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Resolution of Holliday Intermediates in Recombination and DNA Repair: Indirect Suppression of *ruvA*, *ruvB*, and *ruvC* Mutations

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The *ruvA*, *ruvB*, and *ruvC* genes of *Escherichia coli* provide activities that catalyze branch migration and resolution of Holliday junction intermediates in recombination. Mutation of any one of these genes interferes with recombination and reduces the ability of the cell to repair damage to DNA. A suppressor of *ruv* mutations was identified on the basis of its ability to restore resistance to mitomycin and UV light and to allow normal levels of recombination in a *recBC sbcBC* strain carrying a Tn*10* insertion in *ruvA*. The mutation responsible was located at 12.5 min on the genetic map and defines a new locus which has been designated *rus*. The *rus* suppressor works just as well in *recBC sbcA* and *rec*$^+$ *sbc*$^+$ backgrounds and is not allele specific. Mutations in *ruvB* and *ruvC* are suppressed to an intermediate level, except when *ruvA* is also inactive, in which case suppression is complete. In all cases, suppression depends on RecG protein, a DNA-dependent ATPase that catalyzes branch migration of Holliday junctions. The *rus* mutation activates an additional factor that probably works with RecG to process Holliday junction intermediates independently of the RuvAB and RuvC proteins. The possibility that this additional factor is a junction-specific resolvase is discussed.

Damage to DNA is unavoidable, and all organisms have evolved enzymatic systems to promote repair and limit mutation. Repair of UV-damaged DNA has received particular attention, and the mechanisms involved have been studied in some detail in *Escherichia coli*, where a large number of genes have been identified and their products have been purified and characterized in vitro. Most UV-induced lesions are removed by nucleotide excision repair working in conjunction with enzymes that catalyze homologous recombination. Excision repair removes a broad spectrum of lesions in a multistep reaction catalyzed by the Uvr proteins, DNA polymerase I, and DNA ligase (46). The combined action of the UvrA, UvrB, and UvrC proteins leads to incision of the damaged strand on either side of the lesion. The 12- to 13-nucleotide fragment containing the lesion is then released by the helicase activity of UvrD, the gap formed is filled by DNA polymerase I (Pol I), and the new patch is sealed by ligase.

The enzymes of recombination come into play when the replisome encounters a lesion in the template strand and DNA synthesis comes to an abrupt halt. RecA protein allows the cell to recover from this situation by helping the replisome to resume DNA synthesis (7). Part of this process of recovery involves recombinational exchanges with the undamaged sister duplex (9, 32, 33). One possible mechanism is based on the ideas proposed originally by Howard-Flanders and colleagues and modified subsequently to take into account the ability of RecA protein to catalyze pairing and strand exchange reactions between homologous DNA molecules (10, 48, 49). According to this model (Fig. 1), DNA synthesis resumes downstream of the lesion via a mechanism that remains uncertain but which leaves a gap opposite the lesion in the template strand. RecA polymerizes at the gap to form a nucleoprotein filament that promotes homologous pairing and strand exchange with the undamaged sister molecule. Strand transfer past the lesion closes the gap and allows the excision enzymes a further opportunity to repair the DNA. Further extension into duplex regions may result in reciprocal exchange and the formation of a classical Holliday junction (8). Endonuclease cleavage across the point of strand exchange resolves the connection between the sister molecules, leaving DNA polymerase to fill in the gap formed in the parental strand. Otherwise, the molecules could be separated by reversing the direction of branch migration. An alternative mechanism for bypassing the lesion is for the replisome to switch to copying the undamaged daughter strand and to switch back to the parental strand after clearing the lesion. In this model, the recombination complex formed by RecA simply provides the means to switch strands (7).

Although the mechanism(s) of recombinational bypass remains to be established, recent studies have identified activities encoded by the *ruv* and *recG* genes that could process the intermediates generated by RecA into viable products. As with *recA*, mutations in these genes confer sensitivity to UV light, particularly in an excision-deficient background where lesions persist and have to be bypassed at every round of replication (9, 18, 20, 29). Three genes have been identified at the *ruv* locus (36). The *ruvA* and *ruvB* genes encode proteins of 24 and 37 kDa, respectively, that act together to catalyze branch migration of Holliday junctions. RuvA targets RuvB to the junction where the ATPase activity of the latter provides the motor to drive branch migration (11, 12, 31, 38, 44, 45). A third *ruv* gene, *ruvC*, encodes a 19-kDa nuclease that resolves junctions by symmetrical strand cleavage across the point of strand exchange (5, 6, 13).

The sequential action of RecA, RuvAB, and RuvC provides what appears to be a simple enzymatic pathway for the repair of strand gaps. However, the situation is complicated by a functional overlap between *ruv* and *recG*. The *recG*

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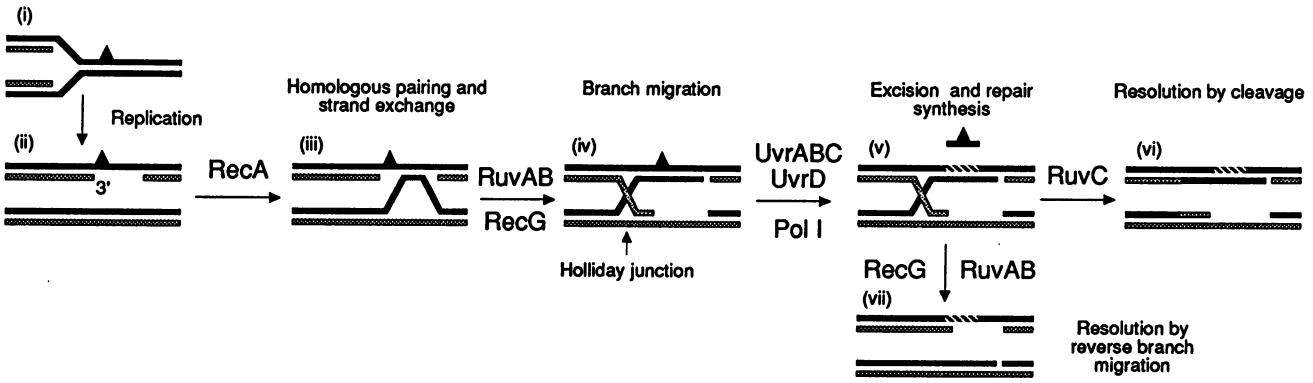

FIG. 1. A model for postreplication repair of UV-damaged DNA. A DNA replication fork encounters a UV-induced pyrimidine dimer (closed triangle) in the template strand (solid line) (i) and resumes DNA synthesis downstream, leaving a gap in the daughter strand (shaded line) (ii). RecA protein polymerizes at the gap and initiates strand exchange with the sister duplex (iii). Branch migration by RuvAB or RecG extends the heteroduplex joint beyond the lesion and leads to a Holliday junction (iv). Excision of the dimer (v) followed by further processing of the junction by RuvC cleavage (vi) or by reverse branch migration with RecG or RuvAB (vii) separates the sister duplexes and allows repair to be completed by filling in the remaining gaps.

locus encodes a 76-kDa protein which has ATPase and DNA-binding activities. It interacts specifically with synthetic Holliday junctions and dissociates these to duplex products indistinguishable from those produced by RuvAB (25). It also catalyzes branch migration of Holliday intermediates made by RecA in vitro (51).

The overlap between RuvAB and RecG is consistent with the fact that both *ruv* and *recG* single mutants produce some 30 to 50% of the normal yield of recombinants in genetic crosses whereas *ruv recG* double mutants produce 0.5% or less (17). Presumably, these proteins provide equally efficient alternative activities for producing recombinants. However, the same cannot be said for repair of damaged DNA since *ruv* mutants are far more sensitive to UV light than *recG* strains. The reason for this difference between recombination and DNA repair is not clear. The *ruvA* and *ruvB* genes form an operon regulated by LexA protein and are induced in response to DNA damage (4, 39, 40). *recG* is a component of the *spoT* operon and is not SOS inducible (14, 24). Perhaps UV-irradiated *ruvAB* mutants have insufficient RecG to cope with all of the lesions generated. A second problem arises from the fact that *ruvC recG* double mutants have the same extreme sensitivity to UV light and severe deficiency in recombination as *ruvA recG* or *ruvB recG* strains (17). There is no evidence that RecG has a nuclease activity that could cleave junctions in the absence of RuvC (25).

In this article, we describe a suppressor of *ruv* mutations that allows both recombination and DNA repair to proceed independently not only of RuvAB but also of RuvC. The properties of the suppressor raise the possibility that *ruvA*, *ruvB*, and *ruvC* mutants are defective for resolution of Holliday junctions and that branch migration proceeds largely unhindered in these strains via RecG. The data presented support models for recombinational bypass of UV lesions that require resolution of Holliday junctions.

MATERIALS AND METHODS

Strains. *E. coli* strains are listed in Table 1. The Δ*ruvA63* allele in N2096 has the same polar effect on *ruvB* as the original *ruvA60*::Tn*10* insertion (36). λNK55 and λ1105 are transposon hop vectors for generating mini-*kan* insertions (41, 47).

Plasmids. pBL125 and pBL135 are *recG*$^+$ derivatives of pBR322 and pTZ18R, respectively (24). pGS751 is a *ruvC*$^+$ derivative of pGEM-7Zf(+) (37). pNK2859 was used to provide the 1.7-kb *Bam*HI probe for the *kan* insertion generated with λ1105 (15). pRS415 is a pBR322-based vector for inserting promoters in front of the *lacZYA* genes (43). pTM101 and pTM102 were made by cloning the 4.1-kb *Eco*RI-*Pvu*II and 4.5-kb *Eco*RI-*Stu*I DNA fragments, respectively, from the *recG* promoter region in pBL125 into pRS415 cut with *Eco*RI and *Sma*I.

DNAs. Synthetic X-junction DNA was made by annealing the oligonucleotides (49- to 51-mer) numbered 1, 2, 3, and 4 described previously (25, 30). Oligonucleotide 1 was ^{32}P end-labelled at the 5′ end before being annealed. DNA concentrations are in nucleotide equivalents. Junction DNA was measured with DNA DipSticks (Invitrogen, San Diego, Calif.), and the measurement is approximate because of the low concentration.

Proteins. *E. coli* RuvA, RuvB, and RecG proteins were purified as described previously (25, 44).

Media and general methods. LB broth and 56/2 salts media have been described (22). The LB medium contained 0.5 g of NaCl per liter except for matings, for which the salt concentration was increased to 10 g/liter. Broth and agar media were supplemented with 20 μg of tetracycline per ml, 40 μg of kanamycin per ml, or 50 μg of ampicillin per ml, as required for strains carrying antibiotic-resistant plasmids or transposons. Plasmid transformations and methods for measuring sensitivity to UV light have been described before (19, 23, 36). UV irradiation was delivered at a dose rate of 1 J/m^2/s. Plate tests for measuring sensitivity to mitomycin used LB agar containing mitomycin at concentrations of either 0.2 or 0.5 μg/ml. Media and methods for propagating phage λ and for selecting tetracycline-sensitive isolates of strains carrying Tn*10* insertions followed the recipes and protocols of Silhavy et al. (42). Assays for β-galactosidase were as described by Miller (27). Methods for analysis of plasmid and chromosomal DNA followed recipes and protocols described by Sambrook et al. (34).

TABLE 1. *Escherichia coli* K-12 strains

Strain	Relevant genotype[a]	Other markers[b]	Source or reference
W3110	*IN(rrnD-rrnE)1*		1
AB1157	*rec*$^+$ *ruv*$^+$ *rus*$^+$	A	1
JC7623	*recB21 recC22 sbcB15 sbcC201*	A	1
CF3324	Δ*spoV1*::*kan* (= Δ*recG263*)		14
AT2538	*pyrE60*	A	CGSC 4518[c]
CV2	*adk-2*	B	J. Cronan (CGSC 4682)
CS85	*ruvC53 eda-51*::Tn*10*	A	41
GS1481	Δ*ruvC64*::*kan*	A	This work
N1585	Δ(*lac-pro*)*XIII*	C	This work
N2057	*ruvA60*::Tn*10*	A	41
N2096	Δ*ruvA63*	A	Tc^s selection on N2057
N2238	*ruvA60 recBC sbcBC*	A	P1.N2057 × JC7623 to Tc^r
N2731	*recG258*::*kan*	A	20
N2973	*recG162*	A	20
N3005	*purE85*::Tn*10*IN(*rrnD-rrnE*)*1*		λNK55 × W3110 to Km^r
N3041	*eda-51* IN(*rrnD-rrnE*)*1*		41
N3105	*purE85*	A	P1.N3005 × AB1157 to Tc^r
N3603	*zbc-2252*::*kan* IN(*rrnD-rrnE*)*1*		λ1105 × W3110 to Km^r
N3605	*zbc-2253*::*kan* IN(*rrnD-rrnE*)*1*		λ1105 × W3110 to Km^r
N3608	*rus-1* Δ*ruvA63 purE85 zbc-2252*	A	P1.N3603 × TNM716 to Km^r
N3617	*rus-1 zbc-2252* IN(*rrnD-rrnE*)*1*		P1.N3608 × W3110 to Km^r
N3684	*ruvA60* Δ*ruvC64*	A	P1.GS1481 × N2057 to Km^r (Tc^r)
RG108	*rus-1 ruvA60 recBC sbcBC*	A	This work
AM547	Δ(ruvA-ruvC)*65*	A	N3684 to Tc^s (and Km^s)
AM549	*ruvA60 recG162*	A	P1.N2057 × N2973 to Tc^r
AM561	Δ*ruvAC65 eda-51*	A	P1.N3041 × AM547 to Tc^r
TNM482	*rus-1 sbcC rpoB his*$^+$ Tc^s	A	—[d]
TNM668	*rus-1 purE85 sbcC*	A	P1.N3105 × TNM482 to Tc^r
TNM716	*rus-1 purE85* Δ*ruvA63*	A	P1.TNM668 × N2096 to Tc^r
TNM718	*recBC sbcBC purE85*	A	P1.N3105 × JC7623 to Tc^r
TNM733	*rus-1*	A	P1.TNM482 × N3105 to Pur$^+$
TNM734	*rus-1 recBC sbcBC*	A	P1.TNM482 × TNM718 to Pur$^+$
TNM759	*rus-1 ruvA60*	A	P1.N2057 × TNM733 to Tc^r
TNM777	*rus-1 ruvC53 eda-51*	A	P1.CS85 × TNM733 to Tc^r
TNM838	*rus-1 recBC sbcBC ruvC53 eda-51*	A	P1.CS85 × TNM734 to Tc^r
TNM839	*recBC sbcBC ruvC53 eda-51*	A	P1.CS85 × JC7623 to Tc^r
TNM844	*rus-1 ruvA60 recBC sbcBC*	A	P1.N2057 × TNM734 to Tc^r
TNM883	*rus-1 recG258*	A	P1.N2731 × TNM733 to Km^r
TNM896	*ruvA60 recG258*	A	P1.N2731 × N2057 to Km^r
TNM897	*rus-1 ruvA60 recG258*	A	P1.N2731 × TNM759 to Km^r
TNM1031	*adk-2 purE85*	B	P1.N3105 × CV2 to Tc^r
TNM1072	Δ*recG263*	A	P1.CF3324 × AB1157 to Km^r
TNM1106	*rus-1 purE85* Δ(*lac-pro*)$_{XIII}$	C	P1.TNM716 × N1585 to Tc^r
TNM1107	*purE85* Δ(*lac-pro*)$_{XIII}$	C	P1.TNM716 × N1585 to Tc^r
TNM1115	*rus-1 ruvA60* Δ*ruvC64*	A	P1.N3684 × TNM733 to Tc^r
TNM1120	*ruvA60* Δ*ruvC64 recBC sbcBC*	A	P1.N3684 × JC7623 to Tc^r
TNM1124	*rus-1* Δ*ruvC64*	A	P1.GS1481 × TNM733 to Km^r
TNM1194	*pyrE60 rus-1 zbc-2252*	A	P1.N3617 × AT2538 to Km^r
TNM1206	*rus-1 ruvA60* Δ*ruvC64 recBC sbcBC*	A	P1.N3684 × TNM734 to Tc^r
TNM1208	*rus-1* Δ*ruvAC65 eda-51*	A	P1.AM561 × TNM733 to Tc^r
TNM1209	*pyrE60 rus-1 zbc-2252* Δ*ruvAC65 eda-51*	A	P1.AM561 × TNM1194 to Tc^r
TNM1215	*recG263 rus-1* Δ*ruvAC65 eda-51*	A	P1.CF3324 × TNM1208 to Km^r
TNM1219	Δ*ruvAC65 recG263 eda-51*	A	P1.CF3324 × AM561 to Km^r
TNM1228	*recG162 rus-1 zbc-2252* Δ*ruvAC65 eda-51*	A	P1.N2973 × TNM1209 to Pyr$^+$
KL548	F′ (F128) *lacI3 lacZ118 proAB*$^+$	D	K. B. Low
3000$_{XIII}$	Hfr (Hayes, PO1) Δ(*proB-lac*)$_{XIII}$		CGSC 5263[c]
KL226	Hfr (Cavalli, PO2A) *relA1 tonA22*		K. B. Low
KL227	Hfr (PO3 of P4X) *relA1 tonA22*		K. B. Low (CGSC 4515[c])
GY2200	Hfr (H, PO1) (λ*ind*)$^+$ *thi-1 relA1*		R. Devoret

[a] After the first full listing, *recB21 recC22 sbcB15 sbcC201* is abbreviated to *recBC sbcBC*, Δ(*ruvA-ruvC*)*65* is abbreviated to Δ*ruvAC65*, and insertions are abbreviated to the gene symbol plus allele number.

[b] A, F$^-$ *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*; B, Hfr (PO2A) *tonA22* Δ*phoA8 ompF627 fadL701 relA1 glpR2 glpD3 pit-10 spoT1*; C, same as that for A except *thr*$^+$ *ara*$^+$ *leu*$^+$Δ(*lac-pro*)$_{XIII}$ (N1585 was made by mating AB1157 to *pro*$^+$ *lac*$^+$ with Hfr KL226 and then to *thr*$^+$ *ara*$^+$ *leu*$^+$ Δ(*pro-lac*)$_{XIII}$ with Hfr 3000$_{XIII}$); D, Δ(*pro-lac*)$_{XIII}$ recA1 rpsE xyl mtl.

[c] Strain supplied from the *E. coli* Genetic Stock Center (CGSC) by B. J. Bachmann.

[d] —, Made by transducing a *thyA* derivative of RG108 to *his*$^+$ *sbcB*$^+$ and then to *thyA*$^+$ *recBC*$^+$ with P1.W3110 before selecting for resistance to rifampin (100 μg/ml) and sensitivity to tetracycline.

Genetic crosses and measures of recombination. F′ and Hfr donors were mated with F^- recipients in high-salt LB broth at 37°C by using procedures described in detail elsewhere (21, 23). Measures of cell viability relate to the number of CFU in the recipient cultures at an A_{650} of 0.4. Transconjugants were selected on 56/2 or LB agar, as appropriate, supplemented with 100 μg of streptomycin per ml to counterselect donor cells. Transductions with phage P1*vir* followed the recipes and protocols described by Miller (27).

Isolation of strain RG108. The *ruvA60 recBC sbcBC* strain, N2238, was grown overnight in LB broth, and 0.05-ml samples were spread on LB agar containing tetracycline to maintain selection for Tn*10* and mitomycin (0.2 μg/ml) to select for revertants. The plates were then irradiated at 30 J/m^2 before being incubated overnight at 37°C. Colonies of mitomycin-resistant survivors were purified and subjected to further tests. RG108 was identified by its resistance to both UV light and mitomycin.

Insertion of mini-*kan* near *purE*. Random insertions of mini-*kan* were generated in strain W3110 by using λ1105 and the media and protocols of Kleckner et al. (15). To identify insertions near *purE*, P1 phage grown on the pooled Km^r colonies was then used to transduce strain N3005 to Pur^+ on glucose minimal agar supplemented with kanamycin.

Deletion of the chromosomal *ruvC* gene and construction of a Δ(*ruvA-ruvC*) strain. pGS751 was digested with *Nde*I and *Stu*I, and after the *Nde*I ends were filled, the larger fragment was ligated to a 1.2-kb *Sma*I *kan* DNA fragment from pUC4-KIXX (Pharmacia) before being transformed into JC7623. Km^r transformants were selected in the absence of ampicillin to allow segregation of plasmid-free cells as described by Oden et al. (28). Recombinants carrying the Δ*ruvC*::*kan* allele (Δ*ruvC64*) inserted in the chromosome were identified by their sensitivity to mitomycin and UV light. The mutation was then transduced to AB1157 to give strain GS1481. The Δ*ruvC64*::*kan* genotype was confirmed by Southern analysis (data not shown). Δ*ruvC64* was transduced into strain N2057 to give the *ruvA60*::Tn*10* Δ*ruvC64* strain N3684, which was identified as a rare transductant resistant to both kanamycin and tetracycline. Deletion of the *ruvA-ruvC* region was achieved by selecting tetracycline-sensitive isolates of N3684 and screening for those clones that had lost resistance to kanamycin. One such isolate (AM547) was shown by Southern analysis (data not shown) to be deleted for most, but not all, of the *kan* sequences. We assume that this deletion, designated Δ*ruvAC65*, has removed the *ruvA* sequences upstream of the original Tn*10* insertion. Complementation studies with cloned *ruv* genes confirmed the *ruvAC* genotype of AM547. They also revealed some expression of *ruvB* but no more than that seen previously in a *ruvA60*::Tn*10* strain (36).

Gel retardation assay. Reaction mixtures (20 μl) contained ^{32}P-labelled synthetic Holliday junction or linear duplex DNA (~0.15 μM) in binding buffer (50 mM Tris-HCl [pH 8.0], 5 mM EDTA, 1 mM dithiothreitol, 100 μg of bovine serum albumin per ml) and various amounts of protein. The proteins were mixed into the reaction mixtures before junction DNA was added. After 15 min on ice, 5 μl of loading buffer (50 mM Tris-HCl [pH 7.5], 4 mM EDTA, 25% glycerol, 400 μg of bovine serum albumin per ml) was added, and the samples were loaded immediately onto 4% polyacrylamide gels in low-ionic-strength buffer (6.7 mM Tris-HCl [pH 8.0], 3.3 mM sodium acetate, 2 mM EDTA). Electrophoresis was carried out at room temperature for 1.75 h at 200 V with continuous circulation of buffer. Gels were dried on Whatman 3MM paper and autoradiographed.

Dissociation of a synthetic Holliday junction. Reaction mixtures (20 μl) contained synthetic Holliday junction (~0.4 μM) in reaction buffer (50 mM Tris-HCl [pH 8.0], 1 mM dithiothreitol, 5 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 5 mM ATP) and various amounts of protein. Reactions were initiated by the addition of RecG. Reaction mixtures were incubated at 37°C for 30 min before the addition of 5 μl of stop buffer (2.5% [wt/vol] sodium dodecyl sulfate, 200 mM EDTA, 10 mg of proteinase K per ml) and then incubated for a further 10 min at 37°C. The DNA products were then electrophoresed at room temperature through a 10% native polyacrylamide gel by using a Tris-borate buffer system (30) and subsequently autoradiographed.

RESULTS

Indirect suppression of a *ruvA*::Tn*10* allele. Strains carrying the *ruvA60*::Tn*10* allele give rise to the occasional revertant with increased resistance to UV light and mitomycin. These are particularly noticeable in a *recBC sbcBC* background where *ruv* mutations confer a more extreme phenotype (18). Strain RG108 is typical of the revertants encountered. It is as resistant to UV light as its *ruv*$^+$ ancestor, JC7623, and produces about 50% as many Pro^+ recombinants in crosses with Hfr KL227. Its immediate *ruvA60* parent strain, N2238, is by comparison very sensitive to UV light and produces recombinants at a 500-fold-lower frequency than JC7623 (Fig. 2a and data not shown). Backcrosses to strains AB1157 and JC7623 confirmed that RG108 retains the Tn*10* insertion in *ruvA* in an unaltered form (data not shown). We conclude that its resistance to DNA-damaging agents is due to an additional mutation which we designate *rus-1* for *ruv* suppression. The suppression is specific to *ruv* mutations. No suppression was detected with mutations in any of the other genes known to affect recombination (data not shown).

Location of the suppressor. Genetic crosses (data not shown) linked *rus-1* to *purE*, which is at 12.2 min on the genetic map (Fig. 3a). *rus-1* single mutants segregating in these crosses were found to be slightly sensitive to mitomycin at 0.5 μg/ml (data not shown). However, they are resistant to UV light (Fig. 2c). To locate *rus* more precisely, we isolated mini-*kan* insertions linked to *purE* and positioned these relative to the Kohara et al. physical map (16) by Southern analysis of chromosomal DNA digests. The probe used was the 1.7-kb *Bam*HI DNA fragment from pNK2859, which carries the same *kan* gene. The results (data not shown) provided an unambiguous *zbc* location for each insertion (Fig. 3b). We then used three-factor crosses to map the *rus* locus relative to *purE*, *adk*, and the two *zbc* insertions. The data (Table 2) gave the order *adk–purE–zbc-2253–rus–zbc-2252*, which places *rus* at about 12.5 min between coordinates 580 and 590 (Fig. 3b).

Suppression in *rec*$^+$ *sbc*$^+$ and *recBC sbc* strains. Although *rus-1* was isolated in a *recBC sbcBC* background, it suppresses *ruv* mutations just as well in *rec*$^+$ *sbc*$^+$, *recBC sbcA*, and *recBC sbcBC* strains (Fig. 2b and data not shown). It also suppresses *ruvA60* in strain W3110 (data not shown), from which we conclude that suppression is not specific to the AB1157 genetic background. We used a *ruvA60 recBC*$^+$ *sbc*$^+$ strain to isolate a number of new suppressors by selecting for resistance to mitomycin. The suppressor was linked to *purE* in every case tested (data not shown).

The *ruvA60* allele severely reduces the number of recombinants recovered in Hfr crosses with *recBC sbcBC* strains and also causes abortive transfer of F-primes (3, 18). *rus-1*

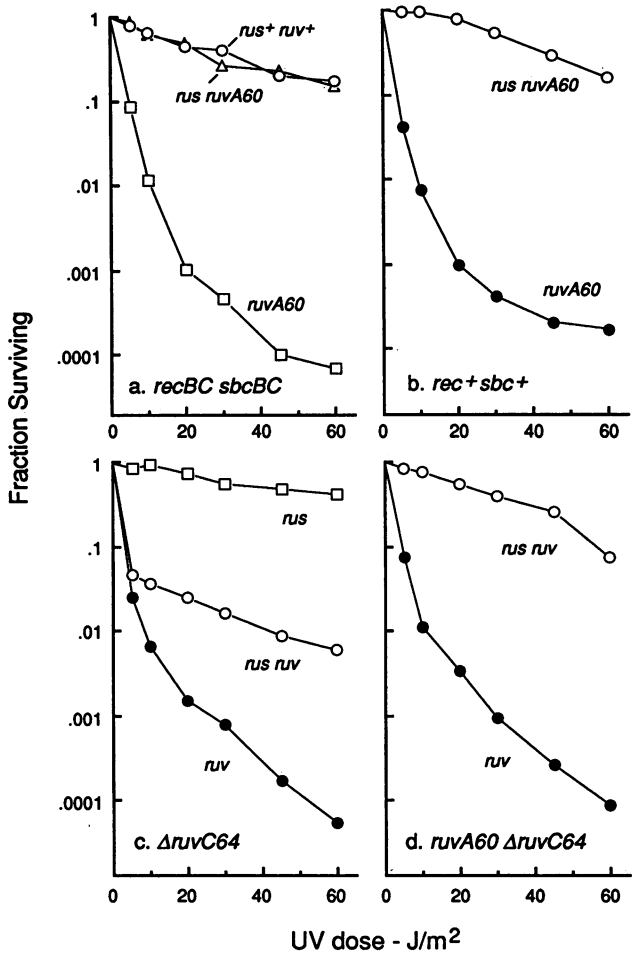

FIG. 2. Suppression of UV sensitivity in *ruv* mutants. The strains used are identified by genotype in each panel: (a) JC7623, RG108, N2238; (b) TNM759, N2057; (c) TNM733, TNM1124, GS1481; (d) TNM1115, N3684. The survival of the *rus-1* strain TNM733 (c) is almost identical to that of the *rus*$^+$ control, AB1157 (data not shown).

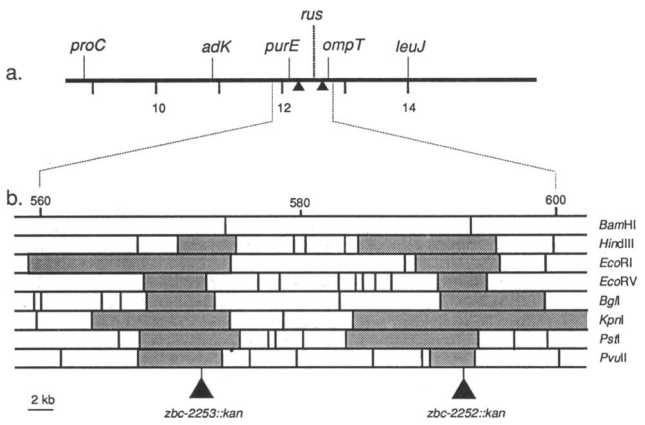

FIG. 3. Map showing location of *rus* on the genetic map (2) (a) and the Kohara et al. physical map (16) (b) of the *E. coli* chromosome. The shaded restriction fragments in panel b are those identified by Southern analysis to be affected by the *zbc-2252* and *zbc-2253* insertions. *Bam*HI digests were not informative since the enzyme cuts the *kan* insertion used at both ends (15).

eliminates these defects (Table 3a). It also eliminates the slight deficiency in recombination caused by *ruvA60* in *rec*$^+$ *sbc*$^+$ and *recBC sbcA* strains (Table 3b and data not shown). The *rus* mutation alone has no obvious effect on recombination. Mutations in *recA* and *recB* reduce recombination by factors of approximately 5,000-fold and 500-fold, respectively, in both *rus ruv* and *rus ruv*$^+$ strains (data not shown), from which we deduce that recombination in a *rus* strain proceeds via a RecA- and RecBCD-dependent mechanism as it does in the wild type. Mutations in *recD*, *recF*, *recJ*, *recN*, *recO*, or *recQ* had no effect on recombination beyond that seen in a *rus*$^+$ strain (data not shown).

Suppression of *ruvB* requires inactivation of *ruvA*. The *ruvA200* allele, a nitrosoguanidine-induced point mutation (35), and *ruvA59*, another Tn*10* insertion (41), are suppressed just as well as *ruvA60* (data not shown). However, *ruvB* mutations are suppressed rather poorly. We examined several different alleles and obtained almost identical results (the data were similar to those obtained with *ruvC* strains [Table 3 and Fig. 2c]). The ability to fully suppress mutations in *ruvA* but not those in *ruvB* was unexpected given that the gene products act together to provide a branch migration activity. Since the polar Tn*10* insertions are fully suppressed, RuvA may be limiting suppression in *ruvB* mutants by preventing an alternative activity like RecG from gaining access to recombination intermediates.

To test this possibility directly, we used a simple band shift assay to examine binding of RecG to a synthetic X-junction in the presence of RuvA. We found that RuvA prevents binding even when RecG is in molar excess over RuvA (Fig. 4). In other experiments, we increased the RecG/RuvA ratio to 10:1. Again, the only complex detected was that formed by RuvA (data not shown). We also examined dissociation of the junction. With 100 nM RecG in the reaction mixture, inhibition was observed with as little as 3.9 nM RuvA (Fig. 5, lane c). Dissociation was eliminated when RuvA was increased to 15 nM (lane e). We conclude that binding of RuvA prevents RecG from gaining access to

TABLE 2. Mapping of *rus* by P1 transductional crosses

P1 donor	Recipient	No.[a]	Marker segregation (% of total)[b]
N3603 (*zbc-2252*::*kan*)	TNM716 (*purE rus ruvA63*)	160	42% *purE rus*$^+$, 52.4% *pur*$^+$ *rus*$^+$, 5% *purE rus*, 0.6% *pur*$^+$ *rus*
N3605 (*zbc-2253*::*kan*)	TNM716 (*purE rus ruvA63*)	148	11.5% *purE rus*$^+$, 47.3% *pur*$^+$ *rus*$^+$, 6.7% *purE rus*, 34.5% *pur*$^+$ *rus*
N3608 (*rus-1 purE zbc-2252*)	W3110 (wild type)	522	44% *pur*$^+$ *rus-1*, 21% *pur*$^+$ *rus*$^+$, 35% *purE rus-1*, 0.02% *purE rus*$^+$
N3603 (*zbc-2252*)	TNM1031 (*adk purE*)	298	5% *pur*$^+$ *adk*$^+$, 50.6% *pur*$^+$ *adk*, 1% *purE adk*$^+$, 43.4% *purE adk*

[a] Selection was for Km^r transductants in each case.

[b] Segregation at *adk* was monitored by growth at 42°C, segregation at *purE* was monitored by the requirement for adenine, and segregation at *rus* was monitored by sensitivity to mitomycin with W3110 as recipient and to both mitomycin and UV light with TNM716.

TABLE 3. Effect of *rus-1* on DNA transfer and recombination in crosses with Hfr and F′ donors

Recipient genotype	Strain no.	Viability	Relative yield of transconjugants[a]			
			× KL548 (F′ Pro$^+$)	× GY2200[b]		× KL227 (Pro$^+$)
				λ	(TL$^+$)	
rec$^+$ *ruv*$^+$ (control)	AB1157	1.0 = 1.8 × 10^{8c}	1.0 = 2.6 × 10^7	1.0 = 1.4 × 10^7	1.0 = 1.7 × 10^7	1.0 = 1.1 × 10^7
(a) *recBC sbcBC*						
rus	TNM734	0.32	1.12	1.70	0.40	1.41
ruvA60	N2238	0.051	0.00048	0.75	0.0021	0.0065
rus ruvA60	TNM844	0.24	1.1	1.77	0.41	1.09
ruvC53	TNM839	0.012	0.00001	0.70	0.00025	0.00011
rus ruvC53	TNM838	0.28	0.001	1.65	0.1	0.032
ruvA60 ΔruvC64	TNM1120	0.188	0.00009	0.86	0.00086	0.0024
rus ruvA60 ΔruvC64	TNM1206	0.37	0.64	2.4	0.35	0.84
(b) *recBC*$^+$ *sbcBC*$^+$						
rus	TNM733	1.13	1.46	1.41	1.18	1.17
ruvA60	N2057	0.48	0.88	1.07	0.24	0.44
rus ruvA60	TNM759	1.07	1.16	1.16	0.98	0.82
recG258	N2731	0.69	1.16	1.16	0.31	0.39
rus recG258	TNM883	0.52	1.10	0.86	0.10	0.16
ruvA60 recG258	TNM896	0.10	0.20	0.83	0.00093	0.002
rus ruvA60 recG258	TNM897	0.35	0.59	1.0	0.035	0.039
ΔruvC64	GS1481	0.73	0.48	0.60	0.18	0.16
rus ΔruvC64	TNM1124	1.34	0.98	0.89	0.64	0.75
ruvA60 ΔruvC64	N3684	0.65	0.49	0.22	0.21	0.49
rus ruvA60 ΔruvC64	TNM1115	0.78	0.76	1.09	0.80	0.86
ruvA60 recG162	AM549	0.47	0.30	0.073	0.00073	0.0067
rus ΔruvAC65 recG162	TNM1228	0.54	0.70	0.66	0.040	0.041
ΔruvAC65 recG263	TNM1219	0.24	0.21	0.88	0.0013	0.002
rus ΔruvAC65 recG263	TNM1215	0.5	0.52	1.18	0.042	0.038

[a] Under this spanner, the values in the first two columns provide different measures of DNA transfer, while values in the second two columns provide measures of recombination. Mating was for 30 (KL548), 40 (Hfr KL227), or 60 (Hfr GY2200) min. The transconjugant class selected is shown in parentheses. The absolute values shown for strain AB1157 are per milliliter of mating mixture. The data for the test strains are means of two to four experiments.

[b] λ, plaques from zygotic induction of the Hfr λ prophage; TL$^+$, Thr$^+$ Leu$^+$.

[c] The absolute value shown for strain AB1157 is per milliliter of recipient culture.

the junction. A similar situation could arise in vivo, especially after SOS induction, and may explain why a multicopy *ruvA*$^+$ plasmid makes *ruv*$^+$ cells extremely sensitive to UV light (36).

Suppression requires RecG. We considered the possibility that *rus-1* might suppress *ruvA(B)* mutations by increasing expression of RecG. To test this possibility, we introduced *recG*$^+$ into a *ruvA60* strain on high-copy-number plasmids (pBL125 and pBL135). There was no increase in resistance to UV light with pBL125. We observed some increase with pBL135, where *recG* is expressed from the vector *lac* promoter. However, the effect was very slight compared with that of *rus-1* and was the same with and without IPTG (isopropyl-β-D-thiogalactopyranoside) induction (data not shown). We also examined transcription from DNA fragments thought to contain the promoters that drive expression of *recG*. Two large sections of the DNA upstream of the *recG* reading frame were cloned in front of the promoterless *lacZYA* genes in pRS415. The fragment cloned in pTM101 contains the putative P2 promoter; that in pTM102 contains both P2 and P3 promoters (14, 24). These constructs were introduced into *rus-1* and *rus*$^+$, Δ(*pro-lac*) strains, and synthesis of β-galactosidase was measured relative to controls carrying pRS415. The data in Table 4 show that the regions cloned contain strong promoters. pTM102 produces slightly more β-galactosidase than pTM101, which suggests that the putative P3 promoter in pTM102 is functional, as suggested previously (24). However, we observed no significant differences between the two strains with either of the plasmids made.

These observations indicate that suppression is unlikely to be due to increased synthesis of RecG. However, RecG is required for suppression. When we introduced a *recG* mutation into a *rus ruvA60* strain, recombination was reduced some 30-fold and sensitivity to UV light was increased to a level typical of a *ruv* mutant (Table 3b and Fig. 6a). We examined a number of different *recG* alleles, including a point mutation (*recG162*), an insertion (*recG258*), and a deletion (*recG263*). All three had the same effect. The loss of *recG* activity has a much greater effect than is seen in *rus*$^+$ *ruv*$^+$ strains. However, the *recG rus ruv* strains are substantially more proficient in recombination and more resistant to UV light than the corresponding *rus*$^+$ strains (Table 3b, Fig. 6a, and data not shown) (17). These observations suggest that *rus-1* activates some gene product to act with RecG in the suppression of *ruv* mutations. If suppression was due simply to increased expression of *recG* or to some activation of RecG protein, then mutation of *recG* would be expected to have the same effect in both *ruv* and *rus ruv* strains.

Suppression of *ruvC*. A possible clue as to the mode of action of *rus-1* came from studies on the suppression of *ruvC*. We observed that *ruvC* mutations were suppressed to the same intermediate extent as *ruvB* mutations. Very similar results were obtained with *ruvC* point mutations and deletions (Fig. 2c, Table 3, and data not shown). To determine whether this incomplete suppression of *ruvC* was due

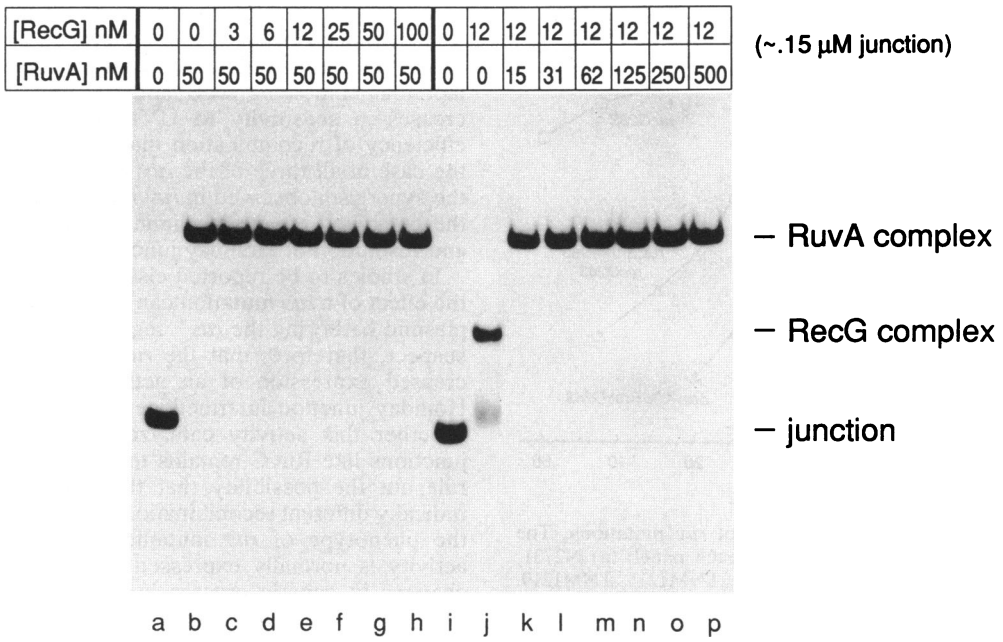

FIG. 4. Effect of RuvA on binding of RecG to a synthetic X-junction. RecG at increasing concentrations and RuvA at a fixed concentration (lanes b to h) or vice versa (lanes j to p) were mixed on ice with 0.4 μM ^{32}P-5′-end-labelled X-junction DNA as described in Materials and Methods. Binding was initiated by the addition of DNA to the reaction mixture. Lanes a and i are controls containing neither RuvA nor RecG. Complexes were separated on a low-ionic-strength gel at room temperature, and labelled DNA was detected by autoradiography.

to the negative effect of RuvA, as it was with *ruvB*, we constructed a strain deficient for both *ruvA* and *ruvC* (see Materials and Methods) and introduced *rus-1*. The resulting strain proved resistant to UV light and proficient in recombination (Fig. 2d and Table 3). Clearly, *rus-1* is able to fully suppress *ruvC* provided that RuvA is inactive. Again, suppression depends on *recG* to the same extent it does in a *rus ruvA60* strain (Fig. 6b, Table 3, and data not shown). It is also worth noting that the *ruvA ruvC* double mutants have the same phenotype as the single mutants, no more and no less.

The ability to suppress a deficiency in *ruvC* in a way that depends on *recG* is highly significant. There is no evidence that RecG has a nuclease activity that can cleave Holliday junctions. We assume, therefore, that suppression depends not only on RecG but also on the activation perhaps of an alternative resolvase to replace RuvC. This is consistent with the finding that *rus-1* makes a *ruvC recG* strain more resistant to UV light (Fig. 6b).

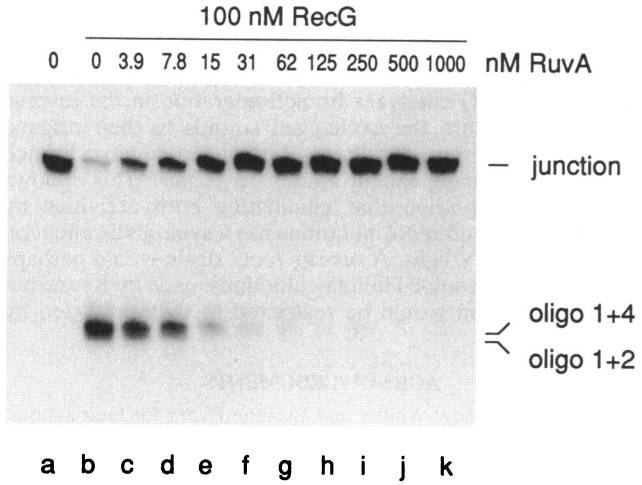

FIG. 5. Inhibitory effect of RuvA on dissociation of a synthetic X-junction by RecG. Reaction mixtures containing 0.15 μM 5′-^{32}P-labelled junction DNA and RecG and RuvA at the indicated concentrations were incubated for 30 min at 37°C. Reactions were stopped and deproteinized as described in Materials and Methods, and the products were analyzed on a 10% polyacrylamide gel.

DISCUSSION

We have shown that *ruv* mutations can be suppressed indirectly by an additional mutation at a new locus called *rus*. With *ruvA* strains, the defects in both recombination and DNA repair are suppressed completely, but with *ruvB* and *ruvC* strains, suppression is incomplete unless *ruvA* is also eliminated. We found that RuvA prevents RecG from gaining access to Holliday junctions in vitro and suspect therefore that it can also interfere with the processing of these structures in vivo when either RuvB or RuvC is absent. This conclusion is consistent with the finding that suppression is largely eliminated by mutation of *recG*.

TABLE 4. Synthesis of β-galactosidase in *rus*$^+$ and *rus-1* strains carrying fusions of the *recG* promoter region to *lacZ*

Strain	Genotype	β-Galactosidase units/*A*650 U		
		pRS415 (vector)	pTM101 (P2)	pTM102 (P2 + P3)
TNM1107	*rus*$^+$	4	1,956	2,423
TNM1106	*rus-1*	5	2,009	2,766

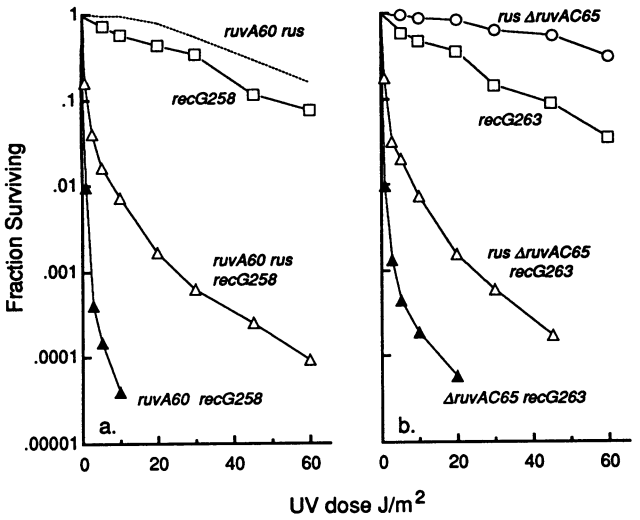

FIG. 6. Effect of *recG* on suppression of *ruv* mutations. The strains used are identified by genotype in each panel: (a) N2731, TNM896, TNM897; (b) TNM1072, TNM1208, TNM1215, TNM1219. The data for *rus-1 ruvA60* from Fig. 2b are included for comparison in panel a (dashed line).

The requirement for RecG fits with the ability of this protein to catalyze branch migration of Holliday junctions in vitro (25, 51). Presumably, RecG replaces RuvAB. However, this immediately raises the question of why *ruvA* and *ruvB* mutants are normally very sensitive to UV light. If RecG can replace RuvAB, they should be resistant. The *recG* locus is rather poorly expressed, and it may be that there is insufficient RecG in a *rus*$^+$ strain to compensate for loss of the SOS-inducible RuvAB proteins (24). If true, then a *rus* mutation might achieve its effect by increasing the level of RecG. There are several reasons why this explanation is probably incorrect. First, we found that multicopy *recG*$^+$ plasmids fail to produce a substantial increase in the survival of UV-irradiated *ruvAB* mutants. Second, *rus-1* does not appear to increase the activity of *recG* promoters. Third, RecG is very much more active than RuvAB in terms of its ability to dissociate synthetic junctions (25, 26). The branch migration activity of RecG is likely to compare very favorably, therefore, with that of RuvAB, even in SOS-induced *rus*$^+$ cells.

If RecG or RuvAB can independently satisfy most of the cell's need for a branch migration activity, it follows that mutation of *ruvA* and *ruvB* must have another effect on repair to account for the sensitivity to UV light. *ruv* single mutants have essentially identical phenotypes regardless of which of the three genes is affected, and as we have demonstrated there is no further decrease in recombination or repair when mutations eliminating both RuvAB and RuvC are combined. The identical phenotype and lack of additivity may reflect the fact that RuvAB and RuvC catalyze consecutive steps in the same repair process. However, given the overlap between RecG and RuvAB, another possibility is that *ruvA*, *ruvB*, and *ruvC* mutants are all deficient in resolution of Holliday junctions, with RecG providing all or most of the activity needed to promote branch migration. This situation would arise if RuvC were able to cleave junctions in vivo only in the presence of RuvAB. The observation that RuvC functions extremely inefficiently in vitro is probably indicative of the fact that it normally works with other proteins (5, 6). The idea is particularly appealing since it explains why *rus* mutations are able to suppress both *ruvAB* and *ruvC* mutations. It also accounts neatly for the fact that combining *recG* and *ruv* mutations leads to increases in sensitivity to UV light and decreases in the efficiency of recombination that are essentially identical in the case of all three of the *ruv* genes (17, 25). Accordingly, the synergism observed in *ruv recG* strains would be due to the combined effects of eliminating both branch migration and resolution of Holliday junctions.

In studies to be reported elsewhere, we have shown that the effect of a *rus* mutation can be mimicked by a multicopy plasmid harboring the *rus*$^+$ region of the chromosome. We suspect, therefore, that the *rus* mutation leads to the increased expression of an activity that helps to process Holliday junction intermediates in association with RecG. Whether this activity catalyzes symmetrical cleavage of junctions like RuvC remains to be determined. We cannot rule out the possibility that the *rus* mutation provides a radically different recombination mechanism. To account for the phenotype of *ruv* mutants, we assume that the *rus* activity is normally expressed sufficiently to promote exchanges in genetic crosses but cannot cope with the increased demands of repair. This hypothesis has a number of clear predictions which we are currently testing. A molecular analysis of the *rus* region is also under way and should reveal exactly how the suppression of *ruv* is achieved.

A further corollary to this work is that the UV sensitivity of a *ruv* mutant provides a measure of the part played by Holliday junction resolution in the repair of damaged DNA. It follows that the increase in sensitivity seen when *recG* is also inactivated provides a measure of the part played by branch migration. From the data presented here and in earlier studies, it would appear that branch migration and resolution are both very important. They support the model presented in Fig. 1. We propose that after DNA replication has left a gap in the newly synthesized DNA opposite the lesion in the template strand, RecA protein binds to the gap and initiates strand exchange as suggested initially by Rupp et al. (33) and West et al. (50). This leads to a Holliday junction that can be branch migrated past the UV lesion by RuvAB or RecG, allowing Uvr(A)BC excinuclease to remove the dimer. After filling in of the excision tract, repair can now be completed in one of two ways. Either RuvC resolves the Holliday junction by cleavage, or, alternatively, RuvAB or RecG catalyzes branch migration in the reverse direction to return the exchanged strands to their original partners. We propose that resolution and reverse branch migration are independent routes to repair. This follows from the observation that eliminating both activities by combining *ruv* and *recG* mutations has a synergistic effect on sensitivity to UV light. A *rus ruv recG* strain would perhaps remain able to resolve Holliday junctions made by RecA, but branch migration would be restricted to that catalyzed by RecA itself.

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