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Modulation of Recombination and DNA Repair by the RecG and PriA Helicases of *Escherichia coli* K-12

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The RecG protein of *Escherichia coli* is a structure-specific DNA helicase that targets strand exchange intermediates in genetic recombination and drives their branch migration along the DNA. Strains carrying null mutations in *recG* show reduced recombination and DNA repair. Suppressors of this phenotype, called *srgA*, were located close to *metB* and shown to be alleles of *priA*. Suppression depends on the RecA, RecBCD, RecF, RuvAB, and RuvC recombination proteins. Nine *srgA* mutations were sequenced and shown to specify mutant PriA proteins with single amino acid substitutions located in or close to one of the conserved helicase motifs. The mutant proteins retain the ability to catalyze primosome assembly, as judged by the viability of *recG srgA* and *srgA* strains and their ability to support replication of plasmids based on the ColE1 replicon. Multicopy *priA*$^+$ plasmids increase substantially the recombination- and repair-deficient phenotype of *recG* strains and confer similar phenotypes on *recG srgA* double mutants but not on *ruvAB* or wild-type strains. The multicopy effect is eliminated by K230R, C446G, and C477G substitutions in PriA. It is concluded that the 3′-5′ DNA helicase/translocase activity of PriA inhibits recombination and that this effect is normally countered by RecG.

Recombination is a fundamental process in biology that serves both to promote genetic diversity and to conserve genome integrity. The enzymology of this process has been dissected in some detail in *Escherichia coli*, where some 20 or more recombination proteins have been identified and linked with specific stages of the molecular reaction (6, 12, 21). The RecBCD pathway, named after one of its principal components, acts at DNA ends and provides the means to generate recombinants in transductional and conjugational crosses, repair DNA double-strand breaks, and renew chromosome duplication when a replication fork has collapsed (1, 6, 14, 43).

Recent studies have focused on the way the RecBCD pathway is able to prime DNA replication and on how this replication seems to be linked intrinsically with the formation of recombinants in genetic crosses (10). Asai et al. have shown that chromosome breaks trigger a particular form of replication, called induced stable DNA replication, which is independent of *oriC* and the initiator protein, DnaA (1). Replication relies instead on the activities of the RecA and RecBCD proteins and of the primosome assembly protein, PriA (1, 3, 26, 30). A break in the chromosome is thought to be processed by RecBCD to expose a 3′-tailed duplex end which then invades an intact homolog through the action of RecA to set up a Holliday junction and at the same time prime leading-strand synthesis (Fig. 1). Lagging-strand synthesis primed by PriA on the displaced strand of the D-loop coupled with resolution of the Holliday junction by the RuvAB and RuvC proteins is proposed to convert the D-loop to a replication fork (1).

Surprisingly, the inactivation of PriA reduces the formation of recombinants in genetic crosses (11, 36). This finding supports the idea that *E. coli* recombination involves extensive replication of DNA, as suggested by Smith (43). Exchanges initiated by RecBCD at the ends of linear DNA fragments transferred during conjugation or transduction could set up two replication forks that duplicate the remaining DNA to generate both wild-type and recombinant copies of the chromosome either as monomers or as a dimer, depending on how the associated Holliday junctions are resolved (1, 10, 43).

A key stage in this model is the branch migration of the invading strand in the 3′-5′ direction into regions of duplex-duplex DNA pairing (Fig. 1). It enables the newly synthesized lagging strand to be joined to the 5′ strand at the end of the invading duplex and sets up a Holliday junction that can be resolved later to give recombinant products. However, RecA polymerizes on single-stranded DNA in the 5′-3′ direction, and it is not immediately obvious therefore how strand exchange extends into the donor duplex. The three-strand junction at the 5′ end of the strand invading the D-loop could be targeted by a branch migration protein such as RecG, as suggested by Whitby and Lloyd (49). RecG is a structure-specific DNA helicase that targets both Holliday junctions and three-strand intermediates in vitro and drives their branch migration along the DNA with a polarity that is dictated by RecA (24, 40, 49–51). It will also target an R-loop, which has some features in common with a D-loop, and remove the RNA (47). Alternatively, the D-loop could be targeted by RuvAB, which has been shown to accelerate strand exchange by RecA and to branch migrate three-strand intermediates (45, 49).

RecG and RuvAB are both needed for normal levels of recombination and DNA repair. Their elimination blocks recombinant formation and confers extreme sensitivity to UV light (17). Either protein could therefore act to promote the early stages of strand exchange, although the RuvAB complex seems rather well designed to interact specifically with Holliday junctions, promoting their branch migration and targeting their resolution by the sequence-specific RuvC resolvase (33, 37, 48). In this report, we describe an investigation of suppressors of *recG* and show that DNA repair and recombination can be modulated in the absence of RecG by changing the activity of PriA. We suggest that the balance between RecG and PriA is critical in establishing Holliday junctions for RuvAB and RuvC.

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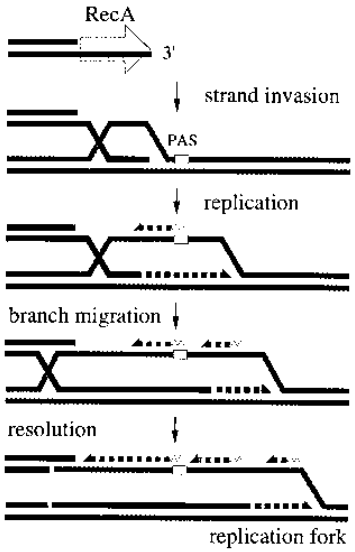

FIG. 1. Model for priming DNA replication by RecA-mediated recombination from a 3′ tailed duplex DNA end (see text for details).

MATERIALS AND METHODS

Strains and plasmids. *E. coli* strains are listed in Table 1. pAM185 is a *priA*$^+$ construct made by cloning an *Eco*RI-*Hin*dIII DNA fragment from Y20RF (31) into the pGEM-7Zf(+) vector (Promega). pAM187 carries *priA*$^+$ from pAM185 inserted between the *Eco*RI and *Pst*I sites in pBR322. The pET3 plasmid constructs expressing wild-type or mutant (K230R, C446G, or C477G) PriA proteins are from K. Marians (54).

Media and general methods. LB broth and 56/2 minimal salts media were as described previously (22) and were supplemented with 100 μg of ampicillin, 20 μg of tetracycline, 25 μg of kanamycin, and 100 μg of streptomycin per ml, as required for selection of antibiotic-resistant strains. Mitomycin (MC) was used in LB agar at 0.2 and 0.5 μg/ml for plate sensitivity tests. Transductions with phage P1*vir* and procedures for determining sensitivity to MC, UV light, and γ-radiation were as described elsewhere (18, 20). Cell density in liquid culture was monitored by measuring the A_{650} and the viable cell number.

Isolation of *recG* suppressors. Cultures of the Δ*recG263*::*kan* strains TNM1072 and N3793 were grown from single colonies to saturation in LB broth. Samples containing ~10^7 cells were spread on LB agar supplemented with 0.2 μg of MC per ml and irradiated with UV light (30 J/m^2) before incubation for 48 h at 37°C. Colonies of survivors were purified and retested to confirm their resistance to UV light and MC. To avoid siblings, only one resistant clone from each culture was taken for further study. Of the 20 *srgA* suppressors studied, *srgA1* and *srgA2* were from selection on TNM1072, and the remainder (see Table 4) were from selection on N3793.

Matings. Methods for mating F-prime and Hfr donors with F$^-$ recipients transformed with recombinant plasmids were generally carried out as described previously (20) except that the donor strains used carried pBR322 in order to confer resistance to the antibiotic in cultures of the recipient. Donor and recipient strains were grown with the same antibiotic selection. DNA transfer and recombination were measured in matings with control and test strains grown in parallel.

DNA manipulation. Purification of chromosomal DNA, plasmid construction, transformation, and general methods for DNA manipulation was done as described previously (35, 42). DNA amplification by PCR used *Taq* DNA polymerase (Perkin-Elmer) in the buffer system provided and was performed on DNA from single colonies (13) or purified chromosomal DNA, using 18- to 25-nucleotide primers based on the target sequence.

Sequencing *srgA* alleles of *priA*. Sections (200 to 900 bp) of the *priA* region in strains carrying *srgA* suppressors of *recG* were amplified by PCR, and the DNA was extracted by using a QIAquick purification kit (Qiagen). The amplified DNA was sequenced directly, using a PRISM cycle sequencing kit (Perkin-Elmer) and a series of (nested) primers, and analyzed on an ABI model 373A automated sequencer. Some sequences were determined by the dideoxynucleotide chain termination method, using T7 sequencing kits (Pharmacia). Template single-stranded DNA was prepared from strain JM101 transformed with pGEM-7Zf constructs carrying the amplified PCR product and infected with the phage M13 KO7.

RESULTS

Suppression of the *recG* mutant phenotype. The inactivation of RecG confers sensitivity to DNA damage (18). We took advantage of this phenotype to search for suppressors in the hope that these might provide further insights into RecG's role within the cell. Derivatives of a *recG* strain that had regained resistance to MC and UV light were selected as described in Materials and Methods. To avoid selecting revertants, we used strains carrying a *recG* deletion tagged with a *kan* insertion. Resistant clones were readily obtained. These strains showed improved growth relative to the parent strain (data not shown). The presence of *recG263* was confirmed by their resistance to kanamycin and by transducing strain AB1157 to Km^r and showing that the transductants were sensitive to UV and MC. The resistant clones were assumed therefore to carry suppres-

TABLE 1. *E. coli* K-12 strains used

Strain	Relevant genotype[a,b]	Reference or source[b,c]
AB1157	F$^-$ *rec*$^+$ *ruv*$^+$ *pri*$^+$	4
UM202	HfrH *thi-1 relA1 katG17*::Tn*10*	44
PN103	*priA2*::*kan*	32
PN105	*priA2*::*kan sulA*	32
W3110	F$^-$ IN(*rrnD-rrnE*)*1*	4
TNM1072	Δ*recG263*::*kan*	28
CS85	*ruvC53 eda-51*::Tn*10*	41
N2057	*ruvA60*::Tn*10*	41
N2096	Δ*ruvA63*	28
N3695	Δ*recG263 srgA1*	MC^r UV^r selection on N3793
N3696	Δ*recG263 srgA2*	MC^r UV^r selection on N3793
N3761	Δ*recG263 recJ284*	P1.(*recJ284*::Tn*10*) × TNM1072 to Tc^r
N3762	Δ*recG263 srgA1 recJ284*	P1.(*recJ284*) × N3695 to Tc^r
N3789	Δ*recG263 ruvA60*	P1.TNM1072 × N2057 to Km^r
N3793	Δ*recG263*	P1.TNM1072 × AB1157 to Km^r
N3937	Δ*recG263 srgA1 ruvA60*	P1.N2057 × N3695 to Tc^r
N3938	Δ*recG263 srgA1 ruvC53*	P1.CS85 × N3695 to Tc^r
AD19	Δ*recG263 srgA1 metB1 arg*$^+$	P1.KL227 × N3695 to Arg$^+$
AD20	Δ*recG263 srgA2 metB1 arg*$^+$	P1.KL227 × N3696 to Arg$^+$
AD239	Δ*recG263 recN262*	P1.(*tyrA16*::Tn*10 recN262*) × N3793 to Tc^r
AD240	Δ*recG263 srgA1 recN262*	P1.(*tyrA16*::Tn*10 recN262*) × N3695 to Tc^r
AD243	Δ*recG263 recF143*	P1.(*tna-300*::Tn*10 recF143*) × N3793 to Tc^r
AD244	Δ*recG263 srgA1 recF143*	P1.(*tna-300 recF143*) × N3695 to Tc^r
AD251	Δ*recG263 recB268*	P1.(*recB268*::Tn*10*) × N3793 to Tc^r
AD252	Δ*recG263 srgA1 recB268*	P1.(*recB268*) × N3695 to Tc^r
AD280	Δ*recG263 recA269*	P1.(*recA269*::Tn*10*) × N3793 to Tc^r
AD281	Δ*recG263 srgA1 recA269*	P1.(*recA269*) × N3695 to Tc^r
JM101	F′ (F128) *proAB*$^+$ *lacI*^{q} *ZΔM15*	52
KL548	F′ (F128) *lacI3 lacZ118 proAB*$^+$	K. B. Low
KL226	Hfr (Cavalli, PO2A)	K. B. Low
KL227	Hfr (PO3 of P4X) *metB1*	K. B. Low
GY2200	Hfr (H, PO1) (λ*ind*)$^+$	R. Devoret

[a] Strains AB1157 and TNM1072 through to AD281 are closely related and are also *thi-1 his-4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 rfbD1*(*?*) *ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31*, except as indicated.

[b] After the first listing, insertion mutations are referred to by the allele number only.

[c] The P1 donors used for constructing the *recA*, *recB*, *recF*, *recJ*, *recN*, and *ruv* strains are from our laboratory collection, and details can be obtained on request.

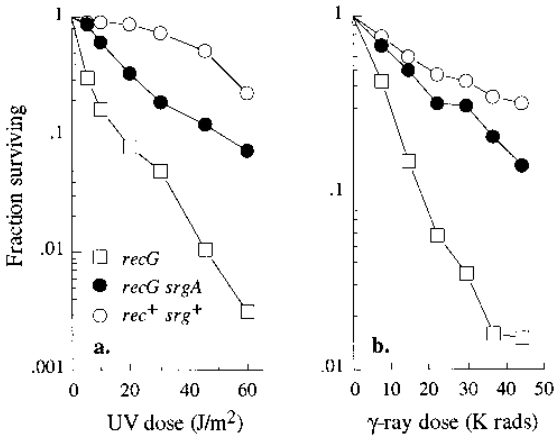

FIG. 2. Restoration of UV and γ-ray resistance to a *recG* strain by *srgA1*. The strains identified by genotype in panel a were AB1157, N3695, and N3793. The data are means of three or more independent experiments.

sors of the *recG* mutant phenotype. Genetic crosses revealed that some carried a new mutation linked to *argE* (see Table 3). The locus concerned was designated *srgA*, for suppressor of *recG*. We examined 24 resistant clones in total and found that 20 fell into this category. All 20 had similar phenotypes (data not shown). The remaining four carried a suppressor located elsewhere which we have not mapped, except it is not linked to *metB* or *recG*.

Effect of *srgA* on recombination and DNA repair. Suppression of *recG* by the *srgA1* allele in strain N3695 was examined in detail. As shown in Fig. 2, it increases substantially resistance to UV light and γ-radiation. It also allows good growth on LB agar containing MC at concentrations that prevent growth of a *recG* srg^+ strain (data not shown) and improves recombination in Hfr crosses (see Table 5). Suppression is eliminated by mutations in *recA*, *recB*, *recF*, *ruvAB*, and *ruvC*. As shown in Table 2, *srgA1* does not improve the radiation resistance of *recG263* strains carrying these mutations. Previous studies revealed that mutation of *recG* increases the sensitivity of *recB*, *ruvAB*, and *ruvC* mutant strains to UV light and in the case of *ruv* strains also blocks recombination (17, 18). The *srgA1* allele does not alleviate these effects (Table 2 and data not shown). However, it does eliminate the synergism between *recG* and *recJ* and between *recG* and *recN*, restoring radiation resistance to the level of *recJ* and *recN* single mutants, respectively (Table 2). From these studies, we conclude that suppression requires the RecA, RecBCD, RecF, RuvAB, and RuvC proteins and is therefore mediated through recombination. However, this recombination does not need RecJ or RecN.

***srgA* is an allele of *priA*.** Three-factor transductional crosses located *srgA* counterclockwise of *metB* (Table 3), which is located at coordinate 4163 on the physical chromosome map (5). All 20 *srgA* alleles showed ~50% linkage to *metB* (Table 3 and data not shown). We sequenced PCR products amplified from open reading frames between *metB* and coordinate 4160.5 and found no mutations (data not shown). We therefore focused on *priA*, the next gene on the map. The *priA* alleles in strains carrying *srgA1* and *srgA4* were sequenced in their entirety and were both found to encode proteins with single amino acid substitutions. Partial sequences were obtained for seven others, and in each case a single amino acid substitution was identified. The mutations are summarized in Table 4. As shown in Fig. 3, all nine substitutions identified are in or very close to one of the conserved helicase motifs in PriA or the zinc finger close to motif IV. An alignment of PriA sequences from four divergent bacterial species (38) revealed that the Y234H substitution affects a conserved hydrophobic region near motif I, while E254K substitutes a basic for an acidic residue next to P253 in motif Ia, a residue conserved in all helicases. L425F and L557P affect conserved hydrophobic residues in motif IV and near motif V, respectively. S305F alters a conserved residue between motifs Ia and II. We conclude that *srgA* suppressors are alleles of *priA*, although we have not sequenced all 20, and probably affect PriA's helicase activity.

Null mutations in *priA* confer sensitivity to radiation, chronic SOS induction, and very low cell viability (32). Our *recG srgA* strains grow as well as the *recG*$^+$ srg^+ strain, AB1157, indicating that the mutant PriA proteins retain at least some activity. However, it was possible that the *recG* mutation was acting as a suppressor of *priA*. The *srgA1* allele was therefore transduced to the *recG*$^+$ genetic background of strain AB1157 by cotransduction with *metB*, and its presence was confirmed by an appropriate backcross and by introducing *recG263*. The *srgA1* single mutant was indistinguishable from AB1157, being just as resistant to UV light and MC and showing no evidence of reduced viability (data not shown). We next introduced *priA2*::*kan* into a *recG162* derivative of AB1157 (18) by transduction from strain PN105. The Km^r transductants obtained were very slow growing and sensitive to UV light and MC (data not shown). We conclude that mutation of *recG* does not suppress the *priA2* mutant phenotype and that *srgA* mutations do not inactivate those PriA functions needed for viability. The ability of *srgA* strains to replicate plasmids based on the ColE1 replicon (see below) supports this view.

Overproduction of wild-type PriA prevents recombination in the absence of RecG. We assumed the *srgA* mutations modified the activity of PriA in some way that allowed recombination and repair to proceed more efficiently. Change-of-function mutations are often dominant. We therefore introduced *priA*$^+$ plasmids into a *recG srgA* strain to test this possibility. As shown in Fig. 4a, the *priA*$^+$ construct pAM187 restored sensitivity to UV light. However, the transformed strain was much

TABLE 2. Effects of *rec* and *ruv* mutations on suppression of *recG* by *srgA*

Strain	Relevant genotype			Fraction surviving[a]			
	recG	*srgA*	Other	UV dose (J/m^2)		γ-Ray dose (kilorads)	
				10	30	14.7	36.8
N3793	*263*	+	+	0.16	0.05	0.15	0.016
N3695	*263*	*1*	+	0.5	0.32	0.49	0.21
AD280	*263*	+	*recA*	0.00011	0.0000043		
AD281	*263*	*1*	*recA*	0.00016	0.0000051		
AD251	*263*	+	*recB*	0.0025	0.00013		
AD252	*263*	*1*	*recB*	0.0026	0.00012		
AD243	*263*	+	*recF*	0.073	0.0047	0.037	0.00082
AD244	*263*	*1*	*recF*	0.069	0.0041	0.059	0.0014
N3761	*263*	+	*recJ*	0.009	0.0002	0.02	0.0006
N3762	*263*	*1*	*recJ*	0.45	0.1	0.2	0.01
AD239	*263*	+	*recN*			0.0059	0.000083
AD240	*263*	*1*	*recN*			0.044	0.0016
N2057	+	+	*ruvA60*	0.0079	0.0008		
N3789	*263*	+	*ruvA60*	0.0001	0.000009		
N3937	*263*	*1*	*ruvA60*	0.00021	0.000014		
CS85	+	+	*ruvC*	0.0077	0.00054		
N3938	*263*	*1*	*ruvC*	0.000094	0.000018		

[a] Values are means from two or three experiments.

TABLE 3. Mapping of *srgA* by P1 transduction

Recipient (Δ*recG*)	P1 donor	Selection	No. tested	Donor marker inheritance (% of total)[a]			
				srgA	*metB*	*katG*	*argE*
N3695 (*srgA1 argE*)	W3110	Arg^+	175	8.0			
	UM202 (*katG*)	Tc^r (*katG*)	313	42.8			47.3
N3696 (*srgA2 argE*)	W3110	Arg^+	173	12.7			
	KL227 (*metB*)	Arg^+	196	7.2	12.3		
	UM202 (*katG*)	Arg^+	198	3.5		30.8	
AD20 (*srgA2 metB*)	UM202 (*katG*)	Met^+	297	52.9		38.0	

[a] Segregation at *srgA* was monitored by sensitivity to MC and UV light, and segregation at *katG* was monitored by sensitivity to tetracycline. The *metB*, *katG*, and *argE* genes are located at coordinates 4163, 4169, and 4188, respectively, of the *E. coli* physical map (5).

more sensitive than a *recG* srg^+ strain carrying the pBR322 vector. pAM187 had a similar effect on the *recG* srg^+ strain, making it much more sensitive than usual. As shown in Table 5, the effect of multicopy $priA^+$ on *recG* and *recG srgA* strains was correlated with a reduction in the efficiency of conjugational recombination to around 3 to 4% of the wild-type level, which is much lower than the 20 to 30% residual activity seen in a *recG* single mutant. This effect of multicopy $priA^+$ appeared to be specific to strains lacking RecG. There was no effect on the UV sensitivity of the $recG^+$ srg^+ control (Fig. 4b), and recombination was if anything slightly improved (Table 5). Likewise, the $priA^+$ plasmid had no effect on the Δ*ruvA63* strain, N2096, which lacks both RuvA and RuvB (28) (Fig. 4b; Table 5). We repeated the study using a $priA^+$ construct, pAM185, from which we expected PriA to be expressed at a level higher than that from pAM187. The results were essentially the same except the *recG* and *recG srgA* strains were even more sensitive to UV light and that the wild-type and *ruvA* strains showed some increased sensitivity at high doses (Fig. 4c and d and data not shown).

Helicase-deficient PriA mutants do not interfere with suppression by *srgA*. PriA has two known activities: it directs assembly of the primosome complex needed to prime lagging-strand synthesis and also acts as a DNA helicase/translocase (15, 16, 31). These activities have been separated by mutation in that helicase/translocase-deficient PriA mutants retain the ability to assemble primosomes (54, 55). As shown in Table 6, plasmids expressing such mutant PriA proteins (K230R, C446G, and C477G) did not enhance the sensitivity of either *recG* or *recG srgA* strains to UV light. Indeed, in the case of the *recG* strain, they increase resistance slightly. This partial suppression is particularly noticeable in plate tests for sensitivity to UV light and MC (data not shown). Given that the K230R mutant is capable of catalyzing the assembly of active primosomes and that the C446G and C477G mutants retain some activity in this respect (54, 55), we conclude that the (dominant) negative effect of $PriA^+$ on *recG* and *recG srgA* strains is related to its ability to act as a helicase/translocase.

TABLE 4. Sequence analysis of *srgA* alleles of *priA*

Allele (of *priA*)	Mutation[a]	PriA substitution
srgA1	$T_{1670} \rightarrow C$	L557P
srgA2	$C_{1273} \rightarrow T$	L425F
srgA4	$T_{1670} \rightarrow C$	L557P
srgA5	$C_{914} \rightarrow T$	S305F
srgA6	$C_{914} \rightarrow T$	S305F
srgA7	$G_{1558} \rightarrow C$	A520P
srgA10	$T_{1460} \rightarrow G$	V487G
srgA21	$T_{700} \rightarrow C$	Y234H
srgA22	$G_{760} \rightarrow A$	E254K

[a] The numbering is from the first nucleotide of the *priA* coding sequence. Mutations were sequenced on both strands of the DNA. The entire sequence of *priA* was determined on both strands for *srgA1* and *srgA4*; 95% was determined for *srgA5*, and 40 to 75% was determined for the others. Our sequences for *priA* matches exactly that of Lee et al. (15), which differs from that of Nurse et al. (31) in having a C instead of a T at position 1946, coding for Ala649 instead of Val649.

DISCUSSION

We have shown that recombination and DNA repair in *recG* mutants depends critically on the activity of PriA. The PriA protein was discovered as a factor necessary for reconstituting in vitro systems for replicating bacteriophage ϕX174 and plasmid ColE1 DNA. It binds single-stranded DNA at specific sequences (primosome assembly sites [PAS]) located near the replication origin and provides an essential component of the primosome assembly complex that loads the replicative helicase, DnaB, and primes lagging-strand synthesis (see references 29 and 53 for reviews). In the ColE1 system, the PAS site is located downstream of the origin. Replication is initiated when a short RNA molecule (RNA II) binds to the origin and is processed by RNase H to provide a 3′-OH for DNA polymerase I to prime synthesis (9). Leading-strand synthesis creates a D-loop and exposes a PAS site for PriA in the displaced strand. PriA is also a DNA-dependent ATPase and after binding at PAS can translocate along DNA in the 3′-5′ direction, using the energy from ATP hydrolysis, unwinding any duplex DNA that it encounters (16, 53).

Insertions in *priA* reduce cell viability, confer sensitivity to DNA damage, inhibit repair of DNA double-strand breaks, prevent priming of DNA replication by the 3′ ends of broken DNA molecules and by R-loops, and reduce recombination in genetic crosses, particularly transduction (11, 30, 32, 36). These phenotypes can be suppressed by introducing in *trans* a mutant PriA protein (K230R) that lacks ATPase and DNA helicase activity but which retains the ability to assemble primosomes (54), implying that primosome assembly is the critical function needed for viability, recombination, and repair.

We have described a new class of *priA* mutations (*srgA*) that

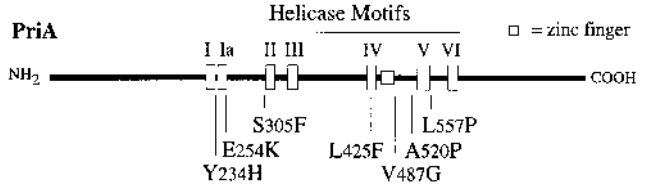

FIG. 3. Locations of substitutions within the 732-amino-acid PriA polypeptide encoded by *srgA* alleles. The conserved helicase motifs are represented by open boxes (not to scale).

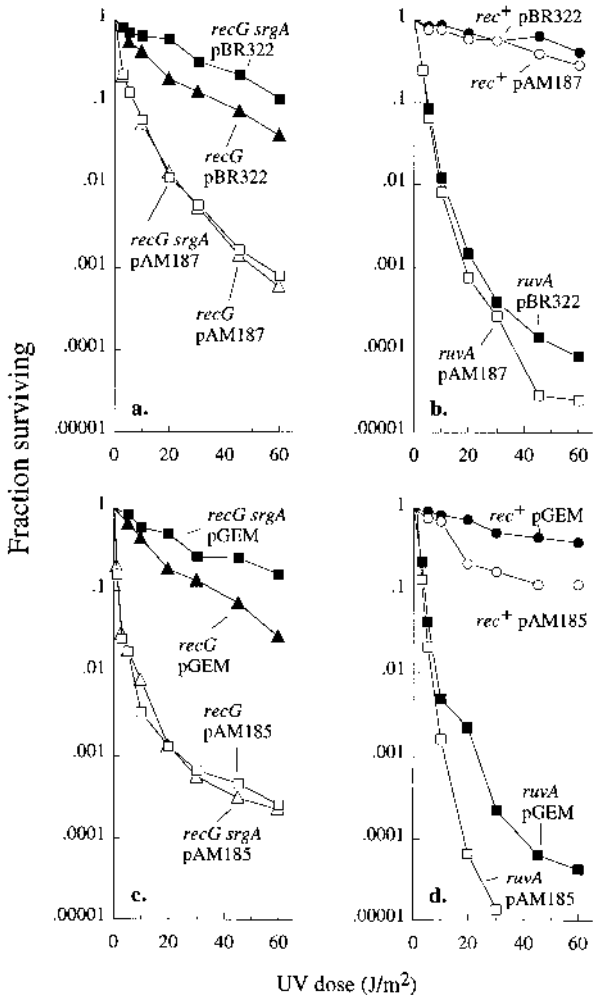

FIG. 4. Effect of *priA*$^+$ plasmids on sensitivity to UV light. The strains identified by genotype in the panels were AB1157, N2096, N3695, and N3793. The data are means of four independent experiments.

confer no obvious phenotype on their own but which have the ability to suppress the deficiency in recombination and DNA repair in *recG* mutant strains. Suppression is not quite complete and is eliminated by inactivation of RecA, RecBCD, RecF, RuvAB, or RuvC, indicating that it relies on RecA-mediated recombination initiated by RecBCD. We sequenced nine *srgA* alleles of *priA* and found in each case a single mutation that results in an amino acid substitution. Two alleles were sequenced in their entirety, and we found no other substitutions. We cannot rule out the possibility that some of the others have additional substitutions.

Each of the substitutions detected is close to one of the conserved helicase motifs or the zinc finger motif between motifs IV and V, which is highly significant, especially since the polarity of DNA translocation and unwinding by PriA is 3′-5′, the same as for RecG (16, 51). This probably means that *srgA* mutations reduce the helicase/translocase activity of PriA and that this allows recombination and DNA repair to proceed more efficiently in the absence of RecG. However, this begs the question of why we did not find more *srgA* alleles with changes directly affecting residues conserved generally among helicases, such as K230 in ATPase motif I. It could be chance, or perhaps a consequence of the selection scheme used, which favored strong suppressors. Removing helicase activity entirely may create a less efficient suppressor. PriA K230R expressed in *trans* suppresses *recG*, but not as well as a *srgA* mutation, though incomplete suppression in this case could also reflect the presence of wild-type PriA made from the chromosome. An alternative possibility is that *srgA* mutations alter PriA's DNA binding affinity, enabling it to target recombination intermediates in place of RecG. We consider this unlikely, given the range of substitutions seen and the finding that only one change is needed to achieve good suppression.

A quite revealing discovery was that multicopy *priA*$^+$ plasmids exert a strong dominant negative effect on recombination and DNA repair in the absence of RecG. This was observed with both *recG* and *recG srgA* strains but not with a *recG*$^+$ strain or if the PriA expressed was deficient in helicase activity (K230R, C446G, or C477G). These observations indicate that RecG counters some PriA helicase or translocase activity that would otherwise interfere with recombination (and repair). They support our contention that *srgA* mutations reduce this activity.

In previous studies, it was shown that *recG ruv* strains are far more sensitive to UV light and much more deficient in recombination than either of the single mutants (17). It is therefore intriguing that increasing the copy number of *priA*$^+$ in the absence of RecG seems to mimic this synergism, as if increasing PriA prevents the RuvAB and RuvC proteins from being

TABLE 5. Effect of a *priA*$^+$ plasmid on conjugational recombination[a]

Strain (genotype)	Viability	Relative yield of transconjugants		
		× F′ KL548 to Pro$^+$ (*rpsL*)	× Hfr GY2200 to Thr$^+$ Leu$^+$ (*rpsL*)	× Hfr KL226 to ProA$^+$ (*rpsL*)
pBR322 strains				
N3793 (Δ*recG*)	0.85 ± 0.07	0.99 ± 0.09	0.30 ± 0.05	0.21 ± 0.02
N3695 (Δ*recG srgA*)	0.94 ± 0.06	1.10 ± 0.13	1.02 ± 0.15	0.68 ± 0.14
N2096 (Δ*ruvA63*)	0.43 ± 0.02	1.04 ± 0.24	0.22 ± 0.06	0.49 ± 0.03
pAM187 (*priA*$^+$) strains				
AB1157 (wild type)	1.03 ± 0.12	1.18 ± 0.16	1.39 ± 0.23	1.38 ± 0.15
N3793 (Δ*recG*)	0.70 ± 0.14	0.65 ± 0.09	0.048 ± 0.007	0.035 ± 0.01
N3695 (Δ*recG srgA*)	0.75 ± 0.3	0.75 ± 0.13	0.054 ± 0.012	0.043 ± 0.014
N2096 (Δ*ruvA63*)	0.61 ± 0.08	0.95 ± 0.19	0.16 ± 0.06	0.45 ± 0.06

[a] Mating was for 30 min (KL548), 40 min (KL226), or 60 min (GY2200) in LB broth containing 10 g of NaCl per liter, with a donor-to-recipient ratio of 1:10. Selection was for the transconjugant class indicated and also for the Tc^r phenotype conferred by the plasmid carried. Values for the mutant strains are given relative to the control strain AB1157 (pBR322) mated in parallel and are means of three independent experiments. The mean actual values for the control were 1.6×10^8 viable cells per ml of the recipient culture and 8.9×10^6 (× KL548), 8.4×10^6 (× Hfr GY2200), and 6.4×10^6 (× KL226) transconjugants per ml of the mating mixture. All three donors carried the pBR322 vector.

TABLE 6. Effects of *priA* plasmids on suppression of *recG* by *srgA*

Strain[a]	Genotype	Plasmid PriA	Fraction surviving UV dose of:	
			30 J/m^2	60 J/m^2
AB1157	*rec*$^+$ *srg*$^+$ (*priA*$^+$)	None	0.67	0.31
		PriA$^+$	0.62	0.31
		K230R	0.65	0.19
		C446G	0.61	0.14
		C477G	0.68	0.17
N3793	Δ*recG263*	None	0.044	0.01
		PriA$^+$	0.0022	0.00068
		K230R	0.13	0.024
		C446G	0.084	0.032
		C477G	0.20	0.07
N3695	Δ*recG263 srgA1*	None	0.19	0.061
		PriA$^+$	0.0094	0.0017
		K230R	0.13	0.025
		C446G	0.048	0.020
		C477G	0.15	0.05

[a] The strains carried the pET3 vector (none) or *priA* derivatives expressing a wild-type or mutant protein as indicated.

able to function. As outlined in Fig. 5a, PriA could achieve this effect by targeting the D-loop formed during the initial stages of recombination and translocating it away from the invading duplex, thus preventing the formation of a Holliday junction. PriA could target the forked structure at the 3′ end of the invading strand and unwind the recipient duplex as it moved 3′-5′ along the template for leading-strand synthesis. However, if unwinding was limited by topological constraints, the net effect could be to translocate the D-loop along the DNA. Alternatively, PriA could target the three-strand junction and use its helicase activity to displace the invading strand and move the D-loop along as the 3′ end of this strand is extended by new DNA synthesis.

RecG could counter PriA by driving the three-strand junction toward the invading duplex to set up a Holliday junction. It could achieve this effect by targeting the three-strand junction directly and driving its branch migration, as suggested by Whitby and Lloyd (49). Alternatively, it could translocate the D-loop toward the invading duplex by targeting the forked structure at the 3′ end and rewinding the recipient strands as it moves 3′-5′ along the displaced strand. Whether RecG is capable of catalyzing a reverse helicase reaction remains to be established. However, the displacement of the invading strand associated with such a reaction would explain why RecG limits DNA replication primed by recombination from broken DNA ends (2). A reverse helicase activity is also consistent with RecG's ability to eject RNA from R-loops (47). We cannot rule out the possibility that the three-strand junction formed by 3′-strand invasion may branch migrate into the region of duplex-duplex pairing to some extent even in the absence of RecG. The RecF, RecO, and RecR proteins, which interact with RecA (46), may assist this reaction by facilitating polymerization of RecA at the 5′ end of the filament.

The model outlined in Fig. 5a assumes that Holliday junctions are set up inefficiently in the absence of RecG, making RuvAB and RuvC somewhat redundant for recombination. The latter is evidently not the case, as *recG ruv* strains are much more deficient in recombination than are *recG* single mutants (17). This difficulty would be overcome if recombination can also initiate with 5′-tailed duplex DNA molecules (34) or, in Hfr crosses, at transient single-strand gaps in the donor DNA (19, 25). The RecA filament assembled 5′-3′ would extend into duplex regions in these cases, enabling strand exchange to lead directly to Holliday junctions without any assistance from RecG (Fig. 5b). However, RecG also targets Holliday junctions and is required for recombinant formation when the normally quiescent RusA resolvase has been activated to replace RuvC (27, 28, 39), or when yet another resolvase activity has been activated in strains deleted for both *rusA* and the *ruv* genes (23). This role of RecG in junction resolution is probably redundant in *ruv*$^+$ strains. Only when both RecG and Ruv proteins are missing would there be a substantial block therefore to recombination.

Recent studies have shown that the RecG and Ruv proteins have opposite effects on the frequency of adaptive reversion of a frameshift mutation in *lacI*, a process that seems to involve a mutagenic form of DNA replication primed from DNA ends processed by RecBCD. RuvAB promotes this type of mutation, while RecG inhibits it (7, 8). To explain this difference, Harris et al. (8) suggested a model for the early stages of recombination in which they proposed that RecG drives 5′-end invasion and aborts 3′-end invasion, while RuvAB does the reverse. This would account for the mutator phenotype of *recG* strains since only 3′ ends are assumed to prime replication. Some of the differences between their model and ours can be reconciled if resolution of Holliday junctions by RuvC were to depend on RuvAB in vivo, as appears to be the case (28, 39). The lack of adaptive revertants in *ruvAB* strains could then be attributed to the inability to resolve Holliday intermediates, which would kill the cell. Inactivation of RecG on the other hand will delay the formation (and hence resolution) of junctions following 3′-strand invasion and tip the balance of activities at the D-loop in favor of PriA and hence of (mutagenic) replication.

To conclude, we have shown that PriA helicase activity is probably much to blame for the failure of *recG* mutants to promote efficient recombination and DNA repair and suggest that the balance between RecG and PriA helicases is critical for the early stages of recombination initiated by RecBCD enzyme. Our model predicts that PriA and RecG target early intermediates in recombination. We are currently testing this possibility.

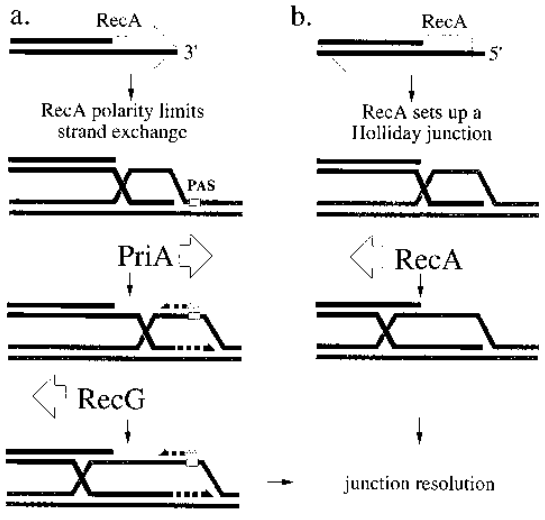

FIG. 5. Model showing effect of RecA polarity on the early stages of recombination initiated by tailed duplex DNA molecules. (a) 3′-end invasion; (b) 5′-end invasion. The large open arrows in panel a are intended to represent the balance of PriA and RecG activities and are not meant to imply sequential steps (see text for details).

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