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UvrD303, a Hyperhelicase Mutant That Antagonizes RecA-Dependent SOS Expression by a Mechanism That Depends on Its C Terminus▽†

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Genomic integrity is critical for an organism's survival and ability to reproduce. In *Escherichia coli*, the UvrD helicase has roles in nucleotide excision repair and methyl-directed mismatch repair and can limit reactions by RecA under certain circumstances. UvrD303 (D403A D404A) is a hyperhelicase mutant, and when expressed from a multicopy plasmid, it results in UV sensitivity (UV^s), recombination deficiency, and antimutability. In order to understand the molecular mechanism underlying the UV^s phenotype of *uvrD303* cells, this mutation was transferred to the *E. coli* chromosome and studied in single copy. It is shown here that *uvrD303* mutants are UV sensitive, recombination deficient, and antimutable and additionally have a moderate defect in inducing the SOS response after UV treatment. The UV-sensitive phenotype is epistatic with *recA* and additive with *uvrA* and is partially suppressed by removing the LexA repressor. Furthermore, *uvrD303* is able to inhibit constitutive SOS expression caused by the *recA730* mutation. The ability of UvrD303 to antagonize SOS expression was dependent on its 40 C-terminal amino acids. It is proposed that UvrD303, via its C terminus, can decrease the levels of RecA activity in the cell.

All organisms must undergo DNA replication to pass their genetic information on to their offspring. Even for a simple organism such as *Escherichia coli*, this is not a trivial task. The coordinated action of many proteins is required to make up a replisome, which must commence DNA replication at the proper time during the cell cycle and at the correct location on the genome. Once this process begins, further challenges must be overcome to continue replication with high processivity and accuracy. Lesions to the template DNA, such as nicks, single-stranded DNA (ssDNA) gaps, abasic sites, double-stranded DNA (dsDNA) breaks, covalent base modifications, or proteins bound to the DNA, may result in stalled or collapsed replication forks, which must be repaired and restarted (13, 33).

The *E. coli* RecA protein is important for recombination and survival after DNA damage. RecA has at least two roles in survival after DNA damage (reviewed in reference 12). First, RecA is needed to induce the SOS response by acting as a sensor and allosteric effector of autoproteolysis of the LexA transcriptional repressor, whose degradation leads to the SOS response. Second, RecA is required for strand invasion during recombinational repair. Both functions require RecA to polymerize onto ssDNA and form a protein-DNA helical filament. This filament acts to increase the rate of LexA autoproteolysis and to perform recombinational repair. It is known that the SOS response increases the rates of transcription of at least 40 genes (11). Two SOS-regulated genes important for this paper are *recA* and *uvrD*.

UvrD (helicase II) is a 3′–5′ helicase with ssDNA-dependent ATPase activity (20, 30). It is part of the SF1 superfamily of helicases that includes Rep. UvrD functions in both nucleotide excision repair (NER) and methyl-directed mismatch repair (MMR) (17, 40, 43). In both processes, the helicase activity of UvrD is required to unwind the DNA strand containing the lesion such that a polymerase and DNA ligase may finish the repair process. Failure to complete these activities in a *uvrD* mutant results in sensitivity to UV light and an increased frequency of spontaneous mutagenesis.

A role for UvrD in regulating homologous recombination has also been established both in vivo and in vitro. In vivo, *uvrD*-null mutants lead to more RecA–green fluorescent protein (GFP) foci per cell in a population, and these foci are, on average, brighter than those in wild-type cells (6). UvrD is also required for the removal of RecA from arrested replication forks in temperature-sensitive *dnaE* and *dnaN* mutants (16). In vitro, UvrD has been shown to disrupt recombination intermediates and to remove RecA from ssDNA (46). This function seems to be conserved, since the *Saccharomyces cerevisiae* homolog of UvrD, Srs2, is capable of removing its cognate recombinase, Rad51, from DNA (24, 47). Failure to perform this antirecombination function in both *E. coli uvrD* and *S. cerevisiae srs2* mutants results in a hyperrecombination phenotype (2, 38, 55).

Previously, Kushner and colleagues constructed a novel allele of *uvrD* called *uvrD303* (53). This mutant has two point mutations (D403A and D404A) in a region of the protein that is thought to regulate helicase activity (5, 7, 26). In vitro, UvrD303 was shown to have a specific helicase activity as much as 10-fold higher than that of the wild-type protein, depending on the substrate (53). When *uvrD303* was expressed from a multicopy plasmid, the cells were UV sensitive (UV^s) and recombination deficient (Rec$^-$) and had a decreased frequency of spontaneous mutagenesis (53).

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TABLE 1. Strains and plasmids used in this work

Strain or plasmid	*recA*	*uvrD*	Other relevant genotype	Source or derivation
Strains				
CAG18491	+	+	*metE3079*::Tn*10*	44
GJ1989	+	+	*lexA3 malB*::Tn*9*	39
JC13509[a]	+	+	+	Lab stock
JC19108	+	+	*lexA71*::Tn*5 malE*::Tn*10*	Lab stock
JC19328	*306*	+	+	Lab stock
JJC145	+	+	*PO66-HfrPK191 srlC*::Tn*10*	4
JJC2457	+	*cat*[h]	+	16
MV1138	+	+	*srlC300*::Tn*10 recAo281*	M. Volkert
SK9041	+	*294*	+	53
SS775	+	+	*lexA3 malE*::Tn*10-9*	GJ1989→JC13509[e]
SS996	+	+	del(*attB*)::*psulA-gfp*	31
SS1054	+	+	*metE3079*::Tn*10*	CAG18491→JC13509[d]
SS1465	+	+	*gal76*::Tn*10* del(*attB*)::*psulA-gfp*	Lab stock
SS2533	+	*cat*	del(*attB*)::*psulA-gfp*	JJC2457→SS996[e]
SS3085	*4136*, *4155*[b]	+	+	37
SS3368	*4136*, *4155*[b]	*cat*	+	6
SS4414	+	+	*lexA3 malE*::Tn*10-9*	SS775/pKD46
SS4629	*730*	+	del(*attB*)::*psulA-gfp*	Lab stock
SS4952	*100*[c]	+	+	3
SS4976	*4142*	+	*recAo1403* del(*attB*)::*psulA-gfp*	Lab stock
SS5112	*730*	+	*metE3079*::Tn*10* del(*attB*)::*psulA-gfp*	CAG18491→SS4629[d]
SS5129	+	+	del(*uvrA*)*100*::*kan*	3
SS5141	+	*303*, *705*	*lexA3 malE*::Tn*10-9*	This study
SS5145	+	*701*, *706*	*lexA3 malE*::Tn*10-9*	This study
SS5191	+	*701*, *706*	+	SS5145→JC13509[f]
SS5402	+	*701*, *706*	+	SS5191/pRC35
SS5428	+	*701*	+	This study
SS5450	+	*701*	+	SS5428→SS1054[g]
SS5482	+	*303*, *705*	+	SS5141→JC13509[f]
SS5489	+	*303*, *705*	+	SS5482/pRC40
SS5496	+	*303*	+	This study
SS5704	+	*303*	+	SS5496→SS1054[g]
SS5711	*4136*, *4155*[b]	*303*	+	SS3085→SS5704[f]
SS5737	+	*701*	*gal76*::Tn*10* del(*attB*)::*psulA-gfp*	SS1465→SS5450[d]
SS5756	*306*	*303*	+	JC19328→SS5704[d]
SS5764	+	*303*	del(*uvrA*)*100*::*kan*	SS5129→SS5704[f]
SS5766	+	+	del(*uvrA*)*100*::*kan*	SS5129→JC13509[f]
SS5769	*306*	+	del(*uvrA*)*100*::*kan*	SS5756→ SS5766[d]
SS5801	*730*	*303*	del(*attB*)::*psulA-gfp*	SS5496→SS5112[g]
SS5802	+	+	*metE3079*::Tn*10* del(*attB*)::*psulA-gfp*	CAG18491→SS996[d]
SS5803	+	*303*	del(*attB*)::*psulA-gfp*	SS5496→SS5802[g]
SS5807	*4142*	+	*metE3079*::Tn*10 recAo1403* del(*attB*)::*psulA-gfp*	CAG18491→SS4976[d]
SS5808	*4142*	*cat*	*recAo1403* del(*attB*)::*psulA-gfp*	JJC2457→SS5807[g]
SS5809	*4142*	*303*	*recAo1403* del(*attB*)::*psulA-gfp*	SS5496→SS5807[g]
SS5907	*100*[c]	+	+	SS4952→JC13509[f]
SS5912	*100*[c]	+	+	SS5907/pLH29
SS5915	*200*[c]	+	+	SS5912/FLP
SS5959	+	+	*lexA71*::Tn*5*	JC19108→JC13509[f]
SS5960	+	*303*	*lexA71*::Tn*5*	JC19108→SS5704[f]
SS5962	+	+	*srlC300*::Tn*10 recAo281*	MV1138→JC13509[d]
SS5963	+	*303*	*srlC300*::Tn*10 recAo281*	MV1138→SS5704[d]
SS5985	+	*303*, *705*	+	SS5482/pRC49
SS5986	+	*303*, *701*	+	This study
SS5987	+	*303*, *701*	+	SS5986→SS1054[g]
SS5988	+	*303*, *701*	del(*attB*)::*psulA-gfp*	SS5986→SS5802[g]
SS5989	*730*	*303*, *701*	del(*attB*)::*psulA-gfp*	SS5986→SS5112[g]
SS5990	*730*	*701*	del(*attB*)::*psulA-gfp*	SS5450→SS5112[g]
SS5993	+	*294*	+	SK9041→JC13509[f]
Plasmids				
pGZK20				53
pGZK31				53
pKD46				14
pKO3				27
pLH29				22
pRC19				This study

Continued on following page

TABLE 1—*Continued*

Strain or plasmid	*recA*	*uvrD*	Other relevant genotype	Source or derivation
pRC32				This study
pRC33				This study
pRC35				This study
pRC40				This study
pRC49				This study
pSJS1488				37

[a] JC13509 has the following genotype: *sulB103 lacMS286* ϕ80d*IIlacBK1 argE3 his-4 thi-1 xyl-5 mtl-1 rpsL31 tsx*. The *lacMS286* ϕ80d*IIlacBK1* genes code for two partial nonoverlapping deletions of the *lac* operon (23, 54).

[b] The full genotype is *recAo1403 ygaD1*::*kan recA4155,4136*::*gfp-901* (37). This is abbreviated to *recA4155,4136* in the table. *recAo1403* is an operator mutation that increases the basal or non-SOS-induced level of transcription twofold (49). *ygaD* is the open reading frame in front of the *recA* gene. It has no known function. *gfp-901* refers to *mut-2* (9) with the additional "monomeric" mutation A206T (52). *recA4155* is a mutant allele of *recA* with an arginine-to-alanine change at codon 28. It does not make storage structures in vivo (37). *recA4136* refers to the specific fusion of *recA* to *gfp*.

[c] The *100* allele (from the Keio collection [3]) essentially replaces the gene in question with a *kan* gene between two FRT sites. The *200* allele is a result of removing the *kan* gene with FLP recombinase, induced by isopropyl-β-D-thiogalactopyranoside (IPTG) and expressed from the chloramphenicol resistance plasmid pLH29 (2). The strain was then grown in the presence of IPTG, and Kan^s Cam^s strains were screened.

[d] Select for tetracycline resistance. Screen by PCR if necessary.

[e] Select for chloramphenicol resistance. Screen by PCR if necessary.

[f] Select for kanamycin resistance. Screen by PCR if necessary.

[g] Select for methionine auxotrophy. Screen by PCR if necessary.

[h] We have sequenced the insertion-deletion mutation originally called del(*uvrD-yigB*)::*cam* in reference 16. We find that the mutation is actually a deletion-insertion mutation of *uvrD* and *yigE*. The endpoints of the deletion are GAAGAAGACGAAG in *uvrD* and CAGGCAAAATCATA in *yigE*. The last sequence in the *uvrD* gene is GAAGAAGACGAAG and the first sequence in the *yigE* gene is CAGGCAAAATCATA (all sequences between those two are deleted). The *cat* gene from pACYC184 (inclusive of the sequences starting with CCAAGCTCGAATT and ending with AAGTTGGAACCT) are in place of the *uvrD* and *yigE* sequences.

Given the hyperhelicase activity of UvrD303 in vitro, it is possible that more RecA removal from DNA and more-efficient MMR activity account for the Rec$^-$ and antimutator phenotypes, respectively, of *uvrD303* mutants. The reason for the UV^s phenotype of these cells, however, is not clear and can be explained by at least two models. One model predicts that despite its hyperhelicase activity, UvrD303 is deficient in NER, perhaps due to failure to interact with other essential upstream proteins in the repair pathway (i.e., UvrB) (1). Alternatively, a second model predicts that through its hyperhelicase activity, UvrD303 inappropriately removes RecA filaments that are required for initiating the SOS response or for recombinational repair of UV-induced DNA damage, or both. One goal of this work was to determine which, if either, of these two models would explain the UV^s phenotype of *uvrD303* mutants.

In this study, the *uvrD303* allele was transferred from a plasmid, where it had been studied previously, to the chromosome in order to avoid copy number effects. The results show that chromosomally encoded *uvrD303* is still Rec$^-$, an antimutator, and UV^s. *uvrD303* is epistatic with *recA* and additive with *uvrA* for the UV^s phenotype, suggesting a defect in RecA-dependent repair rather than in NER. The *uvrD303* mutant is modestly defective in induction of the SOS response after UV treatment, and its UV sensitivity can be suppressed by introducing a *lexA* null mutation. It is shown that *uvrD303* is able to fully suppress the constitutive SOS expression of *recA730* cells. Both of the SOS-antagonistic phenotypes of *uvrD303* mutants are dependent on the protein's 40 C-terminal amino acids.

MATERIALS AND METHODS

Strains and media. All bacterial strains are derivatives of *E. coli* K-12 and are described in Table 1. The protocol for P1 transduction has been described previously (50). All P1 transductants were selected on 2% agar plates; the agar was made with either Luria broth (LB) or 56/2 minimal medium (50) supplemented with 0.2% glucose, 0.001% thiamine, and specified amino acids. The antibiotic used for selection was either kanamycin (50 μg/ml), chloramphenicol (25 μg/ml), or tetracycline (10 μg/ml). All transductants were grown at 37°C and purified on the same type of medium on which they were selected. Assays for conjugal recombination and UV survival have been described elsewhere (8, 41).

Plasmid constructions and transfer of alleles to the chromosome. All DNA-modifying enzymes used (restriction endonucleases, DNA polymerase I (Pol I) Klenow fragment, T4 DNA ligase) were purchased from New England Biolabs and used according to the manufacturer's recommendations.

To construct pRC19, pGZK20 (53) was digested with BsiWI and subsequently treated with DNA Pol I Klenow fragment to fill in the overhanging ends. The reaction was then run on a 1% agarose gel, and the linear fragment was extracted and purified using the QIAEX II kit (Qiagen). Purified DNA was then religated using T4 DNA ligase. The ligation reaction mixture was used to transform DH5α competent cells. Plasmid DNA from ampicillin-resistant colonies was isolated and screened for the presence of a unique SnaBI restriction site. One plasmid containing the site was named pRC19, and this plasmid essentially recreates the *uvrD* del(*40C*) mutation, constructed and characterized previously (32). We have given this mutation the allele number *uvrD701*.

To construct plasmid pRC32, both pGZK31 (53) and pSJS1488 (37) were digested with BsiWI. Digestion reactions were run on a 1% agarose gel. The linearized plasmid pGZK31 and the small fragment of pSJS1488 containing the *kan* gene were gel purified using the QIAEX II kit (Qiagen). Purified DNA fragments were then combined in a ligation mixture containing T4 DNA ligase. The ligation reaction mixture was then used to transform DH5α competent cells. Plasmid DNA from kanamycin-resistant colonies was isolated and screened for the proper restriction pattern by digestion with BamHI and SalI. The resulting plasmid was named pRC32. The linear BamHI-SalI fragment of pRC32 containing the *kan* gene was then transferred to the chromosome by transformation of SS4414 competent cells containing plasmid pKD46 (14). Kanamycin-resistant colonies were purified and screened for ampicillin sensitivity, indicating loss of pKD46. Genomic DNA was then amplified by PCR using primers prSJS708 (5′-TAACAAGCCGCATATCCTGC) and prSJS709 (5′-CGTCGATTAATTC CATAAATCGCTGC) (Sigma). The resulting PCR product was screened for the presence of a BglI site, indicating that the D403A and D404A mutations were on the chromosome. The resulting strain was called SS5141 and contained the *uvrD303* mutations plus a *kan* insertion in the BsiWI site of the *uvrD* gene. We have given this *kan* insertion the allele number *uvrD705*.

pRC33 was generated by digesting pRC19 with BstBI and subsequently treating with DNA Pol I Klenow fragment. pSJS1488 (37) was digested with BsiWI and subsequently treated with DNA Pol I Klenow fragment. DNA fragments were run on a 1% agarose gel. The linear fragment of pRC19 and the small fragment of pSJS1488 containing the *kan* gene were isolated and purified using the QIAEX II kit (Qiagen). Purified DNA fragments were combined in a ligation mixture with T4 DNA ligase. The ligation mixture was used to transform DH5α competent cells. Plasmid DNA from kanamycin-resistant colonies was purified and screened for the proper size by restriction analysis with BamHI and SalI.

One correct plasmid was called pRC33. The linear BamHI-SalI fragment of pRC33 containing the *kan* gene was then transferred to the chromosome by transformation of SS4414 competent cells containing plasmid pKD46 (14). Kanamycin-resistant colonies were purified and screened for ampicillin sensitivity, indicating loss of pKD46. Genomic DNA was then amplified by PCR using primers prSJS714 (5′-GCGCCAGTTCAGCTACAACG) and prSJS716 (5′-GCTCACCGCTGCCTTCCATATTG) (Sigma). The resulting PCR product was screened for the presence of a SnaBI site, indicating that the *uvrD701* mutation was on the chromosome. The resulting strain was called SS5145 and contained the *uvrD701* mutation plus a *kan* insertion in the BstBI site of the *uvrD* gene. This *kan* insertion is given the allele number *uvrD706*.

pRC35 was constructed by digesting both pRC19 and pKO3 (27) with BamHI and SalI. The digested DNA was then run on a 1% gel. The small fragment of pRC35 containing the *uvrD701* mutation and the large linear pKO3 fragments were isolated and purified using the QIAEX II kit (Qiagen). These fragments were then combined in a ligation mixture with T4 DNA ligase. The ligation reaction mixture was then used to transform SS5191 competent cells. Plasmid DNA from chloramphenicol-resistant colonies was isolated, and the resulting plasmid was named pRC35. This plasmid was then used to remove the *kan* gene from the chromosome of this strain (SS5402) by the pKO3-mediated gene replacement method (27). The resulting Kan^s Cam^s strain was called SS5428 and had the *uvrD701* allele on the chromosome, as verified by PCR with prSJS714 and prSJS716 and subsequent digestion with SnaBI.

pRC40 was constructed by digesting pGZK31 (53) and pKO3 (27) with BamHI and SalI. Digestion reactions were run on a 1% agarose gel. The ~1.7-kb BamHI-SalI fragment of pGZK31 and the large fragment of pKO3 were gel purified using the QIAEX II kit (Qiagen). Purified DNA fragments were then combined in a ligation mixture containing T4 DNA ligase. The ligation reaction mixture was then used to transform DH5α competent cells. Plasmid DNA from chloramphenicol-resistant colonies was isolated and screened for the proper restriction pattern by digestion with BglI or SacII. The resulting plasmid was named pRC40. This plasmid was then transformed into SS5482 and used to remove the *kan* gene from the chromosome of this strain by the pKO3-mediated gene replacement method (27). The resulting Kan^s Cam^s strain was called SS5496 and had the *uvrD303* allele on the chromosome, as verified by PCR with prSJS708 and prSJS709 and subsequent digestion with BglI.

pRC49 was generated by digesting pRC40 (containing the *uvrD303* mutations) with BsiWI and subsequently treating with DNA Pol I Klenow fragment. The linear fragment was run on a 1% gel, isolated, and purified using the QIAquick gel purification kit (Qiagen). The purified DNA was then treated with T4 DNA ligase for religation. This ligation mixture was used to transform DH5α competent cells. Plasmid DNA from chloramphenicol-resistant clones was screened for the generation of a new SnaBI site, indicating the presence of the *uvrD701* mutation. One correct plasmid was called pRC49 and contained the *uvrD303,701* mutations. To transfer these mutations to the chromosome, pRC49 was used to transform SS5482 competent cells, and the pKO3-mediated gene replacement method was performed as described above. The resulting Kan^s Cam^s strain was called SS5986 and had *uvrD303,701* on the chromosome, as verified by the presence of a BglI or SnaBI site after PCR using prSJS708 and prSJS709 or prSJS714 and prSJS716, respectively.

Conjugal recombination assays. Experiments were performed essentially as described previously (4). Briefly, donor (JJC145) and recipient strains were grown to mid-exponential phase in LB medium at 37°C with aeration. They were then diluted to an optical density at 600 nm of 0.1, and the donor was allowed to grow for an additional 20 min before 0.2 ml was aliquoted into a tube for each mating reaction. These tubes were placed at 37°C with no shaking to allow growth of pili for 30 min. At this point, 1.8 ml of the donor was added, and mating reactions proceeded for 20 min with no shaking. Reactions were stopped by vortexing vigorously for 1 min and placing tubes on ice. Serial dilutions were made in 56/2 buffer, and 0.1 ml of dilutions was combined with 0.7% top agar and poured onto minimal agarose plates lacking histidine and supplemented with streptomycin (0.1 mg/ml). Recombination efficiencies are reported as the percentage of his^+ recombinants per 100 donors.

Spontaneous mutation frequency assays. Experiments were performed as described previously (18). Results are averages for at least three experiments conducted on three different days.

Preparation of cells for microscopy. Experiments were performed essentially as described previously (31, 37). Briefly, for RecA4155-GFP experiments, cells were grown to mid-log phase in 56/2 glucose minimal medium and then concentrated 10-fold, and 3 μl was placed on a 1% agarose (dissolved in growth medium) pad on a quartz microscope slide. A coverslip was then placed on top of the cells. Images (phase-contrast and fluorescence) were taken before exposure to UV light (zero time point). Then cells were exposed to 10 J/m^2 while on the quartz slide, and the same field of view was photographed again 20 min after exposure. At least six different fields of view (three on two different days) for each time point and strain were analyzed. For SOS experiments, cells were grown to mid-log phase in minimal medium, transferred to a sterile petri dish, and either irradiated or mock irradiated with 5 J/m^2 UV. Cells were then diluted 1:1 in fresh medium and allowed to grow for 1 h with shaking at 37°C. At this point, cells were concentrated 10-fold; 1 μl of internal reference beads (InSpeck Green [505/515] Microscope Image Intensity Calibration kit, 2.5 μm; catalog no. I-7219; Molecular Probes/Invitrogen) was added, and 3 μl of the mixture was added to an agarose pad on a microscope slide. The data shown in this report represent averages of nine fields of view (three on three different days) for each condition and strain. Typically between 1,000 and 3,000 cells are counted.

Microscopy and processing of images. Microscopy and image processing were performed as previously described (31, 37).

RESULTS

***uvrD303* mutants are UV sensitive, recombination deficient, and antimutable.** The *uvrD303* allele was transferred to the *E. coli* chromosome and tested for its ability to participate in NER, homologous recombination, and MMR. Figure 1A shows that the *uvrD303* mutant displayed decreased survival compared to that of the wild-type strain after UV exposure. The mutant was not quite as UV sensitive as shown previously when *uvrD303* was expressed from a plasmid (53). Table 2 shows that the *uvrD303* mutation resulted in about a twofold decrease in conjugal recombination frequency compared to that of $uvrD^+$ cells. Again, this recombination deficiency is slightly milder than that reported previously when *uvrD303* was expressed from a plasmid. By use of a different recombination test, where the formation of Lac^+ papillae is dependent on intrachromosomal recombination, the *uvrD303* cells fail to form papillae (Fig. 2) (54). Lastly, Table 2 shows that the *uvrD303* mutant has a twofold decrease in spontaneous mutation frequency compared to that of the wild-type strain. Except for the magnitudes of the UV survival and conjugational deficiencies, the *uvrD303* allele imparted a similar phenotype whether expressed from a plasmid or the chromosome. It should be noted that in each of these experiments, a *uvrD* deletion strain has a phenotype different from that of the *uvrD303* mutant (Fig. 1 and 2; Tables 2 and 3). It is much more UV sensitive; it has a hyperrecombination rather than a hyporecombination phenotype; and it gives rise to a mutator instead of an antimutator phenotype.

***uvrD303* mutants are defective in RecA-dependent repair (not NER) after UV treatment.** As mentioned above, UvrD functions in both homologous recombination and NER. Both processes contribute independently to survival after UV irradiation. An epistasis analysis was performed to test which of these two processes is affected in the *uvrD303* mutant. It is known that mutations in the *recA* and *uvrA* genes confer sensitivity to UV light and that a *recA uvrA* double mutant displays an additive sensitivity because both repair pathways are affected (8, 21, 34). To test whether *uvrD303* mutations are defective in RecA-dependent repair or in NER, the *recA uvrD303* and *uvrA uvrD303* double mutants were made. If *uvrD303* is defective in RecA-dependent repair, the *recA uvrD303* double mutant should be no more sensitive to UV light than the *recA* single mutant, but the *uvrA uvrD303* double mutant should show an additive sensitivity compared to the *uvrA* single mutant. If *uvrD303* is defective in NER, then the opposite scenarios should be true: the *recA uvrD303* double mutant should

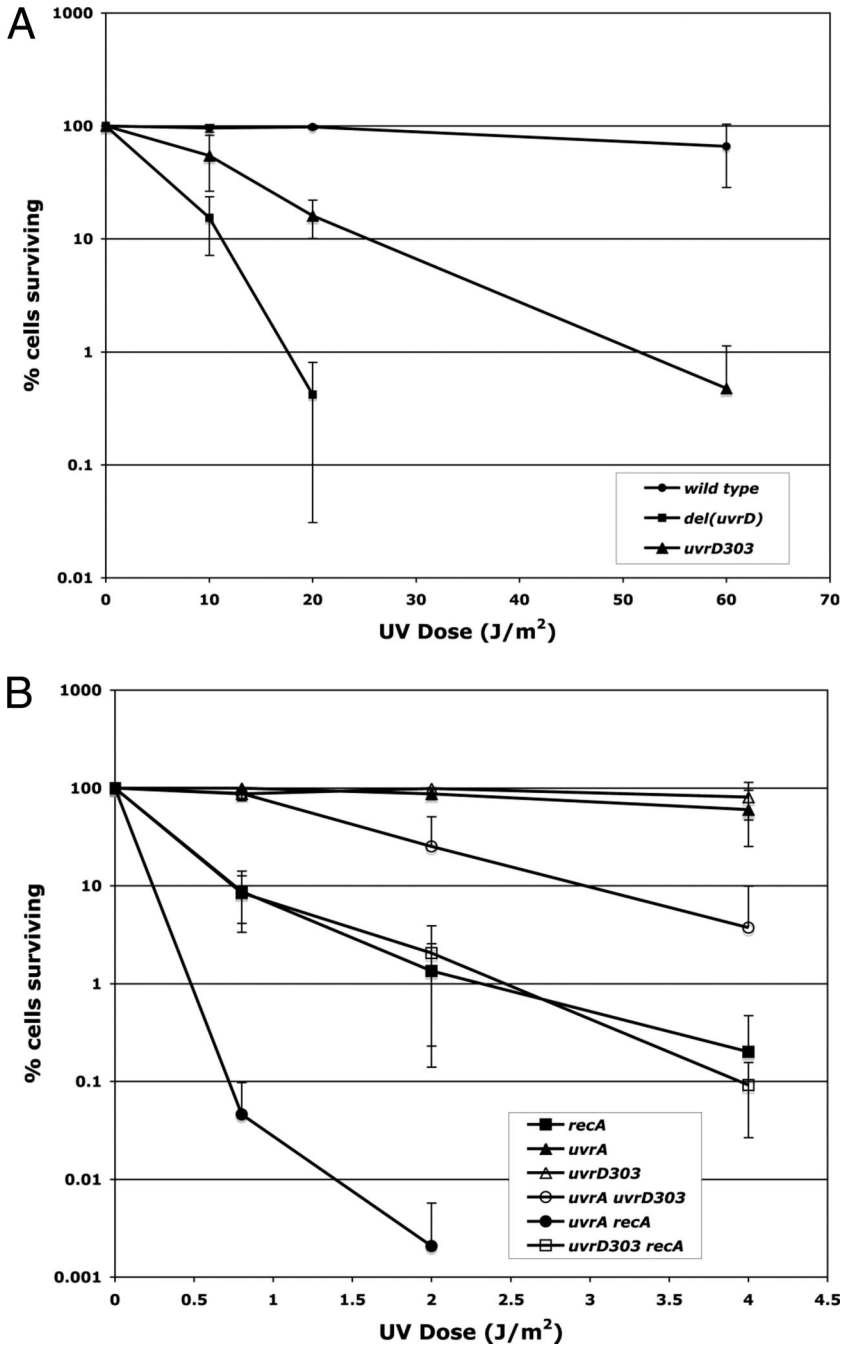

FIG. 1. *uvrD303* cells are defective in RecA-dependent repair (not NER) in response to UV treatment. (A) Circles, wild type (JC13509); squares, del(*uvrD-yigE*)::*cat* (SS3368); triangles, *uvrD303* (SS5704). Survival was measured for untreated cells and at doses of 10, 20, and 60 J/m^2 UV light. (B) Filled squares, del(*recA-srl*)*306*::*Tn10* (JC19328); filled triangles, del(*uvrA*)*100*::*kan* (SS5766); open triangles, *uvrD303* (SS5704); open circles, del(*uvrA*)*100*::*kan uvrD303* (SS5764); filled circles, del(*uvrA*)*100*::*kan* del(*recA-srl*)*306*::Tn*10* (SS5769); open squares, del(*recA-srl*)*306*::Tn*10 uvrD303* (SS5756). Survival was measured for untreated cells and at doses of 0.8, 2, and 4 J/m^2 UV light. Each data point represents the average for at least three independent experiments, and error bars represent standard deviations.

show an additive phenotype, while the *uvrA uvrD303* double mutant should show no more sensitivity to UV light than the *uvrA* single mutant. Figure 1B shows that the *recA uvrD303* double mutant displayed no greater sensitivity to UV light than the *recA* single mutant. The *uvrA uvrD303* double mutant, on the other hand, exhibited an additive phenotype in that it was more sensitive to UV light than either single mutant. These results suggest that *uvrD303* is not defective in NER but rather displays negative effects on RecA-dependent repair of UV-induced damage.

***uvrD303* mutants are defective in SOS induction after exposure to UV light.** While the results of the epistasis experiments

TABLE 2. Conjugal recombination abilities and spontaneous mutation frequencies of some *uvrD* mutants used in this study

Strain	*uvrD*	Conjugal recombination		Spontaneous mutation	
		Avg proficiency per 100 donors	Ratio[a]	Avg frequency	Ratio[a]
JC13509	+	3.58 ± 2.02	1.00	$4.09 \times 10^{-8} \pm 2.92 \times 10^{-8}$	1.00
SS5993	*294*	7.23 ± 2.17	2.02	ND[b]	ND
SS3368	*cat*	ND	ND	$2.01 \times 10^{-6} \pm 8.27 \times 10^{-7}$	49.20
SS5704	*303*	2.02 ± 0.62	0.56	$2.78 \times 10^{-8} \pm 1.82 \times 10^{-8}$	0.68
SS5450	*701*	4.48 ± 3.08	1.25	$5.72 \times 10^{-8} \pm 6.23 \times 10^{-8}$	1.40
SS5987	*303*, *701*	2.68 ± 1.05	0.75	$5.91 \times 10^{-8} \pm 1.51 \times 10^{-8}$	1.44

[a] The ratio of the conjugal recombination proficiency or spontaneous mutation frequency of each strain to that of the wild type is shown.
[b] ND, not determined.

(Fig. 1B) suggested that *uvrD303* negatively affects the *recA* pathway of UV repair, other experiments showed that RecA-GFP focus formation in response to UV treatment was unperturbed in the *uvrD303* mutant relative to *uvrD*$^+$ cells (data not shown). This suggested that the UV^s phenotype of *uvrD303* cells was not directly due to a decreased number of RecA filaments. Since RecA has multiple functions during the repair of UV-induced damage, including catalyzing homologous recombination, inducing the SOS response, and protecting ssDNA at stalled replication forks (10, 28), it is possible that while the RecA filament may still form, one or more of its functions may be decreased in the *uvrD303* background. To test whether the ability of cells to turn on the SOS response to UV-induced damage was affected in the *uvrD303* mutant, SOS expression was measured using fluorescence microscopy of cells expressing *gfp* under the regulation of the *sulA* promoter (31). Table 3 shows that 60 min after exposure to 5 J/m^2 of UV radiation, SOS expression was slightly reduced in the *uvrD303* mutant from that in the wild-type strain. While the wild-type strain had an average relative fluorescence intensity (RI) of about 21, with 99% of cells induced (having an average RI sixfold or more higher than the average RI of the untreated wild-type strain), the *uvrD303* mutant had an average RI of about 12, with a significantly ($P < 0.03$ by Student's t test) lower percentage (80%) of cells induced (Table 3). *uvrD*-deleted cells showed higher basal SOS expression and a robust increase after UV treatment to an average RI of 48, with 99% of cells induced after UV treatment. A broadening of the distribution of cells expressing *gfp* was also seen for the *uvrD*-deleted strain after UV treatment (see Fig. S1 in the supplemental material). The reason for this is currently unclear but may have to do with molecular noise associated with variations in gene expression (15, 35). Taken together, these results suggest that UvrD303 may prevent full SOS induction after UV treatment without disrupting RecA filament formation.

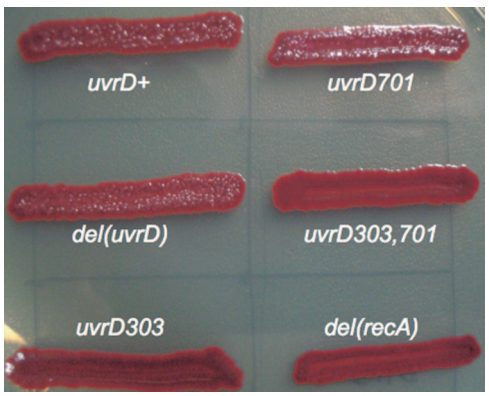

FIG. 2. Qualitative analysis of the abilities of various mutants to perform intrachromosomal recombination. Cells were streaked onto LB plates containing lactose and the indicator dye 2,3,5-triphenyltetrazolium chloride (TTC) and were incubated at 37°C for 48 h. The ability to form *lac*$^+$ (white) papillae depends on intrachromosomal recombination events (54). Strains are as follows: JC13509 (*uvrD*$^+$), SS3368 [del(*uvrD-yigE*)::*cat*], SS5704 (*uvrD303*), SS5450 (*uvrD701*), SS5987 (*uvrD303*,*701*), and SS5987 [del(*recA*)*200*::*frt*].

Constitutive activation of the SOS response restores UV resistance to *uvrD303* cells in a manner that is independent of *recA* expression. To further test whether the *uvrD303* strain's modest effect on SOS expression was the cause of its sensitivity to UV exposure, a *lexA*::Tn*5* mutation was introduced. Cells defective in the LexA transcriptional repressor constitutively express the SOS genes (17). Therefore, if lack of full SOS expression is responsible for the UV^s phenotype of *uvrD303* cells, introduction of the *lexA*::Tn*5* allele should suppress the UV sensitivity of these cells. As seen in Fig. 3, the *lexA*::Tn*5* *uvrD303* double mutant became nearly as resistant to UV light as the *lexA*::Tn*5* single mutant.

One possible explanation for this result was that UvrD303 was preferentially removing RecA from the DNA and that increasing the concentration of RecA with a *lexA*::Tn*5* mutation would shift the equilibrium back to the bound state. To test this idea, a *recAo281 uvrD303* double mutant was constructed. The *recAo281* allele contains a mutation in the operator of *recA* that prevents binding of LexA, resulting in constitutive *recA* overexpression (48). It is seen in Fig. 3 that overexpression of *recA* via the *recAo281* allele did not suppress the UV sensitivity of the *uvrD303* strain.

These results suggest that the UV-sensitive phenotype of the *uvrD303* strain stems from the inability to fully induce the SOS response, although this does not seem to be due to a limitation of available RecA molecules.

***uvrD303* suppresses the constitutive SOS phenotype of *recA730* but not that of *recA4142* mutants.** *recA730* (E38K) mutants constitutively express SOS genes in the absence of DNA damage (25, 51). Since *uvrD303* was shown to have a negative effect on the cell's ability to induce SOS expression after UV treatment, we tested if *uvrD303* would suppress the high levels of SOS expression conferred by the *recA730* mutation. For this purpose, these two mutations were combined into a single strain containing the SOS reporter construct

TABLE 3. UvrD303 prevents complete SOS expression post-UV irradiation through a mechanism that requires its C terminus[a]

Strain	*uvrD*	Before UV irradiation		After UV irradiation	
		Avg RI	% Induction[b]	Avg RI	% Induction
SS996	+	1.00	1.2 ± 1.0	20.8 ± 7.8	98.7 ± 1.7
SS5704	*303*	1.2 ± 0.6	0.7 ± 1.5	12.0 ± 4.9	79.8 ± 8.9
SS5450	*701*	2.3 ± 1.0	2.7 ± 5.2	24.2 ± 9.1	98.6 ± 1.0
SS5987	*303*, *701*	2.9 ± 1.7	4.9 ± 10.2	31.6 ± 13.1	99.1 ± 1.9
SS3368	*cat*	3.9 ± 1.1	8.8 ± 5.7	48.4 ± 21.8	99.0 ± 0.5

[a] Cells were either left untreated or treated with 5 J/m^2 UV light. See Materials and Methods for further details. All values are given with standard deviations.
[b] Percentage of cells with an RI sixfold or more greater than the average RI of the untreated wild-type strain.

TABLE 4. *uvrD303* suppresses the constitutive SOS phenotype of *recA730* cells by a mechanism that is dependent on the UvrD303 C terminus

Strain	*uvrD*	*recA*	Avg RI ±SD	% Constitutive[a] ± SD
SS996	+	+	1.00	0.3 ± 0.5
SS5704	*303*	+	1.2 ± 0.6	0.7 ± 1.5
SS4629	+	*730*	39.2 ± 3.3	93.7 ± 11.0
SS5801	*303*	*730*	1.1 ± 0.1	0.6 ± 0.3
SS5990	*701*	*730*	43.8 ± 2.1	99.1 ± 0.8
SS5989	*303*, *701*	*730*	43.6 ± 2.5	99.2 ± 0.5
SS4976	+	*4142*[b]	17.1 ± 2.4	99.3 ± 0.9
SS5809	*303*	*4142*[b]	17.5 ± 3.8	98.4 ± 1.0

[a] Percentage of cells with an RI sixfold or more greater than the average RI of the wild-type strain (± standard deviation).
[b] All strains containing *recA4142* also have the *recAo1403* operator mutation, which increases the percentage of the population that displays constitutive SOS activity from about 15% to nearly all of the cells (27a).

sulAp-gfp (31). Table 4 shows that 95% of *recA730 uvrD*$^+$ cells were fully induced for SOS expression in a population of exponentially growing cells and that the addition of *uvrD303* markedly decreased the average SOS expression across the population to near-wild-type levels (<1% cells induced >6-fold). Some specificity seemed to be associated with this suppression, however, because *uvrD303* had no effect on the constitutive SOS phenotype of a different *recA* constitutive allele, *recA4142* (F217Y) (45) (Table 4). Potential reasons for this specificity are discussed below.

The C terminus of UvrD303 is required for its antagonistic effects on SOS expression. A C-terminal peptide of the *S. cerevisiae* homolog of UvrD, Srs2, binds tightly to Rad51 (24). Additionally, Srs2 is known to remove Rad51 from the DNA (47). For these reasons, it was of interest to see if the 40 C-terminal amino acids were also important for the ability of UvrD303 to remove RecA730 from the DNA. The 40 C-terminal amino acids had previously been shown to play an essential role in the dimerization of UvrD monomers (32). Although that study, using multiple-turnover kinetics, suggested that these residues were dispensable for the helicase activity of UvrD in vitro (32), a later study, using single-turnover kinetic experiments, contradicted this report and suggested that dimeric UvrD is required for active dsDNA unwinding (29). A third study proposed two possible mechanisms for dsDNA unwinding by UvrD and suggested

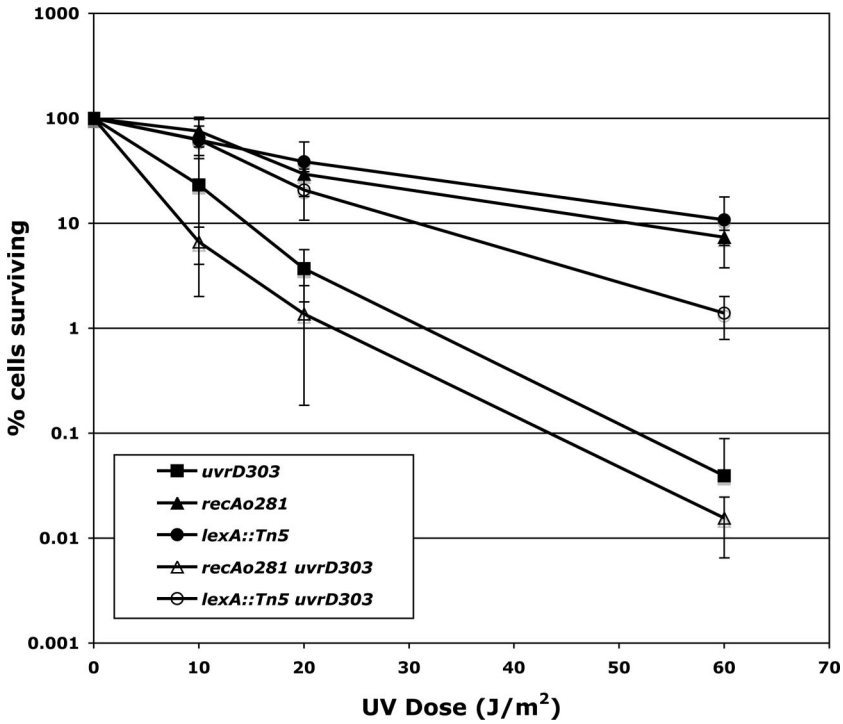

FIG. 3. Constitutive activation of the SOS response, but not overexpression of RecA, restores UV resistance to *uvrD303* cells. Filled squares, *uvrD303* (SS5704); filled triangles, *recAo281* (SS5962); filled circles, *lexA*::Tn*5* (SS5959); open triangles, *recAo281 uvrD303* (SS5963); open circles, *lexA*::Tn*5 uvrD303* (SS5960). Survival was measured for untreated cells and at doses of 10, 20, and 60 J/m^2 UV light. Each data point represents the average for at least three independent experiments. Error bars represent standard deviations.

that multiple monomers (rather than a dimer) may be required for the unwinding of certain substrates (26). Thus, while the role of dimerization in UvrD activity appears to be controversial, it is clear that dimerization depends on the C terminus. Additionally, while UvrD can prevent the formation of recombination intermediates and dissociate RecA from ssDNA in vitro, the highly homologous (40% identical) Rep protein cannot (19, 46). Intriguingly, UvrD is slightly larger than Rep, and alignments between the two show that the 40 C-terminal amino acids of UvrD are not present in Rep (19).

To test whether the 40 C-terminal amino acids of UvrD303 were required for its antagonistic effects on SOS expression, the *uvrD* del(*40C*) mutant (referred to below as the *uvrD701* mutant) was constructed and transferred to the chromosome in place of the endogenous *uvrD* gene. Tables 2 and 3 and Fig. 2 show that *uvrD701* mutants behave very similarly to the wild type. To test whether the C terminus of UvrD303 was important for its antagonistic effects on SOS, the *uvrD303* allele was combined with the *uvrD701* allele and characterized. Table 2 reveals that the *uvrD303*,*701* allele is like the *uvrD303* mutant in that it is slightly Rec^- as measured by conjugal recombination. It appeared more recombination deficient when the Lac^+ papilla recombination test was applied (Fig. 2). The double mutant also showed a slight increase in mutability relative to the wild type (Table 2). However, when the *uvrD303,701* allele was combined in a strain with the SOS reporter construct *sulAp-gfp* and SOS expression was monitored before and after UV treatment, *uvrD303,701* cells, unlike those of the *uvrD303* single mutant, had an induced SOS response 60 min after 5-J/m^2 UV treatment that was intermediate between those for the wild type and the *uvrD* deletion mutant (Table 3). The distribution of cells with binned RIs showed a broadening similar to that seen for a *uvrD* deletion mutant (see Fig. S1 in the supplemental material). The effects on the constitutive SOS expression of the *recA730* strain were also examined in the absence of the C terminus of UvrD303. Table 4 shows that the addition of *uvrD303,701* completely restored the constitutive SOS phenotype of *recA730* cells. The *uvrD701* mutation alone had no effect on the *recA730* phenotype (Table 4).

These results suggest that the C terminus of UvrD303 is required for its antagonistic effects on SOS induction by $recA^+$ cells after UV treatment and by *recA730* cells under normal growth conditions.

DISCUSSION

With some small quantitative differences, a chromosomal copy of *uvrD303* shows recombination-deficient, antimutator, and UV-sensitive phenotypes similar to those shown by *uvrD303* when expressed from a multicopy plasmid (53). This report reveals that the UV sensitivity is likely due to the ability of UvrD303 to interfere with RecA activity and is not due to a defect in NER. This conclusion is further supported by the ability of *uvrD303* to suppress the constitutive SOS phenotype of *recA730* cells and to interfere with normal SOS induction after UV treatment. Although the UV sensitivity of *uvrD303* cells can be suppressed by constitutively activating SOS genes with the *lexA*::Tn*5* mutation, overexpression of at least one SOS-regulated gene, *recA*, is not sufficient to achieve this. This suggests that some other SOS-regulated gene(s) is necessary for suppression. Future work will be necessary to determine which SOS genes are involved. Finally, the two anti-SOS phenotypes are dependent on the presence of the C terminus of UvrD303.

It had been proposed previously that UvrD is able to remove RecA under certain circumstances, as described above. The results presented here suggest that like UvrD, UvrD303 is able to remove RecA from DNA. This allele can do so, however, under a wider set of conditions and/or to a greater extent than the wild type. This ability seems to be specific, because UvrD303 can decrease the constitutive SOS phenotype of *recA730* but has no effect on a different *recA* constitutive SOS allele, *recA4142*. It has been proposed elsewhere that RecA730 binds at replication forks and that RecA4142 binds at dsDNA breaks to induce the SOS response in log-phase cells (27a). Thus, the difference in the ability of UvrD303 to suppress high levels of SOS constitutive expression may be due to the environment in which the RecA resides. It should be pointed out that the ability of UvrD303 to interfere with RecA in some situations is not absolute; UvrD303 decreases RecA activity but does not remove it altogether. For instance, levels of UV resistance and UV-induced SOS expression are much higher in a *uvrD303* strain than in a *recA* deletion strain (37). It is possible that other proteins, such as DinI and RecX, which affect the stability of RecA filaments, may modify the ability of UvrD303 to affect RecA activity in the cell. Future experiments may test this hypothesis.

Previous work has shown that the action of UvrD on RecA filaments occurs during situations where RecA has been loaded in a RecFORJQ-dependent manner, as at a replication fork (6, 16). Additional support for UvrD at replication forks comes from studies of yeast Srs2. This protein removes Rad51 after recruitment to replication forks by a SUMO-modified PCNA processivity clamp during the S phase of the cell cycle (36). While the evidence provided here suggests that the UvrD303 mutant does not completely remove RecA filaments at UV-damaged replication forks, it is likely recruited to these forks and prevents full RecA activity. Given its ability for generating anti-SOS phenotypes (Tables 3 and 4) and the fact that UV induction of the SOS response requires DNA replication (42), it is possible that the C terminus of UvrD303 (or that of wild-type UvrD) may physically interact with RecA or another protein to manifest its effect on RecA activity. Given the observation that the C terminus is also critical for UvrD to form a dimer in vitro (see above), it is possible that a dimeric form of UvrD303 is the functional entity required for these phenotypes in vivo (29).

It is possible that the restoration of high SOS expression observed in the *uvrD303*,*701* mutant post-UV treatment and the *recA730* phenotype (Tables 3 and 4) in the absence of UV irradiation are due to the instability of the double-mutant protein. This would suggest that the *uvrD303*,*701* mutant should have the same phenotypes as the *uvrD*-null mutant. It is seen, however, that the mutability of the double mutant is like that of the wild type (Table 2) and that the *uvrD303*,*701* mutant, like the *uvrD303* mutant, is deficient in the Lac^+ papilla test (Fig. 2). In both cases, the phenotypes are not similar to what one would predict for a partially stable UvrD protein or for the absence of the UvrD protein. Taken

together, these results suggest that *uvrD303,701* folds into an active protein and that this protein is stable enough to produce the phenotypes described above.

How UvrD (or UvrD303) removes RecA at replication forks is still unknown. While it is tempting to speculate that a specific interaction between the C terminus of UvrD and RecA may serve this function, there is no evidence to support this model. Additional experiments will be needed to reveal how the cell determines which RecA filaments are appropriate for UvrD-dependent removal and when this should occur.

This work reveals a new layer of how RecA participates in recombination and regulates the SOS response. While the details of how these processes are initiated (RecA loading) are well understood, less is known about how these processes are "turned off" or completed (dissociation of RecA from the DNA). Previous work has categorized UvrD as an enzyme with involvement in NER and MMR and with a role as an antirecombinase during replication fork arrest (see above). Here it is shown that UvrD303 can counteract the ability of certain RecA filaments to induce the SOS response and that its C terminus may be important for this role. It is intriguing to speculate that UvrD may also play an important role in ending recombination and turning off the SOS response by actively removing RecA filaments from the DNA. Further experiments will be required to test this idea.

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