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# Recombination Is Essential for Viability of an *Escherichia coli dam* (DNA Adenine Methyltransferase) Mutant

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**Double mutants of *Escherichia coli dam* (DNA adenine methyltransferase) strains with *ruvA*, *ruvB*, or *ruvC* could not be constructed, whereas *dam* derivatives with *recD*, *recF*, *recJ*, and *recR* were viable. The *ruv* gene products are required for Holliday junction translocation and resolution of recombination intermediates. A *dam recG* (Holliday junction translocation) mutant strain was isolated but at a very much lower frequency than expected. The inviability of a *dam lexA* (Ind$^-$) host was abrogated by the simultaneous presence of plasmids encoding both *recA* and *ruvAB*. This result indicates that of more than 20 SOS genes, only *recA* and *ruvAB* need to be derepressed to allow for *dam* mutant survival. The presence of *mutS* or *mutL* mutations allowed the construction of *dam lexA* (Ind$^-$) derivatives. The requirement for *recA*, *recB*, *recC*, *ruvA*, *ruvB*, *ruvC*, and possibly *recG* gene expression indicates that recombination is essential for viability of *dam* bacteria probably to repair DNA double-strand breaks. The effect of *mutS* and *mutL* mutations indicates that DNA mismatch repair is the ultimate source of most of these DNA breaks. The requirement for recombination also suggests an explanation for the sensitivity of *dam* cells to certain DNA-damaging agents.**

The *dam* gene of *Escherichia coli* encodes a DNA methyltransferase that methylates adenine in -GATC- sequences in double-stranded DNA (17). Mutant strains lacking this enzyme display a pleiotropic phenotype including increased mutability, hyperrecombination, and increased sensitivity to DNA-damaging agents. In addition, *dam* bacteria have an increased number of single-strand breaks in DNA compared to wild type. The phenotypes displayed by *dam* mutants are consistent with multiple roles of unmethylated, methylated, and hemimethylated -GATC- sequences in cellular physiology. These include regulation of gene expression and strand discrimination during replication-associated DNA mismatch repair (17).

An additional feature of *dam* strains is inviability when combined with mutant alleles of *recA*, *recB*, *recC*, or noninducible (Ind$^-$) *lexA* (19). The *lexA* inviability suggests a requirement for derepression of one or more SOS genes. The SOS response is induced following treatments that damage DNA or inhibit DNA replication (6). About 20 genes (including *recA*, *lexA*, and *ruvAB*) that are negatively regulated by LexA are derepressed following cleavage of the LexA repressor. Treatments that induce the SOS regulon do so by activating the coprotease activity of RecA ("activated RecA"), resulting in LexA cleavage. RecA protein also catalyzes 3′-single-strand invasion of homologous DNA and is, therefore, essential in the recombination process (15).

Peterson et al. (24) showed that *dam* bacteria with a temperature-sensitive *lexA* allele were viable at 42°C but not at 30°C, indicating the requirement for derepressed expression of one or more LexA-regulated SOS genes. In addition, Peterson et al. (24) found higher basal-level expression (two- to sixfold) of several SOS genes (including *recA*, *lexA*, *sulA*, *uvrA*, *uvrB*, *uvrD*, *dinD*, and *recF*) in *dam* mutants than in wild type. However, since other genes are also induced by LexA cleavage, it was not possible to determine which are required for *dam* viability.

In the present communication, the SOS genes required for viability of *dam* strains have been identified. They are *recA* and *ruvAB*, the latter encoding enzymes that translocate Holliday junctions (15, 29). Two other non-SOS genes have also been identified. The *recG* gene product can also catalyze translocation (15), and *dam recG* mutants are probably inviable. It is also shown that expression of the *ruvC* gene product, a Holliday junction resolvase (15, 29), is also required for *dam* mutant viability. The requirement for *recA*, *recB*, *recC*, *recG*, *ruvA*, *ruvB*, and *ruvC* gene expression indicates that recombination is essential for *dam* mutant viability.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** The *E. coli* recipient strains used are derived from AB1157 and are described in Table 1. Annotated descriptions of strains beginning with GM can be found at http://www.ummed.edu/pub/d/dam/dstrains.html. Hfr donors derived from KL14 begin transfer from min 69 and, therefore, transfer the closely linked *dam* and *aroK* genes (min 75) as early markers. Plasmids pGB2 (3) and pGB2*ruvAB* (26), which are derived from pSC101, are compatible with plasmids p*recA* (10) and p*recA*P67W (10), which are derivatives of pBR322.

**Conjugation.** Donors and recipients were grown to logarithmic phase ($1 \times 10^8$ to $2 \times 10^8$/ml) in Difco brain heart (BH; 20 g/liter) broth and mixed at a ratio of 1:10, respectively. After 60 min at 37°C, mating was terminated by vigorous blending and the cells were diluted and plated on BH solidified with 1.6% Difco agar and containing 100 μg of streptomycin per ml and, where necessary, 40 μg of kanamycin per ml or 10 μg of chloramphenicol per ml. When required, spectinomycin was added to 50 μg/ml, and the presence of this agent during mating did not significantly affect the yield of recombinants where donors were sensitive to it. Ampicillin was added to media at 100 μg/ml when required but was not present in the mating mixtures. Plates were incubated for 1 to 2 days at 37°C before scoring. Recombination frequencies are given as number of recombinants per 100 donors. F-*lac* transfer was measured by mating logarithmic-phase cultures at a ratio of one donor to five Lac$^-$ recipients for 60 min at 37°C and determining the percentage of Lac$^+$ recipients by plating on MacConkey (Difco) agar containing 100 μg of streptomycin/ml.

**Plasmid stability.** Cells were diluted to 100 to 200/ml in BH broth and grown to saturation ($1 \times 10^9$ to $2 \times 10^9$/ml) at 37°C in the absence of antibiotics. The cultures were diluted and plated on BH medium with and without ampicillin. A dilution containing 100 to 200 cells was used for the next cycle. This procedure was repeated several times.

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TABLE 1. *Escherichia coli* K-12 strains and plasmids used in this study

| Strain or plasmid | Relevant genotype or description | Source and/or reference |
|---|---|---|
| *E. coli* K-12 strains | | |
| AB1157 | *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 glnV44*(AS) *galK2*(Oc) *hisG4*(Oc) *rfbD1 mgl-51 rpoS396*(Am) *rpsL31*(Str[r]) *kdgK51 xylA5 mtl-1 argE3*(Oc) *thi-1* | E. A. Adelberg |
| AB1874 | F-42 (F-*lac*)/*lac-19* | E. A. Adelberg |
| AM207 | As AB1157 but *recR252*::mTn*10* | R. G. Lloyd |
| AM547 | As AB1157 but Δ*ruvAC65* | R. G. Lloyd |
| DE407 | *lexA3 mal*::Tn*9 sulA211 rpsL31* Δ(*lac-gpt*)*5 mtl-1? thi-1?* | D. Ennis (4) |
| CS81 | As AB1157 but *ruvB52 eda-51*::Tn*10* | R. G. Lloyd |
| GM698 | As KL14 but *dam-13*::Tn*9* | Lab stock |
| GM2807 | As KL14 but *dam-16*::Kan | Lab stock |
| GM2835 | As KL14 but *aroK17*::Cam | Lab stock |
| GM7362 | As DE407 but *mutL218*::Tn*10* | This work |
| GM7363 | As DE407 but *mutS215*::Tn*10* | This work |
| GS1481 | As AB1157 but Δ*ruvC64*::Kan | R. G. Lloyd |
| JC10990 | AS AB1157 but *recF332*::Tn*3* | M. Volkert |
| JC13028 | As AB1157 but *recJ147*(Ts) | S. Lovett |
| KD2250 | As AB1157 but *recQ1803*::Tn*3* | H. Nakayama |
| KL14 | Hfr *relA1 spoT1 thi-1* | K. B. Low |
| KM353 | As AB1157 but *recD1901*::Tn*10* | K. M. Murphy |
| N2057 | As AB1157 but *ruvA60*::Tn*10* | R. G. Lloyd |
| N2446 | As AB1157 but *recJ284*::Tn*10* | R. G. Lloyd |
| N3793 | As AB1157 but Δ*recG263*::Kan | R. G. Lloyd |
| Plasmids | | |
| p*recA* | A pBR322 derivative with the *recA* gene under pTac control, Amp[r] | K. Knight (10) |
| p*recA*P67W | A P67W mutant derivative of p*recA* causing coprotease constitutivity | K. Knight (10) |
| pGB2 | Cloning vector derived from pSC101, Spc[r] | B. Michel (26) |
| pGB2*ruvAB* | pGB2 with cloned *ruvAB* genes | B. Michel (26) |

## RESULTS

**Crosses with *recD*, *recF*, *recJ*, and *recR* strains.** In order to identify which SOS (and other) genes are required for survival of *dam* mutants, mutations in genes that are inviable in a *dam* strain have been sought. As *dam recA* and *dam recBC* double mutants are inviable (21), other genes affecting recombination and repair have been tested. Previous work has shown that *recF*, *umuC*, *dinA*, *dinB*, *dinC*, *recN*, *recO*, and *recQ* derivatives of *dam* mutants could be constructed and are, therefore, not required for viability (23, 24). To extend this spectrum, the results shown in Table 2 indicate that the *dam-13*::Cam allele could be efficiently introduced into *recD*, *recF*, *recR*, and *recJ* mutant recipients by conjugation. The *recF* allele used here is a null mutation in contrast to the *recF143* point mutation used previously (24). The *recD* mutation has been shown to result in a reduced level of RecBCD exonuclease V activity but is fully RecBC recombination proficient (1). The viability of the recombination-proficient *dam recD* double mutant is in contrast to the inviability of *dam recBC* bacteria that are expected to be recombination deficient.

TABLE 2. Effect of various *rec* mutations on recombination frequency in Hfr *dam-13*::Cam × F$^-$ Str[r] crosses and F-*lac* transfer[a]

| Recipient strain | Recombination frequency | F-*lac* transfer (%) |
|---|---|---|
| AB1157 (wild) | 0.45 | 18 |
| KM353 (*recD1901*::Tn*10*) | 1.4 | |
| JC10990 (*recF312*::Tn*3*) | 1.0 | |
| N3793 (Δ*recG263*::Kan) | 0.005 | |
| N2446 (*recJ284*::Tn*10*) | 0.62 | |
| KD2250 (*recQ1803*::Tn*3*) | 1.2 | |
| AM207 (*recR252*::mTn*10* | 1.0 | |
| N2057 (*ruvA60*::Tn*10*) | 0 | 15 |
| CS81 (*ruvB52*) | 0 | 12 |
| GS1481 (Δ*ruvC64*::Kan) | 0 | 9 |
| AM547 (Δ*ruvAC65*) | 0 | 12 |

[a] Conjugation and selection of Cam[r] Str[r] recombinants were carried out as described in Materials and Methods. Recombination frequency is the number of recombinants per 100 Hfr donors. This represents $1.6 \times 10^3$ Cam[r] Str[r] recombinants in 50 μl for the wild-type (AB1157) cross. Zero indicates no recombinants in 50 μl of undiluted mating mixture. The percentage of the recipient population receiving F-*lac* is indicated.

The viability of a *recJ284 dam* double mutant was unexpected, as it was previously shown that a *dam recJ77* double mutant could not be constructed (24). An additional strain, JC13028 [*recJ147*(Ts)], containing a temperature sensitivity mutation, was mated at 32°C with a *dam-16*::Kan donor, and Kan[r] Str[r] recombinants were obtained at a wild-type frequency. None of 100 recombinants tested were temperature sensitive for growth. On balance, it appears that the *recJ77* allele may be anomalous and that *dam recJ* strains are viable. For *Salmonella enterica* serovar Typhimurium, it has also been found that *dam recJ* mutants are viable (27).

**Crosses with a *recG* strain.** The recombination frequency (expressed as the number of recombinants per 100 Hfr donors) of the *dam-13*::Cam donor with the Δ*recG263*::Kan recipient was very low (Table 2). This was unexpected, because a control cross, with the same Hfr donor background but bearing an *aroK17*::Cam mutation (which is closely linked to *dam* [16]), yielded recombinants at a frequency at least 50-fold higher (Table 3). The latter result confirms the observation by Lloyd (14), who noted only about a threefold decrease in recombination frequency in crosses of a *recG* recipient with an HfrH donor. Furthermore, a high frequency of *dam-13*::Cam transfer to the Δ*recG263*::Kan recipient was expected, as the *recG*$^+$ allele (located at min 82 on the genetic map) should be transferred early by the *dam* (min 75) donor. Indeed, 49 of 50 of the

TABLE 3. Effect of various *ruv* mutations on recombination frequency in Hfr *aroK17*::Cam × F⁻ Str[r] crosses[a]

| Recipient | Recombination frequency |
|---|---|
| AB1157 | 1.03 |
| N2057 (*ruvA60*::Tn*10*) | 0.12 |
| CS81 (*ruvB52*) | 0.31 |
| GS1481 (Δ*ruvC64*::Kan) | 0.10 |
| AM547 (Δ*ruvAC65*) | 0.19 |
| N3793 (Δ*recG263*::Kan) | 0.28 |

[a] Conjugation and selection of Cam[r] Str[r] recombinants were carried out as described in Materials and Methods. Recombination frequency is the number of recombinants per 100 Hfr donors.

TABLE 4. Effect of *ruvAB* and *recA* plasmids on recombination frequency in Hfr *dam16*::Kan × F⁻ *ruvA* or *lexA* crosses and F-*lac* transfer[a]

| Recipient strain | Recombination frequency | F-*lac* transfer (%) |
|---|---|---|
| N2057 (*ruvA60*::Tn*10*) | 0 | |
| pGB2/N2057 | 0 | |
| pGB2*ruvAB*/N2057 | 0.5 | |
| DE407 (*lexA3 sfiA211*) | 0.005[b] | 34 |
| pGB2*ruvAB*/DE407 | 0.005[b] | 41 |
| p*recA*/DE407 | 0.007[b] | |
| p*recA*/pGB2*ruvAB*/DE407 | 0.4 | 42 |
| p*recA*P67W/pGB2*ruvAB*/DE407 | 0.19 | 30 |
| GM7362 (DE407 *mutS453*) | 0.1 | |
| GM7363 (DE407 *mutL451*) | 0.15 | |
| p*recA*/pGB2*ruvAB*/GM7362 | 0.5 | |
| p*recA*/pGB2*ruvAB*/GM7363 | 0.5 | |

[a] Conjugation and selection of Kan[r] Str[r] recombinants were carried out as described in Materials and Methods. Recombination frequency is the number of recombinants per 100 donors. This represents $1.04 \times 10^3$ Kan[r] Str[r] recombinants in 50 μl for the wild-type (pGB2*ruvAB*/N2057) cross. Zero indicates no recombinants in 50 μl of undiluted mating mixture. The percentage of the recipient population receiving F-*lac* is indicated.

[b] The few recombinants recovered were all determined to be *lexA*⁺ (Cam[s]) Kan[r] Str[r].

Cam[r] recombinants had the expected *dam recG*⁺ (Cam[r] Kan[s]) genotype.

To test the possibility that a chromosomal duplication encompassing either *dam* or *recG* to form a heteroallelic partial diploid occurred, two of the rare Cam[r] Kan[r] recombinants were grown for about 100 generations in the absence of antibiotics. These isolates exhibited phenotypes identical to those of the same cultures grown in the presence of antibiotics, i.e., sensitivity to 2-aminopurine (a diagnostic test for *dam*) and UV light (a *recG* phenotype) and an equal plating efficiency on media with and without chloramphenicol or kanamycin. This result makes the presence of a chromosomal duplication unlikely. The result also rules out the acquisition of a *mutS* or *mutL* suppressor mutation, since *dam* strains with such suppressors are resistant to 2-aminopurine (21).

**Crosses with *ruv* strains.** No recombinants were obtained by using a *dam* donor with any *ruv* mutant recipient when undiluted mating mixtures were placed on selective media (Table 2). This extends our previous observation that a *dam* derivative of a strain with an uncharacterized *ruv* mutation could not be constructed (24). Recipient strains with mutations in *ruvA*, *ruvB*, or *ruvC* or a deletion removing all three genes failed to yield *dam* recombinants (Table 2). The recombination deficiency in these crosses is not due to lack of genetic transfer, because F-*lac* can be transferred to the *ruv* recipients at near-wild-type frequency (Table 2). The *ruv* strains are recombination proficient when mated with an *aroK17*::Cam donor and show only a 3- to 10-fold reduction in recombination frequency compared to wild type (Table 3). This confirms previous data obtained by Lloyd (14), who noted only a three- to fourfold reduction in recombination frequency with *ruv* mutants with an HfrH donor. The large reduction in recombination frequency with the *dam* donor and *ruv* recipients suggests that these combinations are lethal. The lack of conditional *dam* or *ruv* alleles, however, prevents a direct test of the inviability of these combinations. The probable lethality is of interest because *ruvA* and *ruvB* are LexA-regulated SOS genes and could be candidates for the unknown SOS genes needed for *dam* viability.

The *ruvA60*::Tn*10* mutation in strain N2057 has a polar effect on the contiguous *ruvB* gene, and strains bearing it are RuvAB⁻. Plasmid pGB2*ruvAB* was introduced into N2057 and then mated with GM2807, a Kan[r] *dam* donor. No Kan[r] Str[r] recombinants were obtained with N2057 (*ruvA60*::Tn*10*), but the recombination frequency was increased by over 1,000-fold when pGB2*ruvAB* was present (Table 4). The plasmid, therefore, efficiently complements the *ruvA* mutation for inviability with *dam* and for sensitivity to UV light (data not shown). The level of recombination obtained with the pGB2*ruvAB*/*ruvA60*::Tn*10* strain was used as a control for the remaining crosses in Table 4.

**Crosses with a *lexA3* (Ind−) recipient.** Strain DE407, a distant derivative of AB1157, has the noninducible *lexA3* allele, thereby preventing derepression of the SOS regulon (including the *recA*, *ruvAB*, and *uvr* genes) (4). This strain is, therefore, very sensitive to UV irradiation. A *mal*::Tn*9* (Cam[r]) insertion is closely linked to the *lexA3* allele. Conjugation between DE407 (Str[r]) and the *dam* (Kan[r]) donor produced Kan[r] Str[r] recombinants at a low level (Table 4). Further examination of 100 rare recombinants indicated that they were Cam[s] and not sensitive to UV irradiation. That is, they were *lexA*⁺ due to the transfer of this gene from donor to recipient (the *lexA* gene is located at min 91). This result serves as an internal control showing that some gene transfer and recombination must have occurred. The results above show that no bona fide *dam lexA3* (Kan[r] Str[r] Cam[r]) recombinants were recovered.

The inviability of *dam ruvAB* and *dam recA* bacteria led to the hypothesis that overexpression of these SOS genes might be sufficient to allow *dam lexA3* cells to be viable. To test this idea, plasmids encoding wild-type RecA, RuvA, and RuvB were introduced into strain DE407 (*lexA3*) prior to mating with the *dam* donor. The presence of either p*recA* or pGB2*ruvAB* did not significantly alter the Kan[r] Str[r] recombination frequency compared to that with the plasmidless *lexA3* recipient (Table 4). An almost-1,000-fold increase in Kan[r] Str[r] recombinants was detected, however, when both *ruvAB* and *recA* plasmids were harbored in the *lexA3* recipient (Table 4). One hundred of these recombinants were shown to be Cam[r], indicating the presence of the *lexA3* allele. A similar recombination frequency was obtained when the coprotease constitutive (P67W) RecA-encoding plasmid was substituted for the wild-type *recA*, indicating that recombination ability is not substantially impaired by the mutation.

The differences in recombination frequency of the various plasmid-containing DE407 strains are not due to differential abilities to receive genetic material, because all strains show similar frequencies of transconjugants when mated with an F-*lac* donor (Table 4).

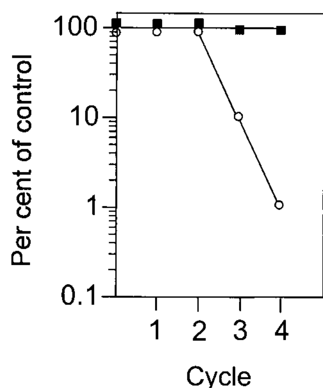


FIG. 1. Loss of the p*recA* plasmid from *dam*$^+$ and *dam* mutant *lexA3* strains. Cells were grown from 100 to 200 per ml to saturation ($1 \times 10^9$ to $2 \times 10^9$/ml) for the indicated number of cycles in the absence of ampicillin. The number of cells growing on BH medium with and without ampicillin is shown. Unfilled circles and filled squares represent the *dam*$^+$ *lexA3* and *dam* mutant *lexA3* strains, respectively.

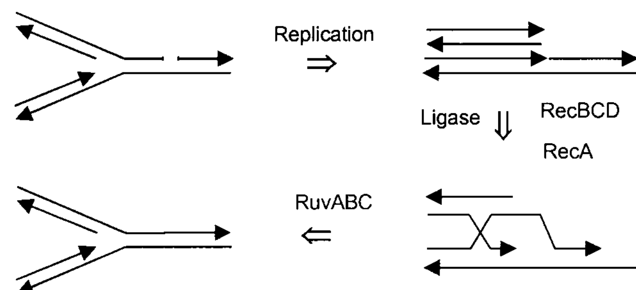


FIG. 2. Model for the generation of double-strand breaks in *dam* bacteria (8, 11). A replication fork approaches a nick releasing a chromosomal arm the end of which becomes a substrate for RecBCD action. The 3′ single strand thus produced is made to synapse by RecA to produce a Holliday junction that can be processed by RuvABC. Resolution of the Holliday junction restores the replication fork. See the work of Kuzminov (11) for further details of this model.

**Mutations affecting mismatch repair suppress *dam lexA3* lethality.** Inactivation of mismatch repair by mutation in either *mutS* or *mutL* allows *dam* derivatives of *recA*, *recB*, or *recC* cells to be constructed (21, 28). To test if a similar situation applies with *lexA3*, *mutS453* (GM7362) and *mutL451* (GM7363) derivatives of DE407 were constructed and used as recipients in matings with a *dam* donor. The results in Table 4 show that *dam lexA3* (Kan$^r$ Str$^r$) recombinants were recovered at frequencies only three- to fourfold less than those of the control. This reduction was consistent from experiment to experiment, and wild-type levels of recombinants were formed only when the *recA* and *ruvAB* plasmids were present in the mismatch repair-deficient *lexA3* recipients (Table 4). One hundred Kan$^r$ Str$^r$ recombinants from each cross above were shown to be Cam$^r$, indicating the presence of the *lexA3* allele. These data indicate that abrogation of mismatch repair removes almost all the cause(s) for *dam lexA3* lethality.

**Plasmid stability.** If the *recA* and *ruvAB* plasmids are essential for viability in a *dam lexA3* strain, then these plasmids should appear to be stable in this strain in the absence of antibiotic selection. The results in Fig. 1 indicate that indeed the *recA* plasmid is stable in the *dam lexA3* strain but much less so in the control *lexA3* parent. There was no significant loss of the *ruvAB* plasmid from either strain during these cycles of growth, presumably due to the presence of the stabilizing *par* function in pGB2 (3) that ensures efficient plasmid segregation into daughter cells.

## DISCUSSION

For a *dam* mutant to be viable, expression of the *recA*, *recB*, *recC*, *ruvA*, *ruvB*, *ruvC*, and most likely *recG* genes is essential. The level of expression from the chromosomal copies of *recB*, *recC*, *recG*, and *ruvC* is sufficient for survival of a *dam* or *dam lexA3* cell, but higher levels of the SOS-regulated RecA and RuvAB proteins are necessary. The precise amount of these proteins required for survival is not yet known, but the level of expression from the plasmids can be estimated. The copy number of the pSC101-based pGB2 vector is about five per cell, and the *ruvAB* genes bear their own promoter. A fivefold increase over the chromosomal level of RuvAB is realistic because attempts to clone the genes in pBR322-based vectors (copy number of 15 to 20) have not been successful (26). The *recA* gene is present in a pBR322 derivative transcribed from the *tac* promoter. The uninduced level of RecA is about 10-fold above that of the wild type (K. Knight, personal communication). The 5- and 10-fold overproduction of RuvAB and RecA, respectively, is similar to the derepressed level from the chromosomal genes in fully SOS-induced wild-type cells (6). Indeed, the induced level of RuvAB and RecA in an SOS-constitutive *recA441* mutant is sufficient to allow a *dam* derivative to be constructed (24).

*ruvABC recG* double mutants are deficient in conjugational recombination, whereas the single mutants are not (14). This suggests an overlap in the function of RuvABC and RecG activity. Both RecG and RuvAB bind specifically to Holliday junctions but appear to have opposing helicase directionality (15). In contrast to conjugal recombination, *dam ruvABC* mutants are inviable (Table 2), indicating that RecG cannot substitute for RuvABC. The processing of recombination intermediates for viability in *dam* cells is, therefore, different from that during conjugation and may require the divergent properties of both RecG and RuvABC. For example, in *dam* mutants perhaps both the 5′- and the 3′-single-strand overhangs that are generated by replication fork collapse (Fig. 2) can be paired with the homologous strand by RecA. To promote strand assimilation by branch migration, the Holliday junction would need to be translocated 5′ to 3′ in one case (the RuvAB polarity) and 3′ to 5′ (the RecG polarity) in the other.

An explanation for the low-level recovery of *dam recG* double mutants is that in the viable recombinants a mutated form of RuvAB can substitute for RecG. This is plausible because the recombination frequency in these experiments approaches the spontaneous mutation frequency. Another possibility is that RuvAB, but not RecG, helps to displace RecA from recombination intermediates. Finally, an unknown suppressor mutation may be present in the viable *dam recG* mutants. As noted in Results, unstable duplications seem a less likely possibility.

Mutations in the *mutS* or *mutL* genes allowed the recovery of *dam* recombinants with strain DE407 (Table 4), suggesting that mismatch repair is instrumental in causing the breaks in DNA of *dam* cells. However, the level of recombinants was lower than that of wild type and was increased to that level by the presence of the *ruvAB* and *recA* plasmids (Table 4). The inability of the *mut* mutations to completely restore the wild-type recombination frequency suggests that, in addition to mismatch repair, some other cellular function might be affecting the level of derepression of the LexA regulon. These data confirm the observation that, although *dam* cells lacking mis-

match repair have fewer nicks in DNA (28), the SOS regulon is still induced (23), suggesting some persistent inducing signal.

Why is recombination of vital importance in a *dam* bacterium? Inactivation of mismatch repair by mutation in either *mutL* or *mutS* allows the construction of *dam recA* (7, 21) and *dam recBC* (28) bacteria and decreases the level of DNA breaks (28). A complex of MutS, MutL, and MutH is required for efficient repair, and a mutation inactivating any one of these proteins results in mismatch repair deficiency (22). The requirement for recombination must be related to the presence of single-strand DNA breaks (19), which arise due to MutH endonuclease activity at unmethylated GATC sites during mismatch repair (2). A simple explanation for the recombination requirement is that occasional double-strand breaks arise due to MutH cleavage at the same unmethylated GATC sequence that contains a nick in the complementary strand (2, 7). The number of such double-strand breaks is expected to be low because their persistence in cells should be lethal. Indeed, they are detected in *dam* bacteria only in the absence of RecBCD and even then at a low frequency (28). The conclusions from the present work indicate that such double-strand break repair requires the *recA*, *recBC*, and *ruvABC* gene products. The model predicts that increasing the level of MutH should increase the frequency of double-strand breaks. The presence of a multicopy plasmid encoding *mutH*, however, does not appear to sensitize *dam* cells for viability (data not shown).

Another or an additional explanation for the role of recombination in *dam* cells involves the frequent single-strand interruptions in DNA and the model proposed by Kuzminov and Stahl (11, 12) and Horiuchi and Fujimura (8) for the collapse and repair of replication forks. Indeed, Kuzminov (11) used the phenotypic properties of *dam* incompatibility with *recA* and *lexA* as a foundation for his model (Fig. 2). When a replication fork encounters a single-strand nick in the DNA of *dam* cells, one of the chromosomal arms dislocates from the chromosome due to the formation of a double-strand break. To reestablish a functional replication fork, the wayward chromosomal arm needs to be recombined back into the chromosome. This requires processing by RecBCD to produce a 3′-invasive strand after encountering a Chi site and the action of RecA to make this strand synapse with its homologue. After formation and translocation of a Holliday junction, resolution by RuvC restores the replication fork (Fig. 2). As multiple replication forks are present in growing bacteria, replication fork collapse is expected to occur frequently, requiring increased recombination capacity. This explains the high subinduced level of SOS genes in *dam* bacteria and the requirement for elevated RecA and RuvAB levels. This model (11) also explains the inviability of *dam* mutants with mutations in either the *polA* or *lig* genes (20) due to the production of excess double-strand breaks as well as the hyperrecombination phenotype of *dam* mutants (18). A further prediction based on this model is that *dam priA* (primosome) mutants should be inviable because replication restart at sites of collapsed replication forks requires reassembly of the primosome complex (13). This prediction is currently being tested.

Seigneur et al. (26) have proposed a model to explain how replication fork arrest in *E. coli rep* mutants, which lack a replicative helicase, leads to formation of double-strand breaks. (Replication fork arrest should be distinguished from replication fork collapse in the model discussed above.) Briefly, the RuvABC proteins are responsible for the formation of double-strand breaks, and the substrate is thought to be a cruciform (Holliday junction) formed by the annealing of the two new DNA daughter strands (Fig. 3). If RecBCD acts on the double-stranded end of the annealed strands before RuvAB, then the breaks are prevented either through the initiation of RecA-RuvABC-dependent recombination or by a recombination-independent resection of the annealed duplex (26). This model is probably not applicable to *dam* mutants because the *rep* and *dam* mutants have different phenotypes (17, 26), a key one being that *rep recA* double mutants are viable while *dam recA* mutants are not. The *repA recA* mutant is viable because of the recombination-independent action of RecBCD referred to above. Although this model may not be applicable during normal growth of *dam* mutants, it may be important when the replication fork is arrested at drug-induced mismatches (see below).

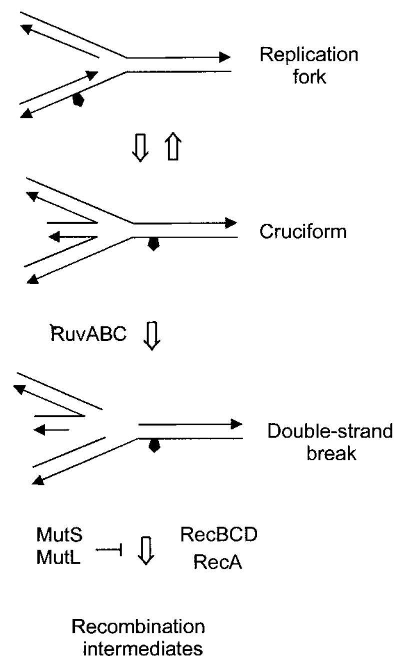


FIG. 3. Origin of double-strand breaks in DNA with drug-induced lesions. DNA containing a lesion (pentagon) such as $O^6$meG is replicated, but the polymerase has stalled while mismatch repair is attempted. Stalling can also occur at any polymerase-blocking lesion. The new DNA strands anneal to form a cruciform structure that can be acted upon by RuvABC, producing a double-strand break (only one of the two possible double-strand break configurations is shown). Alternatively, RecBCD and RecA can act on the tail of the newly synthesized annealed strands of the complete or broken cruciform to form a recombination intermediate. See the work of Seigneur et al. (26) for further details on the formation and processing of cruciform structures.

The recombination requirement for *dam* mutant survival may also explain the increased sensitivity of this strain to DNA damage provoked by alkylating agents (9) and cisplatin (5). DNA damage inflicted by these agents would increase the requirement for repair-associated recombination. Recombination proteins would become limiting, and drug-induced gaps or chromosome breaks would not be repaired, eventually leading to cell death. The importance of recombination pathways in the repair of cisplatin damage has recently been demonstrated (31).

DNA mismatch repair sensitizes *dam* cells to the cytotoxic action of alkylating agents, specifically those that produce $O^6$-methylguanine ($O^6$meG) (9). It was proposed previously (9) that $O^6$meG paired with either C or T is a substrate for mismatch repair recognition. That is, all possible $O^6$meG base pairs are subject to mismatch repair. The specific binding of *E. coli* MutS to $O^6$meG base pairs has been reported previously (25). Consequently, upon replication of the $O^6$meG-containing

strand a futile cycle of mismatch repair ensues. As the replicative polymerase, PolIII, synthesizes mismatch repair tracts (22), this event would cause polymerase stalling. The requirement for recombination in *dam* mutants reported here suggests an additional action at $O^6$meG lesions. The blocked fork could lead to annealing of the newly synthesized strands to produce a cruciform structure (Fig. 3) and production of a chromosome double-strand break by RuvABC as proposed by Seigneur et al. (26). Alternatively, recombination initiated by RecBCD at the tail of the annealed new strands could lead to restoration of the replication fork as described by Seigneur et al. (26). The binding of MutS and MutL to $O^6$meG base pairs in the cruciform structure or in subsequent recombination intermediates (Fig. 3) might effectively abort recombination in a manner similar to that described for base mismatches in phage fd-M13 heteroduplexes (30). This antirecombinogenic action of MutS and MutL would return the DNA to a cruciform configuration where RuvABC could cleave it. In the absence of mismatch repair, the replication fork would not be arrested at $O^6$meG mismatches and the cells would have a greater chance for survival. Experiments are in progress to test the predictions of this model.

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