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Specificity in suppression of SOS expression by *recA4162* and *uvrD303*

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Abstract

Detection and repair of DNA damage is essential in all organisms and depends on the ability of proteins recognizing and processing specific DNA substrates. In *E. coli*, the RecA protein forms a filament on single-stranded DNA (ssDNA) produced by DNA damage and induces the SOS response. Previous work has shown that one type of *recA* mutation (*e.g., recA4162* (*I298V*)) and one type of *uvrD* mutation (*e.g., uvrD303* (D403A, D404A)) can differentially decrease SOS expression depending on the type of inducing treatments (UV damage versus RecA mutants that constitutively express SOS). Here it is tested using other SOS inducing conditions if there is a general feature of ssDNA generated during these treatments that allows *recA4162* and *uvrD303* to decrease SOS expression. The SOS inducing conditions tested include growing cells containing temperature-sensitive DNA replication mutations (*dnaE486, dnaG2903, dnaN159, dnaZ2016* (at 37°C)), a *del(polA)501* mutation and induction of Double-Strand Breaks (DSBs). *uvrD303* could decrease SOS expression under all conditions, while *recA4162* could decrease SOS expression under all conditions except in the *polA* strain or when DSBs occur. It is hypothesized that *recA4162* suppresses SOS expression best when the ssDNA occurs at a gap and that *uvrD303* is able to decrease SOS expression when the ssDNA is either at a gap or when it is generated at a DSB (but does so better at a gap).

Keywords

SOS Response; homologous recombination; DNA repair; DNA replication

INTRODUCTION

DNA damage-inducible responses are found in almost every organism. In eukaryotes, these are often regulated by the ATR and ATM kinases, which activate the signal transduction pathways that coordinate cell division and genome duplication [1]. In *Escherichia coli* (and many other bacteria [2]), the SOS Response is regulated at the level of transcription by the RecA and LexA proteins [3–6]. While many studies on the SOS response have focused on its induction after treatment with DNA damaging agents such as mitomycin C or UV irradiation [7, 8], induction of the SOS response also occurs during conjugation [9], cell

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envelope stress [10] and after treatment with β-lactam antibiotics [11, 12]. The latter is of particular importance because induction of SOS produces mutagenic polymerases that then can increase the likelihood of cells becoming resistant to that antibiotic [13–15]. The SOS response also plays a role in persistence [16, 17], regulation of integrons [18], the induction of bacterial programmed cell death through the activation of toxin-antitoxin systems [19], expression of some drug resistance determinants [20] and is crucial for the pathogenicity of some bacteria [21].

At homeostasis in log phase cells, LexA binds to sites in promoters of at least 40 genes repressing transcription [22, 23]. It also binds at other sites on the chromosome not in promoter regions. The function of these sites, if any, is yet to be determined [24]. It is thought that the processing of DNA damage activates the SOS response by liberating regions of ssDNA to which RecA can bind and polymerize to form a nucleoprotein filament. This filament is an allosteric effector of LexA auto-proteolysis [25, 26]. When the level of LexA decreases sufficiently in the cell, these promoters become active and increase the expression of the SOS genes, which aid in the cell's ability to survive the DNA damage. Eventually, as the damage is repaired, the amount of ssDNA shrinks and the level of LexA rises to turn off SOS and complete the cycle.

The SOS Response has been most studied under conditions of external DNA damaging agents such as UV irradiation where there are typically many lesions per chromosome [7]. It is also known that replication forks routinely encounter "housekeeping" types of DNA damage [27]. These could include damaged bases, nicks in the DNA or protein blocks [28–30]. Although RecA participates in repair of these types of lesions through its ability to form a RecA-DNA filament, it is clear that the SOS response is not usually induced. This is best demonstrated by the observations that about 15–25% of a population of log phase cells have recombination structures at any one time [31–33], yet less than 1% are induced for SOS [34, 35]. Recently, it has been shown that *radA*, the amount of RecA in the cell, and in some cases *recX*, prevent these RecA filaments from inducing the SOS response when presumably fixing housekeeping types of damage [31]. Thus, the cell has the ability to discriminate between types and/or amounts of DNA damage to induce the SOS response. Presumably this depends on when and where RecA can polymerize on ssDNA to produce filaments as well as their duration in the cell and their accessibility to LexA.

Historically, research on SOS regulation has focused on mutants that are defective in this regulation. Two types of regulatory mutants have been described for *recA*. The first type constitutively expresses SOS in the absence of external DNA damage. Several of these types of mutants have been described (reviewed in [36]). It has been shown for two of these mutants, *recA4142* (F217Y) and *recA730* (E38K), that while they both cause SOS constitutive (SOS^C) expression, they do so through different mechanisms [37–39]. SOS^C expression in *recA4142* mutants depends on several genes: *recBCD, ruvAB, recJ* and *sbcB* [37]. It was proposed that RecBCD loads RecA4142 onto the ends of a replication fork that has been reversed by RuvAB and tailored by RecJ and SbcB. SOS^C expression in a *recA730* mutant is not dependent on any of these genes. RecA730 is thought to bind to ssDNA on the lagging strand at a replication fork, although there is no direct data supporting this model.

Another type of SOS regulatory mutant that has been isolated in *recA* is one that genetically suppresses the SOS^C expression of *recA4142* and *recA730*. Two alleles of this type, *recA4162* (I298V) and *recA4164* (L126V), have been isolated ([39] and references therein). They are able to inhibit the SOS^C expression of *recA730* and *recA4142* both intragenically (*in cis*) and extragenically (*in trans*) [39]. This inhibition depends on both *uvrD* and *recX*. These two proteins are known to destabilize RecA-DNA filaments under certain conditions both *in vivo* and *in vitro* [40–46]. It was hypothesized that RecA4162 and RecA4164 better

respond to and/or recruit UvrD and RecX to destabilize the filaments and thus lower SOS expression. It was also shown that *recA4162* and *recA4164* mutants were Rec^+ UV^R and, importantly, were able to induce the SOS response after UV treatment in a manner similar to wild type [39]. The fact that *recA4162* is able to induce the SOS response under some conditions, but not others, is the impetus for this study. Since *recA4162* and *recA4164* behave in a similar fashion, only *recA4162* will be further considered here.

Besides *recA* mutations, other antagonists of SOS expression exist that affect the ability of the cell to induce SOS in a *recA730* mutant. It was shown that *uvrD303* could reduce SOS expression in a *recA730* mutant background and after UV irradiation [47]. *uvrD303* was constructed by Kushner and colleagues [48]. It has two point mutations (D403A, D404A) located in the 2B subdomain of the protein. UvrD is nearly structurally identical to the Rep helicase [49, 50]. In Rep, the 2B domain is not essential for helicase activity [51]. The 2B domain can rotate (by about 130°), is coupled to nucleotide and DNA binding, and is hypothesized to be important for regulation of helicase activity [52, 53]. Since UvrD303 has up to a 10-fold higher helicase activity than wild type depending upon the substrate tested, it was characterized as a “hyperhelicase” [48]. The *uvrD303* mutant is recombination-deficient, UV-sensitive, has lower mutability and can decrease the levels of RecA activity in the cell via a proposed direct interaction between the C-terminus of UvrD303 and RecA [47, 48]. The only instance reported thus far where UvrD303 is unable to decrease constitutive SOS expression is in a *recA4142* mutant [47]. Hence, it would seem that *uvrD303* some specificity.

Several DNA replication mutants cause SOS expression in the absence of external damage (Table 1). These mutants include *dnaE486, dnaG2903, dnaN159* and *dnaZ2106*. These genes encode several of the proteins in the sub-assemblies of a replication fork. All of these mutants are viable at 30°C and inviable at 42°C. All but *dnaG2903* inhibit DNA replication at the non-permissive temperature (Table 1). Of key importance to this study is that all mutants tested show high levels of SOS expression in the absence of external damage at the semi-permissive temperature of 37°C (Table 1). While the reason for this is not known, it has been hypothesized that the DNA replication fork is destabilized and/or disabled and this creates ssDNA to which RecA can bind and induce the SOS response.

polA501 mutations also have high levels of SOS expression [34]. The reason for this could be at least two fold. First, *polA* mutants have defects in processing Okazaki fragments. These mutants are likely to have many more gaps in the newly synthesized lagging strand DNA than wild type. It is also know that *polA501* is synthetically lethal with *recA* and *recB* mutations [54–57]. Therefore it is likely that some of these gaps may be slow to be repaired (repair of gaps required *polA*) and could be converted into Double Strand Breaks (DSBs) by either the action of nucleases or another round of DNA replication [58, 59]. Thus *polA501* mutants could have either gaps or DSB that could be bound by RecA to trigger SOS induction.

In this study we asked whether *recA4162* or *uvrD303* can lower SOS expression in strains that have high levels of SOS expression due to defects at the replication forks or a DSB produced by I-*Sce*I. It is shown that *uvrD303* decreases SOS expression to a large degree (equal to *recA4162*) in all the DNA replication mutants. It also decreases SOS in a *polA501* mutant and after I-*Sce*I treatment producing a DSB, but to a lesser extent. *recA4162*, however, only inhibits SOS expression in the four temperature sensitive DNA replication mutants. It does not suppress expression in the *polA501* strain or at the induced DSB. The results suggest that the ability of *recA4162* to suppress SOS expression in log phase cells is not general, but very specific to RecA loading events at gaps in the immediate vicinity of the replication fork and not at DSBs. The activity of *uvrD303* can remove RecA from SOS

inducing substrates in all regions of the chromosome to a greater or less degree depending on the substrate. UvrD303 seems to have greater success in lowering SOS when the ssDNA is found at gaps rather then when ssDNA is processed from a DSB.

1.1 MATERIALS and METHODS

1.1.1 Strains and Media

All bacterial strains are derivatives of *E. coli* K-12 and are described in Table 2. The protocol for P1 transduction has been described previously [60]. All P1 transductions were selected on 2% agar plates made with either Luria Broth or 56/2 minimal media [60] supplemented with 0.2% glucose, 0.001% thiamine and specified amino acids. Selection with antibiotics either used 50 μg/ml kanamycin, 25 μg/ml chloramphenicol or 10 μg/ml tetracycline. Transductants were grown at either 30°C or 37°C and purified on the same type of media on which they were selected.

1.1.2 Preparation and analysis of cells for microscopy

The cells for SOS expression were prepared as follows. All cells were grown in minimal media. The temperature-sensitive (Ts) cells were grown in minimal medium at 30°C for 1.5 hours, then shifted to 37°C and grown in log phase for 4 hours. If needed the cells were diluted into growth media to maintain log phase growth. Cells that were not temperature-sensitive were grown identically but at 37°C the entire time. 2 microliters of cells were placed on a 1% agarose pad. A coverslip was then applied on top of the agarose pad. Cells were then imaged under identical settings. Images (phase contrast and fluorescent) were taken on 3 different days and 3 different images for each strain each day. The cells were imaged using a 750 msec exposure and a 100× objective (see Figure 1A for micrographs). These images were analyzed by a combination of MicrobeTracker software [61] and Matlab R2011a software (Mathworks, Inc.). The Relative Fluorescence Intensity [62] for each cell was normalized to the average fluorescence intensity of a JC13509 strain (no *gfp*). Typically between 1000 and 3000 cells are counted for each strain. Statistical analysis of the data was performed using Student's T-Test.

2.1 RESULTS

The main assay used in this study is a microscopic fluorescence assay of individual cells containing a *sulAp-gfp* transcriptional fusion reporter system inserted in the *attλ* site. *sulA* is an SOS gene and this reporter system has been used extensively and described in several other studies [31, 34, 35]. Thus, the strategy used here was to introduce the DNA replication mutations into strains having the *sulAp-gfp* reporter system with different alleles of *recA* and *uvrD* to test the ability of *recA4162* or *uvrD303* to observe if SOS expression occurred normally in these mutants or was lowered. In all cases the temperature-sensitive strains were grown in minimal media at 30°C until early log phase and then placed at 37°C for 4 hours before taking images. The temperature stable cells (no *ts* mutation) were grown only at 37°C. These were assayed for the amount of fluorescence in individual cells through microscopic observation. Figure 1A shows an example of images taken from a strain with no reporter system, a wild type strain with the reporter system, *dnaE486, dnaE486 recA4162* and *dnaE486 uvrD303* strains. The data is presented in two ways. First, the level of SOS expression of the population of cells is reported in the tables by measuring and computing the average Relative Fluorescence Intensity (RFI) (compared to a wild type population with no *sulAp-gfp*) of the cells and by the percentage of the population having nine-fold or greater expression than the average cell in a wild type population (the reason why this level has been chosen is explained elsewhere [31]). Second, the SOS expression of cells across

the population is shown by graphing the percentage of cells in the population (y-axis) with a particular level of SOS expression (x-axis) (Figure 1B).

2.1.1 SOS expression profiles of the five DNA replication mutants

As previous studies on the SOS expression of these replication mutants used different methods (and reporter genes) for detection of SOS expression (references contained in Table 1), we first measured the level of SOS expression in these mutants with the same reporter system and under the same conditions. Table 3 shows that all of the replication mutants had elevated levels of SOS expression in the *recA*$^+$ background. They could be divided into two groups: *dnaE486, dnaG2903* and *dnaZ2016* had an average level of SOS expression about 13-fold above background whereas *polA501* and *dnaN159* had about 35-fold above background. Figure 1B shows that the distribution of SOS expression across the population of cells of the different mutants also fell into two groups. The *dnaE486* and *dnaG2903* strains had a more “normal” distribution whereas *dnaN159, dnaZ2016* and *polA501* had a more “spread out” distributions. It is interesting that the *dnaZ2016* did not fit neatly into either group since it had a low average level of expression, but yet a broad distribution. The reason for this is not yet clear.

2.1.2 recA4162 suppresses the SOS expression in all DNA replication mutants but polA501

To determine the ability of *recA4162* to inhibit SOS expression in the DNA replication mutants, *recA4162* was combined with each of the DNA replication mutations *dnaE486, dnaG2903, dnaN159, dnaZ2016*, and *polA501*. Table 3 shows that the addition of *recA4162* causes a significant decrease in SOS expression in all of the DNA replication mutants, but *polA501*. The largest decrease was approximately 4-fold in the *dnaN159* mutant while the *dnaG, dnaE* and *dnaZ* mutants had just over a two-fold decrease. *recA4162* brought the four mutants down to approximately the same level (5–8 fold above background). As mentioned above there was no significant decrease in SOS expression in the *polA501* mutant. Each double mutation (except *polA501 recA4162*) has a distribution curve that looks approximately like WT (Figure 1B). Note that even though the SOS expression was decreased at the semi-permissive temperature of 37°C in each temperature-sensitive DNA replication mutant, the strains remained temperature-sensitive for growth at the non-permissive temperature of 42°C (data not shown). It is concluded that *recA4162* is able to lower SOS expression in all four temperature sensitive DNA replication mutants in a fashion similar to that of the *recA* constitutive mutants, *recA730* and *recA4142*. Unexpectedly, the SOS expression in a *polA501* strain was unaffected.

2.1.3 UvrD303 (hyper-helicase mutant) suppresses SOS expression in all DNA replication mutants

It had been shown previously that *uvrD303* inhibited SOS expression in *recA730*, but not in *recA4142* mutants [47]. Since SOS expression in *recA730* and *recA4142* mutants and most of the DNA replication mutants could be suppressed by *recA4162*, it was of interest to see if *uvrD303* would have the same effect. To test this, *uvrD303* was combined with each DNA replication mutant. Table 3 shows that *uvrD303* significantly decreases the SOS expression in all replisome mutants tested, including *polA501*. Again, while the amount of inhibition was different for each temperature sensitive DNA replication mutant, the levels were similar to that provided by *recA4162*. It is also notable that although *uvrD303* did decrease the SOS expression in the *polA501* mutant, it did bring it down to the same level as it did in the other DNA replication mutants. It is concluded that *uvrD303* is able to suppress SOS expression in all the DNA replication mutants to a greater or lesser extent depending on the mutant.

2.1.4 *uvrD303*, but not *recA4162*, suppresses SOS expression when DSBs are induced by I-*Sce*I

It is known that *recA4162* does not suppress SOS expression after UV treatment whereas *uvrD303* does so partially [39, 47]. We wanted to investigate the specificity of action of these suppressors on SOS induction after treatment that produces a DSB. To test this, we used a system previously described where the I-*Sce*I restriction homing endonuclease of *S. cerevisiae* is expressed in an inducible fashion from a plasmid, creating a DSB at a specific 18bp sequence that has been placed at a specific locus on the *E. coli* chromosome [63, 64]. It was demonstrated that when the I-*Sce*I endonuclease is expressed, a DSB is generated at this specific site and SOS expression is induced in a RecBCD-dependent fashion [65]. To test whether *uvrD303* and/or *recA4162* could inhibit this SOS expression at induced DSBs, either *recA4162* or *uvrD303* were placed into a background containing an I-*Sce*I site at the *argE* locus. The I-*Sce*I endonuclease was then expressed from an arabinose-inducible promoter encoded on a plasmid (pRC38, described in [63]). Results are summarized in Table 4. When *recA4162* was present, we see no inhibition of SOS compared to wild type. When *uvrD303* was present, however, we observe a two-fold inhibition of SOS over wild type. From this we conclude that UvrD303 is able to inhibit SOS expression to some degree at a DSB but RecA4162 acts like $RecA^+$ and allows full SOS expression.

3.1 DISCUSSION

The SOS response in *E. coli* has served as a model system for how a cell can detect, respond to and repair DNA damage on the molecular level. This study tests the hypothesis that novel mutants in *recA* and *uvrD* may be able to differentially inhibit expression of the SOS response depending on the inducing treatment. The inference from this is that these proteins may be able to detect subtle differences in specific ssDNA substrates or in the environment of the ssDNA such that they can inhibit SOS expression in one situation but not another. Table 5 shows that *uvrD303* suppresses SOS expression at least partially under all conditions tested except that of a *recA4142* mutant. This includes several temperature sensitive DNA replication mutants at a semi-permissive temperature, in the absence of *polA501* and at I-*Sce*I-induced DSBs. The decreases observed, however, in the case of the DSBs, in the *polA501* strain and after UV treatment were partial, only about 2-fold. *recA4162*, on the other hand, suppresses SOS expression in *recA730, recA4142* and the temperature sensitive DNA replication mutants and not in the absence of *polA501* or at I-*Sce*I induced DSBs. The ability of *recA4162* and *uvrD303* to inhibit SOS expression in these situations varies from partial to nearly complete. Their additivity was also investigated in the DNA replication mutants by combining the two mutations, but no further decrease of SOS was observed (SM and SS, unpublished results).

Previous characterization of these DNA replication mutants for SOS expression had been done using different reporter systems, in different types of media and different genetic backgrounds (references in Table 1). Here, these different mutants have been tested for SOS expression by using the same method and conditions. It is noted above that the five DNA replication mutants can be divided into two groups: *dnaE486* and *dnaG2903* on one hand having lower SOS expression and somewhat “normal” distributions of SOS expression cells whereas *dnaN159* and *polA501* had much higher levels of expression and a much broader distribution of cells. The *dnaZ2016* mutant had characteristics of both groups: low expression with a broad distribution. These differences in distribution may be the result of a number of factors including the amount of ssDNA generated, the size of the RecA filaments and their relative stability. The distributions suggest that there is heterogeneity in these structures across the population of cells. Regardless of the shape of the initial distribution for each individual mutant, the distribution SOS expression in the presence of either *recA4162* and *uvrD303* was similar to WT (Figure 1B).

It is interesting that on one hand *dnaN159* and *polA501* mutant both have high levels of SOS expression and similar distributions but yet *recA4162* decreases the level of SOS expression in the *dnaN159* mutant 4-fold while it has no effect on the SOS expression in a *polA501* mutant. Sutton hypothesized that the DnaN159 protein leaves an elevated number of single-strand gaps, particularly on the lagging strand (due to faulty interactions with the α subunit of Pol III), leading to heightened SOS expression [66]. Thus, while *dnaN159* and *polA501* mutants both have defects in processing the newly synthesized lagging strand of DNA, they are likely to differ in the type of substrate they may present to RecA for SOS induction. Sutton argues that *dnaN159* produces gaps and it is argued above that *polA501* produces DSBs. These interpretations are also supported by the observations that *polA* mutants are synthetically lethal with *recB* mutations (see above) and *dnaN159* mutants are not [67]. Based upon this argument and that it has been shown that *recA4162* requires both *recX* and *uvrD* for suppression of SOS expression [39], one model for how RecA4162 decreases SOS expression is that it is better able to recruit RecX and UvrD to destabilize and dismantle the RecA filament at gaps, but not at DSBs. This then leads to the prediction that *recA4162* would have no effect on SOS expression when DSBs are generated. Table 4 shows this to be true. The question then arises of why *recA4162* does not decrease SOS expression after UV treatment. This treatment is known to generate gaps in the newly synthesized DNA [68] and requires *recFOR* and DNA replication for SOS Induction [69, 70]. Other studies have suggested that for full SOS Induction after UV treatment, the inducing process is probably best thought of as a two step process: an initial phase where there is RecFOR-dependent loading of RecA at gaps and then a subsequent phase where there is RecBCD-loading of RecA loading at DSB [71]. Thus it is possible that the SOS expression observed in the *recA4162* strain after UV treatment is due to ssDNA generated at the DSBs that occurs in this second phase. An alternate idea suggests that what is important for RecA4162's ability to decrease SOS expression is not strictly limited to gaps but also considers proximity of the ssDNA to the replication fork. This would explain why *recA4162* is able to decrease SOS of the four DNA replication mutants. To then explain why *recA4162* does not suppress SOS expression after UV treatment, one can invoke the requirement of DNA replication in SOS induction of UV treated cells (see above). When the replication fork moves past the lesion (so that a gap can be formed as a prelude to SOS induction and DNA repair), it also moves the replication fork away from the gap. Thus, RecA4162 may not be able to recruit RecX and UvrD under these conditions. It is important to point out that this distance may not be important for wild type RecA to induce SOS, it merely limits RecA4162 from productively recruiting RecX and UvrD to decrease SOS expression.

Lastly, it is worth noting that UvrD303 is able to decrease SOS expression regardless of the DNA replication allele or treatment. There is still some specificity to where or how UvrD303 can do this since it does not decrease the SOS expression produced in a *recA4142* mutant nor does it decrease SOS levels as much after a DSB, UV treatment or in a *polA501* strain as it does for the DNA replication mutants. Since the three conditions where partial suppression (Table 5) is seen are instances where DSBs are thought to be the substrate from which the ssDNA (RecBCD's helicase and nuclease activities on the double strand end of DNA generates the ssDNA) is generated to allow RecA to bind, it suggests that *uvrD303* is better at decreasing SOS expression when the ssDNA is at a gap than when it is generated via a DSB. It is not clear why *uvrD303* does not reduce SOS expression in a *recA4142* strain at least partially since RecA loading at the double strand end produced during replication fork reversal and at a DSB are both RecBCD-dependent. There are likely other differences between these two situations, as far as RecA filament loading and stability are concerned, that are yet to be elucidated.

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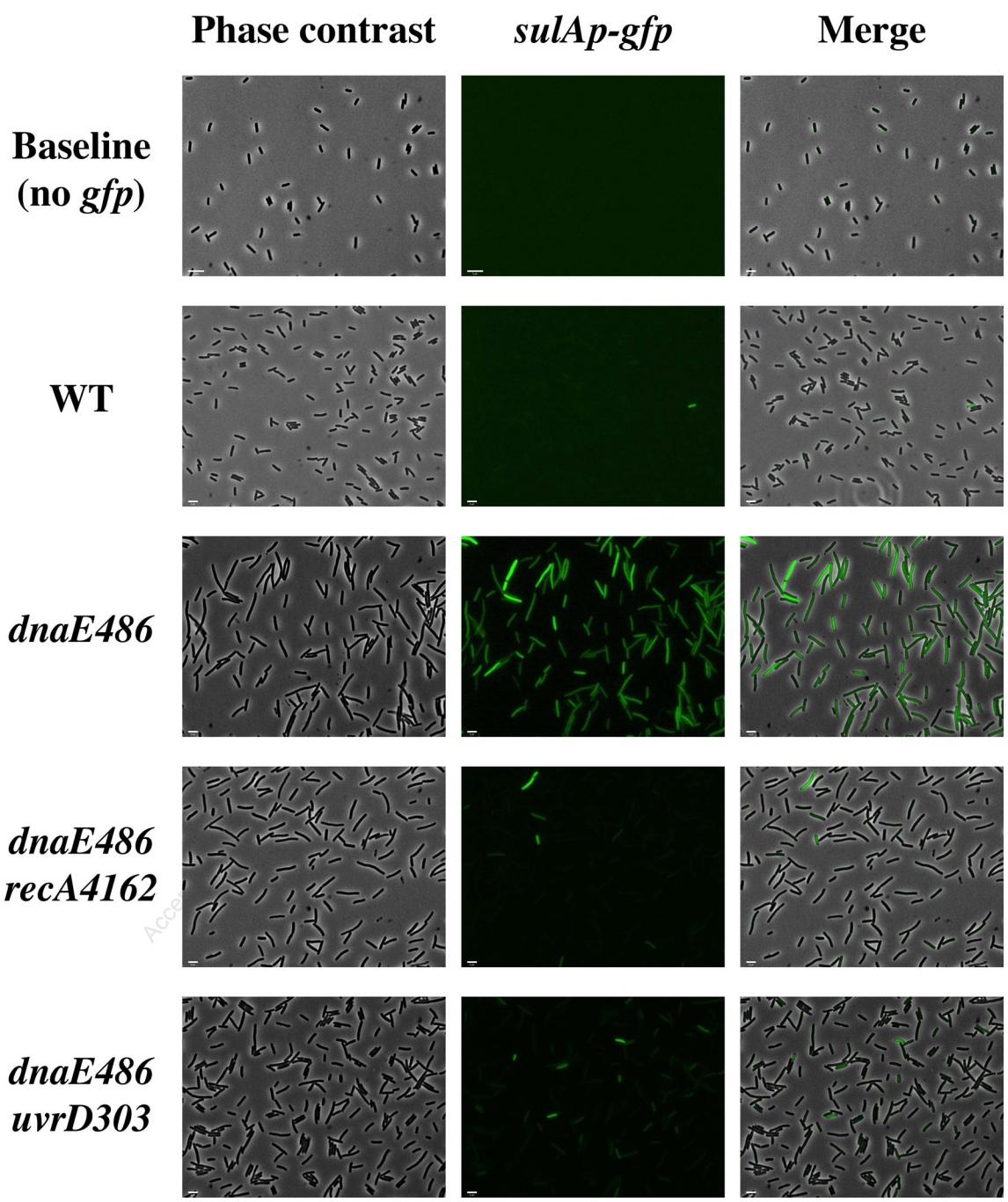
Highlights

- The SOS response can be turned on by a variety of endogenous sources in E. coli
- SOS in DNA replication mutants occurs in the absence of exogenous damage
- Suppressors in recA and uvrD independently can lower this SOS expression
- The recA suppressor is specific to ssDNA at gaps (possibly near the replication fork), not at ssDNA generated at DSBs
- The uvrD suppressor has less specificity, lowering SOS expression at gaps and ssDNA generated at DSBs.
- The uvrD suppressors works better at ssDNA at gaps.



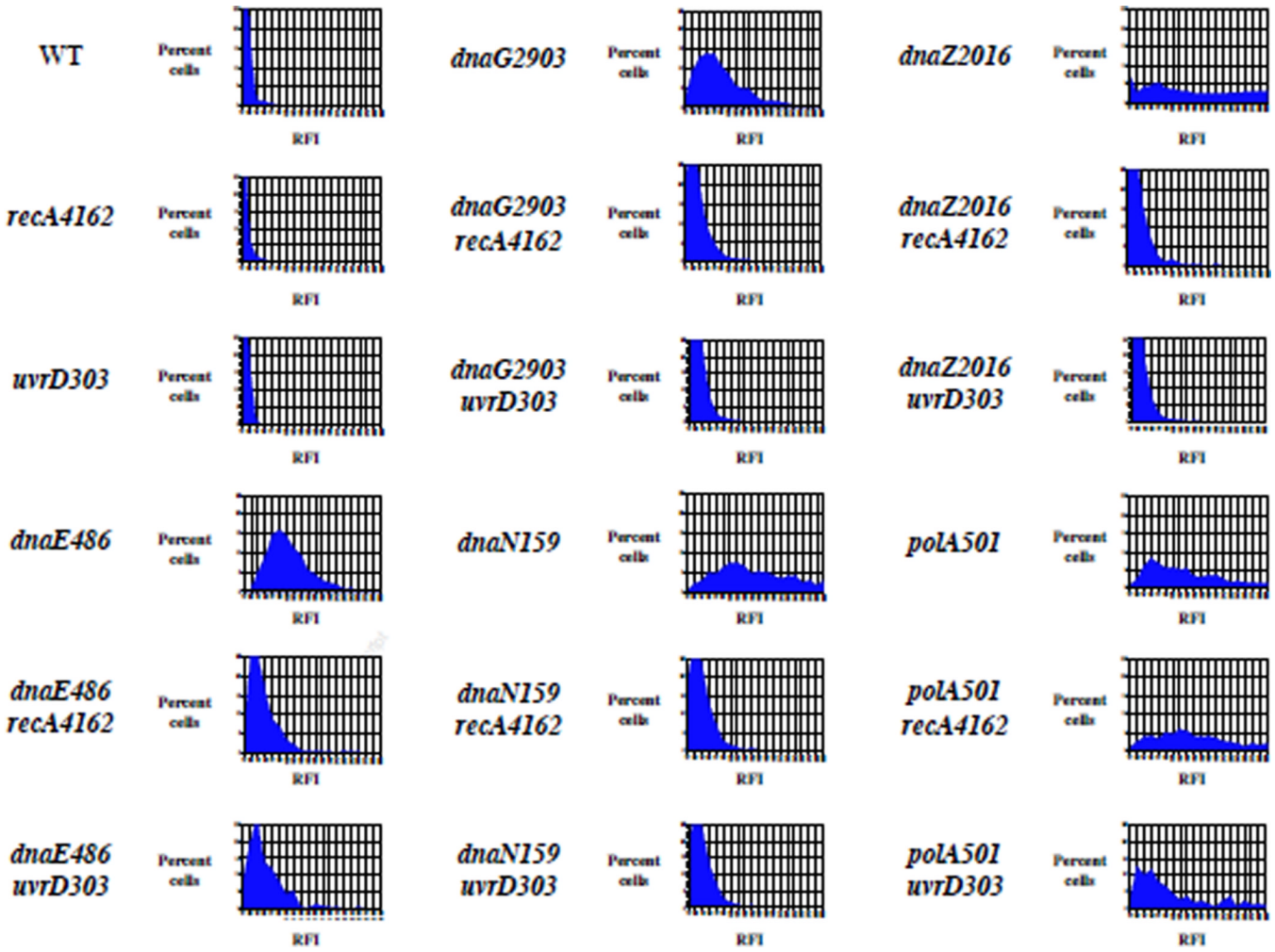


Figure 1. The effects of *recA4162* and *uvrD303* on SOS expression in DNA replication mutants

(A) This figure shows an average snapshot of the SOS induction seen in JC13509 cells (no *gfp*), WT cells and the DNA replication mutant *dnaE486* both alone and under suppression by *recA4162* and *uvrD303*, imaged at 37°C as described in Materials and Methods. **(B)** This figure shows the relative binned data for each strain, with the percentage of cells induced over the relative fluorescence intensity. When combined with *recA4162* or *uvrD303*, each strain but *polA501* shows a decrease in green fluorescence with a concomitant shift in binned data distribution.

Table 1

Summary of the DNA replication mutants used in this study

Mutant gene (amino acid change)	Name of Protein	Function of Protein in DNA Replication	Defects in DNA replication and or Phenotypes for this allele	References
dnaE486 (S885P)	α subunit of DNA Polymerase III	Catalytic subunit of DNA Polymerase III Holoenzyme	Mutator phenotype (Pol V-dependent) and whose interactions with the β clamp may be compromised at high temperatures	[41, 72–74]
dnaG2903 [1] (E567K)	Primase	Primes DNA replication on the lagging strand	Inviable at 42°C, however, no effect on ongoing DNA and RNA primer synthesis. This mutation is located in a poorly conserved region of *dnaG* that mediates interactions with DnaB	[75–78]
dnaN159 (G174A)	β clamp	Processivity subunit	Compromised in interactions with the α subunit at 42°C and is 3-fold more UV^s than wild type	[66, 79]
dnaZ2016 [2] *(aka dnaX2016)* (G118D)	γ subunit clamp loader	Stabilizes interactions between Pol III and DnaB and loads β clamp	Temperature sensitive for DNA replication and cell division (reversible). Defective in ATPase activity and β clamp placement at high temperatures.	[80–84]
del(polA)501	DNA polymerase I	Okazaki fragment maturation	Inviable in rich medium, UV^s, grows poorly.	[34, 85, 86]

[1] Originally known as *dnaP* for phenethyl alcohol resistance [78].

[2] Originally known as *dnaH* [82].

Table 2

Strains used in this work

Strain	*recA*	*dnaG*	*dnaE*	*dnaN*	*dnaZ*	*uvrD*	Other relevant genotype	Source or derivation
AX727	+	+	+	+	*2016*	+		[82]
CAG12152	+	+	+	+	+	+	*zgj-3075::Tn10*	[87, 88]
CAG12152	+	+	+	+	+	+	*zbb-3055::Tn10*	[87, 88]
CAG18436	+	+	+	+	+	+	*zae-502::Tn10*	[87, 88]
CAG18491	+	+	+	+	+	+	*metE3079::Tn10*	[87, 88]
CJ225	+	+	+	+	+	+	*del(polA)501::kan*	[85]
HC120	+	+	+	*159*	+	+		[89]
JC12335	+	+	+	+	+	+	*tnaA300::Tn10*	Lab Strain, [90]
JC13509 *g*	+	+	+	+	+	+		
JC18398	+	+	+	+	*2016*	+	*zbb-3055::Tn10*	CAG12154→AX727 *b*
JC19295	+	+	+	+	+	+	*priA2::kan*	[91]
JW130	+	+	*486*	+	+	+		M. Marinus, [92]
RAB500	+	*2903*	+	+	+	+		[93]
SS996 *e*	+	+	+	+	+	+	*Ωgfp*	[34]
SS1028	+	+	+	*159*	+	+	*tnaA300::Tn10*	JC12235 →HC120 *b*
SS1028	+	+	+	*159*	+	+	*tnaA300::Tn10*	SS1027→JC13509 *b*
SS1054	+	+	+	+	+	+	*metE3079::Tn10*	CAG18491→JC13509 *b*
SS1313	+	+	+	+	*2016*	+	*zbb-3055::Tn10*	JC18398 →JC13509 *b*
SS2306	+	+	+	+	+	+	*del(polA)501::kan Ωgfp*	CJ225→SS996 *d*
SS5268	+	+	+	+	+	+	*del(argE)::I-SceIcs cat Ω gfp*	[63]
SS5457	+	+	+	+	+	+	*del(argE)::I-SceIcs cat Ω gfp*	[63]
SS5496	+	+	+	+	+	*303*		[47]
SS5704	+	+	+	+	+	*303*		SS5496→SS1054 *d*
SS5802	+	+	+	+	+	+	*metE3079::Tn10 Ωgfp*	CAG18491→SS996 *b*
SS5803	+	+	+	+	+	*303*	*Ωgfp*	[47]

Strain	*recA*	*dnaG*	*dnaE*	*dnaN*	*dnaZ*	*uvrD*	Other relevant genotype	Source or derivation
SS6052	*4162*	+	+	+	+	+	*ygaD1::kan Ωgfp*	[39]
SS6222	+	+	*486*	+	+	+	*zae-502::Tn10*	CAG18436→JW130[b]
SS6239	+	+	*486*	+	+	+	*zae-502::Tn10 Ωgfp*	SS6222→SS996[b]
SS6398	*4162*	+	+	+	*2016*	+	*ygaD1::kan zbb-3055::Tn10 Ωgfp*	SS6698→SS6052[b]
SS6399	*4162*	+	+	*159*	+	+	*tnaA300::Tn10 ygaD1::kan Ωgfp*	SS6700→SS6052[b]
SS6656	+	*2903*	+	+	+	+	*Ωgfp*	RAB500[f]→SS996[c]
SS6665	+	*2903*	+	+	+	+	*zgj-3075::Tn10 Ωgfp*	CAG12152→SS6656[b]
SS6668	+	*2903*	+	+	+	+	*zgj-3075::Tn10 Ωgfp*	SS6665→SS996[b]
SS6673	*4162*	*2903*	+	+	+	+	*ygaD1::kan zgj-3075::Tn10 Ωgfp*	SS6668→SS6052[b]
SS6698	+	+	+	+	*2016*	+	*zbb-3055::Tn10 Ωgfp*	SS1313→SS996[b]
SS6700	+	+	+	*159*	+	+	*tnaA300::Tn10 Ωgfp*	SS1028→SS996[b]
SS7719	+	+	*486*	+	+	*303*	*zae-502::Tn10 Ωgfp*	SS6239→SS5803[b]
SS7723	+	+	+	+	*2016*	*303*	*zbb-3055::Tn10 Ωgfp*	SS6698→SS5803[b]
SS7726	+	+	+	*159*	+	*303*	*tnaA300::Tn10 Ωgfp*	SS6700→SS5803[b]
SS7729	+	*2903*	+	+	+	*303*	*zgj-3075::Tn10 Ωgfp*	SS6668→SS5803[b]
SS7742	*4162*	+	*486*	+	+	+	*zae-502::Tn10 Ωgfp*	SS6239→SS6052[b]
SS7765	+	*2903*	+	+	+	*303*	*zgj-3075::Tn10 Ωgfp*	SS6668→SS7756[b]
SS7982	*4162*	+	*486*	+	+	+	*zae-502::Tn10 ygaD1::kan Ωgfp*	SS6239→SS6052 [b]
SS8275	+	+	+	+	+	+	*metE3079::Tn10 del(argE)::I-SceIcs cat Ωgfp*	CAG18491→SS5268 [b]
SS8276	*4162*	+	+	+	+	+	*del(argE)::I-SceIcs cat Ωgfp*	SS6052→SS5268 [c]
SS8277	*4162*	+	+	+	+	+	*del(argE)::I-SceIcs cat Ωgfp*	pRC38→SS8276 [h]
SS8280	+	+	+	+	+	*303*	*del(argE)::I-SceIcs cat Ωgfp*	SS5704→SS8275 [d]
SS8282	+	+	+	+	+	*303*	*del(argE)::I-SceIcs cat Ωgfp*	pRC38→SS8280 [h]
SS8305	+	+	+	+	+	*303*	*Ωgfp*	SS5704→SS5802 [d]
SS8417	*4162*	+	+	+	+	+	*metE3079::Tn10 ygaD1::kan recA4162 Ωgfp*	CAG18491→SS6052 [b]

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Strain	*recA*	*dnaG*	*dnaE*	*dnaN*	*dnaZ*	*uvrD*	Other relevant genotype	Source or derivation
SS8418	+	+	+	+	+	*303*	*metE3079::Tn10 Ωgfp*	CAG18491→SS8305 [b]
SS8474	*4162*	+	+	+	+	+	*del(polA)501::kan ygaD1::kan Ωgfp*	CJ225→SS8417 [d]
SS8477	+	+	+	+	+	*303*	*del(polA)501::kan Ωgfp*	CJ225→SS8418 [d]

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

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

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

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

[e] SS996 has the following genotype: *sulB103 lacMS286* φ80d*IlacBK1* arg*E3 his-4 thi-1 xyl-5 mtl-1 prsL31 tsx del(attB)::psulA-gfp.* The *lacMS286* φ80d*IlacBK1* genes code for two partial nonoverlapping deletions of the *lac* operon.

[f] RAB500 has a kanamycin marker approx. 50% linked (as measured by cotransduction frequency) with *dnaG2903* allele.

[g] JC13509 is the same genotype as SS996, but lacking the *del(attB)::sulAp-gfp.*

[h] Select for ampicillin resistance.

Table 3

Effects of *recA4162* and *uvrD303* on SOS expression in DNA replication mutants

Strain	*recA*	*uvrD*	*dnaE*	*dnaG*	*dnaN*	*dnaZ*	*polA*	Fold SOS expression above background	Percentage of cells with 9-fold or greater SOS expression above background	*p* value [1]
SS996	+	+	+	+	+	+	+	1.9	1.6	
SS6052	*4162*	+	+	+	+	+	+	2.5	2.9	.02
SS5803	+	*303*	+	+	+	+	+	1.4	1.9	<0.001
SS6239	+	+	*486*	+	+	+	+	14.0	94.3	
SS7982	*4162*	+	*486*	+	+	+	+	8.6	43.5	< 0.001
SS7719	+	*303*	*486*	+	+	+	+	7.1	46.4	< 0.001
SS6668	+	+	+	*2903*	+	+	+	13.0	74.7	
SS6673	*4162*	+	+	*2903*	+	+	+	5.2	22.9	< 0.001
SS7729	+	*303*	+	*2903*	+	+	+	5.3	16.5	< 0.001
SS6700	+	+	+	+	*159*	+	+	32.1	95.3	
SS6399	*4162*	+	+	+	*159*	+	+	8.5	54.1	<0.001
SS7726	+	*303*	+	+	*159*	+	+	5.1	28.0	<0.001
SS6698	+	+	+	+	+	*2016*	+	12.9	78.7	
SS6398	*4162*	+	+	+	+	*2016*	+	5.9	19.9	<0.001
SS7723	+	*303*	+	+	+	*2016*	+	4.2	13.4	<0.001
SS2306	+	+	+	+	+	+	*501*	38.0	90.6	
SS8474	*4162*	+	+	+	+	+	*501*	40.8	92.1	<0.001
SS8477	+	*303*	+	+	+	+	*501*	26.8	72.9	<0.001

[1]The *p* value is determined by the Student's T Test where the mutant case is compared to the wild type for each replication mutant. *p* values less than 0.01 are considered significant. In all cases 1000–3000 cells are counted.

Table 4

Effects of *recA4162* and *uvrD303* mutations on SOS created by induced DSBs

Strain	*recA*	*uvrD*	Fold SOS expression above background	Percentage of cells with 9-fold or greater SOS expression above background	*p* value [1]
SS5457 + glucose	+	+	1.7	3.4	
SS5457 + arabinose	+	+	12.3	70.7	< 0.001
SS8277 + glucose	*4162*	+	2.3	4.7	
SS8277 + arabinose	*4162*	+	12.5	78.8	< 0.001
SS8282 + glucose	+	*303*	1.9	4.1	
SS8282 + arabinose	+	*303*	6.6	45.8	< 0.001

[1] These cells were grown in rich media until in early log phase. The cells were then shifted to media with the indicated sugar at 0.2% final concentration for 2 hours. The cells were prepared for imagining directly as indicated in the Materials and Methods. The *p* value is determined by the Student's T Test where the mutant case is compared to the wild type for each replication mutant. *p* values less than 0.01 are considered significant. No significant difference was detected between SS5457 + arabinose and SS8277 + arabinose. Significant differences were detected between SS5457 + arabinose and SS8282 + arabinose ($p < 0.001$). In all cases 1000–3000 cells are counted.

Table 5

Summary of the abilities of *recA4162* and *uvrD303* to suppress SOS expression

		Suppression of SOS Expression?	
Treatment	**Location of ssDNA produced**	***recA4162***	***uvrD303***
recA730	DNA replication fork (?)	Yes	Yes
recA4142	Reversed DNA replication fork	Yes	No
dnaE486	Defect at the DNA replication fork	Yes	Yes
dnaG2903	Defect at the DNA replication fork	Yes	Yes
dnaN159	Defect at the DNA replication fork	Yes	Yes
dnaZ2016	Defect at the DNA replication fork	Yes	Yes
polA	Defect in Okazaki fragment maturation process behind DNA replication fork	No	partial
UV	Gapped DNA behind DNA replication fork	No	partial
DSB	ssDNA produced by RecBCD at I-SceI induced DSB away from DNA replication fork	No	partial