A Sister-Strand Exchange Mechanism For *recA*-Independent Deletion of Repeated DNA Sequences in *Escherichia coli*

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ABSTRACT

In the genomes of many organisms, deletions arise between tandemly repeated DNA sequences of lengths ranging from several kilobases to only a few nucleotides. Using a plasmid-based assay for deletion of a 787-bp tandem repeat, we have found that a *recA*-independent mechanism contributes substantially to the deletion process of even this large region of homology. No *Escherichia coli* recombination gene tested, including *recA*, had greater than a fivefold effect on deletion rates. The *recA*-independence of deletion formation is also observed with constructions present on the chromosome. RecA promotes synapsis and transfer of homologous DNA strands *in vitro* and is indispensable for intermolecular recombination events *in vivo* measured after conjugation. Because deletion formation in *E. coli* shows little or no dependence on *recA*, it has been assumed that homologous recombination contributes little to the deletion process. However, we have found *recA*-independent deletion products suggestive of reciprocal crossovers when branch migration in the cell is inhibited by a *ruvA* mutation. We propose a model for *recA*-independent crossovers between replicating sister strands, which can also explain deletion or amplification of repeated sequences. We suggest that this process may be initiated as post-replicational DNA repair; subsequent strand misalignment at repeated sequences leads to genetic rearrangements.

TANDEMLY repeated sequences are vulnerable to genetic rearrangements leading to deletion or duplication of the repeat and any intervening sequence. Deletions and duplications can also arise between very short homologies of only a few nucleotides in length (ALLGOOD and SILHAVY 1988; MEUTH 1989; EHRLICH 1989). The molecular mechanism(s) of these rearrangements is not clear. Models to explain deletions fall into two main types: recombinational or replicational. Normal homologous recombination pathways may operate on these repeated sequences, leading perhaps to unequal crossing-over on two DNA molecules (Figure 1A). Alternatively, slip-pair models (Figure 1B) presume that strands realign at tandem repeats during replication. These models predict different products. If reciprocal, unequal crossing-over between duplicated sequences yields both deletion and triplication products. If crossovers occur between circular molecules, circular dimers result. By replicational slip-pair, only one strand realigns to yield a deletion product and the other retains the parental duplication. Formation of associated triplications or dimers is not predicted by this mechanism.

We have designed a plasmid-based assay to investigate deletion of tandem repeats of approximately 800 bp in *Escherichia coli*. We have examined the genetic dependence of this process as well as the products formed from such events. Our results confirm previous reports of *recA*-independent deletion of such repeats (MATFIELD, BADAWI and BRAMMAR 1985; DIANOV *et al.* 1991). This *recA*-independent deletion process occurs at high frequency, contrary to the notion that *recA*-independent pathways are intrinsically inefficient. We show that *recA*-independence of deletion is not unique to plasmid-based systems but is also seen for a repeat carried on the *E. coli* chromosome. Most surprising, we have found seemingly reciprocal products associated with *recA*-independent deletion. This supports a recombinational mechanism for at least a large subset of deletion events and we propose a new mechanism for this type of genetic rearrangement.

MATERIALS AND METHODS

Bacterial strains and media: The strains used in this study are derived from AB1157 and listed in Table 1. Strains were grown routinely in LB medium (WILLETTS, CLARK and LOW 1969), with plate media containing 1.5% agar. Plate minimal medium consisted of 56/2 salts (WILLETTS, CLARK and LOW 1969) supplemented with 0.2% glucose, 1 μg/ml thiamine and 50 μg/ml of the appropriate required amino acids. Tetracycline (Tc) and kanamycin (Km) were used at concentrations of 15 and 30 μg/ml, respectively. Selection for plasmids conferring ampicillin (Ap) resistance employed media containing 100 μg/ml ampicillin; chromosomal constructs were selected on media containing 30 μg/ml Ap. Selection for plasmids conferring chloramphenicol (Cm) resistance used 30 μg/ml Cm. P1

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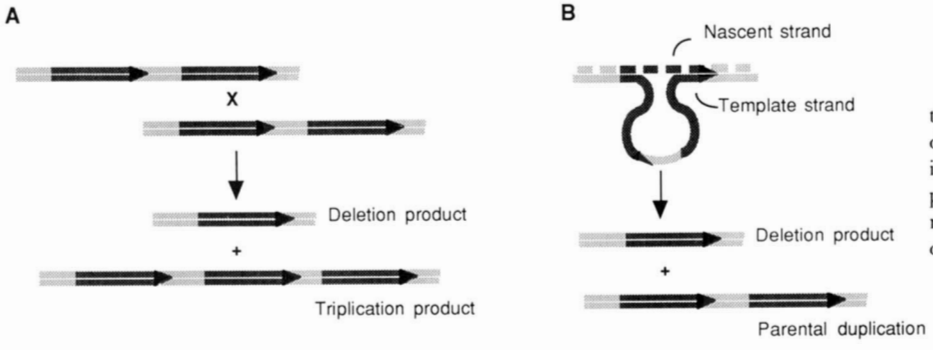

FIGURE 1.—Models for deletion formation. (A) Unequal crossing-over model for deletion formation. If reciprocal, products include deletion and triplication. (B) Slip-pair model for deletion formation. After replication, a deletion and parental duplication result.

TABLE 1

***E. coli* K-12 strains used in this study**

Strain	Genotype	Derivation
A. AB1157 and various isogenic *rec* derivatives used in deletion assays with pSTL55		
AB1157	*rec*$^+$	BACHMANN (1987)
AM207	*recR254*::Tn*10-9*	MAHDI and LLOYD (1989)
CS140	*ruvC53*	R. LLOYD
DM49	*lexA3*	MOUNT, LOW and EDMISTON (1972)
JC5519	*recB21 recC22*	WILLETTS, CLARK and LOW (1969)
JC9239	*recF143*	HORII and CLARK (1973)
JC10287	(*srlR-recA*)*Δ304*	CSONKA and CLARK (1979)
N2096	*ruvAΔ63*	R. LLOYD
N2673	*sbcC201*	D. LEACH
N2731	*recG258*::Tn*10-9*	R. LLOYD
RDK1309	*ssb-113*	KOLODNER, FISHEL and HOWARD (1985)
RDK1541	*recO1504*::Tn*5*	KOLODNER, FISHEL and HOWARD (1985)
RDK1792	*recD1013*	LOVETT, LUISI-DELUCA and KOLODNER (1988)
RDK1899	*sbcB15*	R. KOLODNER
RDK1900	*recQ1*	LOVETT, LUISI-DELUCA and KOLODNER (1988)
SP254	*recN262*	PICKSLEY, ATTFIELD and LLOYD (1984)
STL114	*recJ2003*::Tn*10-9*	This study[a]
STL1144	*ruvAΔ63* (*srlR-recA*)*Δ304*	This study[b]
B. Chromosomal $tetA_{dup}$ constructs and intermediates		
RGS1015	λΩ*lacZ4091*::(*bla305 tetA*$^+$) *cI857 nin5 S100*[c]	KAZIC and BERG (1990)
STL672	*cysC95*::Tn*10*	This study[d]
STL695	*lacZ*::*bla*$^+$ $tetA_{dup}$	This study[d]
STL700	*cysC95*::Tn*10 lacZ*::*bla*$^+$ $tetA_{dup}$	This study[d]
STL753	(*srlR-recA*)*Δ304 lacZ*::*bla*$^+$ $tetA_{dup}$	This study[d]

Except for RGS1015, all strains are derived from AB1157 and carry the additional genotype, unless otherwise indicated, F-*thi-1 hisG4* Δ(*gpt-proA*)*62 argE3 thr-1 leuB6 kdgK51 rfbD1 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rpsL31 rac*$^-$λ$^-$.

[a] Ser$^+$ Km^r Tc^s transductant of RDK1445 (*serA zgb*::Tn*10* derivative of AB1157 provided by R. KOLODNER) with P1 lysate grown on RDK2144 (*recJ2003*::Tn*10*-9 *recB21 recC22 sbcA23* derivative of AB1157). RDK2144 is a recombinant of JC8679 (*recB21 recC22 sbcA23* derivative of AB1157; GILLEN, WILLIS and CLARK 1981) with transformed plasmid pRDK163 cleaved with *Pst*I, *Sal*I and *Eco*RI. pRDK163 is a Tn*10*-9 insertion mutant of plasmid pJC763 (LOVETT and CLARK 1985) isolated as in WAY *et al.* 1988 with λNK1105 provided from N. KLECKNER.

[b] CAG12173 (*cysC95*::Tn*10*, SINGER *et al.* 1989, provided by C. GROSS) was used to transduce recipient N2096, selecting Tc^r Cys$^-$. The resulting strain, STL 1132, was transduced from P1 from JC10287, selecting Cys$^+$ Tc^s Rec$^-$.

[c] This strain also carries chromosomal markers Δ*trpE trpR tna hflA1 supE*$^+$ (?)

[d] Described in MATERIALS AND METHODS.

phage were grown in plate lysates on LCTG medium, LB plates containing 0.2% glucose, 50 μg/ml thymine, 2 mM $CaCl_2$ and 1.2% agar (top agar contained only 0.7% agar) and transductions were performed as in WILLETTS, CLARK and LOW 1969. Plate tests for UV-resistance and recombination-proficiency (CLARK and MARGULIES 1965) were used to score *rec* markers. The construction of the *recJ2003*::Tn*10*-9 insertion on the *E. coli* chromosome was confirmed by Southern blot analysis (AUSUBEL *et al.* 1989) using digoxygenin-labeled pJC763 probe (labeling kit purchased from Boehringer Mannheim).

Plasmids: Deletion assay plasmid pSTL55 was constructed by ligation of *Alw*NI *Nru*I and *Alw*NI *Eco*RV digests of pBR322. This duplicates a 787-bp internal segment of the *tetA* gene, inactivating this gene. pSTL55 confers Ap^r. pSTL56, conferring Cm^r and carrying a *tetA* duplication identical to pSTL55, was constructed similarly by ligation of pACYC184 DNA digested with *Alw*NI EcoRV and *Alw*NI *Nru*I. Synthetic heterodimer pSTL59 was made by ligation of *Hin*dIII digests of pBR322 and pSTL56 and selection for Ap^r Cm^r in subsequent transformation into JC10287. Plasmid DNA was purified by the alkaline-SDS method (BIRNBOIM and DOLY 1979 as modified by AUSUBEL *et al.* 1989). We used the TSS method (CHUNG, NIEMELA and

MILLER 1989) or electroporation (DOWER, MILLER and RAGSDALE 1988) for introducing plasmid DNA into strains. Plasmid DNA was digested with restriction endonucleases and subjected to electrophoresis on 0.8% agarose gels in Tris-acetate EDTA buffer according to standard techniques (AUSUBEL *et al.* 1989).

Chromosomal *tetA* duplication constructions: The *bla*$^+$ and *tetA*$_{dup}$ region of pSTL55 were transferred to the *E. coli* chromosome using a technique and strain provided by D. BERG (KAZIC and BERG 1990). By genetic recombination, the *bla305* and *tetA*$^+$ alleles present on a λΩ*lac*::*bla305 tetA*$^+$ transducing phage in strain RGS1015 were replaced by *bla*$^+$ *tetA*$_{dup}$ sequences on pSTL55. Strain RGS1015 (Ap^s Tc^r λ$^+$) was transformed with pSTL55 selecting Ap^r. This *cI857 S100* λ lysogen was induced by incubation at 42° and a lysate prepared by chloroform treatment (ARBER *et al.* 1983). This lysate was used to infect AB1157 at 30° and Ap^r Tc^s lysogens were selected. The next step was to move the construction from the phage to the *lac* locus by recombination with the *lac* sequences flanking the *bla*$^+$ *tetA*$_{dup}$ region. Ap^r survivors at 42° were selected from the lysogenic strain and screened for lack of λ immunity and conversion to *lacZ*$^-$ using X-gal IPTG plates (MILLER 1992). The resulting *lacZ*::*bla*$^+$ *tetA*$_{dup}$ strain is STL695.

A *recA*Δ derivative carrying the chromosomal *lacZ*::*bla*$^+$ *tetA*$_{dup}$ was constructed in three steps. A *cysC95*::Tn10 derivative of AB1157, STL672, was constructed by transduction with P1 grown on CAG12173 (*cysC95*::Tn*10*, SINGER *et al.* 1989, provided by C. GROSS). Second, the *lacZ*::*bla*$^+$ *tetA*$_{dup}$ allele was moved into STL672 by P1 transduction from STL695, selecting Ap^r and resulting in strain STL700. The (*srlR-recA*)Δ*304* was then introduced into STL700 by P1 transduction from JC10287, selecting Cys$^+$ UV^s. The resulting (*srlR-recA*)Δ*304 lacZ*::*bla*$^+$ *tetA*$_{dup}$ strain is STL753.

Deletion assays: Deletion frequencies were measured by determining the number of Tc^r Ap^r cells in the Ap^r population. Plasmid-borne deletions were assayed by transforming pSTL55 into strain AB1157 and its derivatives (listed in Table 1) which carry additional mutations affecting recombination. All incubations were carried out at 37° except for the *ssb-113* strain, RDK1309, which was grown and assayed at its permissive temperature for growth, 30°. Transformants were streaked on LB + Ap plates to obtain single colonies. This period of growth ensures that subsequent assays of clonal isolates do not represent recombinants preexisting in the transforming DNA preparation or induced by the transformation treatment. (Such recombinants would give a fully Tc-resistant colony and would be excluded from the analysis.) Entire fresh overnight colonies were then resuspended and grown in 1 ml LB+Ap broth for 2 hr. Serial dilutions in 56/2 buffer (WILLETTS, CLARK and LOW 1969) were spotted on LB+Ap and LB+Ap+Tc plates and incubated overnight at 37°. The number of Tc-selected progeny was found to be linear with dilution. Tc^r deletion progeny could also be recovered nonselectively at frequencies similar to that with selection, confirming our expectation that deletion progeny arise preselection and are not induced by selective treatment. Although we took care to examine growing cultures, no difference in deletion frequency was observed with stationary-phase cultures. Multiple independent cultures were assayed for deletion frequency and the number of such cultures is reported in the Tables. Median values do not differ substantially from the mean. Each strain was assayed on 2 or more days, giving similar values.

Deletion rates were calculated by two methods: the method of the mean (LURIA and DELBRÜCK 1943) and the method of the median (LEA and COULSON 1949). Each method has its pitfall (see KENDAL and FROST 1988; STEWART, GORDON and LEVIN 1990): the mean method may overestimate rates (LURIA and DELBRÜCK 1943) and the median method will underestimate rates in certain cases because of large variation in population sizes seen for several of the *rec* mutant strains. Therefore, we have chosen to report values derived from both methods as well as the mean deletion frequencies.

Deletion of chromosome-borne *tetA*$_{dup}$ in strains STL695 and STL753 were preformed as in the plasmid-bearing strains above, except that Ap was used at 30 μg/ml.

Analysis of plasmid deletion products: Entire Tc^r colonies selected from the appropriate strains were grown overnight in LB + Ap. Each colony was selected from an independent clone and therefore represents an independent deletion event. Plasmid DNA was purified, digested and subjected to agarose gel electrophoresis as described above. Electrophoresis of uncut DNA allowed the determination of the dimeric or monomeric plasmid forms. In some cases, extensive nicking of dimeric plasmid forms and electrophoresis with λ *Hin*dIII linear size standards was performed to confirm the dimeric nature of these plasmids, although these data are not shown. Digestion with *Hin*dIII, which cleaves once in pSTL55, was used to determine the nature of pSTL55-derived products: deleted, duplicated or triplicated at *tetA*. All Tc-selected deletion progeny carry a pBR322-sized fragment, indicating that deletion to generate a functional *tet* gene occurred at homologous sequences only. For some triplication products, additional *Eco*RV *Nru*I digests (these sites flank the triplication) confirmed our interpretation but these data are not shown. Only a representative sample of the data is shown in the figures although an identical analysis was performed for all products cited. pSTL55 (nondeleted) plasmid DNA was also isolated and characterized as above from JC10287 and STL1144 strains to ensure that neither plasmid dimerization nor *tet* allele triplication was apparent in the Ap^r Tc^s population.

RESULTS

Deletion frequencies of a plasmid-borne 787 bp repeat in various *rec* mutants: We created a deletion assay plasmid by duplicating a 787 bp internal segment of *tetA* on plasmid pBR322 (Figure 2). Deletion of the repeat in this plasmid, pSTL55, restores tetracycline (Tc) -resistance to the cell. This plasmid was introduced into various mutant derivatives of AB1157 known to have effects on genetic recombination and deletion rates were determined (Table 2). Mean deletion rate was less than 4-fold reduced in *recA*Δ strains relative to *rec*$^+$ cells, indicating that a *recA*-independent deletion pathway contributes substantially to the yield of deletion products.

Mutations in *recF*, *recR* and *recO*, like those in *recA*, also reduced deletion rates 2–5-fold. This group of genes also exhibit effects on a plasmid recombination assay (pRDK41) which detects deletions of 4 kb homologies among other "gene-conversion" events (KOLODNER, FISHEL and HOWARD 1985; MAHDI and LLOYD 1989).The 20–50-fold reduction in recombinant frequencies shown by this class of mutants with the pRDK41 assay is greater than the 2–4-fold reduction that we detected. The other mutants examined

TABLE 2

Relative deletion frequencies and rates of pSTL55 in various isogenic *rec* mutants

Genotype[a]	No. of cultures assayed	Relative deletion frequency[b]	Relative deletion rate–mean[c]	Relative deletion rate–median[d]
A. *rec*$^+$	24	=1 (3.7×10^{-3})	=1 (2.6×10^{-4})	=1 (2.0×10^{-4})
recAΔ304	41	0.31	0.35	0.28
recB21 recC22	24	1.0	1.0	1.0
recD1013	12	3.2	3.2	4.3
recF143	31	0.46	0.50	0.19
recG258	14	2.0	2.0	1.9
recJ2003	13	1.3	1.3	0.72
recN262	8	0.55	0.62	0.45
recO1504	32	0.24	0.26	0.18
recQ1	16	0.39	0.42	0.28
recR254	32	0.26	0.28	0.27
ruvAΔ63	14	0.57	0.62	0.59
ruvC53	13	0.59	0.65	0.58
sbcB15	10	1.1	1.2	1.3
sbcC201	16	0.82	0.88	0.79
lexA3	23	0.83	0.85	0.58
ruvAΔ63 recAΔ304	8	0.15	0.19	0.25
B. *rec*$^+$ (30°)	8	=1 (2.0×10^{-3})	=1 (1.7×10^{-4})	=1 (8.9×10^{-5})
ssb-113 (30°)	14	3.7	3.4	4.4

[a] All strains are derivatives of AB1157 and described in Table 1. The strains in Part A were assayed at 37°. The strains in Part B, the *ssb-113* strain and *rec*$^+$ control, were assayed at 30°, the permissive temperature for this *ts* allele.

[b] The mean deletion frequency relative to that of the *rec*$^+$ control, AB1157. Deletion frequencies were determined by the number of Tc^r cells in the Ap^r population as described in MATERIALS AND METHODS and the mean deletion frequency was determined for the number of independent assays indicated.

[c] The deletion rate relative to that of the *rec*$^+$ control calculated using the method of the mean (LURIA and DELBRÜCK 1943).

[d] The deletion rate relative to that of the *rec*$^+$ control calculated using the method of the median (LEA and COULSON 1949).

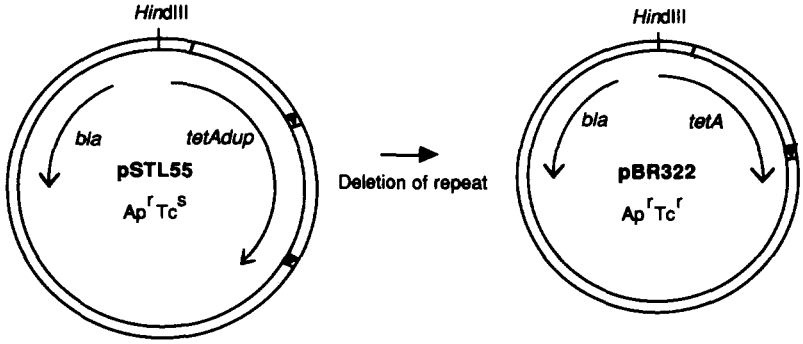

FIGURE 2.—Diagram of plasmid to detect tandem repeat deletion. pSTL55 carries an internal 787-bp duplication of the *tetA* gene, corresponding to the region between the unique *Eco*RV and *Nru*I restrictions sites of pBR322. pSTL55 is 5148 bp in length; pBR322 is 4361 bp.

showed slightly reduced or increased deletion frequencies. A mutation in *recJ*, which causes a very strong 4,000-fold reduction in the pRDK41 assay (KOLODNER, FISHEL and HOWARD 1985), had little effect on deletion assayed in pSTL55. We note, however, that different alleles of *recJ* were used in these experiments. Mutants in *recD*, *recG* and *ssb* exhibited consistently higher levels of deletion in this assay. A mutant in exonuclease I (*sbcB15*) showed very little elevation of deletion rate; mutants in exonuclease I have previously been found to show increased frequencies of deletion at very short homologies (ALLGOOD and SILHAVY 1991).

Deletion of the same 787 bp repeat on the *E. coli* chromosome: Our previous results with a plasmid-borne repeat are consistent with previous reports of only weak *recA*-dependence of deletions of similar-sized tandem homologies (MATFIELD, BADAWI and BRAMMAR 1985; DIANOV *et al.* 1991). However, the deletion of such repeats has been examined only in plasmid-based assays. Of some concern is whether the *recA*-independent pathway is restricted to plasmid replicons. To address this question, we moved the *bla* and tet_{dup} alleles (but not the origin of replication) from plasmid pSTL55 to a site within the *E. coli lac* gene. Deletion rates of this tandem repeat were determined similarly for isogenic *rec*$^+$ (STL695) and *recAΔ* (STL753) strains (Table 3). The rate of deletion, when carried on the *E. coli* chromosome, is about 2 orders of magnitude lower than that determined for the comparable pSTL55 plasmid-bearing strains. Some of this decrease may be accounted for by the lower copy number of the chromosome relative to pBR322-derived plasmids. However, other factors

TABLE 3

Deletion rates of the 787 bp *tetA* duplication placed on the *E. coli* chromosome

Strain[a]	*rec* genotype	No. of independent cultures assayed	Deletion rate (method of mean)[b]	Deletion rate (method of median)[b]
STL695	*rec*$^+$	22	2.5×10^{-6}	2.1×10^{-6}
STL753	*recA*Δ	22	1.9×10^{-6}	7.1×10^{-7}

[a] These AB1157-derived strains carry the 787-bp tandem repeat of *tetA* inserted at the *E. coli lac* locus and are described in Table 1 and MATERIALS AND METHODS.

[b] Deletion rates were calculated by the method of the mean (LURIA and DELBRÜCK 1943) or by the method of the median (LEA and COULSON 1949). These rates were derived from deletion frequencies determined by the number of Tc^r cells in the Ap^r population as described in MATERIALS AND METHODS for the number of independent assays indicated. The rates reported correspond to a mean deletion frequency of 2.6×10^{-5} for STL695 and 1.8×10^{-5} for STL753.

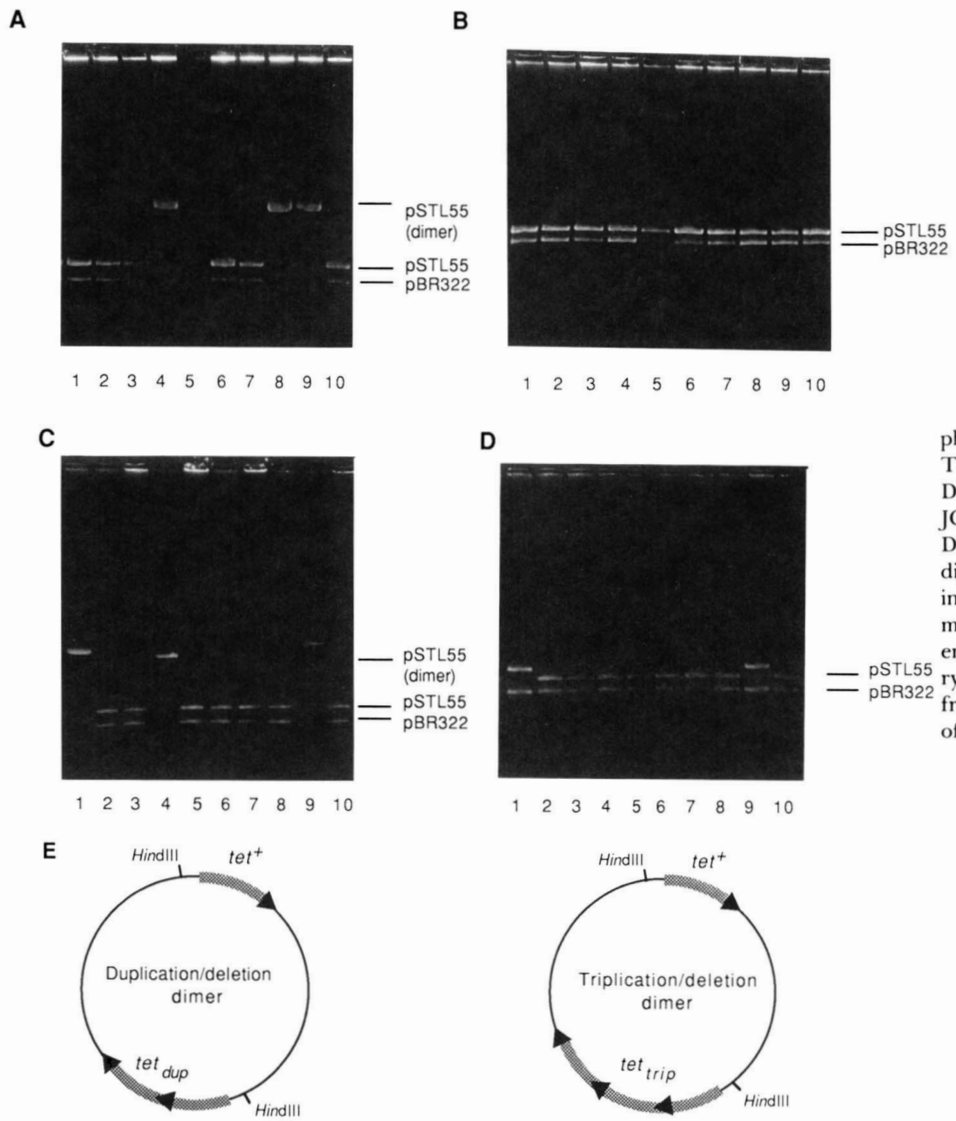

FIGURE 3.—Sample agarose gel electrophoresis of plasmid isolates from selected Tc^r deletion progeny. (A) Uncut plasmid DNA from Tc^r deletion progeny of JC10287 carrying pSTL55. (B) Plasmid DNA preparations from A subjected to digestion with *Hin*dIII, which cleaves once in pBR322 and pSTL55. (C) Uncut plasmid DNA isolated from Tc^r deletion progeny of *ruvA*Δ *recA*Δ strain (STL1144) carrying pSTL55. (D) Plasmid preparations from C digested with *Hin*dIII. (E) Diagram of plasmid dimer types.

such as context or additional selection stringency could also contribute to this difference. Nonetheless, deletion rates of the *tetA* tandem repeat were less than three-fold reduced by a mutation in *recA*.

Analysis of plasmid deletion products in *recA*$^-$ strains: plasmid dimerization accompanies deletion: To gain more insight into the *recA*-independent mechanism for deletion, we examined the Tc-selected deletion products of plasmid pSTL55 in *recA*Δ strains. Agarose gel electrophoresis of these plasmid products (Figure 3A) showed that plasmid dimerization often accompanied the deletion event, as would be predicted by a crossing-over mechanism. 27% (20/75) of the Tc^r deletion progeny from *recA*Δ/pSTL55 isolates carried plasmid dimers (Table 4). That plasmid dimerization is associated with the deletion process is

TABLE 4

Summary of deletion product analysis[a]

Strain (genotype)	Deletion assay plasmid	Plasmid dimers among Tc^r deletion progeny	Triplication/deletion types among Tc^r dimers
JC10287 (*recA*Δ)	pSTL55	20/75	0/20
STL1144(*recA*Δ *ruvA*Δ)	pSTL55	18/80	11/18
JC10287 (*recA*Δ)	pSTL55 pSTL56	29/109	2/29

[a] Deletions were selected and DNA from Tc-resistant deletion isolates was purified and analyzed as described in MATERIALS AND METHODS and shown in Figures 3 and 4. Dimer types are also illustrated in Figure 3. Those dimers that are not of the triplication/deletion type were determined to be of the duplication/deletion type. For the assays with both pSTL55 and pSTL56 co-resident, it was not determined whether identified homodimers were of pSTL55 or pSTL56.

supported by the following observations. First, electrophoretic analysis of *recA*Δ/pSTL55 strains which have not been selected for the deletion suggest that plasmid dimers comprise <5% of the nondeleted population (data not shown). Second, dimers of pSTL55 do not exhibit higher deletion rates when transformed into *recA*Δ strains (data not shown), ruling out the possibility that rare dimers formed are enriched for deletion potential. None of these 20 Tc-selected dimers carried a triplication; all carried one deleted *tet*$^+$ and one parental tet_{dup} allele (Figure 3B, Table 4). Although dimerization may be indicative of a recombinational mechanism for deletions, these dimer products are not characteristic of a reciprocal unequal crossing-over genetic exchange. Plasmid dimerization associated with deletion has been also noted by others (YI, STEARNS and DEMPLE 1988; DIANOV *et al.* 1991; MAZIN. *et al.* 1991).

Reciprocal crossover dimer products revealed in deletion products of *ruvA recA* mutants: In considering a model for unequal exchange that might occur in the absence of RecA function (described in the DISCUSSION below), we reasoned that realignment of a branched intermediate formed between unequally paired chromosomes could destroy the potential for reciprocal products. We therefore examined the products of *recA*-independent deletion of pSTL55 in *ruvA* mutants deficient for a protein that facilitates branch migration. As predicted, the triplication/deletion dimer products were more commonly found in a *ruvA*Δ *recA*Δ mutant strain (Table 4, Figure 3C). Deletion rates in this strain were not grossly changed (Table 2) and 22% (18/80) of the deletion products were plasmid dimers (Table 4). 11 of these 18 dimers carried one *tet*$^+$ and one tet_{trip}; the remainder carried the *tet*$^+$/tet_{dup} alleles (Table 4, Figure 3D). This proportion of dimers carrying triplications in the *ruvA*Δ *recA*Δ mutant strain is significantly higher than that observed in the *recA*Δ strain (by a contingency chi-square test, $P < 0.01$). Electrophoretic analysis of pSTL55 plasmid DNA purified from this *recA*Δ *ruvA*Δ strain allowed us to estimate that <10% of plasmids carry a triplication when the deletion event is not selected. Therefore, the high proportion of triplication alleles in the dimer plasmids is presumed to result from the production of the triplication concomitant with deletion formation.

Such triplication/deletion dimers are diagnostic of a reciprocal unequal crossing-over event (Figure 1A) between two circular plasmid molecules resulting from conservative break-and-join recombination. Unequal crossing-over between the sister strands of a replicating circular molecule also predicts such triplication/deletion dimeric products. Such a product cannot be explained by simple realignment of one strand relative to its complement as proposed by a replicative slip-pair model (Figure 1B). Because RuvA, together with the RuvB protein, promotes branch migration of DNA (IWASAKI *et al.* 1992, TSANEVA, MÜLLER and WEST 1992) and acts as a DNA helicase (TSANEVA, MÜLLER and WEST 1993), we propose that the RuvAB proteins process intermediates that would otherwise lead to reciprocally recombinant dimer products. The uncovering of these reciprocal products suggests that a truly recombinational mechanism can account for at least 20% of *recA*-independent deletion events.

***recA*-independent dimerization associated with deletion is an intramolecular recombination event**: To determine whether dimerization is an intermolecular or intramolecular recombination event, we constructed an additional *tetA* duplication plasmid, pSTL56, from pACYC184, conferring chloramphenicol-resistance (Figure 4A). pBR322 and pACYC184 are compatible plasmids and have unique *Eco*RI restriction sites at different distances relative their common *tetA* gene. Therefore, any heterodimers formed between the two tet_{dup} plasmids could be identified by asymmetric *Eco*RI cleavage. Plasmid DNA analysis of Tc^r deletion progeny of *recA*Δ/pSTL55/pSTL56 strains showed that 27% of the deletion products (29/109) are plasmid dimers (Table 4, Figure 4B). *Eco*RI-cleavage of these dimers showed that all 29 dimers were homodimers of one or the other plasmid replicon (Figure 4C). Of these 29 dimers, two carry a triplication associated with the deletion (Table 4); the remaining 27 are of the duplication/deletion type seen previously. *Hin*dIII *Sca*I digests of a subset of dimer isolates indicated that deletion of the pACYC184 plas-

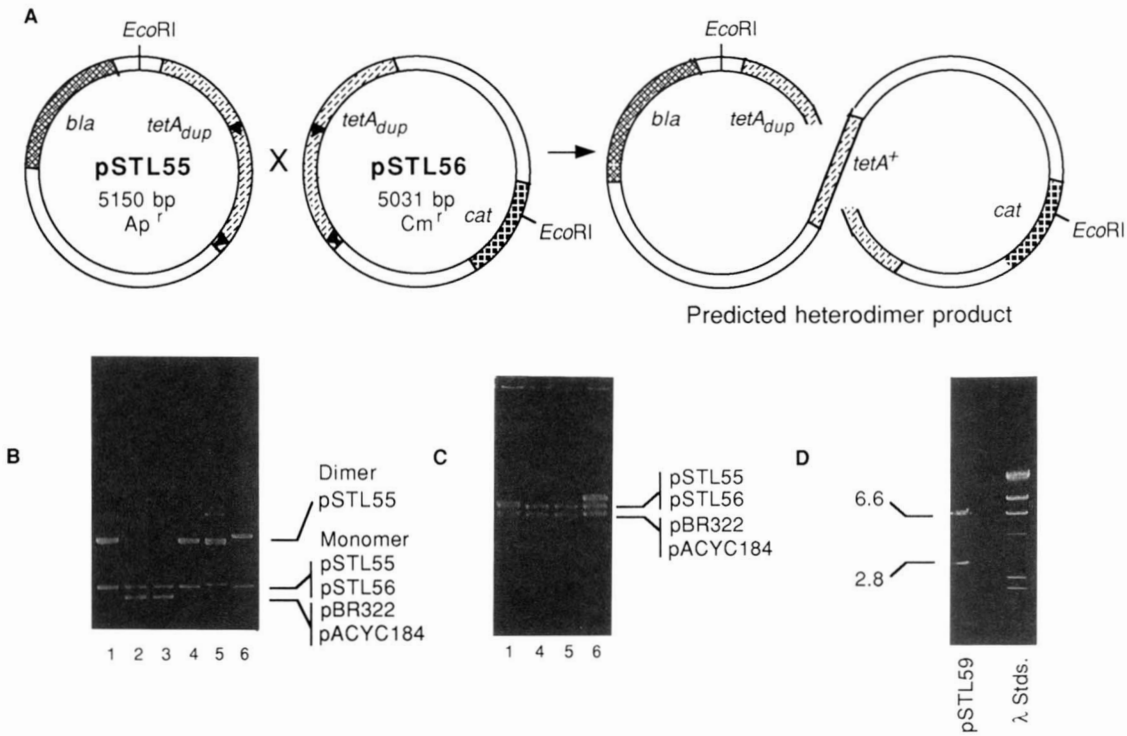

FIGURE 4.—Determination of intramolecular nature of dimerization that accompanies deletion formation. (A) Diagram of the two plasmids, pSTL55 (5148 bp) and pSTL56 (5031 bp), designed to detect intermolecular dimers. (B, C) Representative analysis of plasmid DNA from selected Tc^r deletion progeny of JC10287 carrying both pSTL55 and pSTL56. B Ethidium bromide-stained gels of uncut plasmid DNA. Lanes 1, 4, 5 and 6 show DNA containing dimeric forms. (C) DNA containing dimeric forms (samples 1, 4, 5 and 6 from B) subjected to digestion with *Eco*RI. All dimers are characteristic of homodimer forms. Isolate 6 is a triplication/deletion dimer, one of two such dimers detected in this analysis. (D) Agarose gel electrophoresis of *Eco*RI-digested heterodimer pSTL59 constructed *in vitro* and propagated in JC10287. *Hin*dIII-digested λ size standards are also shown.

mid was slightly less frequent than the pBR322-derived plasmid (9 vs. 16—data not shown). The elusive heterodimer product is not lethal: we could easily construct such a molecule *in vitro* (pSTL59), transform and maintain it in the *recA*Δ strain background (Figure 4D). Therefore, dimer formation that accompanies deletion in *recA*Δ strains results from intramolecular interactions.

DISCUSSION

How general is *recA*-independence of deletion at tandem repeats? We observed that a *recA* mutation reduced deletion rates of a 787-bp tandem repeat by a modest two- to fourfold. This stands in contrast to a greater than 100,000-fold reduction by *recA* mutations of intermolecular recombination measured in conjugational crosses (LOW 1968). A case can be made that all tandem repeats, as least within close chromosomal proximity, can delete by a *recA*-independent mechanism, although the efficiency of this process may vary.

A compilation of data concerning deletion of tandem repeats shows that the effect of *recA* varies from none to 40-fold. Deletion of larger tandem repeats has been examined primarily in plasmid-borne constructions; but our data show that, for the 787 *tetA* repeat we examined, the *recA*-dependence of deletion is not grossly changed when the repeat is on the chromosome. In a previous study, deletion of a 597-bp repeat of *tetA* shows a 20-fold decrease in *recA* mutants whereas a 520-bp repeat in *kan* exhibits no *recA*-dependence of deletion (MATFIELD, BADAWI and BRAMMAR 1985). Other work shows that deletions formed between either 165- or 401-bp repeats are reduced in frequency 8- to 10-fold by *recA* mutations (DIANOV *et al.* 1991); deletions formed between 21- or 42-bp repeats are *recA*-independent. (MAZIN *et al.* 1991). Plasmid recombination assays (COHEN and LABAN 1983; DOHERTY, MORRISON and KOLODNER 1983; KOLODNER, FISHEL and HOWARD 1985) that detect deletions of several kilobase homologies (in addition to other types of recombination events) show only a 30- to 40-fold reduction by mutations in *recA*. Our other plasmid constructions have given results similar to those reported here. We detect no *recA*-effect on a 100-bp *tetA* repeat of pBR322 and less than a twofold-reduction by *recA* for a 217-bp repeat in the *kan* gene of pACYC177 (our unpublished data). Deletion of very short repeats, characteristic of many spontaneous deletion mutations shows, in some assays, no dependence on *recA* (JONES, PRIMROSE and EHRLICH 1982; YI, STEARNS and DEMPLE 1988). However, even deletion between short repeats can exhibit some

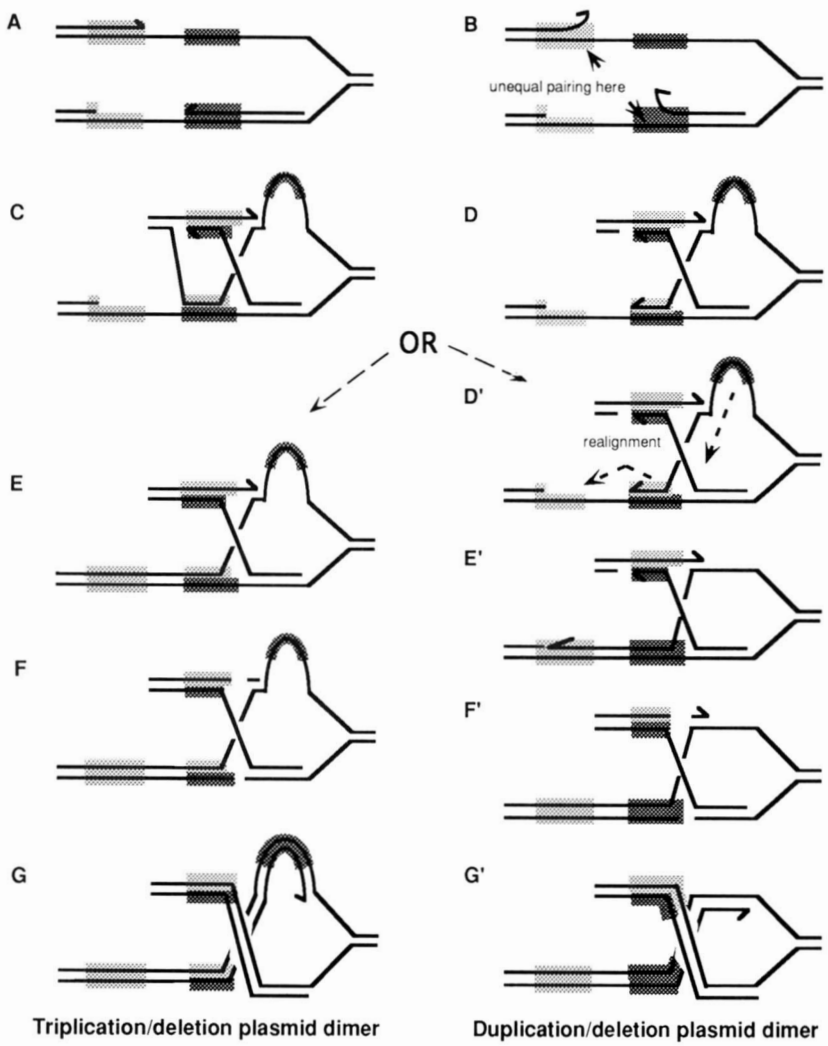

FIGURE 5.—Proposed sister-strand exchange model for deletion events. (A) A stalled replication fork. The repeated sequences are marked with hatching, the left-most repeat with lighter hatching. (B) Displacement of nascent strands in a stalled fork. Unequal pairing in the next step will occur between the repeats marked with arrows. (C) Unequal pairing of two nascent strands and two parental strands producing a crossed-strand Holliday junction structure. (D) Nicking of one crossed parental strand. (E) Repair synthesis and (F) cleavage of parental strand will generate a crossed replication fork, (G) This yields a triplication/deletion dimer product. D′, E′. RuvAB-promoted realignment of parental strands into equally paired configuration, followed by F′, Holliday junction cleavage and repair synthesis produces G′, a crossed fork which will lead to duplication/deletion dimer crossover product.

recA-dependence: another investigation of spontaneous deletions between very short repeats noted a 25-fold reduction by *recA* mutations (ALBERTINI *et al.* 1982).

This variability of the efficiency of the *recA*-independent mechanism relative to the *recA*-dependent deletion mechanism in the numerous examples above may reflect a dependence on the length of the repeat as well as unknown sequence effects. There appears to be a trend toward increasing *recA*-independence of deletion with decreasing repeat length. However, there clearly may be replicon or sequence context effects that govern the contribution of *recA*-independent mechanism for deletion. The 787-bp repeat in *tetA* we have chosen to examine exhibits the highest absolute frequency for *recA*-independent deletion yet reported and argues against the notion that *recA*-independent recombination is intrinsically inefficient relative to *recA*-dependent processes.

RecA-independent recombination is not necessarily nonrecombinational: Because deletion formation depends only weakly on *recA*, recombinational models for deletion formation have generally been discounted. Virtually no intermolecular recombination as measured in conjugational crosses occurs in *recA* mutants (LOW 1968) and *in vitro* RecA displays biochemical properties that support an important role for RecA in the pairing and strand exchange of homologous DNA molecules (WEST 1992). Nevertheless, we have obtained evidence for reciprocal unequal crossing-over products in strains completely deleted for the *recA* gene. These seemingly reciprocal products, a circular plasmid dimer carrying one copy of the selected $tetA^+$ gene and a second *tetA* triplication allele, were rare in $recA^-$ strains but considerably more abundant in *ruvA recA* mutants with an additional block to branch migration (IWASAKI *et al.* 1992; TSANEVA, MÜLLER and WEST 1992). These products are unlikely to be formed by slip-pair mechanisms in which one strand realigns relative to its template and are most easily explained by a recombinational mechanism.

A role for RuvAB: Our observation that reciprocal dimer products are formed more abundantly in *ruvA* mutants is the first observation of a change in the spectrum of recombinational products caused by this

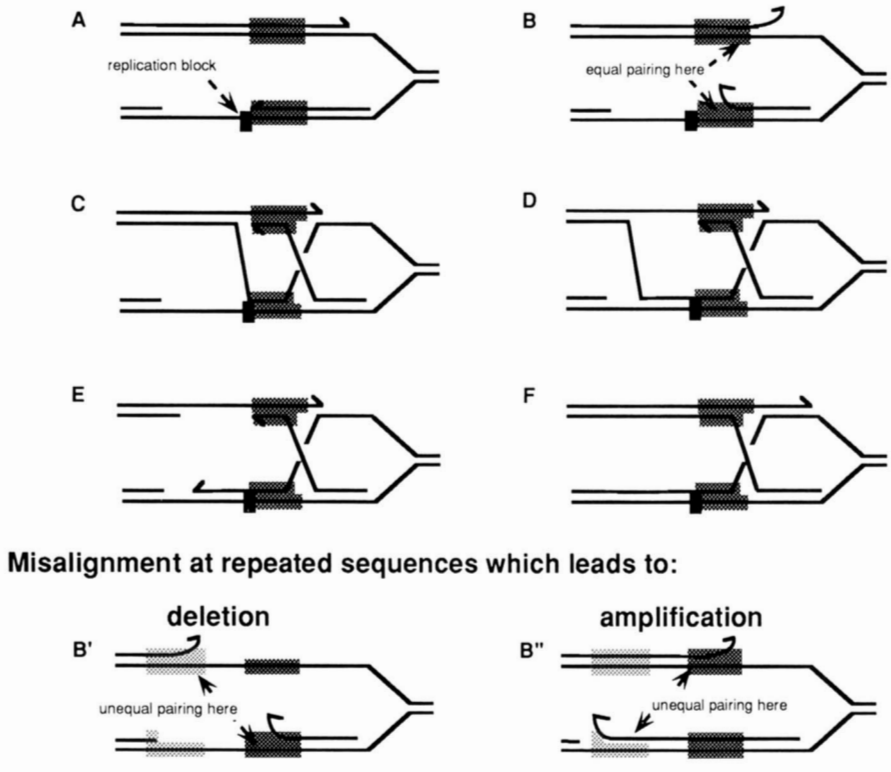

FIGURE 6.—A post-replication gap-filling model: similarity to sister-strand exchange yielding deletions or amplifications. Shown in A through F is sister-strand exchange at *nonrepeated* sequences which bypasses a block to DNA synthesis in the replication fork. (A) A stalled replication fork at a DNA lesion, which blocks DNA synthesis. (B) Nascent strand displacement. Pairing will occur "equally" at the hatched regions. (C) Pairing of the two nascent strands and the two parental strands produces a Holliday structure. (D) Branch migration fills the gap. (E) Cleavage of one crossed strand. (F) Repair synthesis and ligation restores an intact fork with a Holliday junction. If repeated sequences are present in the stalled fork, misalignment at the repeats can occur early in the post-replication repair process, step B above. Shown in B′, unequal pairing between repeats that leads to deletion or B″, unequal pairing between repeats that leads to amplification.

mutation that affects processing of branched DNA. Originally isolated as mutations causing abnormal sensitivity to UV light (OTSUJI, IYEHARA and HIDESHIMA 1974), the *ruvA* and *ruvB* mutations also cause recombination defects but only in combination with other *rec* mutations (*recBC sbcA*, *recBC sbcBC* or *recG*; LLOYD, BENSON and SHURVINTON 1984; LLOYD, BUCKMAN and BENSON 1987; LUISI-DELUCA, LOVETT and KOLODNER 1989; LLOYD 1991). Our sister-strand exchange model for deletions (discussed below) proposes that the helicase activity of the RuvAB protein complex at the Holliday junctions (TSANEVA, MÜLLER and WEST 1993) serves to realign the intermediates to a more favorably paired configuration, which destroys the potential for reciprocal products in our assay. An implication of our result is that one role of RuvAB in the cell may be to destabilize islands of short local pairing in favor of a more extensively paired configuration.

A sister-strand exchange model for deletions: We propose a new model that incorporates elements of both homologous recombination and replicational slip-pair models to explain the dimeric deletion products. (The monomeric deletion products we observe are consistent with the slip-pair model and may be formed therefore by a different mechanism than the dimeric products.) Because the dimers we observed were formed from intramolecular recombination events, we propose an interaction of sister-strands during DNA replication. Although the lack of RecA may exclude other intermolecular genetic recombination events, intramolecular exchange between complementary and partially single-stranded regions may be able to occur in the absence of RecA to accomplish synapsis and strand invasion. (Our observed fourfold stimulation of deletion by a mutation in the *ssb* gene encoding single-strand binding protein, may reflect this need for single-strand DNA.) Like the slip-pair model, our model uses pairing of complementary single-strands to drive the deletion process. However, recombinational features such as strand displacement and branched intermediates are included.

Figure 5 illustrates the steps involving crossing-over between sister DNA molecules adjacent to the replication fork, ultimately leading to the intramolecular dimer products we observed associated with deletions. In addition to the partially single-stranded nature of the replication fork, the close proximity of the interacting molecules may allow this kind of genetic exchange to proceed without RecA protein. (A) The nascent DNA strands are displaced from template strands in a stalled-replication fork. (B) The displaced nascent strands will "unequally" pair with each other. As both leading and lagging nascent DNA strands in *E. coli* DNA replication are thought to be held by a asymmetric polymerase dimer complex (MARIANS 1992), they may be more closely juxtaposed for this type of interaction than is apparent from the illustra-

tion. (C) Unequal pairing of the nascent strand and unequal pairing of parental strands results in a Holliday junction. (D–G) Repair synthesis and resolution of this intermediate leads to a reciprocal triplication/deletion dimer crossover product. However, (D′) strand realignment promoted by RuvAB helicase activity (TSANEVA, MÜLLER and WEST 1993) of one of the duplexes adjacent to the Holliday junction restores the "equal" pairing configuration of the parental strands and removes the potential for the triplication product. E′–G′: this realigned intermediate yields the duplication/deletion dimer crossover product.

Some features of the illustrated model are arbitrary. Although we have suggested that interacting nascent strands are produced by chromosomal replication, they might also come from converging repair DNA synthesis. We have illustrated a scenario in Figure 5 in which leading strand synthesis is impeded relative to the lagging strand; unequal pairing may alternatively arise by in a fork in which leading strand synthesis has proceeded through both repeats, in which case both equal and unequal pairing opportunities exist for the nascent strands. Moreover, the ends of the nascent strands may be created by nucleolytic cleavage rather than representing the point at which DNA synthesis has stopped. Although we have illustrated Holliday junction cleavage to resolve branched structures, such cleavage is not required: an additional round of DNA replication through this structure (Figrue 5E and E′) would produce dimeric products. We have devised this model to accommodate features of the *recA*-independent deletions; however, *recA*-promoted deletion formation may also occur, at least in part, by this mechanism. This mechanism may also account for deletions at very short homologies, so-called "illegitimate" recombination. In this case, the initial pairing of nascent strands (Figure 5B and C) would occur over only a very short region. Accordingly, the frequencies of these events should be much lower than that of larger homologies and that is indeed observed (MAZIN *et al.* 1991). Plasmid dimerization, which may be a hallmark of sister-strand exchange of plasmid-borne repeats, has also been shown to accompany deletions of repeats several nucleotides in length (YI, STEARNS and DEMPLE 1988; MAZIN *et al.* 1991).

This model has several implications. Slowing or stalling of DNA replication (especially of lagging strand synthesis, which allows a free 3′ end to persist longer) should stimulate deletions. DNA lesions or natural pause sites for replication should promote flanking deletions. Deletion frequencies should also show strong dependence on proximity. The proteins that may facilitate these interactions are unknown although they could include DNA helicases or DNA pairing proteins.

Sister-strand deletion—post-replication DNA repair gone awry: Normally, this displacement/pairing process may constitute a post-replication repair mechanism to fill gaps left from DNA lesions that block replication (Figure 6). Note that the steps in this process are similar to those proposed above for deletion: Figure 6A, a stalled fork at DNA damage; (B) displacement of the nascent strands; (C) pairing of nascent strands and pairing of parental strands results in a Holliday junction; (D) branch migration to fill replicational gap past lesion; (E) cleavage of one crossed strand and (F) the replication fork with the gap filled. Resolution of the Holliday junction structure in F will lead to either crossovers or noncrossovers.

An initial misalignment at repeated sequences in an early step of this process (comparable to the post-replication repair step illustrated in Figure 6B) may contribute either to deletion or expansion of the repeated array. In Figure 6B′ the misalignment proposed in our model to initiate deletions is shown again. In addition, an alternative unequal pairing shown in Figure 6B″ could initiate a genetic exchange leading to amplification, producing three copies of a repeat from an initial duplicated sequence. Alternatively, amplifications could arise from rare reciprocal exchange associated with deletions as illustrated in Figure 5. This latter class of genomic rearrangements is of interest because of the association of short repeat amplification with certain human genetic diseases, such as fragile *X* syndrome, Kennedy's disease, myotonic muscular dystrophy and Huntington's disease (RICHARDS and SUTHERLAND 1992; The Huntington's Disease Collaborative Research Group 1993).

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