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# Promoting and Avoiding Recombination: Contrasting Activities of the *Escherichia coli* RuvABC Holliday Junction Resolvase and RecG DNA Translocase

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ABSTRACT

RuvABC and RecG are thought to provide alternative pathways for the late stages of recombination in *Escherichia coli*. Inactivation of both blocks the recovery of recombinants in genetic crosses. RuvABC resolves Holliday junctions, with RuvAB driving branch migration and RuvC catalyzing junction cleavage. RecG also drives branch migration, but no nuclease has been identified that might act with RecG to cleave junctions, apart from RusA, which is not normally expressed. We searched for an alternative nuclease using a synthetic lethality assay to screen for mutations causing inviability in the absence of RuvC, on the premise that a strain without any ability to cut junctions might be inviable. All the mutations identified mapped to *polA*, *dam*, or *uvrD*. None of these genes encodes a nuclease that cleaves Holliday junctions. Probing the reason for the inviability using the RusA Holliday junction resolvase provided strong evidence in each case that the RecG pathway is very ineffective at removing junctions and indicated that a nuclease component most probably does not exist. It also revealed new suppressors of *recG*, which were located to the *ssb* gene. Taken together with the results from the synthetic lethality assays, the properties of the mutant SSB proteins provide evidence that, rather than promoting recombination, a major function of RecG is to curb potentially pathological replication initiated via PriA protein at sites remote from *oriC*.

THE early stages of genetic recombination in *Escherichia coli* associated with initiation of homologous DNA pairing and with strand exchange are well established and can be described in terms of enzymology and reaction pathways (Dillingham and Kowalczykowski 2000, 2008; Singleton *et al.* 2004; Cox 2007a,b). However, later stages associated with resolution of Holliday junction intermediates have proven more difficult to pin down because of what appears at first sight to be a functional overlap between the RuvABC and RecG proteins. RecG also appears to have multiple roles in DNA metabolism that obscure the nature and extent of its involvement in recombination.

The RuvA and RuvB proteins together catalyze branch migration of Holliday junction intermediates and form a complex with RuvC protein that enables the latter to resolve these intermediates by a dual strand cleavage reaction (van Gool *et al.* 1998). RecG is a dsDNA translocase and, like RuvAB, catalyses branch migration of Holliday junctions (Lloyd and Sharples 1993; McGlynn and Lloyd 2001; Singleton *et al.* 2001). Its elimination from *ruv* mutants blocks the recovery of recombinants in genetic crosses and confers extreme sensitivity to genotoxic agents (Lloyd 1991). The strong synergism observed led to the idea that RuvABC and RecG provide partially overlapping pathways for the resolution of Holliday junctions. However, RecG proved to have no intrinsic ability to cleave junctions (Lloyd and Sharples 1993), which raised the possibility that some unidentified nuclease could act with RecG to promote Holliday junction resolution in the way RuvC acts with RuvAB. The RusA protein was a possible candidate (Sharples *et al.* 1994). This homodimeric endonuclease resolves Holliday junctions by a dual strand cleavage mechanism that targets specific DNA sequences (Sharples *et al.* 1994; Bolt and Lloyd 2002). Its expression compensates very effectively for the absence of RuvABC and in a RecG-dependent manner (Mandal *et al.* 1993; Mahdi *et al.* 1996). However, RusA is encoded by a cryptic prophage gene (*rusA*) and is not normally expressed because the gene lacks a promoter. Furthermore, its deletion does not reduce recombination in *ruv* mutant strains (Mahdi *et al.* 1996). Therefore, RusA cannot be the resolvase that operates in strains lacking RuvABC, although it can act as such when activated by a promoter inserted upstream of *rusA* (Mandal *et al.* 1993; Mahdi *et al.* 1996).

To date, our screens for mutations blocking recombination in *ruv* mutants failed to identify an alternative nuclease that could act with RecG, revealing only

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knockouts of the RecA, RecB, or RecC proteins needed to initiate exchanges or of RecG (our unpublished work). This failure could be explained if the requisite activity is needed to maintain viability, at least in the absence of RuvABC or is provided by more than one nuclease. Alternatively, there may be no such nuclease, with RecG alone able to eliminate Holliday junctions simply by driving branch migration and enabling them to merge with replication forks, as has been suggested (Wardrope *et al.* 2009). This possibility would suffice to explain why eliminating RecG has such a strong synergistic effect on *ruv* strains. But recent studies indicate that there may be an additional and perhaps more radical explanation.

RecG unwinds a variety of branched DNA molecules, at least *in vitro* (McGlynn and Lloyd 2000, 2001, 2002b). These include the D loops and R loops that PriA protein could otherwise exploit to initiate stable DNA replication (SDR), a form of chromosome replication that is independent of *oriC* and of the initiator protein DnaA, and which also includes the replication primed by recombination during repair of chromosome breaks (Vincent *et al.* 1996; Fukuoh *et al.* 1997; Kogoma 1997; McGlynn *et al.* 1997). SDR is elevated constitutively in cells lacking RecG (Hong *et al.* 1995) and triggers severe overreplication of the chromosome when increased even further by damage to DNA. This sets in motion a pathological cascade that interferes with the cell cycle and which results in the formation of extraordinarily long filaments that bud off a small cell capable of normal growth and division only after a very long delay (Rudolph *et al.* 2009a,b). Without RuvABC, this problem is likely to be much exacerbated by the failure to resolve Holliday junctions. Thus, *ruv recG* double mutants may be exceptionally sensitive to UV light and other DNA-damaging agents because they are simply overwhelmed by pathological consequences resulting from the increase in SDR.

In this article, we present evidence supporting this hypothesis. The evidence stems from studies in which we had initially exploited a synthetic lethality assay to screen for mutations incompatible with a deletion of *ruvC*. The seven mutations identified inactivated *dam*, *polA*, or *uvrD* (supporting information, File S1 and Figure S1), which previous studies had reported to be inviable with *ruv* (Ishioka *et al.* 1998; Marinus 2000; Flores *et al.* 2005; Magner *et al.* 2007). The *polA* gene encodes Pol I, a DNA polymerase and exonuclease associated with nucleotide excision repair and the processing of Okazaki fragments during DNA replication (Moolenaar *et al.* 2000), whereas the *dam* gene encodes a deoxyadenosine methylase that directs the MutHLS mismatch repair system to the newly synthesized strands (Modrich 1991). Without efficient means to remove RNA primers and complete synthesis, it is thought that *polA* mutants retain gaps in the nascent strands, gaps that trigger fork collapse during the next round of replication (Figure 1A). With no means to distinguish parental strands from nascent strands, the MutHLS proteins initiate mismatch repair indiscriminately in *dam* mutants, which may result in chromosome breakage when repair tracks overlap (Figure 1B). Thus, the viability of both *polA* and *dam* mutants depends on recombination proteins to repair DNA breaks—RecBCD and RecA to initiate exchanges at the broken DNA ends and RuvABC to cleave Holliday junctions (Figure 1, A and B) (Lloyd *et al.* 1974; Hong *et al.* 1995; Kuzminov 1995; Ishioka *et al.* 1998; Marinus 2000).

The *uvrD* gene encodes a DNA helicase associated with both mismatch repair and UvrABC-dependent nucleotide excision repair (Matson and Kaiser-Rogers 1990; Modrich 1991). Recent studies indicate that it also acts to limit recombination when replication forks stall (Flores *et al.* 2005). UvrD has been shown to displace RecA filaments assembled on ssDNA *in vitro* and is thought to do so *in vivo* when RecA is loaded on a region of the nascent lagging strand template exposed at a stalled fork through the actions of RecQ helicase and RecJ exonuclease. With no UvrD present, the RecFOR proteins establish a stable RecA filament on the exposed template (Figure 1C), thus presumably provoking recombination even though this recombination is not essential, as indicated by the fact that inactivating RecA or preventing RecA loading restores viability to *uvrD ruv* cells (Flores *et al.* 2005; Veaute *et al.* 2005; Lestini and Michel 2007; Magner *et al.* 2007). Although UvrD is thought to reduce such pathology by displacing RecA, it might also limit fork stalling by providing a second helicase motor at the fork to help DnaB drive through obstacles (Figure 1C).

What is particularly significant about these observations is that the presence of RecG itself is clearly not sufficient to maintain the viability of *polA*, *dam*, and *uvrD* strains lacking RuvABC. The possibility that the products of these three genes are all essential components of a RecG recombination pathway needed to maintain viability is highly unlikely. None has any activity that might resolve Holliday junctions. Our studies provide strong genetic evidence that the inviability observed when *polA*, *dam*, and *uvrD* strains lack an intact RuvABC system is due in all three cases to the accumulation of Holliday junctions. More importantly, they indicate that RecG is not at all effective in removing Holliday junctions and suggest there is probably no nuclease expressed in wild-type (WT) *E. coli* cells that enables RecG to provide an effective alternative to RuvABC. Taken together, the results presented support the notion that a major function of RecG is to limit PriA-mediated overreplication of the chromosome and its pathological consequences.

## MATERIALS AND METHODS

**Strains:** Bacterial strains are listed in Table S1. All constructs used for synthetic lethality assays are based on *E. coli* K-12

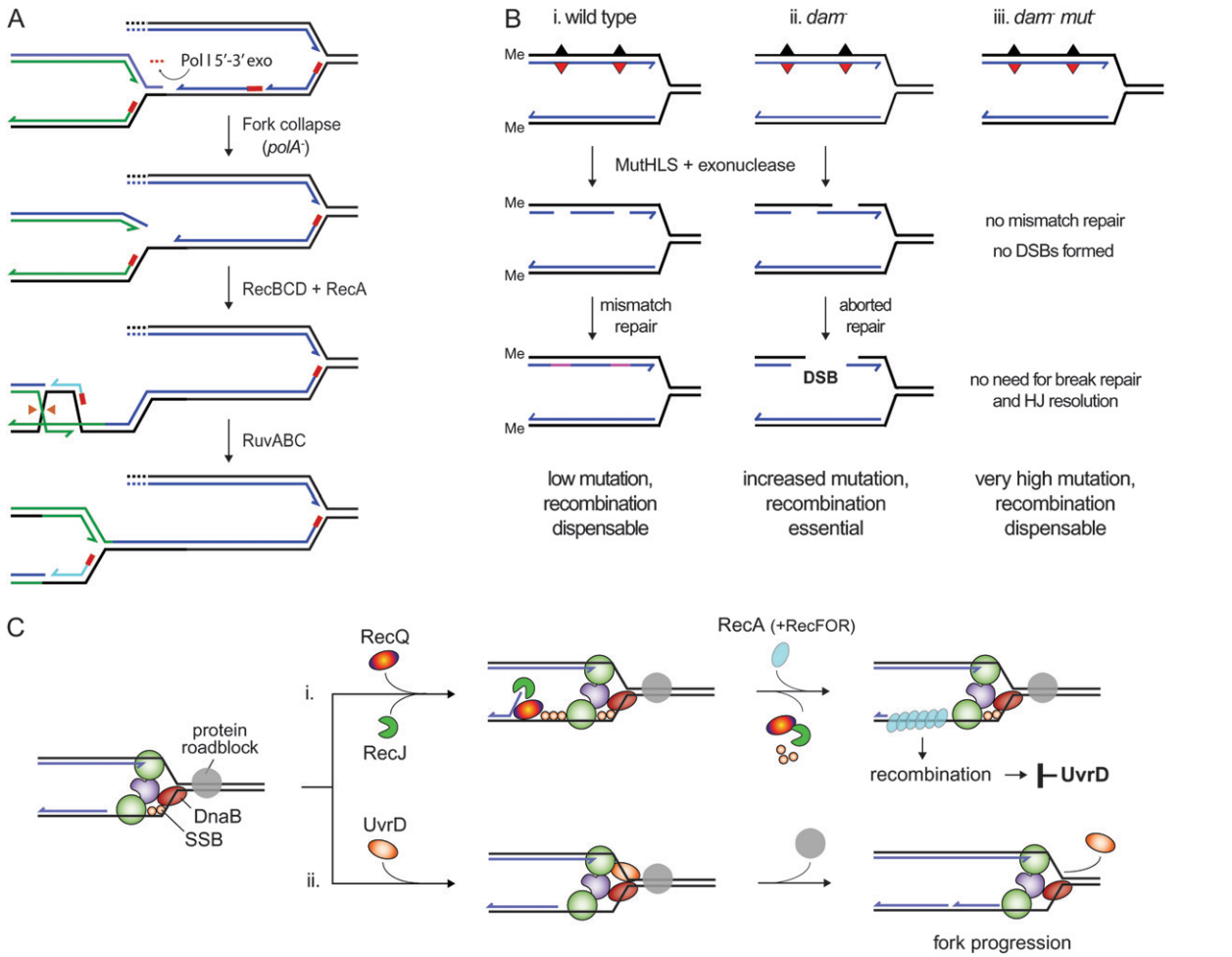


FIGURE 1.—Models of how chromosome replication is affected by *polA*, *dam*, and *uvrD* mutations. (A) Replication fork collapse following incomplete processing of Okazaki fragments in the absence of the polymerase activity of DNA polymerase I. The fork is rescued by recombination, enabling replication to be completed. (B, panel i) MutHLS-mediated mismatch repair in wild-type cells is directed by Dam methylation to the transiently unmethylated nascent strands, enabling replication errors to be eliminated. (panel ii) Chromosome breakage and repair following undirected initiation of mismatch repair in a *dam* mutant. (panel iii) Inactivation of MutHLS prevents chromosome breakage in a *dam* mutant strain and thus eliminates the need for recombination to maintain viability. (C) UvrD prevents recombination during chromosome replication by either (panel i) removing RecA filaments assembled at a stalled fork and/or by (panel ii) helping the replisome to drive through obstacles that might block fork progression.

MG1655 Δ*lacIZYA* (BERNHARDT and DE BOER 2004) carrying pRC7 derivatives. Chromosomal genes were inactivated using Tn*10* or Tn*10kan* insertions, conferring resistance to tetracycline (Tc$^r$) and kanamycin (Km$^r$), respectively, or with deletions tagged with resistance to chloramphenicol (Cm$^r$), kanamycin (Km$^r$), trimethoprim (Tm$^r$), or apramycin (Apra$^r$). Unless referenced otherwise, tagged deletions removed the entire coding sequence of the genes concerned and were generated as described (DATSENKO and WANNER 2000). Further details of the *polA*, *dam*, and *uvrD* mutations are presented in File S1, Figure S1, and Figure S2.

**Plasmids:** The pRC7 construct is a low-copy number, mini-F derivative of pFZY1. It carries the *bla* gene encoding resistance to ampicillin (Ap$^r$) and the *lacZYA* operon under control of *lacI$^q$* (Figure S1A) (BERNHARDT and DE BOER 2004). pAM372 carries the *ruvC*$^+$ coding sequence inserted at the multiple cloning site (MCS) in pRC7 under control of the *lac* promoter. pAM390 carries the wild-type *ruvAB* operon under control of its native promoter inserted at the *Apa*I site in pAM372. pJJ100 and pJJ103 carry the entire *recG*$^+$ gene and some upstream sequences inserted at the *Apa*I site in pRC7 and pAM372, respectively. pAM408 is a derivative of pAM372 carrying the entire *recG*$^+$ gene, some upstream sequences, and a downstream *Bam*HI site inserted at the *Apa*I site. pAM409 is a derivative of pAM408 carrying the *ruvAB* operon under control of its native promoter inserted at the *Bam*HI site downstream of *recG*. The *ruv*$^+$ and/or *recG*$^+$ genes cloned in these pRC7 derivatives restore full resistance to mitomycin C and UV light in strains carrying the relevant *ruv* or *recG* null allele.

pGB066 was constructed by cloning the coding sequence for *priA*$^+$ into the expression vector pT7-7, as described for the *recG*$^+$ construct pAM210 (MAHDI *et al.* 2003). pAM423, pAM425, pAM426, pDIM024, and pDIM025 encode WT SSB protein, or K44E, N14D, R97C or Δ115–144 derivatives, respectively. They carry the coding sequences for *ssb*$^+$, *ssb[A130G]*, *ssb[A40G]*, *ssb[C289T]*, or *ssb[Δ345-434]*, respectively, cloned at the *Nde*I site in pET22b. pCC178 and pCC180 are pET22b derivatives encoding SSB with a P176S substitution of the penultimate residue (*ssb113*) or a deletion of the last 10 residues (Δ*C10*) (CADMAN and MCGLYNN 2004).

**Media and general methods:** Growth media, and methods for monitoring cell growth, P1*vir* transduction, and for determining sensitivity to UV and mitomycin C have been cited (Al-Deib *et al.* 1996; McGlynn and Lloyd 2000; Trautinger *et al.* 2005).

**Synthetic lethality assay:** Cultures of strains carrying pRC7 derivatives were grown overnight in LB broth containing ampicillin to maintain plasmid selection, diluted 80-fold in LB broth, and grown without ampicillin selection to an $A_{650}$ of 0.4 before spreading dilutions on LB agar supplemented with X-gal and IPTG. Plates were photographed and scored after 48 hr at 37°. Where 56/2 minimal salts agar was substituted for LB agar (as indicated in Figure S4B, panel i), the plates were photographed after 72 hr (56/2 agar). Plasmid-free cells forming small white colonies were restreaked on LB agar, to see whether they could be subcultured, and were photographed after 24 hr at 37°. The assay is described in further detail in File S1 and illustrated in Figure S1, A–C.

**Mapping *priA* and *ssb* suppressors of *recG dam*:** Suppressors were isolated as shown in Figure 3B, panel i. Mutations in *priA* were identified by linkage (~90%) to *metB* followed by PCR sequencing. Mutations in *ssb* were identified by first restoring *recG*$^+$ and *dam*$^+$ to the initial strain isolates (JJ1329 and JJ1331), generating strains JJ1489 and JJ1490, respectively (Table S1), both of which remained sensitive to UV light. These were transduced with P1 phage grown on pools of cells carrying random *kan* insertions in the chromosome, generated in strain MG1655 using the EZ-Tn5 ⟨kan-2⟩ Tnp transposome system (Epicentre Technologies). Km$^r$ transductants that were also UV$^r$ were identified. PCR sequencing identified insertions in *yjcB* and *yjbQ*, very close to *ssb*. Further sequencing revealed an A to G transition at bp 130 of the *ssb* coding sequence of JJ1489 and at bp 40 in that of JJ1490. These changes were verified as the suppressors by engineering the same substitution into the chromosome, as described (Datsenko and Wanner 2000), and demonstrating that they conferred sensitivity to UV.

**Proteins:** Wild-type RecG was purified as described (Mahdi *et al.* 2003). Wild-type and mutant SSB proteins were expressed using the relevant *ssb* plasmid transformed into the nuclease-depleted BL21(DE3) derivative, STL5827, or JJ1634 and JJ1635 in the case of N14D and K44E, respectively, and purified broadly as described (Cadman and McGlynn 2004). The molecular mass of the purified SSB proteins was determined by gel filtration on a Superdex 200 10/300 column in 20 mm Tris–HCl 7.5, 150 mm NaCl, and corresponded to that of a tetramer in every case.

For wild-type PriA, cultures of STL5827 transformed with pGB066 were grown to an $A_{650}$ of ~0.6 in Mu broth supplemented with ampicillin before adding IPTG to induce expression of *priA*. After 3 hr of further incubation, the induced cells were collected by centrifugation, broken open by sonication, and PriA purified from the supernatant by passage through heparin- and SP-sepharose columns, followed by gel filtration on a Hiprep 16/60 sephacryl S-200 column, before storing at −80°.

**Physical interaction of SSB with PriA and RecG:** Equimolar mixtures of SSB (tetramer form) and PriA or of SSB and RecG in 20 mm Tris-HCl pH 7.5 were kept on ice for 15 min before adding 2.5 μl 750 g/liter ammonium sulfate solution (final volume, 10 μl). After a further 15 min on ice, the mixtures were centrifuged for 10 min at 14,000 rpm, the supernatants removed, and pellets resuspended in 10 μl 20 mm Tris–HCl. Fractions were then analyzed by SDS–PAGE, using 15% polyacrylamide gels. Controls containing PriA, RecG, or SSB alone, or equimolar mixtures of PriA and SSB, or RecG and SSB proteins without ammonium sulfate precipitation, were analyzed in parallel.

**DNA binding and unwinding assays:** DNA binding was measured by a gel retardation assay using a 50-mer oligonucleotide (5′-ATTCGGCAGCGTTAGCTATCAGAGATCTGTC GTTACAGG-3′) labeled with $^{32}$P at the 5′ end. Binding reactions (20 μl) contained 0.2 nm of labeled oligonucleotide and the indicated SSB proteins at final concentrations of 0.5, 5, and 50 nm in low ionic strength buffer, and were analyzed by electrophoresis on 4% polyacrylamide gels (Lloyd and Sharples 1993). DNA unwinding by PriA helicase, and the effect of SSB on this activity was measured using Fork 2, essentially as described (Cadman and McGlynn 2004), except the ATP and $MgCl_2$ were both at 5 mm.

## RESULTS

Previous studies demonstrated that *polA*, *dam*, and *uvrD* strains lacking RuvABC are inviable and assumed the cells accumulate Holliday junctions that interfere with growth and division (Ishioka *et al.* 1998; Marinus 2000; Flores *et al.* 2005; Magner *et al.* 2007). If true, it would imply that RecG does not provide an efficient alternative resolution pathway. We exploited RusA to investigate whether this is indeed the case, examining its effect on the viability of the relevant double mutant. We used a synthetic lethality assay based on pRC7 for this purpose, a mini-F derivative that lacks F stabilization systems and which is therefore easily lost (Bernhardt and de Boer 2004). It carries the *lac*$^+$ genes and its loss is revealed in a Δ*lac* background by segregation of Lac$^-$ clones. On plates containing the β-galactosidase indicator, X-gal, these clones form white colonies or white sectors within blue (Lac$^+$) colonies, depending on whether plasmid loss occurred before or after plating (Figure S1, A and B) (Bernhardt and de Boer 2004). A *ruv*$^+$ derivative of pRC7 was used to cover a *ruv* deletion in the chromosome (*ruv*$^+$/Δ*ruv*) before introducing a deletion or insertion inactivating *polA*, *dam*, or *uvrD*. Inviability between the covered *ruv* mutation and the uncovered *polA*, *dam*, or *uvrD* allele is revealed by the absence of Lac$^-$ clones and the formation of uniformly blue colonies, or in the case of *uvrD ruv*, by the appearance of rare and rather sickly Lac$^-$ clones (Figure 2, A–D, panels i). Clearly, only those cells retaining the plasmid and therefore expressing RuvABC are capable of robust growth.

**RusA restores viability to *polA*, *dam*, and *uvrD* cells lacking RuvABC:** Parallel constructs were made in which expression of RusA had been activated by *rus-2*, an IS10 insertion upstream of the *rusA* coding sequence (Mahdi *et al.* 1996). Ample growth of plasmid-free, Lac$^+$ clones was detected in each case, demonstrating that RusA confers robust viability on the double mutant cells (Figure 2, A–D, panels ii). Given RusA cleaves Holliday junctions with high specificity and efficiency, and has comparatively little activity on other forms of branched DNAs unless these can adopt a four-way branched configuration mimicking a Holliday junction (Bolt and Lloyd 2002), these observations leave little doubt that unresolved Holliday junctions are responsible

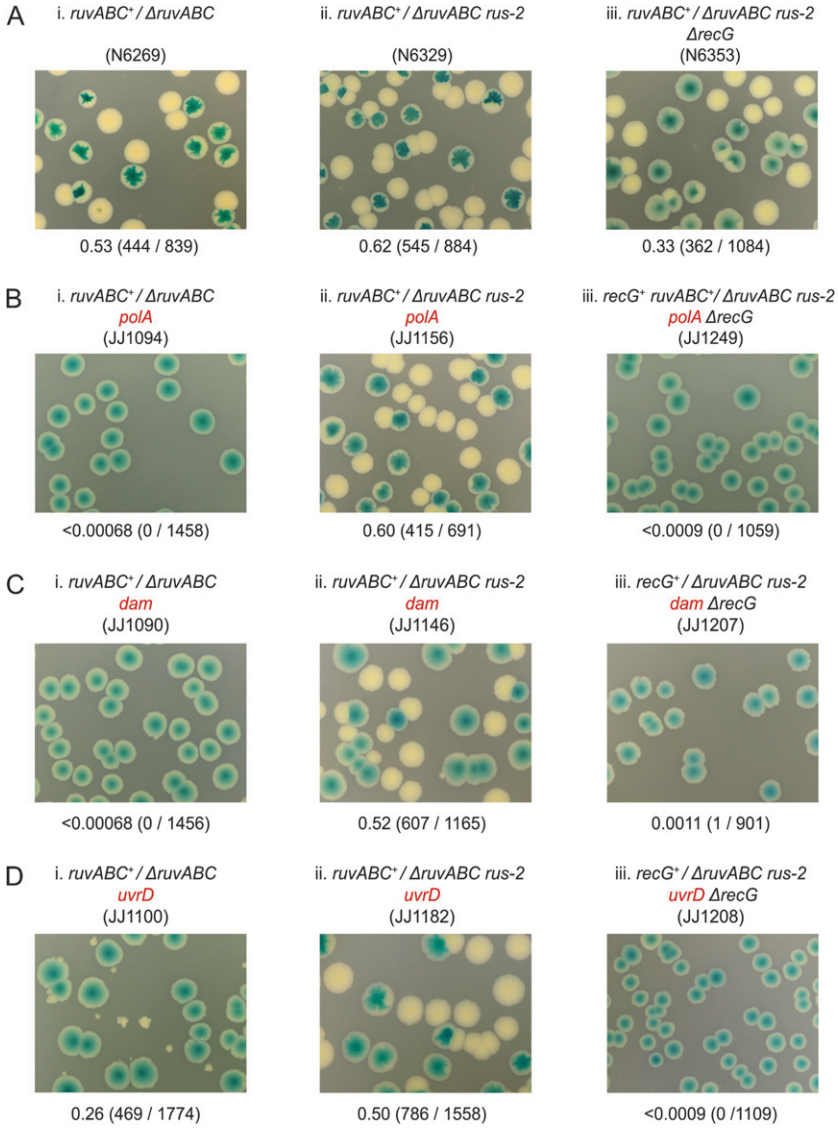


FIGURE 2.—Synthetic lethality assays showing how RusA confers viability on *polA*, *dam*, and *uvrD* cells lacking RuvABC. (A) Control strains. (B) *polA* constructs. (C) *dam* constructs. (D) *uvrD* constructs. The synthetic lethality assay exploited in A–D and in subsequent figures is described in detail in MATERIALS AND METHODS and further illustrated in Figure S1. The relevant genotype of the synthetic lethality construct used is shown *above* each photograph. In each case the relevant plasmid genotype/relevant chromosome genotype (*e.g.*, *ruvABC*$^+$/Δ*ruvABC*) is indicated, along with the strain number in parentheses. The fraction of white colonies is shown *below* with the number of white colonies/total colonies analyzed in parentheses.

for the inviability of *polA*, *dam*, and *uvrD* cells lacking RuvABC.

Previous studies revealed that the ability of RusA to promote recombination and DNA repair in the absence of RuvABC depends on RecG (MANDAL *et al.* 1993; MAHDI *et al.* 1996). Synthetic lethality constructs based on *recG*$^+$ derivatives of pRC7 revealed that the same is true for RusA's ability to confer viability on *polA*, *dam*, and *uvrD* cells lacking RuvABC (Figure 2, A–D, panels iii). This dependence on RecG, coupled with the robust viability observed with RecG present, points very clearly toward the conclusion that any nuclease wild-type *E. coli* may have that is able to act in concert with RecG to resolve Holliday junctions must operate very inefficiently indeed compared with RusA.

**RecG is required to limit the activity of PriA:** Drawing the conclusion from the data in Figure 2 that RecG is needed to support junction resolution by RusA has to be tempered by evidence that RecG itself is required to maintain viability in the case of *polA* and *dam* cells (HONG *et al.* 1995; MARINUS 2000). Synthetic lethality assays based on a *recG*$^+$ derivative of pRC7 confirmed this was so for *polA recG* cells (Figure 3A, panels i and ii). With *dam recG* cells, the assays revealed that viability is much reduced. The double mutant cells form tiny white colonies without the covering plasmid and although these colonies can be subcultured they accumulate suppressors with high frequency, as evident from the emergence of large colony variants (Figure 3B, panels i and ii). The low viability of the *dam recG* cells explains the previous failure to construct the double mutant by conventional crosses (MARINUS 2000).

Previous studies demonstrated that it is possible to construct a *recG uvrD* double mutant (MENDONCA and MATSON 1995). However, a synthetic lethality assay based on a *recG*$^+$/Δ*recG uvrD* construct revealed that eliminating both RecG and UvrD reduces cell viability. This is clear from the smaller size of the colonies formed by plasmid-free segregants. These segregants also accumulate suppressors that appear as larger colony variants

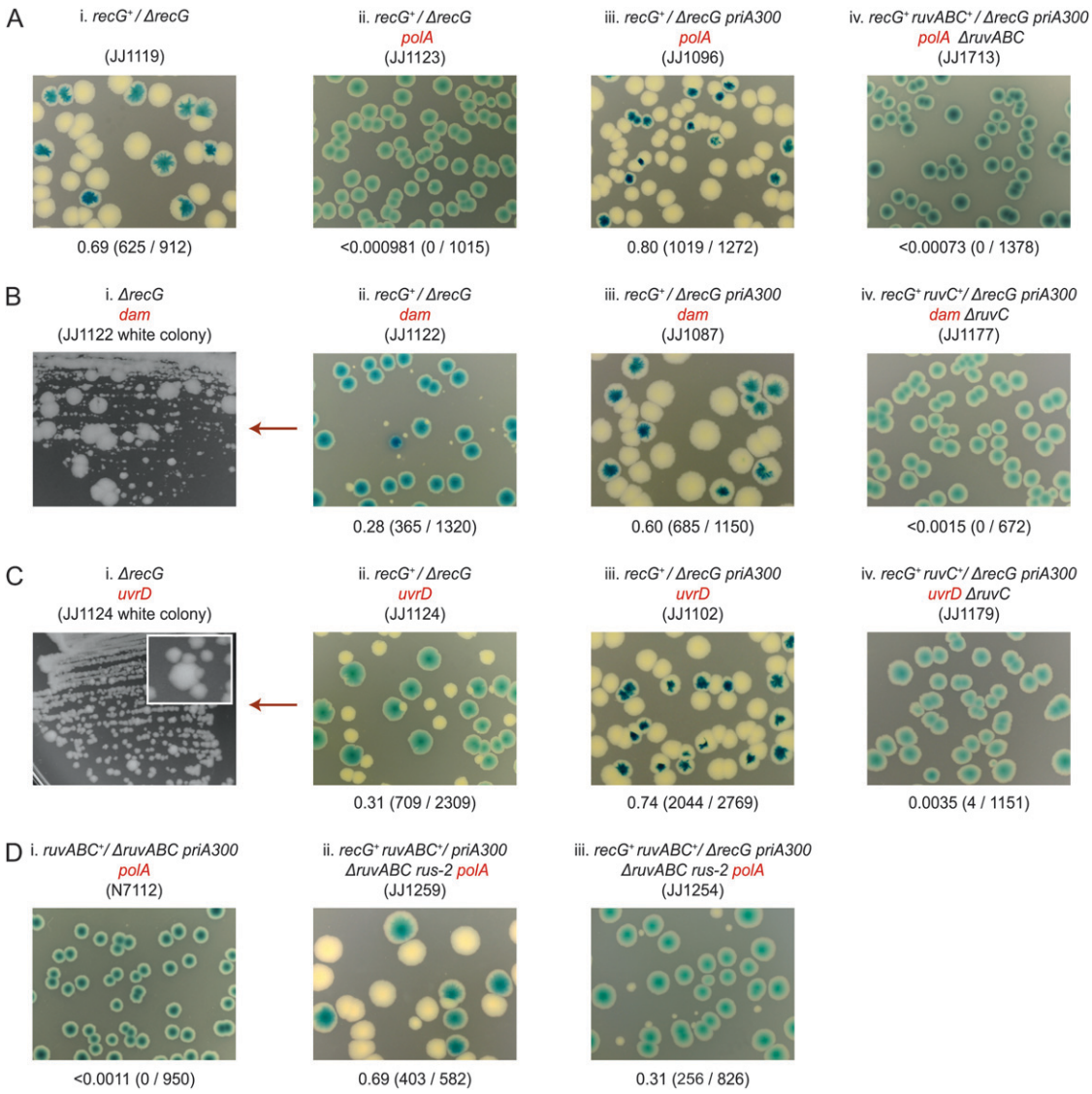


Figure 3.—Synthetic lethality assays showing how $PriA_{K230R}$ confers viability on *polA*, *dam*, and *uvrD* cells lacking RecG or improves their viability. (A and D) *polA* constructs. (B) *dam* constructs. (C) *uvrD* constructs. The overlay inset in C, panel i, is a magnification of a section of the streak underneath showing a large colony variant.

on subculture (Figure 3C, panels i and ii). However, neither effect is as extreme as with *dam recG* cells. The reduced viability of *polA*, *dam*, and *uvrD* cells lacking RecG is surprising given RuvABC is available and our evidence that any RecG pathway for the resolution of Holliday junctions is at best highly inefficient. A recent report also revealed that *recG* cells show little evidence of accumulating Holliday junctions following DNA breakage (Wardrope *et al.* 2009). So, why should RecG be needed to maintain viability?

Previous studies showed that the sensitivity of *recG* cells to mitomycin C and to other DNA damaging agents can be suppressed by mutations reducing or eliminating the helicase activity of PriA, demonstrating that this activity can be detrimental to cell viability when the DNA is damaged and RecG is absent (Al-Deib *et al.* 1996). We considered whether a similar explanation might also account for the reduced viability of *polA*, *dam*, and *uvrD* cells lacking RecG and tested this possibility using *recG*$^+$/Δ*recG priA300* constructs. The *priA300* allele encodes helicase-defective $PriA_{K230R}$ (Zavitz and Marians 1992) and is an effective suppressor of *recG* (Jaktaji and Lloyd 2003). With this allele present, *polA*, *dam*, and *uvrD* derivatives proved quite viable, as evident from the robust growth of plasmid-free Lac$^-$ colonies (Figure 3, A–C, panels iii). However, Δ*ruvC* and Δ*ruvABC* derivatives revealed that this requires the RuvABC system to be intact (Figure 3, A–C, panels iv; data not shown). Furthermore, *priA300* fails to restore viability to equivalent Δ*ruvABC recG*$^+$ and Δ*ruvC recG*$^+$ constructs inactivated for *polA*, *dam*, or *uvrD* (Figure 3D, panel i; data not shown), consistent with previous studies demonstrating that it does not suppress the *ruv* mutant phenotype (Jaktaji and Lloyd 2003). Thus, whereas RecG is dispensable

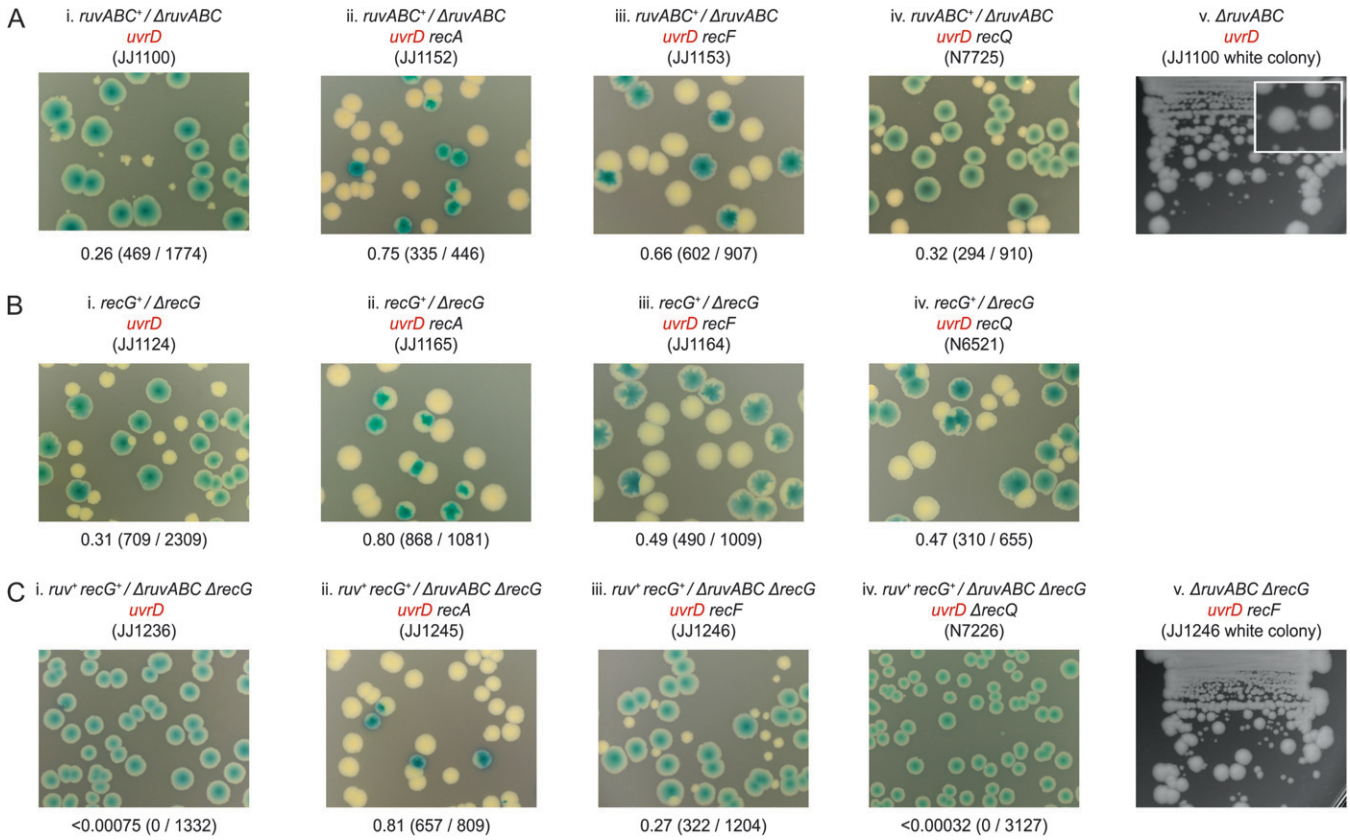


FIGURE 4.—Synthetic lethality assays demonstrating how RecA, RecF, and RecQ affect the viability of *uvrD* cells lacking RuvABC and/or RecG. (A) Cells lacking RuvABC. (B) Cells lacking RecG. (C) Cells lacking both RuvABC and RecG.

once the helicase activity of PriA is eliminated, RuvABC is most certainly not.

This dependence on RuvABC indicates that Holliday junctions still accumulate. We tried to test this directly by building a Δ*recG* Δ*ruvABC priA300 polA* construct in which the recG and *ruvABC* deletions were covered by a *recG*$^+$ *ruv*$^+$ plasmid, and in which RusA was activated by *rus-2*. The construct made segregates white colonies, but these are tiny and grow very poorly on subculture, consistent with RusA being effective only in the presence of RecG (Figure 3D, panels ii and iii; data not shown). Nevertheless, the fact that white colonies do appear supports the notion that the cells accumulate Holliday junctions in the absence of RuvABC, despite the presence of *priA300*.

From these data, it would appear that the viability of *polA*, *dam*, and *uvrD* cells is reduced in the absence of RecG simply because they lack means to counter some deleterious effect of PriA helicase activity. When this is eliminated, RuvABC maintains viability very effectively without any assistance from RecG.

**The absence of RecG provokes recombination:** The plasmid-free cells segregated by a *ruv*$^+$/Δ*ruv uvrD* construct form very sickly colonies that accumulate suppressors, which is not surprising given the mutator phenotype associated with *uvrD*. These suppressors appear as large colony variants on subculture (Figure 4A, panels i and v). Genetic analysis revealed that mutations inactivating RecA, RecFOR, RecJ, or RecQ restore robust viability and account for the suppressors observed (Figure 4A, panels ii–iv; Figure S3, A and B; data not shown). This analysis confirmed previous studies and supports the model outlined in Figure 1C, panel i (FLORES *et al.* 2005; MAGNER *et al.* 2007).

Synthetic lethality assays based on *recG*$^+$/Δ*recG uvrD* constructs revealed a similar improvement in the growth of plasmid-free *recG uvrD* cells following the inactivation of RecA, RecFOR, or RecQ (Figure 4B, panels i–iv; data not shown), suggesting perhaps there might be a common basis for the reduced viability of *ruv uvrD* and *recG uvrD* cells. We tested this by eliminating RecA from *uvrD* cells lacking both RuvABC and RecG. With RecA present, these cells fail to form any colonies without a covering *recG*$^+$ *ruv*$^+$ plasmid (Figure 4C, panel i). Removing RecA restores robust viability (Figure 4C, panel ii). Significantly, removing RecF or RecO is much less effective in this case. It allows plasmid-free cells to form colonies, but these are small relative to those formed by cells retaining the plasmid and accumulate suppressors, demonstrating that their viability is still compromised (Figure 4C, panels iii and v; data not shown). Removing RecQ does not help at all, but neither does it

interfere with the improved viability observed on removing RecA (Figure 4C, panel iv; data not shown). If one accepts that the viability of a strain lacking RuvABC depends on the incidence of recombination mediated by RecA, then these data would suggest that such recombination is more frequent in Δ*ruvABC* cells lacking both UvrD and RecG than in Δ*ruvABC* cells lacking only UvrD. This in turn implies that eliminating RecG itself provokes recombination, and for a reason distinct from that provoked by the absence of UvrD. Since eliminating RecQ does not improve viability at all, and inactivating the RecF or RecO component of RecFOR is only partially effective, we suspect this additional recombination is not triggered by the exposure of ssDNA at stalled forks.

**Identification of *ssb* suppressors of *recG*:** The feeble growth of Δ*recG dam* cells facilitates the identification of suppressors as large colony variants (Figure 3B, panels i and ii). Among the ≥30 independent suppressors analyzed, we identified 11 with mutations in *priA* and 2 with mutations in *ssb*. Others were strong mutators, and reconstructions confirmed that inactivating MutH, MutL, or MutS restores viability (Figure 5, A and B; data not shown). Our analyses demonstrated that the undirected initiation of mismatch repair creates a problem for both *recG* and *ruv* cells.

Given the already established effect of *priA300* (Figure 3B, panel iii), the *priA* suppressors were no surprise. The spectrum of alleles was broader than observed in a previous study that selected directly for *recG* suppressors restoring resistance to mitomycin C (Al-Deib *et al.* 1996). Indeed two were promoter mutations (Figure 5A), indicating that a reduction in the overall level of PriA suffices to improve viability in this case.

The *ssb* mutations proved quite robust suppressors. Both carry A to G transitions in the *ssb* gene (one at position 40 and one at position 130), encoding either an N14D or a K44E substitution in SSB (Figure 5C). In each case, the ability of the altered SSB to confer viability depends on having an intact RuvABC system. The same loss of viability is observed on removing RuvC alone or all three Ruv proteins (Figure 5D and data not shown). Furthermore, $ruv^{+}$/Δ*ruv dam* constructs carrying these *ssb* mutations revealed that neither is a suppressor of *ruv dam* inviability (data not shown). This ability of both *priA* and *ssb* mutations to confer viability on *recG dam* but not on either *recG dam ruv* or *ruv dam* is significant. It demonstrates very clearly that the depletion of RecG and RuvABC creates very different problems for cells initiating mismatch repair indiscriminately during chromosome replication.

**The N14D and K44E derivatives of SSB specifically suppress *recG*:** Both of the *ssb* suppressor strains proved moderately sensitive to UV light. Introducing wild-type alleles for *recG* or *dam*, or both *recG* and *dam*, established that this is a property of the *ssb* alleles (Figure 5E and materials and methods). It also revealed that these mutations specifically suppress *recG*. Despite the sensitivity conferred to UV, these mutations confer no sensitivity to mitomycin C and strongly suppress the mitomycin C sensitivity conferred by a Δ*recG* allele (Figure 5F and data not shown). These findings were confirmed by reconstruction of the relevant genotypes (data not shown). The *ssb* mutations also improve the viability of *recG polA* and *recG* Δ*priA* cells, consistent with being suppressors of *recG* (Figure S4B). Previous studies had demonstrated that *recG* Δ*priA* cells have a low efficiency of plating on LB agar (McCool and Sandler 2001; Gregg *et al.* 2002). Neither of the *ssb* mutations was able to suppress the mitomycin C sensitivity conferred by a Δ*ruv* allele (data not shown), reinforcing the fact that both specifically suppress the *recG* mutant phenotype.

Two other *ssb* mutations were identified previously among suppressors of the very poor viability of Δ*priA dnaC812* strains lacking the DNA binding protein, RdgC (Moore *et al.* 2003). One is a C to T transition (*ssb[C289T]*) encoding an R97C substitution, the other an in-frame deletion of 90 bp (*ssb[Δ345–434]*) removing 30 amino acids from the long C-terminal arms extending from the core of the SSB tetramer (Figure 5C) (Matsumoto *et al.* 2000; Raghunathan *et al.* 2000; Savvides *et al.* 2004). These mutations also improve the viability of *recG dam* cells. However, they are less effective, at least as judged by colony size, consistent with being less effective suppressors of the mitomycin C sensitivity of *recG* (Figure S4A and data not shown). They also confer little or no sensitivity to UV (Figure 5E). Thus, in the case of SSB mutations, the ability to strongly suppress *recG* and, consequently to restore robust viability to *recG dam* cells, seems to come at the price of a reduced ability to survive UV irradiation.

**The SSB mutants retain the ability to bind DNA, PriA, and RecG:** SSB has been shown to interact with several proteins associated with DNA repair and the rescue of replication forks stalled on the template DNA, including RecG and PriA (Curth *et al.* 1996; Cadman and McGlynn 2004; Lecointe *et al.* 2007; Shereda *et al.* 2007; Buss *et al.* 2008). Given *priA* mutations that reduce or eliminate PriA helicase activity suppress *recG*, it is particularly significant that SSB not only binds PriA but also stimulates its helicase activity (Cadman and McGlynn 2004). It raises the possibility that the N14D and K44E mutations eliminate the SSB/PriA interaction. We purified wild-type and mutant SSB proteins to test this directly.

Gel filtration revealed that both mutant proteins migrate with the same molecular mass as wild-type SSB, which forms a stable tetramer in solution (Figure 6A and data not shown). Band-shift assays revealed that they also retain the ability to bind ssDNA, as do the R97C and Δ115-114 proteins (Figure 6B). This is not surprising given that strains expressing these proteins are viable. SSB is an essential protein, being required to

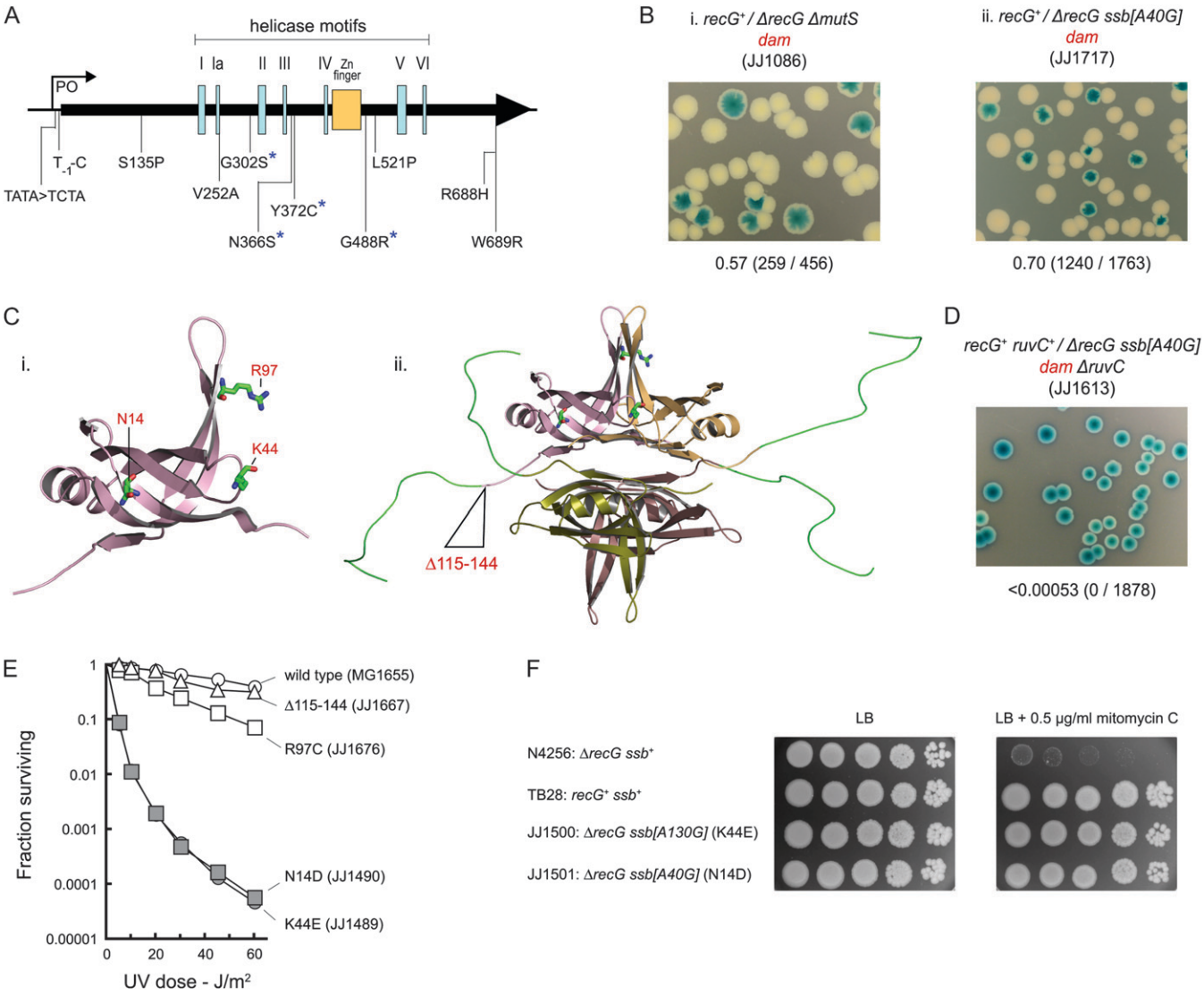


FIGURE 5.—Suppressors of the low viability of *dam recG* cells. (A) Map showing location and identity of *priA* suppressors. Coding mutations are indicated in terms of the changes to PriA. The asterisks identify changes that provide for particularly strong suppression of the mitomycin C sensitivity of a *recG* strain. (B) Synthetic lethality assays illustrating restoration of viability to *recG dam* cells by *mutS* and *ssb* mutations. (C) Structure of an SSB monomer (i) and tetramer (ii) showing the amino acid residues affected by the *ssb* mutations identified. The models were generated using Pymol and SSB crystal coordinates from the PDB database (MATSUMOTO *et al.* 2000; RAGHUNATHAN *et al.* 2000; SAVVIDES *et al.* 2004). (D) Synthetic lethality assay showing that the viability conferred on *recG dam* cells by *ssb[A40G]* depends on RuvC. (E) Effect of *ssb* mutations on sensitivity to UV light. (F) Restoration of mitomycin C resistance to a *recG* strain by *ssb[A40G]* and *ssb[A130G]*. Cultures of the strains indicated were grown in LB broth to an $A_{650}$ of 0.4, diluted in 10-fold steps, and 10-μl aliquots spotted on LB agar without and with mitomycin C, as indicated. The plates were photographed after 24 hr at 37°.

bind the unwound lagging strand template during replication of the chromosome.

To examine the ability to interact with PriA, we exploited the insolubility of SSB in low concentrations of ammonium sulfate that do not precipitate PriA (Figure 6C, lanes a–f) (SHEREDA *et al.* 2007). When the same low level of ammonium sulfate is added to an equimolar mixture of PriA and wild-type SSB (tetramer), most of the PriA is precipitated along with the SSB, with little of either protein remaining in solution (Figure 6D, lanes a–c). Similar coprecipitation was observed when wild-type SSB was replaced with either the N14D or K44E mutant (Figure 6D, lanes g–i; data not shown). These studies also confirmed that SSB interacts with RecG, as reported (BUSS *et al.* 2008; LECOINTE *et al.* 2007), and demonstrated that the mutants retain this property (Figure 6C, lanes g–i; Figure 6D, lanes d–f and j–l).

Next, we examined the ability of the mutant SSB proteins to stimulate the 3′–5′ DNA helicase activity of PriA. McGlynn and co-workers demonstrated that wild-type SSB stimulates PriA to unwind the lagging strand at a fork lacking a leading strand (CADMAN and MCGLYNN 2004). Furthermore, they showed that this activity is

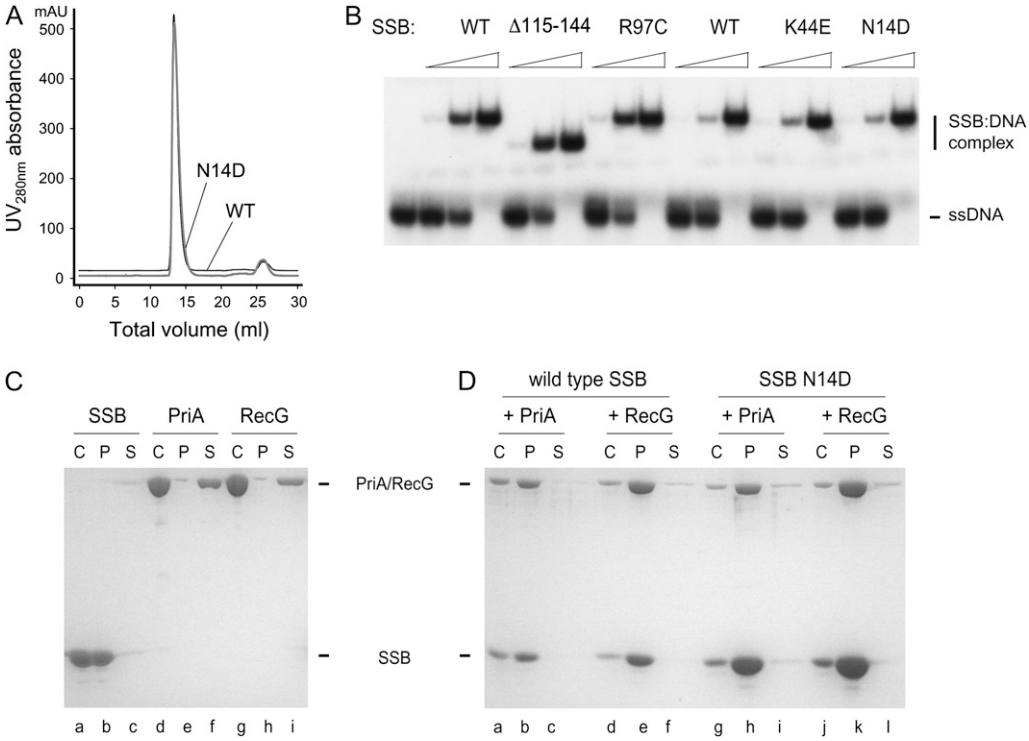


FIGURE 6.—Properties of SSB suppressor proteins. (A) Gel filtration of SSB wild-type and N14D proteins. (B) ssDNA binding activity. Reactions contained 0.2 nM labeled oligonucleotide and the indicated proteins at final concentrations of 0.5, 5, and 50 nM. (C) and (D) SDS–PAGE analysis showing coprecipitation of PriA and RecG with SSB wild-type and N14D proteins, as indicated. C, no ammonium sulfate control; P and S, pellet and supernatant fractions respectively after ammonium sulfate precipitation.

eliminated by a deletion removing the last 10 residues from the C terminus of SSB or by a P176S substitution of the penultimate residue, consistent with the idea that the extended C-terminal projection is associated specifically with protein–protein interactions (SHEREDA *et al.* 2008). We recapitulated these observations using the same fork (Figure 7, A and B, lanes e–h and q–t). We also found that the N14D, K44E, R97C, and Δ115-144 mutants retain the ability to stimulate PriA (Figure 7, A and B, lanes i–p).

SSB protein has a very high affinity for ssDNA and therefore rapidly sequesters any single strands exposed during chromosome replication or repair, preventing loading of RecA and thus establishing a primary defense against unnecessary recombination. When ssDNA is exposed, and recombination called for, the RecFOR proteins are recruited to help load RecA, displacing SSB and stabilizing the RecA nucleoprotein filament (UMEZU *et al.* 1993; MORIMATSU and KOWALCZYKOWSKI 2003; COX 2007a,b). Given the UV sensitivity of cells expressing N14D or K44E derivatives of SSB, we considered the possibility these two proteins might modify one or more interactions needed by RecFOR to load RecA and maintain a stable nucleoprotein filament. Figure 7C shows that an *ssb[A40G]* strain expressing the N14D mutant is substantially more sensitive to UV than a *recF* mutant. The double mutant is exceedingly sensitive, essentially as sensitive as a *recA* null strain at higher doses (Figure 7C), indicating perhaps that the combination of these two mutations effectively blocks RecA-dependent reactions. A similar synergism was observed between *recF* and the *ssb[A130G]* mutation expressing the K44E derivative of SSB (data not shown). Thus it is possible these two mutant SSB proteins are more resistant to displacement by RecA. However, the fact that SSB interacts with so many proteins means we cannot exclude the possibility of some other defect.

## DISCUSSION

The accumulation of unresolved recombination intermediates has been offered previously as an explanation for the inviability or poor viability of *polA*, *uvrD*, and *dam* cells lacking the RuvABC Holliday junction resolvase. However, given the reported redundancy between RecG and RuvABC (LLOYD 1991), it raised the question of why the postulated RecG pathway is unable to cope. We exploited a synthetic lethality assay to address this question, comparing the relative viabilities of *polA*, *uvrD*, and *dam* cells lacking either RuvABC or RecG and examining the ability of the RusA resolvase and PriA helicase deficiency to overcome any reduction in viability. During this work, we also identified mutations in *ssb* that suppress the *recG* phenotype, but not that associated with *ruv* mutations. The main findings are summarized in Table 1.

The synthetic lethality assays revealed that the RusA resolvase confers robust viability on *polA*, *uvrD*, and *dam* cells lacking RuvABC, provided RecG is available (Table 1; Figure 2). Given the reported high specificity of RusA for Holliday junctions (BOLT and LLOYD 2002), this establishes that unresolved junctions are indeed the reason for the inviability, as suspected. However, it also demonstrates very clearly that if the postulated RecG

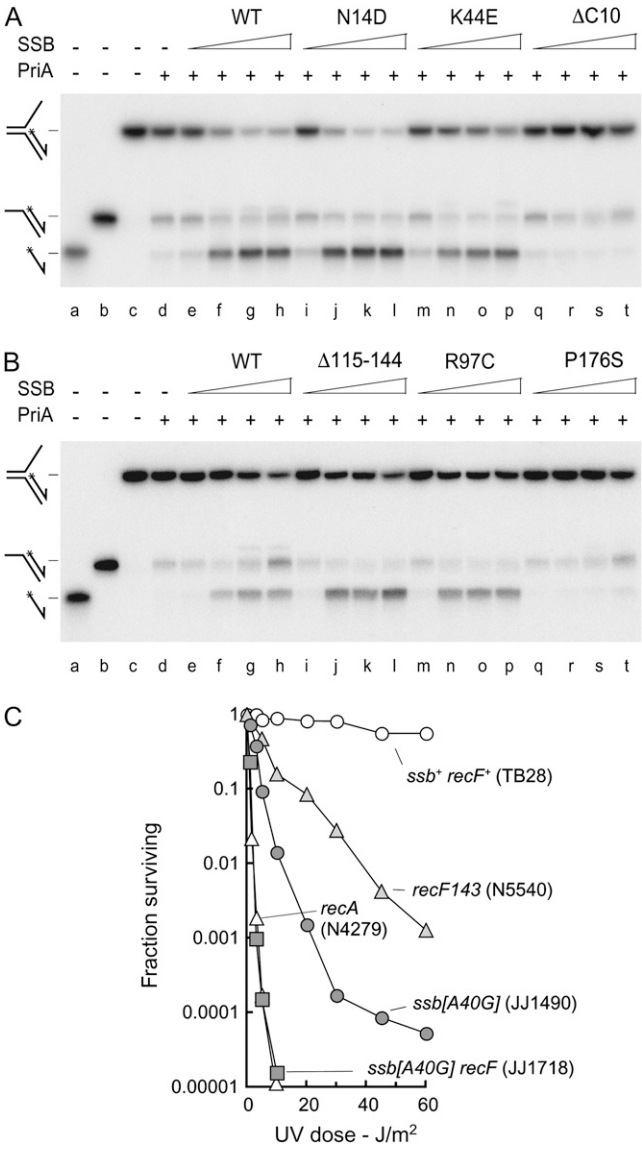


Figure 7.—Effect of SSB proteins on the helicase activity of PriA and effect of SSB N14D on UV repair in *recF* cells. (A and B) Unwinding of a DNA fork by PriA in the presence of SSB. (Lanes a–c) Labeled oligonucleotide, DNA partial duplex, and fork markers. (Lane d) A total of 0.2 nm labeled fork DNA plus 5 nm PriA. (Lanes e–t) A total of 0.2 nm labeled fork DNA, 5 nm PriA, and the indicated SSB protein at 0.2, 2, 20, or 200 nm. (C) Effect of *ssb[A40G]* on the UV sensitivity of a *recF* strain. The strains used are as identified.

pathway involves some nuclease that acts to resolve Holliday junctions by junction cleavage, then this nuclease must operate very inefficiently compared with RusA. Indeed, it strongly suggests that such a nuclease does not exist.

Evidence against the existence of such a nuclease emerged from our analysis of how the viability of *polA*, *uvrD*, and *dam* cells is compromised without RecG (Table 1). Ishioka *et al.* (1997) proposed that the inviability of *recG polA* cells is due to the accumulation of unresolved recombination intermediates and a consequent failure of chromosome segregation. A similar argument was put forward to explain the failure to construct a *recG dam* double mutant (Marinus 2000). However, it is not obvious why RuvABC alone should not suffice to maintain viability in these cases. Our studies with constructs carrying *priA300* proved very informative. They revealed that RuvABC alone is sufficient, provided the helicase activity of PriA is reduced or eliminated (Table 1; Figure 3). Thus, *recG* cells may simply lack means to curb a potentially deleterious effect of PriA helicase activity rather than being partially defective in Holliday junction resolution. Once PriA helicase activity is eliminated, RecG is quite dispensable as far as the viability of *polA*, *uvrD*, and *dam* cells is concerned. RuvABC is then able to cope very well despite the fact that recombination is known to be increased in these mutants (Arthur and Lloyd 1980; Kuzminov 1995; Marinus 2000).

A clue as to why PriA helicase activity might be so detrimental in the absence of RecG emerged from comparisons of how RecA, RecFOR, and RecQ affect the viability of *uvrD ruv*, *uvrD recG*, and *uvrD ruv recG* cells (Figure 4). Our studies confirmed previous reports demonstrating that the absence of UvrD provokes RecA-mediated recombination via a mechanism that could be countered by eliminating RecFOR, RecJ, or RecQ (Flores *et al.* 2005; Magner *et al.* 2007). They revealed in addition that the absence of RecG might also provoke recombination, but for a very different reason as this effect could not be countered efficiently in a *recG ruv uvrD* genetic background by removing RecFOR or RecQ. However, this conclusion requires the assumption that the viability of a strain lacking RuvABC depends on the incidence of recombination mediated by RecA. Since we did not measure recombination directly, we cannot exclude alternative explanations.

Evidence of increased recombination in *recG* cells was reported previously during assays of DNA double strand break repair and of the frequency of exchanges between tandem duplications (Lovett *et al.* 1993; Grove *et al.* 2008). The increased recombination evident from this study is clearly triggered independently of RecQ and RecFOR, indicating that it most probably has little to do with replication fork stalling and exposure of ssDNA as described in Figure 1C. It could be accounted for instead by an increase in RecBCD-mediated loading of RecA at dsDNA ends. As with recombination initiated at ssDNA gaps, UvrD would be expected to limit such exchanges by dissociating the RecA nucleoprotein filaments assembled by RecBCD (Dillingham and Kowalczykowski 2008). However, this begs the question of why the incidence of dsDNA ends should be increased in the absence of RecG.

The ability of RecG to catalyze DNA branch migration is well documented (Lloyd and Sharples 1993; Whitby *et al.* 1993; Whitby and Lloyd 1995; McGlynn and Lloyd 2001), as is its ability to facilitate the recovery

**TABLE 1**

**Viability of *uvrD*, *dam*, and *polA* strains lacking RuvABC or RecG[a]**

| Other genotype | *uvrD*, *dam*, *polA*, *recG* genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *uvrD*$^+$ *dam*$^+$ *polA*$^+$ | | *uvrD*$^+$ *dam*$^+$ *polA*$^-$ | | *uvrD*$^+$ *dam*$^-$ *polA*$^+$ | | *uvrD*$^-$ *dam*$^+$ *polA*$^+$ | |
| | *recG*$^+$ | Δ*recG* | *recG*$^+$ | Δ*recG* | *recG*$^+$ | Δ*recG* | *recG*$^+$ | Δ*recG* |
| None | + | + | + | − | + | * | + | * |
| Δ*ruvABC* | + | + | − | − | − | − | * | − |
| Δ*ruvABC rus-2* | + | + | + | − | + | − | + | − |
| *rus-2* | + | + | + | − | + | * | + | * |
| *priA300* | + | + | + | + | + | + | + | + |
| *priA300* Δ*ruvABC* | + | + | − | − | − | − | − | − |
| *priA300* Δ*ruvABC rus-2* | + | + | + | −[b] | + | − | + | − |
| *ssb[A40G]*[c] | + | + | ND | + | + | + | ND | + |
| *ssb[A40G]* Δ*ruv(AB)C* | + | + | −[d] | − | −[d] | − | * | − |

+, plasmid-free segregants account for >20% of the colonies observed under the conditions employed and form healthy colonies that can be subcultured without difficulty; −, colonies of plasmid-free segregants not detected or form <0.2% of the colonies observed; *, plasmid-free segregants form small or tiny colonies that tend to accumulate suppressors that allow formation of larger colonies on subculture.

[a] As determined using a synthetic lethality assay based on ability of plasmid-free (Lac$^-$) segregants to form colonies on LB agar.

[b] Very tiny colonies of plasmid-free cells detected, but these could not be subcultured, consistent with the idea that RusA needs the presence of RecG to function efficiently.

[c] Identical results were recorded using *ssb[A130G].*

[d] Only Δ*ruvC* tested.

of recombinants in strains lacking RuvABC (Lloyd 1991; Ryder *et al.* 1994; Mahdi *et al.* 1996). It has been suggested that RecG also promotes replication of damaged DNA (McGlynn and Lloyd 2000, 2002a,b), but this has proven more contentious (Donaldson *et al.* 2004; Michel *et al.* 2007). What is not in doubt is the ability of RecG to limit the incidence of DnaA-independent SDR, which is mediated via PriA-dependent loading of DnaB and subsequent replisome assembly at D loops and R loops (Asai and Kogoma 1994a,b; Masai *et al.* 1994; Hong *et al.* 1995). Without RecG to dissociate these branched structures (Vincent *et al.* 1996; Fukuoh *et al.* 1997), replication may initiate wherever they arise, rather than being restricted to DnaA-dependent events at *oriC*. Such initiations may set up replication forks that travel toward *oriC*, breaking the replichore arrangement that otherwise directs fork movement from *oriC* toward the terminus (Reyes-Lamothe *et al.* 2008). This might result in more frequent head-on collisions with RNA polymerase complexes (Rudolph *et al.* 2007), increasing the likelihood of fork breakage and recombination and causing additional difficulties for *polA*, *uvrD*, or *dam* cells. It would also be expected to increase collisions between replication forks as the new forks meet those coming from *oriC* (Rudolph *et al.* 2009b). The replichore arrangement normally restricts fork encounters to a single event within the terminus area each cycle of cell growth and division. An increase in the number of encounters may cause even more difficulties, especially if they take place outside of the control normally exerted by the Tus terminator protein, which appears to limit rereplication (Hiasa and Marians 1994; Krabbe *et al.* 1997; Markovitz 2005).

The ability of *priA300* to restore viability to *polA*, *uvrD*, and *dam* cells lacking RecG is consistent with this idea. Although helicase-deficient PriA proteins retain the ability to assemble a primosome and to complement the DNA repair and growth defects associated with a *priA* null allele (Zavitz and Marians 1992), they reduce SDR quite substantially (Tanaka *et al.* 2003), a fact consistent with genetic data indicating that the K230R derivative may not be able to initiate replication at D loops (Mahdi *et al.* 2006). In doing so, we believe they reduce the chromosome pathology arising as a consequence of unscheduled DNA replication (Rudolph *et al.* 2009a,b).

A reduction in SDR may also account for the *ssb* suppressors of *recG* we identified during this work. Replication is initiated at a D loop, and presumably at an R loop, via the action of PriA, which recognizes and binds to a DNA branch point (McGlynn *et al.* 1997; Liu and Marians 1999; Nurse *et al.* 1999). This leads to the recruitment of a primosome complex composed of PriB, DnaT, DnaB, and DnaG, and finally the assembly of a DNA polymerase III holoenzyme complex that initiates replication in an SSB-dependent manner (Liu and Marians 1999; Liu *et al.* 1999). Strains expressing the mutant SSB proteins we identified as suppressors of *recG* grow well, indicating that normal DNA replication is impaired very little, if at all. Furthermore, the mutant proteins bind ssDNA, interact with PriA, and stimulate its ability to unwind the lagging strand at a fork (Figures 6 and 7). At first sight, this last property would seem to

exclude a simple explanation for their ability to suppress *recG*, namely that they lack the ability to stimulate a potentially toxic helicase activity of PriA. However, the most effective *ssb* mutations confer sensitivity to UV light and in a manner that is synergistic with a *recF* mutation (Figure 7C). This could be explained if the mutant SSB proteins reduce the efficiency of PriA-mediated replisome assembly. If true, it might be expected to reduce the efficiency of replication restart and of inducible SDR after UV irradiation. It might also reduce the basal level of SDR in unirradiated *recG* cells. This leads to the idea that it is perhaps some downstream consequence of PriA-mediated unscheduled replication (SDR) that is toxic for *recG* cells, not the helicase activity of PriA *per se*.

Could increased chromosome pathology associated with SDR explain the much-reduced recovery of recombinants from conjugational and transductional crosses with *ruv recG* double mutants? The idea that *E. coli* has two partially overlapping pathways for the resolution of Holliday junctions, one dependent on RuvABC and the other on RecG, came from studies demonstrating that although *ruv* and single *recG* mutants are reasonably proficient in conjugational recombination, the double mutant is quite deficient (Lloyd 1991). The two new replication forks postulated to be established as each end of the linear donor DNA invades the recipient chromosome (Smith 1991) would likely exacerbate existing difficulties with replication caused by the absence of RecG and may trigger sufficient pathology for the zygotic cell to be no longer viable without RuvABC. The fact that inactivation of RecFOR, which reduces SDR (Kogoma 1997; Rudolph *et al.* 2008), improves the recovery of recombinants by ~10- to 15-fold, lends support to this argument (Ryder *et al.* 1994).

Pathological replication might also provide an explanation for the strong synergism between *ruv* and *recG* mutations with respect to radiation sensitivity and DNA double strand break repair (Lloyd 1991; Meddows *et al.* 2004; Grove *et al.* 2008). However, Grove *et al.* (2008) reported that the RecG-dependent repair pathway produces both crossover and noncrossover products, and in the same proportions as the RuvABC-dependent pathway, consistent with Holliday junction cleavage in either of the two possible orientations. If we accept there is no nuclease that acts with RecG to cleave junctions, then whatever is responsible for the productive repair (recombination) seen without RuvABC would also have to account for the generation of these two alternative resolution products.

Although the results presented argue against a nuclease that could act with RecG to resolve Holliday junctions, we do not rule out the existence of a RecG resolution pathway. Indeed, while the yield of recombinants observed in crosses with *ruv recG* strains lacking RecFOR is much increased, it is still ~20-fold lower than with the equivalent *recG*$^+$ construct (Ryder *et al.* 1994), indicating that RecG may play a part in promoting recombination, at least in the absence of RuvABC. The dsDNA translocase activity of RecG might be sufficient in this case to eliminate a junction, perhaps by driving it to merge with replication forks, as has been suggested (Wardrope *et al.* 2009). However, such activity may fail to cope when the incidence of Holliday junctions is higher, as for instance after UV irradiation or in mutant strains in which DNA repair is compromised and where recombination is therefore elevated. This may not matter in wild-type cells as RuvABC has clearly evolved to resolve junctions with high efficiency. As suggested here, and elsewhere (Rudolph *et al.* 2009a), RecG may have a more basic housekeeping role to limit initiation of replication by PriA, thus reducing the incidence of pathological events that might result from such replication.

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*Note added in proof*: Fonville *et al.* [N. C. Fonville, M. D. Blankschien, D. B. Magner and S. M. Rosenberg 2010 RecQ-dependent death-by-recombination in cells lacking RecG and UvrD. DNA Repair (in press; doi:10.1016/j.dnarep.2009.12.019)] reported that *recG uvrD* is inviable, contrary to our results (Figure 3C). We find that transduction of strain MG1655 Δ*recG*::*apra* to Tc$^r$ with P1 phage from a *metE*::Tn*10 uvrD1*::*kan* donor produces ~17% small Km$^r$ (*i.e.*, *recG uvrD*) colonies that can be subcultured on LB agar. The cotransduction frequency was 16% with MG1655 *recG*$^+$ as a recipient. We do not know the basis of the difference between our observations and those of Fonville *et al.* (2010).

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Supporting Information
http://www.genetics.org/cgi/content/full/genetics.110.114413/DC1

# Promoting and Avoiding Recombination: Contrasting Activities of the *Escherichia coli* RuvABC Holliday Junction Resolvase and RecG DNA Translocase

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**FILE S1**

**Supporting Results**

**Synthetic lethality screen**

We screened for mutations that reduce viability in strains lacking RuvC using a synthetic lethality assay based on pRC7 (Fig. S1A), an unstable mini-F plasmid that is rapidly lost (BERNHARDT and DE BOER 2004). It carries the *lac*$^+$ genes and therefore its loss is revealed in a Δ*lac* background by segregation of Lac$^-$ clones. On plates containing the b-galactosidase indicator, X-gal, these clones form white colonies or white sectors within blue (Lac$^+$) colonies, depending on whether plasmid loss occurred before or after plating. We used a *ruvC*$^+$ derivative of pRC7 to cover a deletion in the chromosome (*ruvC*$^+$/Δ*ruvC*). Compared with a *ruvC*$^+$/*ruvC*$^+$ control, cultures of this strain grown without ampicillin selection contain fewer plasmid-free cells capable of forming white colonies. Also, cells carrying the plasmid form blue colonies with less prominent white sectors (Fig. S1B, panels i-ii). These features reflect the slightly reduced viability of *ruv*$^-$ cells and consequently the survival advantage conferred by retaining the plasmid.

We generated several libraries of Km$^r$ transposon insertions in the *ruvC*$^+$/Δ*ruvC* strain and screened each for mutants forming uniformly dark blue colonies on the basis that this would indicate lethality between the covered Δ*ruvC* allele and the uncovered insertion (Fig. S1C). Libraries of strain N5747 (*ruvC*$^+$/Δ*ruvC*) carrying random *kan* insertions were prepared using λNK1327 (KLECKNER *et al.* 1991) or alternatively the EZ-Tn5 <kan-2> Tnp Transposome system (Epicentre Biotechnologies), using the materials and protocols described. Km$^r$ colonies were selected on LB agar supplemented with ampicillin (Ap) to maintain pAM372, pooled and stored at -20°C. Thawed samples were plated on LB agar supplemented with Xgal and IPTG to yield 50-100 colonies per plate. After 24h at 37°C, the plates were kept at room temperature for a further 2-4 days before streaking any solid blue colonies on fresh indicator plates to confirm the non-sectoring phenotype. P1*vir* was then grown on any clone failing to segregate plasmid-free cells and used to cross the *kan* insertion to the parent strain N5747 and also to strain N5752 (*ruvC*$^+$/*ruvC*$^+$). If the Km$^r$ transductants failed to segregate plasmid-free colonies with the former but did so with the latter, the mutant clone was assumed to carry an insertion synthetically lethal with *ruvC*. Insertions were mapped by PCR and DNA sequencing, using a combination of *kan*-specific and random PCR primers, as described (Epicentre Biotechnologies).

Seven mutant clones were identified following a screening of ~30,000 blue colonies of Km$^r$, insertion mutants. These had insertions in *dam*, *polA* or *uvrD* (Fig. S1E). The two *dam* isolates and the single *polA* isolate produced only blue colonies, consistent with lethality of *ruvC dam* and *ruvC polA* (Fig. S1D, panels i-ii), whereas all four *uvrD* isolates produced a mixture of healthy blue colonies showing little sign of sectoring and a smaller fraction of quite tiny white colonies (Fig. S1D, panel iii and data not shown). These white colonies proved impossible to maintain by subculture on LB agar plates, yielding a mixture of tiny colonies and faster-growing variants carrying suppressors (see main paper Fig. 4A, panel v). Thus, *ruvC uvrD* cells are barely viable. The *uvrD* mutation also increases integration of the pRC7 construct. Such integrants form very dark blue colonies because every progeny cell carries the plasmid and is therefore Lac$^+$ (Fig. S1D, panel iii, arrowed colony). Abortive growth of plasmid-free, *ruvC uvrD* cells dilutes the colour of the colony established by a non-integrant. Backcrossing the insertions to the *ruvC*$^+$/Δ*ruvC* parent established the same mutant phenotype in each case. We also moved the insertions to a *ruv*$^+$/*ruv*$^+$ strain. Healthy white colonies were observed in each case (Fig. S1G and data not shown). Thus, we can rule out any direct effect of the insertions on plasmid maintenance. The *dam* insertions confer a mild mutator phenotype in an otherwise wild type (*ruv*$^+$) and plasmid-free background, but are fairly resistant to irradiation with UV light. The *polA* insertion confers UV sensitivity, whereas the *uvrD* insertions confer both UV sensitivity and a strong mutator phenotype (Fig. S1F, and data not shown). These properties are typical of previously described *dam*, *polA* and *uvrD* mutants.

**Characterisation of the *polA* insertion and construction of *polA* deletions**

DNA polymerase I has an N-terminal 5'-3' exonuclease domain and a C-terminal domain encoding polymerase and 3'-5' proofreading activities (Fig. S2A) (JOYCE and GRINDLEY 1984; PATEL *et al.* 2001). Complete deletion of *polA* results in exceedingly low cell viability in broth media. Mutations inactivating only the polymerase domain have a much milder effect,

but confer sensitivity to UV light (JOYCE and GRINDLEY 1984; MOOLENAAR *et al.* 2000). From the location of the EZ*kan* insertion and the phenotype of the single mutant, we suspected our *polA* isolate we identified during the screen for synthetic lethality with *ruvC* was of the latter type. We engineered a deletion extending from the point of insertion to the 3' end of *polA* to test this possibility, and compared this truncation with a complete deletion of the *polA* coding sequence (Fig. S2A). The truncation confers a UV-sensitive phenotype similar to that of the insertion (Fig. S2B), and does not confer sensitivity to broth, unlike the complete deletion (Fig. S2C). Thus, the *polA*::*kan* allele most likely encodes a protein retaining only the 5'-3' exonuclease activity, which suffices to support viability, except when RuvC is missing.

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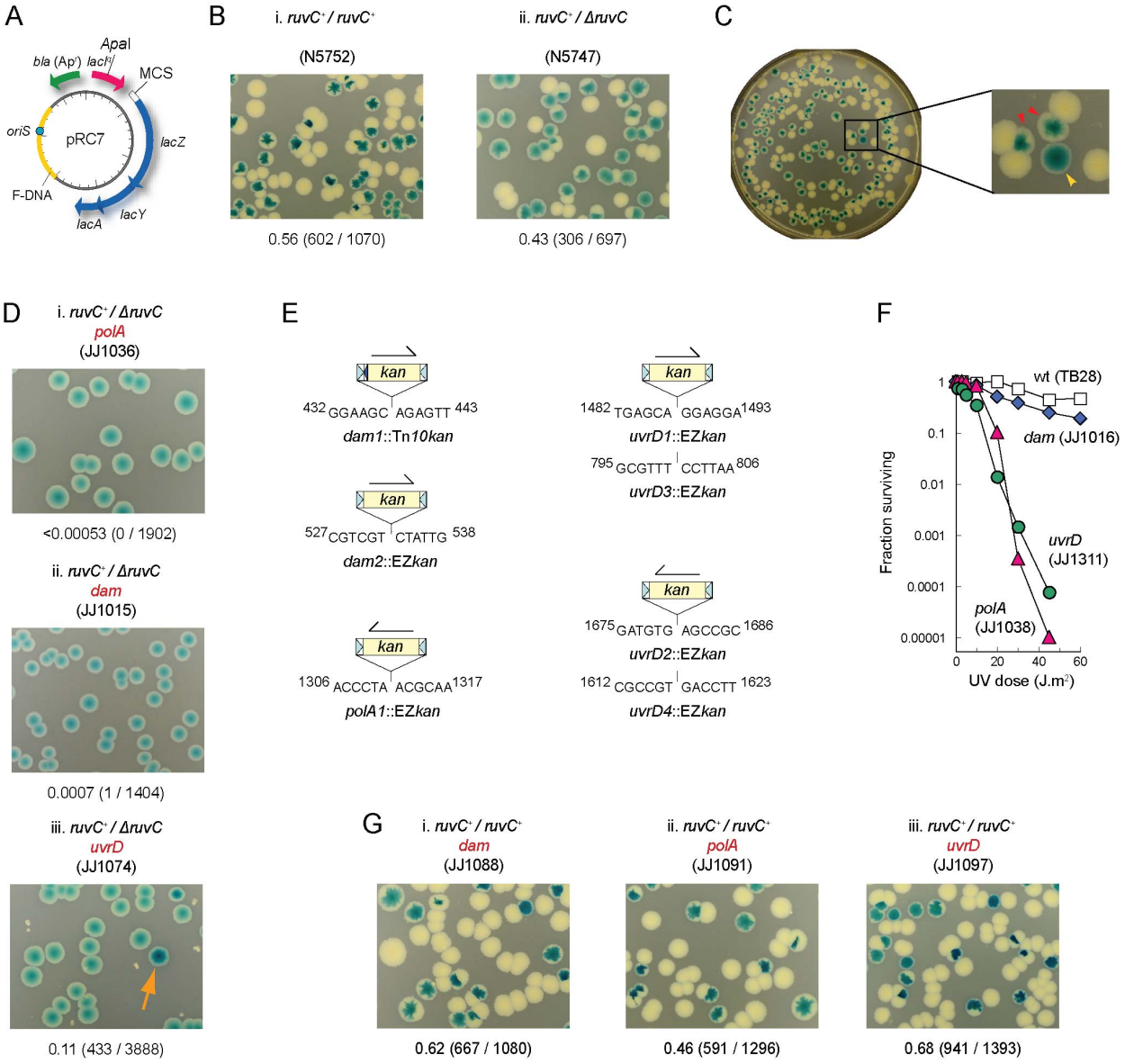


FIGURE S1.—Identification and analysis of chromosome insertions synthetically lethal with Δ*ruvC*. (A) Structure of pRC7. (B) Plate photographs showing segregation of plasmid-free cells (white colonies) from cultures of *ruv*$^+$ and Δ*ruvC* strains carrying the *ruvC*$^+$ construct, pAM372. (C) Plate photograph illustrating the synthetic lethality assay exploited to screen for insertion incompatible with Δ*ruvC* in a library of strain N5747 mutants carrying *kan* insertions. The magnification highlights a non-sectoring colony (yellow arrow) next to two sectoring blue colonies (red arrows). (D) Original mutants identified during the screening as carrying *kan* insertions in *polA*, *dam* or *uvrD*. The orange arrow in panel iii points at a colony formed by a plasmid integrant. The relevant genotype is shown *above* each photograph, with the strain number in parenthesis. The fraction of white colonies is shown *below* with the number of white colonies/total colonies analyzed in parenthesis. (E) Location of *kan* insertions within the coding sequences of *polA*, *dam* and *uvrD*. The region of insertion within each gene is identified by the bp numbers at the beginning and end of the sequences shown (F) Effect of *polA*, *uvrD* and *dam* insertions on sensitivity to UV light. The strains used are indicated in parenthesis. (G) Synthetic lethality assays showing that the *polA*, *dam* and *uvrD* insertions allow segregation of plasmid-free cells in the presence of *ruvC*$^+$ on the chromosome.

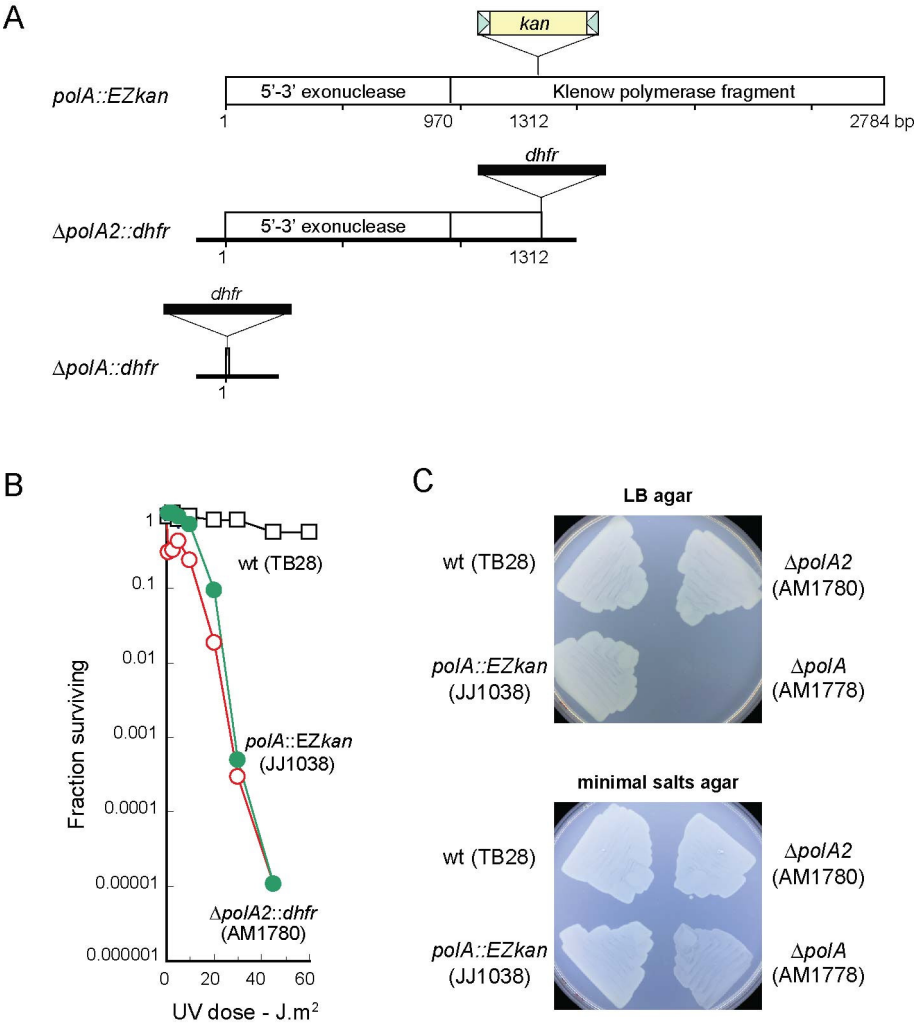


FIGURE S2.—Design and phenotypic analysis of *polA* deletions strains. (A) Location of the *kan* insertion in *polA* relative to the protein domain boundaries and design of partial and complete deletions of the *polA* coding sequence. The sequence deleted in each case is replaced with that coding for resistance to trimethoprim (*dhfr*). (B) Effect of the indicated *polA* mutations on sensitivity to UV light. The strain is identified in parenthesis. (C) Effect of *polA* insertion and deletion mutations on growth. Samples of cultures of the strains indicated grown in LB broth were streaked on LB agar or 56/2 glucose minimal salts agar as indicated. Plates were photographed after 24h at 37C. The strains used are identified in parenthesis.

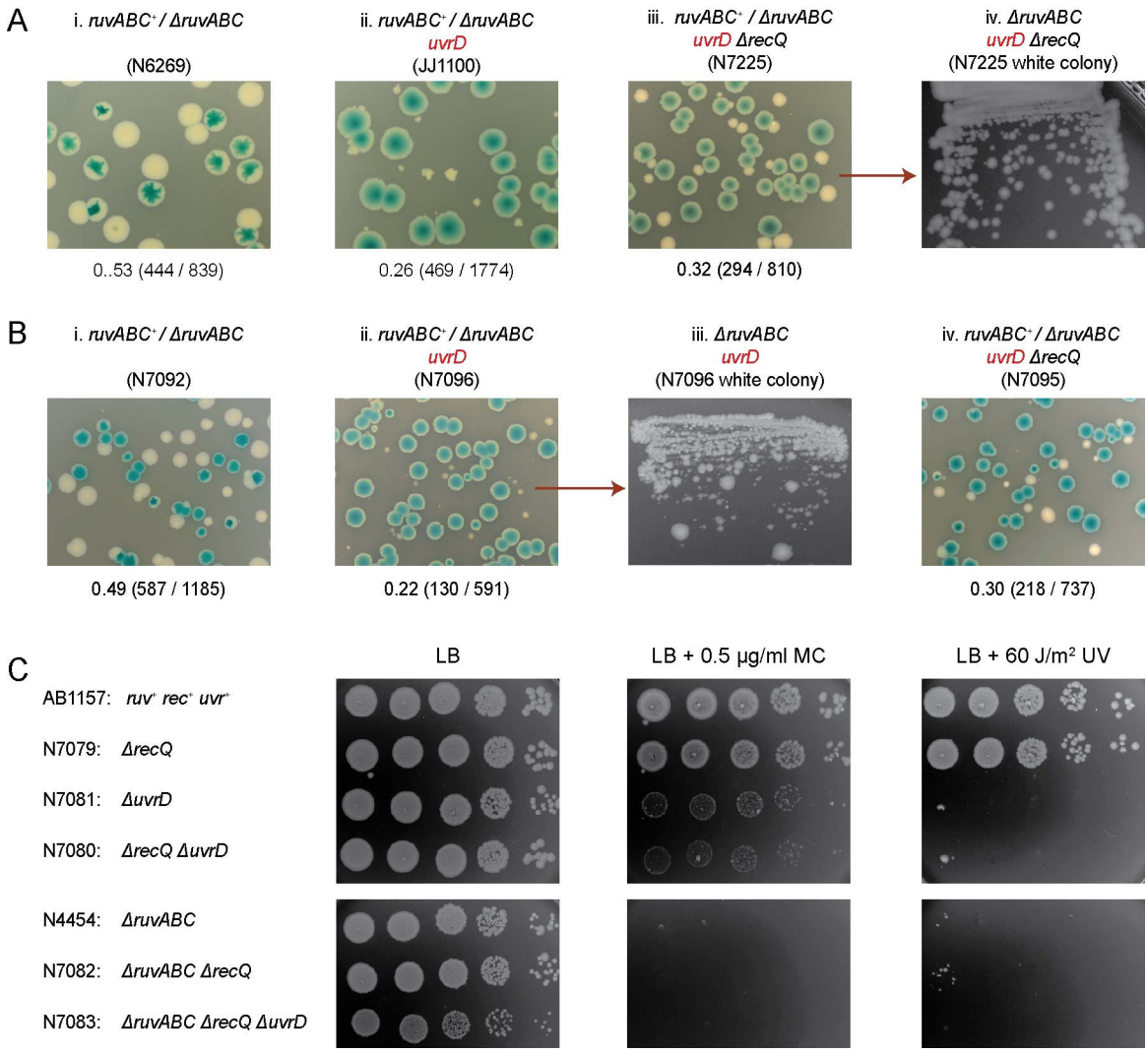


FIGURE S3.—Phenotypic analysis of MG1655 and AB1157 *wrD ruvC* cells, and effect of RecQ. (A) and (B) Synthetic lethality assays comparing *wrD ruv* cells in the MG1655 (A) and AB1157 (B) backgrounds. Note that the elimination of RecQ improves viability in both cases, though the AB1157 and its derivatives generally grow less well than the equivalent MG1655 strains. (C) Growth and sensitivity to mitomycin C and UV light. Cultures of the strains indicated were grown in LB broth to an $A_{650}$ of 0.4, diluted in 10-fold steps in 56/2 salts and 10 μl aliquots of $10^{-1}$ to $10^{-5}$ dilutions spotted on three LB agar plates, one of which contained 0.5 μg/ml mitomycin C, and one of which was irradiated with 60 J/m² UV light. Plates were photographed after 24 h at 37°C.

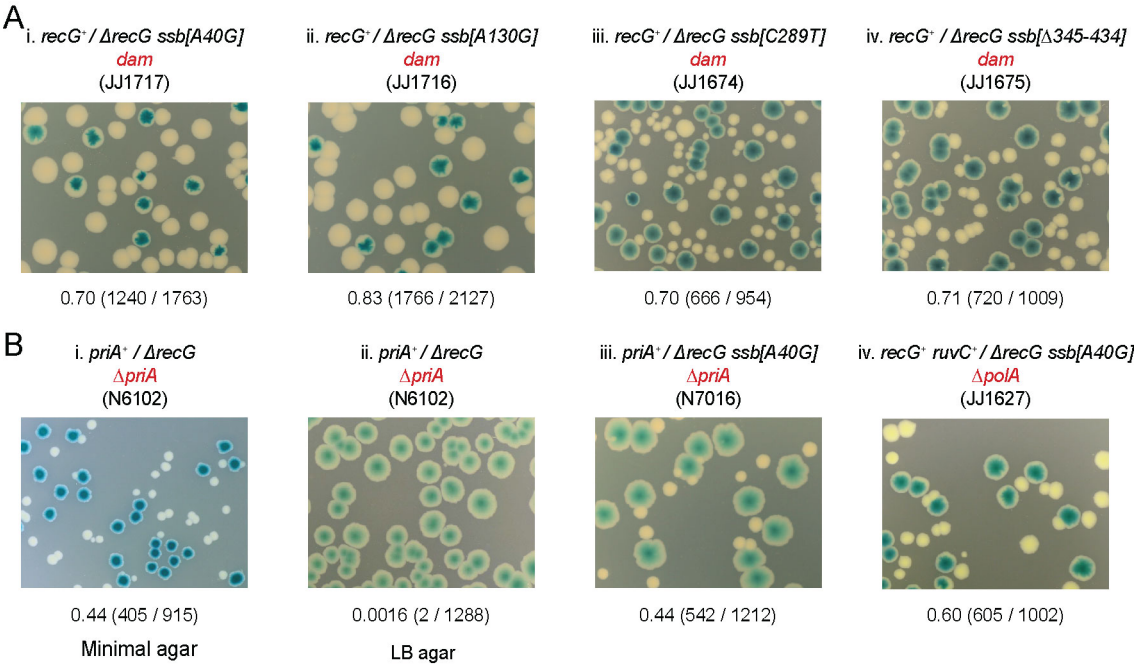


FIGURE S4.—Suppressor properties of mutant SSB proteins. (A) Synthetic lethality assays comparing the ability of SSB mutations to improve the viability of *dam recG* cells. (B) Synthetic lethality assays showing how the N14D substitution encoded by $ssb_{[A40G]}$ improves the viability of deletion *priA recG* and deletion *polA recG* cells. Note that deletion *priA recG* cells grow reasonably well on minimal salts agar (panel i), but very poorly on LB agar, forming very small colonies at a much reduced efficiency (panel ii). The N14D mutation in SSB allows these cells to plate with high efficiency on LB agar (panel iii).

**TABLE S1**

***Escherichia coli* strains used**

| Strain | Relevant genotype | Source or reference |
|---|---|---|
| **MG1655 derivatives (plasmid-free)** | | |
| MG1655 | wild type [a] | (BACHMANN 1996) |
| AM1417 | Δ*pyrE*::*dhfr* | This work |
| AM1655 | Δ*recG*::*apra* | (MAHDI *et al.* 2006) |
| AM1657 | Δ*uvrD*::*dhfr* | This work |
| AM1662 | Δ*recO*::*dhfr* | This work |
| AM1683 | Δ*mutS*::*dhfr* | This work |
| AM1771 | Δ*dam*::*dhfr* | This work |
| AM1776 | Δ*cysG*::*apra* | This work |
| AM1778 | Δ*polA*::*dhfr* | This work |
| AM1780 | Δ*polA2*::*dhfr* | This work |
| AM1967 | Δ*recQ*::*dhfr* | This work |
| JJ1016 | *dam1*::*kan* | P1.JJ1015 × MG1655 to Km$^r$ |
| JJ1038 | *polA*::EZ*kan* | P1.JJ1036 × MG1655 to Km$^r$ |
| JJ1060 | *priA300* Δ*lacIZYA* | White colony segregant of N5933 |
| JJ1075 | Δ*recG*::*apra priA300* Δ*lacIZYA* | P1.AM1655 × JJ1060 to Apra$^r$ |
| JJ1161 | Δ*recG*::*apra polA*::EZ*kan priA300* Δ*lacIZYA* | White colony segregant of JJ1096 |
| JJ1167 | Δ*recG*::*apra* Δ*ruvC*::*cat priA300* Δ*lacIZYA* | P1.N5466 × JJ1075 to Cm$^r$ |
| N4256 | Δ*recG263*::*kan* | (JAKTAJI and LLOYD 2003) |
| N4279 | Δ*recA269*::Tn*10* | P1.N3072 x MG1655 to Tc$^r$ |
| N4560 | Δ*recG265*::*cat* | (MAHDI *et al.* 2006) |
| N4574 | *rus-2* (*orf-56*::IS*10*) Δ*ruvAC65 eda-51*::Tn*10 relA1* Δ*spoT207*::*cat rpoB*35* | (MAHDI *et al.* 2006) |
| N4884 | *rpo*35* Δ*ruvABC*::*cat* | (MAHDI *et al.* 2006) |
| N5466 | Δ*ruvC*::*cat* | (MAHDI *et al.* 2006) |
| N5540 | *tna*::Tn*10 recF143* | P1. JJC12334 × MG1655 to Tc$^r$ |
| N6499 | Δ*recQ*::*kan* Δ*uvrD*::*dhfr priA300* Δ*lacIZYA* | White colony segregant of N6168 |
| TB12 | Δ*lacIZYA*>>*kan*>>FRT | (BERNHARDT and DE BOER 2004) |
| TB28 | Δ*lacIZYA* | (BERNHARDT and DE BOER 2004) |

**TB28 (*ΔlacIZYA*) pAM372 (*lac*$^+$ *ruvC*$^+$) derivatives**

| | | |
|---|---|---|
| JJ1015 | *ΔruvC*::*cat dam1*::*kan* | λNK1327 × N5747 to Km$^r$ |
| JJ1036 | *ΔruvC*::*cat polA*::EZ*kan* | EZTn5 <kan-2> Tnp × N5747 to Km$^r$ |
| JJ1074 | *ΔruvC*::*cat uvrD1*::EZ*kan* | EZTn5 <kan-2> Tnp × N5747 to Km$^r$ |
| JJ1088 | *dam1*::*kan* | P1.JJ1016 × N5752 to Km$^r$ |
| JJ1091 | *polA*::EZ*kan* | P1.JJ1036 × N5752 to Km$^r$ |
| JJ1097 | *uvrD1*::EZ*kan* | P1.JJ1074 × N5752 to Km$^r$ |
| N5747 | *ΔruvC*::*cat* | (TRAUTINGER *et al.* 2005) |
| N5752 | | (TRAUTINGER *et al.* 2005) |

**TB28 (*ΔlacIZYA*)/MG1655 *ΔlacIZYA* pJJ100 (*lac*$^+$ *recG*$^+$) derivatives**

| | | |
|---|---|---|
| JJ1017 | *ΔrecG265*::*cat* | pJJ100 × N5742 to Ap$^r$ |
| JJ1018 | | pJJ100 × TB28 to Ap$^r$ |
| JJ1073 | *ΔmutS*::*dhfr* | P1.AM1683 × JJ1018 to Tm$^r$ |
| JJ1078 | *ΔrecG*::*apra priA300* | pJJ100 × JJ1075 to Ap$^r$ |
| JJ1080 | *ΔrecG*::*apra ΔmutS*::*dhfr* | P1.AM1655 × JJ1073 to Apra$^r$ |
| JJ1086 | *ΔrecG*::*apra ΔmutS*::*dhfr dam1*::*kan* | P1.JJ1016 × JJ1080 to Km$^r$ |
| JJ1087 | *ΔrecG*::*apra dam1*::*kan priA300* | P1.JJ1016 × JJ1078 to Km$^r$ |
| JJ1093 | *polA*::EZ*kan* | P1.JJ1036 × JJ1018 to Km$^r$ |
| JJ1096 | *ΔrecG*::*apra polA*::EZ*kan priA300* | P1.JJ1036 × JJ1078 to Km$^r$ |
| JJ1099 | *uvrD1*::EZ*kan* | P1.JJ1074 × JJ1018 to Km$^r$ |
| JJ1102 | *ΔrecG*::*apra uvrD1*::EZ*kan priA300* | P1.JJ1074 × JJ1078 to Km$^r$ |
| JJ1117 | *ΔrecG*::*apra ΔruvC*::*cat ΔmutS*::*dhfr dam1*::*kan* | P1.N5466 × JJ1086 to Cm$^r$ |
| JJ1119 | *ΔrecG*::*apra* | P1.AM1655 × JJ1017 to Apra$^r$ |
| JJ1122 | *ΔrecG*::*apra dam1*::*kan* | P1.JJ1016 × JJ1119 to Km$^r$ |
| JJ1123 | *ΔrecG*::*apra polA*::EZ*kan* | P1.JJ1036 × JJ1119 to Km$^r$ |
| JJ1124 | *ΔrecG*::*apra uvrD1*::EZ*kan* | P1.JJ1074 × JJ1119 to Km$^r$ |
| JJ1164 | *ΔrecG*::*apra uvrD1*::EZ*kan tna*::Tn*10 recF143* | P1.JJC12334 × JJ1124 to Tc$^r$ |
| JJ1165 | *ΔrecG*::*apra uvrD1*::EZ*kan recA269*::Tn*10* | P1.N3072 × JJ1124 to Tc$^r$ |
| JJ1191 | *ΔrecG*::*apra ΔruvABC*::*cat rus-2* | pJJ100 × JJ1721 to Ap$^r$ |
| JJ1207 | *ΔrecG*::*apra ΔruvABC*::*cat rus-2 dam1*::*kan* | P1.JJ1016 × JJ1191 to Km$^r$ |
| JJ1208 | *ΔrecG*::*apra ΔruvABC*::*cat rus-2 uvrD1*::EZ*kan* | P1.JJ1074 × JJ1191 to Km$^r$ |
| JJ1555 | *ΔrecG*::*apra ssb[A130G]* | pJJ100 × JJ1500 to Ap$^r$ |
| JJ1558 | *ΔrecG*::*apra ssb[A40G]* | pJJ100 × JJ1501 to Ap$^r$ |

| | | |
|---|---|---|
| JJ1674 | Δ*recG*::*apra dam1*::*kan ssb[C289T]* | P1.DIM082 × JJ1122 to Tc$^r$ |
| JJ1675 | Δ*recG*::*apra dam1*::*kan ssb[Δ345-434]* | P1.DIM248 × JJ1122 to Tc$^r$ |
| JJ1696 | Δ*recG*::*apra dam1*::*kan ssb[A130G]* | P1.JJ1016 × JJ1555 to Km$^r$ |
| JJ1699 | Δ*recG*::*apra dam1*::*kan ssb[A40G]* | P1.JJ1016 × JJ1558 to Km$^r$ |
| JJ1716 | Δ*recG*::*apra* Δ*dam*::*dhfr yjcB*::EZ*kan ssb[A130G]* | P1.JJ1507 × N6886 to Km$^r$ |
| JJ1717 | Δ*recG*::*apra* Δ*dam*::*dhfr yjcB*::EZ*kan ssb[A40G]* | P1.JJ1509 × N6886 to Km$^r$ |
| N6500 | Δ*recG*::*apra* Δ*ruvABC*::*cat* | P1.N4884 × JJ1119 to Cm$^r$ |
| N6521 | Δ*recG*::*apra* Δ*uvrD*::*dhfr* Δ*recQ*::*kan* | P1.N6499 × JJ1119 to Km$^r$ |
| N6886 | ΔrecG::*apra* Δ*dam*::*dhfr* | P1.AM1771 × JJ1119 to Tm$^r$ |

**TB28 (Δ*lacIZYA*) pAM390 (*lac*$^+$ *ruvABC*$^+$) derivatives**

| | | |
|---|---|---|
| JJ1090 | Δ*ruvABC*::*cat dam1*::*kan* | P1.JJ1016 × N6269 to Km$^r$ |
| JJ1094 | Δ*ruvABC*::*cat polA* ::EZ*kan* | P1.JJ1036 × N6269 to Km$^r$ |
| JJ1100 | Δ*ruvABC*::*cat uvrD1*::EZ*kan* | P1.JJ1074 × N6269 to Km$^r$ |
| JJ1105 | Δ*ruvABC*::*cat* Δ*mutS*::*dhfr* | P1.AM1683 × N6269 to Tm$^r$ |
| JJ1108 | Δ*ruvABC*::*cat* Δ*mutS*::*dhfr dam1*::*kan* | P1.JJ1016 × JJ1105 to Km$^r$ |
| JJ1111 | Δ*ruvABC*::*cat* Δ*mutS*::*dhfr uvrD1*::EZ*kan* | P1.AM1683 × JJ1100 to Tm$^r$ |
| JJ1138 | Δ*ruvABC*::*cat* Δ*recG*::*apra rus-2* | P1.AM1655× N6329 to Apra$^r$ |
| JJ1146 | Δ*ruvABC*::*cat rus-2 dam1*::*kan* | P1.JJ1016 × N6329 to Km$^r$ |
| JJ1152 | Δ*ruvABC*::*cat uvrD1*::EZ*kan recA269*::Tn*10* | P1.N3072 × JJ1100 to Tc$^r$ |
| JJ1153 | Δ*ruvABC*::*cat uvrD1*::EZ*kan tna*::Tn*10 recF143* | P1. JJC12334 × JJ1100 to Tc$^r$ |
| JJ1156 | Δ*ruvABC*::*cat rus-2 polA* ::EZ*kan* | P1.JJ1036 × N6329 to Km$^r$ |
| JJ1182 | Δ*ruvABC*::*cat rus-2 uvrD1* ::EZ*kan* | P1.JJ1074 × N6329 to Km$^r$ |
| N6269 | Δ*ruvABC*::*cat* | pAM390 × N6268 to Ap$^r$ |
| N6329 | Δ*ruvABC*::*cat rus-2* | pAM390 × N6310 to Ap$^r$ |
| N6353 | Δ*ruvABC*::*cat rus-2* Δ*recG263*::*kan* | P1.N3793 x N6329 to Km$^r$ |
| N7107 | *priA300* Δ*ruvABC*::*cat* | pAM390 × N7106 to Ap$^r$ |
| N7112 | *priA300* Δ*ruvABC*::*cat polA*::EZ*kan* | P1.JJ1160 x N7107 to Km$^r$ |
| N7225 | Δ*ruvABC*::*cat uvrD1*::EZ*kan* Δ*recQ*::*dhfr* | P1.AM1967 × JJ1100 to Tm$^r$ |

**TB28 (Δ*lacIZYA*)/MG1655 Δ*lacIZYA* pJJ103 (*lac*$^+$ *recG*$^+$ *ruvC*$^+$) derivatives**

| | | |
|---|---|---|
| JJ1168 | Δ*recG*::*apra* Δ*ruvC*::*cat priA300* | pJJ103 × JJ1167 to Ap$^r$ |
| JJ1177 | Δ*recG*::*apra* Δ*ruvC*::*cat priA300 dam1*::*kan* | P1.JJ1016 × JJ1168 to Km$^r$ |
| JJ1179 | Δ*recG*::*apra* Δ*ruvC*::*cat priA300 uvrD1*::EZ*kan* | P1.JJ1074 × JJ1168 to Km$^r$ |
| JJ1486 | Δ*recG*::*apra* Δ*ruvC*::*cat* Δ*mutS*::*dhfr dam1*::*kan* | pJJ103 × JJ1483 to Ap$^r$ |

| | | |
|---|---|---|
| JJ1556 | *ΔrecG*::*apra ssb[A130G]* | pJJ103 × JJ1500 to Ap$^r$ |
| JJ1559 | *ΔrecG*::*apra ssb[A40G]* | pJJ103 × JJ1501 to Ap$^r$ |
| JJ1573 | *ΔrecG*::*apra ΔruvC*::*cat ssb[A130G]* | P1.N5466 × JJ1556 to Cm$^r$ |
| JJ1575 | *ΔrecG*::*apra ΔruvC*::*cat ssb[A40G]* | P1.N5466 × JJ1559 to Cm$^r$ |
| JJ1611 | *ΔrecG*::*apra ΔruvC*::*cat Δdam*::*dhfr ssb[A130G]* | P1.AM1771 × JJ1573 to Tm$^r$ |
| JJ1613 | *ΔrecG*::*apra ΔruvC*::*cat Δdam*::*dhfr ssb[A40G]* | P1.AM1771 × JJ1575 to Tm$^r$ |
| JJ1627 | *ΔrecG*::*apra ΔpolA2*::*dhfr ssb[A40G]* | P1.AM1780 × JJ1558 to Tm$^r$ |

**TB28 (*ΔlacIZYA*)/MG1655 *ΔlacIZYA* pAM409 (*lac*$^+$ *recG*$^+$ *ruvABC*$^+$) derivatives**

| | | |
|---|---|---|
| JJ1228 | *ΔrecG*::*apra ΔruwABC*::*cat* | pAM409 × JJ1227 to Ap$^r$ |
| JJ1236 | *ΔrecG*::*apra ΔruwABC*::*cat uvrD1* ::EZ*kan* | P1.JJ1074 × JJ1228 to Km$^r$ |
| JJ1237 | *priA300 ΔruwABC*::*cat rus-2* | pAM409 × N6048 to Ap$^r$ |
| JJ1242 | *ΔrecG*::*apra ΔruwABC*::*cat rus-2* | pAM409 × JJ1241 to Ap$^r$ |
| JJ1245 | *ΔrecG*::*apra ΔruwABC*::*cat uvrD1* ::EZ*kan recA269*::Tn*10* | P1.N3072 × JJ1236 to Tc$^r$ |
| JJ1246 | *ΔrecG*::*apra ΔruwABC*::*cat uvrD1* ::EZ*kan tna*::Tn*10 recF143* | P1. JJC12334 × JJ1236 to Tc$^r$ |
| JJ1247 | *ΔrecG*::*apra ΔruwABC*::*cat uvrD1* ::EZ*kan ΔrecO*::*dhfr* | P1.AM1662 × JJ1236 to Tc$^r$ |
| JJ1249 | *ΔrecG*::*apra ΔruwABC*::*cat rus-2 polA*::EZ*kan* | P1.JJ1038 × JJ1242 to Km$^r$ |
| JJ1254 | *priA300 ΔruwABC*::*cat rus-2 polA*::EZ*kan ΔrecG*::*apra* | P1.AM1655 x JJ1259 to Apra$^r$ |
| JJ1259 | *priA300 ΔruwABC*::*cat rus-2 polA*::EZ*kan* | P1.JJ1038 × JJ1237 to Km$^r$ |
| JJ1560 | *ΔrecG*::*apra ssb[A40G]* | pAM409 × JJ1501 to Ap$^r$ |
| JJ1576 | *ΔrecG*::*apra ΔruwABC*::*cat ssb[A40G]* | P1.N4884 × JJ1560 to Cm$^r$ |
| JJ1712 | *ΔrecG*::*apra priA300 polA* ::EZ*kan* | pAM409 × JJ1161 to Ap$^r$ |
| JJ1713 | *ΔrecG*::*apra ΔruwABC*::*cat priA300 polA*::EZ*kan* | P1.N4884 × JJ1712 to Cm$^r$ |
| N7226 | *ΔrecG*::*apra ΔruwABC*::*cat uvrD1* ::EZ*kan ΔrecQ*::*dhfr* | P1.AM1967 × JJ1236 to Tm$^r$ |

**MG1655 *ΔlacIZYA* pAM374 (*lac*$^+$ *priA*$^+$) derivatives**

| | | |
|---|---|---|
| N5933 | *priA300* | (Mahdi *et al.* 2006) |
| N5942 | *priA300 ΔrecQ*::*kan* | (Mahdi *et al.* 2006) |
| N5944 | *priA300 ΔruwABC*::*cat* | (Mahdi *et al.* 2006) |
| N5971 | *priA300 ΔruwABC*::*cat purE85*::Tn*10* | P1.N3005 x N5944 to Tc$^r$ |
| N5972 | *ΔpriA*::*apra* | (Mahdi *et al.* 2006) |
| N5989 | *priA300 ΔruwABC*::*cat rus-2* | P1.N4574 x N5971 to Pur$^+$ (Tc$^s$) |
| N6102 | *ΔpriA*::*apra ΔrecG*::*cat* | P1.N4560 × N5972 to Cm$^r$ |
| N6168 | *priA300 ΔrecQ*::*kan ΔuvrD*::*dhfr* | P1.AM1657 × N5942 to Tm$^r$ |
| N7016 | *ΔpriA*::*apra ΔrecG*::*cat yjbQ*::EZ*kan ssb[A40G]* | P1.JJ1509 × N6102 to Km$^r$ |

**TB28 (*ΔlacIZYA*)/MG1655 *ΔlacIZYA* plasmid-free derivatives**

| | | |
|---|---|---|
| JJ1160 | *polA*::EZ*kan* | White colony segregant of JJ1093 |
| JJ1227 | *ΔrecG*::*apra ΔruwABC*::*cat* | White colony segregant of N6500 |
| JJ1241 | *ΔrecG*::*apra ΔruwABC*::*cat rus-2* | White colony segregant of JJ1191 |
| JJ1311 | *uvrD1*::EZ*kan* | White colony segregant of JJ1099 |
| JJ1329 | *ΔrecG*::*apra dam1*::*kan ssb[A130G]* | Suppressor of JJ1122 white colony |
| JJ1331 | *ΔrecG*::*apra dam1*::*kan ssb[A40G]* | Suppressor of JJ1122 white colony |
| JJ1476 | *ΔpyrE*::*dhfr dam1*::*kan ssb[A130G]* | P1.AM1417 × JJ1329 to $Tm^r$ Ura- |
| JJ1477 | *ΔpyrE*::*dhfr dam1*::*kan ssb[A40G]* | P1.AM1417 × JJ1331 to $Tm^r$ Ura- |
| JJ1481 | *dam1*::*kan ssb[A130G]* | P1.W3110 × JJ1476 to $Ura^+$ $Tm^s$ |
| JJ1482 | *dam1*::*kan ssb[A40G]* | P1.W3110 × JJ1477 to $Ura^+$ $Tm^s$ |
| JJ1483 | *ΔrecG*::*apra ΔruvC*::*cat ΔmutS*::*dhfr dam1*::*kan* | White colony segregant of JJ1117 |
| JJ1489 | *ΔcysG*::*apra ssb[A130G]* | P1.AM1776 × JJ1481 to $Apra^r$ |
| JJ1490 | *ΔcysG*::*apra ssb[A40G]* | P1.AM1776 × JJ1482 to $Apra^r$ |
| JJ1495 | *ΔcysG*::*apra yjcB*::EZ*kan* | P1.EZ*kan* pool × JJ1489 to $Km^r$ $UV^r$ |
| JJ1496 | *ΔcysG*::*apra yjbQ*::EZ*kan* | P1.EZ*kan* pool × JJ1490 to $Km^r$ $UV^r$ |
| JJ1497 | *ssb[A130G]* | P1.W3110 × JJ1489 to $Cys^+$ $Apra^s$ |
| JJ1498 | *ssb[A40G]* | P1.W3110 × JJ1490 to $Cys^+$ $Apra^s$ |
| JJ1500 | *ΔrecG*::*apra ssb[A130G]* | P1.AM1655 × JJ1497 to $Apra^r$ |
| JJ1501 | *ΔrecG*::*apra ssb[A40G]* | P1.AM1655 × JJ1498 to $Apra^r$ |
| JJ1507 | *ΔcysG*::*apra yjcB*::EZ*kan ssb[A130G]* | P1.JJ1495 × JJ1489 to $Km^r$ ($UV^s$) |
| JJ1509 | *ΔcysG*::*apra yjbQ*::EZ*kan ssb[A40G]* | P1.JJ1496 × JJ1490 to $Km^r$ ($UV^s$) |
| JJ1650 | *ΔrecG*::*apra malE*::Tn*10 ssb[Δ345-434]* | P1.DIM248 × N6576 to $Tc^r$ |
| JJ1661 | *ΔrecG*::*apra malE*::Tn*10 ssb[C289T]* | P1.DIM082 × N6576 to $Tc^r$ |
| JJ1665 | *malE*::Tn*10 ΔpyrE*::*dhfr ssb[Δ345-434]* | P1.AM1417 × JJ1650 to $Tm^r$ Ura-$Apra^s$ |
| JJ1667 | *malE*::Tn*10 ssb[Δ345-434]* | P1.W3110 × JJ1665 to $Ura^+$ $Tm^s$ |
| JJ1668 | *malE*::Tn*10 ΔpyrE*::*dhfr ssb[C289T]* | P1.AM1417 × JJ1661 to $Tm^r$ Ura-$Apra^s$ |
| JJ1676 | *malE*::Tn*10 ssb[C289T]* | P1.W3110 × JJ1668 to $Ura^+$ $Tm^s$ |
| JJ1718 | $ssb_{[A40G]}$ *tna*::Tn*10 recF143* | P1.JJC12334 × JJ1498 to $Tc^r$ |
| JJ1721 | *ΔruwABC*::*cat ΔrecG*::*apra rus-2* | White colony segregant of JJ1138 |
| N5742 | *ΔrecG265*::*cat* | P1.N4452 x TB28 to $Cm^r$ |
| N6048 | *priA300 ΔruwABC*::*cat rus-2* | White colony segregant of N5989 |
| N6268 | *ΔruwABC*::*cat* | (Mahdi *et al.* 2006) |

| | | |
|---|---|---|
| N6310 | *ΔruvABC*::*cat rus-2* | (MAHDI *et al.* 2006) |
| N6576 | *ΔrecG*::*apra* | P1.AM1655 x TB28 to Apra$^r$ |
| N7106 | *priA300 ΔruvABC*::*cat* | White colony segregant of N5944 |

**AB1157 derivatives**

| | | |
|---|---|---|
| AB1157[b] | | (BACHMANN 1996) |
| DIM082 | *priA2*::*kan dnaC212 ΔrdgC*::*dhfr ssb*$_{[C289T]}$ *malE*::Tn*10* | Tim Moore |
| DIM248 | *ΔrdgC*::*dhfr ssb*[Δ345-434] *malE*::Tn*10* | Tim Moore |
| JC12334 | *tna*::Tn*10 recF143* | A.J. Clark |
| N3793 | *ΔrecG263*::*kan* | (MAHDI *et al.* 1996) |
| N4452 | *ΔrecG265*::*cat* | (JAKTAJI and LLOYD 2003) |
| N4454 | *ΔruvABC*::*cat* | (TRAUTINGER *et al.* 2005) |
| N7079 | *ΔrecQ*::*kan* | P1.N6499 × AB1157 to Km$^r$ |
| N7080 | *ΔrecQ*::*kan ΔuvrD*::*dhfr* | P1.N6499 × AB1157 to Km$^r$ Tm$^r$ |
| N7081 | *ΔuvrD*::*dhfr* | P1.AM1657 × AB1157 to Tm$^r$ |
| N7082 | *ΔruvABC*::*cat ΔrecQ*::*kan* | P1.N6499 × N4454 to Km$^r$ |
| N7083 | *ΔruvABC*::*cat ΔrecQ*::*kan ΔuvrD*::*dhfr* | P1.N6499 × N4454 to Km$^r$ Tm$^r$ |
| N7084 | *ΔlacIZYA*>>*kan*>>FRT | P1.TB12 x AB1157 to Km$^r$ |
| N7087 | *ΔlacIZYA* | This work [d] |
| N7089 | pAM390 (*lac*$^+$ *ruvABC*$^+$) / *ΔlacIZYA* | pAM390 × N7087 to Ap$^r$ |
| N7092 | pAM390 (*lac*$^+$ *ruvABC*$^+$) / *ΔlacIZYA ΔruvABC*::*cat* | P1.N4884 × N7089 to Cm$^r$ |
| N7095 | pAM390 (*lac*$^+$ *ruvABC*$^+$) / *ΔlacIZYA ΔruvABC*::*cat ΔrecQ*::*kan ΔuvrD*::*dhfr* | P1.N6499 × N7092 to Km$^r$ Tm$^r$ |
| N7096 | pAM390 (*lac*$^+$ *ruvABC*$^+$) / *ΔlacIZYA ΔruvABC*::*cat ΔuvrD*::*dhfr* | P1.AM1657 × N7092 to Tm$^r$ |

**BL21(DE3) derivatives**

| | | |
|---|---|---|
| BL21 [c] | F$^-$ *ompT hsdS$_B$* (*r$_B^-$ m$_B^-$*) *gal dcm* (DE3) | (MOFFATT and STUDIER 1987) |
| STL5827 | *xonA2 endA*::*tet* | Susan Lovett |
| JJ1634 | *xonA2 endA*::*tet yjcB*::EZ*kan ssb[A130G]* | P1.JJ1507 × STL5827 to Km$^r$ |
| JJ1635 | *xonA2 endA*::*tet yjbQ*::EZ*kan ssb[A40G]* | P1.JJ1509 × STL5827 to Km$^r$ |

**W3110 derivative**

| | | |
|---|---|---|
| N3005 | *purE85*::Tn*10* | (MAHDI *et al.* 2006) |
| N3072 | *recA269*::Tn*10* | (LLOYD *et al.* 1987) |

[a] F$^-$ λ$^-$ *ilvG- rfb-50 rph-1*. MG1655 is the 'wild type' K-12 strain used in this study.

[b] F$^-$ λ$^-$ rac$^-$ *thi-1 hisG4 Δ(gpt-proA)62 argE3 thr-1 leuB6 kdgK51 rfbD1 araC14 lacY1 galK2 xylA5 mtl-1 tsx-33 supE44(glnV44) rpsL31(strR)*

[c] An *E. coli* B strain.

[d] Elimination of the *kan* element in N7084 using pCP20-mediated site-specific recombination as described (BERNHARDT and DE BOER 2004).