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RecA4142 Causes SOS Constitutive Expression by Loading onto Reversed Replication Forks in *Escherichia coli* K-12[▽]

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Escherichia coli **initiates the SOS response when single-stranded DNA (ssDNA) produced by DNA damage is bound by RecA and forms a RecA-DNA filament. *recA* SOS constitutive [*recA*(Con)] mutants induce the SOS response in the absence of DNA damage. It has been proposed that *recA*(Con) mutants bind to ssDNA at replication forks, although the specific mechanism is unknown. Previously, it had been shown that *recA4142*(*F217Y*), a novel *recA*(Con) mutant, was dependent on RecBCD for its high SOS constitutive [SOS(Con)] expression. This was presumably because RecA4142 was loaded at a double-strand end (DSE) of DNA. Herein, it is shown that *recA4142* SOS(Con) expression is additionally dependent on *ruvAB* (replication fork reversal [RFR] activity only) and *recJ* (5′→3′ exonuclease), *xonA* (3′→5′ exonuclease) and partially dependent on *recQ* (helicase). Lastly, *sbcCD* mutations (Mre11/Rad50 homolog) in *recA4142* strains caused full SOS(Con) expression in an *ruvAB*-, *recBCD*-, *recJ*-, and *xonA*-independent manner. It is hypothesized that RuvAB catalyzes RFR, RecJ and XonA blunt the DSE (created by the RFR), and then RecBCD loads RecA4142 onto this end to produce SOS(Con) expression. In *sbcCD* mutants, RecA4142 can bind other DNA substrates by itself that are normally degraded by the SbcCD nuclease.**

The SOS response is a coordinated response of *Escherichia coli* at the level of transcription to DNA damage (10, 18, 26). RecA initiates this response by binding to single-stranded DNA (ssDNA) produced by DNA damage and serving as an allosteric effector for auto-proteolysis of the LexA transcriptional repressor. The transcription of at least 40 genes is increased during the SOS response (16). Some of the induced genes include *recA*, *ruvAB*, *dinI*, and *recX*. The RecA protein also plays a central role in recombinational repair and homologous recombination (8, 11, 22). It participates in all three processes through its ability to polymerize on ssDNA to create a RecA-DNA filament.

Several proteins are known to either help RecA load onto different DNA substrates or regulate the stability of the RecA-DNA filament (reviewed in reference 12). One of the loading complexes is RecBCD. This complex has the ability to load onto a double-strand end (DSE) of DNA. It processes the DNA using helicase, exonuclease, and Chi recognition activities to produce a region of ssDNA with a 3′ end. RecBCD then loads RecA onto this ssDNA, creating a RecA-DNA filament (reviewed in reference 14). Another complex, RecFOR, loads RecA onto ssDNA coated with single-stranded DNA binding protein (SSB) at a gapped DNA substrate (36, 38). After production, DinI stabilizes and RecX destabilizes RecA filaments (29, 37). RecFOR antagonizes the destabilization activity of RecX both *in vivo* and *in vitro* (28, 30).

There are certain mutants of *recA* that turn on SOS expression in the absence of external DNA damage. These are called *recA* constitutive [*recA*(Con)] mutants (reviewed in reference 33). While the specific mechanism of how this type of mutant induces SOS is presently unknown, it is thought that it occurs through RecA binding to ssDNA on the lagging strand at the replication fork. To begin to test this idea, the dependence of proteins known to be important for RecA loading and stability was tested for two *recA*(Con) mutants. One mutant (*recA730*), showed no dependence for any gene tested, while a second mutant [*recA4142*(*F217Y*)] was dependent on *recBCD*, *recFOR*, and the type of medium in which the cells were grown (28). For *recA4142*, it was hypothesized that the role of RecFOR was to regulate the activity of RecX to destabilize the filament and that the role of RecBCD was to load RecA4142 onto a DSE.

The observation that the loading of RecA4142 in log-phase cells is *recBCD* dependent and that almost all cells in the population have constitutive SOS [SOS(Con)] expression suggests that RecA4142 may not simply bind to ssDNA on the lagging strand at the replication fork. One can suggest several different hypotheses to explain this observation. One is that almost all cells have a DSE or DSEs where RecBCD can load RecA4142 to cause SOS(Con) expression. These could be formed at a stalled replication fork where the nascent leading and lagging strands anneal in a process called replication fork reversal (RFR) (40). A reversed replication fork is very similar, if not identical, in structure to a recombinational intermediate called a Holliday junction (27). Thus, RFR is often catalyzed by the RuvAB proteins [as in *rep*, *holD*, and *dnaE*(Ts) mutants], but can also occur independently of RuvAB in some special mutants [i.e., *priA*, *dnaN*(Ts), and *dnaB*(Ts) mutants] (reviewed in references 35, 39, and 40). Another hallmark of RFR is that the reaction is *ruvC* independent. This is because only the ability to form and branch migrate the Holliday junctions is required for RFR (not the ability to resolve them). Another hypothesis is that RecBCD has a yet undiscovered activity that allows RecA loading at substrates other than

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DSEs. While this seems unlikely, it is a formal possibility and may be particular for RecA4142. A third possibility is that is double-strand breaks (DSBs) occur in every cell and that RecBCD uses its well established activities for loading RecA4142 at these sites. This DSB (*vis a vis* one produced by an *I-sceI* cut [34]), however, must not absolutely require RecBCD for repair because *recA4142 recB* mutants are viable.

One way to test between these three ideas is to ask what gene products are necessary for SOS(Con) expression in a *recA4142* mutant. The first hypothesis (loading during RFR) would be *ruvAB* dependent and *ruvC* independent, while the second two would be *ruvAB* independent. In this report, it is shown that SOS(Con) expression is dependent on RuvAB (and only the activity of RuvAB needed to reverse replication forks) and not *ruvC*. This piece of data better supports the first model, but does not exclude the latter two. SOS(Con) expression is also shown to be dependent on two exonucleases, RecJ and XonA. To accommodate these additional dependencies, it is further proposed that RecJ and XonA process the ends so that RecBCD can load RecA4142 onto RuvAB reversed forks. Lastly, it is shown that the absence of the SbcCD nuclease allows SOS(Con) expression, even when RecBCD, RuvAB, RecJ, and RecF are absent. It is proposed that SbcCD nuclease degrades some substrate that RecA4142 can bind in the absence of traditional RecA loading factors.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this work are derivatives of *E. coli* K-12 and are described in Table 1. The protocol for P1 transduction has been described elsewhere (44). All P1 transductions were selected on 2% agar plates containing either minimal or rich media. Where appropriate, plates also contained the following antibiotics at these final concentrations: tetracycline, 10 μg ml^{-1}; chloramphenicol, 25 μg ml^{-1}; or kanamycin, 50 μg ml^{-1}. All transductants were purified on the same type of medium on which they were selected.

Preparation of cells for microscopy and measurements. Cells were prepared for microscopy and other measurements as described in detail with examples elsewhere (28, 31). Cultures were grown in 56/2 minimal medium (44) until mid-log phase (optical density at 600 nm [OD_{600}] of 0.3 to 0.4). Cells were concentrated 10-fold in 56/2 buffer. Approximately 3 to 5 μl was loaded onto fresh agarose pads, and a coverslip was applied. The agarose pads were prepared using a protocol from P. Levin (25). Microscopy was carried out by using an epifluorescent Nikon E600 microscope. An ORCA-ER-cooled charge-coupled device (CCD) camera (Hamamatsu) and Openlabs software (Improvision) were used for all image acquisition. The exposure time was 100 to 250 ms. Approximately nine fields (three on three different days) containing calibration beads were photographed. A phase-contrast image and a fluorescent image of each field were taken. The softwares Openlab 5.0 and Volocity 4.0 (Improvision, Inc.) were used to measure the amount of fluorescence and cell size in individual cells. Calibration of the fluorescence intensity was set by calibration beads [InSpeck Green (505/515) microscope image intensity calibration kit 2.5 μm I-7219 from Molecular Probes]. The relative fluorescence intensity (RFI) value of an individual cell is calculated by dividing the average calibrated pixel value of a particular cell by the average calibrated pixel value of a strain containing Δ*att*λ::*sulAp* Ω*gfp-mut2* (SS996). The RFI values of the population of cells from all three experiments (typically 1,000 to 3,000 cells) are combined and binned according to their RFI. The percentage of cells with a particular RFI is calculated and plotted. The average RFI for each experiment is also calculated. The average for the three experiments and their uncertainties are reported next to the plots in the figures.

RESULTS

In this work, all strains contain a *sulAp-gfp* reporter gene inserted at the *att*λ site. Justification of the use of this construct as a reporter for SOS expression as well as how the measurements are done and reported have been explained in detail elsewhere (28, 31).

SOS(Con) expression is RuvAB dependent and RuvC independent. To begin to test between the three models mentioned above, *ruvAB6203*::*tet* was combined with *recA4142*. Note that all *recA4142* strains discussed here also have *recAo1403*. This is necessary so that all cells in a population have high levels of SOS(Con) expression (28). Figure 1 shows that strains missing RuvAB revealed a large decrease in SOS(Con) expression, suggesting that RuvAB is required for SOS(Con) expression. This result is consistent with the first hypothesis suggesting replication fork reversal as a mechanism to produce the DSE. A further test of this model is that SOS(Con) expression should also be *ruvC* independent. Figure 1 shows that a *ruvC* mutation did not affect the level of SOS(Con) expression. These observations support the hypothesis that RFR may be a mechanism important for high levels of SOS(Con) expression in *recA4142* mutants. When *ruvABC*::*cat* and *ruvA60*::Tn*10* were added to the *recA4142* mutants, the double mutants revealed similar results to when *ruvAB6203*::*tet* was added (data not shown).

To further test if RuvAB and its ability to catalyze RFR were important for high SOS(Con) expression, two additional tests were performed. The first was to test if the addition of pGB2-RuvAB to the *ruvAB* mutant would restore a high level of SOS(Con) expression. Figure 2 shows that this plasmid complemented the ability of the strain to produce high levels of SOS(Con) expression (Fig. 2A) and the UV sensitivity (Fig. 2B) of the strain as compared to that of the pGB2 vector-only control. The second test used a novel *ruvB*(*H198Y*) mutant that is deficient in RFR but maintains the ability to participate in recombination and DNA repair (3, 24). If RFR is responsible for high SOS(Con) expression in the *recA4142* mutant, then addition of the plasmid should not complement the decrease in SOS(Con) expression, but it should complement the increase in UV sensitivity. If RFR were not the required activity for RuvAB to produce high levels of SOS(Con) expression, then one would expect to see full complementation of both the SOS(Con) expression and UV sensitivity. Figures 2A and B show that the addition of pGB2-RuvAB(H198Y) (24) complements the UV sensitivity of the strain, but not the low levels of SOS(Con) expression. From these results, it is concluded that RuvAB's ability to catalyze RFR is required for high levels of SOS(Con) expression in the *recA4142* mutant.

Other work suggests that the RecG helicase may also catalyze replication fork reversal (32, 42). To test if RecG was required for high levels of SOS(Con) expression in the *recA4142* mutant, *recG6200*::*tet* was combined with *recA4142.* Figure 1 shows that this mutation did not change the level of SOS(Con) expression in the *recA4142* mutant. From this, we conclude that RecG is not required for high levels of SOS(Con) expression in the *recA4142* mutant.

RecJ and XonA (SbcB) are required for SOS(Con) expression. As the data presented above support the first model that DSEs may be generated during RFR, other experiments were attempted to further define other gene products that may be important in the loading of RecA4142 by RecBCD at a DSE produced during RuvAB-catalyzed RFR.

RecBCD loads preferentially onto fairly blunt DSEs of DNA (43, 45). Since the DSE produced during RFR by the

TABLE 1. Strains used in this work

Strain	*recA*	*recBCD*	Other relevant genotype	Source or reference
CAG18642	+	+	*zfj-3131*::Tn*10*	41
JC18923	+	+	*recJ284*::Tn*10*	Lab stock
JJC296	+	+	*ruvA60*::*tet*	B. Michel
JJC754	+	+	*ruvABC*::*cat*	B. Michel
JJC783	+	+	*ruvC*::*cat*	B. Michel
KM78	+	*cat*		K. Murphy
SMR839	+	+	*xonA*::*cat*	S. Rosenberg
TP538	+	+	*recG6200*::*tet*	T. Poteete
TP540	+	+	*ruvAB6203*::*tet*	T. Poteete
TP640	+	+	*recQ6218*::*tet*	T. Poteete
SS996[a]	+	+		31
SS4639	*730*	+	*recF4115 tnaA*::miniTn*5 cam*	28
SS4696	*4142*[b]	+	*recF4115 tnaA300*::Tn*10*	28
SS4976	*4142*	+		28
SS5179	+	+	del(*sbcC*)*100*::*kan*	2
SS5303	*4142*	+	*recX*::*cat*[g]	28
SS5305	*4142*	+	*recJ264*::Tn*10*	JC18923→SS4976
SS5312	*4142*	+	*recX*::*cat*[f]	28
SS6021	+	*cat*		KM78→SS996[d]
SS6023	*4142*	*cat*		28
SS6156	*4142*	+	*zfj-3131*::Tn*10*	CAG18642→SS4976[c]
SS7143	*4142*	+	*recG6200*::*tet*	TP538→SS4976[c]
SS7144	*4142*	+	*ruvABC*::*cat*	JJC754→SS4976[d]
SS7160	*4142*	+	*ruvAB6203*::*tet*	TP540→SS4976[c]
SS7161	*4142*	+	*xonA*::*cat*	SMR839→SS4976[d]
SS7162	*4142*	+	*ruvC*::*cat*	JJC783→SS4976[d]
SS7165	+	+	*ruvC*::*cat*	JJC783→SS996[d]
SS7174	*4142*	+	*recQ6218*::*tet*	TP640→SS4976[c]
SS7178	*4142*	+	del(*sbcC*)*100*::*kan*	SS5179→SS996[a]
SS7184	*4142*	+	*recX*::*cat recJ284*::Tn*10*	SS5303→SS5305[d]
SS7186	+	+	*xonA*::*cat*	SMR839→SS996[d]
SS7187	*4142*	+	*ruvA60*::*tet*	JJC296→SS4976[c]
SS7188	+	+	*ruvA60*::*tet*	JJC296→SS996[c]
SS7189	*4142*	+	*ruvAB6203*::*tet*	pRuvAB→SS7160
SS7190	*4142*	+	*ruvAB6203*::*tet*	pGB2→SS7160
SS7191	*4142*	+	*ruvAB6203*::*tet*	pRuvAB H198→SS7160
SS7194	+	+	*recJ284*::Tn*10*	JC18923→SS996[c]
SS7195	+	+	*recQ6218*::*tet*	TP640→SS996[c]
SS7196	+	+	*ruvAB6203*::*tet*	TP540→SS996[c]
SS7197	+	+	del(*sbcB*)*200*::*frt* del(*sbcC*)*200*::*frt*	SS5162→SS7178[e]
SS7198	*4142*	+	del(*sbcB*)*200*::*frt* del(*sbcC*)*200*::*frt*	SS4976→SS7197[b]
SS7404	*4142*	*cat*	del(*sbcB*)*200*::*frt* del(*sbcC*)*200*::*frt*	KM78→SS7198[d]
SS7411	+	+	del(*sbcC*)*100*::*kan*	SS5179→SS996[b]
SS7413	*4142*	+	del(*sbcC*)*200*::*frt*	SS4976→SS7411[b]
SS7417	*4142*	*cat*	del(*sbcC*)*200*::*frt*	KM78→SS7413[d]
SS7418	*4142*	*cat*	*ruvAB6203*::*tet* del(*sbcC*)*200*::*frt*	TP540→SS7413[c]
SS7419	*4142*	+	del(*sbcC*)*200*::*frt recF4115 tnaA300*::Tn*10*	SS5394→SS7413[b]
SS7420	*4142*	+	*recJ284*::Tn*10* del(*sbcC*)*200*::*frt*	JC18923→SS7413[c]
SS7421	*4142*	*cat*	*recF4115 tnaA300*::Tn*10*	SS5394→SS7404[b]
SS7423	*4142*	*cat*	*ruvAB6203*::*tet*	TP540→SS7404[c]
SS7424	+	+	del(*sbcD*)*100*::*kan*	SS5180→SS996[a]
SS7425	*4142*	+	del(*sbcD*)*100*::*kan zfj-3131*::Tn*10*	SS6156→SS7424[c]
SS7429	*4142*	+	*xonA*::*cat* del(*sbcD*)*100*::*kan zfj-3131*::Tn*10*	SMR839→SS7425[d]
SS7430	*4142*	*cat*	del(*sbcD*)*100*::*kan zfj-3131*::Tn*10*	KM78→SS7425[d]
SS7431	*4142*	+	del(*sbcD*)*100*::*kan zfj-3131*::Tn*10 recF4115 tnaA*::miniTn*5 cam*	SS4639→SS7425[d]
SS7432	*4142*	+	*ruvABC*::*cat* del(*sbcD*)*100*::*kan zfj-3131*::Tn*10*	JJC754→SS7425[d]
SS7433	*4142*	+	del(*sbcD*)*100*::*kan zfj-3131*::Tn*10 recJ284*::Tn*10(cat)*	SS4907→SS7425[d]

[a] The genotype for this strain is *sulB103*Δ*att*λ::*sulAp*Ω*gfp-mut2 lacMS286* ϕ80dII*lacBK1 argE3 hi-4 thi-1 xyl-5 mtl-1 rpsL31 tsx.* The *lacMS286* ϕ80dII*lacBK1* genes code for two partial nonoverlapping deletions of the *lac* operon (21, 46), and Δ*att*λ::*sulAp*Ω*gfp-mut2* is the *sulAp-gfp* reporter gene inserted into the *att*λ site (31). All *recA4142* strains used in this work also are *ygaD1*::*kan recAo1403* (28).

[b] Select for Kan[r] and then screen for other marker phenotypically or by PCR.

[c] Select for Tet[r] and then screen for other marker phenotypically or by PCR.

[d] Select for Cat[r] and then screen for other marker phenotypically or by PCR.

[e] This deletion allele was created by first transducing the Kan[r] allele from the Kieo collection into the strain as indicated in the reference column. pLH29, carrying the *flp* gene, was then introduced, and Kan-sensitive derivatives were screened (20).

[f] The Tn*10 tetA*::*cat* insertion deletion mutation was amplified with prSJS690,691 using pACYC184 as a template. The Tn*10 tetA*::*cat* insertion deletion mutation was transferred to the chromosome by the *exo-bet* method (13) into a strain containing *recJ284*::Tn*10*. This original combination of mutants was named and saved as the strain indicated as the donor in this cross.

[g] The full notation for the *recX* mutation is del(*recX*)*4166*::*cat*. The full notation for the *recBCD* mutation is del(*recBCD*)::*cat.* The full notation for the Ω*gfp* mutation is Δ*att*λ::*sulAp*Ω*gfp-mut2.*

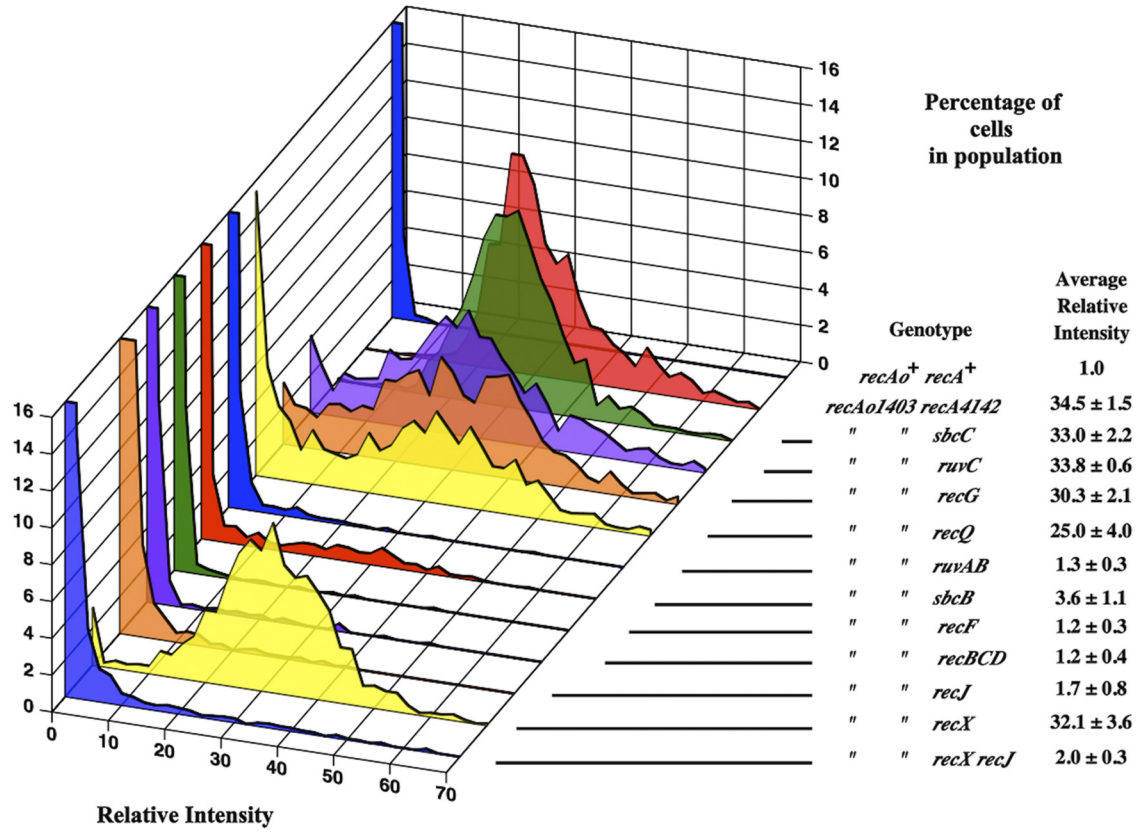

FIG. 1. Distributions of cells with different levels of constitutive SOS expression (detected as GFP fluorescence) expressed as the percentage of cells in the population. The graphs truncate the percentage of cells at 16%. The strains are in order from top of the graph to the bottom, with the relevant part of the genotype in parentheses. All strains were grown in minimal medium at 37°C with aeration. All strains except SS996 have additionally the genotype *recAo1403 recA4142*. The other relevant genotypes are shown in parentheses, and full genotypes are shown in Table 1. The strains are SS996 (wild type, including *recA*$^+$), SS4796 (wild type), SS7413 (*sbcC*), SS7162 (*ruvC*), SS7143 (*recG*), SS7174 (*recQ*), SS7160 (*ruvAB*), SS7161 (*sbcB* or *xonA*), SS4696 (*recF*), SS6023 (*recBCD*), SS5305 (*recJ*), SS5312 (*recX*), and SS7184 (*recX recJ*).

annealing of the nascent leading and lagging stands of DNA may not be blunt (depending on where the DNA polymerases stop), it is possible that the ends produced may need to be processed before RecBCD can load RecA. This processing may be accomplished by exonucleases. It had been shown previously that *xthA*, *xseAB*, *xni*, *tatD*, and *exoX* encode proteins whose activities degrade DNA substrates used by RecBCD to load RecA (6, 28). Therefore, it was not expected that any of these enzymes would be required for SOS(Con) expression in the *recA4142* mutant. However, two other exonucleases, RecJ (5′→3′ exo) and SbcB (also called XonA) (3′→5′ exo), have been shown not to degrade RecBCD/RecA substrates using RecA-green fluorescent protein (GFP) focus formation as an assay (6, 7). Therefore, it was possible that either or both of these proteins might be required for blunting the emerging duplex during RFR so that RecBCD could load RecA4142 to produce SOS(Con) expression. To test this, *recJ* and *sbcB* null mutations were combined with *recA4142*. Figure 1 shows that *recJ* and *sbcB* mutations individually decreased SOS(Con) expression dramatically. Expression of *recJ* from a plasmid (courtesy of S. T. Lovett) in the *recJ* mutant and *sbcB* from a plasmid in the *sbcB* mutant showed complete reversal of the phenotype (data not shown). This suggests that both of these exonucleases are needed to blunt the ends so that RecBCD can load onto the DSE.

As mentioned above, it had been shown that *recFOR* mutations, like *recBCD* mutations, decrease SOS(Con) expression in *recA4142* (28). The reason for the decrease in the *recFOR* mutant, however, was due to an inability to antagonize RecX activity since a *recX* mutation could suppress the decrease seen in the *recFOR* mutant (but not the *recBCD* mutant). Since *recJ* mutations decrease SOS(Con) expression as much as *recFOR* or *recBCD* mutations and RecJ often acts in a pathway with RecFOR, it was possible that RecJ participated in antagonizing RecX like RecFOR. To test this, *recX* and *recJ* mutations were combined in a *recA4142* strain. The *recX recJ* strain showed no increase in SOS(Con) expression relative to the *recJ* strain alone (Fig. 1). It is concluded that RecJ is not involved in antagonizing RecX activity and is likely to help process the DNA substrate required by RecBCD to produce SOS(Con) expression in a *recA4142* strain.

The RecJ exonuclease and RecQ helicase are often thought to interact when processing DNA for the loading of RecA (9, 19). It was therefore of interest to test if RecQ is also required for high levels of SOS(Con) expression. To test this, *recQ6209*::*tet* was combined with *recA4142*. Figure 1

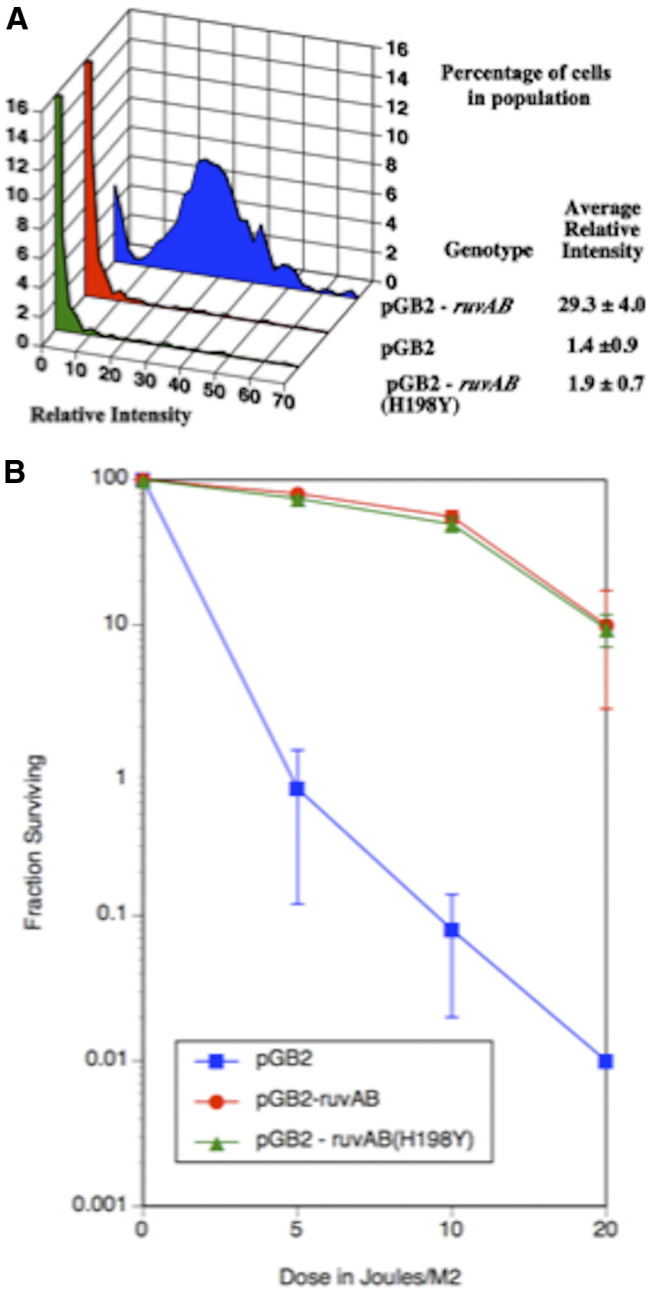

FIG. 2. The three strains used in this figure have the same background: SS7160 (*recAo1403 recA4142 ruvAB6203*::*tet*) with the three different plasmids as indicated. Panel A is the same as Fig. 1. Panel B shows a UV survival curve of the three strains. Strains were grown in minimal media, UV irradiated at a rate of 0.5 J m^2/s, and then serially diluted on to minimal plates. Plates were counted after 36 to 48 h of incubation at 37°C.

shows that the absence of RecQ leads to a 30% decrease in SOS(Con) expression. This suggests that RecQ plays a minor role in RFR that leads to the loading of RecA4142 to produce SOS(Con) expression.

***sbcC* and *sbcD* mutations allow a high level of SOS(Con) expression in a *recA4142* mutant in a *ruvAB*-, *recBCD*-, *recJ*-, and *xonA* (*sbcB*)-independent manner.** The combination of *sbcB* and *sbcC* null mutations is known to suppress the loss of RecBCD enzyme and load the RecA enzyme via the RecFOR pathway of recombination (4). This suggests that *sbcBC* mutations may restore SOS(Con) expression in the *recA4142 recBCD* strain. To begin to test this hypothesis, the *recA4142 recBCD sbcBC* strain was constructed. Figure 3 shows that all cells have a high level of SOS(Con) expression. This supports the initial hypothesis that the RecFOR pathway genes could load RecA4142 during RFR to produce SOS(Con) expression. If true, then mutations in *ruvAB* or *recF* should decrease SOS(Con) expression in the *recA4142 recBCD sbcBC* mutant. Note that *recF* mutations should cause the decrease for two reasons: its ability to load RecA and its ability to antagonize RecX. Contrary to expectation, SOS(Con) expression remained high in both the *ruvAB* and *recF4115* derivatives (Fig. 3). Control experiments were performed to show that the *ruvAB* and *recF4115* mutations had indeed been introduced into the strain by the extreme UV sensitivity they cause (data not shown). *recO* and *recR* derivatives of both the *recBCD sbcBC* mutant and the *sbcBC* mutant were constructed. The *recR* derivatives behaved very similarly to the *recF* mutants. The *recO* mutants showed slightly less SOS(Con) expression than the *recFR* mutants. Still, about 80% of the population had high levels of SOS(Con) expression (data not shown). The reason for this small difference is not clear.

To explain this, it was hypothesized that the *sbcC* null mutation suppressed the absence of *sbcB* and or *recBCD* for SOS(Con) expression in the *recA4142* strain. If true, then when *sbcC* null mutations are added to either *sbcB* or *recBCD* single null mutants, the double-mutant strain should have high SOS(Con) expression. Figure 3 shows all cells in *sbcB sbcC* and *recBCD sbcC* mutants have a high level of SOS(Con) expression. Furthermore, it is shown that addition of an *sbcC* null mutation to a *recA4142* mutant with either an *ruvAB*, *recF*, or *recJ* mutation also restores the high levels of SOS(Con) expression (Fig. 3).

Since SbcC forms a nuclease complex with SbcD, it was tested if an *sbcD* null mutation has the same ability as the *sbcC* mutation to suppress the requirement for *sbcB* (*xonA*), *recBCD*, *ruvAB*, *recF*, and *recJ* mutations for SOS(Con) expression in a *recA4142* mutant. All *sbcD* mutants behaved as their *sbcC* counterparts (data not shown). Lastly, as expected, the ability of the *sbcC* mutation to suppress the low levels of SOS(Con) expression in the *recA4142 sbcC recJ* double mutant is recessive to wild type as the addition of a plasmid with *sbcCD* (courtesy of D. Leach) returns the strain back to low levels of SOS(Con) expression, as seen for a *recA4142 recJ* mutant (data not shown).

It is tentatively concluded that the SbcCD nuclease degrades a DNA substrate that RecA4142 could use to provide SOS(Con) expression. It does not require any of the standard RecA loading enzymes (i.e., RecBCD or RecFOR) to load onto this substrate to yield SOS(Con) expression.

DISCUSSION

This paper provides a mechanistic study of the way one particular *recA*(Con) mutant, the *recA4142* strain, causes SOS(Con) expression. This is important because previously it was assumed that all *recA*(Con) mutants behaved similarly, with the mutant *recA* protein binding to ssDNA on the lagging strand

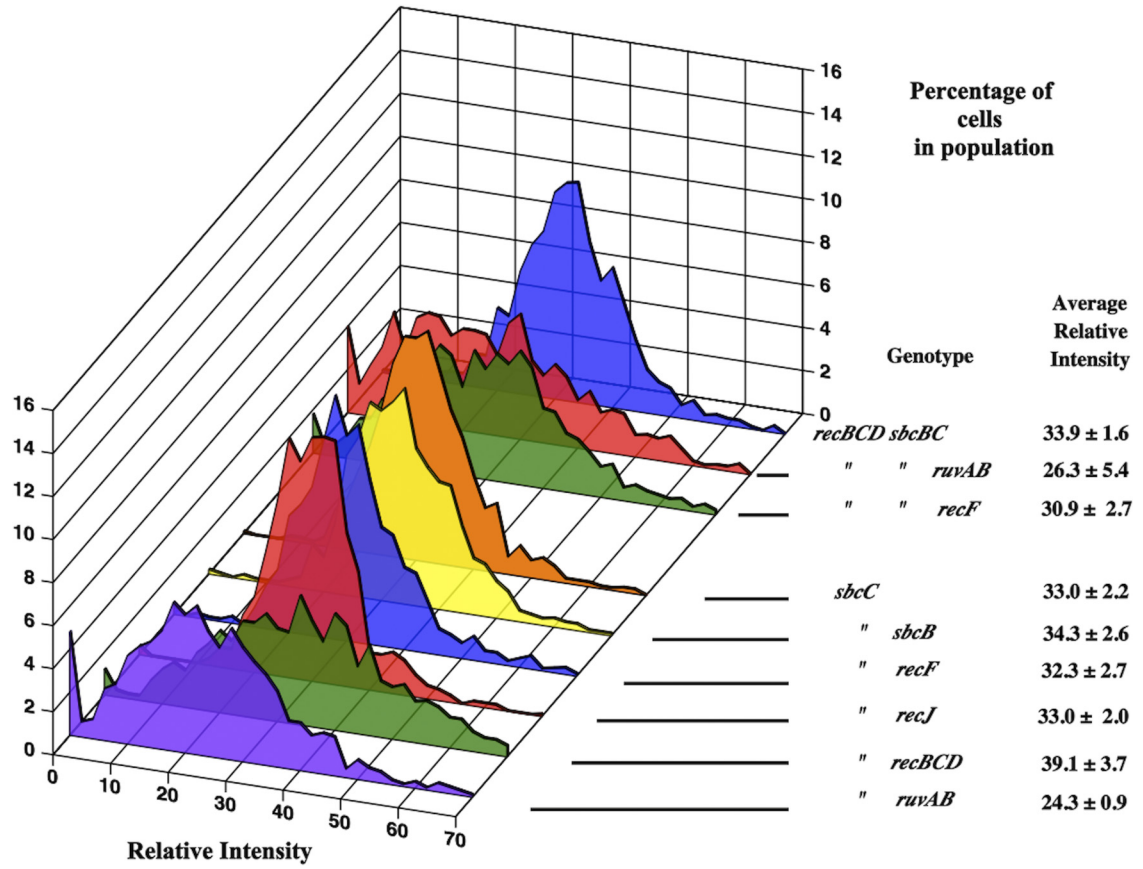

FIG. 3. Same as Fig. 1. The strains are SS7404 (*recBCD sbcBC*), SS7423 (*recBCD sbcBC ruvAB*), SS7421 (*recBCD sbcBC recF*), SS7413 (*sbcC*), SS7198 (*sbcC sbcB*), SS7419 (*sbcC recF*), SS7420 (*sbcC recJ*), SS7417 (*sbcC recBCD*), and SS7418 (*sbcC ruvAB*). All strains also have the genotype *recAo1403 recA4142*.

template at a replication fork. This was probably due to observations with one well-studied mutant, RecA730, that could compete better *in vitro* with SSB for ssDNA than the wild type (15, 23). It is now well established that *recA*(Con) mutants can have different mechanisms for SOS(Con) expression and need not be confined to binding ssDNA on the lagging strand of a replication fork, although that may be true for some mutants. Of the three models proposed at the beginning of this study for how RecA4142 causes RecBCD-dependent SOS(Con) expression, this report supports the first model: that RecBCD loads RecA4142 at a reversed replication fork. The data that SOS(Con) expression is dependent on RuvAB, but not RuvC, and that pRuvAB(H184Y) is able to restore UV resistance to a *ruvAB* mutant, but not its ability to produce SOS(Con) expression, are consistent with this model and not the other two. No experiments were done here, however, that excluded the latter two models. It was further shown that SOS(Con) expression in the *recA4142* mutant was *xonA* and *recJ* dependent. To accommodate these additional observations, the model (Fig. 4) was expanded to suggest that these exonucleases blunt the end of the newly formed duplex of DNA so that RecBCD can load RecA.

Figure 4 suggests that there may be two ways that RecA4142 can produce a stable RecA-DNA filament that can affect LexA autoproteolysis. The first is that it binds to ssDNA produced by Chi-stimulated activities. If this complex is long lived, it may be sufficient to drive down the concentration of LexA. However, given the fact a homologous duplex is in close proximity, it is likely that the RecA-ssDNA will strand invade the homologous duplex, producing a second structure where RecA may be bound to dsDNA. Since dsDNA can also act as a cofactor for LexA cleavage *in vitro* (M. Cox, personal communication), it is possible that this structure is stable in a *recA4142* mutant and could continue catalyzing LexA cleavage until removed, possibly by helicases or by the next round of DNA replication. It is envisioned that wild-type RecA will go through a similar action but will not be stable on the dsDNA after the recombination event. This is the hypothesized difference between RecA4142 and the wild type and the reason why the wild type does not produce SOS expression constitutively in cells where RFR may occur.

Lastly, it was shown that SbcCD mutants provide an alternate substrate for RecA4142 to produce SOS(Con) expression that is independent of *recBCD*, *recFOR*, *recJ*, and *ruvAB*. A simple hypothesis for this mechanism is that since this enzyme has a variety of nucleolytic activities, it could destroy a potential RecA substrate. Interestingly, RecA4142 in an *sbcC* mutant now resembles RecA730 in wild-type cells not requiring any loading functions for SOS(Con) expression (28). However, RecA4142 does require RecA loading functions for DNA re-

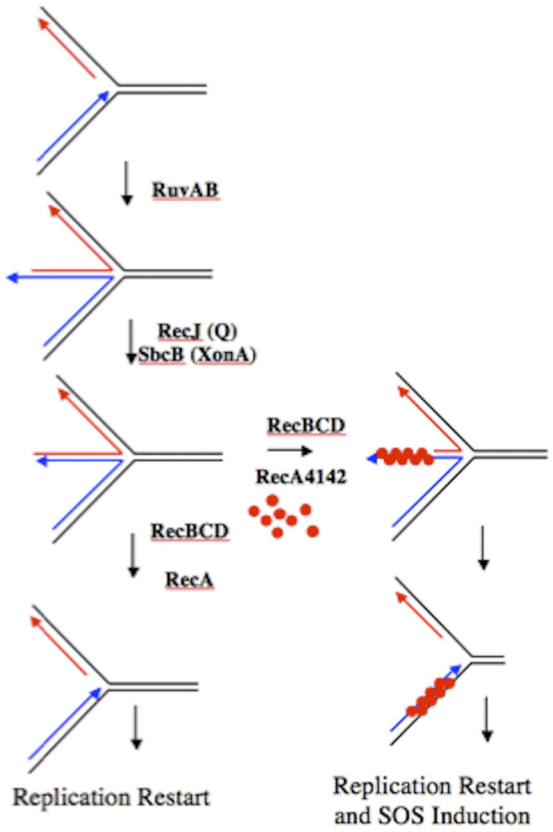

FIG. 4. Model for how RecA4142 can interact with a reversed replication fork with the help of RuvAB, RecJ, SbcB, and RecBCD. The red and blue strands of DNA show the nascent lagging and leading strands of DNA, respectively. The black strands are the parental DNA. The image suggests that RuvAB binds to a stopped fork and reverses it (annealing of the nascent leading and lagging strands [red and blue]). These ends are then processed by the exonuclease activities of RecJ (5′→3′) and SbcB (XonA) (3′→5′). RecBCD can then load onto the DNA, and, depending if there is a Chi site, it will either just degrade newly annealed strands, resetting the fork and allowing for the replication restart proteins to reload the fork, or it can the load RecA4142. Presumably once RecA4142 is loaded, it can start to interact with LexA and help accelerate its auto-cleavage. Since RecA4142 is recombination proficient, it could strand invade the homologous duplex, creating a structure that again is a substrate for the replication restart proteins. If so, RecA4142 may stably persist on the dsDNA and continue to accelerate LexA cleavage until removed.

pair (and presumably recombination): both *recA4142 recBC sbcBC recF* and *recA4142 recBC sbcBC* strains have high SOS(Con) expression, but only the *recA4142 recBC sbcBC recF* strain is slow growing and very UV sensitive (data not shown). Other more complicated models are also possible to explain the SOS(Con) expression in the *sbcC* strains.

How often do reverse replication forks occur when cells are grown in log phase? This has been a difficult question to answer because assays for RFR are indirect, typically requiring that the cells be perturbed in some way (i.e., the addition of a *rep* or DNA replication mutation or some dose of a DNA-damaging agent) and, depending on the assay, that RecBCD also be absent. At face value, the data suggest that RFR occurs in most cells at some point during the cell cycle since almost all *recA4142* cells have high levels of SOS(Con) expression. This suggestion has caveats, however. The first is that RecA4142 may facilitate RFR by causing DNA damage. This seems unlikely since *recA4142* mutants are Rec^+ and UV^r like the wild type (28). Hence, it is probable that RecA4142 only takes advantage of the presence of a reversed fork. The second is the reporter for RFR is GFP that is very stable and has a half-life longer than the cell cycle (1). Therefore, the fluorescence is likely to persist through more than one cell cycle.

It is curious that both SbcB and RecJ exonucleases are needed to process the reversed fork so that RecBCD can load RecA4142. It is easy to imagine that the nascent leading strand would almost always be ahead of the nascent lagging strand, so that SbcB would be needed more often to resect the nascent leading strand and that RecJ would be needed less often to resect the nascent lagging strand. One possibility to explain why both proteins are needed equally is that the two form a complex to process the DSE for RecBCD. In support of this hypothesis, previous work on the networking interactions between *E. coli* proteins has shown a significant interaction between RecJ and SbcB (5).

The ability of *recF* to maintain the high levels of SOS(Con) expression in *recA4142* strains was dependent on the allele of *sbcC* in the strain. If *sbcC* was wild type, then RecF (and RecOR) were required to antagonize the action of RecX. As noted before, the ability of RecX to dramatically decrease SOS(Con) expression was unexpected because it occurred in minimal media and produced such a large effect (until then, RecX effects were only see in rich media and produced subtle changes [28, 37]). If *sbcC* is deleted, then *recF* is not needed for the high levels of SOS(Con) expression. Either some other protein is available to antagonize RecX, or the substrate to which RecA4142 is now bound is different and is no longer susceptible to the destabilizing effects of RecX. In other words, RecX may only be able to destabilize RecA4142 at a specialized structure like a reversed replication fork and not at all structures where RecA4142 is bound on the DNA. Other models may also be possible.

Lastly, this work may be used to try to understand the paradoxical observation that on one hand, *recA4142* cells required RecBCD for high SOS(Con) expression, suggesting that RecBCD was loading RecA at a DSB, and on the other hand, that the *recA4142 recBCD* double mutant was viable. In this work, data are shown that support the hypothesis that a reversed replication fork provides the DSE to which RecBCD loads RecA4142. Hence, one no longer has to hypothesize a DSB on which RecBCD loads RecA. Does this solve the paradox, however, of why the *recA4142 recBCD* mutant is viable? We think it does not, because if RFR is occurring in the *recA4142* strain, then the production of the DSE is still known to be lethal in a *recBCD* mutant (note that although cleavage of the reversed fork by RuvC does produce a DSB, *ruvC* mutations do not rescue the *rep recBCD* synthetic lethality [40]). Therefore, the *recA4142 recBCD* double mutant should still be inviable (but it is not). One way out of this dilemma is to suggest that there is a RecBCD-independent process (via a presently unknown mechanism and possibly specific to *recA4142* cells) that resets the fork and is fast compared with RuvABC binding and RuvC endonucleolytic action (for evidence for the reset of the reversed forks prior to cleavage by RuvABC, see references 17 and 40). Another way

around this dilemma is to hypothesize that the number of RFRs that occur in a "compromised" strain [i.e., *rep* or *dnaE*(Ts)] is greater than the number of RFRs that occur in the recA4142 strain and that this is the reason for the viability. Future research may be able to test these ideas.

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