



Published in final edited form as:

DNA Repair (Amst). 2010 April 4; 9(4): 403–413. doi:10.1016/j.dnarep.2009.12.019.

RecQ-dependent death-by-recombination in cells lacking RecG and UvrD

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Abstract

Maintenance of genomic stability is critical for all cells. Homologous recombination (HR) pathways promote genome stability using evolutionarily conserved proteins such as RecA, SSB, and RecQ, the *E. coli* homologue of five human proteins at least three of which suppress genome instability and cancer. A previous report indicated that RecQ promotes the net accumulation in cells of intermolecular HR intermediates (IRIs), a net effect opposite that of the yeast and two human RecQ homologues. Here we extend those conclusions. We demonstrate that cells that lack both UvrD, an inhibitor of RecA-mediated strand exchange, and RecG, a DNA helicase implicated in IRI resolution, are inviable. We show that the *uvrD recG* cells die a “death-by-recombination” in which IRIs accumulate blocking chromosome segregation. First, their death requires RecA HR protein. Second, the death is accompanied by cytogenetically visible failure to segregate chromosomes. Third, FISH analyses show that the unsegregated chromosomes have completed replication, supporting the hypothesis that unresolved IRIs prevented the segregation. Fourth, we show that RecQ and induction of the SOS response are required for the accumulation of replicated, unsegregated chromosomes and death, as are RecF, RecO, and RecJ. ExoI exonuclease and MutL mismatch-repair protein are partially required. This set of genes is similar but not identical to those that promote death-by-recombination of $\Delta uvrD \Delta ruv$ cells. The data support models in which RecQ promotes the net accumulation in cells of IRIs and RecG promotes resolution of IRIs that form via pathways not wholly identical to those that produce the IRIs resolved by RuvABC. This implies that RecG resolves intermediates other than or in addition to standard Holliday junctions resolved by RuvABC. The role of RecQ in net accumulation of IRIs may be shared by one or more of its human homologues.

Keywords

RecQ; genome instability; homologous recombination; SOS response; mismatch repair; replication fork

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1. Introduction

The RecQ family of DNA helicases is highly conserved throughout evolution, and many RecQ homologues play roles in preventing genomic instability. Humans have five known homologues of *E. coli* RecQ. The loss of three of them, BLM, WRN, and RECQ4, are associated respectively with Bloom, Werner and Rothmund-Thomson cancer-predisposition syndromes (reviewed [1]). Although the other two human RecQ homologues, RECQ1 and RECQ5, are not currently associated with specific syndromes, these proteins also inhibit genome instability and tumorigenesis in mammals [2,3], and so are also probable candidates for human cancer-prevention proteins. How RecQ homologues protect cells from genome instability and resulting cancer susceptibility is an important problem. Though there may be several different cellular processes upon which RecQ homologues impinge, one common one is homologous recombination (HR), which can have both genome stabilizing and destabilizing effects (reviewed [1].)

DNA repair via HR proceeds via a few general steps (reviewed [4,5]). First various proteins expose single-stranded (ss)DNA in a damaged DNA molecule. This can occur at, e.g., a double-strand break or end, or single-strand gap, and is often associated with problems at a replication fork. Next, exposed ssDNA is coated by RecA or a RecA homologue. RecA-coated ssDNA then engages a duplex DNA molecule, usually a sister chromosome, exchanging strands in a region of base complementarity. This strand exchange links the two molecules in an intermolecular HR intermediate (IRI). Finally, IRIs must be “resolved” to produce separate chromosomes so that repaired chromosomes may then be segregated.

Biochemically, RecQ homologues display activities compatible with roles both in forming IRIs and in their resolution. Sometimes the same protein possesses both kinds of activities. For example, *E. coli* RecQ can both promote strand invasion by RecA and disrupt RecA-mediated strand-invasion events *in vitro* [6]. RecQ can also function in a complex with Topoisomerase III and SSB to resolve intermediates formed from converging replication forks *in vitro* [7]. Similarly, the budding yeast homologue of RecQ, Sgs1, and human BLM can form complexes with Top3 to resolve converging recombination intermediates (double Holliday junctions) [8,9]. Human RECQ4 displays ATP-dependent strand-annealing activity, but has no known helicase activity [10], but BLM and WRN appear to have both strand annealing and DNA unwinding (opposing) activities *in vitro* [11].

Given the numerous activities and substrates of RecQ homologues *in vitro*, it has been informative to understand their overall, dominant or net effects on HR in living cells. These fall into two classes depending on whether they promote net accumulation or net reduction of IRIs in cells. For example, although elegant *in vivo* studies demonstrate that one role of yeast Sgs1 is to help expose ssDNA at double-strand ends prior to loading of RecA homologue Rad51 [12–14], a role that leads to formation of IRIs, the net or overall effect of Sgs1 is the opposite. Sgs1 promotes net reduction of IRIs in cells. Cells lacking Sgs1 and the Rad51-removal protein Srs2 are inviable, and their inviability requires Rad51 [15]. This implies that they die from excess accumulation of Rad51-promoted IRIs that prevent chromosome segregation. Similarly, human WRN reduces overall IRI levels, in that WRN-deficient cells also die a RAD51-dependent “death by recombination” in which chromosome segregation fails [16,17]. The death can be averted by production of a bacterial Holliday-junction (IRI) resolvase, indicating excess IRIs as its cause [17]. Also, human BLM protein is thought to reduce IRI levels both because of its biochemical activity in resolving model IRI DNA substrates [18,19] and also because *BLM* cells show increased levels of micronuclei [20] and anaphase bridges which are thought to result from chromosome-segregation defects [21].

Whereas Sgs1, WRN and BLM exemplify one paradigm in HR—net removal of IRIs—*E. coli* RecQ appears to exemplify an opposite paradigm: promotion of net accumulation of IRIs in cells. First, the loss of *recQ* restored viability to $\Delta topB parETs$ cells. When raised to the restrictive temperature, $\Delta topB parETs$ cells die a death that is accompanied by chromosome-segregation failure, and is caused by the loss of an apparent TopoIII-dependent IRI-resolution pathway redundant with RuvABC [22]. Therefore, the fact that loss of *recQ* restored viability to, or relieved the inviability of, *topB parETs* cells implies that the presence of RecQ protein promoted the net accumulation of IRIs in cells (though the data were interpreted otherwise in that paper [22]). Second, cells that lack the demonstrated IRI-resolution system RuvABC and the RecA/IRI-inhibitor protein UvrD die a RecA-dependent “death by recombination” in which unresolved IRIs prevent chromosome segregation [23]. The chromosomes had demonstrably completed replication, implying that their failure to segregate was not from incomplete replication but rather from being joined in unresolved IRIs. Moreover, loss of RecQ allowed $\Delta ruv \Delta uvrD$ cell viability, indicating a role for RecQ in net IRI accumulation [23]. This is a second paradigm for RecQ proteins in HR opposite that of Sgs1, WRN and BLM.

We suggested that organisms with multiple RecQ homologues might possess homologues that affect net IRI levels oppositely, some acting like Sgs1, WRN and BLM, and others acting like *E. coli* RecQ [23]. Such division of labor appears to be the case in Arabidopsis, in which *RECQ4B* appears to promote HR and the accumulation of IRIs, whereas the closely related *RECQ4A* promotes resolution of IRIs that accumulate [24].

The strongest demonstration that RecQ acts oppositely of some other RecQ homologues in cells used the special mutant genetic background of cells lacking *uvrD* and *ruvA* or *ruvB* or *ruvC* Holliday-junction-resolution proteins [23]. In this report, we generalize and extend those conclusions by examining the effect of RecQ in cells that lack *uvrD* and *recG*, a different putative Holliday-junction resolution protein.

RecG is a branch-migration helicase [25], the *in vivo* function of which is less certain than that of the Ruv proteins. RecG is redundant with Ruv in conjugational and transduction HR assays [26], and so was thought to be a Holliday-junction-resolution protein like Ruv. However other functions for RecG in replication-fork management have been suggested such as promoting Holliday-junction formation through replication-fork reversal [27].

The logic of this study is the following. All of recombinational DNA repair proceeds via heteroduplex DNA intermediates often between molecules (IRIs). Because unresolved IRIs kill cells by preventing chromosome segregation, proteins that promote net accumulation of IRIs in cells promote the death of mutants that lack known IRI removal proteins. In this study we identify many proteins responsible for death of IRI-removal-impaired *uvrD recG* cells. This identifies pathways that lead to net accumulation of IRIs in cells, specifically IRIs normally processed by RecG. This was done previously for cells lacking the *uvrD* and *ruvA* or *ruvB* or *ruvC* Holliday-junction-resolution proteins [23]. That study produced a list of proteins, including RecQ, that promote net accumulation of IRIs resolved by Ruv. Because RuvABC resolve Holliday junctions (reviewed [28]), the proteins identified lead to net accumulation specifically of IRIs containing Holliday junctions. In this study we demonstrate that *uvrD recG* cells are also inviable and characterize a large number of proteins that contribute to this inviability. The results confirm, in a different genetic background, that RecQ promotes net accumulation of IRIs and show that this causes “death-by-recombination” of *uvrD recG* cells. Moreover, the proteins identified that promote inviability of *uvrD recG* cells are overlapping but not identical with those identified previously as promoting death of *uvrD ruv* mutants. Therefore, we infer that RecG resolves IRIs not identical to (other than or in addition to) those resolved by Ruv. Moreover, the proteins identified here show the pathways leading to the specific DNA intermediates processed by RecG.

2. Materials and methods

2.1. *E. coli* strains and methods

All strains and plasmids used in this study are described in Table 1. Strains were created using P1 transduction as described [29] and verified using PCR, sensitivity to ultraviolet light and other phenotypic characteristics. Quantitative transductions were performed using concentrations of phage chosen in which transductants were linearly proportional to P1 particles added and transductants per particle was used to assess transduction efficiency. Transductants were incubated at 32°C on Luria Bertani Herskowitz (LBH) plates containing 50 µg/ml kanamycin (Kan) and 0.5% sodium citrate (Citrate) as per [23]. Colony forming units (cfu) were scored after 24 hours of growth using a Microbiology International ProtoCOL counter.

2.2. Cotransduction assay

Cotransductions were performed as in [23]. P1 transductions were performed and cells plated on LBH plates containing 10 µg/ml tetracycline (Tet) and 0.5% Citrate at 32°C, or the temperature stated. Following 48 hours of growth, between 100 and 200 colonies for each genotype were purified by streaking on LBH-Tet-Citrate plates. The transduction plates were then incubated for an additional 48h to confirm that no additional colonies formed. The streaked colonies were incubated for 48h before being replica plated onto LBH-Citrate plates containing 25 µg/ml chloramphenicol (Cam). Cotransductant frequencies are calculated as % Cam^R/Tet^R patches. For cotransduction into recipients containing the *recA200T*s allele, transductions were performed at 30°C to allow recombination, then plating and subsequent incubations were at 42°C. For strains carrying pML104-3, the plasmid was maintained with 100 µg/ml spectinomycin (Spec) at 30°C during growth and cotransduction. Lambda *red gam* induction was performed using 0.4 mM IPTG for one hour prior to cotransduction. The cells were then plated onto medium lacking Spec at 37°C to allow the plasmid to be lost. All Cam^R Tet^R cfu were screened and verified to be Spec^S.

2.3. Temperature-shift assay

Synchronous death of cells upon temperature shift to 30°C was performed by growing cultures to saturation in LBH at 42°C and then plating dilutions on pre-warmed LBH plates at 42°C and 30°C. CfU were scored after 24 hours incubation. Plates were then placed back at 42°C/30°C for an additional 24 hours to verify that no slow-growing colonies appeared.

2.4. Statistical Analyses

Unless otherwise stated, strains indicated as inviable or having restoration of viability are statistically significantly different from the appropriate control strains with $p \leq 0.01$. Statistical analyses were performed using SigmaStat and measured by ANOVA with Fisher LSD post-hoc analysis.

2.5. Microscopy and fluorescence in situ hybridization

Chromosome-segregation analyses were as described [23]. Cells were visualized using a Zeiss Axio Imager microscope equipped with 100× oil objective and DAPI filter and Hamamatsu camera.

Fluorescence In Situ Hybridization (FISH) was as described [23]. Probes were 6 kb DNA fragments PCR amplified (Phusion DNA polymerase, New England Biolabs) from strain MG1655 DNA. Primers for the *ori* and *ter* probes were as described [30]. Probes were visualized using the Oregon Green filter and Rhodamine filter with the above microscope. Foci

were scored on separate channels using ImageJ. Merged images were processed using Axiovision.

3. Results

3.1. Inviability of $\Delta recG \Delta uvrD$ double mutants

We tested whether the net effect in cells of RecG is reduction of IRI levels by determining whether $\Delta recG \Delta uvrD$ cells have a growth defect. If RecG acted similarly to Ruv in IRI resolution [31], then we might expect a $\Delta recG \Delta uvrD$ strain to have a growth defect due to excessive accumulation of IRIs, which can block chromosome segregation leading to death by recombination [23]. To test for synthetic lethality (inviability of the double mutant) between $\Delta recG$ and $\Delta uvrD$, we used phage P1-mediated cotransduction (Fig. 1A; e.g., [23]) which measures the viability of recipient cells cotransduced with two linked antibiotic resistance markers, one of which disrupts a gene of interest, in our case UvrD. The frequency with which both markers are cotransduced is determined by the distance between the markers. When an antibiotic marker disrupts a gene that is synthetically lethal with a mutation in the recipient strain's genome, the frequency of cfu containing both antibiotic resistances will be reduced. We find that a deletion of *uvrD* can be efficiently cotransduced with a linked Tet^R marker into a recombination-proficient (*rec*⁺) background, but cannot be cotransduced into cells lacking RecG, indicating that the $\Delta recG \Delta uvrD$ double mutant is inviable (Fig. 1B).

3.2. Recombination proteins are required for $\Delta recG \Delta uvrD$ inviability

If the synthetic lethality between RecG and UvrD was due to accumulated toxic IRIs, such as D-loops and/or Holliday junctions, then removing proteins that promote formation or longevity of these intermediates might relieve the inviability. RecA is the major recombinase in cells and also activates induction of the SOS DNA-damage response. Because RecA is required for transduction, to test the hypothesis that *recA* deletion might allow viability of $\Delta recG \Delta uvrD$ cells, we used $\Delta recA$ strains carrying *recA*⁺ on plasmid pML104-3, which has a temperature-sensitive origin of replication and is lost at 37°C. The cotransduction was performed at 30°C and then plated at 37°C to switch the cells into a $\Delta recA \Delta recG \Delta uvrD$ state. We found that the *recA* deletion allowed viability of $\Delta recG \Delta uvrD$ cells (Fig. 1B; *p*= 0.02 for the difference between $\Delta recG$ and $\Delta recG \Delta recA$ recipients). Thus RecA function is required for the inviability of $\Delta recG \Delta uvrD$ double mutants.

RecF helps RecA load onto ssDNA such as gaps formed at stalled replication forks (reviewed [32]) and therefore helps promote both the strand-exchange and SOS-induction activities of RecA. We find that functional RecF is also required for inviability of *recG* $\Delta uvrD$ cells (Fig. 1C), as is functional RecO (Fig. 1D), which works in a complex with RecF [33]. That is, $\Delta recG \Delta uvrD$ cells are viable if *recF* (Fig. 1C) or *recO* (Fig. 1D) is also knocked out.

Interestingly, the *recG* $\Delta uvrD$ inviability also requires functional RecQ. That is, *recG* $\Delta uvrD$ $\Delta recQ$ cells are viable (Fig. 2A). Transducing DNAs containing both $\Delta uvrD$ and $\Delta recQ$ were efficiently cotransduced with a linked Tet^R marker into a *recG* strain whereas DNAs that were $\Delta uvrD$ but *recQ*⁺ were not (Fig. 2A). Twenty clones that arose as Cam^R Tet^R colonies when *rec*⁺ cells were cotransduced with the $\Delta uvrD \Delta recQ$ containing P1-lysate and 40 when *recG* was cotransduced were checked by PCR to verify that there was not a high frequency of double recombination that would result in loss of the $\Delta recQ$ mutation. 1/20 Cam^R Tet^R colonies that arose after cotransduction into *rec*⁺ lost the $\Delta recQ$ mutation, but all 40 of the *recG* colonies carried $\Delta recQ$. The requirement for RecQ in the death of *recG* $\Delta uvrD$ cells is similar to that seen previously for RecQ in promoting the net-accumulation of toxic IRIs in $\Delta ruv \Delta uvrD$ cells [23].

RecQ interacts with several proteins either directly or indirectly through an interaction with SSB, single-stranded DNA binding protein [34,35]. Of the proteins thought to interact with RecQ, single-stranded exonucleases RecJ and ExoI (encoded by the *xonA* gene) are of particular interest. RecJ 5' exonuclease could work with RecQ in creating substrates for RecA loading and recombination [36]. We find that either $\Delta recJ$ (Fig. 2B) or $\Delta xonA$ (Exonuclease I; Fig. 2C) allowed viability of *recG* $\Delta uvrD$ cells, though in the case of Exonuclease I the rescue is only partial. The requirement for functional RecJ in the death of *recG* $\Delta uvrD$ cells is similar to that seen for Δruv $\Delta uvrD$ synthetic lethality, and again supports the hypothesis that *recG* $\Delta uvrD$ cells are undergoing death by recombination. The partial restoration of viability to *recG* $\Delta uvrD$ cultures by lox of ExoI is not seen for the Δruv $\Delta uvrD$ synthetic lethality (Fig. 2D). This difference between the *recG* $\Delta uvrD$ and Δruv $\Delta uvrD$ situations is discussed below.

3.3. Role of the SOS response in *recG* $\Delta uvrD$ inviability

Cells that experience DNA damage induce an SOS response. During SOS, more than 40 genes normally repressed by LexA transcriptional repressor are upregulated due to degradation of LexA [37]. We find that a *lexA*(Ind⁻) allele, which keeps the SOS genes repressed, partially restores viability of *recG* $\Delta uvrD$ cultures (Fig. 2E). $\Delta uvrD$ can be cotransduced into a *recG* *lexA*(Ind⁻) recipient strain at an intermediate frequency, indicating that the SOS response contributes to, but is not the only source of, the *recG* $\Delta uvrD$ inviability. Because RecA levels are increased in SOS-induced cells, it was possible that SOS-induced levels of RecA cause the *recG* $\Delta uvrD$ inviability, as was the case for the Δruv $\Delta uvrD$ inviability [23]. To test this hypothesis we placed an operator constitutive allele of RecA (*recAo28I*), which is not repressed by LexA, into the *lexA*(Ind⁻) background. We find the *recAo* allele confers some but not all of the inviability blocked by the SOS-off *lexA*(Ind⁻) mutation (Fig. 2E). We conclude that SOS-induced levels of RecA, and of one or more additional SOS genes account for the role of the SOS response in death of *recG* $\Delta uvrD$ cells. This differs from what was seen for the Δruv $\Delta uvrD$ inviability [23] in which *recA* was the only SOS gene required at SOS-induced levels for the inviability.

One of the proteins induced during the SOS response is Sula, an inhibitor of cell division [4]. We found that Sula plays only a small role in the *recG* $\Delta uvrD$ inviability. $\Delta uvrD$ was cotransduced into *sula* *recG* cells at a frequency of 5% (Fig. 2F) which is lower than into *recG* *lexA*(Ind⁻) (33% cotransduction frequency, Fig. 2E), but still significantly greater than into *recG* alone ($p < 0.05$, ANOVA). Thus, Sula makes a minor contribution to the role of the SOS response in the *recG* $\Delta uvrD$ inviability. RecN is also upregulated by the SOS response, however, $\Delta uvrD$ could not be cotransduced into a *recN* *recG* recipient background (Fig. 2G), indicating that functional RecN is not required for the inviability of *recG* $\Delta uvrD$ cells. We conclude that the SOS response promotes the death of *recG* $\Delta uvrD$ cells by upregulation of RecA, Sula and possibly an additional component(s).

RecQ is required for SOS-induction under certain circumstances [38]. To address whether RecQ promotes death of *recG* $\Delta uvrD$ cells solely by helping turn-on SOS, we examined the frequency with which DNA containing $\Delta uvrD$ $\Delta recQ$ is cotransduced into a *lexA*(Ind⁻) *recG* background. We find that there is a significant increase over the frequency with which $\Delta uvrD$ is cotransduced into *lexA*(Ind⁻) *recG* (Fig. 2I), indicating that RecQ and LexA function in wholly or partially independent pathways promoting inviability of *recG* $\Delta uvrD$ cells. Therefore, RecQ does more in the death pathway than simply helping to activate the SOS response.

3.4. Expression of Rus resolvase does not alleviate the *recG* $\Delta uvrD$ inviability

RusA is a Holliday-junction resolvase encoded in a cryptic prophage which is normally silent in *E. coli* [39]. RusA can compensate for the loss of Ruv *in vitro* [39] and can restore viability

to $\Delta uvrD \Delta ruvC$ strains (RG Lloyd, personal communication). This demonstrates that $\Delta uvrD \Delta ruvC$ cells die from accumulation of IRIs containing either 4-way (Holliday) or 3-way junctions connecting the entangled chromosomes, because these are the DNA substrates cleaved by RusA [40,41]. By contrast, we find that expression of RusA *via* the *rus-1* allele did not relieve the *recG \Delta uvrD* inviability (Fig. 2H). This result suggests that the lethal DNA substrate in *recG \Delta uvrD* strains might be an intermediate that is not recognized and resolved by RusA, as indicated by *in vitro* work [41]. However we cannot rule out the possibility that RusA cannot act on Holliday junctions or other DNA intermediates in the presence of RuvABC which are likely to compete with Rus. RusA has never been shown to have a phenotype in Ruv⁺ cells.

3.5. Role of mismatch repair but not nucleotide excision repair in the SOS pathway of *recG \Delta uvrD* inviability

Apart from removing RecA from ssDNA, UvrD functions in both mismatch repair (MMR), which also requires MutL, MutS and MutH, and nucleotide excision repair (NER), which also requires UvrA, UvrB and UvrC proteins [4]. During MMR, MutS binds the DNA mismatch, MutL binds MutS and coordinates with MutH endonuclease to produce a single-strand nick near the mismatched site, which is then usually unwound by UvrD removing the DNA strand containing the incorrect base [4]. Similarly, UvrABC perform early steps in NER whereas UvrD acts later. We had no difficulty creating the double $\Delta uvrA \text{ recG}$ and $\Delta mutL \text{ recG}$ mutants or the triple $\Delta uvrA \Delta mutL \text{ recG}$ mutant as illustrated by quantitative transduction (Fig. 3A). Therefore, simply lacking the MMR or NER pathways does not cause inviability in *recG* cells.

On the other hand, we find that MutL, but not UvrA, contributes to the $\Delta uvrD \text{ recG}$ inviability (Fig. 3B). $\Delta uvrD$ can be transduced into $\Delta mutL \text{ recG}$ cells, albeit at about half the efficiency as when $\Delta uvrD$ is transduced into *recG*⁺ cells (Fig. 3B). These data imply that incomplete MMR intermediates created by MutSLH but left unresolved in cells lacking UvrD cause some of the inviability of *recG \Delta uvrD* cells. The single-strand-nicked-DNA intermediates created by MutSLH acting upon spontaneous mismatches, perhaps from spontaneous replication errors, appear to underlie some of the problem. Thus, part of the reason for the $\Delta uvrD$ inviability with RecG appears to involve incomplete MMR, whereas part is independent of MutL-dependent MMR. We conclude that a function of UvrD beside MMR or NER, probably its role in opposing RecA filaments, is responsible for about half the synthetic lethality of *recG \Delta uvrD* cells, and that the accumulation of MutL-generated MMR intermediates that accumulate in the absence of UvrD underlies the other half.

Because both the SOS response and incomplete MMR contribute to part of the *recG \Delta uvrD* inviability, we tested whether the loss of these together was sufficient to restore complete viability. We found that the loss of *mutL* did not significantly affect the frequency of $\Delta uvrD$ cotransduction into *lexA*(Ind⁻) *recG* cells (Fig. 3C). These data show that MutL-promoted inviability in *recG \Delta uvrD* cells occurs *via* the SOS-dependent pathway. That is, if SOS induction is blocked, loss of MutL confers no additional growth-enhancing benefit to cultures. This implies that a MutL-generated DNA intermediate retards growth/viability by activating an SOS response. This, plus the fact that RecQ is required for the inviability independently of a role in SOS induction (Fig. 2I), supports death-by-recombination models for part of the inviability.

3.6. DSE processing by RecB does not contribute to the $\Delta recG \Delta uvrD$ inviability

DNA double-strand breaks (DSBs) and ends (DSEs) are recognized by the RecBCD exonuclease complex which also creates substrates for RecA-mediated recombination [42]. To determine whether DSBs/DSEs might be part of the spontaneous DNA damage, failed repair of which kills $\Delta recG \Delta uvrD$ cells, we tested whether RecB contributes to the $\Delta recG \Delta uvrD$

inviability by cotransduction into strains carrying plasmid pML104-3 which, in addition to encoding RecA and having a temperature-sensitive origin of replication, has the lambda recombination genes *red* and *gam* under the control of an IPTG-inducible promoter, allowing recombination. IPTG was added for an hour prior to cotransduction and the cells were plated immediately on Tetracycline plates at 37°C to allow the plasmid to be lost. Fig. 4 shows both the total cotransduction frequency and the adjusted cotransduction frequency of the cells which lost pML104-3 and became Spec^S. We found that $\Delta uvrD$ was cotransduced into a *recB* strain similarly to *rec*⁺, but many of the colonies retained the plasmid indicating that there is some benefit, probably the over-production of RecA by the plasmid, in the absence of RecB. However, $\Delta uvrD$ could not be cotransduced efficiently into $\Delta recG \Delta recB$ cells. We conclude that DNA processing by RecB is not required for the $\Delta recG \Delta uvrD$ inviability. Therefore the DNA substrate recombined during death is not a DSE.

3.7. Chromosome-segregation failure but not failed replication during death of *recG uvrD* cells

If *recG uvrD* cells die a death by recombination, we would expect this death to be accompanied by chromosome-segregation failure. We constructed *recA(Ts) recG uvrD* cells, which are viable at the restrictive temperature (42°C), due to the absence of RecA function, then shifted these cells to permissive temperature to watch them die en masse. When grown at the non-permissive temperature (42°C), *recA(Ts) recG uvrD* cells are viable and 75% show normal chromosome segregation (Fig. 5A, B). When the cells are switched to the permissive temperature (30°C) for 4 hours, the cells die synchronously (Fig. 5A). During this death, the cells become filamented (elongated) with only 20% having normal chromosome segregation whereas the majority (64%) have unsegregated chromosomes (Fig. 5B). Removing RecF, ExoI, RecJ or RecQ from the cells rescues the chromosome-segregation defect, restoring the cells to $\geq 80\%$ having normal chromosome segregation (Fig. 5B). These data support the hypothesis that RecA, RecQ and RecF promote net accumulation of IRIs in *recG uvrD* cells.

RecG is implicated not only in dissociating D-loops but also in regressing stalled replication forks [27]. We therefore wanted to test the possibility that the death seen in *recG uvrD* cells is not death by recombination but rather due to replication failure. An accumulation of stalled replication forks that remain unregressed in the absence of RecG would be expected to cause the chromosome-segregation defect seen. To test this possibility, we used fluorescence in situ hybridization (FISH) with probes to the *E. coli* replication origin and terminus to determine whether or not replication is completed normally. If chromosome replication were incomplete then switching *recA(Ts) recG uvrD* cells to the permissive temperature should result in an increase in the ratio of origins:termini in cells from the approximately 2:1 seen in normally replicating cells, such as wild-type or *recA(Ts) recG uvrD* cells at the restrictive temperature. Instead, we see that switching the cells to the permissive temperature did not alter the ratio of origin:terminus foci significantly. All populations of cells showed 1.8 *ori:ter* as expected of normally replicating cells and seen for wild-type cells (Fig. 5C). We conclude that chromosome replication is completed normally during RecA-, RecQ-, RecF-dependent death of *recG uvrD* cells. The observation of failed chromosome segregation with completed chromosome replication supports a death by accumulation of unresolved IRIs.

4. Discussion

4.1. RecQ promotes death by recombination in *recG uvrD* cells

We have shown that cells lacking RecG and UvrD are inviable due to a chromosome-segregation defect (Fig. 5B) that occurs despite completion of chromosome replication (Fig. 5C), implicating a build-up of unresolved IRIs as a cause of their death. Although an SOS response is partially required for the death (Fig. 2E), RecA (Fig. 1B) and RecQ (Fig. 2A)

promote this death-by-recombination in roles independent of SOS induction (Fig. 2E and I), strongly supporting the idea that RecQ promotes a net accumulation in cells of unresolved IRIs. Whereas this conclusion was drawn previously from results in cells lacking RuvABC and UvrD [23], the current data generalize this conclusion to a different strain background showing that it is general property of RecQ not peculiar to the $\Delta ruv \Delta uvrD$ mutant strain background.

The synthetic lethality of *recG* and $\Delta uvrD$ is similar to what was seen in cells lacking Ruv and UvrD, but some differences in genetic requirements imply some differences in the mechanisms. Unlike death of $\Delta ruv \Delta uvrD$ cells, we observed that death of *recG* $\Delta uvrD$ cultures requires an SOS-response component in addition to RecA (Fig. 2E), and partially requires Exo I (Fig. 2C) and MutL (Fig. 3B, C). This death is also not blocked by RusA activation (Fig. 2H). Together these data indicate that there are some differences from the death-by-recombination of $\Delta ruv \Delta uvrD$ cells. Presumably, some different DNA substrates accumulate in cells lacking RecG and UvrD than in cells lacking Ruv and UvrD.

In Fig. 6 we show an elaboration on the general model for death-by-recombination previously presented by Magner *et al.* [23], adapted for RecQ-promoted death of *recG* $\Delta uvrD$ cells. This model shows RecF, RecO, RecA, RecQ and RecJ working to promote the net accumulation of IRIs in cells. The IRIs can become toxic if they remain unresolved. UvrD acts to oppose RecA [43] and thus effect a net reduction in IRIs in cells. It is unknown whether RecQ promotes net accumulation of IRIs by assisting the formation of IRIs, or alternatively, by opposing a Ruv- and RecG-independent IRI resolution pathway. This is an important aspect of RecQ mechanism of action in HR that awaits future experiments.

4.2. Mismatch repair intermediates promote death via SOS in *recG* cells

The model in Fig. 6 also accommodates our findings that part of the death of *recG* $\Delta uvrD$ cells requires MutL (Fig. 3B), and acts via the SOS component of the death pathway (Fig. 3C). We suggest that MMR intermediates initiated by MutL and left unresolved because of the absence of UvrD lead to SOS induction, which contributes to death in *recG* cells. This was observed only in *recG* $\Delta uvrD$ cells (here) and not in *ruv* $\Delta uvrD$ cells [23]. Cells lacking *recG* might be particularly sensitive to death by enhanced SOS induction because they are already partially SOS induced [44]. The loss of UvrD and accumulation of MMR intermediates might push them past a threshold of SOS-induction beyond which cell proliferation is impaired.

4.3. RecG role in net IRI reduction

The data presented here also suggest that different classes (structures) of IRIs can accumulate in cells, some of which are recognized and resolved by RuvABC, others of which are recognized/resolved by RecG. These IRIs accumulated in the absence of UvrD are either too numerous to be resolved by the limiting capacity of RuvABC present in the *recG* $\Delta uvrD$ cells or are unable to switch between resolution pathways. This could be either because they are intermediates not recognized by the Ruv and Rus (Fig. 2H) enzymes (i.e. not Holliday or three-way junctions), or possibly they are bound and sequestered, though not resolved, by the Ruv proteins which are at their capacity for resolution. Although the inability to restore viability to *recG* $\Delta uvrD$ cells with RusA suggests that the toxic substrate is not a Holliday junction, the caveat to this interpretation is that RusA may be unable to act in the presence of RuvABC. To date, all of its phenotypes have been documented in cells lacking Ruv [40,41]. The Ruv proteins might compete with RusA for their joint substrate. It is possible that some proteins that promote the $\Delta recG \Delta uvrD$ inviability such as MutL and ExoI, or even RecQ and RecJ do so by preventing switching between classes of IRIs. Because RecG has been hypothesized to process D-loops [45–47], one possibility for the $\Delta recG \Delta uvrD$ inviability is that D-loops form but cannot be branch-migrated into a substrate recognizable by RuvABC or unwound and reannealed to the original strand in a synthesis-dependent strand annealing (SDSA) pathway

[4]. The failure to process D-loops might cause them to accumulate and the IRIs joined by them to become toxic.

Although there is no known eukaryotic homolog of *E. coli* RecG it is likely that there are analogues throughout phylogeny. Most of DNA recombination and repair proteins are highly conserved (notably RecQ and RecA) and it would be surprising if this were not also true of RecG. Like RecQ, RecG has been studied but its role *in vivo* is still unknown. It was thought to have significant redundancy with other helicases present in *E. coli* [26]. The differences between the $\Delta recG \Delta uvrD$ and $\Delta ruv \Delta uvrD$ inviabilities: partial rescue by *sula*, $\Delta xonA$ and $\Delta mutL$ that are not seen for $\Delta ruv \Delta uvrD$ [23], may indicate that different DNA substrates, or different IRIs, accumulate in the $\Delta ruv \Delta uvrD$ than in the $\Delta recG \Delta uvrD$ background, all of which can become toxic if unresolved. This suggests that RecG is not simply a redundant helicase in *E. coli*, but also, or in addition, resolves IRIs that are poorly recognized/resolved by RuvABC.

4.4. Role of RecQ in net IRI accumulation

RecQ has now been shown to promote death-by-recombination in both the $\Delta ruv \Delta uvrD$ [23] and the $\Delta recG \Delta uvrD$ (here) backgrounds. Models presented previously show RecQ acting at its preferred substrate, a 5' DNA end at a replication fork, to produce a substrate for RecA binding, thus leading to IRI formation [23]. It is possible that RecQ, acting in this manner could promote the regression of a stalled replication fork, a substrate that RecG might then be able to recognize and have a role in resetting. RecA and RecF are able to stabilize regressed replication forks [48]. However, our data do not support this as the role for RecQ in $\Delta recG \Delta uvrD$ inviability because we saw no evidence for incomplete replication in the dying $\Delta recG \Delta uvrD$ cells (Fig. 5C). Though the net effect of RecQ remains an increase in the accumulation of IRIs in cells, how it causes this effect and the substrate(s) it recognizes *in vivo* remain uncertain. Homologues of *E. coli* RecQ may be involved in one or both paradigms of RecQ action. As more information comes to light on the different, opposing roles of RecQ homologues *in vivo*, understanding the RecQ-promoted death-by-recombination pathway(s) and the proteins involved will illuminate how RecQ homologues prevent genomic instability.

Acknowledgments

We thank David Bates for advice and the generous use of the Zeiss microscope, RG Lloyd for communication of unpublished results, and PJ Hastings for comments on the manuscript. This work was supported by National Institutes of Health grant R01-CA85777.

Abbreviations

cfu	colony-forming units
FISH	fluorescence in situ hybridization
HR	homologous recombination
IRI	intermolecular recombination intermediate
ssDNA	single-stranded DNA

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A Cotransduction Assay

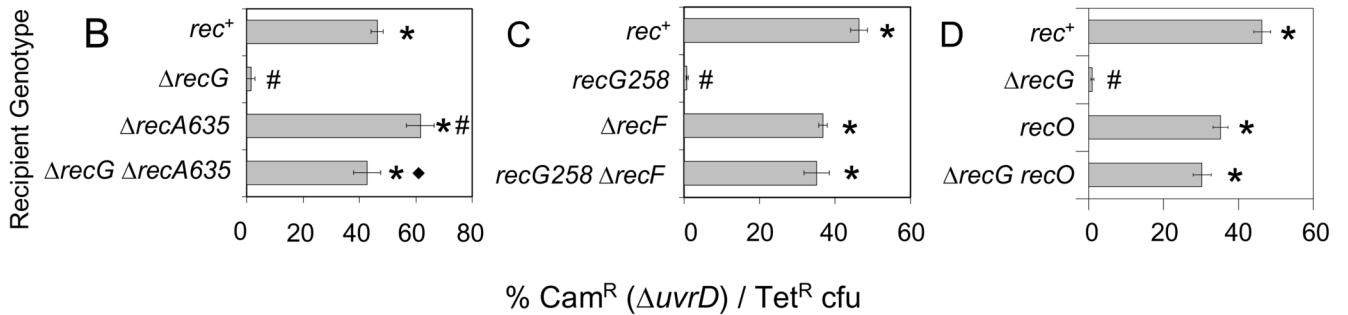
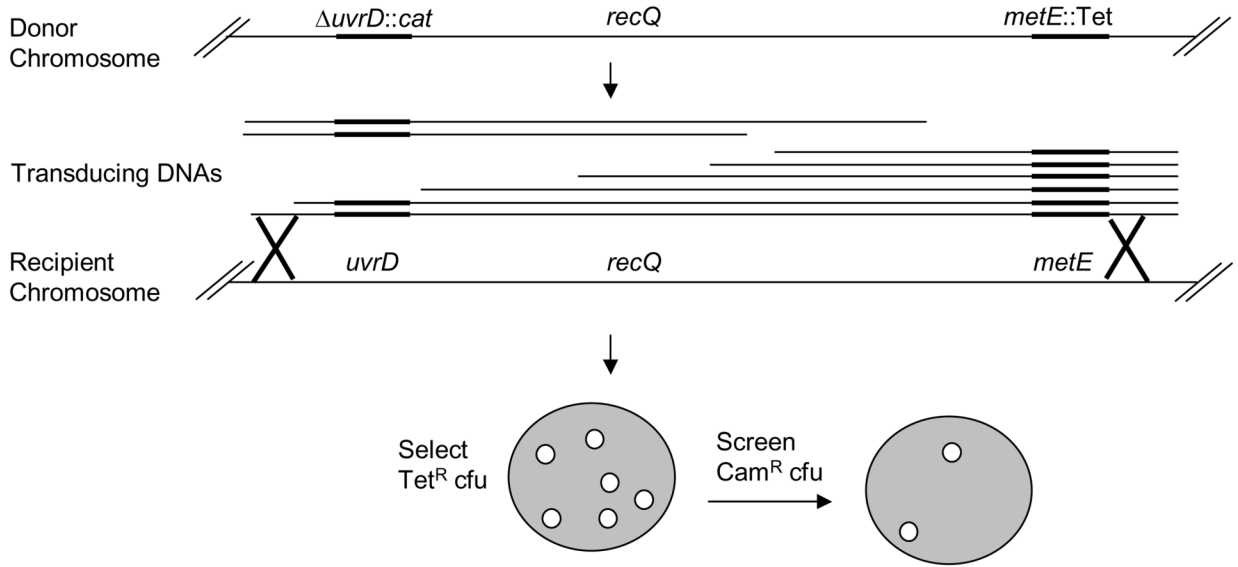
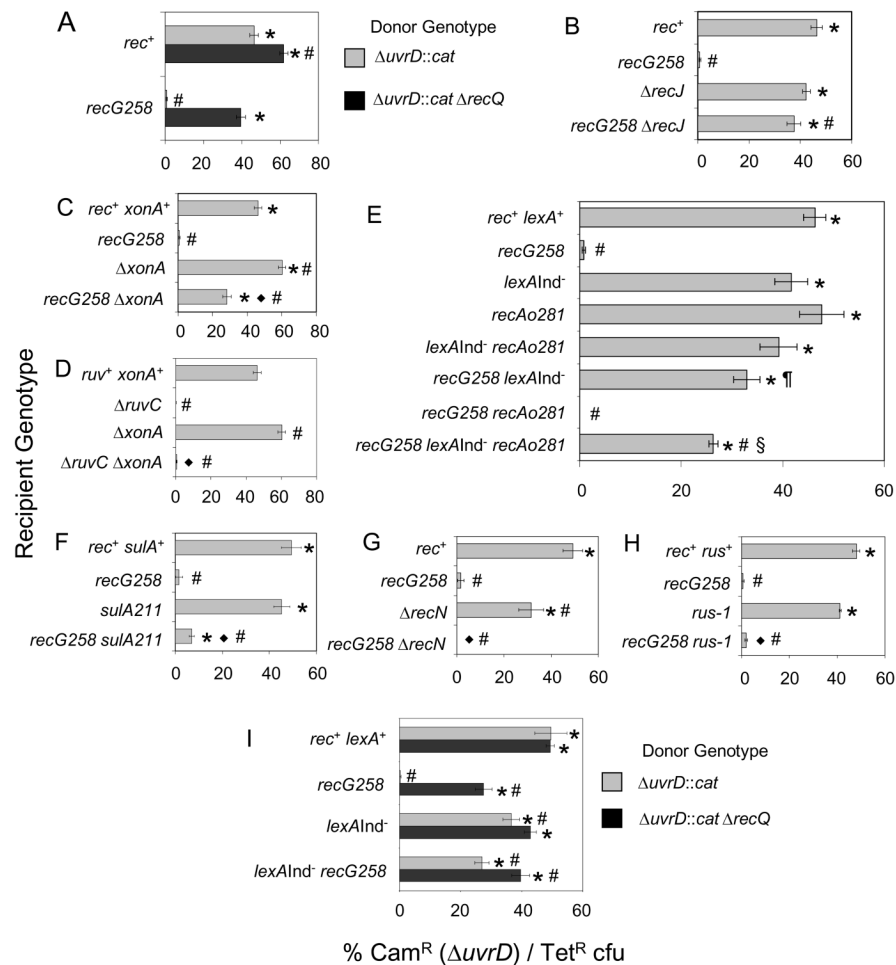


Fig. 1.

Demonstration of inviability of $recG \Delta uvrD$ double mutants by cotransduction and requirements for RecA, RecF and RecO for the inviability. (A) Cotransduction assay. Phage P1 grown on a donor strain with two linked antibiotic resistances (SMR9811) recombine into a recipient strain. Transductants are selected for the non-lethal marker (Tet^R) and screened for those that also contain the potentially lethal marker ($\Delta uvrD::cat$). $recQ$ is located between $uvrD$ and the linked Tet marker, so for examining a requirement for RecQ (Fig. 2) P1 grown on SMR9812, a strain that is $\Delta recQ$, is used. (B) $\Delta recG \Delta uvrD$ cells are inviable and RecA is required for their inviability. $\Delta uvrD$ cannot be cotransduced into a $\Delta recG$ recipient (SMR10698), but can into rec^+ (SMR10424), $\Delta recA$ (SMR10427) and $\Delta recA \Delta recG$ (SMR10428) recipients carrying pML104-3. (C) RecF is required for $recG \Delta uvrD$ inviability. $\Delta uvrD$ cannot be cotransduced into $recG258$ (SMR10419), but can into rec^+ (SMR6319), $\Delta recF$ (SMR10423) and $\Delta recF recG258$ (SMR10437) recipients. (D) RecO is required for $\Delta recG \Delta uvrD$ inviability. $\Delta uvrD$ cannot be cotransduced into $\Delta recG$ (SMR11133), but can into $recO$ (SMR11134) and $recO \Delta recG$ (SMR11135) recipients. Mean \pm SEM of 3 experiments for B, C and D. * indicates a significant difference from $recG$ (B–D). # indicates a significant difference from rec^+ (B–D); ♦ indicates a significant difference from the $\Delta recA$ single mutant (B, $p = 0.02$) of the double mutant indicated.

**Fig. 2.**

Roles of recombination proteins and the SOS response in the death of *recG uvrD* cells. Data are results of co-transduction experiments as described in Fig. 1. (A) RecQ is required for *recG ΔuvrD* inviability. $\Delta uvrD$ and $\Delta recQ$ alleles can be cotransduced together into *recG258* recipient cells (SMR10419, donor strain SMR9812), but the $\Delta uvrD$ mutation alone (donor SMR9811) cannot. *rec*⁺ positive-control recipient, SMR6319. (B) RecJ is required for *recG ΔuvrD* inviability. $\Delta uvrD$ can be cotransduced into both $\Delta recJ$ (SMR10434) and $\Delta recJ recG258$ (SMR10439) recipients. (C) ExoI is partially required for *recG ΔuvrD* inviability. $\Delta uvrD$ can be cotransduced into a $\Delta xonA$ recipient (SMR10395) efficiently, and a $\Delta xonA recG258$ recipient (SMR10438) with intermediate efficiency. (D) ExoI is not required for inviability of $\Delta ruvC \Delta uvrD$ cells. $\Delta uvrD$ cannot be cotransduced into a $\Delta xonA \Delta ruvC$ recipient (SMR10417). $\Delta xonA$ positive control, SMR10395; *ruvC* negative control, SMR10408. (E) The SOS response, SOS-induced levels of RecA, and induction of another SOS gene(s) promote *recG ΔuvrD* inviability. Efficient cotransduction of $\Delta uvrD$ into “SOS-off” *lexA*(Ind⁻) (SMR9801), *recAo281* (SMR9809) cells which produce SOS-induced levels of RecA, *lexA*(Ind⁻) *recAo281* (SMR9805), and *lexA*(Ind⁻) *recG258* (SMR10440) recipients, indicates that shutting off the SOS response via the *lexA*(Ind⁻) mutation partially restores viability to *recG ΔuvrD* cultures. Cotransduction efficiency is partially reduced from the level in *lexA*(Ind⁻) *recG258* in a *lexA*(Ind⁻) *recAo281 recG258* recipient (SMR10441), but is not abolished as in *recAo281 recG258* (SMR10442) and *recG258* (SMR10419). Therefore, SOS-induced levels of RecA account for some but not all of the contribution of the SOS response to *recG ΔuvrD*

inviability. (F) Sula contributes little to *recG* Δ *uvrD* inviability. Δ *uvrD* is cotransduced poorly into a *sulA211 recG258* recipient (SMR10443). *sulA211* positive control, SMR9837. (G) RecN is not required for *recG* Δ *uvrD* inviability. Δ *uvrD* cannot be cotransduced into a Δ *recN recG258* recipient (SMR10734). Δ *recN* positive control, SMR10730. (H) Activation of expression of Rus endonuclease by the *rus-1* mutation does not restore viability to *recG* Δ *uvrD* cells. Δ *uvrD* cannot be cotransduced into *recG258 rus-1* recipients (SMR10746). *rec⁺ rus⁺* control, SMR10743; *rus-1* positive control, SMR10744; *recG258* negative control, SMR10745. (I) RecQ promotes *recG* Δ *uvrD* inviability wholly or partly independently of induction of the SOS response. Δ *uvrD* and Δ *recQ* alleles confer greater viability when cotransduced together into *recG258 lexA(Ind⁻)* recipient cells (SMR10440, donor strain SMR9812) than the Δ *uvrD* alone (donor SMR9811), implying that Δ *recQ* promotes *recG* Δ *uvrD* inviability wholly or partly independently of the LexA/SOS response. Mean \pm SEM of 3 experiments (A–I). * indicates a significant difference from *recG*; # indicates a significant difference from *rec⁺* (A–I). \blacklozenge indicates a significant difference of each double mutant tested from the following isogenic single mutant: Δ *xonA* (C, D), *sulA* (F), *recN* (G), and *rus-1* (H). In (A) *recG* cotransduced with Δ *uvrD* Δ *recQ* is significantly different from *rec⁺* cotransduced with same. In (E), the § indicated strain is significantly different from all others in the panel while the ¶ indicated strain is significantly different from the *lexA(Ind⁻)* single mutant among mutants carrying the *lexA(Ind⁻)* allele. In (I) *lexA(Ind⁻) recG* cotransduced with Δ *uvrD* Δ *recQ* is significantly different from the same strain cotransduced with Δ *uvrD* alone. It is also significantly different from *recG* cotransduced with the Δ *recQ* Δ *uvrD* donor.

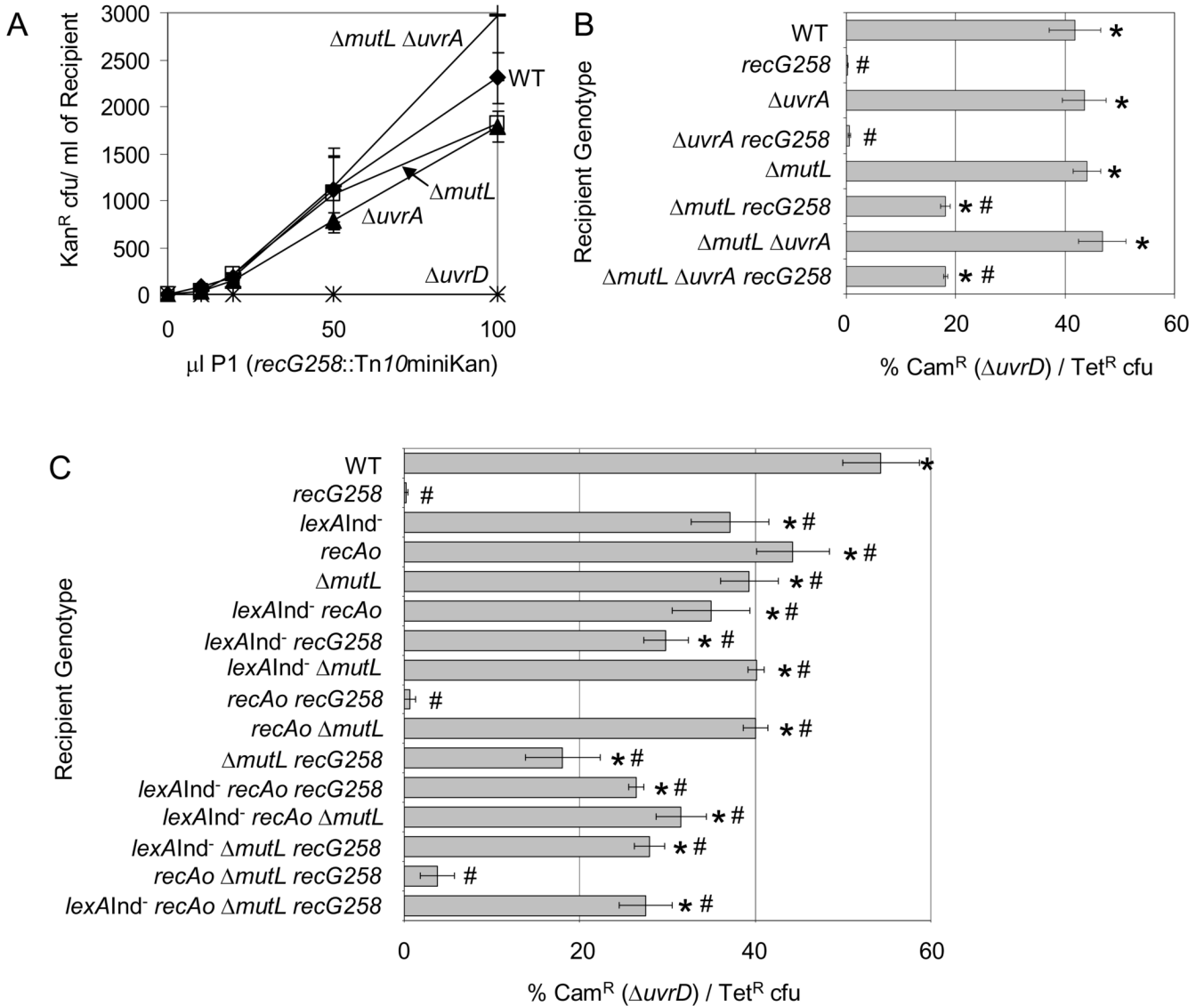
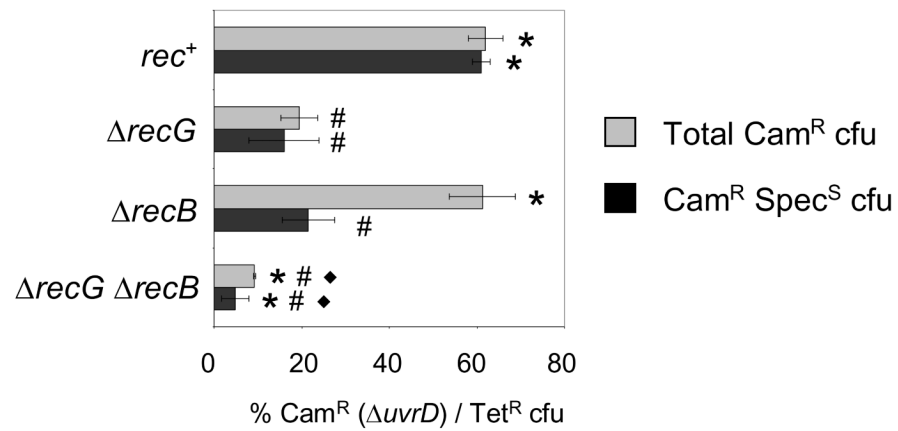
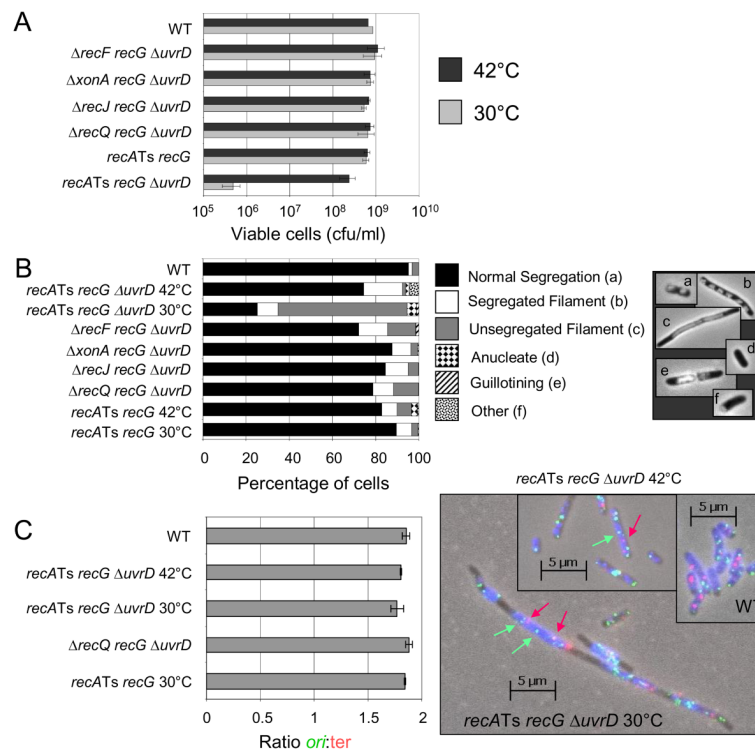


Fig. 3. Incomplete mismatch repair and not nucleotide excision repair contributes to death of *recG* $\Delta uvrD$ cells. (A) Simple loss of either MMR or NER does not cause inviability of *recG* cells, in that quantitative transduction of *recG258* (SMR9932 donor) into strains mutant for proteins involved in NER ($\Delta uvrA$, SMR8977), MMR ($\Delta mutL$, SMR8982) or both ($\Delta uvrA \Delta mutL$, SMR8986) is not impaired relative to MMR- and NER-proficient cells (SMR6319). Therefore, blocking formation of the MMR and NER intermediates created by MutL and UvrA does not create an inviability with *recG*. $\Delta uvrD$ negative control, SMR8976. (B) Although simple loss of either MMR or NER does not cause inviability of *recG* cells, loss of the MutL step in MMR relieves some of the inviability of *recG* $\Delta uvrD$ cultures. This implies that MMR intermediates initiated by MutL can be lethal to *recG* cells if UvrD is not present to complete repair and remove those intermediates. $\Delta uvrD$ can be cotransduced with intermediate efficiency into a $\Delta mutL recG258$ recipient (SMR11116) indicating a partial requirement for MutL, but not UvrA, in the death of *recG* $\Delta uvrD$ cultures. *recG258* SMR10419; $\Delta uvrA recG258$ SMR11115; MMR- and NER-proficient cells SMR6319 (“WT”); $\Delta uvrA$ SMR8977; $\Delta mutL$ SMR8982;

$\Delta uvrA \Delta mutL$ SMR8986; and $\Delta mutL \Delta uvrA recG258$ SMR11117 recipient cells. (C) The combination of incomplete MMR and SOS-induction does not account for all of the *recG* $\Delta uvrD$ inviability. When combined, the partial requirement for MutL and the SOS response does not restore more viability to *recG* $\Delta uvrD$ cells than *lexA*(Ind⁻) or $\Delta mutL$ alone, or in the presence of SOS-induced levels of RecA. MMR- and SOS-proficient cells SMR6319; *recG258* SMR10419; *lexA*(Ind⁻) SMR9801; *recAo281* SMR9809; $\Delta mutL$ SMR11323; *lexA*(Ind⁻) *recAo281* SMR9805; *lexA*(Ind⁻) *recG258* SMR10440; *lexA*(Ind⁻) $\Delta mutL$ SMR11318; *recAo281 recG258* SMR10442; *recAo281* $\Delta mutL$ SMR11320; $\Delta mutL recG258$ SMR11116; *lexA*(Ind⁻) *recAo281 recG258* SMR10441; *lexA*(Ind⁻) *recAo281* $\Delta mutL$ SMR11319; *lexA*(Ind⁻) $\Delta mutL recG258$ SMR11325; *recAo281* $\Delta mutL recG258$ SMR11329; *lexA*(Ind⁻) *recAo281* $\Delta mutL recG258$ SMR11327. Mean \pm SEM of 3 experiments (A–C). * indicates a significant difference from *recG*; # indicates a significant difference from WT (B, C). In (B) the triple $\Delta mutL \Delta uvrA recG258$ mutant is significantly different from all of the constituent single and double mutants except for $\Delta mutL recG258$ ($p=0.99$). In (C) the quadruple mutant *lexA*(Ind⁻) *recAo281* $\Delta mutL recG258$ is significantly different from *recAo281*; *lexA*(Ind⁻) $\Delta mutL$; *recAo281 recG258*; *recAo281* $\Delta mutL$ ($p=0.01$); $\Delta mutL$ ($p=0.02$), and *recAo281* $\Delta mutL recG258$. For WT compared to *recAo*, $p=0.02$.

**Fig. 4.**

RecB is not required for death of Δ *recG* Δ *uvrD* cells. Δ *uvrD* cannot be cotransduced into Δ *recG* (SMR11188) or Δ *recB* Δ *recG* (SMR11190), but can into *rec*⁺ (SMR10424) and Δ *recB* (SMR11189). All strains carry pML104-3 therefore all Tet^R cfu were also tested for Spec^R to assay the loss of pML104-3 at the restrictive temperature, 37°C. Note that the somewhat higher cotransduction into *recG* cells seen here is an apparent effect of the altered temperature regimen with higher temperature allowing greater viability (data not shown). Mean \pm SEM of 3 experiments. * indicates a significant difference from *recG*, # indicates a significant difference from WT, and ♦ indicates a significant difference from Δ *recB*. For Δ *recG* compared to Δ *recG* Δ *recB* $p \leq 0.05$.

**Fig. 5.**

Chromosome-segregation defects but completed replication during synchronous death of *recG ΔuvrD* cells. (A) Upon shift of *recA(Ts) recG ΔuvrD* cells (SMR10740) switched to the permissive temperature (30°C, RecA⁺ phenotype), from the restrictive temperature (42°C, RecA⁻ phenotype), cells die synchronously and show (B) chromosome-segregation defects as indicated by an increased percentage of filamented cells with unsegregated nucleoids. The chromosome-segregation defect is also rescued by the additional mutation of $\Delta recF$, $\Delta xonA$, $\Delta recJ$, or $\Delta recQ$ (Strains SMR10735, SMR10736, SMR10737 and SMR10738 respectively). Control *recA(Ts) recG* cells (SMR10739) do not show chromosome-segregation defects at the permissive temperature. Mean of 3 experiments. SEM is < 5% of cells/category for all genotypes and temps. (C) Completion of chromosome replication in dying *recA(Ts) recG ΔuvrD* cells shown by fluorescence in situ hybridization (FISH) to *ori* (green foci) and terminus-proximal (red foci) chromosomal sequences. Left, the ratios of *ori:ter* foci in cells are unchanged during synchronous death of *recA(Ts) recG ΔuvrD* (SMR10740) cells at permissive or restrictive temperatures from those of the non-dying wild-type (*rec*⁺, SMR6319) control. The ratio of *ori:ter* is not different from that of *rec*⁺ or *recA(Ts) recG* (SMR10739) cells, or of $\Delta recQ recG \Delta uvrD$ (SMR10738) cells. Right, representative example of FISH data. Images are overlays of phase contrast (blue), red (*ter* hybridization) and green (*ori* hybridization) exposures. Red arrows, examples of red *ter* foci; green arrows, examples of green *ori* foci. Mean ± SEM of 3 experiments for B and C; ≥ 500 cells scored/genotype/temperature/experiment.

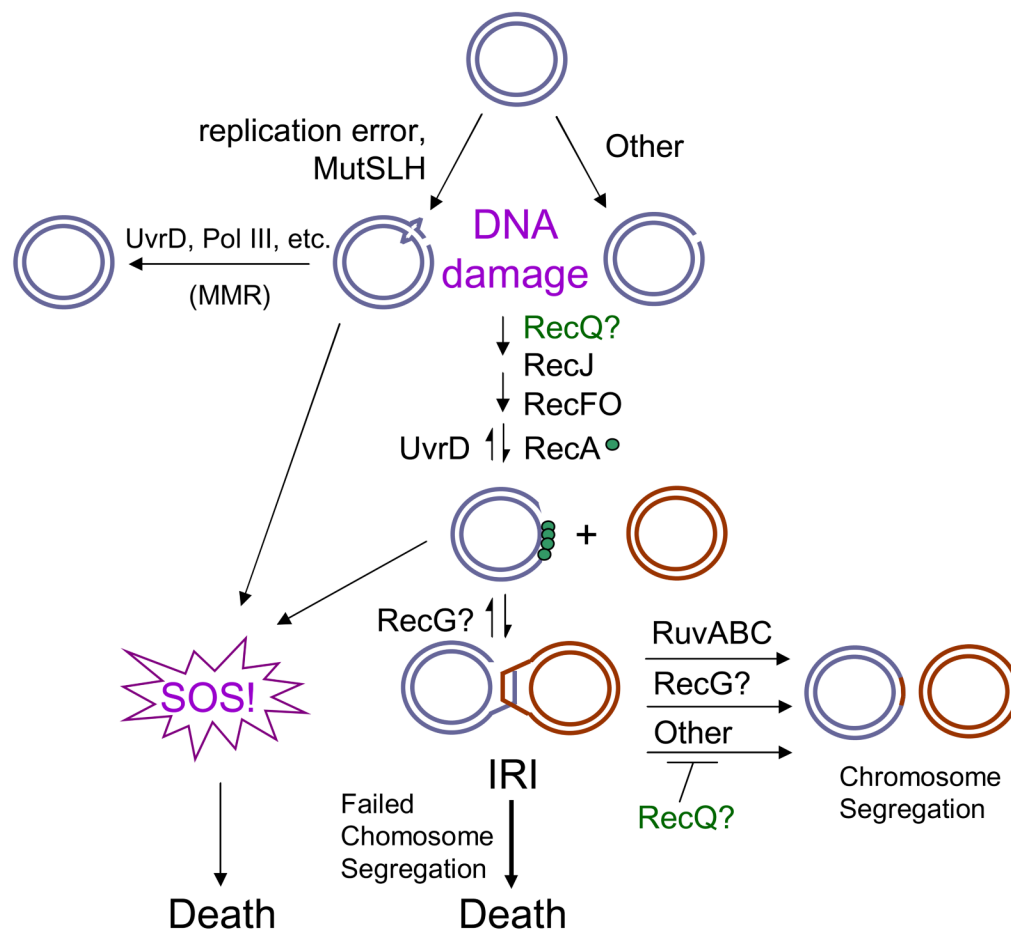


Fig. 6. Models for death of $\Delta recG \Delta uvrD$ cells. DNA damage arises from replication errors (mismatches) which are recognized and processed by MutSLH or from other sources. In the presence of UvrD, MMR can be completed to give intact dsDNA. In the absence of UvrD, the damaged DNA is processed by homologous recombination proteins. RecQ and RecJ may function together at this stage to create ssDNA gaps which are substrates for RecF, O and RecA, which can promote both the induction of SOS and the initiation of recombination (formation of IRIs). IRIs are then resolved in multiple pathways, primarily through the action of RuvABC, but also *via* the action of RecG. Both the persistence of unresolved IRIs and the SOS response can lead to loss of viability (death.) RecG might branch migrate D-loops or IRIs to contribute to their elimination. RecQ might either promote the formation of ssDNA required for IRI formation or inhibit an alternative resolution pathway to Ruv and RecG. Blue and Red circles represent dsDNA.

Table 1

Escherichia coli K-12 strains and plasmids used in this study.

Plasmid/Strain	Relevant genotype	Source or reference
pCP20	FLP recombinase vector, temperature-sensitive origin, Amp ^R , Cam ^R	[49]
pML104-3	<i>red⁺ gam⁺</i> under IPTG promoter, temperature-sensitive origin, Spec ^R , RecA ⁺	S. Elledge
BW26355	Δ <i>recA635</i> ::FRTKanFRT	CGSC7651 ^a ; [49]
JW0525	Δ <i>intD</i> ::FRTKanFRT	[50]
JW1852	Δ <i>ruvC</i> ::FRTKanFRT	[50]
JW1993	Δ <i>xonA</i> ::FRTKanFRT	[50]
JW2597	Δ <i>recN</i> ::FRTKanFRT	[50]
JW3627	Δ <i>recG</i> ::FRTKanFRT	[50]
JW4128	Δ <i>mutL</i> ::FRTKanFRT	[50]
MG1655	sequenced wild-type <i>E. coli</i> K-12	[51]
N2731	<i>recG258</i> ::Tn10miniKan	[44]
RDK1541	<i>recO1504</i> ::Tn5	[52]
RTC0013	Δ <i>recB</i> ::Kan	[53]
RTC0016	Δ <i>recG</i> ::Kan	[53]
SMR6319	594 <i>hsdrK⁻ mK⁺</i>	[23]
SMR8976	SMR6319 Δ <i>uvrD404</i> ::FRTcatFRT	[23]
SMR8977	SMR6319 Δ <i>uvrA402</i> ::Gen	[23]
SMR8982	SMR6319 Δ <i>mutL482</i> ::FRT	[23]
SMR8986	SMR6319 Δ <i>mutL482</i> ::FRT Δ <i>uvrA402</i> ::Gen	[23]
SMR8987	Δ <i>recF1904</i> ::FRTcatFRT	[23]
SMR8991	SMR6319 <i>recA200</i> (Ts)	[23]
SMR9801	SMR6319 <i>lexA3</i> (Ind ⁻)	[23]
SMR9805	SMR6319 <i>recAo281 lexA3</i> (Ind ⁻)	[23]
SMR9809	SMR6319 <i>recAo281</i>	[23]
SMR9811	SMR6319 Δ <i>uvrD404</i> ::FRTcatFRT <i>metE163</i> ::Tn10	[23]
SMR9812	SMR6319 Δ <i>uvrD404</i> ::FRTcatFRT Δ <i>recQ1906</i> ::FRT <i>metE163</i> ::Tn10	[23]
SMR9837	SMR6319 <i>sulA211</i>	[23]
SMR9847	SMR6319 Δ <i>recJ</i> ::FRTKanFRT	[23]
SMR9932	MG1655 <i>recG258</i> ::Tn10miniKan	MG1655 x P1(N2731)
SMR10394	SMR6319 Δ <i>xonA</i> ::FRTKanFRT	SMR6319 x P1(JW1993)
SMR10395	SMR6319 Δ <i>xonA</i> ::FRT	SMR10394 x pCP20
SMR10401	SMR6319 Δ <i>recG</i> ::Kan	SMR6319 x P1(RTC0016)
SMR10407	SMR6319 Δ <i>ruvC</i> ::FRTKanFRT	SMR6319 x P1(JW1852)
SMR10408	SMR6319 Δ <i>ruvC</i> ::FRT	SMR10407 x pCP20
SMR10411	SMR6319 Δ <i>recF1904</i> ::FRTcatFRT	SMR6319 x P1(SMR8987)
SMR10416	SMR6319 Δ <i>ruvC</i> ::FRT Δ <i>xonA</i> ::FRTKanFRT	SMR10408 x P1(JW1993)
SMR10417	SMR6319 Δ <i>ruvC</i> ::FRT Δ <i>xonA</i> ::FRT	SMR10416 x pCP20

Plasmid/Strain	Relevant genotype	Source or reference
SMR10419	SMR6319 <i>recG258::Tn10</i> miniKan	SMR6319 x P1(SMR9932)
SMR10423	SMR6319 Δ <i>recF::FRT</i>	SMR10411 x pCP20
SMR10424	SMR6319 [pML104-3]	SMR6319 x pML104-3
SMR10425	SMR6319 Δ <i>recA635::FRT</i> KanFRT	SMR6319 x P1(BW26355)
SMR10426	SMR6319 Δ <i>recA::FRT</i>	SMR10425 x pCP20
SMR10427	SMR6319 Δ <i>recA::FRT</i> [pML104-3]	SMR10426 x pML104-3
SMR10428	SMR6319 Δ <i>recA::FRT</i> Δ <i>recG::Kan</i> [pML104-3]	SMR10427 x P1(RTC0016)
SMR10434	SMR6319 Δ <i>recJ::FRT</i>	SMR9847 x pCP20
SMR10437	SMR6319 Δ <i>recF::FRT</i> <i>recG258::Tn10</i> miniKan	SMR10423 x P1(SMR9932)
SMR10438	SMR6319 Δ <i>xonA::FRT</i> <i>recG258::Tn10</i> miniKan	SMR10395 x P1(SMR9932)
SMR10439	SMR6319 Δ <i>recJ::FRT</i> <i>recG258::Tn10</i> miniKan	SMR10434 x P1(SMR9932)
SMR10440	SMR6319 <i>lexA3</i> (Ind ⁻) <i>recG258::Tn10</i> miniKan	SMR9801 x P1(SMR9932)
SMR10441	SMR6319 <i>recAo281</i> <i>lexA3</i> (Ind ⁻) <i>recG258::Tn10</i> miniKan	SMR9805 x P1(SMR9932)
SMR10442	SMR6319 <i>recAo281</i> <i>recG258::Tn10</i> miniKan	SMR9809 x P1(SMR9932)
SMR10443	SMR6319 <i>sulA211</i> <i>recG258::Tn10</i> miniKan	SMR9837 x P1(SMR9932)
SMR10678	<i>ruvA60</i> <i>rus-1</i> Δ <i>intD::FRT</i> KanFRT	TNM759 x P1(JW0525)
SMR10698	SMR6319 Δ <i>recG::Kan</i> [pML104-3]	SMR10401 x pML104-3
SMR10729	SMR6319 Δ <i>recN::FRT</i> KanFRT	SMR6319 x P1(JW2597)
SMR10730	SMR6319 Δ <i>recN::FRT</i>	SMR10729 x pCP20
SMR10734	SMR6319 Δ <i>recN::FRT</i> <i>recG258::Tn10</i> miniKan	SMR10730 x P1(SMR9932)
SMR10735	SMR6319 Δ <i>recF::FRT</i> <i>recG258::Tn10</i> miniKan Δ <i>uvrD::FRT</i> <i>cat</i> FRT	SMR10437 x P1(SMR9811)
SMR10736	SMR6319 Δ <i>xonA::FRT</i> <i>recG258::Tn10</i> miniKan Δ <i>uvrD::FRT</i> <i>cat</i> FRT	SMR10438 x P1(SMR9811)
SMR10737	SMR6319 Δ <i>recJ::FRT</i> <i>recG258::Tn10</i> miniKan Δ <i>uvrD::FRT</i> <i>cat</i> FRT	SMR10439 x P1(SMR9811)
SMR10738	SMR6319 <i>recG258::Tn10</i> miniKan Δ <i>uvrD::FRT</i> <i>cat</i> FRT Δ <i>recQ::FRT</i> <i>metE163::Tn10</i>	SMR10419 x P1(SMR9812)
SMR10739	SMR6319 <i>recA200</i> (Ts) <i>recG258::Tn10</i> miniKan	SMR8991 x P1(SMR9932)
SMR10740	SMR6319 <i>recA200</i> (Ts) <i>recG258::Tn10</i> miniKan Δ <i>uvrD::FRT</i> <i>cat</i> FRT	SMR10739 x P1(SMR9811)
SMR10741	SMR6319 Δ <i>intD::FRT</i> KanFRT	SMR6319 x P1(JW0525)
SMR10742	SMR6319 Δ <i>intD::FRT</i> KanFRT <i>rus-1</i>	SMR6319 x P1(SMR10678)
SMR10743	SMR6319 Δ <i>intD::FRT</i>	SMR10741 x pCP20
SMR10744	SMR6319 Δ <i>intD::FRT</i> <i>rus-1</i>	SMR10742 x pCP20
SMR10745	SMR6319 Δ <i>intD::FRT</i> <i>recG258::Tn10</i> miniKan	SMR10743 x P1(SMR9932)
SMR10746	SMR6319 Δ <i>intD::FRT</i> <i>rus-1</i> <i>recG258::Tn10</i> miniKan	SMR10744 x P1(SMR9932)
SMR11115	SMR6319 Δ <i>uvrA402::Gen</i> <i>recG258::Tn10</i> miniKan	SMR8977 x P1(SMR9932)
SMR11116	SMR6319 Δ <i>mutL482::FRT</i> <i>recG258::Tn10</i> miniKan	SMR8982 x P1(SMR9932)
SMR11117	SMR6319 Δ <i>mutL482::FRT</i> Δ <i>uvrA402::Gen</i> <i>recG258::Tn10</i> miniKan	SMR8986 x P1(SMR9932)
SMR11132	SMR6319 Δ <i>recG::FRT</i> KanFRT	SMR6319 x P1(JW3627)
SMR11133	SMR6319 Δ <i>recG::FRT</i>	SMR11132 x pCP20
SMR11134	SMR6319 <i>recO1504::Tn5</i>	SMR6319 x P1(RDK1541)
SMR11135	SMR6319 Δ <i>recG::FRT</i> <i>recO1504::Tn5</i>	SMR11133 x P1(RDK1541)

Plasmid/Strain	Relevant genotype	Source or reference
SMR11186	SMR6319 $\Delta recB::Kan$	SMR6319 x P1(RTC0013)
SMR11187	SMR6319 $\Delta recG::FRT \Delta recB::Kan$	SMR11133 x P1(RTC0013)
SMR11188	SMR6319 $\Delta recG::FRT$ [pML104-3]	SMR11133 x pML104-3
SMR11189	SMR6319 $\Delta recB::Kan$ [pML104-3]	SMR11186 x pML104-3
SMR11190	SMR6319 $\Delta recG::FRT \Delta recB::Kan$ [pML104-3]	SMR11187 x pML104-3
SMR11301	SMR6319 $lexA3(Ind^-) \Delta mutL::FRT KanFRT$	SMR9801 x P1(JW4128)
SMR11303	SMR6319 $recAo281 lexA3(Ind^-) \Delta mutL::FRT KanFRT$	SMR9805 x P1(JW4128)
SMR11305	SMR6319 $recAo281 \Delta mutL::FRT KanFRT$	SMR9809 x P1(JW4128)
SMR11318	SMR6319 $lexA3(Ind^-) \Delta mutL::FRT$	SMR11301 x pCP20
SMR11319	SMR6319 $recAo281 lexA3(Ind^-) \Delta mutL::FRT$	SMR11303 x pCP20
SMR11320	SMR6319 $recAo281 \Delta mutL::FRT$	SMR11305 x pCP20
SMR11323	SMR6319 $\Delta mutL::FRT KanFRT$	SMR6319 x P1(JW4128)
SMR11325	SMR6319 $lexA3(Ind^-) \Delta mutL::FRT recG258::Tn10miniKan$	SMR11318 x P1(SMR9932)
SMR11327	SMR6319 $recAo281 lexA3(Ind^-) \Delta mutL::FRT recG258::Tn10miniKan$	SMR11319 x P1(SMR9932)
SMR11329	SMR6319 $recAo281 \Delta mutL::FRT recG258::Tn10miniKan$	SMR11320 x P1(SMR9932)
TNM759	<i>rus-1 ruvA60</i>	[39]

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