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Suppression of Constitutive SOS Expression by *recA4162* (I298V) and *recA4164* (L126V) requires UvrD and RecX in *Escherichia coli* K-12

Jarukit Edward Long[1], **Nicholas Renzette**[2], and **Steven J. Sandler**[1,2,*]

[1] Department of Microbiology, Morrill Science Center IV N203, University of Massachusetts at Amherst, Amherst, MA 01003

[2] Molecular and Cellular Biology Graduate Program, Morrill Science Center, University of Massachusetts at Amherst, Amherst, MA 01003

Abstract

Sensing DNA damage and initiation of genetic responses to repair DNA damage are critical to cell survival. In *E. coli*, RecA polymerizes on ssDNA produced by DNA damage creating a RecA-DNA filament that interacts with the LexA repressor inducing the SOS Response. RecA filament stability is negatively modulated by RecX and UvrD. *recA730* (E38K) and *recA4142* (F217Y) constitutively express the SOS Response. *recA4162* (I298V) and *recA4164* (L126V) are intragenic suppressors of the constitutive SOS phenotype of *recA730*. Herein, it is shown that these suppressors are not allele specific and can suppress SOS^C expression of *recA730* and *recA4142 in cis* and *in trans*. *recA4162* and *recA4164* single mutants (and the *recA730* and *recA4142* derivatives) are Rec^+, UV^R and are able to induce the SOS response after UV treatment like wild type. UvrD and RecX are required for the suppression in two (*recA730,4164* and *recA4142,4162*) of the four double mutants tested. To explain the data, one model suggests that $recA^C$ alleles promote SOS^C expression by mimicking RecA filament structures that induce SOS and the suppressor alleles mimic RecA filament at end of SOS. UvrD and RecX are attracted to these latter structures to help dismantle or destabilize the RecA filament.

Keywords

RecA; SOS Response; Recombination; DNA Repair

INTRODUCTION

Organisms have the ability to detect and respond to DNA damage. In *Escherichia coli*, RecA and LexA regulate, at the level of transcription, the cellular reaction to DNA damage called the SOS Response. RecA is the sensor for the response and LexA is the repressor (Walker, 1996). RecA also repairs DNA damage through recombination (Lusetti and Cox, 2002). The SOS Response is activated when DNA damage generates ssDNA to which RecA binds and forms a protein-DNA filament. The RecA-DNA filament then increases the rate of LexA auto-proteolysis (Little, 1991) and this, in turn, increases the rate of transcription of SOS genes (Courcelle *et al.*, 2001; Fernandez De Henestrosa *et al.*, 2000). The loading of RecA onto DNA requires either the RecBCD enzyme or the RecFOR complex depending on the substrate (Clark and Sandler, 1994). RecA filaments are dynamic structures and grow by

*Corresponding author: Telephone Number: (413)-577-4391, FAX Number: (413)-545-1578, sandler@microbio.umass.edu.

adding monomers at the 3′ end and losing them from the 5′ end (Bork *et al.*, 2001; Shan *et al.*, 1997).

The stability of the RecA-DNA filaments is of critical importance for its ability to function in recombination, DNA repair and induction of the SOS response. Of several proteins known to influence RecA filament stability (reviewed in (Cox, 2007)); RecX and UvrD both have negative impacts on the stability of RecA-DNA filaments and both are part of the SOS regulon. Biochemical (Drees *et al.*, 2004a; Drees *et al.*, 2004b; Lusetti *et al.*, 2006; Stohl *et al.*, 2003) and structural analysis (Mishra *et al.*, 2003; Ragone *et al.*, 2008; VanLoock *et al.*, 2003) suggest that RecX binds either at the 3′ end of the RecA filament and or in the major groove where it promotes local dissociation, creating new 5′ and 3′ ends, while inhibiting the addition of monomers at the 3′ end. RecX mediated destabilization is further complicated by the ability of RecFOR to antagonize this activity (Long *et al.*, 2008; Lusetti *et al.*, 2006). Recently, it has been suggested that ssDNA binding may also be critical for RecX function (Baitin *et al.*, 2008). The *recX* gene is co-transcribed with the *recA* gene (Pages *et al.*, 2003).

The UvrD helicase can remove RecA from ssDNA *in vitro* (Veaute *et al.*, 2005). RecA and UvrD's eukaryotic homologs, RAD51 and SRS2, share a similar relationship (reviewed in (Macris and Sung, 2005) and (Veaute *et al.*, 2003)). The absence of these helicases often leads to hyper-recombinogenic phenotypes under conditions of normal growth and the inability to suppress stressful situations caused by recombination (Kerrest *et al.*, 2009; Zieg *et al.*, 1978). In *E. coli*, *uvrD* mutants have two-fold more RecA-GFP foci and these foci are on average two-fold brighter than wild type strains (Centore and Sandler, 2007). The cell uses UvrD to remove RecA from the DNA. For instance, UvrD removes RecA from stopped replication forks in $dnaN^{ts}$ and $dnaE^{ts}$ strains where RecA's activity is detrimental ((Flores *et al.*, 2005) and reviewed in (Michel *et al.*, 2007)).

recA constitutive ($recA^{C}$) mutants have lost the ability to properly regulate the SOS Response and show high levels of SOS expression in log phase cells in the absence of external DNA damage {(Kirby *et al.*, 1967; Nastri *et al.*, 1997; Skiba and Knight, 1994; Tessman and Peterson, 1985; Witkin *et al.*, 1982) and reviewed in (McGrew and Knight, 2003)}. Two $recA^{C}$ mutants discussed here are *recA730* (E38K) and *recA4142* (F217Y) (McGrew and Knight, 2003; Skiba and Knight, 1994; Witkin *et al.*, 1982). Structurally these two mutations change amino acids in different parts of the RecA protein (Figure 1). *recA730* changes an amino acid located on the outside of the RecA-DNA filament and *recA4142* changes an amino acid at the monomer-monomer interface. These two mutants have been recently characterized for the mechanism by which they cause constitutive SOS (SOS^{C}) expression in log phase cells (Long *et al.*, 2008). Several differences were seen. The first is that SOS^{C} expression in *recA4142* mutants is sensitive to mutations in RecA loading and stability factors: *recBCD*, *recX* and *dinI*; whereas the SOS^{C} expression in a *recA730* mutant is not. Another difference is that SOS^{C} expression in a *recA730* strain is sensitive to the hyper-helicase mutant *uvrD303*, whereas the SOS^{C} expression in a *recA4142* mutant is not (Centore *et al.*, 2009). The initial level of transcription in *recA4142* cells (and not *recA730* cells) is critical for SOS^{C} expression. In $recAo^{+}$ *recA4142* mutants, about 8% of a population of cells are constitutive for SOS (SOS^{C}) expression. This can be increased to 100% by the *recAo1403* operator mutation which increases the basal rate of transcription 2–3 fold (Long *et al.*, 2008; Wertman and Mount, 1985). In this report, all strains with *recA4142* also have *recAo1403* as well and for simplicity will be referred to simply as *recA4142*. In summary, the results are consistent with a model whereby RecA4142 is loaded by RecBCD at double strand ends that exist in log phase cells and that RecA730 binds to other ssDNA in the cell, possibly at replication forks, in a manner yet to be determined.

Historically, two intragenic suppressor mutations for the SOS^C phenotype of *recA730* were found. The suppressor mutations are named herein *recA4162* (I298V) and *recA4164* (L126V) so that the paper is clear when it discusses these suppressor mutations in the presence of other *recA*^{C} alleles or by themselves. The *recA4162* suppressor was originally identified as one of the mutations in the *recA* allele first called *tif-1* for temperature-inducible filamentation (Kirby *et al.*, 1967). This allele was later shown to contain two mutations (E38K and I298V) and re-named *recA441* (Knight *et al.*, 1984). This allele showed the SOS^C phenotype in a temperature dependent manner (at 42°C, not at 30°C). We named this allele *recA730,4162*. The other suppressor, *recA4164*, was isolated as one of the mutations in an allele originally called *recA718* (contains both *recA730* and *recA4164*) (McCall *et al.*, 1987; Witkin *et al.*, 1982). Unlike *recA730,4162*, SOS^C expression is suppressed in *recA730,4164* cells at all temperatures. The positions of the codons mutated in *recA4162* and *recA4164* (as well as *recA730 and recA4142*) are different from one another (Figure 1). The codon position mutated in *recA4162* is in the carboxy-terminal domain and the codon position mutated in *recA4164* is close to the face of the protein thought to be on the inside of the RecA-DNA helical filament near the DNA (Chen *et al.*, 2008).

Even though these two suppressor mutations have been known for many years, the mechanism by which they function is not yet clear. Intragenic suppression is sometimes found to be allele specific. When it is allele specific, the suppression mechanism often proposed is that the first mutation locally distorts the structure of the protein and the second mutation restores it. *A priori*, in the case of *recA730,4162* or *recA730,4164*, this mechanism of suppression is unlikely to explain these situations since the two suppressors mutations are not in proximity of the "offending" *recA730* mutation even if one considers the location of the mutations in adjacent monomers in a filament (Figure 1). Nonetheless, it is possible that the suppressor mutations restore local structure by some indirect effect. If the suppressors do not restore the structure in an allele specific manner, then it is possible that these mutations affect some general property of the RecA protein such that they no longer support SOS^C expression. It is also possible that the suppression does not occur at the level of individual monomers, but at the level of a filament.

It is shown that suppression of the SOS^C phenotype by *recA4162* and *recA4164* is not allele specific since they suppress another *recA*^{C} allele, *recA4142, in cis*. It is then shown that *recA4162* or *recA4164* are able to suppress (and are dominant to) to both *recA730* and *recA4142* for the ability to produce SOS^C expression. This inability to allow SOS^C expression is not due to an inability to properly interact with the DNA or properly induce the SOS response since all strains with either of the two suppressor mutations individually or in combination with *recA730* and *recA4142* are able to induce the SOS Response after UV treatment like wild type and are Rec$^+$ and UV^R. Lastly, it is shown that both RecX and UvrD are required for suppression in *recA730,4164* and *recA142,4162* mutants, but not in *recA730,4162* and *recA4142,4164* mutants. Several UvrD missense mutants are tested for their ability to aid in the suppression of SOS^C expression and differential abilities are seen. It is suggested that these two suppressor mutations inhibit SOS^C expression by mimicking the structure of the RecA filament that occurs at the end of the SOS Response. This structure is better at recruiting and or responding to the proteins that destabilize or dissolve the RecA filament.

RESULTS

recA4162 and *recA4164* suppress the SOS^C expression of *recA4142*

The suppression provided by *recA4162* and *recA4164* to *recA730* could be due to specific interactions (allele specific) within the *recA730* protein. If the suppression is not due to some special interactions, then the mutations should be able to suppress the SOS^C

expression of other *recA*^{C} allele such as *recA4142*. As indicated above, *recA4142* was picked for this study because the mutation causing the *recA*^{C} phenotype is in a different part of RecA and its requirements for SOS^{C} expression is different as compared to *recA730* (Figure 1 and see above). To test this, *recA4142* was combined with each of the suppressor mutations on the chromosome and measured for its ability to produce SOS^{C} expression in log phase cells. The level of SOS expression was measured using the *sulAp-gfp* transcriptional reporter system as has been described previously (Long *et al.*, 2008;McCool *et al.*, 2004). All measurements were done with log phase cells grown in minimal medium.

The average Relative Fluorescence Intensity (RFI) and the percentage of cells in a population expressing SOS^{C} six-fold above wild type for the *recA730* single mutant and *recA730* with the two suppressor mutations are shown in Table 1. *recA730* mutants show that nearly all their cells have a RFI greater than six-fold above wild type and that the average RFI of the population is about 40-fold above wild type. As expected, the addition of *recA4162* and *recA4164* to the *recA730* mutant decreased both the percentage of cells in the population that had high levels of SOS^{C} expression and the average RFI across the population to just 2-fold above wild type (or a 20 fold decrease from *recA730*). Table 1 shows that the series of *recA4142* strains with and without the suppressors mutations reveal a pattern very similar to the *recA730* series. It is notable that *recA4142,4162* displayed no SOS^{C} expression at 42°C unlike its *recA730* counterpart (data not shown). It is possible that the low levels of SOS^{C} expression in the *recA4142,4162* and *recA4142,4164* were due the inactivation of the RecA protein. Table 2 shows that these double mutants are all active as wild type for conjugal recombination, survival after UV irradiation (DNA repair) and induction of the SOS response after UV treatment. Therefore, we conclude that the suppression provided by *recA4162* and *recA4164* is not allele specific since they can suppress the SOS^{C} expression of at least two different *recA*^{C} alleles that produce SOS^{C} expression by very different mechanisms and that the suppression is not due to inactivation of the RecA protein for its normal functions.

recA4162 or recA4164 suppress SOS^{C} expression in trans

RecA polymerizes on ssDNA in a head to tail fashion to create a RecA-DNA filament. Given this type of arrangement, it is possible that the mechanism of suppression of SOS^{C} expression by RecA4162 and RecA4164 is not due to suppression within individual RecA monomers but is manifested at the level of the filament; possibly through interaction of adjacent monomers or some conformation of the filament.

To test if these suppressors can act *in trans*, a 4.5 kb *Bam* HI fragment carrying the *recA4162* and *recA4164* genes and their surrounding regions (this includes *ygaD1::kan* upstream of *recA*, the wild type *recA* promoter before the *recA* gene and the *recX* gene that follows immediately) were cloned into a low-copy vector pDPT429 (Taylor and Cohen, 1979). These plasmids were used to transform cells containing *recA730* or *recA4142*. Table 1 shows that when *recA4162* or *recA4164* is expressed *in trans* in a *recA730* mutant, an eight-fold decrease in RFI is seen. This suggests that both suppressor mutations are dominant to *recA730*. It is possible that the decrease is not due to a specific property of the suppressor mutation and that a copy of *recA*$^{+}$ on a plasmid would also decrease SOS^{C} expression. To test this, *recA*$^{+}$ was expressed *in trans* on a plasmid with *recA730* on the chromosome. Table 1 shows that no significant decrease in RFI is seen. This suggests that the decrease is specific to the suppressor mutations and that *recA730* is dominant to wild type for this phenotype.

These series of experiments were then repeated for cells containing *recA4142*. Similar results are seen when the *recA4162* or *recA4164* plasmids were placed in the *recA4142* strain: an eight-fold decrease in RFI (Table 1). A difference, however, was seen when *recA*$^{+}$

is expressed *in trans* with *recA4142*. Here a two-fold decrease in the RFI is seen when compared to the *recA4142* strain with vector alone. These results suggest that the two suppressor mutations are also dominant to *recA4142* and that although $recA^+$ can achieve some degree of suppression of the SOS^C phenotype when expressed *in trans*, full suppression requires the suppressor mutation.

It is possible that the decrease seen occurs because the suppressor genes on the plasmids are expressed at a higher level than that of the chromosomal $recA^C$ alleles and thus their phenotype dominates. Therefore the situations were reversed; *recA730* or *recA4142* were cloned and expressed from the low copy vector and *recA4162* and *recA4164* were expressed from the chromosome. With one minor exception, similar patterns of SOS^C expression and suppression were seen (Table 1). The minor exception is that the plasmid containing *recA4142* in a *recA* deletion strain showed a population of cells that were only 28-fold above background instead of 34-fold and the percentage of cells having six-fold above background RFI was only 81% instead of 100%. Although the cause of this small decrease is not known, it is possible that this is due to the fact that the plasmid version of *recA4142* contains $recAo^+$ instead of *recAo1403*. Nonetheless, when this plasmid was placed in strains with *recA4162* and *recA4164* on the chromosome, SOS^C expression was found to be similar to background levels (Table 1).

It is possible that the level of SOS^C expression seen in the *in trans* experiment is due to the fact that the suppressor alleles by themselves are inactive or that when the two alleles are mixed (suppressor and $recA^C$), they inactivate one another. These ideas were tested. Table 2 shows that two mutants harboring the suppressor allele are as active for recombination, DNA repair and induction of SOS after UV treatment as wild type. Surprisingly, these strains show a low level of SOS^C expression by themselves (Table 1). Additionally, the recombination proficiency and UV-induced SOS expression was tested for all four *in trans* combinations of suppressor and $recA^C$ allele. They were found to be Rec^+ and have UV-induced SOS expression like wild type (data not shown).

These results show that when *recA4162* or *recA4164* are expressed *in trans* with either *recA730* or *recA4142*, suppression of constitutive SOS expression is seen suggesting that the mechanism of suppression occurs at the level of the filament.

The combination of RecX and UvrD are required for suppression of SOS^C expression by *recA4162* and *recA4164*

The *in trans* experiments above suggested that suppression should be occurring at the level of the RecA filament and not at the level of the RecA monomer. It is known that at least two proteins interact with RecA filaments and destabilize the filaments or remove RecA from the DNA. These are RecX and UvrD respectively. To test if RecX and or UvrD are required to suppress the SOS^C expression produced by *recA730* and *recA4142*, the four double mutants (*recA730,4162*, *recA730,4264*, *recA4142,4162* and *recA4142,4164*) were combined singly with either a *del(recX)* or a *del(uvrD)* mutation. If these gene products are required to suppress the SOS^C expression, then their removal should result in an increase in SOS^C expression. Table 3 shows that individually, deletion of just *recX* had little effect. Removal of *uvrD*, however, revealed a modest 2–4 fold increase in RFI in *recA730,4162*, *recA4142,4162* and *recA4142,4164* over a $recA^+$ strain. Removal of both *uvrD* and *recX* had a much larger, about an 8-fold increase in *recA730,4164* and *recA4142,4162* strains over a $recA^+$ strain. Table 3 shows that the *recA730,4162* and *recA4142,4164* mutants were not additionally affected. From this we can tentatively conclude that at least two of the suppressor mutations require the action of the UvrD and RecX proteins to inhibit the SOS^C expression of RecA730 and RecA4142. There may, however, be other proteins and or mechanisms that are also required for inhibition since two of the mutants showed only small,

if any, increases in the absence of RecX and UvrD and none of the four mutants showed full levels of SOS^C expression (*e.g.*, equal to that of a *recA730* mutant).

Differential ability of *uvrD* missense mutant to aid in suppression

UvrD deletion mutant are UV^S, hyper-recombinogenic and mutators. Biochemically, UvrD has been shown to have ATPase activity, helicase/translocase activity, participate in Nucleotide Excision Repair (NER) reactions, Methyl-Directed Mismatch (MMR) repair reactions and remove RecA from ssDNA (Atkinson *et al.*, 2009; Matson and Robertson, 2006; Veaute *et al.*, 2005). *uvrD3* (E387K), *uvrD252* (G30D), *uvrD701* (deletion of the C-terminal 40 aa) and *uvrD307* (R284A) were combined with either *recA730,4162* or *recA4142,4164* to test if they can aid the suppression of SOS^C expression in the absence of *recX*. Figure 2 shows that *uvrD307* and *uvrD252* revealed the highest levels of SOS^C expression of the four *uvrD* alleles tested. SOS^C expression in the *uvrD3* and *uvrD701* strains were very low like $uvrD^+$ for both *recA* mutants, whereas *uvrD307* and *uvrD252* showed higher levels. In general, higher levels were seen with *recA4142,4164* than with *recA730,4162*. Interestingly, when compared to the null mutant, only the *uvrD252* allele showed nearly the same average RFI, but that the percentage of cells in the population producing SOS expression six-fold above background was much lower (57% compared to 95%). From these we conclude that different *uvrD* alleles have differential ability to aid in the suppression.

Other mutations in genes required for NER and MMR do not increase SOS^C expression in the *recA recX* double mutants like *del(uvrD)*

Since it is known that UvrD participates in two DNA repair processes in the cell: NER and MMR and that cells deficient in these processes presumably have higher backgrounds of DNA damage, this in turn could be responsible for the increase in SOS^C expression we observed in the *recA730,4164 del(recX) del(uvrD)* and *recA4142,4164 del(recX) del(uvrD)* mutants. To test this, we combined null mutations in *uvrA*, *uvrB*, *mutH*, *mutL* and *mutS* with *recA730,4164 del(recX)* and *recA4142,4164 del(recX)* and measure the level of SOS^C expression. Table 4 shows that, relative to the $recA^+$ $recX^+$ strains, there are not any significant increases in SOS^C expression when any of the five mutations are added to the two mutants above with the exception of *uvrA* mutation in the *recA730,4164 del(recX)* strain. Here, about a two-fold increase is seen. In summary, across the 10 strains (the five pairs of mutants), there is much less SOS^C expression than when *del(uvrD)* is added to these two combinations of mutations (Table 3 and Figure 2). This supports the idea that the increase in SOS^C expression seen in *recA730,4164 del(recX) del(uvrD)* and *recA4142,4164 del(recX) del(uvrD)* mutants is due to absence of UvrD's activity in removing RecA from the DNA and not from an increase in background levels of DNA damage.

DISCUSSION

Intragenic suppressors of the SOS^C phenotype of *recA730* have been known for many years, but very little was known about how these mutations suppressed SOS^C expression. The amino acid changes of *recA4162* (I298V) and *recA4164* (L126V) mapped to different parts of RecA and it was not clear if they worked by similar or different mechanisms. This paper shows that the ability of these suppressor mutations to inhibit SOS^C is not allele specific, they can suppress *in cis* or *in trans* and that they are likely to operate at the level of the filament possibly to better attract and or respond to UvrD and RecX. The results also showed, however, that all alleles were not equally affected by the absence of UvrD and RecX. *recA730,4164* and *recA4142,4162* revealed little or no increase when either UvrD or RecX were removed, but when both were removed, a fairly large increase in SOS^C expression is seen suggesting that the two gene products act independently to suppress

SOS^C expression. This was not seen, however, with *recA4142,4164*. Here, *recA4142,4164* showed an increase in SOS^C expression when UvrD was removed, but did not show an additional increase when RecX was also removed. Lastly, *recA730,4162* showed very little increase when either or both UvrD and RecX were removed. Thus, it is very difficult to unambiguously and systematically categorize all these changes since all four *recA* mutants did not respond equally to the absence of either RecX or UvrD alone or both at once. One reason why all four *recA* alleles may not have behaved the same with both the *uvrD* and *recX* mutations is that some other mechanism may also be operating to inhibit full SOS^C expression. This idea is supported by the observations that all four *recA* mutants did have SOS^C expression at the level of a *recA730* or *recA4142* mutant.

The study of suppressors, whether intragenic or extragenic, has long been a potent tool to investigate molecular mechanisms. The suppressors here serve to suppress the ability of two *recA^C* mutants to turn on the SOS Response when they should not. The suppressor mutations by themselves, or in conjunction with the *recA^C* mutations, do not inhibit RecA's normal ability to function in any way that we have tried to measure (Table 2 and data not shown). Thus, the only function of the suppressor is to inhibit the ability of the *recA^C* mutation to turn on the SOS Response when it should not. These suppressors, however, are very selective in their ability to repress SOS^C expression, they only do so in log phase cells in the absence of external DNA damage. When the cells are treated with UV light, SOS is normally induced in the suppressor strains, whether they are single mutants, *in cis* or *in trans* with the *recA^C* allele. Their ability to discriminate between the two situations suggests that *recA^C* mutants binding to DNA *in vivo* is somehow different from that when normal SOS is induced. Perhaps the DNA substrate, its location or context in the cell is different.

How might the suppressors function? Evidence supports the notion that not all RecA filaments in the cell induce the SOS Response. It is known that about 15% of cells in a log phase population have RecA filaments as measured by RecA-GFP formation or the effects of RecA-mediated of recombination, but less than about 0.5% of the population are induced for SOS ((McCool *et al.*, 2004; Pennington and Rosenberg, 2007; Renzette *et al.*, 2005; Steiner and Kuempel, 1998) and summarized in (Long *et al.*, 2008)). Thus, there must be some difference between the filaments that induce the SOS response and those that form in log phase cells presumably to fix DNA damage caused by normal metabolic functions. While the specific differences are not known, it has been hypothesized that the SOS inducing filaments could be longer or have a slightly different conformation. Here, it is further suggested that RecA filaments may have slightly different forms as the cell progresses through the stages of the SOS response. In particular, *recA^C* mutants may resemble those filaments that induce the SOS Response (after UV treatment) and this is the reason why when they are loaded onto the DNA, they promote LexA auto-cleavage. As the damage is repaired, the SOS Response should decrease. A necessary part of this is the disassembly of the RecA filaments. It is possible that at this point, the RecA filaments adopt a slightly different structure making it more susceptible to the destabilizing and dismantling effects of RecX and UvrD. The fact that *uvrD* and *recX* are induced during the SOS response and would be in higher quantities at the end of the SOS response may also aid in this process. Since the suppressor mutations (*recA4162* and *recA4164*) are dominant to the *recA^C* mutations (*recA730* and *recA4142*) *in cis* or *in trans*, it suggests that turning off SOS is dominant to turning it on. This seems like an appropriate way to prevent SOS expression unless it is absolutely necessary.

There were significant differences between the abilities of the different alleles of *uvrD* to aid in the suppression. Each of the *uvrD* mutants tested has been characterized for standard *uvrD* phenotypes (the *uvrD* deletion mutant is UV^S, hyper-rec and a mutator; see above). *uvrD701* is missing the C-terminal forty amino acids and behaves as a monomer instead of a

dimer. It is has wild type activity both *in vitro* and *in vivo* (Centore *et al.*, 2009; Mechanic *et al.*, 1999). *uvrD307* (R284A) is mutant in a highly conserved helicase motif close to the P-loop motif. Genetic studies show that this mutant is UV^S, hyper-rec and a mutator like a *uvrD* deletion mutant (Hall and Matson, 1997; Zhang *et al.*, 1997). *In vitro*, it has ability to bind DNA like wild type, but has a greatly decreased ability to bind ATP and almost no helicase activity. The crystal structure of UvrD (Lee and Yang, 2006) shows that *uvrD252* (G30D) (also called *recL152* (Rothman and Fried, 1984)) is located very close to the *uvrD307* (R284A) mutation and the P-loop motif. *In vivo*, *uvrD252* mutants are UV^S. The literature is, however, controversial on the mutator and hyper-rec phenotypes of *uvrD252*. It has reported that *uvrD252* strains have the ability to act with wild type (Washburn and Kushner, 1991), low (Arthur and Lloyd, 1980) or high (Marinus, 1980) mutator activity. Similarly, the hyper-rec phenotype has been reported to be either low ((Zieg *et al.*, 1978) and our unpublished results) or high (Arthur and Lloyd, 1980). Although here, the methods for measuring the hyper-rec phenotype were quite different. In the former case, recombinants were measured and in the latter case, transcription from recombinational intermediates was measured. This mutant also maintains the ability to ameliorate the negative effects of recombination proteins at certain types of stopped replication forks (Lestini and Michel, 2007, 2008). *In vitro*, it has reduced ATP binding and helicase activity (Washburn and Kushner, 1993). *uvrD3* (E387K) is located in the 2B domain thought to be important in the regulation of the helicase activity (Brendza *et al.*, 2005). *uvrD3* (E387K) mutants are dominant, UV^S and do not display a mutator, but have a hyper-rec phenotype (Maples and Kushner, 1982; Marinus, 1980; Ogawa *et al.*, 1968; Zieg *et al.*, 1978). Thus the data is consistent with the interpretation that *uvrD3* is a partial activity mutant that can still undo SOS^C RecA filaments, but cannot undo RecA filaments that lead to the hyper-rec phenotype. *UvrD252* is then a mutant of the opposite type; it can still undo recombinational intermediates (this assumes that *uvrD252* is not hyper-rec and that Arthur and Lloyd measured some other phenotype of *uvrD252* with their assay), but not SOS^C RecA filaments. Lastly, UvrD307 has lost both abilities. Biochemically, the inability to aid *recA4162* or *recA4164* in suppression of SOS^C expression correlates with the low levels of helicase activity, the inability to bind ATP and the region of the protein that binds ATP. It is therefore possible that UvrD exerts its destabilizing effect through its helicase activity or through a special conformation it might adopt when bound with a nucleotide.

Lastly, it was shown that an inability to do NER or MMR through mutations in *uvrA*, *uvrB*, *mutH*, *mutL* and *mutS* does not increase the ability of *recA730,4164 del(recX)* and *recA4142,4164 del(recX)* to produce SOS^C expression like a *del(uvrD)* mutation. This supports the idea that UvrD has a specific role in repression of the SOS^C expression and this increase does not correspond to a lack of DNA repair capacity in cells lacking the NER and MMR pathways. It is also known that UvrAB and MutL can aid UvrD in loading onto certain substrates and augment its activity (reviewed in (Matson and Robertson, 2006) and (Atkinson *et al.*, 2009)). Since *uvrA*, *uvrB* and *mutL* mutants do not display high levels of SOS^C like *del(uvrD)* mutants, one can speculate that either UvrD has an UvrAB-MutL-independent method to load onto the DNA or it does not need to load onto the DNA to repress SOS^C expression. Further experiments will be necessary to test these ideas.

EXPERIMENTAL PROCEEDURES

Bacterial strains

All bacterial strains used in this work are derivatives of *E. coli* K-12 and are described in Table 5. The protocol for P1 transduction has been described elsewhere (Willetts *et al.*, 1969). 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 media on which they were selected. When necessary, the *recA* alleles were placed on the chromosome in the place of *recA*$^+$ as previously described (see below). Table 2 shows the characterization of these mutants for their survival to UV irradiation, ability to inherit markers during conjugation and the ability to induce the SOS response. Specific protocols for these tests have been previously described (Sandler *et al.*, 1996).

Construction of plasmids

All plasmids used in this work are derivatives of a low copy number vector called pDPT429 (Taylor and Cohen, 1979). All plasmids have a 4.5 kb *Bam*HI fragment cloned into the *Bam*HI site of pDPT429 in the same orientation. The *Bam*HI fragment contain *ygaD1::kan* {*kan* gene inserted at *Bsi*WI site (Renzette *et al.*, 2005)}, *recA* (some contain different alleles as indicated) and *recX* genes. In all cases where the construction called for PCR amplification, these sequences were confirmed by DNA sequence analysis.

To create the plasmid pNR122 (containing *recA*$^+$), pNR117 and pDPT429 was restricted with *Bam*HI. pNR117 is a derivative of pBR322 with the 4.5 kb *Bam*HI fragment containing *ygaD1::kan recAo*$^+$ *recA*$^+$ *recX*$^+$. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA*+. This plasmid is called pNR122.

To create the plasmid pNR123 (containing *recA4162*), pRecAN99 (gift from Steve Kowalczykowski) (Mirshad and Kowalczykowski, 2003) and pSJS1373 (like pNR117 but with *recA803*) were first restricted with *Rsr*II and *Pme*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA803*, *N99.* This plasmid is called pNR64. The *recAI298V* fragment was then amplified using prSJS453 (5′ GAAATCTACGGACCGGAATCTTCCGG3′) and prSJS472 (5′ TCTTCTCCTTTACTGATGCTCCCAAAATCTTCGTTAGTTTCTGC3′) with SS5296 as the template DNA. The resulting fragment was then restricted with *Rsr*II and *Kpn*I and ligated into the same sites of pNR64 to produce a plasmid containing *ygaD1::kan recA803,4162*. This plasmid is called pNR102. pNR102 and pNR115 was then restricted with *Rsr*II and *Kpn*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA4162.* This plasmid is called pNR118. pNR118 and pDPT429 were then restricted with *Bam*HI. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA4162.* This plasmid is called pNR123.

To create the plasmid pNR124 (containing *recA4164*), pSJS1337 (Renzette and Sandler, 2008) and pJC869 (Madiraju *et al.*, 1988) was first restricted with *Hind*III and *Sph*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *recA803,2201.* This plasmid is called pNR53. pNR53 and pSJS1373 was then restricted with *Rsr*II and *Pme*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA803,2201.* This plasmid is called pEL12. The *recA4164* fragment was then amplified using pSJS453 and prSJS472 with SS5292 as the template DNA. The resulting fragment was then restricted with *Rsr*II and *Blp*I and ligated into the same sites of pEL12 to produce a plasmid containing *ygaD1::kan recA803,4164.* This plasmid is called pNR106. pNR106 and pNR115 were then restricted with *Rsr*II and *Kpn*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA4164.* This plasmid is called pNR119. pNR119 and pDPT429 were then restricted with *Bam*HI. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA4164.* This plasmid is called pNR124.

To create pEL16 (containing *recA4142*), pSJS1373 and pDPT429 were restricted with *Bam*HI. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA803.* This plasmid is called pNR105. pNR105 and pNR115 (Long *et al.*, 2008) was then restricted with *RsrII* and *KpnI*. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA recX.* This plasmid is called pNR117. pNR115 and pNR117 was then restricted with *Bpi*I and *Rsr*II. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA4142 recX*. This plasmid is called pEL15. pEL15 and pDPT429 were then restricted with *Bam*HI. The appropriate fragments were isolated, mixed and treated to produce a plasmid containing *ygaD1::kan recA4142 recX*. This plasmid is called pEL16.

To create the plasmid pNR127 (containing *recA730*), pEAW305 (gift from Mike Cox) and pSJS1373 were restricted with *Nco*I and *Pme*I. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA730*. This plasmid is called pNR59. pNR59 and pDPT429 were then restricted with *Bam*HI. The appropriate fragments were isolated, mixed and treated with DNA ligase to produce a plasmid containing *ygaD1::kan recA730.* This plasmid is called pNR127.

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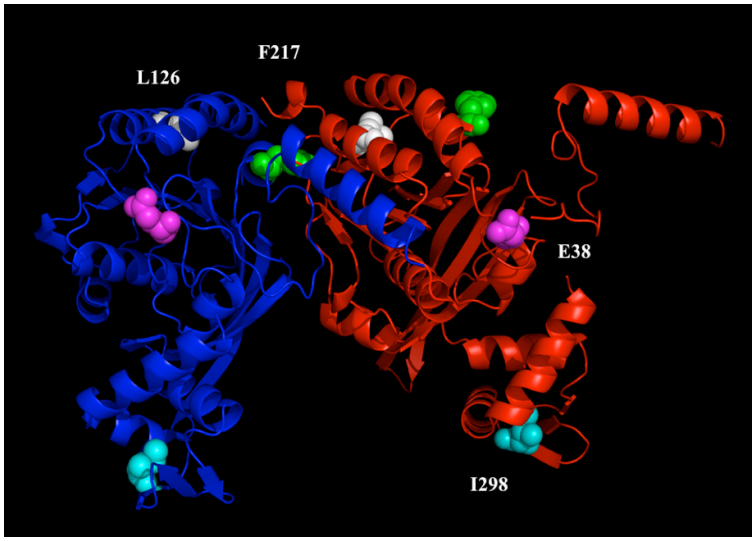

Figure 1.
This figure shows a depiction of two adjacent RecA molecules (red and blue) as they may appear in a helical filament based on the crystal structure without DNA (Story and Steitz, 1992; Story *et al.*, 1992). The green residues represent the positions of F217Y, the magenta residues show the positions of E38K, the white residues are L126V and the cyan residues are I298V.

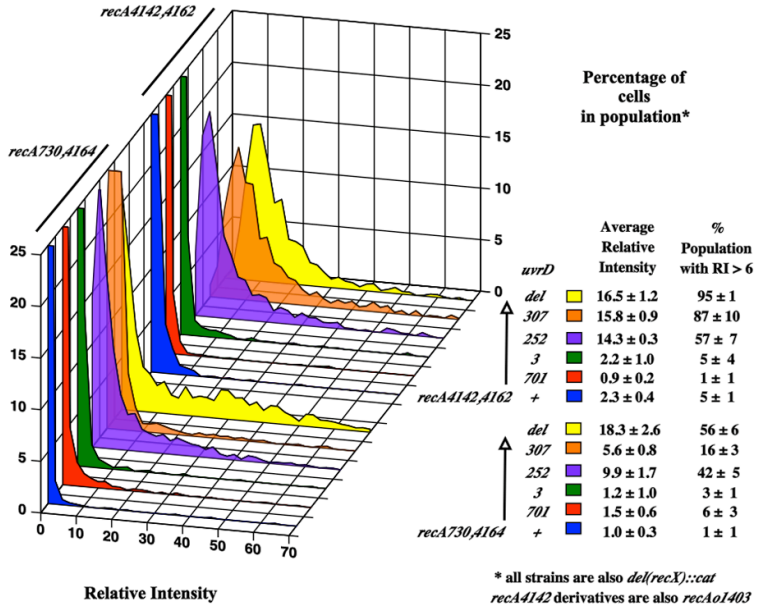

Figure 2.

This figure shows the 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 25%. The strains are in order from top of the graph to the bottom. Unless otherwise indicated, all strains were grown in minimal medium at 37°C with aeration. The strains are: SS7450 {*recA4142,4162 del(recX)::cat del(uvrD)}*, SS7457 (*recA4142,4162 del(recX)::cat uvrD307)*, SS7459 (*recA4142,4162 del(recX)::cat uvrD252)*, SS7489 (*recA4142,4162 del(recX)::cat uvrD3)*, SS7451 (*recA4142,4162 del(recX)::cat uvrD701)*, SS7449 *(recA4142,4162 del(recX)::cat)*, SS7478{*recA730,4164 del(recX)::cat del(uvrD)}*, SS7488 (*recA730,4164 del(recX)::cat uvrD307)*, SS7487 (*recA730,4164 del(recX)::cat uvrD252)*, SS7485 (*recA730,4164 del(recX)::cat uvrD3)*, SS7486 (*recA730,4164 del(recX)::cat uvrD701)*, SS7475 *(recA730,4164 del(recX)::cat)*. The SOS expression ratio of the *recA*$^{+}$ *del(recX)::cat* versions of these *uvrD* mutants are on the order of 1–2 RFI depending on the particular set of mutants (data not shown).

Table 1

Suppression of constitutive SOS expression by *recA4162* and *recA4164 in cis* and *in trans* with *recA730* and *recA4142*

Strains	*Chr.recAo*	*Chr.recA*	*Plasmid recA*	RFI ratio[a]	% of cells expressing SOS^C ≥ 6-fold
SS996	+	+		1.0 ± 0.0	0.2 ± 0.1
SS4629	+	*730*		40.4 ± 7.1	99.6 ± 0.2
SS6013	+	*4142*		3.6 ± 0.7	7.6 ± 2.8
SS6052	+	*4162*		1.8 ± 0.1	3.6 ± 2.9
SS6027	+	*4164*		2.0 ± 0.1	2.0 ± 1.3
SS4630	+	*730,4162* [b]		1.0 ± 0.6	0.3 ± 0.2
SS5292	+	*730,4164*		0.8 ± 0.2	1.0 ± 0.1
SS4976	*o1403*	*4142*		34.5 ± 1.5	100 ± 0.0
SS6089	*o1403*	*4142,4162*		1.0 ± 0.2	0.5 ± 0.4
SS6062	*o1403*	*4142,4164*		2.2 ± 0.2	1.9 ± 1.0
SS5376	+	*730*	pDPT429	56.1 ± 4.3	98.9 ± 1.7
SS6074	+	*730*	*recA*	48.0 ± 8.3	99.8 ± 0.2
SS6077	+	*730*	*recA4162*	7.8 ± 1.9	13.3 ± 1.5
SS6079	+	*730*	*recA4164*	7.1 ± 2.0	18.9 ± 7.3
SS5377	*o1403*	*4142*	pDPT429	34.0 ± 8.9	89.3 ± 18.5
SS6075	*o1403*	*4142*	*recA*	14.9 ± 2.8	81.8 ± 13.1
SS6076	*o1403*	*4142*	*recA4162*	7.0 ± 2.6	15.5 ± 6.7
SS6078	*o1403*	*4142*	*recA4164*	6.6 ± 1.3	10.1 ± 2.0
SS6118	+	*del*	*730*	33.6 ± 2.7	99.2 ± 1.0
SS6086	+	*recA4162*	*730*	1.6 ± 0.5	1.64 ± 1.4
SS6085	+	*recA4164*	*730*	1.0 ± 0.6	1.38 ±0.5
SS6100	+	*del*	*4142*	28.2 ± 1.9	81.5 ± 7.0
SS6081	+	*recA4162*	*4142*	1.7 ± 0.4	3.95 ± 2.0
SS6082	+	*recA4164*	*4142*	2.3 ± 0.7	2.66 ± 2.9

[a] See Materials and Methods for specific protocols. All Strains were grown in minimal medium at 37°C in log phase before sampling. All numbers are the average of three independent measurements. For each strain approximately 1000–2000 cells were counted. All strains having *recA4142* on the chromosome also have *recAo1403*. Plasmid versions of *recA4142* are $recAo^+$. RFI stand for Relative Fluorescence Intensity and is a measure of the SOS^C expression.

[b] *recA730,4162 (recA441)* are sampled at 30°C.

Table 2

Summary of phenotypic analysis of *recA* mutants used in this study[a]

Strain	*recAo*	*recA*	Relative Recombination[b]	% Survival at 5J/m of UV	Average RFI after 5J of UV
SS996	+	+	1.09 ± 0.26	80.0 ± 3.77	8.7 ± 2.8
SS391	+	*938::cat*	0.0006 ± 0.0002	<0.001	ND [d]
SS4629	+	*730*	1.50 ± 0.14	78.0 ± 2.00	ND
SS6013	+	*4142*	1.79 ± 0.34	87.8 ± 6.27	11.1 ± 1.8
SS6052	+	*4162*	1.38 ± 0.36	76.4 ± 4.31	8.1 ± 2.2
SS6027	+	*4164*	1.15 ± 0.16	78.0 ± 2.64	6.7 ± 0.9
SS4630	+	*730,4162*	0.82 ± 0.18[c]	82.2 ± 6.29	8.3 ± 0.81
SS5292	+	*730,4164*	0.79 ± 0.30	83.3 ± 4.32	12.3 ± 3.1
SS4976	*1403*	*4142*	1.11 ± 0.28	83.1 ± 5.61	ND
SS6089	*1403*	*4142,4162*	1.32 ± 0.35	87.1 ± 9.89	10.0 ± 1.1
SS6062	*1403*	*4142,4164*	0.97 ± 0.25	83.6 ± 1.59	11.4 ± 2.0

[a] See Materials and Methods for specific protocols. All numbers are the average of at least three independent measurements.

[b] Relative to JC13509.

[c] The Hfr mating for this Recombination test done at 37°C. The same test done at 30°C is 0.08±0.01.

[d] ND is Not Determined.

Table 3

The effect of *recX* and *uvrD* deletion mutations on the ability of *recA* constitutive allele with suppressor to produce SOS^C expression [a]

Strain	*recA*	*recX*	*uvrD*	RFI ratio[a]	% of cells expressing $SOS^C \geq$ 6-fold
SS996	+	+	+	1.0	0.3
SS4630	*730,4162*[b]	+	+	1.0 ± 0.6	0.3 ± 0.2
SS5292	*730,4164*	+	+	0.8 ± 0.2	1.0 ±0.1
SS6089	*4142,4162*	+	+	1.0 ± 0.2	0.5 ± 0.4
SS6062	*4142,4164*	+	+	2.2 ± 0.2	1.9 ±1.0
SS6080	+	*del*	+	1.7 ± 0.2	1.5 ± 0.6
SS7474	*730,4162*	*del*	+	1.5 ± 0.5	2.5 ± 1.2
SS7475	*730,4164*	*del*	+	1.0 ± 0.3	1.1 ± 1.0
SS7449	*4142,4162*	*del*	+	2.3 ± 0.4	5.3 ± 0.6
SS7476	*4142,4164*	*del*	+	1.5 ± 0.4	4.2 ± 1.8
SS7139	+	+	*del*	1.8 ± 0.6	4.8 ± 1.0
SS7464	*730,4162*	+	*kan*	3.3 ± 0.5	8.6 ±2.5
SS7463	*730,4164*	+	*del*	1.6 ± 0.2	3.4 ± 0.7
SS7434	*4142,4162*	+	*del*	6.2 ± 1.1	25.8 ± 6.8
SS7465	*4142,4164*	+	*del*	7.7 ± 1.5	39.1 ± 10.6
SS7467	+	*del*	*del*	2.0 ± 0.4	7.2 ± 1.5
SS7477	*730,4162*	*del*	*del*	2.6 ± 1.1	5.1 ± 1.9
SS7478	*730,4164*	*del*	*del*	18.3 ± 2.6	56.0 ± 6.2
SS7450	*4142,4162*	*del*	*del*	16.5 ± 1.2	94.5 ± 1.3
SS7479	*4142,4164*	*del*	*del*	5.9 ± 1.6	29.0 ± 13.6

[a] Same as Table 1. All strains having *recA4142* also have *recAo1403*.

[b] All *recA730,4162* strains were measured at 30°C.

Table 4

The effect of NER and MMR mutations on the ability of *recA* constitutive allele with suppressor to produce SOS^C expression[a]

Strain	*recA*	*recX*	*other*	SOS^C expression ratio[a]	% of cells expressing SOS^C ≥ 6-fold
SS5752	+	+	*uvrA*	1.2 ± 0.2	2.9 ± 0.5
SS5767	+	+	*uvrB*	0.9 ± 0.1	0.5 ± 0.4
SS5949	+	+	*mutL*	1.2 ± 0.3	3.4 ± 1.6
SS7604	+	+	*mutH*	1.2 ± 0.3	4.3 ± 2.2
SS7605	+	+	*mutS*	1.4 ± 0.5	3.0 ± 1.7
SS7615	*4142,4162*	*cat*	*uvrA*	1.2 ± 0.6	4.4 ± 1.8
SS7612	*4142,4162*	*cat*	*uvrB*	0.8 ± 0.2	1.5 ± 1.1
SS7611	*4142,4162*	*cat*	*mutL*	1.2 ± 0.2	4.5 ± 1.4
SS7619	*4142,4162*	*cat*	*mutH*	1.6 ± 0.6	3.1 ± 2.2
SS7617	*4142,4162*	*cat*	*mutS*	1.4 ± 0.4	2.6 ± 0.9
SS7616	*730,4164*	*cat*	*uvrA*	1.8 ± 0.6	6.2 ± 0.5
SS7614	*730,4164*	*cat*	*uvrB*	1.3 ± 0.2	2.1 ± 0.8
SS7613	*730,4164*	*cat*	*mutL*	1.5 ± 0.4	2.4 ± 0.2
SS7620	*730,4164*	*cat*	*mutH*	1.1 ± 0.3	3.7 ± 1.2
SS7618	*730,4164*	*cat*	*mutS*	1.0 ± 0.1	2.1 ± 1.6

[a]Same as Table 1. All strains having *recA4142* also have *recAo1403*.

Table 5

Strains used in this work

Strain	*ygaD*	*recAo*	*recA*	*RecX*	*uvrD*	*attλ*	Other relevant genotype	Origin of reference
CAG18491	+	+	+	+	+	+	*metE3079::Tn10*	*E.coli* Stock Center
DM1187	+	+	*730,4162 (recA441)*	+	+	+		D. Mount
GY8382	+	+	*938::cat*	+	+	+		B. Michel
JC13509[a]	+	+	+	+	+	+		Lab Stock
JJC7643	+	+	+	+	*307*	+	*yigE::cat*	B. Michel
K253	+	+	*730,4164 (recA718)*	+	+	+	*srlC300::Tn10*	M. Volkert
KL880	+	+	+	+	*252*	+		B. Low
N14–4	+	+	+	+	*3*	+		B. Low
N3055	+	+	+	+	+	+	*uvrA277::Tn10*	R.G. Lloyd
SS391	+	+	*938::cat*	+	+	+		GY8382→JC13509[c]
SS996	+	+	+	+	+	Ω*gfp*		(McCool *et al.*, 2004)
SS1054	+	+	+	+	+	+	*metE3079::Tn10*	(Centore *et al.*, 2009)
SS1436	+	+	*938::cat*	+	+	Ω*gfp*		(McCool *et al.*, 2004)
SS4070	+	+	+	+	+	+	*del(mutL)100::kan*	(Baba *et al.*, 2006)
SS4073	+	+	+	+	+	+	*del(mutH)100::kan*	(Baba *et al.*, 2006)
SS4539	+	+	+	+	*252*	+		KL880→SS1054[g]
SS4540	+	+	+	+	*3*	+		N14–4→SS1054[g]
SS4558	+	+	+	+	*252*	Ω*gfp*	*gal-76::Tn10*	SS1465→SS4539[c]
SS4559	+	+	+	+	*3*	Ω*gfp*	*gal-76::Tn10*	SS1465→SS4540[c]
SS4570	+	+	+	+	*kan*	+		(Baba *et al.*, 2006)
SS4626	+	+	+	+	+	Ω*gfp*	*zfj-3131::Tn10 alaS5*	(Long *et al.*, 2008)
SS4629	+	+	*730*	+	+	Ω*gfp*		(Long *et al.*, 2008)
SS4630	+	+	*730,4162*	+	+	Ω*gfp*		DM1187→SS4626[e]
SS4976	*kan*	*o1403*	*4142*	+	+	Ω*gfp*		(Long *et al.*, 2008)
SS5130	+	+	+	+	+	+	*del(uvrB)100::kan*	(Baba *et al.*, 2006)
SS5802	+	+	+	+	+	Ω*gfp*	*metE3079::Tn10*	CAG18491→SS996[c]
SS5292	+	+	*730,4164*	+	+	Ω*gfp*	*srlC300::Tn10*	K253→SS996[c]

Strain	*ygaD*	*recAo*	*recA*	*RecX*	*uvrD*	*attλ*	Other relevant genotype	Origin of reference
SS5296	*kan*	+	*803,4162*	+	+	Ω*gfp*		Lab Stock
SS5376	+	+	*730*	+	+	Ω*gfp*		pDPT429→SS4629[d]
SS5377	*kan*	*o1403*	*4142*	+	+	Ω*gfp*		pDPT429→SS4976[d]
SS5737	+	+	+	+	*701*	Ω*gfp*	*gal-76::Tn10*	(Centore *et al.*, 2009)
SS5752	+	+	+	+	+	Ω*gfp*	*uvrA277::Tn10*	N3055→SS996[c]
SS5753	+	+	+	+	*307*	Ω*gfp*	*yigE::cat*	JJC2643→SS5802[g]
SS5767	+	+	+	+	+	Ω*gfp*	*del(uvrB)100::kan*	SS5130→SS996[b]
SS5949	+	+	+	+	+	Ω*gfp*	*del(mutL)100::kan*	SS4070→SS996[b]
SS5958	+	+	*del*(*recA*)*100::kan*	+	+	Ω*gfp*		SS4952→SS996[b]
SS6013	*kan*	+	*4142*	+	+	Ω*gfp*		SS6009→SS996[h]
SS6089	*kan*	*o1403*	*4142,4162*	+	+	Ω*gfp*		SS5323→SS996[b, h]
SS6027	*kan*	+	*4164*	+	+	Ω*gfp*		SS6026→SS996[b, h]
SS6052	*kan*	+	*4162*	+	+	Ω*gfp*		SS6430→SS4630[b, h]
SS6062	*kan*	*o1403*	*4142,4164*	+	+	Ω*gfp*		SS6444→SS996[b, h]
SS6074	+	+	*730*	+	+	Ω*gfp*		pNR122→SS4629[d]
SS6075	*kan*	*o1403*	*4142,4164*	+	+	Ω*gfp*		pNR122→SS4976[d]
SS6076	*kan*	*o1403*	*4142,4164*	+	+	Ω*gfp*		pNR123→SS4976[d]
SS6077	+	+	*730*	+	+	Ω*gfp*		pNR123→SS4629[d]
SS6078	*kan*	*o1403*	*4142,4164*	+	+	Ω*gfp*		pNR124→SS4976[d]
SS6079	+	+	*730*	+	+	Ω*gfp*		pNR124→SS4629[d]
SS6080	+	+	+	*cat*	+	Ω*gfp*		(Long *et al.*, 2008)
SS6081	*kan*	+	*4162*	+	+	Ω*gfp*		pEL16→SS6052[d]
SS6082	*kan*	+	*4164*	+	+	Ω*gfp*		pEL16→SS6027[d]
SS6085	*kan*	+	*4164*	+	+	Ω*gfp*		pNR127→SS6027[d]
SS6086	*kan*	+	*4162*	+	+	Ω*gfp*		pNR127→SS6052[d]
SS6118	+	+	*del*	+	+	Ω*gfp*		pNR127→SS5958[d]
SS6100	+	+	*del*	+	+	Ω*gfp*		pEL16→SS5958[d]

Strain	*ygaD*	*recAo*	*recA*	*RecX*	*uvrD*	*attλ*	Other relevant genotype	Origin of reference
SS7125	+	+	+	+	*kan*	Ω*gfp*		SS4570→SS996[b]
SS7139	+	+	+	+	*del*	Ω*gfp*		SS7134[i]
SS7434	*kan*	*1403*	*4142,4162*	+	*del*	Ω*gfp*		SS5323→SS7139[b]
SS7449	*kan*	*1403*	*4142,4162*	*cat*	+	Ω*gfp*		SS7444→SS996[f]
SS7450	*kan*	*1403*	*4142,4162*	*cat*	*del*	Ω*gfp*		SS7444→SS7139[f]
SS7451	*kan*	*1403*	*4142,4162*	*cat*	*701*	Ω*gfp*	*gal-76::Tn10*	SS7444→SS5737[f]
SS7457	*kan*	*1403*	*4142,4162*	*cat*	*307*	Ω*gfp*	*yigE::cat*	SS7444→SS5753[f]
SS7459	*kan*	*1403*	*4142,4162*	*cat*	*252*	Ω*gfp*		SS7444→SS4558[f]
SS7463	+	+	*730,4164*	+	*del*	Ω*gfp*	*srlC300::Tn10*	K253→SS7139[c]
SS7464	+	+	*730,4162*	+	*kan*	Ω*gfp*		SS4570→SS4630[b]
SS7465	*kan*	*1403*	*4142,4164*	+	*del*	Ω*gfp*		SS6444→SS7139[b]
SS7467	+	+	+	*cat*	*del*	Ω*gfp*		SS6080→SS7139[f]
SS7468	+	+	+	*cat*	*252*	Ω*gfp*		SS6080→SS4558[f]
SS7470	+	+	+	*cat*	*701*	Ω*gfp*		SS6080→SS7139[f]
SS7474	+	+	*730,4162*	*cat*	+	Ω*gfp*		SS7471→SS996[j, f]
SS7475	+	+	*730,4164*	*cat*	+	Ω*gfp*	*srlC300::Tn10*	SS7472→SS996[j, f]
SS7476	*kan*	*1403*	*4142,4164*	*cat*	+	Ω*gfp*		SS7473→SS996[j, f]
SS7477	+	+	*730,4162*	*cat*	*del*	Ω*gfp*		SS7471→SS7139[f]
SS7478	+	+	*730,4164*	*cat*	*del*	Ω*gfp*	*srlC300::Tn10*	SS7472→SS7139[f]
SS7479	*kan*	*1403*	*4142,4164*	*cat*	*del*	Ω*gfp*		SS7473→SS7139[f]
SS7480	+	+	+	*cat*	+	Ω*gfp*	*metE3079::Tn10*	CAG18491→SS6080[c]
SS7481	+	+	+	*cat*	*307*	Ω*gfp*	*yigE::cat*	JJC7643→SS7480[g]
SS7485	+	+	*730,4164*	*cat*	*3*	Ω*gfp*		SS7472→SS4559[f]
SS7486	+	+	*730,4164*	*cat*	*701*	Ω*gfp*		SS7472→SS5737[f]
SS7487	+	+	*730,4164*	*cat*	*252*	Ω*gfp*		SS7472→SS4558[f]
SS7488	+	+	*730,4164*	*cat*	*307*	Ω*gfp*	*yigE::cat srlC300::Tn10*	SS7472→SS5753[c]
SS7489	*kan*	*1403*	*4142,4162*	*cat*	*3*	Ω*gfp*		SS7444→SS4559[f]

Strain	*ygaD*	*recAo*	*recA*	*RecX*	*uvrD*	*attλ*	Other relevant genotype	Origin of reference
SS7604	+	+	+	+	+	*Ωgfp*	*del(mutH)100::kan*	SS4073→SS996[b]
SS7605	+	+	+	+	+	*Ωgfp*	*del(mutS)100::kan*	SS4882→SS996[b]
SS7611	*kan*	*o1403*	*4142,4162*	*cat*	+	*Ωgfp*	*del(mutL)100::kan*	SS7444→SS5949[j, f]
SS7612	*kan*	*o1403*	*4142,4162*	*cat*	+	*Ωgfp*	*del(uvrB)100::kan*	SS7444→SS5767[f]
SS7613	+	+	*730,4164*	*cat*	+	*Ωgfp*	*del(mutL)100::kan srlC300::Tn10*	SS7472→SS5949[j, f]
SS7614	+	+	*730,4164*	*cat*	+	*Ωgfp*	*del(uvrB)100::kan srlC300::Tn10*	SS7472→SS5767[f]
SS7615	*kan*	*1403*	*4142,4162*	*cat*	+	*Ωgfp*	*del(uvrA)100::kan*	SS7444→SS5752[f]
SS7616	+	+	*730,4164*	*cat*	+	*Ωgfp*	*del(uvrA)100::kan srlC300::Tn10*	SS7472→SS5752[f]
SS7617	*kan*	*1403*	*4142,4162*	*cat*	+	*Ωgfp*	*del(mutS)100::kan*	SS7444→SS7605[f]
SS7618	+	+	*730,4164*	*cat*	+	*Ωgfp*	*del(mutS)100::kan srlC300::Tn10*	SS7472→SS7605[f]
SS7619	*kan*	*1403*	*4142,4162*	*cat*	+	*Ωgfp*	*del(mutH)100::kan*	SS7444→SS7604[f]
SS7620	+	+	*730,4164*	*cat*	+	*Ωgfp*	*del(mutH)100::kan srlC300::Tn10*	SS7472→SS7604[f]

[a] JC13509 has the following genotype: *sulB103 lacMS286 φ 80dIIlacBK1 argE3 hi-4 thi-1 xyl-5 mtl-1 rpsL31 tsx*. The *lacMS286φ80dIIlacBK1* code for two partial non-overlapping deletions of the *lac* operon (Konrad, 1977; Zieg and Kushner, 1977). Full notation for *ygaD* mutation is *ygaD1::kan*. Full notation for *Ωgfp* mutation is *Δattλ::sulApΩgfp-mut2*.

[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 Amp^R

[e] Select for $AlaS^+$

[f] Select for Cat^R

[g] Select for Met^+

[h] These *recAo* or *recA* mutations were first constructed on a plasmid as described in the Materials and Methods. They were then transferred to the chromosome using the method of Datsenko and Wanner (Datsenko and Wanner, 2000) using a strain that was *lexA3 malE::Tn10* in a JC13509 background with pKD46 encoding *exo* and *bet*. This original combination of mutants were named and saved as the strain indicated as the donor in this cross.

[i] This deletion allele was created by first transducing the kan resistant derivative from the Keio collection into the strain as indicated in the reference column. pLH29, carrying the *flp* gene, was then introduced and Kan sensitive derivatives were screened.

[j] *recX::cat* was amplified with prSJS748,749 using pACYC184 (New England Biolabs) as a template. *recX::cat* was transferred to the chromosome using the *exo-bet* method next to the *recA* allele indicated. This original combination of mutants were named and saved as the strain indicated as the donor in this cross.