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RecA-independent recombination: Dependence on the *Escherichia coli* RarA protein

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Abstract

Most, but not all, homologous genetic recombination in bacteria is mediated by the RecA recombinase. The mechanistic origin of RecA-independent recombination has remained enigmatic. Here, we demonstrate that the RarA protein makes a major enzymatic contribution to RecA-independent recombination. In particular, RarA makes substantial contributions to intermolecular recombination and to recombination events involving relatively short (<200 bp) homologous sequences, where RecA-mediated recombination is inefficient. The effects are seen here in plasmid-based recombination assays and in vivo cloning processes. Vestigial levels of recombination remain even when both RecA and RarA are absent. Additional pathways for RecA-independent recombination, possibly mediated by helicases, are suppressed by exonucleases ExoI and RecJ. Translesion DNA polymerases may also contribute. Our results provide additional substance to a previous report of a functional overlap between RecA and RarA.

KEYWORDS

in vivo cloning, Mgs1, RarA, RecA, recombination, translesion DNA polymerases

1 | INTRODUCTION

Most of the homologous recombination that occurs during normal growth in bacteria is closely tied to replication (Cox et al., 2000; Kowalczykowski, 2000; Kuzminov, 1999; Michel, 2000). Replication forks can stall when encountering roadblocks, such as DNA lesions, template-strand breaks, or DNA-bound proteins. Stalling, particularly when it involves an encounter with a template strand break, may lead to a double strand break, fork collapse, and replisome dissociation (Cox, 2001; Cox et al., 2000; Heller & Marians, 2006; Klein & Kreuzer, 2002; Kowalczykowski, 2000; Kuzminov, 1999; Kuzminov, 2001; Lopes et al., 2001; Merrikh et al., 2012; Michel, 2000; Michel et al., 2007). Although estimates vary, replication forks in bacteria may stall or collapse as often as once per cell generation during normal growth conditions (Courcelle et al., 2015; Cox, 2001; Cox, 2002; Cox et al., 2000; Kuzminov, 1995; Mangiameli et al., 2017; McCool et al., 2004; Michel et al., 2001; Michel et al., 2004; Romero et al., 2019; Romero et al., 2020; Syeda et al., 2014). Most of the adverse replication-fork encounters are resolved using a variety of pathways that do not introduce mutations, particularly recombinational DNA repair (Aguilera & Garcia-Muse, 2013; Cox, 2001; Cox, 2002; Cox et al., 2000; Heller & Marians, 2006; Klein & Kreuzer, 2002; Kowalczykowski, 2000; Kuzminov, 1999; Kuzminov, 2001; Lopes et al., 2001; Merrikh et al., 2012; Michel, 2000; Michel et al., 2007; Mirkin & Mirkin, 2007).

When bacterial cells are stressed by conditions that increase levels of DNA damage, the SOS response is induced. In the early stages of SOS, nonmutagenic pathways for DNA repair still predominate. However, if the SOS response is prolonged, a different set of pathways for DNA damage tolerance becomes more prominent. These pathways involve specialized DNA polymerases that carry out translesion DNA synthesis (TLS) (Fuchs, 2016; Gabbai et al., 2014; Goodman, 2002; Goodman, 2016; Goodman et al., 2016; Goodman & Woodgate, 2013; Gruber et al., 2015; Ikeda et al., 2014; Indiani et al., 2013; Jeiranian et al., 2013; Jiang et al., 2009; Kath et al., 2016; Kath et al., 2014; Mallik et al., 2015; Margara et al., 2016; Robinson

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et al., 2015). In *E. coli*, TLS is carried out by DNA polymerases II, IV, and V (Goodman & Woodgate, 2013). Under normal aerobic conditions, TLS can become important when nonmutagenic pathways for replication-fork repair are blocked (Naiman et al., 2016).

Replication forks do not always stall when encountering a template lesion. In at least some cases, the replisome skips over lesions, leaving them behind in what is termed a postreplication gap. In bacteria, the postreplication gap is closed by either translesion DNA synthesis, template switching, or by recombinational DNA repair via the RecFOR pathway (Fuchs, 2016; Fujii et al., 2006; Laranjo et al., 2017; Lovett, 2017; Naiman et al., 2016; Romero et al., 2019; Romero et al., 2020). Failure to close a gap inevitably leads to a double strand break when the gap is encountered in the next replication cycle (Cox et al., 2000).

Whereas most detectable homologous recombination events in bacteria depend upon the RecA recombinase, a substantial level of RecA-independent recombination can be documented under some conditions (Albertini et al., 1982; Andersson & Hughes, 2009; Bi & Liu, 1994; Bzymek & Lovett, 2001; Bzymek et al., 1999; Chédin et al., 1994; Dianov et al., 1991; Dutra et al., 2007; Goldfless et al., 2006; Lovett, 2017; Lovett et al., 1993; Lovett & Feschenko, 1996; Lovett et al., 1994; Lovett et al., 2002; Mazin et al., 1991; Morag et al., 1999; Saveson & Lovett, 1997; Zienkiewicz et al., 2013). Much of the RecA-independent events can be traced to a particular form of template switching. Template switching involves the pairing of DNA through alignment, either with itself, with a parental strand or with another sister nascent strand. As a term, "template switch" has a complex history. It has been associated with at least three types of substrate processing, only one of which suits the process we will be discussing here. The first of these is the fork reversal template switching that sometimes occurs to overcome leading strand barriers to rescue replication (Lovett, 2017; Poole & Cortez, 2017; Vujanovic et al., 2017). Based on biochemical evidence, this process might be mediated by RecA, RuvAB, or RecG (McGlynn & Lloyd, 2000).The second process sometimes described as template switching is the recombinational DNA repair mediated by RecA or Rad51 family recombinases (Branzei & Szakal, 2017; Jalan et al., 2019; Pietrobon et al., 2014).

In this report, we focus only on a third process: recombinase (RecA)-independent template switching, primarily in gaps behind the replication fork. Rearrangements between repeated sequences, even though they are homology-dependent, can occur independently of RecA. The rate of recombination depends on the size of repeats and the distance between them (Dutra et al., 2007; Lovett, 2017; Lovett et al., 2002). These RecA-independent rearrangements are stimulated in mutants that affect the DNA polymerase III core, clamp loader complex or other proteins involved in DNA replication and restart pathways (Lovett et al., 1994; Saveson & Lovett, 1997). Homology-dependent but RecA-independent recombination, therefore, can result in genetic rearrangements during DNA replication that are associated with crossovers between sister chromosomes. However, lack of information about the frequency with which RecA-independent template switching occurs and the proteins involved in this pathway makes it difficult to understand its contribution to cell maintenance. RecA-independent recombination is sufficient to render satellite DNA (large arrays of tandemly repeating, noncoding DNA) unstable even when cloned in plasmid vectors and are grown in Δ*recA* strains (Brutlag et al., 1977).

We have recently initiated the study of several proteins that may function in the repair of gaps. The current study explores the function of the RarA protein. The *Escherichia coli* RarA protein is an AAA^+ ATPase (447 amino acid residues; 49.6 kDa). RarA is part of a family with close homologs in eukaryotes (Mgs1 in yeast, WRNIP1 in humans). Sequence conservation within the family is extensive, with RarA sharing roughly 40% identity and 56%–58% similarity with its *Saccharomyces cerevisiae* (Mgs1) and *Homo sapiens* (WRNIP1) homologs (Barre et al., 2001; Sherratt et al., 2004). This extensive homology suggests a conserved function in DNA metabolism. RarA also shares considerable sequence homology with the τ, δ, and δ′ subunits of the DNA polymerase III clamp-loader complex, placing RarA in the clamp-loader AAA^+ clade. The protein has also been referred to as MgsA, a reference to its homology with the yeast protein Mgs1 (Shibata et al., 2005). As the RarA designation was proposed first (Barre et al., 2001), and to avoid confusion with the *mgsA* acronym previously assigned to the gene encoding methylglyoxal synthase (Totemeyer et al., 1998), we use the *rarA* nomenclature. It is well documented that the RarA family of proteins is involved in the maintenance of genome stability in cells, but its function and mechanism of action remain uncertain in spite of nearly two decades of work.

Several dozen studies have now been published on the RarA/Mgs1/WRNIP1 protein family. Although these have yielded a complex, and sometimes contradictory plethora of observations, several themes are evident. First, RarA family members localize to the replisome through interactions with either the single-stranded DNA binding protein, SSB (RarA), or ubiquitinated processivity clamp PCNA (Mgs1 and WRNIP1) (Barre et al., 2001; Bish & Myers, 2007; Costes et al., 2010; Crosetto et al., 2008; Lau et al., 2003; Page et al., 2011; Saugar et al., 2012; Sherratt et al., 2004). Second, the sequence and structure of RarA (and by extension the other members of this family) place it in the clamp-loader clade of AAA^+ ATPases (Page et al., 2011). However, it appears to function as a tetramer rather than having the usual pentameric structure (Page et al., 2011). Third, RarA has an effect on replisome stability and somehow promotes TLS (Kelman & O'Donnell, 1995; Michel & Sinha, 2017; Shibata et al., 2005; Vandewiele et al., 2002; Yoshimura et al., 2017). Fourth, RarA, Mgs1, and WRNIP1 all exhibit a DNA-dependent ATPase activity in vitro that specifically targets duplex DNA ends and gap boundaries (Branzei et al., 2002b; Hishida et al., 2001; Kim et al., 2005; Page et al., 2011; Stanage et al., 2017; Tsurimoto et al., 2005). Fifth, RarA function appears to complement a range of DNA damage tolerance pathways (Branzei et al., 2002a; Branzei et al., 2002b; Hayashi et al., 2008; Hishida et al., 2001; Hishida et al., 2002; Motlagh et al., 2006; Sherratt et al., 2004; Shibata et al., 2005; Yoshimura et al., 2006; Yoshimura et al., 2009). These genetic results suggest that RarA does not belong to any currently defined repair pathway.

Monitoring of template switching events is made possible by engineering genetic events that can be traced to postreplication gaps. Lovett and colleagues (Lovett et al., 2002) developed an assay to

detect crossing over events between two replicons sharing varied lengths of the homologous regions. Efficient RecA-dependent recombination requires a homology threshold of around 200bp (Bi & Liu, 1994; Dutra et al., 2007; Lovett et al., 2002). Recombination events involving shorter regions of homology are largely RecA-independent. This assay permits identification of proteins involved in either recombination or processing of recombination intermediates in the RecA-independent pathway.

Shinagawa and colleagues (Shibata et al., 2005) have previously reported evidence for an apparent overlap between RecA and RarA function. That theme is evident in the current work. We have made use of several assays of intramolecular and intermolecular recombination in vivo, all of which are linked with events that occur in postreplication gaps. In particular, a class of RecA-independent recombination events has been associated with postreplication gaps (Lovett, 2017). Here, we demonstrate that much of this RecA-independent recombination is dependent on RarA.

2 | RESULTS

2.1 | RecA-independent recombination is dependent on RarA

Much of this study makes use of strains having a deletion of the *rarA* gene. In unpublished work, we have determined that the end of the *rarA* gene encoding the C-terminal 41 amino acid residues of the RarA protein includes sequences that affect expression of the downstream *serS* gene. As expression of *serS* directly affects the stringent response (Tosa & Pizer, 1971), a complete deletion of *rarA* generates growth and survival phenotypes that reflect the stringent response rather than a lack of *rarA* function. We have constructed a deletion of the first 406 of the 447 codons of RarA (Δ*rarA N406*), which avoids all effects on *serS*. This is the *rarA* deletion strain used in all the work that follows. As a further control using P1 transduction, we have been able to cross this N406 deletion back into WT (MG1655) cells with no measurable effect on growth.

We suspected that RarA is involved in some aspect of RecA-independent recombination in gaps, due to the DNA binding and flap creation properties of this protein. Once gaps are generated, RarA might be promoting the RecA independent template switching process or facilitating the activity of another protein. We examined the effect of deleting *rarA* on RecA-independent intermolecular recombination utilizing the plasmid-based assay developed by Lovett and colleagues (Lovett et al., 2002). Two sets of plasmids sharing the homology length of 104 bp or 411 bp were used. Recombination efficiencies were tested separately in strains with deletions in *rarA*, *recA*, or both. Deletion of the *rarA* gene alone decreased recombination between 104 bp homologous segments slightly but did not hamper recombination between 411 bp homology sharing regions to a significant extent. Loss of *recA*, however, shows a small reduction in recombination at the homology length of 104 bp compared to WT and a larger approximately 10-fold reduction at 411 bp homology length (Figure 1). This result agrees

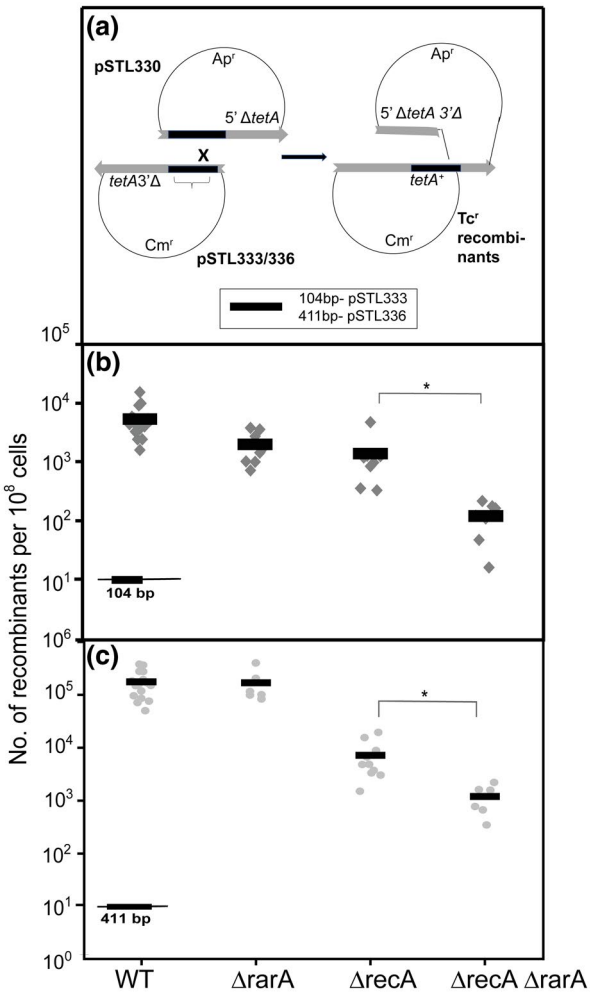


FIGURE 1 Deletion of *rarA* decreases intermolecular recombination in a Δ*recA* cell. (a) Illustration of an intermolecular recombination mechanism between two plasmids as used in this study, which is largely RecA-independent (Lovett et al., 2002). (b and c) The effect of RarA function on RecA-independent recombination was analyzed. The number of recombinants per 10^8 cells is shown for wild-type, along with mutant strains lacking *recA*, *rarA*, or both at different length homologies. Statistical analysis was conducted using pair-wise *t* tests along with Bonferroni correction. Cases were compared at the significance levels of $\alpha = 0.05$ and 0.008 represented in *and ** symbols, respectively. Each point on the graph represents a value obtained from a single independent experiment (n ≥ 6 for each strain used) [Colour figure can be viewed at wileyonlinelibrary.com]

with previous studies (Lovett et al., 2002), indicating RecA-dependent recombination predominates when homologous segments are over 200 bp in length (Bi & Liu, 1994). Analysis of the recombination frequency in the double mutant Δ*recA* Δ*rarA* showed a significantly decreased recombination rate, by a factor of 11- and 6-fold at 104 bp and 411 bp homology sharing regions plasmids respectively, when compared to the Δ*recA* strain (Figure 1). This demonstrates a significant dependence of RecA-independent intermolecular recombination on RarA protein at both homology lengths, although the distinction is amplified with the shorter homology since RecA-dependent events on this substrate are less efficient.

2.2 | RarA requires its ATPase activity to mediate RecA-independent recombination

To determine the functional role of the RarA ATPase activity in RecA-independent recombination, we analyzed the changes in recombination efficiency when the RarA K63R variant is present in *recA*-deficient cells (Figure 2). An ATPase-dead RarA, RarA K63R (Page et al., 2011) showed a decrease in recombination equivalent to a Δ*rarA* strain in the Δ*recA* background at all homology lengths tested. This observation indicates that the ATPase activity of RarA is required in whatever role RarA has in promoting RecA-independent recombination.

2.3 | RarA mediates little intramolecular crossing over in RecA-deficient cells

Having explored intermolecular recombination events, we next focused on the effect of a RarA deletion on intramolecular repeat deletion events associated with RecA-independent template switching in gaps. We utilized a multicopy plasmid pSTL57 carrying 101 bp direct repeats adjacent to each other. A successful deletion event confers resistance to tetracycline, allowing ready detection. Deletion of *rarA* did not significantly reduce the deletion events in a Δ*recA* background with the plasmids carrying repeats adjacent to each other (Figure 3a). This overall pattern remained consistent even with the use of different plasmids where repeats were separated either by larger distances or a stem loop structure (Figure S1). The results suggest that RarA contributes little to recombination events involving nearby repeated sequences on the same DNA molecule.

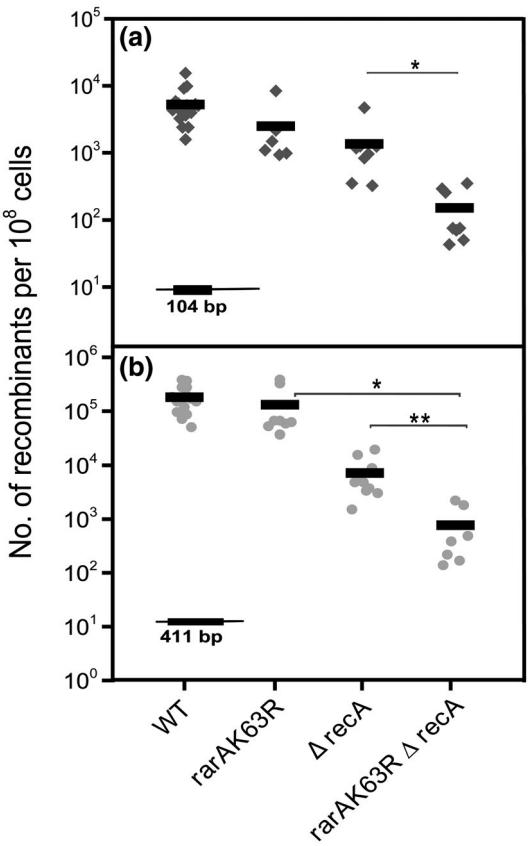


FIGURE 2 RarA requires its ATPase activity to mediate RecA-independent recombination. Effects of RarA mutants, deficient in ATP hydrolysis, on RecA-independent recombination were analyzed. Recombinants per 10^8 cells of different mutant strain are reported at 104 bp (a) and 411 bp (b) homologies lengths. Values were compared at the significance levels of $\alpha = 0.05$ and 0.008 reported in symbol *and **, respectively. n ≥ 6 for each strain used

However, an involvement of RarA in intermolecular recombination is still evident. Gel analysis of the plasmids isolated from the tetracycline resistant survivor cells in these strains revealed an important effect of RarA—facilitation of crossing over. Plasmids isolated from tetracycline resistant colonies of WT and Δ*rarA* strains exhibited a combination of deleted, normal sized, and large sized plasmids. These large species are primarily dimers, with perhaps a few larger species or replication intermediates mixed in. These are presumably generated by crossing over mediated at least in part by RarA. In the Δ*recA* strain, these larger plasmid species were prominent in 9 of 17 randomly chosen isolates, with a representative sample of seven of these shown in Figure 3b. Monomeric sized plasmids were totally absent in those nine isolates. In contrast, when RarA function was also missing (Δ*rarA*Δ*recA* cells), the large-sized plasmids almost disappeared. None of 10 randomly chosen isolates lacked monomeric plasmids, with six examples shown in Figure 3b. The plasmids analyzed in Figure 3b come from Tet^R survivors of the selection, which can be generated by both intramolecular and intermolecular recombination. When RarA is available, the intermolecular events become more prominent.

This direct visualization of the plasmids suggests that RarA is responsible either for crossover-mediated dimer and multimer formation in Δ*recA* strains or in the long-term maintenance of these larger plasmid products after they are formed. A subset of RecA-independent rearrangements is dependent on RarA and are associated with replicon dimerization. Deletion does not necessarily accompany those events. Hence, no significant reduction was observed upon the elimination of RarA in these cells.

2.4 | Potential involvement of other proteins in RecA-independent recombination

RecA-independent recombination cannot be entirely attributed to RarA. In the absence of RecA and RarA, there is still a small background of recombination that we wished to explore further. For the following sets of experiments, the intermolecular recombination assay described in Figure 1 was utilized.

Exonucleases. Lovett and colleagues (Dutra et al., 2007) reported that the recombination frequency in the absence of *recA* is equal to the frequency in $recA^+$ cells if exonucleases were deleted. We aimed to determine the effect of *rarA* deletion on the efficiency of recombination when both 3′→5′ and 5′→3′ exonucleases are deleted in Δ*recA* strain. We found that the deletion of *recJ* (5′→3′ exonuclease) and *exoI* (3′→5′ exonuclease) in a Δ*recA* background increases the

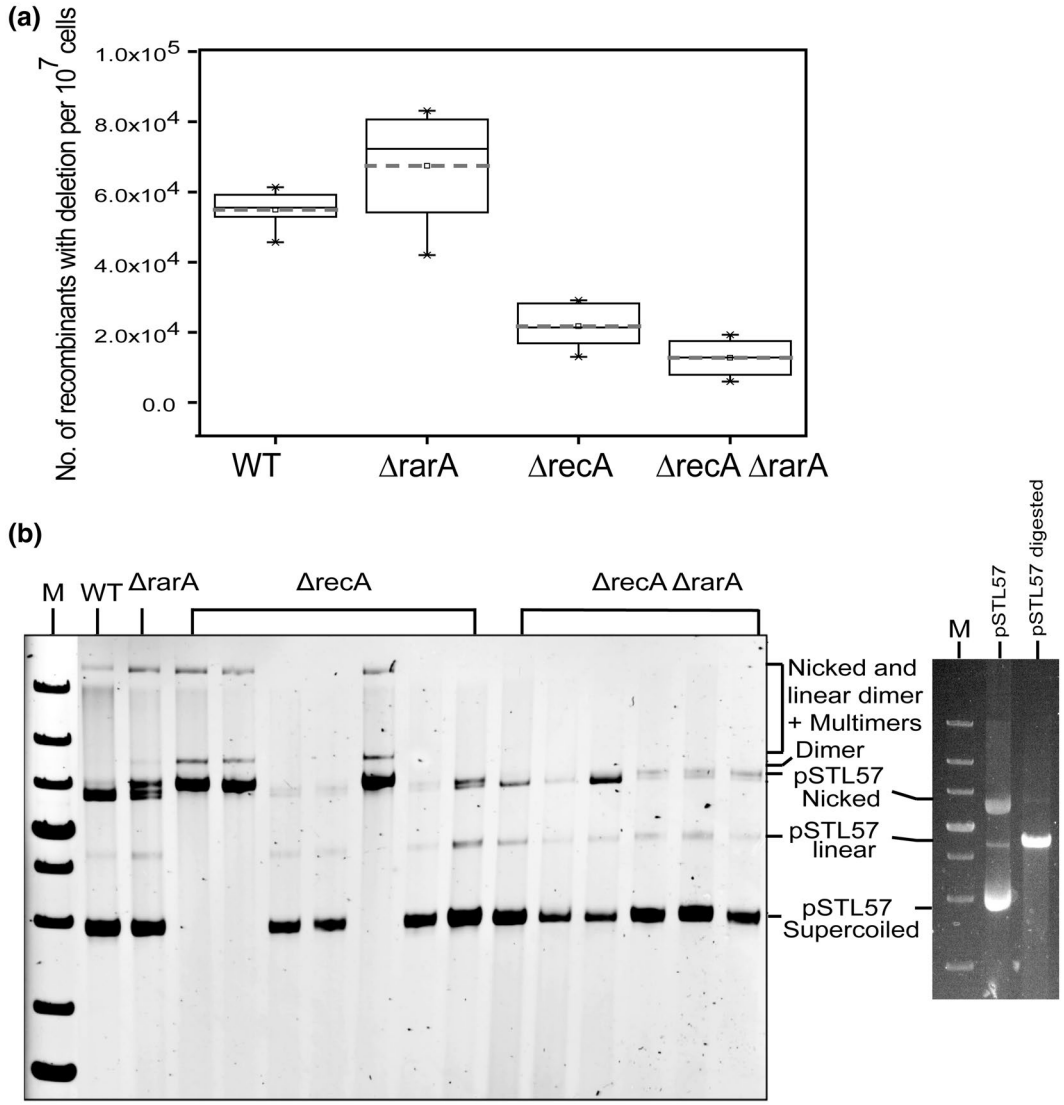


FIGURE 3 RarA mediates limited intramolecular crossing over in RecA-deficient cells. (a) DNA deletion frequencies were assayed using the pSTL57 plasmid bearing direct 101 bp Tet repeats adjacent to each other in WT, *recA*, *rarA*, and *recArarA* strains. Successful deletion events result in resistance to tetracycline. Error bars represent the standard deviation of at least five experiments. (b) Plasmids were isolated from Tet^R recombinants from the assay in panel A. Gel electrophoresis was carried out as described in Methods. The control gel at right displays the purified pSTL57 plasmid and its cleaved linear form. The set of DNA markers is identical to that used in the data gel on the left

recombination frequency by 4-fold and 10-fold, respectively, when a shorter homology length (104 bp; Figure 4) or longer homology region (411 bp; Figure S2) was assayed and compared to a Δ*recA* strain. Differences were statistically significant at $\alpha = 0.083$ value. With the shorter homology, the pattern continued even when *rarA* was deleted. The results suggest that intermediates for an alternative recombination pathway are generated, even in the absence of RecA or RarA. These intermediates are destroyed by the exonucleases. With the longer homology, recombination again increased when the exonucleases were deleted. However, in this case, the loss of both *recA* and *rarA* caused about a 15-fold decline relative to the recombination seen in the Δ*recJ* Δ*exoI* strain, albeit not to the low level seen when exonuclease function was intact.

RecF, RecO, and RecR. A series of experiments, presented in Figure 4 (in combination with exonuclease deletions) and in Figure 5, were carried out to explore the effects of the RecFOR proteins. Due to many phenotypic similarities, these three proteins are often associated in an epistasis group involved in the loading of RecA protein into gaps and in gap repair (Clark & Sandler, 1994; Madiraju et al., 1992; Sawitzke & Stahl, 1992; Smith & Wang, 1989; Wang et al., 1993; Whitby & Lloyd, 1995). However, recent studies have identified phenotypic and spatiotemporal distinctions between *recF* and *recO* (Grompone et al., 2004; Henrikus et al., 2019; Sandler, 1994; Sandler & Clark, 1993; Sandler & Clark, 1994). RecF and RecO both form complexes with RecR, but not with each other (Bork et al., 2001; Henrikus et al., 2019; Honda et al., 2006; Webb et al., 1995; Webb et al., 1997). The effects of *recFOR* deletions in *rarA*/*recA* backgrounds were first explored in exonuclease-deficient strains. RecFOR works along with RecA to repair single-stranded gaps. In almost all cases, deletion of *recO* or *recF* in exonuclease and

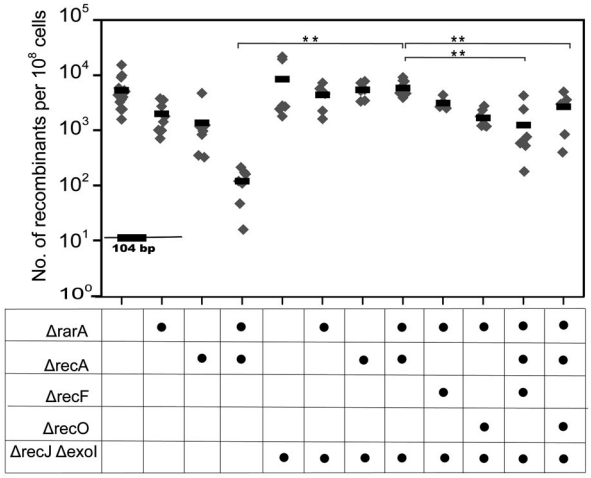


FIGURE 4 Exonucleases suppress the alternative pathways of RecA-independent recombination. Effects of deletion of exonucleases on RecA-dependent and RarA-dependent recombination rates involving 104 bp regions of homology were analyzed. Statistical differences between recombination rates of different strains are reported at the significance levels of $\alpha = 0.05$ and 0.008 reported in symbol *and **, respectively. n ≥ 6 for each strain used. Genotypes are summarized in the table at bottom

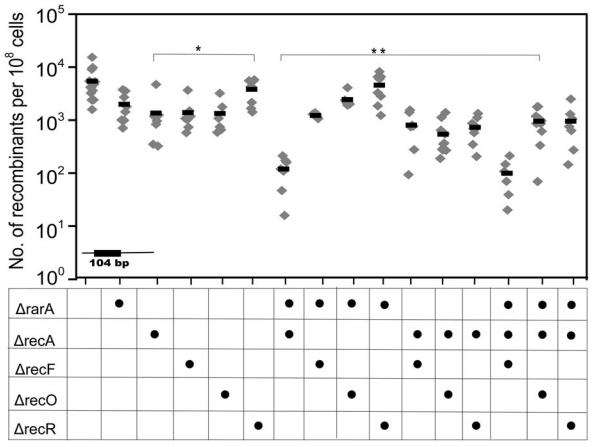


FIGURE 5 RecF and RecO could contribute to RecA-independent recombination pathways. Effects of RecFOR initiation pathway on intermolecular RecA-mediated and RecA-independent crossing over were observed at a homology length of 104 bp. Statistical differences between recombination rates of different strains are reported at the significance levels of $\alpha = 0.05$ and 0.008 reported in symbol *and **, respectively. n ≥ 6 for each strain used

recA/*rarA*-deficient backgrounds resulted in a reduction in the recombination rate (Figure 4). The result indicates that RecO and RecF may have some role in generating or processing the intermediates that lead to recombination when exonucleases do not remove them, a role that is independent of RecA or RarA. One result obtained with the longer (411 bp; Figure S3) homology was otherwise notable. When *rarA*, *recJ*, and *exoI* were all deleted, a further deletion of *recA* and *recF* resulted in a significantly greater decline in recombination frequency than a further deletion of *recO*. In vitro, RecO has been more directly implicated in RecA protein loading than RecF (Hobbs et al., 2007; Lenhart et al., 2014; Shan et al., 1997; Umezu & Kolodner, 1994).

We also explored the effects of *recFOR* deletions in strains that possess the exonucleases but lack *rarA*, *recA*, or both (Figure 5). In this case, the cells lacking one of the *recFOR* genes exhibit recombination deficits that generally paralleled those seen for *recA* deletions, with some notable exceptions. For recombination involving the shorter (104 bp) homology, deleting *recR* has the least effect of the *recFOR* trio. A further deletion of *rarA* has little effect on these results except when it is paired with a deletion of *recA*, a pairing that consistently produced the largest decline in recombination frequency. Pairing *recA* deletions with deletions of one of the *recFOR* proteins elicited an apparent additional decline in recombination frequency, albeit small. When *rarA* and *recA* are both missing, a further deletion of either *recO* or *recR* actually permitted a significant increase in recombination, although deleting *recF* did not.

The same set of experiments was carried out with the larger (411 bp) homology (Figure S3), where RecA-mediated recombination plays a larger role in the overall observed frequency. In this case, the decline observed when *recFOR* deletions were paired with *recA* deletions were clearly greater than seen with *recA* alone, again suggesting *recA*-independent roles for the RecFOR proteins. The declines were comparable in this case to those seen in the Δ*recA*Δ*rarA* strain. In the Δ*recA*Δ*rarA* background, a deletion of *recO* (but in this case not *recR*) again produced an increase in recombination. RecO (and to some extent RecR) appears to have an activity that interferes with the rare recombination that might occur in the absence of RarA and RecA.

2.4.1 | TLS polymerases

A similar set of experiments was carried out in strains lacking one of the three TLS DNA polymerases, Polymerases II, IV, and V. For recombination involving either homology length, no effect was seen when a TLS polymerase deletion was paired with a *rarA* deletion. Deleting one of the TLS polymerases further decreased the recombination frequency when paired with a *recA* deletion, approaching the declines noted in the Δ*recA*Δ*rarA* background (Figure 6 and Figure S4 for substrates with 104 and 411 bp homologies, respectively). This result suggests that TLS polymerases may play a role in RecA-independent recombination, possibly even in the same pathway as RarA.

2.5 | Elimination of the RarA-dependent and RecA-independent recombination pathway improves the growth of XerD-deficient cells

XerD is a site-specific tyrosine recombinase, which along with XerC, is required to resolve chromosome dimers into monomers to facilitate cell segregation. Many of these dimers arise as a result of DNA

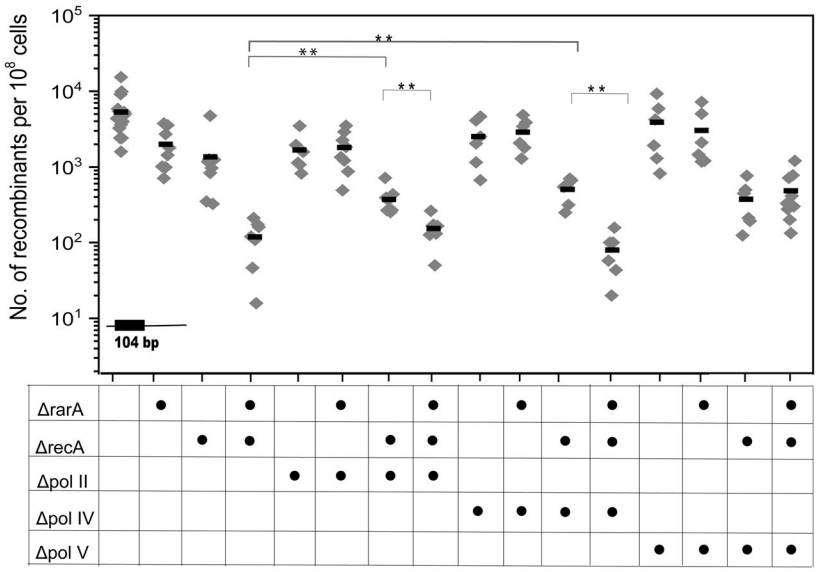


FIGURE 6 TLS polymerases may play a role in RarA-dependent RecA-independent recombination. Effects of deletion of TLS polymerases on RecA-dependent and RarA-dependent recombination rates involving 104 bp of homology was analyzed. Statistical differences between recombination frequencies of different strains are reported at the significance levels of $\alpha = 0.05$ and 0.008 reported in symbol *and ** respectively. $n \geq 6$ for each strain used

crossovers that occur during replication, behind the replication fork in postreplication gaps. When dimer resolution falters, cell growth is impacted. We were, therefore, interested in the effects of *recA* and *rarA* deletions on cell growth in the absence of *xerD*, reasoning that a reduction in recombination in postreplication gaps would reduce dimer formation. Growth curves are presented in Figure 7. As expected, XerD-deficient cells exhibit significant growth defects when cells are grown under normal conditions in LB media, reflected primarily in the final cell density at which the cultures enter stationary phase. As seen before, deletion of *recA* also causes growth defects. The effects of *rarA* deletions are generally minimal. However, the deletion of both *rarA* and *recA* genes completely rescues the growth defect of Δ*xerD* cells. The results suggest that a pathway involving RarA creates crossover products independently of RecA under normal conditions that then require the XerCD pathway for resolution of the resulting dimers. Eliminating both RarA and RecA reduces chromosomal dimer formation sufficiently to restore optimal growth.

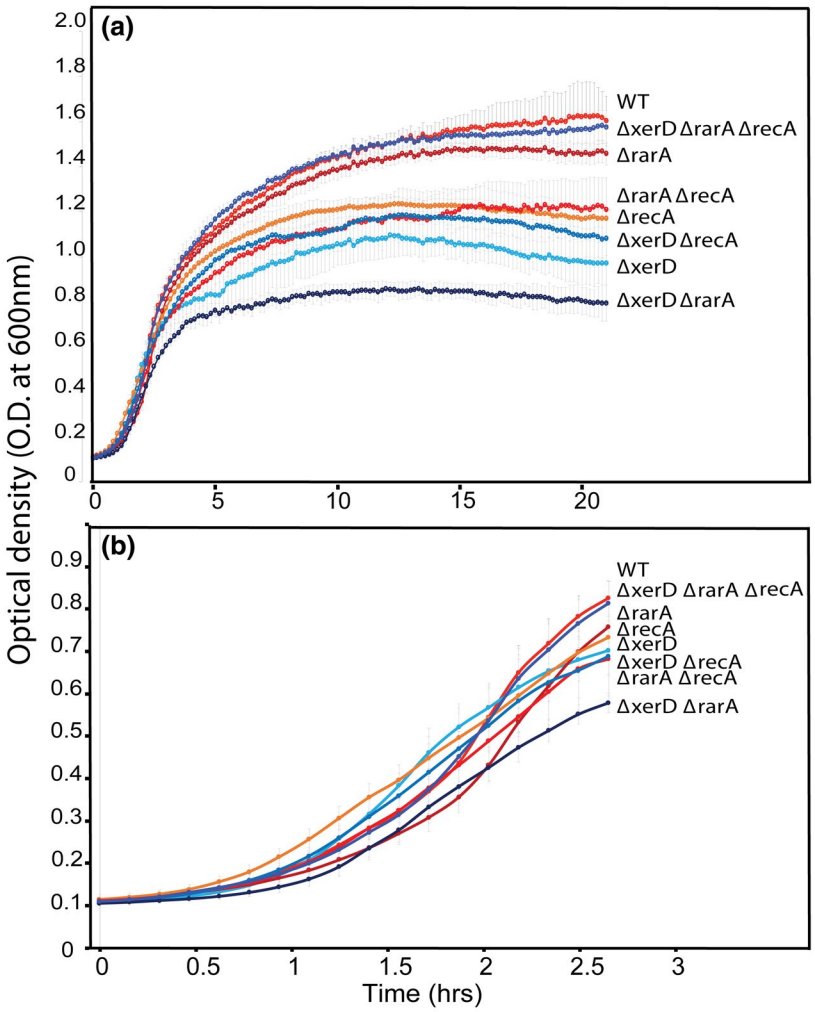
2.6 | Loss of RarA increases the sensitivity of RecA-deficient cells to UV but not to other drugs

We explored the sensitivity of cells lacking *rarA*, *recA* or both to different DNA damaging agents. Loss of *rarA* itself does not exhibit sensitivity to UV, Ciprofloxacin, NFZ, Coumermycin, MMC, or H_2O_2. Loss of *rarA*, however, in Δ*recA* cells slightly increases the sensitivity of *recA* cells to UV. This effect is limited to UV damage. Subtraction of RarA in Δ*recA* cells does not affect its sensitivity to any of the other agents tested (Figure S5). The results indicate that the recombination pathway involving RarA may be somewhat more prominent when damage is in the form of pyrimidine dimers. As mentioned above, deletion of *xerD* improves the growth of Δ*rarA*Δ*recA* cells (and vice versa). However, a *xerD* deletion does not affect the sensitivity of this strain to UV. This indicates no significant role of XerD in DNA damage suppression or repair pathways in Δ*rarA*Δ*recA* cells.

2.7 | In vivo cloning in *E. coli* is partially dependent on RarA

The cloning of a DNA segment into a circular plasmid in vitro has been a common procedure used in recombinant DNA technology (Kaufman & Evans, 1990; Scharf et al., 1986). In contrast to the common and standard in vitro recombination protocols, studies have found that some strains of *E. coli* can assemble linear DNA fragments to form a plasmid in vivo (Bubeck et al., 1993; García-Nafría et al., 2016; Huang et al., 2017; Kostylev et al., 2015; Oliner et al., 1993). Linear DNAs including vector and engineered segments, containing terminal sequences with as little as 20 bp of homology, are co-transformed into an appropriate *E. coli* strain to obtain a recombined plasmid. This process is independent of RecA-based recombination. The prophage RecET recombinase was initially identified as an important contributor (Bubeck et al., 1993; Jacobus & Gross, 2015; Kostylev et al., 2015; Oliner et al., 1993). However, recent work indicates that RecET is not required and that exonuclease XthA plays a key role (Nozaki & Niki, 2019). The detailed mechanism of in vivo cloning remains uncertain.

We wished to determine if RarA affected RecA-independent in vivo cloning efficiency (Figure 8). Cloning was performed in Δ*hsdR* (WT) and Δ*hsdR* Δ*rarA* cells as detailed in the methods section and shown in Figure 8a and b. The *hsdR* gene encodes for a type I restriction enzyme, EcoKI, which cleaves DNA at specific recognition sequence 5'-AACN6GTGC-3' (Sain & Murray, 1980). The Δ*hsdR* background was chosen to prevent degradation of pUC19 DNA, which contains an EcoKI recognition sequence. Plasmids isolated from colonies that were resistant to both chloramphenicol (Cm) and ampicillin (Amp) antibiotics were checked on an agarose gel (Figure 8c).



Strains	WT	Δ xerD	Δ recA	Δ rarA	Δ rarA Δ recA	Δ xerD Δ recA	Δ xerD Δ rarA	Δ xerD Δ rarA Δ recA
Generation time	61.7 ±5.8	142.5 ±27.1	97.1 ±11.0	50.9 ±2.7	86.4 ±10.6	100.4 ±8.6	90.8 ±5.7	65.7 ±4.5

FIGURE 7 Elimination of RarA and RecA improves the growth of XerD-deficient cells. (a) Growth curves of WT, *recA*, *rarA*, *xerD*, *recArarA*, *xerDrecA*, *xerDrarA*, and *xerDrarArecA* mutants in LB medium. OD_{600} versus time traces of each strain shown in comparison to a wild-type control (in pink). (b) Growth rate of different bacterial strains grown at 37°C for 2 hr. Error bars represent the standard deviation of at least four independent repeats carried out on the same day in the same microtiter plate. Each experiment was also repeated on three different days with consistent results to confirm the phenotype. Using the data from the exponential (log) phase of these growth curves, the generation time of the different strains was calculated and is presented as a table in Figure 7B

Recombined plasmids were consistently present in the Cm/Amp resistant colonies. In vivo *E. coli* cloning efficiency was calculated by dividing recombination efficiency (no. of colonies resistant to Cm/Amp) to transformation efficiency. This eliminated the probability of false detection due to low DNA uptake or plasmid maintenance. A deletion of *rarA* reduced the in vivo cloning efficiency by 20%–60% relative to a strain with the same genetic background but with a WT *rarA* gene. This indicates that RarA, unlike RecA, may facilitate recombination with short (20 bp) homologous regions in *E. coli* cells.

3 | DISCUSSION

Our major conclusion is that RarA plays a major role in RecA-independent recombination in *E. coli* (Figure 9). This role is especially prominent for intermolecular recombination events involving short (<200 bp) homologies. The role of RarA in recombination is largely obscured when RecA function is present, as *rarA* deletions have little effect on measured levels of recombination on their own. The pathways in which RarA participates thus function at a relatively low level in wild-type cells. However, that level is sufficient for RarA to make a significant contribution to in vivo cloning protocols and can affect the stability of cloned DNA with repeated sequence elements. Shinagawa and colleagues previously published evidence for a functional overlap between RarA and RecA (Shibata et al., 2005). The current study supports and expands upon this idea. In addition, the results provide broader insight into the molecular origins of RecA-independent recombination. For cloned DNA that is unstable in *E. coli* with a *recA* deletion, addition of a *rarA* deletion (preserving the last 41 codons to preserve normal *serS* expression) may well improve stability.

The actual enzymatic activity that RarA engages in has only been partially defined. RarA promotes strand separation at duplex DNA ends or gap ends (Stanage et al., 2017). A close RarA homologue, Mgs1, promotes DNA annealing (Hishida et al., 2001). Our working hypothesis is that those two activities together constitute a DNA strand invasion activity somewhat similar to RecA. This would readily explain the apparent overlap in function in vivo (Shibata et al., 2005).

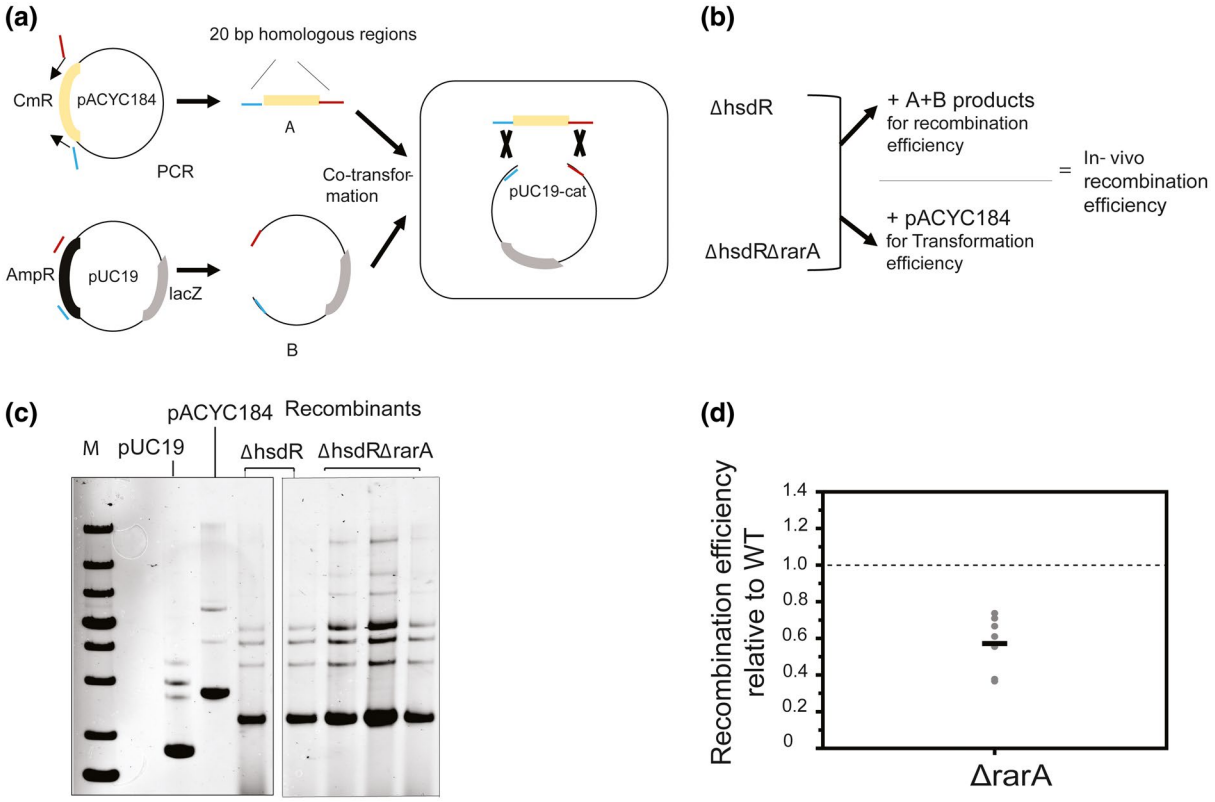


FIGURE 8 In vivo cloning in *E. coli* is partially dependent on RarA. (a and b) Schematic representation of in vivo cloning procedure involving assembly of two DNA fragments in a cell. Assays were carried out as described in Methods. (c) Recombinant plasmids were isolated from the indicated strains and run on a 1% of agarose gel. pUC19 and pACYC184 plasmids were used as a control. Δ*hsdR* background is used to prevent degradation of an unmethylated Hsd recognition sequence in pUC19 DNA. (d) In vivo cloning efficiency of Δ*hsdR* Δ*rarA* (represented as Δ*rarA)* is reported relative to Δ*hsdR* (represented as WT) [Colour figure can be viewed at wileyonlinelibrary.com]

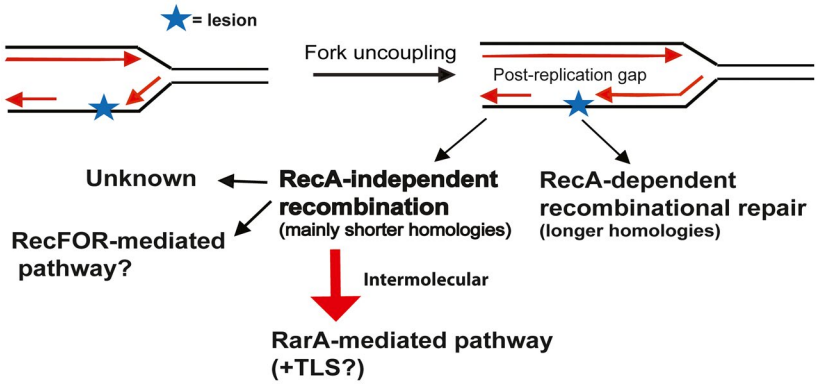


FIGURE 9 RecA-dependent and RecA-independent recombination pathways. Schematic outlining recombination pathways facilitated by RarA, independent of RecA, as revealed in this study

Additional proteins (a TLS polymerase like Pol IV?) might facilitate such a reaction. Alternatively, RarA could promote key steps in a single strand annealing recombination pathway. The results in Figures 3 and 7 suggest that RarA is somehow involved in the creation of crossovers that require the intervention of XerD to facilitate chromosomal segregation, adding to the implications of a RarA contribution primarily to intermolecular recombination events. To date, reconstitution efforts in vitro have been incomplete at best and do not yet provide a platform for more detailed mechanistic understanding.

The results in the current study define contributions to RecA-independent recombination that extend beyond RarA alone. Increases in RecA and RarA-independent recombination seen when the exonucleases ExoI and RecJ are deleted speak to the background generation of recombination intermediates that these exonucleases destroy. We note that all cellular helicases remain operative in these cells. As suggested previously (Bhargava et al., 2016; Lovett, 2017), single-stranded DNA generation could lead to a single strand annealing pathway of recombination. Although the effects of RecF and RecO deletions were generally minor, a few experiments suggest the presence of an additional recombination pathway that these proteins could contribute to (or in some cases interfere with) even in the absence of RecA. The effects of losing TLS DNA polymerases

in these assays are small but suggest a possible contribution of TLS polymerases to RecA-independent recombination.

Although we have postulated a role for RarA in the metabolism of DNA gaps and the effects of RarA on recombination are evident, the normal function of the RarA pathway in wild-type cells is not yet well-defined. On its own, a deletion of the *rarA* gene has little effect on cell physiology or growth other than conferring a modest sensitivity to UV. The question of RarA function must be viewed in the context of a protein family (RarA, Mgs1, and WRNP1) that is among the most widespread and conserved of any family with a putative role in DNA repair (Barre et al., 2001; Bish & Myers, 2007; Branzei et al., 2002a; Branzei et al., 2002b; Crosetto et al., 2008; Hayashi et al., 2008; Hishida et al., 2001; Hishida et al., 2002; Hishida et al., 2006; Kawabe et al., 2006; Kim et al., 2005; Lestini & Michel, 2007; Nomura et al., 2012; Page et al., 2011; Sherratt et al., 2004; Shibata et al., 2005; Stanage et al., 2017; Yoshimura et al., 2006; Yoshimura et al., 2009). RarA family proteins contribute to genome stability in all cells in ways that give some urgency to a better functional understanding.

4 | EXPERIMENTAL PROCEDURES

4.1 | Strain construction

All strains are *E. coli* MG1655 derivatives and are listed in Table 1. Δ*rarAN406* and *rarAK63R* strains were made using a *galK* selection-based recombineering method described by Warming and Copeland (Warming et al., 2005). All other strains were constructed using Lambda Red recombination as described by Datsenko and Wanner (Datsenko & Wanner, 2000). All chromosomal mutations were confirmed using Sanger sequencing. When required, antibiotic resistance of a given strain was eliminated using FLP recombinase encoded by the pLH29 plasmid as described previously (Huang et al., 1997). Standard transformation protocols were used to generate strains harboring the plasmids (Lovett et al., 1993; Lovett et al., 2002) as indicated in Figures 1–6.

4.2 | Two-plasmid recombination assay

Strains were made chemically competent and transformed with the indicated pSTL330 + pSTL333 and pSTL330 + pSTL336 plasmids, followed by plating on LB plates containing chloramphenicol (20 μg/ml) and ampicillin (50 μg/ml) and incubating at 37°C overnight. Transformants obtained were then picked and inoculated in 3 mL LB/Chloramphenicol + Ampicillin (Chl + Amp) overnight for 12 hr at 37°C. Overnight culture were then diluted 1:100 in fresh LB/Chl + Amp medium and grown at 37°C until the OD_{600} reached to 0.5. Cultures were then serially diluted in PBS to 10^{-6} and 100 μl of different dilutions were plated on Chl + Amp and Chl + Amp+Tet plates. The ratio of Chl + Amp+Tet to Chl + Amp colonies were determined at the dilutions that gave 30 to 300 colonies and recombination frequencies were calculated.

4.3 | Statistical analysis

The difference between recombination efficiencies of different strains used in Figures 1–2 And 4–6 were statistically compared. Pair-wise *t* tests at a significance level of $\alpha = 0.05$ were employed. To control for Type I error in this multiple testing scenario, the Bonferroni correction was used. Bonferroni correction was used to perform multiple comparisons, which adjusts the significance level of each comparison to $\alpha m = \alpha/m$, where *m* is the number of comparisons being made.

However, each case has a different number of variables (and consequently a different number of tests). Therefore, to make the comparison level consistent, Figure 1 was chosen as our baseline as it is present in all of the figures. Here, m = 6, and consequently, $\alpha m = \alpha/m = 0.05/6 = 0.00833$. The results are displayed in Figures 1–2 and 4–6.

4.4 | Plasmid repeat deletion assay

Standard transformation protocols were used to generate strains harboring the indicated pSTL57 plasmid. The transformants were selected on LB plates containing ampicillin (100 μg/ml) and the single transformants obtained were then streaked on Amp/LB plates and incubated at 37°C overnight. Colonies obtained were then picked and inoculated in 3 ml LB/Ampicillin overnight for 12 hr at 37°C. Overnight culture were diluted 1:100 in fresh LB medium and grown at 37°C till OD_{600} reached 0.2. Cultures were then serially diluted in PBS to 10^{-6} and 100 μl of different dilutions were plated on Amp and Amp/Tet plates. The ratio of Amp/Tet to Amp colonies were determined at the dilutions that gave 30 to 300 colonies and repeat deletion frequencies were calculated.

4.5 | Plasmid size gel electrophoresis

Tetracycline resistant colonies (10–17 in total) were chosen at random from each strain containing pSTL57 and grown at 37°C for 16 hr. Plasmids were purified using the Promega Wizard® SV Minipreps DNA Purification Systems kit and stored in 25 μl TE buffer at −20°C. DNA concentrations of each plasmid preparation were normalized and approximately, 100 ng was loaded on a 0.8% agarose Tris-acetate EDTA (TAE) gel. The gel was run at 100 V for 4 hr, stained and photographed.

4.6 | Growth curves

Overnight cultures of indicated strains were initiated from freezer stocks. Overnight cultures were then diluted 1:100 in fresh LB medium and 100 μl of each culture was added to a 96 well plate. Absorbance at OD_{600} were recorded at every 10 min for 22 hr at 37°C while shaking in a H1 Synergy Biotek plate reader.

TABLE 1 List of strains used in this study

Strain	Genotype	Parent strain	Source/Technique
MG1655	*rarA + recA + exoI + recJ + recF + recO + recR + polB + dinB + umuDC+*		George Weinstock
EAW20	Δ*recA*	MG1655	Lambda RED recombination
EAW974	Δ*rarA*	MG1655	Gal K + recombineering
EAW1131	Δ*recA* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW20
EAW907	*rarAK63R*	MG1655	Gal K + recombineering
KJ401	*rarAK63R* Δ*recA*	EAW907	Transduction of EAW907 with P1 grown on EAW20
EAW1149	Δ*recJ* Δ*exoI*	EAW820	Transduction of EAW820 with P1 grown on EAW326
EAW1165	Δ*recA* Δ*recJ* Δ*exoI*	EAW1149	Transduction of EAW1149 with P1 grown on EAW20
EAW1162	Δ*rarA* Δ*recJ* Δ*exoI*	EAW1147	Transduction of EAW1147 with P1 grown on EAW326
EAW1166	Δ*rarA* Δ*recA* Δ*recJ* Δ*exoI*	EAW1162	Transduction of EAW1162 with P1 grown on EAW20
EAW1326	Δ*recF* Δ*rarA* Δ*recJ* Δ*exoI*	EAW1162	Transduction of EAW1162 with P1 grown on EAW629
EAW1325	Δ*recO* Δ*rarA* Δ*recJ* Δ*exoI*	EAW1162	Transduction of EAW1162 with P1 grown on EAW114
EAW1344	Δ*recA* Δ*recF* Δ*rarA* Δ*recJ* Δ*exoI*	EAW1326	Transduction of EAW1326 with P1 grown on EAW20
EAW1349	Δ*recA* Δ*recO* Δ*rarA* Δ*recJ* Δ*exoI*	EAW1325	Transduction of EAW1325 with P1 grown on EAW20
EAW629	Δ*recF*	MG1655	Transduction of MG1655 with P1 grown on EAW277
EAW114	Δ*recO*	MG1655	Lambda RED recombination
EAW669	Δ*recR*	MG1655	Lambda RED recombination
EAW989	Δ*recF* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW629
EAW984	Δ*recO* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW114
KJ508	Δ*recR* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW669
KJ380	Δ*recF* Δ*recA*	EAW629	Transduction of EAW629 with P1 grown on EAW20
KJ379	Δ*recO* Δ*recA*	EAW114	Transduction of EAW114 with P1 grown on EAW20
KJ511	Δ*recR* Δ*recA*	EAW669	Transduction of EAW669 with P1 grown on EAW20
KJ483	Δ*recF* Δ*rarA* Δ*recA*	EAW989	Transduction of EAW989 with P1 grown on EAW20
EAW1370	Δ*recO* Δ*rarA* Δ*recA*	EAW984	Transduction of EAW984 with P1 grown on EAW20
KJ512	Δ*recR* Δ*rarA* Δ*recA*	KJ508	Transduction of EAW984 with P1 grown on EAW669
EAW21	Δ*pol II*	MG1655	Lambda RED recombination
EAW983	Δ*pol II* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW21
KJ288	Δ*pol II* Δ*recA*	EAW21	Transduction of EAW21 with P1 grown on EAW20

TABLE 1 Continued

Strain	Genotype	Parent strain	Source/Technique
KJ287	Δ*pol II* Δ*rarA* Δ*recA*	EAW983	Transduction of EAW983 with P1 grown on EAW20
EAW18	Δ*pol IV*	MG1655	Lambda RED recombination
EAW979	Δ*pol IV* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW18
KJ286	Δ*pol IV* Δ*recA*	EAW18	Transduction of EAW18 with P1 grown on EAW20
KJ287	Δ*pol IV* Δ*rarA* Δ*recA*	EAW979	Transduction of EAW979 with P1 grown on EAW20
EAW17	Δ*pol V*	MG1655	Lambda RED recombination
EAW982	Δ*pol V* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW17
KJ290	Δ*pol V* Δ*recA*	EAW17	Transduction of EAW17 with P1 grown on EAW20
KJ291	Δ*pol V* Δ*rarA* Δ*recA*	EAW982	Transduction of EAW982 with P1 grown on EAW20
EAW1133	Δ*xerD*	MG1655	Lambda RED recombination
KJ543	Δ*xerD* Δ*recA*	EAW1133	Transduction of EAW 1,133 with P1 grown on EAW20
EAW1125	Δ*xerD* Δ*rarA*	EAW974	Transduction of EAW974 with P1 grown on EAW1133
KJ491	Δ*xerD* Δ*rarA* Δ*recA*	EAW1125	Transduction of EAW 1,125 with P1 grown on EAW20

4.7 | DNA damage sensitivity assay

Overnight cultures of indicated strains were diluted 1:100 in fresh LB medium. Cultures were grown at 37°C with aeration and shaking until the OD_{600} measured 0.2. Aliquots (1 ml) were taken from each culture, serially diluted in 1X PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, 1 mM CaCl2, and 0.5 mM MgCl2) to 10^{-6}. A 10 µl of each dilution were spot plated on agar plates containing the indicated DNA damaging agents. Plates were incubated overnight at 37°C and imaged the next day using a 700 FOTO/Analyst Apprentice Digital Camera System (Fotodyne, Inc.)

4.8 | In vivo cloning assay

4.8.1 | Preparation of PCR products for transformation

We used Phusion polymerase for PCR. The thermal cycler program was as follows: 98°C for 30 sec, followed by 30 cycles of 98°C for 10 s, 55°C for 20 s, and 72°C for 3 min, and a final extension of 72°C for 10 min. pUC_F and pUC_R and cat (pUC25)_F and cat(pUC_25)_R oligonucleotides sets were used to amplify desired regions of pUC19 and pACYC184 respectively as indicated below (Nozaki & Niki, 2019).The final concentration of the template DNA in each reaction mixture was adjusted to 0.0147 ng/µl for pACYC184 and 0.028 ng/ul for pUC19 plasmids. The cat (chloramphenicol resistance) gene was amplified from pACYC184 DNA. PCR amplified DNA were digested by Fast digest DpnI enzyme for 20 min at 37°C.

pUC_F: 5′-GTTTTCCCAGTCACGACGTT-3′.

pUC_R: 5′-GCCTGATGCGGTATTTTCTC-3′.

cat (pUC25) F: 5′-GTAAGGAGAAAATACCGCATCAGGC TGTGACGGAAGATCACTTCG-3′.

cat (pUC_25) R: 5′-TTTACAACGTCGTGACTGGGAAAAC GGGCACCAATAACTGCCTTA-3′.

4.8.2 | Transformation

We used the modified TSS method as described (Nozaki & Niki, 2019) to introduce PCR amplified fragments into *E. coli* cells. In order to make competent cells, four overnight cultures, two of WT MG1655 and two of EAW974 (Δ*rarA* N406) were set by inoculating a few cells from a single colony in 1 ml LB media in sterile eppendorf tubes and incubating at 37°C for 20 hr without shaking. The OD_{600} of each overnight culture was normalized to approximately 1.4 by dilution with LB media to a total final volume of 1 ml. The culture tubes were chilled on ice for 10 min and cells were pelleted at 5,000 × *g* for 1 min at 4°C. The supernatant was removed and the cell pellet was dissolved in 100 µl of an ice-cold solution consisting of 50 uL of LB, 40 µl of 2 × TSS media

(20% (wt/vol) polyethylene glycol 8,000 (PEG 8,000), 100 mM $MgSO_4$, and 20% (vol/vol) glycerol in LB media), and 10 μl dimethyl sulfoxide (DMSO). Control pACYC184 supercoiled DNA, 10 ng, was added to one each of the WT and Δ*rarA* prepared cell suspensions. Amplified linear DNA fragments, including 0.008 g of the CmR fragment derived from pACYC184 and 0.0165 g of vector fragment derived from pUC19, was added to the remaining prepared samples of WT and Δ*rarA* cells. The four mixtures were frozen immediately for one minute in liquid nitrogen. The tubes were then incubated on ice for 10 min, vortexed, and incubated on ice for another 10 min. LB (1 ml) was then added to each tube and incubated at 37°C for 45 min. The cells were centrifuged, supernatant was poured off, and the pellet was resuspended in the small amount of remaining supernatant. Each sample containing linear DNA fragments was plated on a single LB plate containing 20 μg/ml chloramphenicol and 50 μg/ml ampicillin. Each sample transformed with circular pACYC184 was plated on a single LB place containing 20 μg/ml chloramphenicol. The plates were incubated at 37°C for 16 hr, and the colonies were counted. Colonies per gram of added DNA was calculated for each plate. Recombination efficiency was defined as colonies/g for the chloramphenicol/Ampicillin plates (cells transformed with the linear fragments) divided by the colonies/g for the chloramphenicol plates (cells transformed with circular pACYC184). On each given day, the recombination efficiency for the Δ*rarA* cells was divided by the recombination efficiency for the WT cells to obtain the values plotted in Figure 8. This experiment was repeated eight times on eight different days.

4.8.3 | Assay of iVEC activity

PCR amplified fragments, linearized pUC19 with 20-bp homologous overlapping ends and an antibiotic resistance gene from pACYC184 were introduced into *E. coli* cells following the modified TSS method described above. A 5 ul of PCR amplified and Dpn1-treated cat fragment (Cm^R) from pACYC184 and linearized pUC19 (Amp^R) were used for the transformation of Δ*hsdR* and Δ*hsdR* Δ*rarA* strains. The number of colonies resistant to both ampicillin and chloramphenicol after simultaneous introduction of the cat fragment and linearized pUC19 into indicated strains was calculated. The in vivo recombination efficiency of each strains was normalized by dividing the number of Cm^R Amp^R recombinants to their transformation efficiency (Cm^R). The relative in vivo transformation efficiency of Δ*hsdR* Δ*rarA* with respect to Δ*hsdR* were then plotted in Figure 8b

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

K.J. and M.M.C. designed research; K.J. and E.A.W. performed experiments; K.J., Z.J.R., and M.M.C. analyzed data; and K.J. and M.M.C. wrote the paper.

DATA AVAILABILITY STATEMENT

All data associated with this study are available within the manuscript and supplementary materials.

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REFERENCES

Aguilera, A. & Garcia-Muse, T. (2013) Causes of genome instability. *Annual Review of Genetics*, *47*, 1–32.

Albertini, A.M., Hofer, M., Calos, M.P. & Miller, J.H. (1982) On the formation of spontaneous deletions: the importance of short sequence homologies in the generation of large deletions. *Cell*, *29*, 319–328.

Andersson, D.I. & Hughes, D. (2009) Gene amplification and adaptive evolution in bacteria. *Annual Review of Genetics*, *43*, 167–195.

Barre, F.X., Soballe, B., Michel, B., Aroyo, M., Robertson, M. & Sherratt, D. (2001) Circles: The replication-recombination-chromosome segregation connection. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8189–8195.

Bhargava, R., Onyango, D.O. & Stark, J.M. (2016) Regulation of single-strand annealing and its role in genome maintenance. *Trends in Genetics: TIG*, *32*, 566–575.

Bi, X. & Liu, L.F. (1994) *recA*-independent and *recA*-dependent intramolecular plasmid recombination. Differential homology requirement and distance effect. *Journal of Molecular Biology*, *235*, 414–423.

Bish, R.A. & Myers, M.P. (2007) Werner helicase-interacting protein 1 binds polyubiquitin via its zinc finger domain. *Journal of Biological Chemistry*, *282*, 23184–23193.

Bork, J.M., Cox, M.M. & Inman, R.B. (2001) The RecOR proteins modulate RecA protein function at 5' ends of single-stranded DNA. *Embo Journal*, *20*, 7313–7322.

Branzei, D., Seki, M., Onoda, F. & Enomoto, T. (2002a) The product of *Saccharomyces cerevisiae* WHIP/MGS1, a gene related to replication factor C genes, interacts functionally with DNA polymerase delta. *Molecular Genetics and Genomics*, *268*, 371–386.

Branzei, D., Seki, M., Onoda, F., Yagi, H., Kawabe, Y. & Enomoto, T. (2002b) Characterization of the slow-growth phenotype of *S. cerevisiae whip/mgs1 sgs1* double deletion mutants. *DNA Repair*, *1*, 671–682.

Branzei, D. & Szakal, B. (2017) Building up and breaking down: Mechanisms controlling recombination during replication. *Critical Reviews in Biochemistry and Molecular Biology*, *52*, 381–394.

Brutlag, D., Fry, K., Nelson, T. & Hung, P. (1977) Synthesis of hybrid bacterial plasmids containing highly repeated satellite DNA. *Cell*, *10*, 509–519.

Bubeck, P., Winkler, M. & Bautsch, W. (1993) Rapid cloning by homologous recombination *in vivo*. *Nucleic Acids Research*, *21*, 3601–3602.

Bzymek, M. & Lovett, S.T. (2001) Evidence for two mechanisms of palindrome-stimulated deletion in *Escherichia coli*: Single-strand annealing and replication slipped mispairing. *Genetics*, *158*, 527–540.

Bzymek, M., Saveson, C.J., Feschenko, V.V. & Lovett, S.T. (1999) Slipped misalignment mechanisms of deletion formation: *in vivo* susceptibility to nucleases. *Journal of Bacteriology*, *181*, 477–482.

Chédin, F., Dervyn, E., Dervyn, R., Ehrlich, S.D. & Noirot, P. (1994) Frequency of deletion formation decreases exponentially with distance between short direct repeats. *Molecular Microbiology*, *12*, 561–569.

Clark, A.J. & Sandler, S.J. (1994) Homologous genetic recombination: the pieces begin to fall into place. *Critical Review in Microbiology*, *20*, 125–142.

Costes, A., Lecointe, F., McGovern, S., Quevillon-Cheruel, S. & Polard, P. (2010) The C-terminal domain of the bacterial SSB protein acts as a DNA maintenance hub at active chromosome replication forks. *PLoS Genetics*, *6*, e1001238.

Courcelle, J., Wendel, B.M., Livingstone, D.D. & Courcelle, C.T. (2015) RecBCD is required to complete chromosomal replication: Implications for double-strand break frequencies and repair mechanisms. *DNA Repair*, *32*, 86–95.

Cox, M.M. (2001) Historical overview: Searching for replication help in all of the rec places. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8173–8180.

Cox, M.M. (2002) The nonmutagenic repair of broken replication forks via recombination. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, *510*, 107–120.

Cox, M.M., Goodman, M.F., Kreuzer, K.N., Sherratt, D.J., Sandler, S.J. & Marians, K.J. (2000) The importance of repairing stalled replication forks. *Nature*, *404*, 37–41.

Crosetto, N., Bienko, M., Hibbert, R.G., Perica, T., Ambrogio, C., Kensche, T. et al (2008) Human Wrnip1 is localized in replication factories in a ubiquitin-binding zinc finger-dependent manner. *Journal of Biological Chemistry*, *283*, 35173–35185.

Datsenko, K.A. & Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences*, *97*, 6640–6645.

Dianov, G.L., Kuzminov, A.V., Mazin, A.V. & Salganik, R.I. (1991) Molecular mechanisms of deletion formation in *Escherichia coli* plasmids. *Molecular and General Genetics MGG*, *228*, 153–159.

Dutra, B.E., Sutera, V.A. & Lovett, S.T. (2007) RecA-independent recombination is efficient but limited by exonucleases. *Proceedings of the National Academy of Sciences*, *104*, 216–221.

Fuchs, R.P. (2016) Tolerance of lesions in *E. coli*: Chronological competition between translesion synthesis and damage avoidance. *DNA Repair*, *44*, 51–58.

Fujii, S., Isogawa, A. & Fuchs, R.P. (2006) RecFOR proteins are essential for Pol V-mediated translesion synthesis and mutagenesis. *Embo Journal*, *25*, 5754–5763.

Gabbai, C.B., Yeeles, J.T.P. & Marians, K.J. (2014) Replisome-mediated translesion synthesis and leading strand template lesion skipping are competing bypass mechanisms. *Journal of Biological Chemistry*, *289*, 32811–32823.

García-Nafría, J., Watson, J.F. & Greger, I.H. (2016) IVA cloning: A single-tube universal cloning system exploiting bacterial *In Vivo* Assembly. *Scientific Reports*, *6*, e27459.

Goldfless, S.J., Morag, A.S., Belisle, K.A., Sutera, V.A. Jr & Lovett, S.T. (2006) DNA repeat rearrangements mediated by DnaK-dependent replication fork repair. *Molecular Cell*, *21*, 595–604.

Goodman, M.F. (2002) Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annual Review of Biochemistry*, *71*, 17–50.

Goodman, M.F. (2016) Better living with hyper-mutation. *Environmental and Molecular Mutagenesis*, *57*, 421–434.

Goodman, M.F., McDonald, J.P., Jaszczur, M.M. & Woodgate, R. (2016) Insights into the complex levels of regulation imposed on *Escherichia coli* DNA polymerase V. *DNA Repair*, *44*, 42–50.

Goodman, M.F. & Woodgate, R. (2013) Translesion DNA Polymerases. *Cold Spring Harbor Perspectives in Biology*, *5*, a010363.

Grompone, G., Ehrlich, D. & Michel, B. (2004) Cells defective for replication restart undergo replication fork reversal. *EMBO reports*, *5*, 607–612.

Gruber, A.J., Erdem, A.L., Sabat, G., Karata, K., Jaszczur, M.M., Vo, D.D. et al (2015) A RecA protein surface required for activation of DNA polymerase V. *PLoS Genetics*, *11*, e1005066.

Hayashi, T., Seki, M., Inoue, E., Yoshimura, A., Kusa, Y., Tada, S. et al (2008) Vertebrate WRNIP1 and BLM are required for efficient maintenance of genome stability. *Genes and Genetic Systems*, *83*, 95–100.

Heller, R.C. & Marians, K.J. (2006) Replisome assembly and the direct restart of stalled replication forks. *Nature Reviews Molecular Cell Biology*, *7*, 932–943.

Henrikus, S.S., Henry, C., Ghodke, H., Wood, E.A., Mbele, N., Saxena, R. et al (2019) RecFOR epistasis group: RecF and RecO have distinct localizations and functions in *Escherichia coli*. *Nucleic Acids Research*, *47*, 2946–2965.

Hishida, T., Iwasaki, H., Ohno, T., Morishita, T. & Shinagawa, H. (2001) A yeast gene, MGS1, encoding a DNA-dependent AAA(+) ATPase is required to maintain genome stability. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8283–8289.

Hishida, T., Ohno, T., Iwasaki, H. & Shinagawa, H. (2002) *Saccharomyces cerevisiae* MGS1 is essential in strains deficient in the RAD6-dependent DNA damage tolerance pathway. *Embo Journal*, *21*, 2019–2029.

Hishida, T., Ohya, T., Kubota, Y., Kamada, Y. & Shinagawa, H. (2006) Functional and physical interaction of yeast Mgs1 with PCNA: Impact on RAD6-dependent DNA damage tolerance. *Molecular and Cellular Biology*, *26*, 5509–5517.

Hobbs, M.D., Sakai, A. & Cox, M.M. (2007) SSB protein limits RecOR binding onto single-stranded DNA. *Journal of Biological Chemistry*, *282*, 11058–11067.

Honda, M., Inoue, J., Yoshimasu, M., Ito, Y., Shibata, T. & Mikawa, T. (2006) Identification of the RecR Toprim domain as the binding site for both RecF and RecO. A role of RecR in RecFOR assembly at double-stranded DNA-single-stranded DNA junctions. *Journal of Biological Chemistry*, *281*, 18549–18559.

Huang, F., Spangler, J.R. & Huang, A.Y. (2017) *In vivo* cloning of up to 16 kb plasmids in *E. coli* is as simple as PCR. *PLoS One*, *12*, e0183974.

Huang, L.C., Wood, E.A. & Cox, M.M. (1997) Convenient and reversible site-specific targeting of exogenous DNA into a bacterial chromosome by use of the FLP recombinase: The FLIRT system. *Journal of Bacteriology*, *179*, 6076–6083.

Ikeda, M., Furukohri, A., Philippin, G., Loechler, E., Akiyama, M.T., Katayama, T. et al. (2014) DNA polymerase IV mediates efficient and quick recovery of replication forks stalled at N-2-dG adducts. *Nucleic Acids Research*, *42*, 8461–8472.

Indiani, C., Patel, M., Goodman, M.F. & O'Donnell, M.E. (2013) RecA acts as a switch to regulate polymerase occupancy in a moving replication fork. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 5410–5415.

Jacobus, A.P. & Gross, J. (2015) Optimal cloning of PCR fragments by homologous recombination in *Escherichia coli*. *PLoS One*, *10*, e0119221.

Jalan, M., Oehler, J., Morrow, C.A., Osman, F. & Whitby, M.C. (2019) Factors affecting template switch recombination associated with restarted DNA replication. *ELife*, *8*, e41697.

Jeiranian, H.A., Schalow, B.J., Courcelle, C.T. & Courcelle, J. (2013) Fate of the replisome following arrest by UV-induced DNA damage in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 11421–11426.

Jiang, Q., Karata, K., Woodgate, R., Cox, M.M. & Goodman, M.F. (2009) The active form of DNA polymerase V is UmuD'2C•RecA•ATP. *Nature*, *460*, 359–363.

Kath, J.E., Chang, S., Scotland, M.K., Wilbertz, J.H., Jergic, S., Dixon, N.E. et al (2016) Exchange between *Escherichia coli* polymerases II and III on a processivity clamp. *Nucleic Acids Research*, *44*, 1681–1690.

Kath, J.E., Jergic, S., Heltzel, J.M., Jacob, D.T., Dixon, N.E., Sutton, M.D. et al (2014) Polymerase exchange on single DNA molecules reveals processivity clamp control of translesion synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 7647–7652.

Kaufman, D.L. & Evans, G.A. (1990) Restriction endonuclease cleavage at the termini of PCR products. *BioTechniques*, *9*, 304–306.

Kawabe, Y.I., Seki, M., Yoshimura, A., Nishino, K., Hayashi, T., Takeuchi, T. et al (2006) Analyses of the interaction of WRNIP1 with Werner

syndrome protein (WRN) *in vitro* and in the cell. *DNA Repair*, *5*, 816–828.

Kelman, Z. & O'Donnell, M. (1995) DNA polymerase III holoenzyme: Structure and function of a chromosomal replicating machine. *Annual Review of Biochemistry*, *64*, 171–200.

Kim, J.H., Kang, Y.H., Kang, H.J., Kim, D.H., Ryu, G.H., Kang, M.J. et al (2005) *In vivo* and *in vitro* studies of Mgs1 suggest a link between genome instability and Okazaki fragment processing. *Nucleic Acids Research*, *33*, 6137–6150.

Klein, H.L. & Kreuzer, K.N. (2002) Replication, recombination, and repair: going for the gold. *Molecular Cell*, *9*, 471–480.

Kostylev, M., Otwell, A.E., Richardson, R.E. & Suzuki, Y. (2015) Cloning should be simple: *Escherichia coli* DH5α-mediated assembly of multiple DNA fragments with short end homologies. *PLoS One*, *10*, e0137466.

Kowalczykowski, S.C. (2000) Initiation of genetic recombination and recombination-dependent replication. *Trends in Biochemical Sciences*, *25*, 156–165.

Kuzminov, A. (1995) Collapse and repair of replication forks in *Escherichia coli*. *Molecular Microbiology*, *16*, 373–384.

Kuzminov, A. (1999) Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. *Microbiology and Molecular Biology Reviews*, *63*, 751–813.

Kuzminov, A. (2001) DNA replication meets genetic exchange: Chromosomal damage and its repair by homologous recombination. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8461–8468.

Laranjo, L.T., Gross, S.J., Zeiger, D.M. & Lovett, S.T. (2017) SSB recruitment of Exonuclease I aborts template-switching in *Escherichia coli*. *DNA Repair*, *57*, 12–16.

Lau, I.F., Filipe, S.R., Soballe, B., Okstad, O.A., Barre, F.X. & Sherratt, D.J. (2003) Spatial and temporal organization of replicating *Escherichia coli* chromosomes. *Molecular Microbiology*, *49*, 731–743.

Lenhart, J.S., Brandes, E.R., Schroeder, J.W., Sorenson, R.J., Showalter, H.D. & Simmons, L.A. (2014) RecO and RecR are necessary for RecA loading in response to DNA damage and replication fork stress. *Journal of Bacteriology*, *196*, 2851–2860.

Lestini, R. & Michel, B. (2007) UvrD controls the access of recombination proteins to blocked replication forks. *Embo Journal*, *26*, 3804–3814.

Lopes, M., Cotta-Ramusino, C., Pellicioli, A., Liberi, G., Plevani, P., Muzi-Falconi, M. et al (2001) The DNA replication checkpoint response stabilizes stalled replication forks. *Nature*, *412*, 557–561.

Lovett, S.T. (2017) Template-switching during replication fork repair in bacteria. *DNA Repair*, *56*, 118–128.

Lovett, S.T., Drapkin, P.T., Sutera, V.A. Jr & Gluckman-Peskind, T.J. (1993) A sister-strand exchange mechanism for *recA*-independent deletion of repeated DNA sequences in *Escherichia coli*. *Genetics*, *135*, 631–642.

Lovett, S.T. & Feschenko, V.V. (1996) Stabilization of diverged tandem repeats by mismatch repair: Evidence for deletion formation via a misaligned replication intermediate. *Proceedings of the National Academy of Sciences of the United States of America*, *93*, 7120–7124.

Lovett, S.T., Gluckman, T.J., Simon, P.J., Sutera, V.A. Jr & Drapkin, P.T. (1994) Recombination between repeats in *Escherichia coli* by a *recA*-independent, proximity-sensitive mechanism. *Molecular and General Genetics MGG*, *245*, 294–300.

Lovett, S.T., Hurley, R.L., Sutera, V.A. Jr, Aubuchon, R.H. & Lebedeva, M.A. (2002) Crossing over between regions of limited homology in *Escherichia coli*. *RecA-dependent and RecA-independent pathways*. *Genetics*, *160*, 851–859.

Madiraju, M.V., Lavery, P.E., Kowalczykowski, S.C. & Clark, A.J. (1992) Enzymatic properties of the RecA803 protein, a partial suppressor of *recF* mutations. *Biochemistry*, *31*, 10529–10535.

Mallik, S., Popodi, E.M., Hanson, A.J. & Foster, P.L. (2015) Interactions and localization of *Escherichia coli* error-prone DNA polymerase IV after DNA damage. *Journal of Bacteriology*, *197*, 2792–2809.

Mangiameli, S.M., Merrikh, C.N., Wiggins, P.A. & Merrikh, H. (2017) Transcription leads to pervasive replisome instability in bacteria. *ELife*, *6*, e19848.

Margara, L.M., Fernandez, M.M., Malchiodi, E.L., Argarana, C.E. & Monti, M.R. (2016) MutS regulates access of the error-prone DNA polymerase Pol IV to replication sites: A novel mechanism for maintaining replication fidelity. *Nucleic Acids Research*, *44*, 7700–7713.

Mazin, A.V., Kuzminov, A.V., Dianov, G.L. & Salganik, R.I. (1991) Mechanisms of deletion formation in *Escherichia coli* plasmids. II. Deletions mediated by short direct repeats. *Molecular and General Genetics MGG*, *228*, 209–214.

McCool, J.D., Long, E., Petrosino, J.F., Sandler, H.A., Rosenberg, S.M. & Sandler, S.J. (2004) Measurement of SOS expression in individual *Escherichia coli* K-12 cells using fluorescence microscopy. *Molecular Microbiology*, *53*, 1343–1357.

McGlynn, P. & Lloyd, R.G. (2000) Modulation of RNA polymerase by (p) ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell*, *101*, 35–45.

Merrikh, H., Zhang, Y., Grossman, A.D. & Wang, J.D. (2012) Replication-transcription conflicts in bacteria. *Nature Reviews Microbiology*, *10*, 449–458.

Michel, B. (2000) Replication fork arrest and DNA recombination. *Trends in Biochemical Sciences*, *25*, 173–178.

Michel, B., Boubakri, H., Baharoglu, Z., LeMasson, M. & Lestini, R. (2007) Recombination proteins and rescue of arrested replication forks. *DNA Repair*, *6*, 967–980.

Michel, B., Flores, M.J., Viguera, E., Grompone, G., Seigneur, M. & Bidnenko, V. (2001) Rescue of arrested replication forks by homologous recombination. *Proceedings of the National Academy of Sciences of the United States of America*, *98*, 8181–8188.

Michel, B., Grompone, G., Flores, M.J. & Bidnenko, V. (2004) Multiple pathways process stalled replication forks. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 12783–12788.

Michel, B. & Sinha, A.K. (2017) The inactivation of *rfaP*, *rarA* or *sspA* gene improves the viability of the *Escherichia coli* DNA polymerase III *holD* mutant. *Molecular Microbiology*, *104*, 1008–1026.

Mirkin, E.V. & Mirkin, S.M. (2007) Replication fork stalling at natural impediments. *Microbiology and Molecular Biology Reviews*, *71*, 13–35.

Morag, A.S., Saveson, C.J. & Lovett, S.T. (1999) Expansion of DNA repeats in *Escherichia coli*: effects of recombination and replication functions. *Journal of Molecular Biology*, *289*, 21–27.

Motlagh, N.D.V., Seki, M., Branzei, D. & Enomoto, T. (2006) Mgs1 and Rad18/Rad5/Mms2 are required for survival of *Saccharomyces cerevisiae* mutants with novel temperature/cold sensitive alleles of the DNA polymerase delta subunit, Pol31. *DNA Repair*, *5*, 1459–1474.

Naiman, K., Pages, V. & Fuchs, R.P. (2016) A defect in homologous recombination leads to increased translesion synthesis in *E. coli*. *Nucleic Acids Research*, *44*, 7691–7699.

Nomura, H., Yoshimura, A., Edo, T., Kanno, S., Tada, S., Seki, M. et al (2012) WRNIP1 accumulates at laser light irradiated sites rapidly via its ubiquitin-binding zinc finger domain and independently from its ATPase domain. *Biochemical and Biophysical Research Communications*, *417*, 1145–1150.

Nozaki, S. & Niki, H. (2019) Exonuclease III (XthA) Enforces In Vivo DNA Cloning of *Escherichia coli* To Create Cohesive Ends. *Journal of Bacteriology*, *201*, e00660-e618.

Oliner, J.D., Kinzler, K.W. & Vogelstein, B. (1993) *In vivo* cloning of PCR products in *E. coli*. *Nucleic Acids Research*, *21*, 5192–5197.

Page, A.N., George, N.P., Marceau, A.H., Cox, M.M. & Keck, J.L. (2011) Structure and biochemical activities of *Escherichia coli* MgsA. *Journal of Biological Chemistry*, *286*, 12075–12085.

Pietrobon, V., Fréon, K., Hardy, J., Costes, A., Iraqui, I., Ochsenbein, F. et al (2014) The chromatin assembly factor 1 promotes Rad51-dependent template switches at replication forks by counteracting

D-loop disassembly by the RecQ-type helicase Rqh1. *PLOS Biology*, *12*, e1001968.
Poole, L.A. & Cortez, D. (2017) Functions of SMARCAL1, ZRANB3, and HLTF in maintaining genome stability. *Critical Reviews in Biochemistry and Molecular Biology*, *52*, 696–714.
Robinson, A., McDonald, J.P., Caldas, V.E.A., Patel, M., Wood, E.A., Punter, C.M. et al (2015) Regulation of mutagenic DNA polymerase V activation in space and time. *PLoS Genetics*, *11*, e1005482.
Romero, Z.J., Armstrong, T.J., Henrikus, S.S., Chen, S.H., Glass, D.J., Ferrazzoli, A.E. et al (2019) Frequent template switching in postreplication gaps: suppression of deleterious consequences by the *Escherichia coli* Uup and RadD proteins. *Nucleic Acids Research*, *48*, 212–230.
Romero, Z.J., Chen, S.H., Armstrong, T., Wood, E.A., van Oijen, A., Robinson, A. et al (2020) Resolving Toxic DNA repair intermediates in every *E. coli* replication cycle: critical roles for RecG, Uup and RadD. *Nucleic Acids Research*, *48*, 8445–8460.
Sain, B. & Murray, N.E. (1980) The *hsd* (host specificity) genes of *E. coli* K 12. *Molecular and General Genetics MGG*, *180*, 35–46.
Sandler, S.J. (1994) Studies on the mechanism of reduction of W-inducible *sulAp* expression by *recF* overexpression in *Escherichia coli* K-12. *Molecular and General Genetics MGG*, *245*, 741–749.
Sandler, S.J. & Clark, A.J. (1993) Use of high and low level overexpression plasmids to test mutant alleles of the *recF* gene of *Escherichia coli* K-12 for partial activity. *Genetics*, *135*, 643–654.
Sandler, S.J. & Clark, A.J. (1994) RecOR suppression of *recF* mutant phenotypes in *Escherichia coli* K-12. *Journal of Bacteriology*, *176*, 3661–3672.
Saugar, I., Parker, J.L., Zhao, S.K. & Ulrich, H.D. (2012) The genome maintenance factor Mgs1 is targeted to sites of replication stress by ubiquitylated PCNA. *Nucleic Acids Research*, *40*, 245–257.
Saveson, C.J. & Lovett, S.T. (1997) Enhanced deletion formation by aberrant DNA replication in *Escherichia coli*. *Genetics*, *146*, 457–470.
Sawitzke, J.A. & Stahl, F.W. (1992) Phage lambda has an analog of *Escherichia coli recO*, *recR* and *recF* genes. *Genetics*, *130*, 7–16.
Scharf, S.J., Horn, G.T. & Erlich, H.A. (1986) Direct cloning and sequence analysis of enzymatically amplified genomic sequences. *Science*, *233*, 1076–1078.
Shan, Q., Bork, J.M., Webb, B.L., Inman, R.B. & Cox, M.M. (1997) RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *Journal of Molecular Biology*, *265*, 519–540.
Sherratt, D.J., Soballe, B., Barre, F.X., Filipe, S., Lau, I., Massey, T. et al (2004) Recombination and chromosome segregation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, *359*, 61–69.
Shibata, T., Hishida, T., Kubota, Y., Han, Y.W., Iwasaki, H. & Shinagawa, H. (2005) Functional overlap between RecA and MgsA (RarA) in the rescue of stalled replication forks in *Escherichia coli*. *Genes to Cells*, *10*, 181–191.
Smith, K.C. & Wang, T.-C.-V. (1989) *recA*-dependent DNA repair processes. *BioEssays*, *10*, 12–16.
Stanage, T.H., Page, A.N. & Cox, M.M. (2017) DNA flap creation by the RarA/MgsA protein of *Escherichia coli*. *Nucleic Acids Research*, *45*, 2724–2735.
Syeda, A.H., Hawkins, M. & McGlynn, P. (2014) Recombination and Replication. *Cold Spring Harbor Perspectives in Biology*, *6*, a016550.
Tosa, T. & Pizer, L.I. (1971) Effect of Serine Hydroxamate on the Growth of *Escherichia coli*. *Journal of Bacteriology*, *106*, 966–971.
Totemeyer, S., Booth, N.A., Nichols, W.W., Dunbar, B. & Booth, I.R. (1998) From famine to feast: the role of methylglyoxal production in *Escherichia coli*. *Molecular Microbiology*, *27*, 553–562.
Tsurimoto, T., Shinozaki, A., Yano, M., Seki, M. & Enomoto, T. (2005) Human Werner helicase interacting protein 1 (WRNIP1) functions as a novel modulator for DNA polymerase delta. *Genes to Cells*, *10*, 13–22.
Umezu, K. & Kolodner, R.D. (1994) Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *Journal of Biological Chemistry*, *269*, 30005–30013.
Vandewiele, D., Fernandez de Henestrosa, A.R., Timms, A.R., Bridges, B.A. & Woodgate, R. (2002) Sequence analysis and phenotypes of five temperature sensitive mutator alleles of dnaE, encoding modified alpha-catalytic subunits of *Escherichia coli* DNA polymerase III holoenzyme. *Mutation Research*, *499*, 85–95.
Vujanovic, M., Krietsch, J., Raso, M.C., Terraneo, N., Zellweger, R., Schmid, J.A. et al. (2017) Replication Fork Slowing and Reversal upon DNA Damage Require PCNA Polyubiquitination and ZRANB3 DNA Translocase Activity. *Molecular Cell*, *67*, 882–890.
Wang, T.-C.-V., Chang, H.-Y. & Hung, J.-L. (1993) Cosuppression of *recF*, *recR* and *recO* mutations by mutant *recA* alleles in *Escherichia coli* cells. *Mutation Research/DNA Repair*, *294*, 157–166.
Warming, S., Costantino, N., Court, D.L., Jenkins, N.A. & Copeland, N.G. (2005) Simple and highly efficient BAC recombineering using *galK* selection. *Nucleic Acids Research*, *33*, e36.
Webb, B.L., Cox, M.M. & Inman, R.B. (1995) An interaction between the *Escherichia coli* RecF and RecR proteins dependent on ATP and double-stranded DNA. *Journal of Biological Chemistry*, *270*, 31397–31404.
Webb, B.L., Cox, M.M. & Inman, R.B. (1997) Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell*, *91*, 347–356.
Whitby, M.C. & Lloyd, R.G. (1995) Altered SOS induction associated with mutations in *recF*, *recO* and *recR*. *Molecular and General Genetics MGG*, *246*, 174–179.
Yoshimura, A., Seki, M. & Enomoto, T. (2017) The role of WRNIP1 in genome maintenance. *Cell Cycle*, 1–7.
Yoshimura, A., Seki, M., Hayashi, T., Kusa, Y., Tada, S., Ishii, Y. et al (2006) Functional relationships between Rad18 and WRNIP1 in vertebrate cells. *Biological and Pharmaceutical Bulletin*, *29*, 2192–2196.
Yoshimura, A., Seki, M., Kanamori, M., Tateishi, S., Tsurimoto, T., Tada, S. et al. (2009) Physical and functional interaction between WRNIP1 and RAD18. *Genes and Genetic Systems*, *84*, 171–178.
Zienkiewicz, M., Kern-Zdanowicz, I., Carattoli, A., Gniadkowski, M. & Cegłowski, P. (2013) Tandem multiplication of the IS26-flanked amplicon with the *blaSHV-5* gene within plasmid p1658/97. *FEMS Microbiology Letters*, *341*, 27–36.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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