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UvrD Limits the Number and Intensities of RecA-Green Fluorescent Protein Structures in *Escherichia coli* K-12▽†

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RecA is important for recombination, DNA repair, and SOS induction. In *Escherichia coli*, RecBCD, RecFOR, and RecJQ prepare DNA substrates onto which RecA binds. UvrD is a 3′-to-5′ helicase that participates in methyl-directed mismatch repair and nucleotide excision repair. *uvrD* deletion mutants are sensitive to UV irradiation, hypermutable, and hyper-rec. In vitro, UvrD can dissociate RecA from single-stranded DNA. Other experiments suggest that UvrD removes RecA from DNA where it promotes unproductive reactions. To test if UvrD limits the number and/or the size of RecA-DNA structures in vivo, an *uvrD* mutation was combined with *recA-gfp*. This *recA* allele allows the number of RecA structures and the amount of RecA at these structures to be assayed in living cells. *uvrD* mutants show a threefold increase in the number of RecA-GFP foci, and these foci are, on average, nearly twofold higher in relative intensity. The increased number of RecA-green fluorescent protein foci in the *uvrD* mutant is dependent on *recF*, *recO*, *recR*, *recJ*, and *recQ*. The increase in average relative intensity is dependent on *recO* and *recQ*. These data support an in vivo role for UvrD in removing RecA from the DNA.

Inheritance of a bacterial chromosome is a complex process. It demands that the initiation of DNA replication occur at an origin at the proper time during the cell cycle. Once initiated, replication must be carried out quickly and with high fidelity to ensure that each daughter cell receives a complete copy of the genome in a timely manner. It has become apparent, however, that replication forks will stop at various types of DNA damage that are often the result of standard metabolic reactions. In order for genomic integrity to be maintained, the DNA damage must be repaired, and the replication forks must be reconstructed and restarted. Depending on the type of DNA damage and/or obstruction, homologous recombination is generally considered an important cellular tool for fixing these forks and the DNA damage (reviewed in references 6, 14, and 19).

The process of homologous recombination involves many gene products (15). The first step in recombination is tailoring the DNA to liberate regions of single-stranded DNA (ssDNA) onto which RecA can bind. RecBCD and RecFOR protein complexes help RecA load onto DNA at double strand breaks and gapped DNA, respectively (reviewed in references 3 and 13). At replication forks that have stopped at damage induced by UV treatment, RecJ and RecQ have been proposed to provide a different method, one in which RecQ and RecJ cooperate to unwind and degrade the newly synthesized lagging strand and liberate ssDNA for RecA to bind (5). In each case, binding of RecA to the DNA creates a protein-DNA filament. It is this filament that serves to repair the DNA by searching the sister chromosome for a homologous region and then exchanging the strands of DNA.

UvrD was initially found as a mutant sensitive to UV irradiation (21). Also called helicase II, UvrD has 3′-to-5′ helicase activity (17). Roles for UvrD have been established in both nucleotide excision repair (NER) and methyl-directed mismatch repair (MMR) pathways (reviewed in references 11, 26, and 28). In vitro experiments show that MutL can load UvrD onto DNA during MMR (18). Once loaded on the DNA, it is thought that UvrD uses its helicase activity to dissociate a fragment of ssDNA from its complementary strand. *uvrD* mutants are also hyper-rec (1, 8, 30, 37). This phenotype has been thought to be a consequence of incomplete NER or MMR, leading to more nicks and gaps onto which RecA may bind and initiate recombination (1).

A different idea to explain the hyper-rec phenotype of *uvrD* mutants, however, is supported by the studies of Petit and colleagues (23, 30). They suggested that the role of UvrD may be to limit protein-DNA structures made by RecA. This was based on two types of observations. The first was that in *Bacillus subtilis*, mutations in *recF*, *recO*, or *recR* could rescue the lethality of a *pcrA* (an *uvrD* homolog) mutation and that similarly, in *E. coli*, *recF*, rec mutations could rescue the synthetic lethality of *uvrD* and *rep* mutations (23). The second observation was that in vitro, UvrD could both prevent the formation of recombination intermediates and dissociate RecA from existing RecA-ssDNA filaments (20, 30).

Other experiments by Flores and colleagues also support a model by which UvrD removes RecA from DNA. They showed that UvrD is necessary to remove RecA at certain types of stopped replication forks where RecA-mediated recombination is inappropriate (9, 10). Their experiments showed that mutations in the *recFOR*, *recJ*, and *recQ* (*recJQ*) genes having presynaptic roles in the loading of RecA (see above) would

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TABLE 1. Strains used in this work

Strain	Presence or description of gene		Other relevant genotype	Origin or reference
	recA	*uvrD*		
JC13509[a]	+	+		Laboratory stock
JC18825	+	+	*recF4115 tnaA300*::Tn*10*	27
JC18923	+	+	*recJ284*::Tn*10*	Laboratory stock
JJC2457	+	*cat*	del(*uvrD-yigB*)::*cat*	B. Michel
SS2683	*4155,4136*[b]	+	*recF4115 tnaA300*::Tn*10 ygaD1*::*kan*	25
SS2684	*4155,4136*	+	del(*recO*)*6218*::*tet ygaD1*::*kan*	25
SS3047	*4155,4136*	+	*ygaD1*::*kan*	25
SS3085	*4155,4136*	+	*ygaD1*::*kan*	25
SS3150	*4155,4136*	+	*recR252*::Tn*10-9 ygaD1*::*kan*	25
SS3306	*4155,4136*	+	*zfi-3131*::Tn*10 ygaD1*::*kan*	CAG18642 Π SS3085[e]
SS3307	*4155,4136*	*tet*	*ygaD1*::*kan*	SK6786 Π SS3085[e]
SS3317	*4155,4136*	+	del(*recQ*)*6216*::*tet ygaD1*::*kan*	TP640 Π SS3085[e]
SS3368	+	*cat*		JJC2457 Π JC13509[c]
SS3372	*4155,4136*	*cat*	*recF4115 tnaA300*::Tn*10 ygaD1*::*kan*	JJC2457 Π SS2683[c]
SS3373	*4155,4136*	*cat*	del(*recO*) *6218*::*tet ygaD1*::*kan*	JJC2457 Π SS2684[c]
SS3374	*4155,4136*	*cat*	*recR252*::Tn*10-9 ygaD1*::*kan*	JJC2457 Π SS3150[c]
SS3378	*4155,4136*	*cat*	*ygaD1*::*kan*	SS3047 Π SS3368[d]
SS3379	*4155,4136*	*cat*	del(*recQ*)*6216*::*tet ygaD1*::*kan*	TP640 Π SS3378[e]
SS3380	*4155,4136*	*cat*	*recJ284*::Tn*10 ygaD1*::*kan*	JC18923 Π SS3378[e]
SS3394	*4155,4136*	+	*recJ284*::Tn*10 ygaD1*::*kan*	JC18923 Π SS3085[e]
TP640	+	+	del(*recQ*)*6216*::*tet*	A. Poteete
TP641	+	+	*recO6218*::*tet*	24

[a] JC13509 has the following genotype: *sulB103 lacMS286* Φ80dII-*lacBK1 argE3 his-4 thi-1 xyl-5 mtl-1 rpsL31 tsx*. *lacMS286* Φ80dII- *lacBK1* codes for two nonoverlapping deletions of the *lac* operon (12, 36).

[b] The full genotype is *recAo1403 ygaD1*::*kan recA4155,4136*::*gfp-901* (25). 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 by twofold (31). *ygaD* is the open reading frame upstream of the *recA* gene. It has an unknown function. *gfp-901* refers to *mut-2* (4) with the additional "monomeric" mutation A206T (33). *recA4155* is a mutant allele of *recA* with an arginine-to-alanine change at codon 28. It does not make storage structures in vivo (25). *recA4136* refers to the specific fusion of *recA* to *gfp*.

[c] Select for Cat[r] and then screen for other markers phenotypically or by PCR if necessary.

[d] Select for Kan[r] and then screen for other markers phenotypically or by PCR if necessary.

[e] Select for Tet[r] and then screen for other markers phenotypically or by PCR if necessary.

suppress the lethal effects caused by the absence of UvrD in *dnaE*(Ts) and *dnaN*(Ts) mutants (10). These authors proposed that the cell could survive either through the action of UvrD removing RecA from the inappropriate substrate or by mutating genes that code for proteins that load RecA at these substrates.

Previously, we characterized a molecular tool that visualizes the location of RecA in live log-phase cells through the use of a *recA-gfp* translational fusion gene. The formal genotype for this strain is *ygaD1*::*kan recAo1403 recA4136*::*gfp-901* and is explained in detail in the footnote of Table 1. This *recA-gfp* fusion was placed at *recA*'s normal location in the chromosome. It has been shown to be able to recombine, repair DNA, and induce the SOS response at near-wild-type levels (25). In vivo, two types of RecA-GFP structures were identified: those that are on the DNA (about 50% of the structures) and those that are not (the remaining 50%). The latter are presumably storage structures (16, 25, 29). It has been shown that *recA*(*R28A*) is proficient for recombination and DNA repair in vivo but does not make storage structures in vitro (7). When this mutation was transferred to *recA-gfp*, the new allele, called *recA4155-gfp*, was as Rec$^+$ UV^r as *recA-gfp* but had about half as many foci as *recA-gfp*. This suggested that *recA4155-gfp* does not make storage structures in vivo. Analysis shows that 13% of *recA4155-gfp* cells have foci, all of the foci are on the DNA, and the majority of the foci are *recB* dependent. Measurements of the locations of these foci in cells show that they are found at positions where the DNA replication factories are likely to occur (25). It should be noted, however, that while RecA foci could be associated with stopped replication forks, they could also be associated with ssDNA located away from a replication fork. It was additionally shown that the intensities of RecA-GFP foci can vary over a 20-fold range (25).

In this report, we test the model that UvrD removes RecA from DNA in growing log-phase cells by combining an *uvrD* deletion mutation with a *recA4155-gfp* translational fusion gene. The model predicts that *uvrD* mutants should either have on average more RecA-GFP foci (per cell) and/or that the foci should have a higher average relative intensity. This increase should then be dependent on the *recFOR* and *recJQ* genes.

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 (32). All P1 transductions were selected on 2%-agar plates made with either Luria broth or 56/2 minimal medium (32) supplemented with 0.2% glucose, 0.001% thiamine, and specified amino acids. Selection using antibiotics used 50 μg/ml kanamycin, 25 μg/ml chloramphenicol, or 10 μg/ml tetracycline. All transductants were grown at 37°C and purified on the same type of medium on which they were selected. The complete nucleotide sequence for the *recA-gfp* construct is given under GenBank accession number AY994192.

Preparation of cells for microscopy. The cells were grown in 56/2 glucose minimal medium at 37°C with aeration until in log phase. One milliliter of cell culture was then briefly centrifuged and then resuspended in a 1/10 volume of growth medium. Two microliters of cells were then placed on a thin layer of 1% agarose (dissolved in growth medium) on a microscope slide. A coverslip was then placed on the agarose surface.

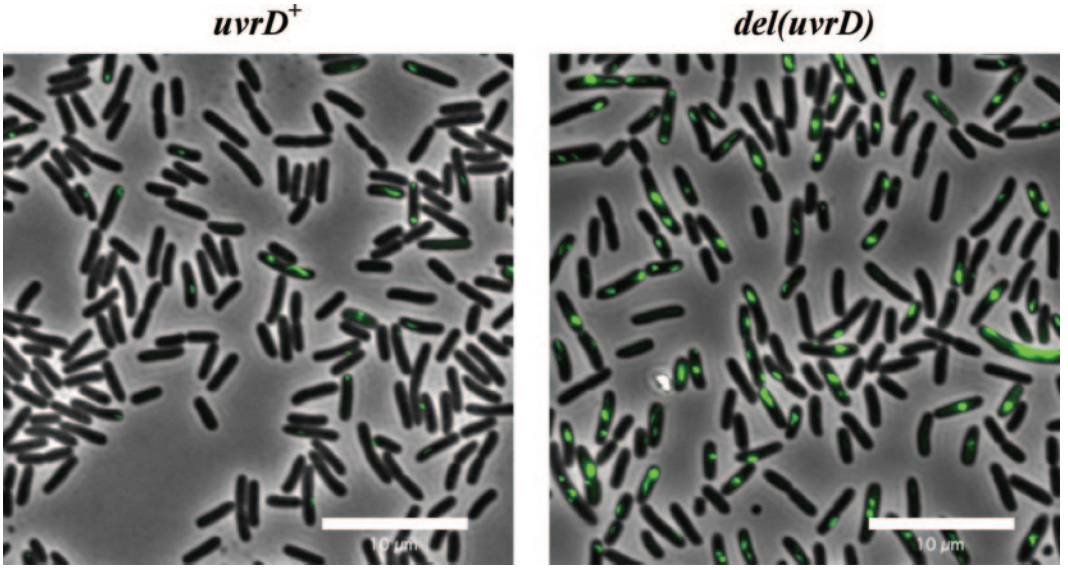

FIG. 1. These images are overlay images of phase-contrast and fluorescent images of live wild-type and *uvrD* mutant cells containing *recA4155-gfp*. Cells were grown to log phase in minimal medium, and the phase-contrast and fluorescent images were taken as explained in Materials and Methods.

Microscopy and processing of images. Cells and foci are visualized by light and fluorescence microscopy, respectively, using a Nikon 600 Eclipse microscope equipped with a *Z*-axis focus drive with an ORCA-ER camera. Shutters and filters on the microscope are automated and controlled by Openlab 5.0 software (Improvision). A no. 86013 fluorescein isothiocyanate filter (Chroma) with excitation and emission maxima of 484 ± 14 and 517 ± 30 nm, respectively, was used. A *Z*-stack of *x-y* planes was taken for each fluorescent image. A typical *Z*-stack comprises 15 to 20 ordered images taken from 2.5 to 3 μm below to 2.5 to 3 μm above the focal plane of the phase-contrast image in 0.3-μm steps. Each *x-y*-plane fluorescent image was taken with a 250-ms exposure using a single neutral density filter. The images were then deconvolved using Volocity 4.0 software (Improvision). Single *x-y* planes were then selected and merged with each other and the phase-contrast image to produce the images shown in Fig. 1 or analyzed. The images were then analyzed for distributions of foci in cells, and total fluorescence of cells and foci using Openlab 5.0 software. The minimal focus is defined as four (2 by 2) adjacent pixels that are all fourfold above the background fluorescence for that cell. Calibration of the fluorescence intensity was set by the internal reference beads (InSpeck Green 505/515 microscope image intensity calibration kit, 2.5 mm, no. I-7219; Molecular Probes) contained within each field analyzed. The average pixel intensities of the foci were determined on deconvolved, merged images. The number of foci per area of cell was determined by measuring the cell area from the phase-contrast image. The total number of foci (determined from the fluorescent image) was then divided by the total area of cells (determined from the phase-contrast image). The intensities of the foci form a somewhat continuous distribution between the relative intensities indicated in the tables. These intensities are grouped into equally spaced bins to facilitate the comparison and statistical analysis used.

RESULTS

***uvrD* mutants have a higher number and average relative intensity of RecA-GFP foci than the wild type.** If UvrD has a role in removing RecA from the DNA in vivo, then *uvrD* mutants should have an increased number and/or increased average relative intensity of RecA-GFP foci compared to the wild type. To test this idea, wild-type and *uvrD* cells containing *recA4155-gfp* were grown in minimal medium into log phase at 37°C. Cells were combined with calibration beads and prepared for microscopy (see Materials and Methods).

Figure 1 shows a sample image of wild-type and *uvrD* mutant strains. Tables 2 and 3 show the distribution of foci within cells in a population and the distribution of relative intensities of the foci, respectively. It is seen that the *uvrD* mutant has about threefold more foci per area of cell than the wild type (Table 2). Table 3 shows that the average relative intensity also increases by nearly twofold (from 0.24 to 0.39). In both cases, the chi-square test for homogeneity for an $r \times c$ contingency table shows that the difference between the wild-type and *uvrD* distributions is highly significant ($a < 0.001$). Similar results were seen for cells grown in Luria broth (data not shown). It is

TABLE 2. Effect of single mutations on distribution of RecA-GFP foci[a]

Strain no. (mutated gene)	% of cells with no. of foci					No. of foci/area[b]	Count[c]	α value[d]
	0	1	2	3	4			
SS3085 (None)	86	11	3	0	0	78	2,072	
SS3378 (*uvrD*)	67	23	8	2	0	228	700	<0.001
SS2683 (*recF*)	91	7	1	1	0	49	873	0.25
SS2684 (*recO*)	90	8	2	0	0	64	594	0.7
SS3150 (*recR*)	89	8	2	1	0	65	796	0.6
SS3394 (*recJ*)	90	7	2	0	0	70	1,373	0.5
SS3317 (*recQ*)	88	9	2	1	0	85	1,369	0.8

[a] All strains contain *recA4155-gfp* and were grown in minimal medium. These strains contain the del(*uvrD-yigB*)::*cat*, *recF4115*, del(*recO*)*6218*::*tet*, *recR252*::Tn*10-9*, *recJ284*::Tn*10*, and del(*recQ*)*6216*::*tet* alleles, respectively, and their derivations are described in Table 1. The values for *recF* (SS2683), *recO* (SS2684), and *recR* (SS3150) have been previously published (25) and are provided here for ease of comparison.

[b] Total number of foci divided by the total square area of the cell times a factor of 100 to make the number an integer.

[c] Total number of cells counted.

[d] The *a* values are for a "chi-square test of homogeneity for an $r \times c$ contingency table" ($r \times c$ χ test) (22) that compares the distributions of foci in the population. This does not use the calculated values for the foci per area of cell or the average intensities of the foci (see Tables 3 and 4) in the statistical test. The wild-type or singly mutated strain is compared with the single mutant or double mutant, respectively. An "*a*" value of 0.01 means that there is a 1 in 100 hundred chance this data could have occurred by chance. Values of 0.05 or below are considered significant.

TABLE 3. Effects of single mutations on relative intensities of RecA-GFP foci[a]

Strain no. (mutated gene)	% of foci with relative intensity of:											Avg[b]	Count	*a* value
	<0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	1.1	>1.2			
SS3085 (None)	39.4	34.9	18.9	4.9	1.1	0.5	0.1	0.0	0.1	0.0	0.1	0.24	2,544	
SS3378 (*uvrD*)	7.9	25.5	28.3	18.1	10.0	5.1	2.7	1.5	0.5	0.1	0.1	0.39	6,040	<0.001
SS2683 (*recF*)	52.7	34.9	8.7	2.6	0.5	0.3	0.3	0.0	0.1	0.0	0.0	0.22	1,483	0.3
SS2684 (*recO*)	11.8	54.7	25.5	5.0	1.8	0.7	0.5	0.1	0.0	0.0	0.1	0.29	1,070	<.001
SS3150 (*recR*)	41.0	35.2	17.5	3.9	1.5	0.5	0.1	0.3	0.0	0.0	0.0	0.24	1,094	0.9
SS3394 (*recJ*)	14.8	61.4	16.9	4.1	2.1	0.3	0.3	0.0	0.1	0.0	0.0	0.26	1,509	0.001
SS3317 (*recQ*)	14.5	62.3	18.0	4.2	0.7	0.3	0.0	0.0	0.0	0.0	0.0	0.27	1,547	0.001

[a] See footnote *a* of Table 2.
[b] Average relative intensity of all foci measured.

formally possible that the *uvrD* mutation restores the ability of *recA4155-gfp* to form storage structures and that this phenomenon is responsible for the observed increase in focus number. To test this idea, the number of foci was determined in the presence of 4,6-diamidino-2-phenylindole. This chemical competes with RecA for binding to DNA (34). Like the wild type strain, less than 16% of the original number of foci per area was seen in the presence of 4,6-diamidino-2-phenylindole, leaving less than 6% of the cells with a single focus (data not shown). This further supports the view that the majority of the foci visualized in the *uvrD recA4155-gfp* strain are on the DNA and that *uvrD* mutations do not restore the ability of *recA4155* strains to form storage structures. We conclude that in live, log-phase cells, an *uvrD* deletion mutation increases both the number and intensity of RecA-GFP foci.

***recF*, *recO*, *recR*, *recJ*, and *recQ* are required for the high number of RecA-GFP foci in an *uvrD* mutant.** As mentioned above, it was shown that mutations in *recFOR* and *recJQ* could suppress negative effects of an *uvrD* mutation in certain mutants (9, 23). These results predicted that mutations in the *recFORJQ* genes should decrease the number and/or the intensity of RecA-GFP foci in *uvrD* mutants. To test this, mutations in *recF*, *recO*, *recR*, *recJ*, and *recQ* were introduced into the *uvrD recA4155-gfp* mutant.

The first step in testing this was to measure the distributions of foci and their intensities for the *recFORJQ* single mutants. Table 2 and Table 3 show that none of the single mutants varied significantly from the wild type in their distributions of foci within cells in a population. Table 3 shows that while none of the *recFORJQ* mutations change the average relative intensity by more than 17% (compare the wild type with the *recO* mutant), the focus intensity distributions of the *recO*, *recJ*, and *recQ* mutants vary significantly from that of the wild type.

Table 4 shows the distributions of foci in log-phase *uvrD recA4155-gfp* cells grown in minimal medium with a mutation in either *recF*, *recO*, *recR*, *recJ*, or *recQ*. Mutations in each of these five genes cause a 40 to 60% decrease in the number of RecA-GFP foci of the *uvrD* mutant. The changes in distribution are significant for all mutants. The *recO*, *recR*, and *recQ* mutants show a large decrease of about 60%, while the *recF* and *recJ* mutants show a smaller decrease of about 40%.

Table 4 shows that only *recO* and *recQ* mutations decrease the average relative intensity of the foci from the high level of the *uvrD* mutant back to wild-type levels. The *recF* and *recJ* mutants decrease the levels slightly, and the levels for the *recR* mutant are unchanged. If one compares the binned distributions of the focus intensities (see Table S1 in the supplemental material), however, it is seen that *recFORJQ* mutations each change the distribution from that of the *uvrD* single mutant in a significant way.

It is concluded that mutations in any of the *recFORJQ* genes decrease the number of foci and change the distribution of focus intensity significantly from those of the *uvrD* mutant. However, a large decrease in average relative intensity of foci is seen only in *recO* and *recQ* mutants.

DISCUSSION

This work shows that RecA loading and/or its stability on DNA is increased in an *uvrD* mutant in log-phase cells grown

TABLE 4. Effects of *recF*, *recO*, *recR*, *recJ*, and *recQ* mutations on distribution of RecA-GFP foci and their intensities in *uvrD* mutants[a]

Strain no. (*uvrD* mutation, other mutated gene)	% of cells with no. of foci					No. of foci/area	Count	α value	α value[b]	Summary of avg RI of foci[c]	
	0	1	2	3	4					Avg RI	α value[d]
SS3085 (None, none)	86	11	3	0	0	78	2,072			0.24	
SS3378 (*cat*, none)	67	23	8	2	0	228	700			0.39	
SS3372 (*cat*, *recF*)	82	11	4	2	1	158	2,141	0.025	0.05	0.33	0.04
SS3373 (*cat*, *recO*)	88	9	2	1	0	105	2,007	<0.001	0.8	0.25	<0.001
SS3374 (*cat*, *recR*)	85	12	2	1	0	100	2,033	0.004	0.95	0.38	0.04
SS3380 (*cat*, *recJ*)	82	13	3	1	0	135	1,836	0.01	0.2	0.32	<0.001
SS3379 (*cat*, *recQ*)	85	12	3	0	0	93	1,692	0.002	0.95	0.25	0.005

[a] See footnote *a* of Table 2.
[b] α value for comparison with the wild type.
[c] RI, relative intensity.
[d] α value for comparison of the distributions of intensities from Table S1 in the supplemental material with that for the *uvrD* single mutant.

in minimal medium. This was demonstrated by measuring the number and intensity of RecA-GFP foci. Previous results showed that the synthetic lethality between *uvrD* and *rep* mutations could be suppressed by mutations in *recFOR* (23), and the negative effects of *uvrD* mutations on *dnaE*(Ts) and *dnaN*(Ts) mutants could be suppressed by mutations in *recFORJQ* (10). These authors proposed and then further supported the model that UvrD removed RecA from DNA where its function was inappropriate. Consistent with this model, it is shown here that in log-phase cells grown in minimal medium, the high number of RecA-GFP foci for an *uvrD* mutant is partially dependent on the *recFOR* and *recJQ* genes.

It is also shown that the distributions of focus intensities changed significantly when a mutation in *recF*, *recO*, *recR*, *recJ*, or *recQ* was added to an *uvrD* mutant. Only mutations in *recO* and *recQ* decreased the average relative intensity back to wild-type levels. Thus, although there are significant changes in all strains, the simple change of decreasing the average relative intensity was seen in only two cases. The reason for this difference is not yet clear.

It is noticeable that the *uvrD* strains with additional mutations in *recFORJQ* do not lose all ability to form RecA-GFP foci. Previous work had shown that in wild-type cells, nearly all RecA-GFP foci were *recB* dependent (25). This is consistent with the presence of RecBCD in the strain loading RecA. This suggests that in *uvrD* mutants there are two types of RecA-GFP foci formed: the ones that would normally form and be loaded by RecBCD and those that are additionally loaded by RecFOR. The latter foci are likely not to occur in an otherwise wild-type strain, since *recFOR* mutations do not significantly change the distribution of RecA-GFP foci in a population (Table 2). This suggests that the antirecombinase activity of UvrD seems to be specific to certain situations. A further suggestion of this idea is that there may be some DNA structure or protein complex remaining after the RecA loading event that signals UvrD to remove RecA at this location. We were not able to test whether there are RecBCD-dependent foci in an *uvrD* mutant, because *recB* and *uvrD* mutations are synthetically lethal (2; R. Centore and S. Sandler, unpublished results).

At the onset of this work, two models for why *uvrD* mutants are hyper-rec were suggested. One proposed that more RecA loading events occurred at places where NER was incomplete, and the other suggested that UvrD had a role in removing RecA from places where it was inappropriately recombining DNA. The experiments presented here support both models. It is likely that some of the increase in RecA-GFP foci in the *uvrD* mutant is due to nicks and gaps left by incomplete NER. When replication forks encounter these, they are converted to double strand breaks, and RecA loading is then RecBCD dependent. This may explain the *uvrD-recB* synthetic lethality. The remaining part of the increase is due to RecFORJQ loading events and can be interpreted to be instances when UvrD would normally remove RecA from the DNA (Table 4). The characterization of a novel *uvrD* mutant, *uvrD303*, further supports the latter model (35). It was shown that a strain with *uvrD303* on a plasmid is UV^s, like an *uvrD* deletion mutant, but it was also shown to be Rec^- and nonmutable (unlike an *uvrD* deletion mutant's phenotypes; see above). Additionally, it was shown that UvrD303 has 10-fold-higher helicase activity than the wild type. In agreement with Zhang et al.'s suggestion that the UvrD303 enzyme has antirecombinogenic properties, the results here would suggest that UvrD303's hyper-helicase activity may remove RecA not only in inappropriate situations but also in appropriate ones, resulting in the UV^s and Rec^- phenotypes. The nonmutable phenotype suggests that UvrD303 still functions in MMR and NER. This combination of results predicts that *uvrD303* mutants, in contrast to the deletion mutants, should have fewer RecA-GFP foci than the wild type. This idea is currently being tested.

Bidnenko et al. showed that *uvrD recO* double mutants are much more hyper-rec than *uvrD* single mutants, as measured by conjugal recombination (2). These results need not be in conflict with the results presented here indicating that *recO* mutations decrease the number of RecA filaments in *uvrD* mutants. This could be due to differences in either the DNA substrates (conjugating DNA versus replicating chromosomal DNA) or the assays used.

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