at 40 °C for three hours or stressed and allowed to recover for three hours was compared with infection of cells maintained at 37 °C throughout.
Mild heat stress caused a consistent reduction in Adenovirus 5 late gene expression. In FACS, there was a clear reduction in the number of hexon expressing cells in the stressed and recovered groups. RTqPCR showed reduced hexon mRNA levels which were accompanied by decrease in WB protein levels, confirmed by a reduction in the percentage of cells showing late protein staining by IF. In contrast, analysis of viral DNA replication showed no significant difference between the stressed and control cells. Thus both virus entry and the early phase of infection are unaffected by prior stress to the cells. Initial data from equivalent experiments in PML-knockdown cell lines suggest that PML is important for this inhibition of the late phase of infection.

LI15/15

Transient activation of human cytomegalovirus lytic gene expression during latency allows cytotoxic T cell killing of infected cells

Benjamin Krishna, Betty Lau, Sarah Jackson, Mark Wills, John Sinclair, Emma Poole

Cambridge University, Cambridge, UK

During human cytomegalovirus (HCMV) latency in the myeloid lineage, histone modifications around the major immediate-early promoter (MIEP) result in a repressive chromatin structure and the profound inhibition of lytic major immediate-early (IE) gene expression. We now show this chromatin-mediated repression of the MIEP is maintained, in part, by a latency-associated increase in expression of cellular histone deacetylase 4 (HDAC4) and that this occurs in a TGF-ß and hsa-miR206 dependent manner. Our data also show that pharmacological inhibition of HDAC4 by the Class II HDAC inhibitor MC1568 relieves repression of the MIEP resulting in transient induction of viral immediate-early gene expression. Interestingly, this de-repressive effect of MC1568 on the MIEP is only transient, in that the viral MIEP becomes inactive in the absence of drug. However, this transient expression of IE offers a window of opportunity for untimely IE gene expression which we show results in these latently infected cells becoming targets for elimination by IE-specific cytotoxic T cells. These data suggest that transient induction of viral major IE expression by histone deacetylase inhibitors, such as MC1568, may offer a novel therapeutic strategy to help purge latently infected cells by immune targeting of cells

Please note: Abstracts are published as received from the authors and are not subject to editing.

expressing transiently induced IE antigen

LI15/16

Characterising Murid Herpesvirus-4 host entry in the lung

Ricardo Milho[1], Janet May[1], Philip Stevenson[2], Stacey Efstathiou[1]

1University of Cambridge, Department of Pathology, Cambridge, UK, 2University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, Australia

Herpesviruses are ubiquitous pathogens responsible for a significant burden of disease in the human population. While herpesviruses have been studied extensively *in vitro*, how they cross differentiated epithelia *in vivo* remains unclear. Here we tracked host entry in the lung by the lymphotropic rhadinovirus Murid Herpesvirus-4 (MuHV-4), a mouse model of the human gammaherpesviruses Epstein-Barr virus (EBV) and Kaposi's Sarcoma-associated herpesvirus (KSHV). At the peak of acute replication MuHV-4 infected large clusters of alveolar type I pneumocytes. But during early lung infection, incoming virions targeted almost exclusively alveolar macrophages (CD11c+ F4/80+). Using a loxP-tagged reporter virus to infect LysM-Cre mice revealed that almost all the virus recovered from the lungs had passed through LysM+ myeloid cells. Like many herpesviruses, MuHV-4 binds to heparan sulfate (HS). We detected HS expression on the luminal surface of type I pneumocytes, but not on alveolar macrophages. We surmise that MuHV-4 colonises the lung in three distinct stages: incoming virions first bind to HS on the surface of type I pneumocytes; these virions are captured by alveolar macrophages; finally the macrophage-derived viral progeny infects adjacent pneumocytes, perhaps submucosally. We are now looking to test this hypothesis using MuHV-4 glycoprotein mutants that lack HS binding.

LI15/17

Identification of HCMV microRNA targets using CLASH technology

Stephen Chiweshe, Natalie Reynolds, Jon Pavelin, Pip Beard, David Tollervey, Finn Grey

University of Edinburgh, Edinburgh, UK

Human cytomegalovirus (HCMV) encodes at least 22 miRNAs whose targets are largely unknown. Previously, we have used RISC immunoprecipitation techniques to systematically identify targets of HCMV miRNAs. However this technique is constrained by limits of microarray coverage. Furthermore, no information is gained on the specific target site or the associated targeting miRNA. Advances in miRNA target analysis, including HITS-CLIP and PAR-CLIP, have taken advantage of deep sequencing to generate greater coverage as well as information on target positions in transcripts. Recently, CLASH technology has been developed which builds on HITS-CLIP by including an RNA/RNA intra-molecular ligation step that results in hybrids between the target mRNA clip and the associated miRNA. In brief, this technique can be broken down into 4 steps: 1) Cross-linking of miRNA and target transcripts to RISC complexes from HCMV infected cells; 2) 2-step affinity pull-down of RISC complexes; 3) Incomplete digestion of RNA, ligation of miRNAs to target transcripts, and attachment of primers to allow PCR amplification; 4) Isolation of RNA, reverse transcription and deep sequence analysis. This study will generate a global understanding of HCMV miRNA targets and provide the basis for understanding the functional role of miRNAs in the context of virus infections.

LI15/18

Impairment of autophagy by African swine fever virus

Christopher Netherton[1], Thomas Wileman[2], Geraldine Taylor[1]

[1]The Pirbright Institute, Woking, UK, [2]University of East Anglia, Norwich, UK

African swine fever virus (ASFV) causes a lethal haemorrhagic disease of domestic pigs and is endemic in sub-Saharan Africa. There is also an ongoing outbreak of the disease in European Russia that has led to the death of over 400,000 animals since late 2007. There is currently no vaccine available, however prior infection with attenuated strains of ASFV can protect against infection with closely related virulent strains. Autophagy is a highly conserved, essential process that regulates a number of pathways that are critical for mounting an effective immune response to an invading pathogen. Recent experiments have shown that inhibiting the ability of viruses to regulate autophagy can lead to enhanced immune responses. We have shown that ASFV does not require autophagy for replication and that ASFV can inhibit autophagosome formation in cultured cell lines and primary porcine macrophages. The observation that ASFV does not require autophagy for replication shows that deletion of autophagy modulators from the ASFV genome in order to improve the immunogenicity of an attenuated ASFV vaccine candidate should be feasible. Therefore, we predict that low virulent strains of ASFV that lack autophagy modulators will provide enhanced immunity against virulent isolates of the virus.

LI15/19

Pathogenesis of the Ovine Herpesvirus 2 in their reservoir and susceptible hosts

Dashty Amin[1], Anja Kipar[1,2], James Stewat[1]

[1]Infection and Global Health, University of Liverpool, Liverpool, UK, [2]Institute of Veterinary pathology, Zurich University, Zurich, Swaziland

Ovine Herpesvirus-2 (OvHV-2) is a γ-herpesvirus which is endemic in sheep subclinically but when transmitted to cattle it induces malignant catarrhal fever cattle, a frequently fatal lymphoproliferative disease. The pathogenesis and site of OvH-2 is unknown in both species. In this study we tried to find the precise cellular location of OvHV-2 in sheep and cattle by riboprobe In situ hybridisation technique using respiratory and GI tract and lymphoid organs of random healthy animals beside MCF diseased cattle. Two different probes have been used for lytic and latent transcripts. Results showed probe hybridisation with viral mRNA in sheep in Lung BALT lymphocytes, tongue mucosal epithelium, spleen zonal macrophages and mediastinal lymph nodes. But interestingly in clinically healthy cattle results were very similar like in sheep in more than 12 cattle (of 12). We also optimised a direct quantitative real time PCR technique to find as low as one viral copy per reaction, using Taqman® probe, and detected viral DNA. This experiment show for the first time that viral location is found in sheep and interestingly cattle harbour the virus in a most possibly latent profile

Please note: Abstracts are published as received from the authors and are not subject to editing.

without showing disease.

LI15/20

Characterization of a rhesus cytomegalovirus expressing Ebola virus glycoprotein: towards a disseminating vaccine strategy to prevent Ebola virus transmission from great apes

Aisling A. Murphy[1], Andrea Marzi[2], Chris Parkins[3], Matthew Emery[1], Ilhem Messaoudi[4], Heinz Feldmann[2], Michael A. Jarvis[1]

[1]Department of Biomedical and Healthcare Sciences, Plymouth University, Plymouth, UK

[2]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA [3]Vaccine and Gene Therapy Institute, Oregon Health & Science University, Beaverton, Oregon, USA 4Division of Biomedical Sciences, University of California, Riverside, USA

Ebolavirus (EBOV), a Category A pathogen, causes rapidly progressing hemorrhagic fever in infected humans (mortality rates >90%). Great apes (chimpanzees and gorillas) are similarly highly susceptible to EBOV, and are a major transmission source during human EBOV outbreaks in Africa. A number of effective EBOV vaccines have been developed. However, these vaccines require direct inoculation for induction of EBOV immunity. Therefore, their ability may be limited for providing high levels of coverage for inaccessible wild great ape animal populations. We hypothesize that a novel 'disseminating' vaccine based on cytomegalovirus (CMV) vectors expressing EBOV antigens may achieve the desired levels of coverage. In this scenario, the capacity of the 'disseminating' vaccine to spread through the targeted population by animal-to-animal contact following inoculation of a few 'founder' animals is used to confer EBOV-specific immunity at the population level. We recently showed the capacity of a CMV-based EBOV vaccine to provide durable protective immunity to EBOV lethal challenge in mice (Tsuda et al. 2011). In the present study, we will determine the capacity of a CMV-based vaccine expressing full-length EBOV glycoprotein to provide protective immunity in the EBOV macaque model, which is regarded as the 'gold standard' model for EBOV vaccination studies.

LI15/21

Examining the effect of murine γ-herpesvirus-68 lytic infection on key post-transcriptional steps associated with mRNA maturation

Bahram Ebrahimi, Igor Morozov, Kathleen Cheung and Mark Caddick

University of Liverpool, UK

Targeting of bulk mRNA for degradation is a first key casualty after infection with most pathogenic viruses leading to a phenomenon termed host shutoff. In -herpesviruses, a virally encoded protein termed SOX (BGLF5 in EBV) has been associated with mediating host shutoff. In spite of numerous efforts over the past decade, the fate of cellular mRNAs which succumb to host shutoff remains elusive. Using the murine model, MHV-68, we sought to map different populations of cellular mRNA (i.e. capped, decapped) in cells infected with MHV-68. Moreover, we analysed possible alterations to 3'UTR for example inclusion of non-canonical base, and poly(A) tail length of these transcripts before and after MHV-68 infection. Using molecular techniques, we demonstrate that capping and poly(A) tailing are not affected in MHV-68 infected cells. Our data strongly suggest that MHV-68-mediated mRNA degradation taps into the normal physiological RNA turn over during lytic infection and provide preliminary evidence as to where mRNA degradation may be taking place.

LI15/22

Diversity in the polyadenylation and decay of cellular microRNAs during Vaccinia virus infection

Amy Buck[2], Alasdair Ivens[2], Katrina Gordon[2], Nicky Craig[1], Alexandre Houzelle[1], Alice Roche[1], Neil Turnbull[1], Pip Beard[1]

[1]The Roslin Institute, Edinburgh, UK, [2]Centre for Immunity, Infection and Evolution, Edinburgh, UK

Vaccinia virus (VACV) is a large cytoplasmic DNA virus that causes dramatic alterations to many cellular pathways including microRNA biogenesis. We used small RNA sequencing to quantify comprehensively the impact of VACV infection on the expression of endogenous small non-coding RNAs (sncRNAs) at early (6 h) and late (24 h) times post infection. The most notable changes occurred to the miRNA family of sncRNAs which displayed a drop in abundance of unmodified miRNAs, concomitant with an increase in miRNAs modified by 3' polyadenylation. Interestingly, our detailed analysis of individual miRNAs revealed extensive diversity in the extent of degradation and modification that occurs, particularly at the earlier (6 h) time point. In addition, conditions which restricted viral replication (using the chemical inhibitor AraC, a non-permissive cell type or an attenuated VACV strain) had little to no effect on 3' polyadenylation but significantly hampered miRNA decay, indicating that optimal VACV-induced miRNA decay requires a complete viral life cycle. Our results suggest that intrinsic features of cellular miRNAs cause them to be differentially polyadenylated and degraded during VACV infection.

LI15/23

MCPyV-induced changes in the actin cytoskeleton: implications for the metastatic potential of Merkel cell carcinoma

Gabriele Stakaityte, Laura Knight, Adrian Whitehouse

University of Leeds, Leeds, UK

Merkel cell carcinoma is a rare but aggressive skin cancer. Recently, Merkel cell polyomavirus (MCPyV) has been implicated as the causative agent. The MCPyV genome encodes large T (LT) and small T antigens (ST), which are alternatively spliced oncogenic proteins. We have recently demonstrated that ST expression induces cell motility and invasiveness. To explore the molecular mechanisms underlying this ST-induced phenotype, quantitative proteomic analysis was utilised and showed an upregulation of actin-associated proteins involved in actin nucleation and the formation of motile structures, namely Arp3, N-WASP and WAVE. Using immunofluorescence, we show that ST induces the formation of actin protrusions. Co-localisation of actin-associated proteins and the effects of Rho-family GTPases have defined these protrusions as filopodia. ST is known to function through its interactions with cellular protein phosphatases 2A and 4C (PP2A and PP4C). Using dominant-negative mutants of PP2A and PP4C and mutants of ST

that do not interact with these enzymes we observe a reduction in filopodia formation and decreased cell motility. We are working to determine the stages where ST affects the cell motility pathway in hopes of blocking these interactions.

LI15/24

Latency, luciferase and long non-coding RNA: use of a reporter virus in vivo model to study the impact of the HSV-1 latency-associated transcripts on latent virus gene expression

Michael Nicoll, William Hann, Viv Connor, Heather Coleman, Stacey Efstathiou

University of Cambridge, Cambridge, UK

Following lytic replication in the oral mucosa, herpes simplex virus type-1 (HSV-1) establishes a lifelong latent infection in sensory neurons of the trigeminal ganglia (TGs), from which periodic reactivation and transmission occur. In contrast to an extensive profile of virus gene expression during lytic replication, the HSV-1 genome is maintained as a transcriptionally quiescent episome during latency. During this latter phase of infection, the only abundant viral gene products are the latency-associated transcripts (LATs); a set of co-linear long non-coding RNAs expressed from diploid regions of the genome. We have previously demonstrated that the latent neuronal population decreases in number throughout infection in the absence of LAT expression. These data suggest a role for LATs in the maintenance of latency - a role that may be conducted by silencing the virus genome. In this study we report the use of firefly luciferase-expressing reporter viruses to quantify latent gene expression from whole tissues. Up to five-fold higher luciferase activity was detected from TGs infected with LAT-negative recombinants. Furthermore, analysis of luciferase expression 120 days post-infection demonstrated a loss of signal during LAT-negative virus latency. These data support a role for the LATs in HSV-1 genome silencing and the maintenance of latency.

LI15/25

KSHV lytic infection causes changes in host cell miRNA expression levels

Christopher Owen, Adrian Whitehouse

University of Leeds, Leeds, West Yorkshire, UK

Kaposi's Sarcoma associated herpesvirus is the causative agent of Kaposi's Sarcoma, Multicentric Castleman's Disease and Primary Effusion Lymphoma. The virus encodes more than 2 dozen microRNAs (miRNAs), many of which play well categorised roles during the latent and lytic stages of virus infection. However, little is known about the cellular miRNAs regulated by KSHV during lytic infection. Using a combination of RNAseq and miRNA analysis tools we have identified altered expression of 22 cellular miRNAs at different stages of KSHV lytic infection, none of which have previously been implicated in the virus life cycle. Work is currently being carried out to determine the roles these miRNAs are playing in KSHV replication.

LI15/26

Nuclear sequestration of Hsc70 protein during Kaposi's sarcoma-associated herpesvirus (KSHV) infection

Belinda Baquero, Adrian Whitehouse

University of Leeds, Leeds, UK

KSHV is the causative agent of a highly vascular tumour of endothelial lymphatic origin, termed Kaposi's saroma. KSHV has two distinct life cycles, latency and lytic replication, both are essential for KSHV-mediated tumorigenesis. To identify novel cellular proteins which play a key role during lytic KSHV replication, SILAC-based quantitative proteomics was performed comparing purified unreactivated (harbouring latent virus) and KSHV-reactivated (harbouring lytic virus) nuclear envelope fractions. Results revealed that the cellular chaperone heat shock cognate 71 kDa (Hsc70) was present at significantly levels in the nuclear envelopes of reactivated cells. Immunofluorescence labelling of endogenous Hsc70 protein confirmed an accumulation of Hsc70 in the nuclei of reactivated HEK-293T cells, in contrast, Hsc70 was equally distributed between the cytoplasm and nuclei of unreactivated cells. Importantly, the same differential Hsc70 localization was also observed in the TREx BCBL-1 cell line, a B cell line that closely resembles the natural cellular target of KSHV, the B lymphocytes. Further studies are currently ongoing to determine the function of Hsc70 during KSHV infection.

LI15/27

Elephant Endotheliotropic Herpesvirus 1 (EEHV1) in the Asian elephant

Laura Bennett, Lisa Yon, Stephen Dunham, Rachael Tarlinton

School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, UK

Infection with Elephant endotheliotropic herpesvirus 1 (EEHV-1) is potentially fatal in Asian elephants. Young elephants aged 1-4 years are particularly susceptible, with a mortality rate approaching 85%. Like other betaherpesviruses, EEHV latency occurs in leucocytes. During latency, virus is shed at very low levels and signs of clinical disease do not occur. As part of an ongoing project to establish the prevalence of EEHV-1 in captive European elephants, trunk wash samples were collected from 4 female elephants located at a Zoo in Europe, biweekly over a 3 month period. DNA was extracted using a Nucleospin kit (Macherey Nagel) and the presence of EEHV detected using qPCR (Stanton et al., 2010). Low levels of EEHV were found during the study. This adds to the body of data that is accumulating that suggests that the majority of captive Asian elephants in European zoos are infected with EEHV1. Further samples will need to be collected to determine whether the negative samples are due to intermittent shedding in the affected animal.

STANTON, J. J. et al, 2010. American Journal of Veterinary Research, 71, 925-933.

LI15/29

Latent expression profiles of Murine gammaherpesvirus 68 miRNAs *in vivo*

Amr Bayoumy, Dr Finn Grey, Dr Bernadette Dutia

Roslin Institute, University of Edinburgh, UK

MicroRNAs (miRNAs) regulate gene expression post-transcriptionally by altering the stability of their target mRNA. Murine gammaherpesvirus 68 (MHV-68) encodes at least 15 miRNAs that

Please note: Abstracts are published as received from the authors and are not subject to editing.

are located in a genomic region known to encode latency-associated transcripts, but their functions are unknown. Strict species specificity has limited research on the human gammaherpesviruses mainly to in vitro studies. Infection of mice with MHV-68 provides a unique animal model to investigate in-vivo pathogenic features that are difficult to assess in humans. During latency, MHV-68 expresses a protein encoded by ORF73 named MHV-68 latency-associated nuclear antigen (mLANA). In this study, a recombinant MHV-68 virus containing beta-lactamase gene fused to ORF73 was used to detect and isolate latently infected splenocytes by flow cytometry. mLANA positive splenocytes were isolated at day-14 and day-21 post infection. ORF73 was detected, but viral DNA polymerase was undetectable in mLANA+ cells using qRT-PCR, implying viral latency in these cells. We have characterised the expression profile of eight miRNAs in this cell population over time. There are changes in the relative levels of expression of a number of miRNAs at different time points during the establishment of latent infection

LI16

Virology workshop: positive strand RNA viruses

LI16/01

The role of AKT activation during avian infectious bronchitis virus infection

Ambalika Batra[1], Helena Maier[1], Paul Britton[1], Julian Hiscox[2], Mark Fife[1]

[1]The Pirbright Institute, Compton, UK, [2]Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

The PI3K/AKT signalling pathway plays a crucial role in the regulation of many cellular processes and is widely studied for its role in cancer. Many studies have investigated the effect of viral infection on the mammalian pathway, however few have looked at the effect in avian cells. One of the most economically damaging diseases to the poultry industry is infectious bronchitis. Infectious bronchitis is caused by infectious bronchitis virus (IBV), a gammacoronavirus. We have examined the effect of IBV infection on the PI3K/AKT pathway in avian cells. Activation of AKT has been found to occur during infection with IBV, however the mechanism by which the virus interacts with the pathway is not yet known. Whilst AKT and its isoforms have been extensively studied in mammalian cells, there is little known of their characteristics and tissue specificity in avian cells. The relative expression of AKT in different avian tissues has therefore also been studied in an attempt to understand the importance and function of AKT within avian cells.

LI16/02

Competitive fitness in coronaviruses is not correlated with size or number of double membrane vesicles

Hawaa A-Mulla, Benjamin Neuman

University of Reading, Reading, UK

Positive-stranded viruses synthesize their RNA in membrane-bound organelles, but it is not clear how this benefits the virus or the host. For coronaviruses, these organelles take the form of double-membrane vesicles (DMVs) interconnected by a convoluted membrane network. We used electron microscopy to identify murine coronaviruses with mutations in nsp3 and nsp14 that replicated normally while producing smaller and half the normal amount of DMVs. Viruses with mutations in nsp5 and nsp16 produced small DMVs but also replicated normally. Quantitative RT-PCR confirmed that the most strongly affected of these, the nsp3 mutant, produced more viral RNA than wild-type virus. Competitive growth assays were carried out in both continuous and primary cells to better understand the role of DMV formation in viral fitness. Surprisingly, several viruses that produced fewer or smaller DMVs showed a higher relative fitness compared to wild-type virus, suggesting the size and number of DMVs is not associated with a replication advantage. For the first time, this directly demonstrates that replication and organelle formation may be, at least in part, studied separately during positive-stranded RNA virus infection.

LI16/03

Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes; updated criteria and assignment web resource

Donald Smith[1], Jens Bukh[2], Carla Kuiken[3], Scott Muerhoff[4], Charles Rice[5], Jack Stapleton[6], Peter Simmonds[1]

[1]University of Edinburgh, Edinburgh, UK, [2]University of Copenhagen, Copenhagen, Denmark, [3]Los Alamos National Laboratory, Los Alamos, New Mexico, USA, [4]Abbott Diagnostics, Abbott Park, Illinois, USA, [5]The Rockefeller University, New York, New York, USA, [6]University of Iowa, Iowa City, Iowa, USA

We present a major update to the 2005 consensus proposal for the classification of hepatitis C virus (HCV) into genotypes and subtypes by incorporating additional sequence information derived from over 1300 (near-) complete genome sequences of HCV available on public databases in May 2013. Analysis resolved several nomenclature conflicts between genotype designations and using consensus criteria created a classification of HCV into seven confirmed genotypes and 67 subtypes. There are 22 additional complete coding region sequences of unassigned subtype. The study additionally describes the development of a web resource hosted by the International Committee for Taxonomy of Viruses (ICTV) that maintains and regularly updates tables of reference isolates, accession numbers and annotated alignments (http://talk.ictvonline.org/links/hcv/hcv-classification.htm). Those who need to check or propose new genotypes or subtypes of HCV to contact the Flavivirus Study Group in advance of publication to avoid nomenclature conflicts appearing in the literature. While the criteria for assigning genotypes and subtypes remain unchanged from previous consensus proposals, changes are proposed in the assignment of provisional subtypes, subtype numbering beyond "w" and the nomenclature of inter-genotypic recombinant.

LI16/04

Identifying RNA sequences in the HCV genome that specifically interact with NS5A

Zsofia Igloi[1], Simon White[1], Sally Harrison[2], Eric Dykeman[3], Reidun Twarock[3], Mark Harris[1]

[1]University of Leeds, Leeds, UK, [2]The Leeds Institute of Molecular Medicine (LIMM), Leeds, UK, [3]University of York, York, UK

Hepatitis C virus (HCV) is a global health burden affecting 180 million

people worldwide. One of the viral non-structural proteins, NS5A, has been shown to be an indispensable component of the HCV genome replication and assembly machineries. We and others have previously shown that NS5A possesses RNA binding activity and binds to both the 5' and 3' UTR of the HCV genome *in vitro,* however the details of the binding specificity remain to be established. To determine exactly what RNA sequences are bound by NS5A we used two complementary approaches. Firstly, we selected RNA aptamers from a randomised library that bound bacterially expressed NS5A with high affinity, using the process of Systematic Evolution of Ligands by Exponential Enrichment (SELEX) coupled with high throughput sequencing. In a second approach, a modified CLIP (UV crosslinking and immunoprecipitation) analysis was undertaken whereby *Strep*-tagged NS5A was affinity purified from Huh7 cells harbouring a JFH-1 derived subgenomic replicon or infected with JFH-1 virus. These complementary techniques aim to define the sequence specificity of NS5A-RNA binding both *in vitro* and *in vivo* leading to deeper understanding of the virus lifecycle and creating potential drug targets.

LI16/05

The norovirus capsid shell domain contributes to capsid stability and regulates viral RNA-dependent RNA polymerase activity

Jia Lu, Lucy Thorne, Frédéric Sorgeloos, Ian Goodfellow

University of Cambridge, Cambridge, UK

Norovirus RNA synthesis has been previously shown to be modulated by an interaction between the viral RNA-dependent RNA polymerase (RdRp) and the major capsid protein, VP1. More specifically, the interaction is mediated by the odd numbered loops 1, 3, 5 and 7 of the shell domain of VP1, which are sufficient for increasing cognate RdRp activity but not that of a heterologous polymerase. In this study, recombinant murine noroviruses (MNVs) with VP1 chimeras of the odd numbered loops of the human norovirus (HuNoV) VP1 were characterized in the context of infection. Since loop 3 is identical between MNV and HuNoV, single, double and triple loop swap mutants of loop 1, 5 and 7 were generated. In single loop swap mutants, MNV with HuNoV loop 1 (L1) or loop 7 (L7) displayed delayed growth properties while loop 5 (L5) appeared to confer faster replication at early stages of infection with less capsid stability. Some double and a triple loop swap abolished MNV replication. All loop mutants were blind passaged after recovery to identify compensatory mutations in VP1 or in the RdRp to reveal which residues in the two proteins may mediate the interaction and are responsible for enhancing RdRp activity.

LI16/06

Bluetongue virus serotype 26 (BTV-26) cannot replicate in the Culicoides sonorensis (KC) cell line - association with particular genome segments

Gillian Pullinger, Marc Guimera, Kyriaki Nomikou, Mark Boyce, Peter Mertens

The Pirbright Institute, Pirbright, Woking, Surrey, UK

Bluetongue virus (BTV) is transmitted between its ruminant hosts by adult Culicoides (biting midges). The BTV genome consists of ten segments of linear dsRNA. Two new serotypes of BTV (BTV-25 and BTV-26) have recently been identified. BTV-26 unusually cannot replicate in a Culicoides sonorensis cell line (KC-cells), although it does replicate in mammalian (BSR) cells. To investigate the role of particular viral proteins in the insect-cell replication defect, reverse-genetics was used to generate mono-reassortants containing individual genome segments of BTV-26, in a BTV-1 'background'. The only mono-reassortant that was not recovered in BSR cells, was one containing BTV-26 genome-segment 2 (encoding outer-capsid protein VP2). However, a triple-reassortant containing BTV-26 segments 2, 6 and 7 (encoding outer-capsid proteins VP2 and VP5, and outer-core protein VP7, respectively) was generated successfully in BSR cells. Further growth studies showed that this triple-reassortant is unable to replicate in KC cells, while the mono-reassortant containing BTV-26 segment 7 replicated slowly. Since VP2 and VP7 are both involved in viral entry into insect cells, it is possible that BTV-26 is impaired in this process. Two other mono-reassortants, containing BTV-26 segment 1 (viral polymerase), or segment 3 (the sub-core-shell protein) also showed severely impaired replication in insect cells.

LI16/07

Isolation and analysis of recombinants arising from a mixed virus infection using bioinformatics and next generation sequencing

Fadi Alnaji, Jonathan Moore, Andrew Woodman, David Evans

School of Life Sciences, University of Warwick, Coventry, UK

Viruses with RNA genomes constitute the majority of the significant global pathogens. Of these, viruses with positive-sense, single-stranded genomes generally have small genomes and, related to this, error-prone RNA dependent RNA polymerases.

During replication the mutations caused by the polymerase generate diversity, an evolutionary mechanism which enables the viruses to survive under changing environmental pressures. More extensive genetic variation is achieved through recombination between co-infecting viruses. In studies from our laboratory, recombination is proposed to be a biphasic process in which the initial crossover event is imprecise (promiscuous) and is followed by a further process (resolution), which selects for viruses with enhanced fitness. From previous studies, either or both of the nucleotide identity and local RNA secondary structure influence recombination. Although the molecular determinants remain poorly understood.

In this project, a combination of reverse genetic approaches with next generation sequencing to investigate the both initial products and subsequent resolution events involved in recombination in enteroviruses. A bioinformatics pipeline was developed and rigorously tested using simulated NGS data containing known and pre-defined junctions. Currently investigating the analysis of NGS data from co-transfected and co-infected cells to determine whether recombination and/or the secondary resolution events are sequence or structure specific.

LI16/08

Highly pathogenic porcine reproductive and respiratory syndrome virus N protein- PARP-1 interaction- a novel antiviral target

Long Liu[2], Zoe Lear[1], David Hughes[1], Weining Wu[3], En-

Please note: Abstracts are published as received from the authors and are not subject to editing.

min Zhou[2], Adrian Whitehouse[1], Hongying Chen[2], Julian Hiscox[3]

[1]University of Leeds, Leeds, UK, [2]Northwest A&F University, Yangling, China, [3]University of Liverpool, Liverpool, Liverpool, UK

Anti-viral therapy is conventionally targeted to disrupt the function of unique viral proteins that have no cellular equivalent. For viruses with RNA genomes the application of anti-viral therapy acts as a strong selection pressure for genotypes/phenotypes that are resistant to the drug therapy. Identifying and targeting the function of cellular proteins that are critical for virus biology may provide an attractive alternative for therapeutic intervention. Porcine reproductive and respiratory syndrome virus (PRRSV) is a pathogen of global importance to the swine industry. To identify a list of potential target proteins, proteomics was used to determine the cellular interactome of the PRRSV N protein. Poly [ADP-ribose] polymerase 1 (PARP-1) was identified as an interaction partner of N protein. Use of the PARP-1 small molecule inhibitor, 3-AB, in PRRSV infected cells demonstrated that PARP-1 was required for virus biology and acted as a pro-viral factor. Serial growth of PRRSV in different concentrations of 3-AB did not yield mutant viruses that were able to grow with wild type kinetics. This study provides further evidence that cellular proteins, which are critical for virus biology, can be targeted to ablate virus growth and provide a high barrier for the emergence of drug resistance.

LI16/09

Development of a GBV-B neutralisation assay

Emma St Clair Pearce[1], Giada Mattiuzzo[1], Nicola Rose[1], Angray Kang[2], Mark Page[1]

[1]National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK, [2]Queen Mary University of London, London, UK

Hepatitis C virus (HCV) has become a global health concern with over 170 million people chronically infected and 350,000 deaths every year from HCV-related liver diseases. GBV-B infection in tamarins is a surrogate model for HCV infection. Whilst HCV infection becomes chronic in 80% of those infected, GBV-B is naturally cleared. To better understand this natural clearance, we wish to study the humoral immune response in this model. However, there is no available GBV-B neutralisation assay for antibodies identified through this model. Pseudotyped virus expressing the envelope proteins of GBV-B was produced through co-transfection of HEK 293T cells with 3 plasmids containing a lentiviral backbone, Green Fluorescent Protein (GFP) and GBV-B E1E2 envelope proteins. Virus is then added to target cells for 48h and fluorescence is measured by FACS analysis. Different cell lines will be tested to find suitable target cells and once established, archived sera from previously infected tamarins will be tested in this system. As GBV-B and HCV share significant sequence homology, any antibodies identified could be mapped to epitopes which may be common to both GBV-B and HCV with the potential to be incorporated into vaccine development.

LI16/10

Mushroom Virus X - Developing an early warning system

Edward Dobbs[1], Greg Deakin[1,2], Julie Bennett[1], Helen Grogan[2], Kerry Burton[1]

[1]East Malling Research, East Malling, Kent, UK, [2]Teagasc Food Research Centre, Ashtown, Dublin, Ireland

Mushroom Virus X (MVX) is a disease that causes serious commercial damage to the cultivated white mushroom industry, an industry worth $4.7bn per annum.

Detecting MVX in mushroom compost before it is used by growers is a vital step in managing this disease. Currently two problems preclude development of the test:

(1) Mushroom compost is a chemically complex substrate containing high levels of humic substances, the products of partially degraded straw and manure, making molecular extraction from the fungal mycelia very difficult.

(2) MVX is a complex of numerous viruses and only partial sequence information is available for some of the viruses.

Here we describe the development of a cheap, fast and highly sensitive RNA extraction method for mushroom compost, along with Next Generation Sequencing of MVX which has allowed identification of >30 RNA species associated with the complex. 18 novel fungal viruses were discovered as well as 10 novel satellites.

The new extraction method, coupled with the viral sequences, means that a qPCR-based detection method can now be used to test for MVX in both fruit bodies and mushroom compost. This will be used to link MVX symptoms to individual viruses and allow routine MVX testing of compost.

LI16/11

High throughput quantitative proteomic analysis of dengue virus - host cell interactions

Amjad Yousuf, Han-Chen Chiu, Holger Hannemann, David Matthews, Andrew Davidson

University of Bristol, Bristol, UK

Dengue virus (DENV) causes the most significant arthropod-borne viral disease of humans, with an estimated 400 million infections annually, worldwide. We are using a combination of high throughput mass spectrometry based proteomic approaches to identify human proteins that interact with DENV proteins to either promote or restrict virus replication.

Initially, nuclear and cytoplasmic fractions from DENV-2 infected A549 cells were analyzed. Bioinformatic analysis of the data led to the identification of cellular pathways and protein functional groups altered in response to DENV infection. Alterations in the amounts of representative proteins were confirmed by Western blotting and immunofluorescence assay. Stable inducible over-expression of two proteins (PRAF2 and ERC1), that were almost undetectable after DENV infection was found to inhibit virus infection.

More recently, comparative analysis of DENV-2 and -4 infected Huh-7 cells has been done. Total cell lysates and fractions containing an active replicase complex were analyzed, leading to the identification of proteins that are significantly increased in the replicase containing fraction. The effect on DENV replication of depleting these proteins is currently being investigated. Collectively the studies have identified common and cell specific pathways and proteins that are modulated by DENV infection and may be targets for intervention strategies.

LI16/12

Development of a methodology for amplification of West Nile virus coding sequences directly from clinical samples

Claire Jeffries[1], George Carnell[2], Karen Mansfield[1], Anthony Fooks[1], Nicholas Johnson[1]

[1]Animal Health and Veterinary Laboratories Agency, New Haw, Surrey, UK, [2]London School of Hygiene and Tropical Medicine, Keppel Street, London, UK

West Nile virus (WNV) is a mosquito-borne flavivirus of public health and veterinary importance. The worldwide distribution of WNV has increased dramatically over the past few decades, becoming endemic in several novel regions.

A methodology for amplification of WNV coding sequences directly from clinical samples reduces the need for virus culture, thus reducing the time and safety requirements associated with live virus work. This allows focus to be placed on downstream processes such as studies targeted at understanding the epidemiology and biology of the virus as well as vaccine development.

Primers were designed using a multiple sequence alignment of WNV strains. Reverse transcription and amplification methodologies were assessed and optimised using WNV RNA extracted from tissue culture, as well as WNV positive avian clinical samples, and specificity and sensitivity was evaluated. Restriction digests were performed to confirm correct sequences of amplicons produced and a unique restriction site was found, allowing rapid discrimination between lineage 1 and 2 strains. Sequencing of amplicons was performed to further confirm amplification results.

This methodology has the potential to be useful for a wide range of downstream applications, and allows the rapid generation of sequence data from clinical samples without the need for viral culturing.

LI16/13

Dissection of genetic diversity within virus populations: comparisons between the seven FMDV serotypes using a novel PCR-free NGS approach

Grace Logan[1,2], Donald P. King[1], Daniel T. Haydon[2], Eleanor M. Cottam[1]

[1]The Pirbright Institute, Surrey, UK, [2]The University of Glasgow, Glasgow, UK

Understanding how viruses generate diversity is critical to studying their spread and controlling disease. Foot-and-mouth disease virus (FMDV) is very diverse and exists as a swarm of genetically related variants. This enables the virus to adapt rapidly to new environments and evade immune responses. Diversity is created through spontaneous mutation and recombination.

Precise mutation rates are unknown for FMDV serotypes. Previous studies for RNA viruses indicate an approximate figure for mutations per nucleotide per site per genome between 10-3 and 10-5 (Drake and Holland 1999). These estimates are limited to averages opposed to site specific mutation rates, and conclusions have not been drawn concerning types of mutation.

Mutation rate estimates can also be hampered by errors introduced in sample preparation and sequencing. For example PCR introduced artefacts are common and can be wrongly identified as mutations. We have developed a novel PCR-free NGS method to dissect the genetic diversity within virus populations of the seven different FMDV serotypes and estimated site specific mutation frequencies.

Drake, J. W., and J. J. Holland. 1999. Mutation rates among RNA viruses. Proc. Natl. Acad. Sci. U. S. A. 96:13910-13913.

LI16/14

Development and validation of Bluetongue virus particles expressing fluorescent NS1 protein as a tool for in vivo studies

Alice Muntzer[2,1], Anthony Wilson[2], Andrew Shaw[1], Pippa Hawes[2], Peter Mertens[2], Massimo Palmarini[1]

[1]University of Glasgow Centre for Virus Research, Glasgow, UK, [2]The Pirbright Institute, Pirbright, UK

Arthropods vector some of the most economically and medically important viruses, including Bluetongue virus (BTV), a segmented double-stranded RNA (dsRNA) virus, which causes a severe haemorrhagic disease of ruminants called bluetongue. BTV is transmitted via the saliva of infected Culicoides spp. biting midges. Despite the importance of arthropods for virus transmission, there is little information concerning the dynamics of viral replication and dissemination within the arthropod vector. A number of intrinsic barriers are known to inhibit BTV infection of Culicoides spp. midges. Efforts to first identify these barriers, manipulate factors affecting their operation, and quantify their effect on viral titre have been impeded by the lack of tools capable of visualising infectious virus in midges in vivo. Here, we describe the design and rescue of recombinant BTV serotype-1 (BTV-1) particles that express BTV non-structural NS1 protein fused to fluorescent reporter, EGFP (BTV-1_NS1/mEGFP), using reverse genetics. Data indicate that BTV-1_NS1/mEGFP provides an accurate model of wtBTV-1 in vitro and will be a useful tool for in vivo studies of NS1 protein localisation. We determine the temperature-dependency of EGFP fluorescence as an indicator of BTV polymerase fidelity.

LI16/15

Towards the eradication of polio: developing a novel polio vaccine

Oluwapelumi Adeyemi, James Kelly, Clare Nicol, Morgan Herod, Nicola Stonehouse, David Rowlands

School of Molecular and Cellular Biology, Faculty of Biological Sciences and Astbury Cerner for Structural Molecular Biology, University of Leeds, Leeds, UK

Poliomyelitis is a highly infectious viral disease, caused by poliovirus (PV). It can result in acute flaccid paralysis and may lead to death. Massive integrated global immunisation programmes have been, and are, operating with the goal of eradicating the disease using an attenuated virus vaccine (OPV) and/or an inactivated virus vaccine (IPV). Despite the success of OPV, the possibility of reversion to virulence makes this unsafe as a post-eradication vaccine. IPV has limitations in its inability to produce a mucosal immune response and a high production cost. The use of virus-like particles (VLPs) as vaccines against hepatitis B virus and human papillomaviruses has demonstrated the potential of this approach to vaccine development. However, PV VLPs are unstable and readily convert to an altered antigenic form which is ineffective for vaccine purposes when generated in vitro. In this study, we have sought to increase PV VLP stability by selecting thermo-stable poliovirus mutants at elevated temperatures. Using this approach we have identified mutations in the structural proteins which result in substantial increases in thermo-stability of both virus infectivity and native antigenicity. Ongoing studies are examining whether empty particles (and hence VLPs) produced from these mutant viruses are similarly thermo-stable.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI16/16

Immunogenicity of African horse sickness virus VP7 crystals

Laura Bailey, Peter Mertens, Javier Castillo-Olivares

The Pirbright Institute, Surrey, UK

African horse sickness (AHS) is a highly lethal arthropod borne disease of equidae, of which there are nine serotypes. In the absence of effective treatment, vaccination plays an important role in the control of AHS. Concerns associated with the usage of the commercially available live attenuated vaccine have stimulated the development of novel recombinant vaccines. VP7 is a highly immunogenic serogroup specific AHSV antigen that can be used as a vaccine delivery molecule. Replication of AHSV in cell culture, and over expression of VP7 in insect cells results in the generation of bi-dimensional hexagonal crystals of approximately 6µm in size. We have carried out a vaccination experiment in ponies, to study the immunogenicity of VP7 in relation to protein dose and size of crystals. For this study, four groups of two Welsh mountain ponies were vaccinated with either of the following: a) 25µg VP7 crystals; b) 150µg VP7 crystals; c) 25µg sonicated VP7 crystals; and d) 150µg sonicated VP7 crystals. Ponies were vaccinated on days 0 and 28, serum samples collected and antibodies responses analysed. The results of this study will be presented and discussed.

LI16/18

A pony vaccination study using subdomains of the outer capsid protein VP2 of African Horse Sickness virus expressed by MVA vaccines

Nicola Manning[1,2], Sarah Gilbert[2], Peter Mertens[1], Javier Castillo-Olivares[1]

[1]Pirbright Institute, Surrey, UK, [2]Oxford University, Oxford, UK

African Horse Sickness virus (AHSV) causes a highly lethal disease in horses, and is transmitted by biting midges of the genus Culicoides. Although considered endemic in Africa, outbreaks have historically occurred within Europe. In the absence of treatment, vaccination is of major importance.The AHSV outer capsid protein VP2 is the main target of neutralising antibodies and AHSV-VP2 based recombinant vaccines have been shown to be fully protective in challenge models. Neutralising conformational and linear epitopes have been mapped to VP2 (aa 279-503). However a virus with a VP2 truncation in this region outgrows full-length VP2 AHSV in cell culture suggesting that cell binding domains and possibly other neutralising epitopes exist outside the truncated region. To test this hypothesis we generated six recombinant modified vaccinia Ankara (MVA) viruses expressing different VP2 subdomains: a) full-length AHSV-VP2; b) VP2 (aa 279-503); c) VP2 (aa 1-354); d) VP2 (355-707); e) VP2 (708-1060); and f) truncated VP2 (deletion of aa 279-503). Ponies were immunised with either of these constructs, serum samples collected and antibody responses analysed by virus neutralising test and immune-blotting. These results will be presented and discussed.

LI16/19

Whole-Genome Sequence Analysis of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Simon Watson[1], Alimuddin Zumla[5,6], Hatem Makhdoom[2], Anne Palser[1], Swee Hoe Ong[1], Abdullah Al Rabeeah[4], Rafat Alhakeem[4], Abdullah Assiri[4], Jaffar Al-Tawfiq[11], Ali Albarrak[8], Mazin Barry[12], Atef Shibl[12], Fahad Alrabeah[7], Sami Hajjar[7], Hanan Balkhy[10], Hesham Flemban[9], Andrew Rambaut[3,13], Paul Kellam[1,5], Ziad Memish[4]

1Wellcome Trust Sanger Institute, Hinxton, UK, 2Jeddah Regional Laboratory, Ministry of Health, Jeddah, Saudi Arabia, 3Institute of Evolutionary Biology, Ashworth Laboratories, Edinburgh, UK, 4Global Centre for Mass Gatherings Medicine, Ministry of Health, Riyadh, Saudi Arabia, 5Division of Infection and Immunity, University College London, London, UK, 6Department of Medical Microbiology, University College London Hospitals NHS Foundation Trust, London, UK, 7King Faisal Specialist Hospital, Riyadh, Saudi Arabia, 8Prince Sultan Military Medical City, Riyadh, Saudi Arabia, 9Alhada Military Hospital, Riyadh, Saudi Arabia, 10King Abdulaziz Medical City, Riyadh, Saudi Arabia, 11Saudi Aramco Medical Services Organization, Saudi Aramco, Dhahran, Saudi Arabia, 12King Saud University, Riyadh, Saudi Arabia, 13Fogarty International Center, NIH, Bethesda, USA

The Middle East Respiratory Syndrome coronavirus (MERS-Cov) is a single-stranded RNA betacoronavirus of obscure origin first documented in the Kingdom of Saudi Arabia (KSA) in September 2012. In addition to 26 MERS-CoV genomes generated previously, we have sequenced a further 32 genomes collected from the KSA between July and September 2013, bringing the number of publically-available MERS-CoV genomes to 65, to better understand the spread, circulation and evolution of the virus. The MERS-Cov evolutionary rate was estimated to be 1.12 x 10-3 substitutions per site per year, narrowing its time to most recent common ancestor (tMRCA) to March 2012. Phylogenetically there is a mixture of clades with definable geographical range and small clades without clear spatial or temporal range. The appearance of phylogenetically-related MERS-CoV in geographically distant locations must be taken into account in efforts to identify the animal source and transmission pattern of the virus. Codon-level analysis of 42 epidemiologically-unlinked genomes reveals codon 1020 in the viral spike protein, located in a domain required for cell entry, to be under positive selection. Work is currently underway to generate MERS-CoV spike protein pseudotyped lentivirus to ascertain the mutation's biological relevance.

LI16/20

Genetic Determinants of Semliki Forest virus neuroinvasion

Mhairi Ferguson[1], Sirle Saul[2], Rennos Fragkoudis[1,3], Sabine Weisheit[1], Andres Merits[2], John Fazakerley[1]

[1]The Pirbright Institute, Surrey, UK, [2]Institute of Technology, University of Tartu, Tartu, Estonia, [3]The Roslin Instituteq, Edinburgh, UK

Alphaviruses cause serious epidemics in humans and equines. The molecular clone of Semliki Forest virus (SFV), SFV4, provides a well characterised model to study alphavirus infection. SFV4 was derived from a strain of SFV designated Prototype virus, which is closely related to the L10 strain. L10 is efficiently neuroinvasive following intraperitoneal (IP) infection of adult mice, while SFV4 is not detected in the brain. To determine the genetic differences between these two viruses, Solexa (Illumina) sequencing was carried out. The genome sequences of SFV4 and L10 were very

similar with only 12 nucleotide differences. Six differences that altered the amino acid sequence were predicted to affect the neuroinvasive ability of SFV. A clone of L10, termed SFV6, was generated from SFV4 by incorporation of these six changes. SFV6 was efficiently neuroinvasive. To determine which difference determined neuroinvasion, a panel of SFV4 mutants was created. These mutant viruses were IP inoculated into BALB/c mice and virus titres determined in the blood and brain. Change of a single amino acid in envelope glycoprotein 2 at position 162 or 247 converted the neuroinvasive ability of SFV4 to that of SFV6. This change in neuroinvasion was attributed to differential binding to heparan sulfate.

LI16/21

Autophagy and Flavivirus replication

Emily Hopkins[1], Bertalan Bicksack[2,1], James Stewart[1], Sareen Galbraith[1,3]

[1]University of Liverpool, Liverpool, UK, [2]University of East Anglia, East Anglia, UK, 3Leeds Metropolitan University, Leeds, UK

Autophagy is the process of cellular recycling, in which damaged and unwanted proteins are broken down into their components, to free resources for necessary processes. In this way it acts to keep cells alive during stress and starvation. Autophagy is utilised by many viruses in order to enhance viral growth. One viral group in particular that utilises this process are the flaviviruses, which cause viral encephalitis within those they infect. Within this group are viruses of medical importance, including Dengue, West Nile virus and Japanese encephalitis virus, the latter being the most important cause of viral encephalitis in Eastern and Southern Asia with disability-adjusted life years estimated at 709,000. It is now thought that the differential regulation of autophagy within the cells of the central nervous system plays a role in the pathology of the viruses. By inducing and inhibiting autophagy in cells infected with Semliki Forest Virus and recording the resulting viral titre, the link between the autophagy and flavivirus infection may be established. This in turn may lead to the development of the first therapeutic treatment available for encephalitis, and the development of vaccines against flaviviruses, which are currently either unavailable or unsuitable, due to cost.

LI16/22

organelle stress and the dysregulation of lipid metabolism during hepatitis c virus replication

Michael McGarvey, Charles Burch

Imperial College London, London, UK

It has been shown that infection of hepatocytes with hepatitis C virus (HCV) may lead to glucose intolerance and a dysregulation of lipid metabolism, indicated by the incidence of insulin resistance and hepatosteatosis. A panel of genes were selected based on the re-evaluation of data collected from a previous microarray study in which Huh7 cells were infected with the JFH-1 isolate of HCV. Using a sub-genomic replicon model (genotype 2a) to examine the effect of viral non-structural proteins on host gene expression over a time course of 48 hours, we show that genes concerned with lipid metabolism (TXNIP, PCK2, IRS2) and organelle stress (STC2, CYCS) are significantly upregulated during the first 24 hours of HCV replication. TXNIP, a key component in the dysregulation of lipid metabolism is induced by the cellular uptake of glucose via the transcriptional activity of Mlx and subject to the regulatory control of the microRNA, miR-17, which, in turn, is disabled by the endoribonuclease activity of the IRE-1α arm of the UPR following ER stress. Here, we demonstrate knockdown of TXNIP by using an inhibitor (STF-083010) to target IRE-1α and thereby impair the replication of viral genes.

LI16/23

Role of VF1 in Murine Norovirus persistence *in vivo*

Constantina Christodoulou[1], Frédéric Sorgeloos[1], Lucy Thorne[1], Dalan Bailey[2], Ian Goodfellow[1]

[1]University of Cambridge, Cambridge, UK, [2]University of Birmingham, Birmingham, UK

Viruses have developed numerous mechanisms to evade or subvert the host response to infection. Our group has previously identified the Virulence Factor 1 (VF1) protein encoded by ORF4 of murine norovirus (MNV), and we have shown it to be an accessory protein that increases replication efficiency in the host yet is dispensable *in vitro*. Lack of VF1 expression led to increased expression of antiviral genes including interferon-β, and increased caspase 3/7 activation in cell culture, suggesting that VF1 plays a role in delaying apoptosis and antagonising the innate immune response.
The role of VF1 in viral persistence was examined using MNV3 in immunocompetent mice. Mice inoculated with MNV3 lacking the ability to express VF1 via the introduction of a stop codon showed reduced viral shedding but VF1 expression was restored by day 5 post infection. The introduction of additional stop codons to prevent reversion reduced viral loads to a greater degree, demonstrating the importance of VF1 in overall viral fitness. Our data would indicate that while VF1 is not essential for viral persistence in vivo, it contributes to viral fitness and to viral loads. Ongoing work focuses on the mechanism of action of VF1 and it's contribution to norovirus pathogenesis.

LI16/24

Prevelence of Bovine Astrovirus in Scotland

Bill Gregory[1], Colin Mason[2], Pip Beard[1], Colin Sharp[1]

[1]The Roslin Institute, University of Edinburgh,, Easter Bush, Midlothian, EH25 9RG, UK, [2]SAC Commercial Limited- Veterinary Services, Peter Wilson Building, King's Buildings, King's Buildings, West Mains Road, Edinburgh EH9 3JG, UK

Astroviruses are small, non-enveloped positive single stranded RNA viruses known to infect mammals and birds. Infections are often associated with gastroenteritis. While bovine astrovirus was amongst the earliest astroviruses to be discovered it's prevalence and population structure remain largely uncharacterised. In contrast, large studies in pigs have shown a high diverse population at the sequence level and prevalence levels varying between 2 and 20%. Here we report the presence of astrovirus in calves from Dumfries and Ayrshire. Using primers, capable of amplifying viral RNA-dependent polymerase (RdRp) prevalence rates were 74% for scouring calves and 65% for non-scouring calves across farms across both counties. This suggests that astrovirus may be an incidental finding in calf faeces and not directly correlated with disease. However, although the virus is detected in both scouring and non-scouring calves, higher viral loads are associated with scouring. Rotavirus was detected in 78% of scouring and 22% of

Please note: Abstracts are published as received from the authors and are not subject to editing.

non-scouring calves. Sequencing of the RdRp products has shown the presence of five sequence groups. In some instances the strain of astrovirus detected in the faeces of the same calf varied from week to week. There was no correlation between a specific astrovirus strain and disease.

LI16/25

Facilitation of alphavirus transmission and dissemination by mosquito bite-induced innate immune response

Marieke Pingen[1], Esther Schnettler[1], Alain Kohl[1], John Fazakerley[2], Gerry J Graham[1] and Clive S. McKimmie[1].

[1]Institue of Infection, Immunology and Inflammation, University of Glasgow, UK.

[2]Institute for Animal Health, Pirbright, UK

Increasing migration and global warming facilitate a rise in viral infections transmitted by mosquitoes, such as the alphaviruses West Nile Virus and Chikungunya. We aimed to characterize the early events in alphavirus transmission by mosquitoes that enable virus dissemination in their mammalian host.
We defined dissemination of the model alphavirus Semliki Forest Virus (SFV) in mice. In the presence of a mosquito bite, needle inoculation of SFV resulted in higher levels of virus replication in skin, elevated viremia and accelerated disease with higher mortality. To investigate the underlying mechanism of this correlation between early virus burden and morbidity, the impact of mosquito bites on skin was investigated in more detail. Gene expression profiles of mosquito-bitten skin demonstrated a rapid upregulation of the inflammatory cytokine IL-1 and neutrophil-attracting chemokines such as CXCL2 in bites compared to virus-infected skin.
In the draining lymph node, viral replication was higher in the absence of a mosquito bite. Medullary sinus macrophages, which monitor the lymph for pathogens, seem to be the first cells infected by SFV in the lymph node.
In conclusion, we have developed a relevant and tractable *in vivo* model system to investigate the impact of mosquito bites on arbovirus transmission. Our data suggests that the innate immune response to mosquito bites facilitates viral dissemination.

LI16/26

RNA binding and unwinding mechanism of an avian reovirus non-structural protein σNS

Alex Borodavka[1,2], Peter G Stockley[1,2], Roman Tuma[1,2]

[1]University of Leeds, Astbury Centre for Structural Molecular Biology, Leeds, UK, [2]University of Leeds, School of Molecular and Cellular Biology, Leeds, UK

Double-stranded RNA viruses of the Reoviridae family are important human, animal and plant pathogens. Their virions contain up to 11 distinct genomic segments, each of which is essential for virus replication. The mechanism by which one of each segments is packaged into virions is not known. Non-structural RNA binding proteins may play key role in the process. We have characterized a non-structural RNA binding protein σNS from avian reovirus. Using analytical ultracentrifugation, small angle X-ray scattering and native ion-mobility mass-spectrometry we have demonstrated that the protein forms stable, elongated hexamers. The hexamer binds ssRNA with high affinity, but no sequence specificity. By using the energy from RNA binding the hexamer removes secondary structure from viral RNA transcripts and unfolds stem-loops. The hexamer exhibits multiple binding sites for RNA which brings together several strands and assists RNA-RNA interactions between segments during assortment and packaging.

LI17

Virology workshop: negative strand RNA viruses

LI17/01

Preclinical evaluation of engineered influenza A virus for pancreatic ductal adenocarcinoma virotherapy

Matteo Samuele Pizzuto[1,2], Samantha Kasloff[1], Micol Benussi[3], Vincenzo Ciminale[3], Ilaria Capua[1], Wendy Barclay[2]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, 1OIE/FAO and National Reference Laboratory for Avian Influenza Virus,Viale dell'Università 10, 35020, Legnaro (PD), Italy, [2]Influenza Group, Imperial College London, St. Mary's Campus, Norfolk Place, W2 1PG London, UK, [3]Department of Oncology and Surgical Sciences, University of Padua,Via Gattamelata, 64,, 35128 Padova, Italy

Pancreatic ductal adenocarcinoma (PDA) is the most common and aggressive form of pancreatic cancer. Therapies proven successful in other tumor types have shown little efficacy in treating PDA and the need for alternative treatments is crucial. Virotherapy uses natural or engineered oncolytic viruses (OVs) to selectively kill tumor cells. Recently, our group demonstrated the ability of influenza viruses (IV) to infect and induce high levels of apoptosis in a variety of human-origin PDA lines, including BxPC-3 cells previously shown resistant to different oncolytic viruses. To increase the specificity of this approach, a series of genetically engineered influenza viruses were generated altered in the NS1 and PB1-F2 viral genes, that are associated with interferon evasion, apoptosis modulation and virus replication. Here, the oncolytic potential of a conditionally replicating H7N3 NS1-77 PB1-F2 L75H avian influenza A virus was tested in BxPC-3 cells in vitro and in xenograft model. The results strengthen the idea that research in the area of oncolytic virology should not be focused on a short list of candidates, but rather on a broad range of viruses characterized by specific cancer cell permissiveness, and that influenza virus could play a role in further studies on treatment of PDA.

LI17/02

An assessment of H17 HA species tropism

Fiona Ryrie[1], Elena Robinson[2], Ian Jones[2], Holly Shelton[1]

[1]The Pirbright Institute, Compton, UK, [2]University of Reading, Reading, UK

In 2009 genetic material from a novel subtype of Influenza virus, H17N10 was isolated in bats. Structural studies indicate the N10 neuraminidase possesses no sialidase activity and the H17 Haemagglutinin utilises a different receptor than the usual sialic acid preferred by the first 16 HA subtypes. To assess the host range of this virus we generated recombinant H17 HA protein using a Baculovirus vector. The recombinant protein was glycosylated and secreted. It was used to probe *ex vivo* tissue sections taken from a range of economically important livestock and humans. *Ex vivo* sections have an advantage over glycan arrays since all potential

receptors, including non sialic acid and proteins are represented allowing a true assessment of H17 HA for attachment. Attachment is a first step in zoonosis and therefore fundamental to an assessment of the natural tropisms of this novel subtype. The H17 pattern of binding was found to be distinct when compared to an avian H5. H17 bound respiratory and enteric tract tissue taken from two different chicken breeds but not to duck tissue or tracheal sections from horse, pig, dog, ferret or humans. This is the first report of binding to any biological substrate by H17 HA.

LI17/03

Isolation and characterisation of the positive-sense replicative intermediate of a negative-strand RNA virus

Ashley York, Narin Hengrung, Frank Vreede, Juha Huiskonen, Ervin Fodor

University of Oxford, Oxford, UK

Negative-strand RNA viruses represent a significant class of important pathogens that cause substantial morbidity and mortality in human and animal hosts worldwide. A defining feature of these viruses is that their single-stranded RNA genomes are of opposite polarity to messenger RNA and are replicated through a positive-sense intermediate. The replicative intermediate is thought to exist as a complementary ribonucleoprotein (cRNP) complex, however, the isolation of such complexes from infected cells has never been accomplished. We report the development of an RNA-based affinity-purification strategy for the isolation of cRNPs of influenza A virus from infected cells. This technological advance enabled the structural and functional characterisation of this elusive but essential component of the viral RNA replication machine. The cRNP exhibits a filamentous double-helical organisation with defined termini, containing the viral RNA-dependent RNA polymerase (RdRP) at one end and a loop structure at the other end. *In vitro* characterisation of cRNP activity yielded mechanistic insights into the workings of this RNA synthesis machine. We propose a model of influenza virus genome replication that relies on the *trans*-activation of the cRNP-associated RdRP. The described purification strategy should be applicable to other negative-strand RNA viruses and will promote studies into their replication mechanisms.

LI17/04

Identification of cellular serine/threonine-protein phosphatase 6 (PP6) as a regulator of the influenza A virus RNA-dependent RNA polymerase

Ashley York, Edward Hutchinson, Ervin Fodor

University of Oxford, Oxford, UK

Reversible phosphorylation, on serine, threonine or tyrosine residues, is one of the most significant and well-studied post-translational protein modifications. Phosphorylation has been shown to be critical in the regulation of numerous cellular processes including the cell cycle and signal transduction pathways. It also has an important role in the influenza virus life-cycle. Here we report the identification of cellular serine/threonine-protein phosphatase 6 (PP6) as an interaction partner and regulator of the influenza virus RNA-dependent RNA polymerase (RdRP). We use affinity-tagging, purification and mass spectrometry to determine the physical interactions between the influenza virus RdRP and host proteins in infected HEK 293T cells. Using a mass spectrometry interaction statistics (MiST) quantitative scoring system, we identified over 200 candidate proteins that interact with the influenza RdRP, with the highest-scoring protein being identified as PP6. PP6 was confirmed to interact with the PB2 subunit of the influenza RdRP. Knockdown of PP6 in human cells significantly attenuated virus growth and viral RNA synthesis. Additionally, nuclear-export of genomic viral ribonucleoproteins was impaired. These data facilitate a more comprehensive and detailed understanding of the role of the host proteome during the virus life-cycle and contribute to our emerging knowledge of phosphorylation in influenza biology.

LI17/05

Regulation of influenza virus RNP complex formation

Lauren Turrell, Frank Vreede, Ervin Fodor

Oxford University, Oxford, UK

The RNA genome of influenza A virus consists of eight single stranded negative sense vRNA segments. The vRNA segments are bound by viral RNA polymerase at the 5' and 3' ends and the remaining viral RNA is associated with nucleoprotein (NP) to form a viral ribonucleoprotein (vRNP) complex. These vRNP complexes carry out both viral transcription and replication. NP, in the RNP complex, forms oligomers through an interaction between the tail-loop and groove of neighbouring monomers. However, little is known about the regulation of NP oligomerisation. A phosphorylation site within the groove of NP has been identified in both influenza A and influenza B viruses. This study shows that constitutive phosphorylation at this site, using a phospho-mimic, disrupts NP oligomerisation and leads to a significant decrease in both viral transcription and replication. In contrast, NP oligomerisation is not disrupted if phosphorylation at the site is prevented by alanine substitution, allowing both transcription and replication to occur. However, a virus with this substitution in NP has a significantly lower titre compared to wild type virus. Overall, these findings suggest that NP oligomerisation via the tail-loop/groove interaction may be regulated by phosphorylation and that this regulation is essential for viral fitness.

LI17/06

Development of an A549 GFP Reporter Cell-line to Monitor Interferon Signalling

Zoe Gage, Andri Vasou, Richard Randall, Catherine Adamson

University of St Andrews, St Andrews, Fife, UK

Virus infection triggers the cellular interferon (IFN) response to elicit an anti-viral state. Virus infection induces IFNβ expression. Secreted IFNβ binds to non-infected cells activating the IFN signalling cascade via the Jak-STAT pathway. Leading to activation of the IFN-Stimulated Response Element (ISRE) in the promoter of IFN-stimulated genes (ISGs). Up-regulation of hundreds of ISGs with antiviral activites creates the antivrial state. We have previously generated an A549 reporter cell-line with GFP under the control of the IFNβ promoter to facilitate detection of IFN induction. We have now created a second A549 reporter cell-line with GFP under the control of an ISRE element to detect IFN signalling. Lentivirus

Please note: Abstracts are published as received from the authors and are not subject to editing.

technology was used to create a stable cell-line that expresses GFP following stimulation with purified IFN. Multiple rounds of lentiviral infections followed by FACS was required to generate an optimal cell-line. Cell-line characterization demonstarted that GFP expression is significantly reduced upon (i) addition of a known Jak1 inhibitor or (ii) expression of viral IFN antagonists which inhibit IFN signalling. These reporter cell-lines provide valuable tools to further understand the IFN response and how it is counteracted by viruses or to develop assays to identify inibitors of the IFN response.

LI17/07

The role of the M2 protein in influenza virus assembly and budding

Agnieszka Martyna, Jeremy Rossman

University of Kent, Canterbury, UK

Influenza A virus is an enveloped, negative sense RNA virus which causes annual epidemics and major pandemics. Assembly and budding of new viral particles is a complex and multistep process, of which, many aspects remain unclear, despite many years of research. Influenza virus M2 protein is a homotetrameric transmembrane protein, containing three domains: ecto domain, transmembrane domain and cytoplasmic tail (CT). In the final stage of budding it has been shown that M2 mediates membrane scission through an amphipathic helix, which is formed by the first 17 amino acids of the protein's CT, however the exact mechanism by which membrane scission is triggered is not known. The aim of this project is to define the molecular mechanisms of influenza virus assembly and budding as mediated by the M2 protein. Using Giant Unilamellar Vesicles and Giant Plasma Membrane Vesicle- SUPER templates we are investigating the functions of the M2 cytoplasmic tail and amphipathic helix in viral assembly and budding. Current results indicate that the polar face of the M2 amphipathic helix and the specific lipid composition of the membrane are both important for viral budding.

LI17/08

Determining the genetic and phylogeographic origin of highly pathogenic avian influenza virus (H7N3) in Mexico

Lu Lu[1], Samantha J Lycett[2], Andrew J Leigh Brown[1]

[1]University of Edinburgh, Edinburgh, UK, [2]University of Glasgow, Glasgow, UK

To explore the origin of the recent H7N3 highly pathogenic avian influenza virus (HP AIV) outbreaks in poultry in Mexico (first reported in June 2012 and the latest one was reported on 31st Aug, 2013 by OIE), time resolved phylogenetic trees were generated from eight segments of North America AIV sequences, with location, subtype, avian host species being modelled as discrete traits upon the trees. A hierarchical phylogenetic model (HPM) was performed which allowed trait rates to be jointly inferred across different segments. Our results indicated the novel AIV was derived from the large North America AIV pool of low pathogenic (LP) AIV through complicated reassortment events. Phylogeographic analysis indicated that the predecessor of the HPAI H7N3 was not from the adjacent regions but is a reassortant with different segments being contributed by wild waterfowl from different flyways. Five segments (HA, NA, NP, M, NS) were introduced from wild birds migrating along the central North American flyway, while PB2, PB1 and PA were introduced via the western flyway. These results highlight a potential role for Mexico as a hotspot of virus reassortment as it is where wild birds from different migration routes mix during the winter.

LI17/09

The Distribution and Roles of Mitochondrial PB2 in Influenza Infections

Joshua Long, Ervin Fodor

Sir William Dunn School of Pathology, University of Oxford, UK

Influenza viruses infect a wide range of hosts including humans, waterfowl, poultry and swine. The viral polymerase consists of three subunits PB1, PB2 and PA, which act in concert in the nucleus to catalyse viral transcription and replication. In line with this, PB2 proteins of all influenza viruses exhibit nuclear localisation. However, PB2 proteins of a large proportion of human seasonal strains were also found to localise to the mitochondria. This is due to the presence of an asparagine at position 9 of PB2 in place of an aspartic acid encoded in other strains. Mitochondrial association of PB2 has been linked to the regulation of innate immune responses and the stabilisation of the mitochondrial membrane potential in virus infected cells. In order to understand the functional implications of the mitochondrial association of PB2 in more detail we are investigating the intra-mitochondrial distribution of PB2 and its effect on mitochondrial morphology using electron microscopy and the recently-developed APEX-technology. Moreover, we have also developed a protocol to purify mitochondria from virus and mock-infected cells to allow the comparison of mitochondrial proteomes of cells infected with viruses encoding mitochondrial or non-mitochondrial PB2 using mass spectrometry.

LI17/10

Exploring the antigenic architecture of H9 haemagglutinin of avian influenza

Thomas Peacock[1,2], Kolli Reddy[1], Holly Shelton[1], Wendy Barclay[2], Munir Iqbal[0]

[1]The Pirbright Institute, Compton, Berkshire, UK, [2]Imperial College London, London, UK

Avian influenza subtype H9N2 is a threat to both global food security and human health. Efficacious vaccines and diagnostics are vital tools in minimising the impact of this virus. Like other influenza strains, genetic drift of the major antigen, haemagglutinin, leads to vaccine failure in the field. Our aim is to elucidate amino acid motifs responsible for the antigenic differences seen in H9N2 viruses in order to better understand the antigenicity of the virus. We also aim to be able to produce more broadly reacting vaccines, effective against antigenically distinct viruses. We have identified several genetically similar H9N2 strains which are antigenically distinct. These selected strains will be used to understand which haemagglutinin motifs are responsible for H9 Haemagglutinin antigenic diversity. To address this, mutant viruses have been produced differing in single or multiple amino acid residues using reverse genetics methodologies. The antigenic properties of these mutant viruses were characterised using monoclonal antibodies and chicken post-infection antisera. Additionally, monoclonal antibodies will be used for classical 'escape mutant' studies. Such studies will lead to a more evidence based approach to future vaccine seed-

strain selection and the design of novel recombinant vaccines to combat H9N2 Influenza.

LI17/11

Characterisation of neutralising epitopes of currently circulating influenza A (H3N2) viruses using monoclonal antibodies

Kerstin Beer, Rodney Daniels, John McCauley

MRC National Institute for Medical Research, London, UK

Influenza virus A (H3N2), the predominant strain in seasonal influenza, can cause a disease associated with severe complications. Infection is preventable by vaccination with influenza surface proteins, including haemagglutinin (HA) - the main target of neutralising antibodies (Abs). During the evolution of human H3N2 viruses, changes in antigenic properties of HA have resulted in markedly reduced binding to sialic acid receptors.

To examine the correlation between changes in neutralising epitopes and function of recent H3N2 viruses a reassessment of epitope diversity needs to be carried out. As a first step to analysing the immune response to vaccination and infection characterisation of a new panel of H3N2 neutralizing monoclonal Abs (mAbs) is being undertaken.

Mice were immunized with H3N2 virus A/Victoria/361/2011 and their spleen cells fused with myeloma cells to generate Ab-producing hybridoma cells. A virus neutralising assay was applied to identify hybridoma cells producing neutralising Abs and then to determine the ability of purified mAbs to neutralise different viruses. A haemagglutination inhibition assay was applied to measure the ability of mAbs to inhibit receptor binding.

Several neutralising mAbs against H3N2 have been generated and their neutralising ability against a variety of viruses will be discussed. Further structural analyses are planned.

LI17/12

Investigating the role of the influenza A virus PB1-F2 protein in poultry

Joe James[1,2], Manuela Mura[2], Olivier Moncorgé[2], Holly Shelton[1], Wendy Barclay[2]

[1]The Pirbright Institute, Compton, UK, [2]Imperial College London, London, UK

The PB1-F2 protein is translated from the +1 frame of the genetic segment that encodes for the Influenza virus polymerase protein, PB1. Work in mammalian systems has shown that PB1-F2 protein has a range of effects dependent upon cell type and viral strain including apoptosis, innate immune modulation and regulation of viral polymerase activity. The PB1-F2 protein is truncated or missing in many isolates recovered from mammalian hosts, however 96% of all avian influenza isolates possess a full length PB1-F2 gene suggesting an important function in the avian host. To date, only limited studies have been undertaken in avian systems.

Here we show that PB1-F2 from avian influenza A viruses does not impact upon viral polymerase activity in chicken cells using a mini-replicon assay system. However expression of PB1-F2 did antagonise the chicken type I interferon system. A preliminary *in vivo* study where chickens were infected with isogenic viruses that differed only in the length of PB1-F2 indicated that truncation of PB1-F2 to 57 amino acids resulted in increased pathogenicity. We speculate that PB1-F2 modulates the innate immune response during virus infection in chickens and loss of the C-terminus results in dysregulation and consequently a more severe outcome of infection.

LI17/13

Genetic heterogeneity of mumps vaccines

Edward Mee, Philip Minor, Silke Schepelmann

NIBSC-MHRA, South Mimms, UK

Standard regulatory tests assess MMR vaccine potency against a reference but do not quantify the genetic diversity of vaccines. We analysed 12 batches of mumps-containing vaccine, comprising 2-4 batches from each of four manufacturers to assess genetic heterogeneity. Replicate RNA samples were amplified by sequence-independent RT-PCR and sequenced using Illumina MiSeq. Reads were assembled against mumps reference strains and non-synonymous changes analysed. Distinct patterns of heterogeneity were observed in different vaccines. Vaccine A comprised a known mixture of two strains, but a very limited spectrum of variant amino acids. Vaccine B comprised a single strain with multiple high-frequency variants that were largely consistent between batches. Vaccine C contained multiple high-frequency variants that exhibited batch-to-batch variation in frequency. Vaccine D, a single mumps vaccine, comprised a single strain exhibiting multiple variant residues with batch-to-batch variation in frequency. Differences in variant frequencies between replicates are likely explicable by the random nature of the RT-PCR - the total number of variants may therefore be higher than observed. The impact of variants on vaccine safety and efficacy remains unknown, but further work to establish the range of batch-to-batch variation will allow for identification of irregularities, e.g. due to changes in manufacturing processes.

LI17/14

A minireplicon system for Oropouche orthobunyavirus

Natasha Tilston-Lunel[1,3], Daisy da Silva[2], Richard Elliott[3]

[1]Biomedical Sciences Research Complex, School of Biology, University of St Andrews, Fife, UK, [2]Center for Technological Innovation, Instituto Evandro Chagas, Ananindeua, Brazil, [3]MRC - University of Glasgow, Centre for Virus Research, Glasgow, UK

Oropouche orthobunyavirus (OROV) causes frequent outbreaks of a febrile illness in the Northern parts of Brazil. However, despite being the cause for an estimated half a million human infections since its first isolation in 1955 our knowledge of this virus is extremely limited. Here we present the establishment of a T7 RNA polymerase-driven minireplicon system for OROV. The minireplicon comprises viral untranslated regions (UTR) flanking the reporter gene *Renilla* luciferase; all viral proteins are expressed from plasmids under the control of the T7 promoter. Our results show that the viral polymerase is fully functional and that the viral glycoproteins are capable of forming virus-like particles (VLP). The results also indicate that the L-segment UTR contains a stronger promoter than that of the M-segment, in contrast to what we see with other bunyaviruses that have been studied. We also show that the viral non-structural protein (NSs) inhibits viral polymerase activity, and when expressed on its own NSs reduces reporter activity from a CMV-driven plasmid indicating a possible affect on cellular RNA polymerase II.

Please note: Abstracts are published as received from the authors and are not subject to editing.

LI17/15

Schmallenberg virus diagnostic: latex agglutination test

Thomas O'Shea Brown, Janet Daly, Barnabas King

University of Nottingham, Nottingham, UK

Latex agglutination (LA) testing has been used to identify various analytes since 1956. In veterinary medicine this invaluable diagnostic tool is commonly used to identify pathogens such as avian influenza and feline panleukopenia virus. This form of testing gives the clinician a rapid result for the sample being tested, at a cheaper price for the customer, when compared to other diagnostic methods. With this in mind, a rapid and reproducible LA test was developed to detect antibodies specific to Schmallenberg virus (SBV)   a novel arbovirus of ruminants linked with congenitally malformed neonates and a transient non-specific febrile syndrome in adult cattle. Currently there are no rapid pen-side diagnostic tests available to test samples for the presence of SBV-specific antibodies. The buffer in which the viral protein was adsorbed to the latex beads was optimised, which ultimately led to agglutination being observed macroscopically post-adsorption.

Further testing and refinement of this test will allow individuals to understand the seroprevalence throughout their animal group. Knowing this would, in due course, lead to a more accurate vaccination programme and so a potential saving of money.

LI17/16

Lyophilisation of lentiviral pseudotypes for the development and distribution of a virus neutralisation assay kit for influenza, Marburg and rabies viruses

Stuart Mather[1], Simon Scott[1], Edward Wright[2], Nigel Temperton[1]

[1]Viral Pseudotype Unit (Medway), School of Pharmacy, University of Kent, Chatham Maritime, Kent, UK, [2]Viral Pseudotype Unit (Fitzrovia), Faculty of Science and Technology, University of Westminster, London, UK

Some conventional serological assays can accurately quantify neutralising antibody responses raised against epitopes on virus glycoproteins, enabling vaccine evaluation and serosurveillance studies to take place. However, these assays often necessitate the handling of wild-type virus in expensive high-biosafety laboratories, which restricts the scope of their application, particularly in resource-deprived areas. A solution to this issue is the use of lentiviral pseudotype viruses (PVs) - chimeric, replication-deficient virions that imitate the binding and entry mechanisms of their wild-type equivalents. Pseudotype virus neutralisation assays (PVNAs) bypass high biosafety requirements and yield comparable results to established assays. This study explores PV lyophilisation as a cost-effective, alternative means for production, distribution and storage of a PVNA-based diagnostic kit. Influenza H5, rabies and Marburg virus pseudotypes were subjected to freeze-drying before incubation at a variety of temperatures and humidities, and employment in neutralisation assays following reconstitution. High levels of PV titre were retained post-lyophilisation, with significant levels of virus activity maintained even after medium-term storage at tropical conditions. Also, the performance of PVs in neutralisation assays was unaffected by the lyophilisation process. These results confirm the viability of a freeze-dried PVNA-based diagnostic kit, which could considerably facilitate in-field serology for a wide portfolio of viruses.

LI17/17

Influenza A/Anhui/1/13 (H7N9) virus replicates well and transmits efficiently by contact in the guinea pig model

John D. Gabbard[2], Daniel Dlugolenski[2], Debbie Van Riel[3], Nicole Marshall[1], Summer E. Galloway[1], Elizabeth W. Howerth[2], Patricia J. Campbell[1], Cheryl Jones[2], Scott Johnson[2], Lauren Leotis-Byrd[1], David A. Steinhauer[1], Thjys Kuiken[3], S. Mark Tompkins[2], Ralph Tripp[2], Anice C. Lowen[1], John Steel[1]

[1]Emory University, Atlanta, GA, USA, [2]University of Georgia, Athens, GA, USA, [3]Erasmus Medical Center, Rotterdam, The Netherlands

To date, 188 cases of avian influenza A (H7N9) virus infection have been reported in humans. We report on the characterization of A/Anhui/1/2013 (H7N9) [An13] influenza A virus, *in vitro* and in the guinea pig model. Comparison with A/Panama/2007/99 (H3N2) [Pan99], and A/rhea/NC/93 (H7N1) [rhea93], allowed evaluation of the degree of mammalian adaptation exhibited by An13. The H7N9 strain displayed low ID50, grew robustly in the upper respiratory tract, and transmitted efficiently to contact exposed animals in the guinea pig model. The viral phenotypes were indistinguishable from the human seasonal Pan99 strain, while rhea93 grew less well, and did not transmit. In contrast, An13 HA displayed a high pH of fusion, typical of avian strains, and resembled avian viruses in its pattern of attachment to fixed tissues derived from guinea pig respiratory tract. Moreover, analysis of virus binding to sialic acids demonstrated a phenotype intermediate to that of the human and avian viruses, with binding to α-2,3 and α-2,6 linked sialic acids.

In sum, An13 retained the fusion properties of an avian influenza virus, demonstrated attachment properties intermediate between typical human and avian strains, and displayed growth and transmission phenotypes in the guinea pig model indicative of mammalian adaptation.

LI17/18

Early events of Bluetongue virus infection in small ruminants

Eleonora Melzi[1,3], Marco Caporale[1,2], Luigina Di Gialleonardo[2], Mara Rocchi[3], Gary Entrican[3], Massimo Palmarini[1]

[1]MRC - University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical Veterinary and Life Sciences, University of Glasgow, Glasgow, UK, [2]Zooprofilattico Sperimentale dell'Abruzzo e del Molise G. Caporale, Teramo, Italy, [3]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, UK

Bluetongue virus (BTV) is the etiological agent of Bluetongue, a haemorrhagic disease that affects ruminants, responsible for significant economic losses in sheep. The clinical outcome of BTV infection is highly variable and depends on virus strain/serotype and the species/breed of the infected animal. We hypothesised that in the mammalian host the early events of BTV infection are critical to determine the clinical spectrum of the disease.

The aim of this study was to dissect the early events of BTV infection, by identifying the cell populations targeted during the first phases of virus colonisation of the skin and the peripheral lymph nodes.
We used confocal microscopy to identify and characterise infected cells in sheep and goats experimentally infected with highly virulent and attenuated BTV-8 strains. Samples were analysed at various timepoints after infection.
Interestingly, we observed that BTV infects different cell populations in sheep compared to goats. The latter are susceptible to BTV infection but rarely develop clinical disease. We also identified micro-anatomical modifications in the lymph nodes potentially associated with an altered or delayed immune response. Thus, there might be a relationship between the severity of Bluetongue and the first events taking place soon after virus infection.

LI17/19

Bacterial perturbation of epithelial barriers: implications for viral infection

Elizabeth K Benedikz, Nicola F Fletcher, David Mason, Adam F Cunningham, Jane A McKeating

University of Birmingham, Birmingham, UK

Epithelial cells provide a shield that restricts pathogen entry into the host. Whilst viruses contain the mechanisms sufficient to breach this barrier, other factors may also contribute, such as host genetic defects or exposure to the microflora or bacterial pathogens. Although there are well documented examples of the latter influencing viral infections, the mechanisms underlying such activities are incompletely understood. For instance, bacteria may directly damage the epithelial cell and inter-cell contacts or alternatively may induce an inflammatory response that alters barrier integrity indirectly, with none of these elements mutually exclusive. Nevertheless, the net outcome could be the enhanced infectivity of a viral pathogen. Using multiple *in vitro* models of infection involving lung and gut epithelial cells and Lassa and Vesicular Stomatitis virus pseudoparticles we show that transient epithelial cell exposure to live *Salmonella* Typhimurium is sufficient to markedly enhance pseudoparticle entry into polarized epithelial cells. We show this is not due to depolarization of the epithelial monolayer but is associated with the induction of NF-κB signalling pathways and levels of pro-inflammatory cytokines released. These data indicate that epithelial cell receptiveness to viral infection can be modulated through prior, short-term exposure to bacteria.

LI17/20

A novel vaccine against Crimean-Congo Haemorrhagic Fever protects 100% of animals against lethal challenge in a mouse model

Karen R. Buttigieg, Stuart D. Dowall, Stephen Findlay-Wilson, Aleksandra Miloszewska, Emma Rayner, Roger Hewson, Miles W. Carroll

Public Health England, Porton Down, Wiltshire, UK

Crimean-Congo Haemorrhagic Fever (CCHF) is a severe tick-borne disease, endemic in many countries in Africa, the Middle East, Eastern Europe and Asia. Depending on the mode of infection and medical care, approximately 10-70% of confirmed cases are fatal. There is no licensed vaccine or effective treatment available. In the present study, the attenuated poxvirus vector, Modified Vaccinia virus Ankara, was used to develop a recombinant vaccine expressing the CCHF virus glycoproteins. Cellular and humoral immunogenicity was confirmed in two mouse strains, including type I interferon receptor knockout mice, which are susceptible to CCHF disease. Our vaccine protected all recipient animals from lethal disease in a challenge model adapted to represent infection via a tick bite. Histopathology and viral load analysis of protected animals confirmed they had been exposed to challenge virus, even though they did not exhibit clinical signs. This is the first demonstration of the efficacy of a CCHF vaccine. Future work will focus on elucidating the nature of the protective immunity developed by this vaccine candidate.
All animal work was done in accordance with Home Office Guidelines and with local ethical committee approval.

LI17/21

Regulation of Rift Valley fever virus NSs expression in mosquito cells

Stephen Welch, Agnieskza Szemiel, Benjamin Brennan, Richard Elliott

MRC University of Glasgow Cebtre for Virus Research, Glasgow, UK, Glasgow, UK

Rift Valley fever virus is a zoonotic virus capable of causing severe disease in both humans and animals. The virus can be transmitted by mosquitos and epidemics of the disease are most commonly associated with increased numbers of mosquitos following high rainfall in endemic areas. Whilst the virus interactions within a mammalian host are well characterized, this understanding is not as well defined in mosquitos. The main virulence factor in mammalian hosts is the non structural protein NSs, and we have been investigating its role in mosquito cells, and how transcription and expression of the NSs mRNA are regulated in those cells. In addition we have rescued the siRNA response in *Aedes albopictus* C6/36 cells, which natively have a dysfunctional response due to a large deletion in the *dcr2* gene, and assessed the effect of NSs expression. We have also investigated the importance of proteasome directed degradation of NSs when it is expressed

LI17/22

The role of Influenza A structural proteins in filamentous Virus Like Particle (VLP) formation

Christopher Wilson, Scott Roddy, Jeremy Rossman

University of Kent, Canterbury, UK

The influenza A virus is an enveloped, negative strand RNA virus that is responsible both for seasonal outbreaks and pandemics. The virus displays both spherical and filamentous morphology, the prevalence of each being influenced by a variety of factors including strain, growth medium and point mutations. A higher proportion of filamentous virions is commonly observed in clinical isolates, whereas strains passaged in the laboratory more commonly produce only spherical virions. This suggests a fitness advantage for filamentous forms of the virus in vivo, however the reason for this advantage has yet to be determined. Using a plasmid based Virus Like Particle (VLP) system in Human Embryonic Kidney 293 cells we are investigating the ability of influenza A structural proteins to produce filamentous VLPs. Our investigations have revealed a key role of Neuraminidase (NA) in this process, as single protein expression has been repeatedly observed to produce filamentous

Please note: Abstracts are published as received from the authors and are not subject to editing.

VLPs through immunofluorescence microscopy and transmission electron microscopy. Furthermore we show that the addition of other structural proteins including M1 and Nucleoprotein affects the frequency and robustness of filamentous VLPs. This suggests that filamentous morphology is influenced by interplay between several structural proteins, with NA playing a key role.

LI17/23

Vaccines against rabies virus: Assessment of neutralising epitopes on the lyssavirus glycoprotein

Jennifer S Evans[1,2], Edward Wright3, Andrew J Easton[2], Anthony R Fooks[1,4], Ashley C Banyard[1]

[1]Virology Department, Animal Health and Veterinary Laboratories Agency, New Haw, Surrey, UK, [2]School of Life Sciences, University of Warwick, Coventry, West Midlands, UK, [3]School of Life Sciences, University of Westminster, London, UK, [4]National Consortium for Zoonosis Research, University of Liverpool, Liverpool, Wirral, UK

The lyssaviruses are a group of genetically divergent viruses capable of causing fatal encephalitis in mammals. Vaccines and post exposure treatments that protect against classical strains of rabies have been used globally for decades. In the assessment of vaccine protection, both serological and molecular models have defined several highly immunogenic epitopes within the glycoprotein that represent key areas targeted by neutralising antibodies. Currently, the lyssavirus genus is divided, based on genetic and antigenic data, into phylogroups indicating the level of protection afforded by rabies vaccines: licensed vaccines afford protection against all phylogroup I viruses but induce no neutralising antibodies against phylogroup II or III lyssaviruses. Here we demonstrate that swapping key antigenic regions between glycoproteins from different phylogroups can greatly alter the ability of hyperimmune sera to neutralise recombinant lyssavirus pseudotype viruses. We describe the effects of glycoprotein mutation on both the functionality and neutralisation of recombinant lyssavirus pseudotypes and comment on the effect of both inter and intra epitope exchange on neutralisation profiles from pseudotype viruses representing the different lyssavirus phylogroups. Finally, utilising reverse genetics techniques we assess the effect of glycoprotein mutation on the ability to rescue and grow recombinant lyssaviruses containing mutated glycoproteins.

LI17/24

Cellular interacting partners of Lujo Virus nucleoprotein, a recently identified haemorrhagic fever virus of the Arenaviridae

Andrew Buckley[1], Rebecca Surtees[1], Julian Hiscox[2], Roger Hewson[3], John Barr[1]

[1]University of Leeds, Leeds, West Yorkshire, UK, [2]University of Liverpool, Liverpool, Merseyside, UK, [3]Public Health England, Salisbury, Wiltshire, UK

The Arenaviridae family of negative sense RNA viruses comprise the majority of haemorrhagic fever viruses, contributing to significant human disease worldwide, with associated mortality between 15-80%. No vaccines or effective treatments are available for any of these serious human pathogens. Most arenaviruses are grouped into old world (OW) or New World (NW) clades based on sequence homology and serology, which correlates with their geographic location of initial isolation. Lujo virus (LUJV) was recently isolated from Zambia as the causative agent of a haemorrhagic fever with a mortality rate of 80%. Interestingly, LUJV exhibits significant sequence divergence from the classic OW isolates, placing it closer to the NW clade despite its geographical origin. To better understand the molecular and cellular biology of Lujo virus, we examined the interaction between the viral nucleoprotein (NP) and cellular factors required for the LUJV life cycle. We used an immunoprecipitation protocol based on the EGFP-Trap procedure to pull down cellular protein that associated with NP expressed in mammalian cells. Identified proteins included chaperone proteins, trafficking proteins and some involved in immune regulation, such as HSP70, actin and PCBP2. Characterisation of the interacting partners' role in replication may identify therapeutic targets to combat arenavirus-mediated disease.

LI17/25

Accumulation of human adapting mutations during the first 3 years of circulation of pH1N1 2009 in humans

Ruth Elderfield[1], Simon Watson[2], Alex Godlee[1], MOSAIC investigators, Paul Kellam[2] and Wendy Barclay[1].

[1] Imperial College London, London, UK. [2]Wellcome Trust Sanger Institute, Hinxton, Cambridge.

In spring 2009 a novel H1N1 influenza virus emerged from pigs and caused a pandemic. There were two additional waves of infection in the UK during the following winter seasons.
Genetic differences accumulated in all 8 genome segments in the later waves. We compared in detail two prototypic strains representing, the pandemic virus in 2009 as it emerged from pigs (England/195/2009, 1st wave) and a strain collected as part of the MOSAIC project in the winter of 2010/2011 (England/687/2010, 3rd wave), by measuring replication in human airway cells (HAE) and mice, interferon induction *in vivo* and *in vitro*, and polymerase mini-genome assays. Reverse genetic systems allowed the rescue of viruses with defined genomes.
Mutations in 3rd wave viruses altered receptor binding, enhanced polymerase activity and three changes in the NS1 protein enhanced the ability to suppress interferon induction. However a recombinant 1st wave virus with the 3rd wave NS mutations was attenuated in mice compared to the prototypic pandemic virus.
We postulate that the balance between virus replication, triggering of the interferon response and the virus' ability to counter that signal was altered as the virus adapted to its new human host in ways that affected the disease phenotype.

LI17/26

Elucidating the biochemical determinants for restriction of influenza virus polymerase activity by PB2 627E

Duncan Paterson, Aartjan te Velthuis, Frank Vreede, Ervin Fodor

Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Most avian influenza viruses do not replicate efficiently in human cells. This is partly due to the low activity of the RNA polymerase of avian influenza viruses in mammalian cells. An E→K adaptive

mutation at residue 627 of the PB2 subunit of the polymerase increases the activity of avian-derived virus polymerases in mammalian cells. Accordingly, viral ribonucleoprotein (RNP) reconstitution assays show that a viral polymerase containing PB2 627E characteristic of avian influenza viruses exhibits impaired activity in mammalian cells compared to a viral polymerase that contains PB2 627K characteristic of mammalian-adapted influenza viruses. In contrast, purified viral polymerases containing either PB2 627E or PB2 627K show comparable levels of activity in transcription assays that require no RNP assembly. To reconcile these conflicting observations, we used an NP-independent cell based transcription/replication assay to assess polymerase activity. We found that PB2 627E polymerase inhibition in mammalian cells is independent of NP expression, but is dependent on the length of the viral RNA template. In addition, restriction of PB2 627E polymerase was overcome by mutations specific to the viral RNA template promoter sequence. Consequently, we propose that PB2 627 affects recruitment of the viral RNA promoter by the viral polymerase in human cells.

LI17/27

The nuclear export function of influenza virus NEP is regulated by phosphorylation

Duncan Paterson, Ervin Fodor

Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

Influenza viruses replicate and transcribe their genomes within the nucleus of infected cells. Consequently, incorporation of newly synthesised viral genomic RNA into progeny virions is dependent on the nuclear export of genomic RNA, in the form of viral ribonucleoprotein complexes (vRNPs). To accomplish this, the virus encodes nuclear export protein (NEP) which acts as an adapter between vRNPs and the cellular nuclear export machinery in a manner that is likely to be tightly regulated. NEP has recently been shown to undergo phosphorylation at a highly conserved serine residue, 24S. We demonstrate that substitution of this residue with a phospho-mimetic amino acid specifically up-regulates nuclear export of NEP. In addition, biophysical characterisation of purified NEP indicates that phosphorylation of 24S may alter the tertiary conformation of NEP. In agreement with these findings, recombinant viruses with mutations to the NEP phosphorylation site were severely attenuated in replication. Accordingly, we propose that phosphorylation of NEP 24S results in a structurally distinct form of NEP that favours interaction with the host nuclear export machinery, thereby regulating the nuclear export of vRNP complexes.

LI17/28

Studying the immunogenicity of a novel vaccine against Crimean-Congo Haemorrhagic Fever

Aleksandra Miloszewska, Karen R. Buttigieg, Stuart D. Dowall, Miles W. Carroll, Roger Hewson

Public Health England, Porton Down, Wiltshire, UK

Crimean-Congo Haemorrhagic Fever (CCHF) is the most widespread tick-borne human viral disease endemic in the Balkans, Asia and Africa. The wide distribution of CCHF virus, the severity of the disease and the high fatality rate make CCHF a major public health concern. There is no licensed vaccine or effective treatment available.

Modified Vaccinia virus Ankara (MVA) is an attenuated poxvirus with a proven safety record. Despite its growth deficiency in most mammalian cells, MVA promotes high-level expression of recombinant genes. Its potent immunogenicity elicits cellular and humoral immunity and avoids the requirement for an adjuvant to be co-administered.

A viral-vectored vaccine based on recombinant MVA expressing the major CCHF glycoproteins was constructed. Three different strains of mice were vaccinated in a homologous prime-boost regimen. Antibody and cellular responses to CCHF antigens were analysed. The novel vaccine candidate induced T-cell and antibody responses in mice with various genetic backgrounds, including those with an abrogated type I interferon response which are susceptible to infection. Protective efficacy of the vaccine candidate will be studied to learn which arm of the immune response is required for protection from lethal disease

LI18

Virology workshop: clinical virology

LI18/02

To Investigate Whether Biliary Atresia has an Infectious Aetiology in a Paediatric Cohort

Apsara Kandanearatchi[1], Elke Ruttenstock[2], Mark Zuckerman[1], Richard Thompson[2], Mark Davenport[2], Melvyn Smith[1]

[1]South London Specialist Virology Centre King's College Hospital, London, UK, [2]Institute of Liver Studies King's College Hospital, London, UK

Background

Biliary atresia (BA) results from obstruction of extrahepatic bile ducts leading to chronic hepatic inflammation and fibrosis. Treatment strategies involve a Kasai procedure, if unsuccessful liver transplantation. The condition is grouped into four subtypes based on the clinical disease phenotype: Isolated (Type 1), Syndromic BA (Type 2), Cystic BA (Type 3) and CMV associated (Type 4). 75% of patients have Type 1 BA which is of unknown aetiology. An infectious aetiology has been suggested in a number of recent publications.

Methods

The aim of this investigation is to determine whether the disease is associated with a novel infectious agent or an as yet unidentified host-response.

An anonymised database was established and 169 samples, including bile, liver tissue and blood collected, additionally, there is an archive of approximately 250 samples. Samples are being analysed by SDS-PAGE and LC-MS/MS and next generation sequencing using small RNA libraries to maximize the pathogen-to host nucleotide ratio.

Results/Conclusion

Protein analyses show a number of potentially significant host and pathogen sequences. Results from the sequence analysis have identified several host miRNA and piwi RNA species, together with pathogen sequences that may correlate with the protein data. Further analyses are underway and the results will be presented.

LI18/03

Please note: Abstracts are published as received from the authors and are not subject to editing.

A novel high-throughput luciferase-based microneutralization assays for the diagnosis of bluetongue virus infection

Sandro Filipe Nunes[1], Gil Raven[2], Marco Caporale[1,3], Andrew Shaw[1], Massimo Palmarini[1]

[1]Centre for Virus Research, University of Glasgow, Glasgow, UK, [2]Inholland University of Applied Sciences,, Amsterdam, The Netherlands, [3]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy

Bluetongue virus (BTV) is a major infectious disease of ruminants with a worldwide distribution. Currently, 26 distinct serotypes of BTV (BTV-1 to BTV-26) have been identified showing varied levels of antibody cross-reactivity. Neutralization assays are typically used for the serological diagnosis of BTV infection. These assays are time-consuming and subjected to a high operator-dependent variability as they rely on the identification of viral cytopathic effect in reporter cells at 5 days post-infection.
In order to develop a reliable high-throughput BTV neutralization assay, we generated by reverse genetics a panel of synthetic BTV (sBTV) reassortants expressing a NanoLuc® Luciferase reporter gene. These sBTV viruses share a common BTV-1 "backbone" with heterologous outer core proteins of different serotypes (VP2/VP5). This system allows for similar replication kinetics in cell culture of sBTV of different serotypes and reduces variability as the output (luciferase luminescence) is quantified automatically avoiding operator bias. This novel assay is more time effective than traditional neutralization assays and can be easily automated for high-throughput in a BTV diagnostics setting.

LI18/04

HEV: an uncommon cause of cirrhosis in advanced HIV

Bhagteshwar Singh[1], Joel Paul[2], Ed Wilkins[1]

[1]Monsall Unit, Department of Infectious Diseases and Tropical Medicine, North Manchester General Hospital, Manchester, UK, [2]Department of Microbiology and Virology, The Royal Oldham Hospital, Oldham, UK

Background: Chronic hepatitis E virus (HEV) infection (viraemia >6 months) has been reported in immunosuppression, including HIV. A case of chronic HEV in a patient with advanced HIV and decompensated liver cirrhosis is presented, as is the Unit's HEV testing practice.
Methods: The patient's clinical, imaging and laboratory records were reviewed. Laboratory records were searched for all HEV tests in 2013.
Results: A 52 year old man was admitted with urinary sepsis, decompensated cirrhosis and uncontrolled HIV (diagnosed 1993). Anti-HEV (IgG and IgM) and HEV-RNA were positive for genotype 3 HEV. Ribavirin and highly active antiretroviral therapy (HAART) were commenced, but he developed recurrent bacteraemia and further decompensation of cirrhosis, dying of gastrointestinal haemorrhage. Stored samples confirmed positive serology and RNA for HEV over 14 months. In 2013, 24 HEV serology requests on HIV patients from this Unit yielded eight positive results; all for our reported patient.
Conclusion: Current guidance suggests testing for HEV in HIV if other causes of hepatic dysfunction have been excluded, and use of HAART and ribavirin in persistent infection, as was tried unsuccessfully in the reported case. This case highlights the importance of considering chronic HEV as an uncommon cause of liver disease in HIV.

LI18/05

HIV diagnosis in the older patient: Don't miss the opportunity!

Theodoros Vrouchos[1], Michael Przybylo[2], Joel Paul[3]

[1]Royal Oldham Hospital, Oldham, UK, [2]Royal Oldham Hospital, Oldham, UK, 3Royal Oldham Hospital, Oldham, UK

As HIV enters its 4th epidemic decade, an increase in this disease burden is being observed among the elderly population. According to the Public Health England, the number of new HIV diagnoses in patients over 50 years in the UK almost doubled between 1998 and 2013, and a higher proportion of this age group were diagnosed late in the disease as compared with younger adults.
Several factors account for this increase, including lack of targeted HIV prevention campaigns for this age group and risk awareness by frontline healthcare workers, including GPs and care homes.
We present a case of a 67 year old female in whom multiple opportunities for HIV diagnosis had been missed after non-specific clinical presentations including reoccurring shingles, stroke, and bilateral paraparesis. HIV testing was only considered when a diagnosis of cerebral Toxoplasmosis was made. At diagnosis, her CD4 count was 26 and HIV viral load was 750,000 copies per ml.
We believe she would have been diagnosed with HIV earlier if she presented with similar symptoms as a younger patient.
This case reiterates that HIV testing should be actively considered in the workup of elderly patients.

LI18/06

No longer any need to extract? – M-swab a simple option for processing swabs for herpes simplex virus and varicella zoster virus.

Daniel Guerendiain, Alison Hardie, Jenny Dove, Sandeep Ramalingam, Kate Templeton

Specialist Virology Centre, Department of Laboratory Medicine, Royal Infirmary, Edinburgh, UK.

The M-swab (Copan) is a newly designed kit to collect, transport and process samples for real-time PCR. No purification is required prior to PCR. Currently, viral transport medium (VTM) is the most commonly used transport medium for viral detection and it first requires extraction prior to PCR. This is a process which takes an hour and costs £3 per specimen. The aim was to compare the performance of M-Swab with VTM (UTM, Copan) and to assess if feasible for routine diagnosis.
Five positive samples collected in UTM for herpes simplex virus (HSV) and varicella zoster virus (VZV) were identified. In each case, 4 further 1:10 dilutions were made in UTM to identify lower limit of detection. Testing of each dilution was performed on day 0, 2, 7 and 28. The performance of the PCR was compared with either prior extraction, 3 minute heating of M-Swab at 98oC or no extraction.
The M-swab achieved the same end point sensitivity for HSV and VZV. The comparable dilutions generally had a lower Ct (1-2 Cts) with the prior extraction. Samples tested with no extraction performed poorly and missed 70% of positive results. M-Swab could be a simple alternative for processing swabs prior to PCR.

LI18/07

Parvovirus B19 infections in pregnant women in Edinburgh in 2012 -2013

Heli Harvala, Intisar Al Shukri and Kate Templeton

Specialist Virology Centre, Royal Infirmary Edinburgh, UK

Parvovirus B19 (B19V) infections are common, but under-investigated and under-reported. Here we describe the characteristics of acute B19V infections observed in pregnant women during the last epidemic season.
Increased numbers of B19V infections were noted in the Edinburgh area in 2012 - 2013, with a total of 123 acute infections (33 pregnant women, 76 non-pregnant women and 14 men). The majority of non-pregnant females (86%) and all males presented with symptoms including arthralgia, rash and anaemia, whereas only one pregnant woman was symptomatic (3%). An overall infection rate of 18% was measured in pregnant women who were tested following B19V exposure (26/141). Furthermore, a 7% seroconversion rate was recorded in non-immune pregnant women who were re-tested after exposure (7/104). The highest rate of foetal loss (25%; 3/12) was observed in those who had acute B19V infection in early pregnancy (<11 weeks), whereas no pregnancies were lost if acute infection was recorded after the gestation age of 12 weeks (0/21).
These results indicate that effort should be targeted towards investigation of suspected B19V infections in early pregnancy. In addition, better-structured virological and public health data collection on acute B19V infections and associated foetal losses would be beneficial for planning further interventions.

LI18/08

Offered paper – Characterizing the neutralization profile of diverse, patient derived, Hepatitis C Virus samples to broadly neutralizing antibodies

Sushant Saluja, Alexander Tarr

University of Nottingham, Nottingham, UK

Hepatitis C virus (HCV) replicates through interaction of its surface glycoprotein E2 with various receptors on hepatocytes. An effective antibody-based vaccine must block this interaction by eliciting potently neutralizing antibodies. In order to inform vaccine design, however, the breadth of resistance to broadly neutralizing anti-HCV antibodies (BNAb) needs to be determined. Therefore, we characterized the neutralization profile of 10 highly infectious HCV isolates to five BNAbs. Furthermore we sequenced the E1E2 genes of a subset of HCV samples to identify any selective pressures in the antibody binding regions, and to determine what produces the infectious and non-infectious phenotypes.
Reactivity of antibodies was tested against HCV pseudoparticles, which were created by incorporating full-length, patient-derived, HCV glycoproteins E1 and E2 onto retroviral core particles.
These pseudoparticles demonstrated different susceptibilities to neutralization and could be divided into neutralization-sensitive and-resistant phenotypes. Sequence analysis showed mutations in key regions implicated in antibody binding within the E1E2 gene of antibody-resistant pseudoparticles.
Comparison of the highly conserved epitope 436GWLAGLFY443 and certain other residues showed that residues known to mediate HCV infectivity were not conserved in the non-infectious samples.
This study has identified patient-derived HCV isolates at the extremes of neutralization sensitivity. These can potentially be used to test an antibody's therapeutic potential to inform vaccine design.

LI18/09

Anti-pathogen associated gene up-regulation following blood-feeding in a mosquito vector for West Nile virus, *Culex pipiens*

Karen Mansfield[1], Charlotte Cook[1], Richard Ellis[1], Nicholas Johnson[1], Anthony Fooks[1,2]

[1]Animal Health and Veterinary Laboratories Agency, New Haw, Surrey, UK, [2]National Centre for Zoonoses Research, Liverpool, UK

Culex pipiens mosquitoes constitute a vector for a number of pathogens, including the mosquito-borne flavivirus West Nile virus (WNV). WNV is a neuroinvasive virus, causing encephalitic disease in humans and other vertebrates, including horses and birds. Mammalian infection stimulates an innate immune response with a cytokine profile typical of other neurological viruses. However the immune response within the mosquito vector is not clearly understood. The emergence of WNV in Europe, and the prevalence of *Culex pipiens* mosquitoes in the UK, suggests that this virus has the potential to emerge in the UK. The aim of this study was to identify mosquito genes stimulated by blood-feeding which may be associated with an antiviral response.
Adult *Culex pipiens* mosquitoes hatched from eggs collected in Surrey, UK, were fed fresh horse blood. RNA was extracted from homogenates of unfed and fed mosquitoes, and further processed to enable Illumina sequencing. Through comparison with an existing *Culex pipiens quinquefasciatus* genomic index, sequence analysis enabled identification of mosquito genes up-regulated following blood-feeding. Along with up-regulation of many metabolic and developmental genes, blood-feeding induced up-regulation of genes with an anti-pathogen function. These results provide an insight into the mosquito immune response, and act as a useful control for future experiments, investigating the effect of flavivirus infection on gene expression in the mosquito.

LI18/10

Evaluation of a pseudotyped lentivirus-based neutralisation assay for potency testing of post exposure prophylaxis rabies immunoglobulins

Giada Mattiuzzo[1], Edward Wright[2], Carl Dolman[3], Phil Minor[1], Mark Page[1]

[1]NIBSC-MHRA, Division of Virology, South Mimms-Potters bar, UK, [2]University of Westminster, London, UK, [3]NIBSC-MHRA, Division of Biotherapeutics, South Mimms, Potters Bar, UK

Rabies infection is still a burden in developing countries, causing acute viral encephalitis, for which the prognosis is almost always fatal. Vaccination and prompt administration of post exposure prophylaxis (PEP) are highly effective in preventing disease. PEP involves a combination of rabies vaccine and rabies immunoglobulins (RIG). Biological potency of RIG is currently tested using the Rapid Fluorescent Focus Inhibition Test (RFFIT), a cell-based neutralisation assay performed using wild type rabies virus CVS-11, which requires a biological containment level 4 (CL4). Pseudotyped lentivirus-based neutralisation assay offers the advantages of being a safer and more accessible test. RIG samples

Please note: Abstracts are published as received from the authors and are not subject to editing.

were tested in a neutralisation assay using a HIV-based virus expressing the envelope of rabies virus strain CVS-11. The potency of the RIG is evaluated against the WHO 2nd International Standard for anti-rabies immunoglobulin, and the results compared with those obtained using RFFIT. The adoption of the pseudotyped lentivirus-based neutralisation assay will preclude the need to handle highly contagious wild type rabies virus under CL4 facilities, allowing for a safer and more accessible potency assay for RIG.

LI18/11

Human Enterovirus 68: a polio mimic in children?

By Suzanne English[1], Surendra Parmar[1], Alasdair Parker[2] Deepa Krishnakumar[2] Robert Ross-Russell[3], Kathryn Rolfe[1], Hamid Jalal[1], Chris Smith[1], Hongyi Zhang[1]

[1]Department of Medical Virology, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ. [2]Child Development Centre, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ. [3]Department of Paediatrics, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ.

Since the mid-2000s, there have been increasing numbers of reported outbreaks of a rare human enterovirus, human enterovirus 68 (hEV68), associated with moderate to severe respiratory disease in children. However, in 2013-2014 a report of up to 20 paediatric cases from California has suggested an additional association between hEV68 and acute flaccid paralysis mimicking polio, following a short respiratory illness. We report the first UK case of persistent, partial paralysis in a 10 month old boy following severe bronchiolitis and contemporaneous hEV 68 positivity by PCR on nasopharyngeal aspirate but not on cerebrospinal fluid (CSF) analysis. The lack of CSF positivity close to the onset of paralysis makes a direct, polio-like infection of the central nervous system unlikely but does not exclude an immune-mediated process.

LI19

Virology workshop: retroviruses

LI19/01

Replication kinetics and persistence of a conditionally live attenuated SIV (SIVrtTA) *in vivo* confers protection against homologous and heterologous wild-type challenge in macaques

Neil Berry[1], Maria Manoussaka[2], Claire Ham[1], Deborah Ferguson[1], Mark Page[1], Richard Stebbings[1], Atze Das[1], Martin Cranage[3], Neil Almond[1], Ben Berkhout[1]

[1]NIBSC, South Mimms, UK, [2]St Georges, University of Lond, London, UK, 3Amsterdam Medical Centre, Amsterdam, UK

Live-attenuated SIV vaccination confers potent protection against wild-type (wt) SIV in macaques. A novel live-attenuated SIVmac239Δnef vaccine (SIVrtTA), dependent on doxycycline (dox) for replication, was evaluated for protective efficacy *in vivo.* Two vaccine studies have been performed using this vaccine: one in Indian rhesus macaques (RM) that were vaccinated with SIVrtTA for 6 months before homologous challenge with wild type SIVmac239and the second in Mauritian cynomolgus macaques (MCM) vaccinated for between 3 and 20 weeks before challenge with the heterologous SIVsmE660 virus. Two of 8 vaccinated rhesus macaques challenged with SIVmac239 and 10/18 challenged with SIVsmE660 were protected against detectable infection with wild type virus. The kinetics of replication and persistence of the vaccine virus appears to unify these apparently distinct results and predict protective outcome. The potential mechanisms by which vaccine persistence contribute to superior protection will be presented.

LI19/02

Viral induction of tumours in precision-cut lung slices

Chris Cousens, Charline Alleaume, Esther Bijsmans, Henny Martineau, Patricia Dewar, Jeanie Finlayson, Mark Dagleish, David Griffiths

Moredun Research Institute, Edinburgh, UK

Ovine pulmonary adenocarcinoma (OPA) is a lung cancer of sheep caused by jaagsiekte sheep retrovirus (JSRV). Previous studies have shown that the JSRV Env protein has oncogenic activity and triggers neoplastic transformation by activating a number of cellular signalling pathways. OPA therefore provides a unique model for investigating early events in lung cancer pathogenesis. Here, we used precision-cut lung slices (PCLS) to study JSRV infection and transformation *in vitro*.

PCLS prepared from ovine lung were infected with JSRV and maintained in culture for up to 3 weeks. Increasing amounts of JSRV RNA were detectable in culture supernatants and multiple foci of virus-positive cells were identified within the tissue slices, indicating that JSRV can replicate in this system. Infected cells were also positive for cellular markers of type 2 pneumocytes. A proportion of JSRV-infected cells also showed positive labelling for P-Akt and P-ERK1/2, indicating that these molecules are activated in JSRV-infected PCLS. These pathways were also activated in natural and experimentally-induced lung tumours from sheep.

These results validate PCLS as a novel tool for studying the early events of JSRV infection in the lung. Future work will examine the role of additional signalling molecules and pathways in JSRV-induced transformation.

LI19/03

Retromer interacts with the HIV-1 envelope glycoprotein and regulates its intracellular trafficking

Elisabetta Groppelli*, Luke Granger, Alice Len and Clare Jolly

The envelope glycoprotein (Env) of HIV-1 is a critical determinant of viral infectivity and is the main target for humoral responses. Correct intracellular localization of Env and incorporation into virions is required for efficient viral assembly and spread; however, little is known about how Env trafficked in infected cells. Retromer is a conserved complex that associates with endosomal membranes and directs endosome-to-Golgi retrieval of a select group of cargo proteins. Here we identify retromer a novel and important cellular factor regulating Env trafficking. Using immunofluorescence microscopy, RNAi and functional assays we show that Env colocalises with retromer and that inactivating retromer alters

Env trafficking, impairs Golgi retrieval and impacts on virion assembly. Notably, mutagenesis and binding studies reveal that the determinants of retromer-dependent Env trafficking map to the Env cytoplasmic tail (EnvCT). Our data suggest a model in which retromer complex plays a key role in regulating endosomal sorting of Env that is mediated by interactions between retromer and the EnvCT. We thus describe retromer as previously unknown cellular factor regulating Env trafficking and identify Env as a new member of a select family of retromer-sorted cargo proteins.

LI20

Virology workshop: respiratory viruses

LI20/01

Developing a pseudotype virus neutralisation assay for West Nile virus

Barnabas King[1], Edward Wright[2], Stuart Mather[3], Simon Scott[3], Nigel Temperton[3], Daniel Horton[4], Janet Daly[1]

[1]School of Veterinary Medicine and Science, University of Nottingham, Nottingham, UK, [2]Faculty of Science and Technology, University of Westminster, London, UK, [3]Viral Pseudotype Unit, Medway School of Pharmacy, Medway, UK, [4]Wildlife Zoonoses and Vector-Borne Diseases Research Group, Animal Health and Veterinary Laboratories agency, Weybridge, UK

West Nile virus (WNV) is a mosquito-borne virus that circulates in wild birds and causes incidental infections in humans and horses. WNV infection of humans and horses is usually asymptomatic but it can cause a neuro-invasive disease termed West Nile encephalomyelitis, which can range from fever through to paralysis and sudden death.

The UK is currently free of WNV, however spread of the vector into new regions of Eastern Europe and the Mediterranean and expanding global trade increase the likelihood of infected vectors reaching the UK.

WNV produces low levels of viraemia so diagnosis relies upon detection of virus-specific antibodies. The plaque reduction neutralisation test (PRNT) is more specific than ELISA, but diagnosis can take up to 14 days; WNV infections are typically cleared or develop into West Nile encephalomyelitis within 10-14 days post-infection. In addition; PRNT is high cost, low through-put and requires BSL 3 facilities.

Pseudotype viruses (PVs) can be used at BSL 1, detect virus-specific neutralising antibodies and provide quantitative out-puts for more rapid, standardised diagnosis. We have pseudotyped the WNV prM and E glycoproteins on a lentivirus backbone with the aim of developing a pseudotype virus neutralisation assay for diagnosis of WNV infection.

LI20/02

Different forms of M2e vaccines provided cross-protection against H5N1 influenza in mice

BJ Zheng, VHC Leung, KY Yuen

The University of Hong Kong, Hong Kong, Hong Kong

In this study, we aims to develop universal vaccine based on different forms of M2 vaccine candidates against H5N1 infection. We designed and prepared mM2e (synthetic monomer M2e peptide), M2e-MAP (tetra-branched multiple antigen peptide), fusion protein M2e-ASP-1 (1 M2e fused with ASP-1) and M2e3-ASP1 (3 M2e fused with ASP-1) and evaluated their immune responses and protective effects.

Our results showed that (1) M2e-MAP, M2e-SAP-1 and M2e3-ASP-1 elicited much stronger M2e-specific antibody responses than mM2e; (2) M2e-MAP and M2e3-ASP-1 provided better protection and cross-protection against viral challenges of different clades/strains of H5N1 virus and H1N1 virus than M2e-ASP-1, whereas mM2e did not show obvious protection against viral challenge of H5N1 virus; (3) consistently, the mice vaccinated with M2e-MAP and M2e3-ASP-1 showed much lower viral load detected and less inflammation found in lung tissues; (4) cross-protective effect of M2e-based vaccines might be related to the identity of the M2e sequence between the vaccine and the H5N1 virus.

Taken together, this study has demonstrated that both M2e-MAP and M2e3-ASP-1 can provide potent cross-protection against infections of some heterologous strains of H5N1 virus and H1N1 virus.

LI20/03

Prevalence and risk factors associated with entamoeba histolytica/dispar/moshkovskii infection among selected local government in zone c senatorial district of yobe state

Adamu Galadima Dagona

Yobe State University, Damaturu, Nigeria

This study was carried out to determine the incidence and risk factors associated with infection of E. histolytica/E. dispari among three local governments, in Zone C senatorial district of Yobe. Of 500 Faecal samples were examined by formalin-ether sedimentation and trichrome staining techniques. Of which, 18.5% (38/205) of Nguru, 14.8% (21/155) of Machina, and 30.71% (43/140) of Jajimaji were positive for E. histolytica/E. dispar moshkovskii respectively. The infection showed an age-dependency relationship, with higher rates observed among those aged above 13 years in all local government studied. Multivariate analysis confirmed that not washing hands and other family members infected with E. histolytica/E. dispar were significant risk factors of infection among all local government. However, eating with hands, the consumption of raw vegetables, and close contact with domestic animals were identified as significant risk factors in Jajimaji. The findings highlighted that E. histolytica/E. dispar parasites are still prevalent in the zone Further studies using molecular approaches to distinguish the morphologically identical species of pathogenic, E. histolytica from the non-pathogenic, E. dispar are needed. The establishment of such survey will be useful in the planning and implementation of specific prevention and control strategies of this infection in zone C senatorial zone.

LI20/04

Using label free proteomics to investigate human respiratory syncytial virus and the effects of the antiviral ribavirin

Waleed Aljabr[1], Olivier Touzelet[1], Stuart Armstrong[1], David Matthews[3], John Barr[2], Julian Hiscox[1]

[1]University of Liverpool, Liverpool, UK, [2]University of Leeds, Leeds, UK, 3University of Bristol, Bristol, UK

Human respiratory syncytial virus (HRSV) causes severe lower respiratory tract infection in infants worldwide. Costly antiviral

Please note: Abstracts are published as received from the authors and are not subject to editing.

therapies to HRSV are of limited efficacy. Ribavirin is one of the most commonly used antiviral drugs licensed for the treatment of many different virus infections including HRSV. The precise mechanism of how ribavirin disrupts HRSV biology is unknown and both error catastrophe and defects in cellular RNA metabolism have been proposed. This project used a combination of label free quantitative proteomics and deep sequencing to investigate the potential mechanism of action of ribavirin in the inhibition of HRSV. Different treatments with ribavirin were analyzed for their effect on HRSV biology. Selected examples were further characterized using the omic approaches. Over 1500 cellular proteins were identified and quantified by LC-MS/MS and pathway analysis showed significant changes in proteins associated with RNA metabolism. Quasi species analysis of the viral RNA genome suggested that the frequency of nucleotide changes over the assay period was no different to when the virus was passaged on a different cell type or treated with an alternative inhibitor of HRSV polymerase function. The data suggests that the effect of ribavirin on HRSV is cellular mediated rather than directly anti-viral.

LI20/05

Biophysical characterisation of Influenza A (H3N2) virus receptor binding

Lauren Parker, Stephen Martin, Rodney Daniels, John McCauley

MRC National Institute for Medical Research, London, UK

Influenza A virus (subtype H3N2) is a cause of seasonal influenza in humans and is included as a component of seasonal flu vaccines. These vaccines are generally propagated in eggs, and this commonly results in the introduction of amino acid substitutions into the haemagglutinin (HA) glycoprotein. These mutations in HA can affect virus receptor binding but in addition, can alter the antigenic properties of the virus and so compromise the choice of candidate vaccine virus strains.

To evaluate the effects of egg-adaptive changes seen in recent H3N2 vaccine viruses on sialic acid receptor binding, we have carried out quantitative measurement of virus receptor binding using surface biolayer interferometry. Included in these studies were a panel of mutant H3N2 viruses generated by reverse genetics to represent mutations seen in both vaccine (egg-propagated) and circulating wild-type (cell-propagated) viruses.

The results demonstrated a measurable decrease in binding to sialic acid-containing receptor analogues in all viruses where mutations that arose due to egg-propagation were reverted back to wild-type sequence. Conversely, receptor binding increased in viruses where egg adaptation mutations were introduced into cell-propagated virus. Antigenic analyses of these viruses are also being carried out to correlate changes in receptor avidity with antigenic properties.

LI20/06

Characterisation and safety testing of an A(H7N9) candidate vaccine virus

Kate Guilfoyle[1], Sarah Roseby[1], Carolyn Nicolson[1], Michael Bennett[2], Lynne Whittaker[2], Rod Daniels[2], John McCauley[2], Robert Newman[1], Ruth Harvey[1], Othmar Engelhardt[1], Diane Major[1]

[1]National Institute for Biological Standards and Control, Potters Bar, UK, [2]MRC National Institute for Medical Research, London, UK

Following the emergence of A(H7N9) viruses in China in spring 2013, a number of laboratories generated candidate vaccine viruses (CVVs) that can be used for the production of vaccines. We generated two CVVs using reverse genetics technology. One of these, derived from wt virus A/Anhui/1/2013 and termed NIBRG-268, was fully characterised according to WHO guidelines. The virus was found to be antigenically equivalent to its parental wt virus. Safety testing included assessment of pathogenicity in ferrets. We found that the wt virus, A/Anhui/1/2013, did not cause overt signs of disease in infected ferrets but replicated efficiently in the lower respiratory tract. The CVV NIBRG-268 replicated in the upper respiratory tract, but we could not detect infectious virus in the lungs of infected animals. Thus, we concluded that NIBRG-268 was attenuated relative to its parental wt virus and that it met the specifications of a CVV that can be used by vaccine manufacturers under BSL2 enhanced conditions.

LI20/07

The study of protease-mediated haemagglutinin activation using influenza A virus pseudotypes

Francesca Ferrara[1], Eleonora Molesti[1], Eva Böttcher-Friebertshäuser[2], Stefan Pöhlmann[3], Davide Corti[4], Giovanni Cattoli[5], Simon Scott[1], Nigel Temperton[1]

[1]Viral Pseudotype Unit, School of Pharmacy, University of Kent, Chatham Maritime, Kent, UK, [2]Institute of Virology, Philipps University Marburg, Marburg, Germany, [3]Infection Biology Unit, German Primate Center, Göttingen, Germany, [4]Institute for Research in Biomedicine, Bellinzona, Switzerland, [5]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy

The monomer of influenza haemagglutinin (HA) is synthesized as a polypeptide precursor that during maturation is cleaved by proteases into two active subunits. Recent studies have demonstrated the role of the human transmembrane protease serine 2 (TMPRSS2), the human transmembrane protease serine 4 (TMPRSS4) and the human airway trypsin-like protease (HAT) in HA activation by cleavage. As a model of activation of all influenza subtypes we have used influenza HA lentiviral pseudotypes. Influenza pseudotypes were obtained by co-transfecting human embryonic kidney HEK293T/17 cells using plasmids coding for the influenza HA, HIV gag-pol and a lentiviral vector incorporating firefly luciferase. In order to investigate the role of proteases in HA activation, a plasmid expressing protease was co-transfected during pseudotype production. The influenza pseudotypes produced in the presence of proteases were then tested for their ability to transduce HEK293T/17 cells and were compared to pseudotypes produced in the absence of proteases, and after trypsin treatment to mediate HA cleavage and activation.

The results obtained show that TMPRSS2, TMPRSS4 and HAT can activate, *in vitro*, with differential specificity both the HA of human seasonal influenza and also other avian HA influenza strains in a pseudotype production system.

LI20/08

The study of heterosubtypic antibody responses against Influenza A viruses elicited by seasonal vaccination using a pseudotype neutralization

assay

Francesca Ferrara[1], Eleonora Molesti[1], Eva Böttcher-Friebertshäuser[2], Davide Corti[3], Simon Scott[1], Emanuele Montomoli[4], Nigel Temperton[1]

[1]Viral Pseudotype Unit, School of Pharmacy, University of Kent, Chatham Maritime, Kent, UK, [2]Institute of Virology, Philipps University Marburg, Marburg, Germany, [3]Institute for Research in Biomedicine, Bellinzona, Switzerland, [4]Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy

The evaluation of the ability of vaccines to elicit heterosubtypic antibody responses to confer broad protection against different influenza subtypes is an important facet of pandemic preparedness. Classical serological assays, such as haemagglutination inhibition and microneutralization, have demonstrated low sensitivity for the detection of cross-neutralizing antibodies, especially those directed against epitopes in the haemagglutinin HA2 stalk region. Influenza pseudotypes represent safe tools to study the neutralizing antibody response since they are replication-defective viruses and they harbour on their envelope only the haemagglutinin that is the major target of this response.

We have generated a panel of human and avian influenza A pseudotypes and have employed them as surrogate antigens in neutralization assays to study the presence and magnitude of heterosubtypic neutralizing antibody responses in human sera collected before and after the 2007-2008 seasonal influenza vaccination.

In the human sera tested using a pseudotype neutralization assay, heterosubtypic neutralizing antibody responses are detected and the neutralizing antibody end-point titres are boosted after seasonal vaccination. The increased sensitivity of the pseudotype neutralization assay performed using a panel of influenza A pseudotypes permits the detection of heterosubtypic antibody responses before and after seasonal influenza vaccination.

LI20/09

Investigating the role of chaperones in HRSV infection and their potential as antiviral targets

Diane Munday[1], Weining Wu[1], Nikki Smith[2], Jenna Fix[3], Paul Digard[2], John Barr[4], Andrew Easton[5], Jean-François Eléouët[3], Julian Hiscox[1]

[1]University of Liverpool, Liverpool, UK, [2]University of Edinburgh, Edinburgh, UK, [3]French National Institute for Agricultural Research, Jouy-en-Josas, France, [4]University of Leeds, Leeds, UK, 5University of Warwick, Warwick, UK

Human respiratory syncytial virus (HRSV) is a major cause of serious, lower respiratory tract infection in infants. Infections recur throughout life and no efficacious treatment or vaccine is available. Anti-viral compounds face the challenge of high mutation rates. Targeting a cellular protein might therefore aid the design of anti-viral compounds with a broader spectrum of activity which may be less prone to the development of drug resistance. Molecular chaperones such as HSP70 and HSP90, and their co-chaperones present such an opportunity. Byassisting in the folding and/or assembly of macromolecular structures such as viral protein-protein interactions, such processes could be disrupted. Here, the pro-viral role of chaperones in HRSV infection was explored in the context of wild type infection and through the use of a mini-replicon system to focus on replication. Immunoprecipitation, combined with quantitative proteomics, was used to identify cellular proteins which potentially interacted with viral proteins involved in replication. Ablation of chaperone function was achieved using small molecule inhibitors or siRNA knock-down that abrogated various aspects of virus biology. This study demonstrates that the function of cellular proteins such as chaperones could be targeted to develop a virus-specific therapy which targets a specific virus-host cell protein interaction.

LI20/10

Production of Influenza B haemagglutinin lentiviral pseudotype particles and their use in neutralization assays

Francesca Ferrara[1], Eva Böttcher-Friebertshäuser[2], Davide Corti[3], Simon Scott[1], Paul Kellam[4], Sarah C Gilbert[5], Nigel Temperton[1]

University of Kent, UK

Influenza B (FluB) viruses cause respiratory disease epidemics in human populations. Furthermore an Influenza B strain is routinely included in seasonal vaccination and recently a quadrivalent vaccine containing two FluB strains was developed. Serological methods permit the evaluation of Influenza epidemiological distribution and are additionally used to evaluate vaccine efficacy. However the haemagglutination inhibition assay has been shown to be relatively insensitive for the detection of antibodies against FluB viruses. The use of replication-defective viruses, such as pseudotypes, in microneutralization assays is an accepted and safe alternative approach to study antibody responses elicited by natural infection or vaccination. We have produced a panel of Influenza B haemagglutinin (HA) pseudotypes using a standard pseudotype-producing transfection protocol. To activate FluB HA we have explored the use of two proteases, the transmembrane protease serine 2 and the human airway trypsin-like protease, by adding the relevant encoding plasmid to the transfection mixture. When tested for their ability to transduce target cells, the influenza B pseudotypes exhibit high transduction titers. With these newly developed reagents, which have hitherto not been reported in the literature, we are currently investigating the feasibility of using Influenza B pseudotypes as surrogate antigens in neutralization assays

LI20/11

Investigation of neutralising antibody responses to outbreak and vaccine strains of equine influenza using pseudotyped viruses

Rebecca Kinsley[1], Stuart Mather[1], Debra Elton[2], Alana Kilby[2], Janet Daly[3], Nigel Temperton[1], Simon Scott[1]

[1]Viral Pseudotype Unit, School of Pharmacy, University of Kent, Chatham Maritime, Kent, UK, [2]Animal Health Trust, Newmarket, Suffolk, UK, [3]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK

In 1979, influenza caused a major epidemic amongst horses across Europe including Newmarket, UK. Subsequently, vaccines were produced using multiple outbreak strains including A/equine/Newmarket/79 and A/equine/Fontainebleau/1/79 (both subtype H3N8) which prevented further outbreaks until 1989 when a new antigenic drift variant emerged. The A/equine/Sussex/89 (H3N8) strain came from one of the affected regions in the UK where both

Please note: Abstracts are published as received from the authors and are not subject to editing.

unvaccinated and, notably, vaccinated horses were affected. The accumulation of mutations within important antigenic epitopes of the virus surface glycoprotein haemagglutinin (HA) can lead to a decrease in the efficiency of antibody recognition. Three mutations, previously identified within five major epitopes of human influenza HA, have been incorporated into equine influenza pseudotyped lentiviruses (PVs). The PVs were generated via co-transfection of HEK293T cells with four plasmids expressing the equine influenza HA surface glycoproteins, HIV gag-pol, firefly luciferase reporter gene and TMPRSS2 endoprotease (to cleave the HA). The resulting viruses were harvested and pseudotype virus antibody neutralisation assays (PVNAs) against Newmarket/79 and Sussex/89-specific sera were performed. Results showed that specific single amino acid mutations in the putative major epitope sites altered the ability of the sera to neutralise the PVs, indicating their importance in vaccine protection.

LI20/12

Development of a pseudotype-based neutralisation assay for seal influenza virus

Kyriaki Tsalouchidou, Rebecca Kinsley, Stuart Mather, Francesca Ferrara, Nigel Temperton, Simon Scott

Viral Pseudotype Unit, University of Kent, School of Pharmacy, Chatham Maritime, Kent, UK

Influenza is a classic zoonotic infection, with transmission to humans from avian (e.g. poultry) and mammalian sources (e.g. swine). This has led to limited outbreaks, seasonal epidemics and occasional pandemics varying in severity. The primary reservoir for influenza virus is in the wild bird population, particularly waterfowl. Recently, an outbreak of influenza-mediated pneumonia killed hundreds of seals along the New England coast. Sequence analysis revealed specific amino acid differences between the seal isolate (A/harbour seal/Massachusetts/1/2011; H3N8 subtype) and circulating North American waterfowl strains. In order to establish a tool for future serological studies we have developed a pseudotype virus neutralisation assay (PVNA). The replication-defective pseudotype virus (PV) particles generated contained a lentivirus 'core' and an envelope containing the seal influenza surface glycoprotein, haemagglutinin (HA). HA is able to elicit neutralising antibody responses which can prevent virus attachment or endosomal fusion activity in target cells. The PV produced was used to measure neutralising antibody levels in a series of H3 haemagglutininsubtype reference sera (human, avian, equine) and naïve or pre-vaccination controls. The PVNA was able to clearly differentiate between these serum sets, and thus provides a valuable experimental assay for future research on seal populations (e.g. sero-surveillance, sero-epidemiology, antigenic drift).

LI20/13

Structural Studies of Respiratory Syncytial Virus

Saskia Bakker[1], Joanne Power[1], Swetha Vijayakrishnan[1], Hussain Jaffery1, Colin Loney[1], Jude Short[2], David Bhella[1]

[1]MRC - University of Glasgow Centre for Virus Research, Glasgow, UK, [2]MRC Laboratory of Molecular Biology, Cambridge, UK

Human Respiratory Syncytial Virus (hRSV) is an important cause of pneumonia and bronchiolitis in infants, as well as the elderly and immunocompromised. No vaccine or effective anti-viral therapies are available for hRSV. Difficulties in purification of hRSV virions have resulted in a lack of structural information about the virion and delayed the developments of new treatment. To circumvent the purification process, cells were cultivated and infected directly on electron microscopy grids and plunge-frozen in situ. Although irregular and spherical virions are also observed, data collected from these samples show some virions exhibit filamentous morphology with an apparently regular matrix layer and regularly spaced surface glycoproteins, which suggests the glycoproteins and matrix may be linked. The filamentous virions form the basis for helical 3D reconstruction using Iterative Helical Real-Space Reconstruction (IHRSR) and Fourier-Bessel methods. Tomograms have also been collected and sub-tomogram averaging of the surface glycoproteins is in progress.

LI20/14

Using Mass Spectrometry to Identify Interacting Partners of IFITM3

Sarah Smith[1], Rachael Wash[1], Mercedes Pardo[1], Jyoti Choudhary[1], Paul Kellam[1,2]

[1]Wellcome Trust Sanger Institute, Hinxton, UK, [2]5MRC/UCL Centre for medical molecular virology, Division of Infection and immunity, University College London, London, UK

Interferon-inducible transmembrane protein 3 (IFITM3) is an effector protein of the innate immune system, which confers potent, cell-intrinsic resistance to infection by diverse enveloped and non-enveloped viruses, both *in vitro* and *in vivo*, including influenza virus, West Nile virus, dengue virus and reovirus.

IFITM3 prevents cytosolic entry of these viruses by blocking complete virus fusion with cell endosome membranes. There is some evidence that IFITM3 prevents fusion by increasing the levels of cholesterol inside the endosomes but it is still unclear how this mechanism works or how many interacting partners there are for this protein.

We have established stable cell lines (A549s) over-expressing HA-C-terminally-tagged human IFITM3 for Stable Isotope Labelling by Amino Acids in Cell culture (SILAC) to identify interacting partners of IFITM3 that are not present in the non-infective state. Influenza infections were carried out in the carbon-13 labelled cell lines and unlabelled cells were used as a non-infective control. Co-immunoprecipitations were carried out in tandem and the eluates pooled before analysis by Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry.

Such a 'top-down' methodology is needed to identify novel protein-protein interactions of host antiviral restriction factors.

LI20/15

The nucleo-cytoplasmic trafficking of human respiratory syncytial virus matrix protein is differentially regulated between strains from A and B subgroups.

Olivier Touzelet, Julian Hiscox

University of Liverpool, Liverpool, Merseyside, UK

Human respiratory syncytial virus (HRSV), of which there are two subgroups (A and B), is a major cause of morbidity and mortality in humans. There is still no vaccine or therapeutic option available. Its matrix protein (M) can traffic in and out of the nucleus of infected

cells via its nuclear localization (NLS) and nuclear export signals (NES). Interestingly, both the NLS and the NES have two amino acid changes between A and B strains that are conserved within each subgroup. Therefore, we hypothesized that these differences may differentially regulate the nucleo-cytoplasmic trafficking of M between subgroups. To address this, A549 cells were infected with a strain from each subgroup (A2 and B8/60), which led to a reduced nuclear localization of M following infection with B8/60. Furthermore, using GFP-tagged constructs of M from each strain we showed that these amino acid changes within the nuclear transport signals are sufficient to modify the sub-cellular localization of the protein. In order to swap signals between A and B subgroups, site-directed mutagenesis of the NLS/NES on the GFP-tagged constructs are ongoing, as well as generation of recombinant viruses. Thus, deciphering the mechanisms regulating M nucleo-cytoplasmic trafficking may open new therapeutic strategies against HRSV.

LI20/16

Extending the repertoire of anti-influenza compounds: biological and structural analysis of two new classes of neuraminidase inhibitor.

Philip Kerry[1], Sankar Mohan[2], Nicole Bance[2], Masahiro Niikura[2], Mario Pinto[2]

[1]University of St Andrews, St Andrews, Fife, UK, [2]Simon Fraser University, Burnaby, British Colombia, Canada

Influenza viruses remain a major respiratory pathogen, causing both periodic pandemics and seasonal epidemics. Neuraminidase (NA) inhibitors have become the standard antiviral therapy to combat the effects of influenza viruses, but resistance to the current inhibitors is increasingly prevalent. To overcome the problems of resistance we have synthesized two new classes of inhibitor based upon the scaffold of the existing NA inhibitor Oseltamivir. In the first series, guanidino or triazole groups were added in place of the original amino group at position 4 of the cyclohexene ring. X-ray crystallographic analysis of these compounds in complex with NA demonstrated the additional groups project from the active site towards the 150-cavity (formed by residues 146-152) giving inhibitors greater selectivity. Furthermore, the lead compound from this series demonstrated reduced susceptibility to the common H274Y mutation in a cell culture assay. As part of the optimization of this lead compound, we serendipitously discovered a second class of inhibitor with a novel spirolactam structure. The most potent of these new inhibitors showed comparable inhibitory properties to the Oseltamivir in tissue culture assay. Crystallographic analysis of this series in complex with NA gave further insight into the binding mode.

LI20/17

The N-terminus of the influenza B virus nucleoprotein is essential for virus viability, nuclear localization and is required for optimal transcription and replication of the viral genome

Lee Sherry, Matt Smith, Sophie Davidson, David Jackson

University of St Andrews, St Andrews, UK

The nucleoprotein (NP) of influenza viruses is a multifunctional protein with essential roles throughout viral replication. Influenza A and B virus NP proteins show a relatively high level of sequence conservation. However influenza B virus NP (BNP) contains an evolutionarily conserved N-terminal 50 amino acid extension that is lacking from influenza A virus NP. There is conflicting evidence as to the functions of the BNP extension, therefore we used reverse genetics to assess the significance of this region on the functions of BNP and virus viability. Truncation of more than three amino acids prevented virus recovery suggesting that the N-terminal extension is essential for virus viability. Mutational analysis indicated that multiple regions of BNP are involved in nuclear localization, with the entire N-terminal extension required for this to function efficiently. Viruses containing mutations in the first 10 residues of BNP demonstrated significantly attenuated phenotypes, characterised by a reduction in viral mRNA transcription, whereas mutations further downstream resulted in a significant enhancement of genome replication. Overall our results demonstrate that the N-terminal extension of BNP is essential for virus viability not only for directing nuclear localization of BNP, but also for regulating viral mRNA transcription and genome replication.

LI20/18

Revisiting the action of ribavirin on human respiratory syncytial virus – an omic approach

Julian Hiscox

University of Leeds, UK

Human respiratory syncytial virus (HRSV) causes severe lower respiratory tract infection. Antiviral therapies to HRSV are of limited efficacy. Ribavirin is one of the most commonly used antiviral drugs licensed for the treatment of many different virus infections including HRSV. The precise mechanism of how ribavirin disrupts HRSV biology is unknown and both error catastrophe and defects in cellular RNA metabolism have been proposed. This project used a combination of label free quantitative proteomics and deep sequencing to investigate the potential mechanism of action of ribavirin in the inhibition of HRSV in cell culture. The inclusion of ribavirin different times post-infection was analyzed for their effect on HRSV biology. Selected examples were further characterized using the omic approaches. Over 1500 cellular proteins were identified and quantified by LC-MS/MS and pathway analysis showed significant changes in proteins associated with RNA metabolism. Quasi species analysis of the viral RNA genome suggested that the frequency of nucleotide changes over the assay period was no different to when the virus was passaged on a different cell type or treated with an alternative inhibitor of HRSV polymerase function. The data suggests that the effect of ribavirin on HRSV is cellular mediated rather than directly anti-viral

Please note: Abstracts are published as received from the authors and are not subject to editing.