Pathways for Homologous Recombination Between Chromosomal Direct Repeats in *Salmonella typhimurium*

Timothy Galitski and John R. Roth

Department of Biology, University of Utah, Salt Lake City, Utah 84112

Manuscript received June 21, 1996
Accepted for publication March 25, 1997

ABSTRACT

Homologous recombination pathways probably evolved primarily to accomplish chromosomal repair and the formation and resolution of duplications by sister-chromosome exchanges. Various DNA lesions initiate these events. Classical recombination assays, involving bacterial sex, focus attention on double-strand ends of DNA. Sexual exchanges, initiated at these ends, depend on the RecBCD pathway. In the absence of RecBCD function, mutation of the *sbcB* and *sbcC* genes activates the apparently cryptic RecF pathway. To provide a more general view of recombination, we describe an assay in which endogenous DNA damage initiates recombination between chromosomal direct repeats. The repeats flank markers conferring lactose utilization (Lac^+) and ampicillin resistance (Ap^R); recombination generates Lac^- Ap^S segregants. In this assay, the RecF pathway is not cryptic; it plays a major role without *sbcBC* mutations. Others have proposed that single-strand gaps are the natural substrate for RecF-dependent recombination. Supporting this view, recombination stimulated by a double-strand break (DSB) in a chromosomal repeat depended on RecB function, not RecF function. Without RecBCD function, *sbcBC* mutations modified the RecF pathway and allowed it to catalyze DSB-stimulated recombination. Sexual recombination assays overestimate the importance of RecBCD and DSBs, and underestimate the importance of the RecF pathway.

TRADITIONAL studies of bacterial homologous recombination exploit the sexuality of these organisms (LEDERBERG and TATUM 1946). In typical conjugal or generalized transduction crosses, recombination occurs between an injected linear donor DNA molecule (with two recombinagenic double-strand ends) and a homologous recipient chromosome. Recombination-proficient recipient strains integrate the donor DNA and give rise to recombinant organisms that can be detected by positive selection. Classical analysis of sexual recombination revealed recombination pathways. These were defined by mutants with reduced ability to serve as a recipient in conjugal crosses (reviewed in CLARK 1971, 1973). In wild-type bacteria, the RecBCD pathway is the major sexual recombination pathway (reviewed in SMITH 1988); *recB* mutants and *recC* mutants are Rec^-. In *recB* or *recC* mutant strains (abbreviated *recBC*), the combined effects of two extragenic suppressor mutations, *sbcB* and *sbcC*, restore recombination ability and DNA-damage resistance (KUSHNER *et al.* 1971; LLOYD and BUCKMAN 1985). In these suppressed mutants, a previously cryptic alternative to the RecBCD pathway operates. In the Rec^+ triple mutants, *recBC sbcB sbcC*, recombination depends on the functions of the RecF pathway (HORII and CLARK 1973). The RecBCD and RecF pathways are not strict alternatives for sexual recombination. The RecF pathway has come to be regarded as a minor cryptic recombination pathway that can be activated in the absence of the primary RecBCD pathway. Consequently, it has received less attention.

In spite of the close association of homologous recombination and sexuality, it seems likely that the principal purposes for which recombination pathways evolved are the immediately valuable processes of chromosomal repair and the formation and resolution of gene duplications. The severe viability problems and characteristic DNA-damage sensitivities of various *rec* mutants support this view. The need for recombinational DNA repair and the occurrence of chromosomal rearrangements seems to be universal, whereas the relative contribution of sexual recombination to genetic variation differs from species to species. This spectrum ranges from primarily asexual species with a clonal population structure to species with an obligate sexual stage of the life cycle. Among bacterial species, population structures range from panmictic, in which frequent sex randomizes allele combinations, to clonal, in which significant linkage disequilibrium (nonrandom allele associations) is observed (MAYNARD SMITH *et al.* 1993). It is clear that some prokaryotes, including the enterics *Escherichia coli* (OCHMAN and SELANDER 1984) and *Salmonella typhimurium* (BELTRAN *et al.* 1988), exist in clonal populations that engage in relatively infrequent sexual episodes. For all organisms, the repair of single-

Corresponding author: Timothy Galitski, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.
E-mail: galitski@wi.mit.edu

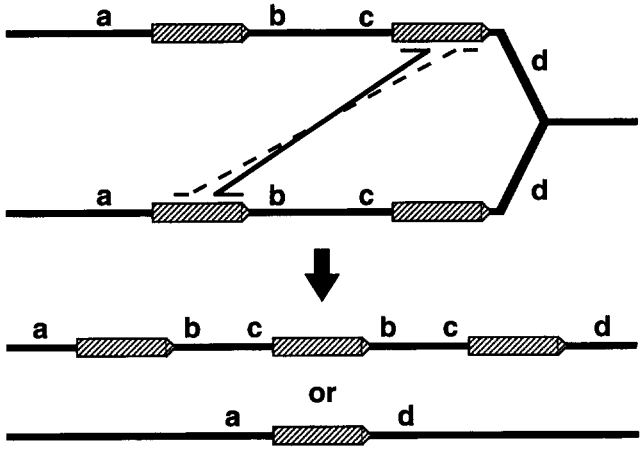

FIGURE 1.—Chromosomal rearrangement by unequal recombination. DNA replication (growing fork at right) produces sister chromosomes. Striped boxes represent repeated sequence elements in the same orientation. A nonreciprocal exchange (one pair of flanks rejoined) between ectopic repeats can result in duplication (—) or deletion (---) of the intervening loci (b and c). A reciprocal exchange gives both products.

and double-strand DNA lesions through sister-chromosome exchanges probably represents the most frequent uses of homologous recombination.

The direct tandem duplication of chromosomal intervals results from unequal sister-chromosome exchanges initiated by spontaneous DNA lesions. Recombination between ectopic homologies in direct orientation (*e.g.*, *rrn* loci, transposable elements, Rhs elements, REP sequences, duplicated intervals) results in a duplication and/or a deletion of the intervening material (Figure 1). One can observe the very frequent duplication, and further amplification or the return to haploidy, of many large (>1 min or ~47 kb) chromosomal intervals in *S. typhimurium* (ANDERSON and ROTH 1981). Duplication frequencies fall in the range 10^{-4} to 10^{-3} for most loci. For loci mapping between *rrn* operons, the duplication frequency is a few percent. The transposable element IS*200* provides another important source of ectopic repeats used to form spontaneous duplications in *S. typhimurium* (HAACK and ROTH 1995). The high frequencies of chromosomal duplications and the conservation of repeated elements suggest an important role for chromosomal duplications in bacterial survival and evolution.

The duplication and amplification of chromosomal intervals may be an important source of genetic variation that can increase fitness by reversibly increasing gene dosage (reviewed in ROTH *et al.* 1996). Various laboratories have shown that specific adverse conditions select for bacterial variants harboring specific duplications or amplifications that arise by recombination between direct repeats. Because these rearrangements are readily reversed by recombination, they disappear from the population when selection is removed. So, the presence of duplications in a population can facilitate the exploitation of available resources and enhance the survival of a clone under adverse conditions. The high frequency of gene duplications and their reversibility suggest that this ability is used essentially as a gene regulatory mechanism.

If the main use of homologous recombination is to perform sister-chromosome exchanges needed for DNA repair and duplication formation and segregation, then it seems reasonable to use these events in an assay system. Here, we describe a system for observing recombination between direct repeats of a chromosomal segment. The properties that distinguish this system from standard sexual recombination assays are the following:

1. All recombining sequences and recombination enzymes are internal to an intact bacterium. Recombinants arise within bacterial clones without the participation of bacteriophage or plasmid functions that contribute to sexual exchanges.
2. Initiating DNA structures (single-strand gaps, double-strand breaks, etc.) form spontaneously by endogenous processes, and they can be induced experimentally.
3. Both spontaneous and experimentally induced recombination events are very frequent. Selection for rare recombinant genotypes is not required.
4. The recombinants scored can arise by a single intramolecular nonreciprocal exchange between direct chromosomal repeats. They also can result from more complex events such as multiple exchanges, intermolecular events (between sister chromosomes), and reciprocal exchanges.

Using this system, we provide evidence that both the RecBCD and the RecF pathways play major roles in recombination. Furthermore, whereas the substrate of the RecBCD pathway is the chromosomal double-strand break or the double-strand ends of a donated DNA fragment, the natural substrate of the RecF pathway is neither of these. In the absence of RecBCD function, *sbcB sbcC* suppressor mutations allow the RecF pathway to catalyze recombination initiated by double-strand breaks. These results support the idea that single-strand gaps in the bacterial chromosome are the natural substrate for RecF pathway recombination in wild-type cells.

MATERIALS AND METHODS

Growth media and genetic methods: The E medium of VOGEL and BONNER (1956) supplemented with 0.2% glucose was used as the defined minimal medium. Growth on alternative carbon sources was in NCE salts, described by BERKOWITZ *et al.* (1968), supplemented with 0.2% of the appropriate carbon source. The complex medium was nutrient broth, NB, (8 g/liter, Difco Laboratories) with added NaCl (5 g/liter).

Solid media contained BBL agar at 1.5%. Auxotrophic requirements on minimal media were satisfied with supplements at final concentrations recommended by DAVIS *et al.* (1980). Phage plates used to score plaque morphologies contained 1% Bacto tryptone (Difco), 0.8% NaCl, and 1.2% agar. Phage top agar was the same except it contained 0.7% agar. Final concentrations of antibiotics were as follows: tetracycline hydrochloride, Tc, at 20 μg/ml; kanamycin sulfate, Km, at 50 μg/ml; chloramphenicol, Cm, at 20 μg/ml; sodium ampicillin, Ap, at 30 μg/ml for single-copy elements (MudA) or at 100 μg/ml for multicopy elements (plasmids). The chromogenic β-galactosidase substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) was obtained from Diagnostic Chemicals Ltd. and used at a final concentration of 25 μg/ml. The nonmetabolizable inducer of the LacI repressor protein, isopropyl-β-D-thiogalactopyranoside (IPTG), was used at a final concentration of 1 mM. All incubations were at 37°. Sterile 0.85% NaCl (saline) was used to dilute cultures except as noted.

Transductional crosses, mediated by the phage mutant P22 HT105/1 *int-201* (SCHMIEGER 1971), were performed as described by ROTH (1970).

Construction and verification of duplication strains: Strains carrying direct-order duplications of predetermined chromosomal intervals were constructed and verified as described by HUGHES and ROTH (1985). These merodiploids are the progeny of triparental P22-mediated crosses involving two donors, each with a simple MudA (HUGHES and ROTH 1984) insertion that will define one of the duplication endpoints, and a haploid recipient. In this method, recombination between MudA elements (during transduction) results in the duplication of the intervening loci. The duplicated sequences flank a MudA element located at the duplication join point. The MudA element of these strains carries an ampicillin-resistance determinant and the *lacZYA* genes transcriptionally fused to an adjacent chromosomal operon. Thus, duplication-bearing strains have Lac^+ and Ap^R phenotypes that are lost when an exchange occurs between the flanking repeats.

The duplications used here, DUP1731[(*leuA1179*)*MudA*(*nadC220*)] and DUP1732[(*pyrB2694*)*MudA*(*thr-469*)], include the region from *leuA* to *nadC* (3′ to 4′) and the region from *pyrB* to *thr* (97′ to 100′), respectively (Table 1). For each duplication, we confirmed the diploidy of a locus within the duplication and the haploidy of two loci mapping outside the duplication. These three test loci are *guaC* (inside DUP1731, outside DUP1732), *serB* (inside DUP1732, outside DUP1731), and *nadA* (outside both DUP1731 and DUP1732). These tests were done by transduction of an auxotrophic Tn*10* (tetracycline-resistant, Tc^R) insertion for each test locus. If the recipient was diploid for a test locus, all Tc^R transductants were prototrophic. If the recipient was haploid for a test locus, all Tc^R transductants were auxotrophic. When haploid (Ap^S Lac^-) segregants were isolated from prototrophic tetracycline-resistant duplication strains, the segregants fell into two classes, tetracycline-resistant auxotrophs and tetracycline-sensitive prototrophs (data not shown). Thus, these merodiploid heterozygous transductant strains produce haploid segregants carrying one of two alleles present in the parent.

The MudA join point of a strain carrying DUP1732 was converted to MudJ (CASTILHO *et al.* 1984). The MudJ element is the same as the MudA element except it carries a kanamycin-resistance determinant instead of the ampicillin-resistance gene. Using strain TT12319 (*purA2158*::MudJ) as a donor, strain TT18939 (DUP1732 with a MudA join point) was transduced to kanamycin resistance on E glucose kanamycin plates. Inheritance of the *purA*::MudJ element causes purine auxotrophy; these transductants do not grow on the minimal medium. Recombination between the donor and recipient Mud elements replaces the MudA join point with a MudJ join point, and gives rise to prototrophic kanamycin-resistant transductants. A Lac^+ Km^R (unstable) Ap^S Pur^+ transductant was saved as strain TT18947 {DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)]}.

Construction of *rec* derivatives of duplication strains: Various *rec* mutations were introduced into wild-type strain LT2, and into rec^+ duplication strains TT18931 (DUP1731) and TT18939 (DUP1732). Insertion mutations with a drug resistance, such as *recB503*::Tn*10* (MAHAN and ROTH 1989a), *recD541*::Tn*10*dCm (MIESEL and ROTH 1994), *recF521*::Tn*5* (RUDD and MENZEL 1987), *recJ504*::MudJ (MAHAN *et al.* 1992), and *recN555*::Tn*10*dCm (T. GALITSKI, unpublished data), were transduced by selecting on NB plates with the appropriate antibiotic. To construct derivatives carrying the *recA1* mutation (WING *et al.* 1968), the *srl-203*::Tn*10*dCm mutation of strain TT11183 (*srl-203*::Tn*10*dCm) was introduced by transduction selecting Cm^R. Then, the srl^+-linked *recA1* mutation was cotransduced into these derivatives by selecting a Srl^+ phenotype on NCE sorbitol plates. Transductants carrying *recA1* were identified by their UV sensitivity. All the *rec* alleles used here are recessive to the wild-type allele and presumably confer null phenotypes. Note that *recJ504*::MudJ is a Lac^- insertion of the *lac* operon fusion element MudJ in the *recJ* gene; the *lac* genes are wild type but they are presumably in the wrong orientation for transcription. Blue Xgal staining of duplication strains carrying this insertion is due to the *lac* fusion at the duplication join point, not the MudJ element at the *recJ* locus.

The *S. typhimurium recN* mutation used was isolated starting with a DNA-damage-inducible MudJ insertion that was shown to map at the *recN* locus closely linked to the *pheA* gene at 58′ (T. GALITSKI and D. THALER, unpublished results). A *recN*::Tn*10*dCm insertion was identified based on close linkage to the original MudJ insertion, on its mitomycin-C sensitivity, and on its Rec^- phenotype when placed in a *recB sbcB sbcC* strain.

Tests of UV sensitivity and phage-plaque morphology: To verify strain genotypes and to investigate the responses of previously untested genotypes, UV sensitivity and phage-plaquing phenotypes of bacterial strains were assessed. Sensitivity to ultraviolet light (UV) was scored by spotting 10-μl aliquots of serial 100-fold dilutions of overnight cultures on two nutrient agar plates. One of the plates was exposed to a UV dose that reduced survival of wild-type strains to about one-third of unirradiated controls. Ratios of UV survivors to unirradiated controls were determined. Phage-plaquing phenotypes of bacterial strains were scored by spotting serial 100-fold dilutions of wild-type P22, P22 *erf* am (H1173), and P22 *c2-5 h21* Δ327 (*abc1 abc2*) lysates on freshly poured bacterial lawns in soft agar on phage plates. When the spots dried, the plates were incubated overnight and scored. Bacteriophage strains were supplied by N. BENSON.

Colony-sectoring recombination screen: Recombination between chromosomal direct repeats was scored qualitatively in colonies by plating duplication strains with *lac* fusions at the join point on NB Xgal plates and incubating these for 4 days. Haploid (Lac^- Ap^S) recombinant clones form white sectors within the colonies. Photographs of such colonies were taken using 400 ASA color print film in a Canon AE-1 camera mounted on an Olympus SZH-ILLD microscope with indirect transillumination and 15× magnification.

Quantitative recombination assay: Rates of recombination between chromosomal direct repeats were determined by ob-

serving the frequencies of Lac$^-$ segregants in broth cultures of duplication strains. For each strain assayed, an overnight NB Ap culture, started from a single colony, was diluted 10^6-fold in fresh NB Ap and grown with aeration to incipient turbidity. Immediately, the culture was diluted 10^4-fold in fresh NB and multiple 1 ml NB cultures (without antibiotic to allow the growth of Ap^S Lac$^-$ segregants) were started with a 10 μl inoculum (typically fewer than 10 cells) from the 10^4-fold dilution. These NB cultures were incubated for 24 hr (except as noted). The inoculum size was determined by measuring the cfu/ml in the 10^4-fold dilution from which inocula were derived. These platings were on NB Xgal to verify simultaneously the Lac$^+$ phenotype of cells in the inocula (data not shown); thus, segregants found in the multiple 1 ml NB cultures arose independently. For each strain assayed, 20 independent nonselective cultures were prepared. These cultures were diluted 10^6-fold in saline; an appropriate volume of this dilution (0.1 ml for *rec*$^+$ and *recF* strains, 0.2 ml for *recA* strains, 0.3 ml for *recB* and *recB recF* strains) was spread to each of four NB Xgal plates (40 plates for *recA* and *recB recF* strains). The plates were incubated for 2–4 days. Colonies were scored as either Lac$^+$ at plating (some blue staining), or Lac$^-$ at plating (no blue staining) and the total population size was noted. The Lac$^-$ segregant frequency of each culture is the ratio of Lac$^-$ colonies to total colonies (segregants/cell). The segregation rate was calculated using the median Lac$^-$ segregant frequency. The number of generations was calculated from the final and initial population size. Segregation rate is the final segregant frequency divided by elapsed generations (segregants/cell/generation).

Plasmid constructions: Plasmids allowing the regulated expression of various alleles of the IS*10* transposase gene were constructed. The 1300-bp *Eco*RI-*Hin*dIII fragment of plasmid pMJR1560 (STARK 1987) contains the *lacI*^{q} gene, expressing elevated levels of the LacI repressor. This fragment was ligated to the 2700-bp *Eco*RI-*Hin*dIII fragment of plasmid pGC2 (MYERS *et al.* 1985). The resulting plasmid, pZT379, has a polylinker upstream of a complete *lacI*^{q} gene, followed by an M13 origin, a pBR322-derived origin, and an ampicillin-resistance determinant. Between the *Cla*I and *Bam*HI polylinker sites of vector plasmid pZT379, fragments (1300-bp *Cla*I-*Bam*HI) containing IS*10* transposase alleles and the LacI-repressor-sensitive Ptac promoter were introduced. These fragments were derived from plasmids, kindly provided by D. HANIFORD and N. KLECKNER, including pDH10, pDH12, pDH10-GD163, and pDH10-PS167 (HANIFORD *et al.* 1989). The resulting plasmids are pZT380 (wild-type transposase, Tnp$^+$), pZT381 (a transposase null allele with an internal in-frame deletion between two *Nco*I sites, TnpΔ), pZT382 and pZT383 (expressing transposase mutants that catalyze the excision, but not the transposition, of Tn*10*-derived elements). For the experiments presented, we used plasmid pZT382 expressing transposase mutant GD163, designated Tnp*. This plasmid allows the introduction of a double-strand break at a defined time (the addition of IPTG) and at a single defined site (a Tn*10* insertion) in the chromosomes of a bacterial population.

Construction of double-strand-break-assay strains: These strains differ from previously described duplication strains in three respects. They possess a single Tn*10* element (providing a DSB site), and they carry a plasmid providing expression of a mutant IS*10* transposase (catalyzing excision of the Tn*10*). In addition, a MudJ duplication join point with Km^R (not the Ap^R of MudA) was used to allow selective maintenance of the transposase plasmids that confer ampicillin resistance. The MudJ duplication join point (and consequently the entire duplication) of strain TT18947 {DUP1732[(*pyrB2-694*)*MudJ*(*thr-469*)]; described above} was transduced to strain SA2337 (*purC7*) to form merodiploid strain TT18948 {*purC7* DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)]}. The duplication DUP1732 spans the region from *pyrB* (97′) to *thr* (100′). This interval includes the *serB* locus, mutations of which cause serine auxotrophy. The *serB* gene is ~125 kb from *pyrB* (unlinked by P22 transduction) and ~25 kb from *thr* (15% linked). The *serB1466*::Tn*10*dTc mutation was transduced into duplication strain TT18948 by selecting Tc^R. Since this strain has two copies of *serB*, these transductants were Tc^R Ser$^+$. The Tn*10*dTc insertions of these transductants could be in either copy of *serB*. Because *serB* is linked to *thr*, it was possible to determine which copy carried the Tn*10*dTc insertion. An insertion in the counterclockwise copy would be linked to the duplication join point (Km^R). An insertion in the clockwise copy would be linked to *thr*$^+$. Strain TT18949 had the serB1466::Tn*10*dTc insertion (DBS site) in the counterclockwise copy (data not shown); it was used for this study. A control strain was constructed also. This strain, TT18950, carries the *nadA379*::Tn*10*dTc insertion, not the *serB1466*::Tn*10*dTc insertion. The *nadA* locus maps at 17′ (far from DUP1732).

In the construction of mutant Rec$^-$ derivatives of strain TT18949 {*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)]}, we avoided insertion mutations in *rec* genes, especially Tn*10* insertions, both because of a lack of available drug resistances and because Tn*10* insertions would provide additional sites of double-strand breakage. All *rec* alleles were introduced by the same general strategy (described above) used to introduce the *recA1* allele to duplication strains; in each case a mutant phenotype generated by an insertion mutation was corrected in a transduction cross in which the *rec* allele was coinherited. The *recB10* allele (EISENSTARK *et al.* 1969) and the *recD561* allele (MIESEL and ROTH 1994) were introduced by linkage to the *argA* locus. The *recF558* allele (T. GALITSKI, unpublished data) was introduced by repair of a *dgo* mutation. The deletion DEL1792 (*phs-sbcB*) (BENSON and ROTH 1994) was introduced by linkage to the *his* operon. The *sbcCD8* allele (BENSON and ROTH 1994) was introduced by linkage to *proC*. All of these *rec* and *sbc* alleles are recessive to the correponding wild-type alleles. Table 1 lists the complete strain genotypes. Note that insertions of Tn*10*dCm were commonly used as linked markers for the introduction of *rec* and *sbc* alleles. These insertions were removed in the course of the constructions as described above for *srl-203*::Tn*10*dCm and *recA1*. Since Tn*10*dCm is a defective element (ELLIOTT and ROTH 1988), and since no active Tn*10* elements were used during these constructions, the final strains harbor no IS*10* insertions providing unwanted double-strand break sites.

The *recF558* allele was isolated (after hydroxylamine-induced local mutagenesis described by HONG and AMES 1971) and verified during the course of this work. The phenotype and map position of the *recF558* allele is identical to that of the *recF521*::Tn*5* insertion. Both cause UV sensitivity in a wild-type background and a Rec$^-$ phenotype in a *recBC sbcB sbcC* background. Both are transductionally linked to the *dgo* locus (data not shown; see RUDD and MENZEL 1987). These *recF558* phenotypes are fully complemented (data not shown) by plasmid pMAB4, a minimal *E. coli recF*$^+$ clone (kindly provided by R. MYERS; BLANAR *et al.* 1984).

The introduction of transposase-expressing plasmids was the final step in all constructions. For each strain, two plasmids were introduced by transductions selecting Ap^R. One of the plasmids, pZT382, expresses Tnp*, the mutant IS*10* transposase that catalyzes double-strand breakage but not transposition. The other plasmid, pZT381, expresses TnpΔ, a deletion mutant transposase that catalyzes neither double-

strand breakage nor transposition. Strains with plasmid pZT381 served as negative controls that show the spontaneous frequencies of haploid Lac$^-$ segregants. These spontaneous frequencies were subtracted from those observed with the strains carrying plasmid pZT382, providing double-strand breaks at the Tn*10*dTc insertion site. This difference is the frequency of haploid Lac$^-$ segregants induced by a double-strand break as presented in Figure 7. In Figure 6, the uncorrected numbers are presented to illustrate the nature of the assay system.

Assays of recombination stimulated by double-strand breaks: The stimulatory effect of transposase-induced DSBs was tested in various *rec* backgrounds. The frequency of Lac$^-$ recombinants in cultures of DSB-assay strains was measured. Independent (started from separate single colonies) starter cultures of assayed strains were grown to saturation in NB Km Ap. Each culture was diluted 10-fold in 4.5 ml saline. From each of these dilutions, a 0.01-ml aliquot was transferred to each of three 2-ml tubes of NB Ap with IPTG. The IPTG induces expression of the mutant transposase that catalyzes excision of the Tn*10*dTc, leaving a double-strand break in the chromosome. Some control cultures had no IPTG. These experimental cultures were incubated overnight. Both the starter cultures and the experimental cultures were diluted appropriately in saline to provide 0.1-ml aliquots containing at least 50 colony-forming units. These 0.1-ml aliquots were spread on NB Xgal plates (four for each culture), incubated for 2 days, and scored. Colonies were scored as either all white (Lac$^-$ at plating) or blue with white sectors (Lac$^+$ at plating). The Lac$^-$ segregant frequency of each culture is the ratio of white (Lac$^-$) colonies to total colonies. For each strain, the Lac$^-$ segregant frequency is that of the experimental culture minus the low segregant frequency in the starter culture from which it was derived.

RESULTS

Detection of recombination between extensive direct-order chromosomal repeats: Duplication strains were used to detect recombination between extensive direct-order chromosomal repeats. In these strains the duplicated regions flank a MudA element, conferring Ap^R and Lac$^+$ phenotypes. The chromosomal structure of one of these duplication strains, TT18939 {DUP1732 [(*pyrB2694*)*MudA*(*thr-469*)]}, is diagrammed in Figure 2. The full genotypes of merodiploid strains TT18931 (DUP1731) and TT18939 (DUP1732) are listed in Table 1. The duplication DUP1731[(*leuA1179*)*MudA*(*nadC220*)] is a one minute (~40 kb) direct-order duplication of the region from the *leuA* locus at 3′ to the *nadC* locus at 4′ with a transcriptional fusion of the MudA *lac* operon to *leuA* at the join point. Similarly, DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] is a three minute (~150 kb) direct-order duplication from the *pyrB* locus at 97′ to the *thr* locus at 100′ with a transcriptional *lac* fusion to *thr* at the join point. Note that DUP1731 and DUP1732 do not overlap; observations made with both strains are not due to peculiarities of a shared interval. The construction and verification of these duplications is described in MATERIALS AND METHODS.

An exchange between the direct-order chromosomal repeats results in the segregation of a haploid recombinant that has lost the join-point markers; these recombinants are Lac$^-$ and ampicillin sensitive (Figure 2). Though Figure 2 illustrates an intermolecular nonreciprocal exchange, intramolecular and reciprocal exchanges also will produce Lac$^-$ Ap^S recombinants. These segregation events occur spontaneously; the assay system provides neither DNA damage nor fragment ends to initiate recombination. Presumably, endogenous processes provide the initiating DNA lesions. The observed segregation events occur very frequently and can be observed without selection for recombinants.

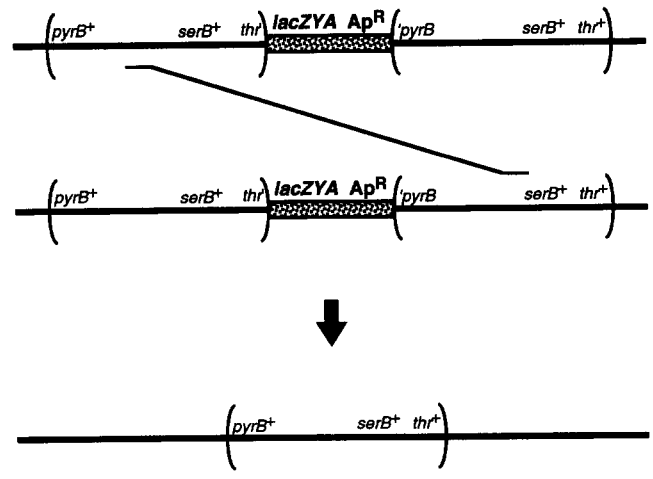

FIGURE 2.—A screen for recombination between direct chromosomal repeats. The merodiploid strain TT18939 {DUP1732[(*pyrB2694*)*MudA*(*thr-469*)]} carries a 150-kb duplication of loci from *pyrB* at 97′ to *thr* at 100′. These direct repeats flank a MudA join-point element conferring Lac$^+$ and Ap^R phenotypes. An exchange between the repeats leads to the segregation of haploid Lac$^-$ Ap^S recombinants.

Properties of the *rec* genotypes investigated: To both verify strain genotypes and investigate the responses of previously untested genotypes, UV sensitivity and phage-P22 plaquing phenotypes of mutant *rec* strains were assessed. The data of Table 2 summarize observations made with a *rec*$^+$ strain and its *rec* derivatives. These properties are generally identical to the behavior of analogous *E. coli* mutants, tested with phage lambda.

UV survival, relative to unirradiated controls, was assayed at a UV dose that reduces survival of *rec*$^+$ strains to about one-third. As expected, a *recD* mutation did not confer UV sensitivity (MIESEL and ROTH 1994). Mutant *recA* strains showed a UV hypersensitivity. Strains with a mutant allele of either *recB*, *recF*, or *recJ* showed a less extreme UV sensitivity. Of particular note is the severe UV sensitivity of *recB recF* double mutant strains, and the *recA*-like UV sensitivity of *recB recJ* double mutants previously reported by MAHAN *et al.* (1992). Moreover, *recB recJ* double mutants were difficult to construct, maintain, and study due to viability problems and very slow growth.

Since growth of some mutant bacteriophage P22

strains requires host recombination functions, these phage strains were used to verify or phenotypically characterize *rec* genotypes. Wild-type P22 was used as a control. Normally, the formation of lysogens by wild-type P22 produces turbid plaques; most of the host strains investigated gave this result. However, wild-type P22 produced clear plaques on *recB recJ* double mutants. This observation, combined with the extremely poor viability of these double mutants, might indicate an SOS-constitutive phenotype due to a general defect in the repair of potentially lethal chromosomal lesions: SOS constitutivity precludes lysogen formation. The phage P22 *erf* amber mutant lacks the essential recombination function needed for growth on *recA* strains (BOTSTEIN and MATZ 1970). The Erf function is involved in recircularization by recombination at the terminal redundancies of linear injected P22 genomes. In these tests, *recA* mutants, of course, and *recB recJ* double mutants, curiously, were nonpermissive hosts for P22 *erf* am. Also, this phage produced only small plaques at a reduced efficiency on *recJ* mutants; this defect was reported by MAHAN *et al.* (1993). Phage P22 *abc c2-5* is a clear-plaque phage lacking an anti-RecBCD function that inhibits the exonuclease V activity (POTEETE *et al.* 1988) of the host RecBCD enzyme. Exonuclease V degrades rolling-circle replication intermediates. The P22 *abc* mutant forms pinpoint plaques on *recBCD*$^+$ strains, but it can form large plaques on *recB, recC,* or *recD* mutant hosts. The total nonpermissivity of *recJ* mutants for P22 *abc,* and the suppression of this effect by *recB* mutations, has been reported by MAHAN *et al.* (1992).

The data summarized by Table 2 confirm the *rec* genotypes of the duplication strains used in this study. Some novel aspects of these observations contribute to the interpretation of recombination data presented below.

The colony-sectoring recombination assay: The segregation of Lac$^-$ recombinant clones within colonies forms the basis of a screen for recombination defects. Homologous recombination between the direct repeats of duplication strains like TT18931 and TT18939 results in the segregation of haploid Lac$^-$ clones (Figure 2). These events are detected on Xgal plates as white (Lac$^-$) sectors within blue (Lac$^+$) colonies (Figure 3A). The white sectors reflect recombination events that cause loss of the duplicated region and the join point. The cells in these white sectors are Ap^S (no join point) and haploid (no duplication) (data not shown). Furthermore, the appearance of these sectors requires homologous recombination functions (described below). Thus, they arose by a recombination event like that depicted in Figure 2, not a mutational event affecting LacZ function.

Each sector is assumed to be a recombinant clone arising from a single event. This assumption is supported by characterization of very rare single Lac$^-$ sectors in *recA* derivatives. These rare sectors arise by *rec*-independent deletion events. Some of these rare sectors are homogeneous populations of bacteria with an unusual phenotype (*e.g.,* Lac$^-$ Ap^R; data not shown). The best explanation for these observations is that each sector contains descendants of a single recombinant cell.

Effects of *rec* mutations: To investigate the recombination-pathway dependence of the events scored in the colony-sectoring recombination assay, various *rec* derivatives of duplication strains TT18931 and TT18939 were tested. For each genotype, three independent isolates were observed in the colony-sectoring assay described in MATERIALS AND METHODS. Essentially, duplication strains are streaked for single colonies on NB Xgal plates. After 4 days colonies were scored for the presence of white (Lac$^-$) sectors. Figure 3 shows representative colonies of strain TT18939 (DUP1732 *rec*$^+$) (A), and derived *rec* mutants: *recA1* (B), *recB503*::Tn*10* (C), *recF521*::Tn*5* (D), *recJ504*::MudJ (E), and *recB503*::Tn*10 recF521*::Tn*5* (F). The same results were obtained with strain TT18931 (DUP1731 *rec*$^+$) and its *rec* derivatives (Table 1; data not shown). The *recF* mutants and *recJ* mutants could not be distinguished from *rec*$^+$ strains in this assay. The same was true of a *recD* mutant (data not shown). Typical *recA* colonies showed no sectors. Strains with a *recB* mutation showed only a slight defect. The proficiency of *recB* strains in duplication segregation has been observed in *E. coli* by SCLAFANI and WECHSLER (1981) and in Salmonella by SEGALL (1987). However, *recB recF* double mutants exhibited an obvious defect in recombination between chromosomal direct repeats. Sickly *recB recJ* double mutants are not shown in Figure 3; after extended incubation, they formed irregular colonies with many white sectors suggesting that they are able to support duplication segregation. A *recN* mutation had no effect in the colony-sectoring assay either as a single mutant, in double mutant combinations with *recB* or *recF,* or as part of a triple mutant combination with *recB* and *recF* (data not shown).

The colony-sectoring assay of recombination is a convenient qualitative screen for recombination defects. The validity of the colony-sectoring assay was affirmed, and its sensitivity was calibrated by quantitating duplication segregation rates during growth in liquid nutrient broth.

Quantitative recombination assay: A duplication segregation rate, segregants per cell per generation, can be calculated from the segregant frequency in independent cultures that have grown for a known number of generations (see MATERIALS AND METHODS). Twenty independent segregant frequencies were observed in broth cultures of strain TT18931 (DUP1731 *rec*$^+$) and *rec* derivatives thereof. As an example of the results obtained, Table 3 lists these frequencies in ascending order. The median frequency, the mean of the 10th and

11th values, was chosen to represent each strain to avoid disproportionate contributions by "jackpots." The quantitative data of Table 3 paralleled the results of the colony-sectoring assay. Data from 10 cultures of strain TT18933 (DUP1731 *recA1*) are not tabulated. Of 144,904 colonies of this strain, three, from two cultures, were Lac$^-$. This yields an overestimate of 10^{-5} for its median segregant frequency. Other than *recA* mutants, only *recB recF* double mutants showed a substantial recombination defect.

Cultures of *recB recF* double mutant strains grew slowly and, since all cultures in Table 3 were incubated for 24 hr, spent less time in stationary phase. One could argue that the relative dearth of segregants in *recB recF* cultures was due to shorter incubation time in stationary phase. This possibility was eliminated by the observation that 10 cultures of the *recB recF* double mutant gave the same distribution of Lac$^-$ segregant frequencies after an additional 24-hr period of incubation. The results of extended stationary phase incubation are consistent with the expectation that duplication segregation occurs mainly during exponential growth.

The median Lac$^-$ segregant frequency and the number of generations are two pieces of information needed to calculate a duplication segregation rate. The number of generations was calculated from the initial and final numbers of colony-forming units in the cultures (see MATERIALS AND METHODS). For each strain, the Lac$^-$ segregation rate, a (segregation events per cell per generation), is

$$a = 2f_s/g,$$

where f_s is the median segregant frequency, and g is the number of generations (Table 4).

Spontaneous recombination between chromosomal direct repeats: The results of the colony-sectoring assay, the Lac$^-$ segregation rates, and the relative recombination proficiencies (the ratio of the Lac$^-$ segregation rate to that of the *rec*$^+$ parent) of various *rec* genotypes are summarized in Table 4. All of the single mutant types, except *recA*, were Rec$^+$. The most striking observations are the recombination proficiency of *recB* mutants and *recF* mutants, and the recombination deficiency of the *recB recF* double mutant. Whereas sexual recombination assays show a strong dependence on the RecA and RecBC functions, but no dependence on RecF function (except in a *recBC sbcB sbcC* mutant background), recombination between chromosomal direct repeats requires RecA plus either RecBC or RecF function. This result indicated that some spontaneously initiated events depend on RecBCD-pathway recombination, whereas some depend on recombination by a RecF pathway that is active in wild-type cells.

A model and predictions: The genetics of spontaneous recombination between chromosomal direct repeats suggested a model that accounts for the recombination pathway dependence of both sexual and asexual recombination (Figure 4). This model is elaborated more fully in DISCUSSION.

The main feature of this model is the different substrate specificities of the RecBCD and RecF recombination pathways. It is proposed that the primary initiating substrate of the RecF pathway is a single-strand gap, whereas a double-strand break is the substrate of the RecBCD pathway (Figure 4A). This general idea has been proposed and independently supported previously (*e.g.*, WANG and SMITH 1983; LLOYD and THOMAS 1984). Both types of lesions occur spontaneously in the chromosome and stimulate repair and rearrangement events. Either pathway can produce an exchange between direct repeats. Eliminating one pathway does not result in a substantial defect. However, the elimination of both pathways causes a Rec$^-$ phenotype. In classical sexual recombination assays (conjugation or transduction; Figure 4B), the donor DNA has double-strand ends. Thus, the inheritance of a donor marker requires RecBCD function but not RecF function. In the absence of the RecBCD pathway, suppressor mutations combined with RecJ exonuclease activity allow RecF pathway enzymes to operate on the ends of donor DNA. This might be due to processing of a double-strand end to generate a single-strand tail that approximates the features of a single-strand gap, the RecF pathway's primary substrate.

This model predicts that recombination stimulated by a specific type of DNA lesion will depend on a specific pathway. For example, if recombination between direct repeats were induced above spontaneous levels by the introduction of a DSB in one of the repeats, this increase in recombination will depend on RecB function, but not on RecF function. Furthermore, *sbc* (suppressor of *recBC*) mutations will restore DSB-induced recombination to a *recB* mutant by allowing the RecF pathway to substitute. In wild-type strains, DSB-induced recombination will be indifferent to a *recF* mutation, whereas in *recB sbcB sbcC* strains DSB-induced recombination will depend on RecF function. These predictions are tested below.

Induction of a specific DNA double-strand break: A system was developed for the regulated induction of a single predetermined double-strand break at the site of a Tn*10* insertion in the bacterial chromosome. This system exploits an IS*10* transposase mutant isolated and characterized by HANIFORD *et al.* (1989). This mutant transposase (Tnp*) catalyzes the excision of an element with IS*10* ends from a donor site. Excision leaves a DSB and causes a transient induction of the SOS regulon of DNA-damage-induced genes. However, this mutant transposase is defective for target site interactions. Excision produces primarily a flush cut double strand end and a free abortive excised fragment bearing bound transposase. Both genetic (BENDER *et al.* 1991)

TABLE 1

Bacterial strains

Strain[a]	Genotype[b]	Source[c]
LT2	Wild type	Lab collection
TT11183	*srl-203*::Tn*10*dCm	Lab collection
SB2452 = TR2246	*HfrB2 strA metA22 recA1*	J. WYCHE
TT14556	*recB503*::Tn*10*	Lab collection
TT16812	*recD541*::Tn*10*dCm	Lab collection
KRS2322 = TT11293	*recF521*::Tn*5*	K. RUDD
TT15278	*recJ504*::MudJ	Lab collection
TT18831	*recN555*::Tn*10*dCm	
TT12319	*purA2158*::MudJ	Lab collection
SA2337 = TR7206	*purC7*	K. SANDERSON
TR5124	*recB10 hisD1447*	Lab collection
TT17766	*recD561*del	Lab collection
TT18923	*ara-9 recF558*	
TT19082	*recA1*/pMAB4 (pBR322 *recF*$^+$)	Lab collection
TT18924	fels2$^-$ *leuA414*am DEL1792(*phs-sbcB*)	Lab collection
TT17582	fels2$^-$ *leuA414*am *recB497*::MudJ *sbcB1 sbcCD8*	Lab collection
TT17427	*recA1*/pZT379 (vector, Ap^R, *lacI*^{q})	
TT17428	*recA1*/pZT380 (*tnp*$^+$)	
TT17429	*recA1*/pZT381 (*tnp*Δ)	
TT17430	*recA1*/pZT382 (*tnp*GD163)	
TT17431	*recA1*/pZT383 (*tnp*PS167)	
TT18925	*recA1*	
TT18926	*recB503*::Tn*10*	
TT19079	*recD541*::Tn*10*dCm	
TT18927	*recF521*::Tn5	
TT18928	*recJ504*::MudJ	
TT18929	*recB503*::Tn*10 recF521*::Tn*5*	
TT18930	*recB503*::Tn*10 recJ504*::MudJ	
TT18931	DUP1731[(*leuA1179*)*MudA*(*nadC220*)]	
TT18933	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recA1*	
TT18934	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recB503*::Tn*10*	
TT19080	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recD541*::Tn*10*dCm	
TT18935	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recF521*::Tn*5*	
TT18936	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recJ504*::MudJ	
TT18937	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recB503*::Tn*10 recF521*::Tn5	
TT18938	DUP1731[(*leuA1179*)*MudA*(*nadC220*)] *recB503*::*recJ504*::MudJ	
TT18939	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)]	
TT18941	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recA1*	
TT18942	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recB503*::Tn*10*	
TT19081	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recD541*::Tn*10*dCm	
TT18943	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recF521*::Tn*5*	
TT18944	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recJ504*::MudJ	
TT18945	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recB503*::Tn*10 recF521*::Tn*5*	
TT18946	DUP1732[(*pyrB2694*)*MudA*(*thr-469*)] *recB503*::Tn*10 recJ504*::MudJ	
TT18947	DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)]	
TT18948	*purC7* DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)]	
TT18949	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)]	
TT18950	*purC7* DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)] *nadA379*::Tn*10*dTc	
TT18964	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)]/pZT381	
TT18965	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)]/pZT382	
TT18966	*purC7* DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)]/pZT382	
TT18967	*purC7* DUP1732[(*pyrB2694*)*MudJ*(*thr-469*)] *nadA379*::Tn*10*dTc/pZT382	
TT18968	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recA1*/pZT381	
TT18969	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recA1*/pZT382	
TT18970	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10*/pZT381	
TT18971	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10*/pZT382	
TT18972	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recD561*/pZT381	

TABLE 1

Continued

Strain[a]	Genotype[b]	Source[c]
TT18973	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recD561*/pZT382	
TT18974	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recF558*/pZT381	
TT18975	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recF558*/pZT382	
TT18976	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10* DEL1792(*phs-sbcB*) *sbcCD8*/pZT381	
TT18977	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10* DEL1792(*phs-sbcB*) *sbcCD8*/pZT382	
TT18978	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10* DEL1792(*phs-sbcB*) *sbcCD8 recF558*/pZT381	
TT18979	*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc)*MudJ*(*thr-469*)] *recB10* DEL1792(*phs-sbcB*) *sbcCD8 recF558*/pZT382	

[a] All strains are derivatives of *S. typhimurium* LT2.
[b] The chromosomal structure of strain TT18939 is diagrammed in Figure 2.
[c] Except where noted, all strains were constructed during the course of this work.

and biochemical evidence (HANIFORD *et al.* 1991) argue strongly against rejoining of the donor site following cleavage. Thus, the fate of the donor site (the DSB) is either degradation or repair by homologous recombination. The source of the template homology for this repair could be either an intact sister chromosome or an ectopic repeat of the sequence flanking the break site.

The DSB system requires only a plasmid, pZT382, to provide regulated expression of the Tnp* protein (excision-proficient transposition-defective transposase) and a transposition-defective Tn*10* element (with the ends of IS*10*) at any chromosomal site. The plasmid carries the transposase allele under the expression of the Ptac promoter. It also carries the *lacI*^{q} gene (see MATERIALS AND METHODS and Figure 5). The expression of Tnp* protein is repressed without IPTG and induced in the presence of IPTG. The Tnp* mutant enzyme catalyzes the excision of the Tn*10* from the chromosomal site and leaves a double-strand break.

Stimulation of recombination by a specific DSB: In this assay, recombination stimulation requires expression of Tnp* (the transposase that makes breaks) and a Tn*10*dTc (the break site) in one of the repeats (see Figure 6). Cultures of DSB assay strains (described in MATERIALS AND METHODS) were diluted from selective medium (counterselecting Lac$^-$ Ap^S segregants) into nonselective medium containing IPTG to induce transposase expression. The cultures were incubated and their Lac$^-$ segregant frequencies were determined as described above. The uninduced Lac$^-$ segregant frequency was ~0.08. The stimulation of Lac$^-$ segregation above this level required a Tn*10*dTc (Figure 6, no Tn*10*), the plasmid expressing Tnp* (Figure 6,

TABLE 2

UV-survival and phage-plaquing phenotypes of *rec* mutants

Strain[a]	Genotype	UV survival[b]	P22 plaque morphologies[c]		
			P22 wild type	P22 *erfam*	P22 *abc c2-5*
LT2	*rec*$^+$	0.3	+++ turbid	+++ turbid	+ clear
TT18925	*recA*	10^{-6}	+++ turbid	0	+ clear
TT18926	*recB*	10^{-2}	+++ turbid	+++ turbid	+++ clear
TT19079	*recD*	0.3	+++ turbid	+++ turbid	+++ clear
TT18927	*recF*	2×10^{-2}	+++ turbid	+++ turbid	+ clear
TT18928	*recJ*	10^{-2}	+++ turbid	++ turbid	0
TT18929	*recB recF*	5×10^{-4}	+++ turbid	+++ turbid	+++ clear
TT18930	*recB recJ*	10^{-5}	+++ clear	0	+++ clear

[a] The data tabulated here compare isogenic haploid strains. Data for the two *rec*$^+$ merodiploid strains TT18931 and TT18939 and their *rec* derivatives (Table 1) were similar. In all cases, identical results were obtained for each of three independent isolates from the crosses performed to construct these strains.
[b] Relative survival of UV-irradiated bacteria to unirradiated controls. See MATERIALS AND METHODS.
[c] Plaque morphology is indicated as follows: +++, large; ++, small; +, pinpoint; 0, no plaques. See MATERIALS AND METHODS for the full genotypes of the P22 strains.

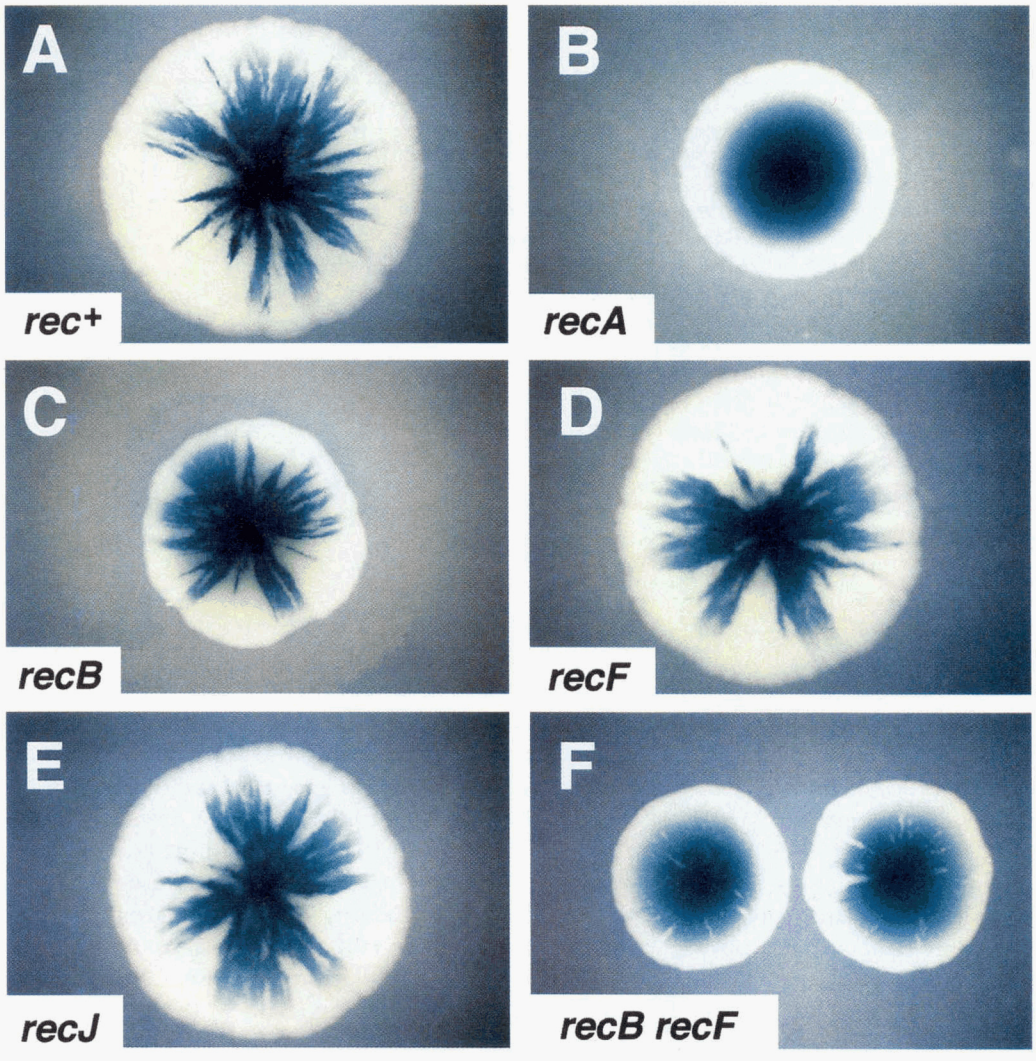

FIGURE 3.—Colony-sectoring recombination assay. Recombination between the direct chromosomal repeats of strain TT18939 {DUP1732 [(*pyrB2694*)*MudA*(*thr-469*)]} generates Lac$^-$ clones within colonies. These recombinants appear as white sectors on NB Xgal plates. (A) *rec*$^+$ (TT18939). (B) *recA1* (TT18942). (C) *recB503*::Tn*10* (TT18943). (D) *recF5221*::Tn*5* (TT18944). (E) *recJ504*::MudJ (TT18944). (F) *recB503*::Tn*10 recF521*::Tn*5* (TT18945).

TnpΔ), and IPTG to induce Tnp* expression (Figure 6, no IPTG). Also, the stimulation required a Tn*10*dTc in one of the repeats; a Tn*10*dTc far from the repeats (Figure 6, Tn*10* at *nadA*) had no effect. Strains lacking any essential component show no stimulation of recombination above background spontaneous frequencies. Strains with all these elements and a *rec*$^+$ genotype show a substantial increase in Lac$^-$ segregant frequency due to double-strand breakage of one of the repeats (Figure 6, Tn*10* at *serB*).

The absolute increase in the Lac$^-$ recombinant frequency above the background level (seen in Figure 6) is of interest; the relative increase is arbitrary. Under the specified conditions (MATERIALS AND METHODS) the background Lac$^-$ segregant frequency is ~0.08. These spontaneous segregants (background) accumulate during exponential growth (see above); the background Lac$^-$ segregant frequency can be controlled arbitrarily by varying the number of generations of exponential growth (data not shown). The induction of a transposase-catalyzed DSB in one of the repeats (Figure 6, Tn*10* at *serB*) increases the segregant frequency from ~0.08 to ~0.4; one-third of the cells (0.4 minus 0.08) are DSB-stimulated Lac$^-$ recombinants. This increase above background does not depend on the duration of exponential growth. This is because transposase does not work continuously during growth; it appears to induce DSBs in bursts, one upon initial exposure to IPTG, and one near the end of exponential phase growth (data not shown). The efficiency of double-strand breakage must be at least as high as the frequency of DSB-stimulated Lac$^-$ recombinants (one-third); some double-strand breaks will be repaired either using the same repeat on a sister chromosome (not an ectopic repeat), or using an ectopic repeat but without the exchange of flanking markers.

Induction of transposase activity in the strains tested shows very little effect on the course of exponential growth; there is at most a brief cessation of growth (data not shown). This probably reflects the fact that transposase cutting is not 100% efficient and many introduced breaks can be efficiently repaired.

TABLE 3

Lac⁻ segregant frequencies in cultures of a *rec*⁺ duplication strain and *rec* derivatives[a]

Culture	*rec*⁺	*recB*	*recF*	*recB recF*
1	0.058	0.016	0.035	0.0053
2	0.060	0.022	0.041	0.0054
3	0.062	0.026	0.044	0.0062
4	0.065	0.030	0.053	0.0067
5	0.067	0.033	0.053	0.0067
6	0.067	0.036	0.053	0.0068
7	0.068	0.037	0.054	0.0069
8	0.069	0.037	0.056	0.0073
9	0.071	0.043	0.060	0.0074
10	0.071	0.047	0.061	0.0080
Median[b]	0.071	0.048	0.061	0.0082
11	0.071	0.048	0.061	0.0084
12	0.073	0.048	0.068	0.0088
13	0.073	0.048	0.069	0.0088
14	0.083	0.049	0.076	0.0092
15	0.083	0.059	0.077	0.0096
16	0.084	0.060	0.078	0.0120
17	0.084	0.063	0.080	0.0140
18	0.085	0.063	0.091	0.0140
19	0.087	0.220	0.096	0.0180
20	0.100	0.350	0.120	0.7200

[a] The full genotypes of strain TT18931 (DUP1731 *rec*⁺) and its *rec* derivatives, TT18934 (DUP1731 *recB*), TT18935 (DUP1731 *recF*), and TT18937 (DUP1731 *recB recF*), are listed in Table 1.

[b] The median Lac⁻ segregant frequency from 20 independent cultures sorted in ascending order (see MATERIALS AND METHODS).

Effects of *rec* mutations on DSB-stimulated recombination: For each *rec* genotype studied, two strains were tested in assays of DSB-stimulated recombination. A strain with the pZT381 plasmid (TnpΔ) served as a negative control to show the background frequency of spontaneous Lac⁻ recombinants. An isogenic strain with the pZT382 plasmid (Tnp*) showed a Lac⁻ recombinant frequency comprised of this background frequency of spontaneous events plus the frequency of events stimulated by a double-strand break. Thus, for each *rec* genotype, the DSB-stimulated Lac⁻ recombinant frequency was the Lac⁻ frequency of the strain with Tnp* minus the Lac⁻ frequency of the strain with TnpΔ (Figure 7).

The effects of various *rec* mutations on DSB-stimulated recombination between direct repeats support the recombination model. Figure 7 shows that recombination initiated by a double-strand break depends primarily on RecB function. A *recF* mutation had a twofold effect on the frequency of DSB-stimulated events, whereas a *recB* mutation nearly eliminated this stimulation. As expected, *sbc* suppressor mutations restored DSB-stimulated recombination to a *recB* mutant to levels approaching the wild type. As predicted by the model, this restored DSB-stimulated recombination activity in a *recB sbcB sbcC* strain depended on RecF function. As was observed for spontaneous recombination events, DSB-stimulated recombination depends completely on RecA function. Essentially no Lac⁻ segregants are observed in a *recA* mutant under any tested conditions. The effect of a *recD* mutation, eliminating the exonuclease V activity (double-stranded DNA exonuclease) of the RecBCD enzyme, was a twofold decrease in DSB-stimulated recombination. These findings are discussed below.

DISCUSSION

Recombination between chromosomal direct repeats: We have studied homologous recombination events between intact extensive chromosomal direct repeats flanking join-point markers. The detected events

TABLE 4

Effect of *rec* mutations on spontaneous recombination between chromosomal direct repeats

Strain	*rec* genotype	Colony-sectoring assay[a]	Lac⁻ segregation rate[b]	Relative recombination proficiency[c]
TT18931	*rec*⁺	+	4.9×10^{-3}	1
TT18933	*recA*	0	$<4.6 \times 10^{-6}$	<0.00094
TT18934	*recB*	+	3.4×10^{-3}	0.69
TT19080	*recD*	+	ND	ND
TT18935	*recF*	+	4.4×10^{-3}	0.90
TT18936	*recJ*	+	ND	ND
TT18937	*recB recF*	0	5.9×10^{-4}	0.12

[a] Strains were scored for the presence of white Lac⁻ segregant sectors in colonies on NB Xgal plates as illustrated in Figure 3.

[b] Lac⁻ segregation rate, segregants per cell per generation (2 × median segregant frequency × generations^{-1}). The number of culture generations was calculated from determinations of the final and initial colony-forming units in cultures. ND, no data.

[c] Relative recombination proficiency is the ratio of the Lac⁻ segregation rate to that of strain TT18931 (DUP1731 *rec*⁺).

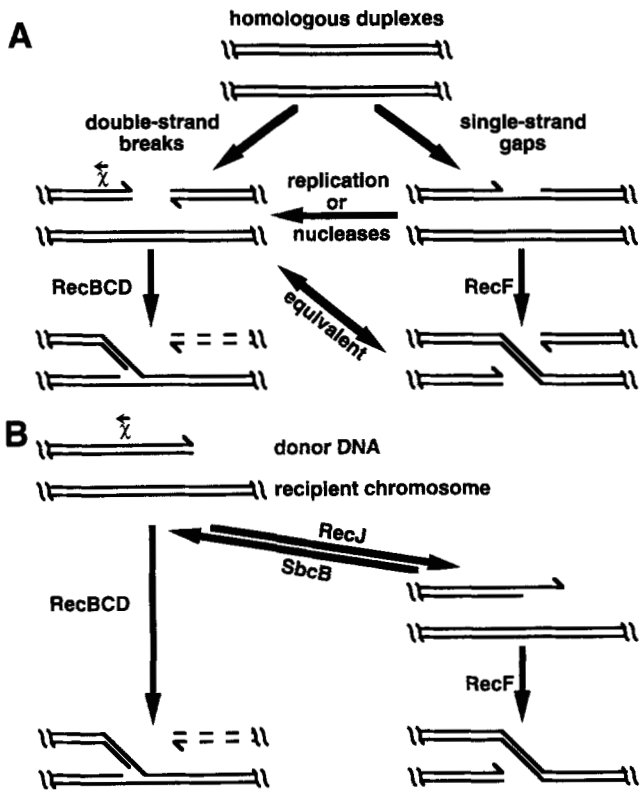

FIGURE 4.—A model for bacterial homologous recombination pathways. DNA strand polarities are indicated with a half arrow at the 3′ end. Strands that are degraded or lost are represented by dashed lines. χ sites and their orientations are indicated. (A) Chromosomal repair and rearrangement through homologous recombination initiated by double-strand breaks or single-strand gaps. The RecBCD pathway and the RecF pathway are alternatives that operate on different initiating DNA lesions. (B) Sexual recombination by integration of donor DNA at the homologous region of the recipient chromosome. Events depicted are at one end of the donated fragment. Because the donor DNA has double-strand ends, these events depend primarily on RecBCD function. In the absence of RecBCD function, RecJ exonuclease produces a suitable substrate for RecF pathway recombination; this substrate can be stabilized by loss of SbcB exonuclease activity.

eliminate the join-point markers located between the repeats. A DNA lesion (either spontaneous or experimentally induced) in one of the repeats provides a substrate that initiates homologous strand invasion at the other repeat followed by resolution to exchange flanking sequences.

An alternative model for the recombination events studied here involves resection and single-strand annealing (SYMINGTON *et al.* 1985). For example, a double-strand break might allow rather extensive RecBCD catalyzed degradation such that the duplication join point (Lac^+) is lost and complementary single-strand tails derived from the two repeats are available to pair. Simple single-strand annealing (not homologous strand invasion) followed by gap filling and ligation would give the Lac^- recombinants. For the reasons outlined below, we think this possibility is unlikely.

1. This alternative model might predict that the homologous strand invasion catalyst, the RecA protein, would be superfluous. However, both spontaneous and DSB-stimulated events observed here depended absolutely on RecA activity.
2. The resection and single-strand annealing model predicts that recombination should depend on exonuclease activities. The exonuclease V activity of the RecBCD enzyme would be the best candidate since it is the major exonuclease of *E. coli* and *S. typhimurium* (reviewed in KOWALCZYKOWSKI *et al.* 1994). This is especially true for recombination events stimulated by a DSB, the RecBCD substrate. Loss of the RecD subunit of RecBCD eliminates exonuclease V activity, but not RecBC-mediated recombination (AMUNDSEN *et al.* 1986; LOVETT *et al.* 1988). Contrary to the predictions of the single-strand annealing model, a *recD* mutation eliminated neither spontaneous nor DSB-stimulated recombination between chromosomal direct repeats.
3. Recombinant frequencies are independent of the length of material between the repeats; this was tested by comparing segregant frequencies of identical duplications, one with a MudJ (10 kb) and one with a MudA (38 kb) at the join point (data not shown). The single-strand annealing model predicts a decrease with increasing distance between repeats.

We conclude that the recombination events scored in this system occur through homologous strand invasion and exchange of flanking markers.

Two major recombination pathways: Classical sexual recombination assays (in which double-strand ends are provided as substrates) revealed a major RecBCD pathway in wild-type strains and a minor or cryptic RecF pathway that includes most of the known recombination functions. This curious state of affairs suggests that recombination pathways can be studied more profitably using an assay system that more closely represents their natural roles. The recombination functions of *E. coli* and *S. typhimurium* probably evolved primarily to accomplish DNA repair, and the formation and reversion of gene duplications. The assay presented here closely approximates this inferred natural role of homologous recombination.

The effects of *rec* mutations on recombination between chromosomal direct repeats suggest that both the RecBCD pathway and the RecF pathway are major recombination pathways in wild-type bacteria. Both *recB* mutants and *recF* mutants are Rec^+ by this assay; *recB recF* double mutants are Rec^-. Thus, recombination between direct repeats can occur by two alternative equally significant routes, one RecB-dependent and one RecF-dependent. Furthermore, in this assay, the

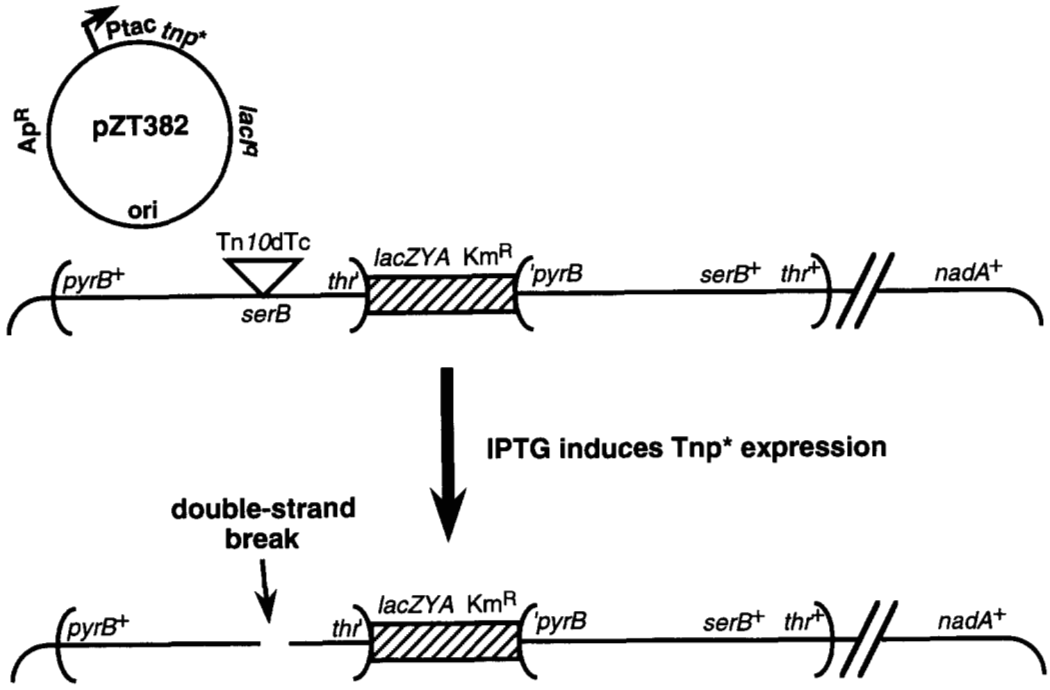

FIGURE 5.—A system for the regulated induction of a single predetermined double-strand break in the bacterial chromosome. Plasmid pZT382 carries a mutant Tn*10* transposase gene, *tnp**. This gene is transcribed from the Ptac promoter that is repressed by LacI repressor protein encoded by the *lacI*^{q} gene (which overproduces LacI protein). The addition of IPTG inactivates LacI and thereby induces Tnp* protein expression. This mutant Tnp* transposase catalyzes the excision of a Tn*10*dTc element from the bacterial chromosome and leaves a double-strand break. Because Tnp* protein is defective for transposition, excision also produces an abortive excised transposon fragment (not shown).

RecF-dependent alternative does not depend on suppressors (*sbcB* and *sbcC*) needed to activate this pathway for sexual recombination. One should note, also, that the recombination defect of *recB recF* double mutants is not as severe as that of a *recA* mutant. Some homologous recombination activity remains in *recB recF* double mutants. The source of this could be either a distinct minor recombination pathway or partial activity of one of the major pathways. Partial activity could be due to functional overlap with a related enzyme.

The RecF pathway redefined: The RecF pathway as observed in this work not only has an elevated status, it depends on a different set of enzymes. The classically defined RecF pathway functions include the RecJ single-strand exonuclease and the RecN protein of unknown function. Mutations of either *recF*, *recJ* (HORII and CLARK 1973) or *recN* (LLOYD *et al.* 1983) knock out sexual recombination in *recBC sbcB sbcC* strains. However, in the direct-repeat assay, neither a *recJ* mutation nor a *recN* mutation has the same effect as a *recF* mutation. Whereas a *recF* mutation combined with a *recB* mutation causes a Rec^{-} phenotype in the direct-repeat assay, *recB recJ* double mutants and *recB recN* double mutants are Rec^{+}. Thus, RecF-dependent sexual recombination and RecF-dependent recombination between chromosomal direct repeats are not equivalent. We propose that the defining difference is the nature of the initiating DNA substrate. In the sexual assay, the RecJ and RecN functions may be needed to allow use of double-strand end substrates in a *recBC sbcB sbcC* strain.

Substrate specificities: The biochemistry and genetics of the RecBCD enzyme and the RecF protein suggest that the primary substrate of the RecBCD pathway is a double-strand break or the double-strand ends of donor DNA, whereas the primary substrate of the RecF pathway is a chromosomal single-strand gap or single-strand tails of donated DNA. An extensive literature (reviewed in KOWALCZYKOWSKI *et al.* 1994; MYERS and STAHL 1994) documents the activity of the RecBCD enzyme at DNA double-strand ends. Recently, the biochemical interactions of RecF protein and DNA have been studied (GRIFFIN and KOLODNER 1990; MADIRAJU and CLARK 1991; HEGDE *et al.* 1996). The RecF protein binds preferentially to double-stranded DNA with a single-strand gap. This affinity exceeds that for either double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA). This implies that RecF binds to dsDNA-ssDNA junctions. Thus, RecF is poised to catalyze recombination initiated at DNA gaps, whereas the RecBCD enzyme is known to produce ssDNA tails after loading at a double-strand end and degrading the DNA until encountering a χ site (DIXON and KOWALCZYKOWSKI 1993; KUZMINOV *et al.* 1994).

The functional dependencies of double-strand-break-induced recombination between chromosomal direct repeats support the proposed substrate specificities of

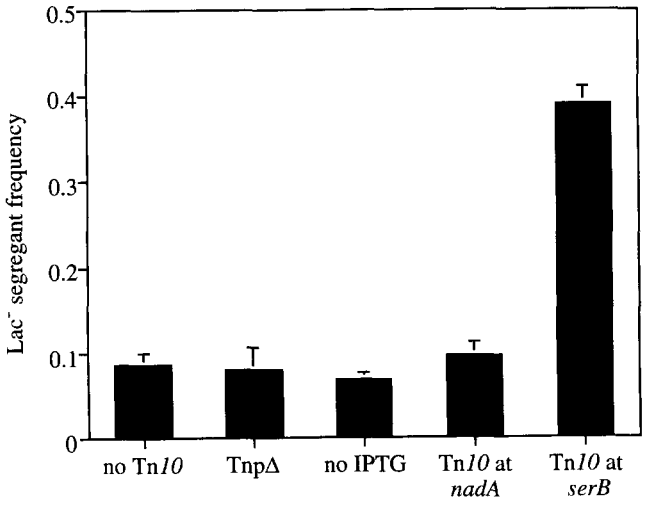

FIGURE 6.—Characterization of the double-strand break system. In a strain carrying direct-order chromosomal repeats flanking a Lac$^+$ join-point element, a DSB in one of the repeats (Tn*10* at *serB*, TT18965; see Figure 5) stimulates the segregation of Lac$^-$ recombinants. Background spontaneous levels of recombination are observed in strains (1) without a DSB site (no Tn*10*, TT18966), (2) expressing a null transposase allele (TnpΔ, TT18964), (3) in the absence of Tnp* expression (no IPTG, TT18965), and (4) with a DSB site far from the duplicated interval (Tn*10* at *nadA*, TT18967).

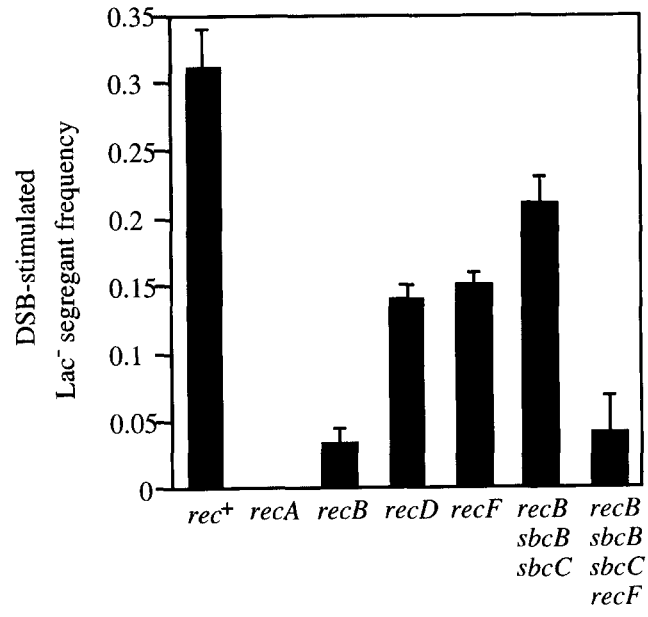

FIGURE 7.—Effects of *rec* mutations on recombination stimulated by a double-strand break. Mutant derivatives of the strain depicted in Figure 5 were constructed. The frequencies of Lac$^-$ segregation events stimulated by a double-strand break (see MATERIALS AND METHODS) are shown for the *rec*$^+$ parent, TT18965 {*purC7* DUP1732[(*pyrB2694 serB1466*::Tn*10*dTc) *MudA*(*thr-469*)]/pZT382}, and derivatives TT18969 (*recA1*), TT18971 (*recB10*), TT18973 (*recD561*), TT18975 (*recF558*), TT18977 [*recB10* DEL1792(*phs-sbcB*) *sbcCD8*], and TT18979 [*recB10* DEL1792(*phs-sbcB*) *sbcCD8 recF558*].

the RecBCD and RecF pathways. A specific DSB in one of the repeats stimulates recombination that depends primarily on RecB function, not RecF function. The RecB dependence of DSB-stimulated recombination is due to the recombinase activity of the RecBC enzyme, not the exonuclease activity of the RecBCD enzyme. This in indicated by the greater severity of the *recB* mutant defect compared to that of a *recD* mutant. A *recF* mutation caused a minor defect in DSB-stimulated recombination; this may reflect conversion of some flush double-strand ends to ends with a single-strand overhang, which can serve as a RecF substrate. This conversion may be more efficient, or the single-strand tail may be more stable in a *recB sbcB sbcC* strain. Thus, a *recB sbcB sbcC* strain showed a restoration of DSB-stimulated recombination; this recombination activity depended on RecF function.

Throughout this article we have discussed mutations that cause a reduced frequency of duplication segregation and we have assumed that the defect is due to a failure of recombinational repair. However, it is possible that a mutation could reduce the frequency of duplication segregation by increasing the fidelity of recombination, that is, reducing unequal sister strand exchanges in favor of equal sister strand exchanges. We think this alternative is unlikely to explain the defect seen in *recB, recF* single or double mutants, because these mutations generally cause a defect in recombination regardless of the assay used. However a *recD* mutation stimulates sexual recombination; thus the minor *recD* defect in double-strand-break-induced duplication segregation (Figure 7) could reflect an increase in equal sister-chromosome exchanges.

Control experiments demonstrated that the stimulation of recombination by a DSB was due to the initiation of recombination at the site of the DSB. One might have predicted that the stimulation of recombination is due to secondary effects of a DSB such as the SOS induction caused by Tn*10*/IS*10* excision (ROBERTS and KLECKNER 1988). However, tests showed that the stimulation was a *cis* effect (at the site of the DSB), not a *trans* effect (like SOS induction); there was no stimulation of recombination if the DSB site was far from the recombining repeats. The same conclusion was reached by HAGEMANN and CRAIG (1993) studying the effects of Tn*7* transposition (through a similar mechanism leaving a double-strand break) on recombination. Furthermore, the fact that induced DSBs increase the proportion of recombinants indicates that the frequency of initiating DNA lesions limits the frequency of recombination events.

A recombination model: The key feature of the recombination model presented in Figure 4 is that different pathways operate on different initiating DNA structures. The experiments and model described here do not address the final processing of these substrates to mature recombinant products. Figure 4A depicts spon-

taneous recombination between generic homologous sequences. These sequences could be intermolecular or intramolecular; they could be ectopic or at the same position on sister chromosomes. Figure 4B shows the sexual recombination of a linear homologous donor DNA and an intact recipient chromosome.

DNA double-strand ends may be generated directly, or indirectly through a single-strand gap intermediate. The mechanisms of rolling circle replication, endonuclease cleavage, transposition, transduction, and conjugation produce DNA molecules with double-strand ends. Indirectly, single-strand gaps may be converted to double-strand breaks through DNA replication, nuclease activity, or recombination.

It has been pointed out previously that a replication fork encountering a single-strand gap in the template molecule will collapse; this leaves one intact daughter chromosome and one terminating at a double-strand end (reviewed in ASAI *et al.* 1994; KUZMINOV 1995). The RecBCD enzyme can load at the broken end and degrade the molecule until encountering a χ site where it will start producing single-stranded DNA through its helicase activity (DIXON and KOWALCZYKOWSKI 1993). This single-stranded DNA is a substrate for RecA-catalyzed homologous strand invasion of the intact molecule. The resulting D-loop structure with an invading 3′ end may serve as a reinitiation site for DNA replication.

Nuclease activities could convert a single-strand gap to a double-strand break with flush ends. BENSON and ROTH (1994) have proposed that the SbcB protein has two nuclease activities, its known 3′ to 5′ exonuclease activity and a putative endonuclease activity. The putative endonuclease activity cuts the continuous strand opposite a gap at the junction with the 3′ end thereby generating one flush double-strand end and one with a 3′ single-strand tail. The exonuclease I activity (3′ to 5′ single-strand exonuclease; KUSHNER *et al.* 1972) of SbcB degrades this tail to produce a second flush double-strand end. Thus, SbcB might have two activities that serve to prepare flush double-strand ends, known substrates for the RecBCD enzyme.

Nonreciprocal recombination by the RecF pathway (or any pathway) would produce one recombinant molecule and one broken molecule. If the ends of this molecule are not flush, single-strand exonucleases like RecJ (5′ to 3′; LOVETT and KOLODNER 1989) or SbcB (3′ to 5′; KUSHNER *et al.* 1972) can make them flush. The broken molecule will then be a substrate for a subsequent recombination event mediated by RecBCD. The first recombinant molecule could serve as a target for the second exchange. This scenario resembles the two-step model for recombination proposed by MAHAN and ROTH (1989b).

Gaps may be converted easily to chromosome breaks. Thus, *recF* mutants, which fail to repair gaps, have good viability (data not shown) because the RecBCD pathway ultimately can repair the damage. Failure to repair a double-strand break may be a more life-threatening problem. The poor viability of *recB* mutants (data not shown) supports this. However, the occurrence of a double-strand break is not always a terminal condition in the absence of RecBCD activity. This is indicated by the genetics of sexual recombination and DSB-stimulated recombination. In *recB* mutants, residual recombination initiated at double-strand ends can be restored to high efficiency by the addition of suppressor mutations, *sbcB* and *sbcC* (KUSHNER *et al.* 1971, 1972; Figure 7). Conventional thought on this is that exonucleases like RecJ can degrade one strand leaving a recombinagenic 3′-ended single-strand tail, allowing RecF-mediated recombination. The elimination of SbcB's 3′ exonuclease activity stabilizes this end. Alternatively, we propose that the effect is not strictly the production and stabilization of a single-strand tail; rather, it is the generation and stabilization of a dsDNA-ssDNA junction, a suitable RecF substrate. The role of the RecF protein may be either to catalyze strand separation or to facilitate RecA filament formation on a single-stranded region.

The phenotypes of *recB recJ* double mutants further support the existence of alternatives to RecBCD for repair of double-strand breaks. RecJ may be a RecF pathway function in sexual assays only because it is needed to convert a flush double-strand end to an end with a recessed single-strand (a dsDNA-ssDNA junction) for RecF binding. This idea predicts that repair of DSBs depends primarily on RecBCD and secondarily (in the absence of RecBCD) on RecJ, whereas recombination initiated at single-strand gaps depends on neither of these. This prediction is supported by the following. (1) The *recB recJ* double mutants show SOS-constitutivity and extremely low viability, lower than that of either *recB* mutants or *recJ* mutants. (2) The *recB recJ* double mutants are nonpermissive for P22 *erf*. The injected linear P22 genome of this mutant phage requires recircularization by host-mediated recombination at double-strand-ended terminal redundancies; this occurs in single *recB* or *recJ* mutants but not in the *recB recJ* double mutant. (3) The *recB recJ* double mutants, although sick, are still recombination proficient in the colony-sectoring assay. We suggest that these exchanges are stimulated by single-strand gaps that continue to be effective in initiating recombination in a *recB recJ* double mutant.

The assay system described here provides a way to score recombination events that are initiated by structures other than double-strand breaks. This is unlike sexual recombination assays that all provide substrates with double-strand ends. The single-strand gap is likely to be the predominant, recombination-initiating alternative to a double-strand break. Single-strand gaps are formed when a replication fork traverses a region con-

taining damaged (non-pairing) bases in one strand. Single-strand excision tracts formed during the repair of damaged DNA bases also may serve as recombination substrates. We have used the colony-sectoring assay as a screen for mutants with low rates of recombination. Preliminary characterization of such mutants indicates that oxidative DNA damage may be the major source of endogenous recombination-initiating lesions.

We thank N. BENSON, R. DAWSON, K. HAACK, D. HANIFORD, N. KLECKNER, A. KUZMINOV, R. MYERS, L. MIESEL, G. SMITH, F. STAHL, and D. THALER for sharing strains, plasmids, and influential discussions. This work was supported by grant GM-27068 (J.R.R.) and predoctoral training grant 5 T32 GM07464-18 (T.G.) from the National Institutes of Health.

LITERATURE CITED

AMUNDSEN, S. K., A. F. TAYLOR, A. M. CHAUDHURY and G. R. SMITH, 1986 *recD:* a gene for an essential third subunit of exonuclease V. Proc. Natl. Acad. Sci. USA **83:** 5558–5562.

ANDERSON, P., and J. R. ROTH, 1981 Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. Proc. Natl. Acad. Sci. USA **78:** 3113–3117.

ASAI, T., D. B. BATES and T. KOGOMA, 1994 DNA replication triggered by double-strand breaks in *E. coli:* dependence on homologous recombination functions. Cell **78:** 1051–1061.

BELTRAN, P., J. M. MUSSER, R. HELMUTH, J. J. FARMER, W. M. FRERICHS *et al.*, 1988 Toward a population genetic analysis of *Salmonella*: genetic diversity and relationships among strains of serotypes *S. choleraesuis, S. derby, S. dublin, S. enteritidis, S. heidelberg, S. infantis, S. newport,* and *S. typhimurium.* Proc. Natl. Acad. Sci. USA **85:** 7753–7757.

BENDER, J., J. KUO and N. KLECKNER, 1991 Genetic evidence against intramolecular rejoining of the donor DNA molecule following IS*10* transposition. Genetics **128:** 687–694.

BENSON, N. R., and J. R. ROTH, 1994 Suppressors of *recB* mutations in *Salmonella typhimurium.* Genetics **138:** 11–29.

BERKOWITZ, D., J. M. HUSHON, H. J. J. WHITFIELD, J. ROTH and B. N. AMES, 1968 Procedure for identifying nonsense mutations. J. Bacteriol. **96:** 215–220.

BLANAR, M. A., S. J. SANDLER, M. E. ARMENGOD, L. W. REAM and A. J. CLARK, 1984 Molecular analysis of the *recF* gene of *Escherichia coli.* Proc. Natl. Acad. Sci. USA **81:** 4622–4626.

BOTSTEIN, D., and M. J. MATZ, 1970 A recombination function essential to the growth of bacteriophage P22. J. Mol. Biol. **54:** 417–440.

CASTILHO, B. A., P. OLFSON and M. J. CASADABAN, 1984 Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158:** 488–495.

CLARK, A. J., 1971 Toward a metabolic interpretation of genetic recombination. Annu. Rev. Microbiol. **25:** 437–464.

CLARK, A. J., 1973 Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7:** 67–86.

DAVIS, R. W., D. BOTSTEIN and J. R. ROTH, 1980 *Advanced Bacterial Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

DIXON, D. A., and S. C. KOWALCZYKOWSKI, 1993 The recombination hotspot χ is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. Cell **73:** 87–96.

EISENSTARK, A., R. EISENSTARK, J. VAN DILLEWIJN and A. RORSH, 1969 Radiation-sensitive and recombinationless mutants of *Salmonella typhimurium.* Mutat. Res. **8:** 497–504.

ELLIOTT, T., and J. R. ROTH, 1988 Characterization of Tn*10d*-Cam: a transposition-defective Tn*10* specifying chloramphenicol resistance. Mol. Gen. Genet. **213:** 332–338.

GRIFFIN, T. J., and R. D. KOLODNER, 1990 Purification and preliminary characterization of the *Escherichia coli* K12 RecF protein. J. Bacteriol. **172:** 6291–6299.

HAACK, K. R., and J. R. ROTH, 1995 Recombination between chromosomal IS*200* elements supports frequent duplication formation in *Salmonella typhimurium.* Genetics **141:** 1245–1252.

HAGEMANN, A. T., and N. L. CRAIG, 1993 Tn*7* transposition creates a hotspot for homologous recombination at the transposon donor site. Genetics **133:** 9–16.

HANIFORD, D. B., A. R. CHELOUCHE and N. KLECKNER, 1989 A specific class of IS*10* transposase mutants are blocked for target site interactions and promote formation of an excised transposon fragment. Cell **59:** 385–394.

HANIFORD, D. B., H. W. BENJAMIN and N. KLECKNER, 1991 Kinetic and structural analysis of a cleaved donor intermediate and a strand transfer intermediate in Tn*10* transposition. Cell **64:** 171–179.

HEGDE, S. P., M. RAJAGOPALAN and M. V. V. S. MADIRAJU, 1996 Preferential binding of *Escherichia coli* RecF protein to gapped DNA in the presence of adenosine (γ-thio) triphosphate. J. Bacteriol. **178:** 184–190.

HONG, J-S., and B. N. AMES, 1971 Localized mutagenesis of any small region of the bacterial chromosome. Proc. Natl. Acad. Sci. USA **68:** 3158–3162.

HORII, Z. I., and A. J. CLARK, 1973 Genetic analysis of the RecF pathway of genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80:** 327–344.

HUGHES, K. T., and J. R. ROTH, 1984 Conditionally transposition-defective derivative of Mud1 (Amp Lac). J. Bacteriol. **159:** 130–137.

HUGHES, K. T., and J. R. ROTH, 1985 Directed formation of deletions and duplications using Mu*d*(Ap, *lac*). Genetics **109:** 263–282.

KOWALCZYKOWSKI, S. C., D. A. DIXON, A. K. EGGLESTON, S. D. LAUDER and W. M. REHRAUER, 1994 Biochemistry of homologous recombination in *Escherichia coli.* Microbiol. Rev. **58:** 401–465.

KUSHNER, S. R., H. NAGAISHI, A. TEMPLIN and A. J. CLARK, 1971 Genetic recombination in *Escherichia coli*: the role of exonuclease I. Proc. Natl. Acad. Sci. USA **68:** 824–827.

KUSHNER, S. R., H. NAGAISHI and A. J. CLARK, 1972 Indirect suppression of *recB* and *recC* mutations by exonuclease I deficiency. Proc. Natl. Acad. Sci. USA **69:** 1366–1370.

KUZMINOV, A., 1995 Collapse and repair of replication forks in *Escherichia coli.* Mol. Microbiol. **16:** 373–384.

KUZMINOV, A., E. SCHABTACH and F.W. STAHL, 1994 χ sites in combination with RecA protein increase the survival of linear DNA in *Escherichia coli* by inactivating exoV activity of RecBCD nuclease. EMBO J. **13:** 2764–2776.

LEDERBERG, J., and E. L. TATUM, 1946 Gene recombination in *Escherichia coli.* Nature **158:** 558.

LLOYD, R. G., and C. BUCKMAN, 1985 Identification and genetic analysis of *sbcC* mutations in commonly used *recBC sbcB* strains of *Escherichia coli* K12. J. Bacteriol. **164:** 836–844.

LLOYD, R. G., and A. THOMAS, 1984 A molecular model for conjugational recombination in *E. coli* K12. Mol. Gen. Genet. **197:** 328–336.

LLOYD, R. G., S. M. PICKSLEY and C. PRESCOTT, 1983 Inducible expression of a gene specific to the RecF pathway for recombination in *Escherichia coli* K12. Mol. Gen. Genet. **190:** 162–167.

LOVETT, S. T., and R. D. KOLODNER, 1989 Identification and purification of a single-stranded-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli.* Proc. Natl. Acad. Sci. USA **86:** 2627–2631.

LOVETT, S. T., C. LUISI-DELUCA and R. D. KOLODNER, 1988 The genetic dependence of recombination in *recD* mutants of *Escherichia coli.* Genetics **120:** 37–45.

MADIRAJU, M. V. V. S., and A. J. CLARK, 1991 Effect of RecF protein on reactions catalyzed by RecA protein. Nucleic Acids Res. **19:** 6295–6300.

MAHAN, M. J., and J. R. ROTH, 1989a *recB* and *recC* genes of *Salmonella typhimurium.* J. Bacteriol. **171:** 612–615.

MAHAN, M. J., and J. R. ROTH, 1989b Role of RecBC function in formation of chromosomal rearrangements: a two-step model for recombination. Genetics **121:** 433–443.

MAHAN, M. J., J. CASADESUS and J. R. ROTH, 1992 The *Salmonella typhimurium* RecJ function permits growth of P22 *abc* phage on *recBCD*$^+$ hosts. Mol. Gen. Genet. **232:** 470–478.

MAHAN, M. J., A. GARZON and J. CASADESUS, 1993 Host RecJ is re-

quired for growth of P22 *erf* bacteriophage. J. Bacteriol. **175:** 288–290.

MAYNARD SMITH, J., N. H. SMITH, M. O'ROURKE and B. G. SPRATT, 1993 How clonal are bacteria? Proc. Natl. Acad. Sci. USA **90:** 4384–4388.

MIESEL, L., and J. R. ROTH, 1994 *Salmonella recD* mutations increase recombination in a short sequence transduction assay. J. Bacteriol. **176:** 4092–4103.

MYERS, R. S., and F. W. STAHL, 1994 χ and the RecBCD enzyme of *Escherichia coli*. Annu. Rev. Genet. **28:** 49–70.

MYERS, R. M., L. S. LERMAN and T. MANIATIS, 1985 A general method for saturation mutagenesis of cloned DNA fragments. Science **229:** 242–247.

OCHMAN, H., and R. K. SELANDER, 1984 Evidence for clonal population structure in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81:** 198–201.

POTEETE, A. R., A. C. FENTON and K. C. MURPHY, 1988 Modulation of *Escherichia coli* RecBCD activity by the bacteriophage λ Gam and P22 Abc functions. J. Bacteriol. **170:** 2012–2021.

ROBERTS, D., and N. KLECKNER, 1988 Tn*10* transposition promotes RecA-dependent induction of a λ prophage. Proc. Natl. Acad. Sci. USA **85:** 6037–6041.

ROTH, J. R., 1970 Genetic techniques in studies of bacterial metabolism. Methods Enzymol. **17A:** 3–35.

ROTH, J. R., N. BENSON, T. GALITSKI, K. HAACK, J. LAWRENCE *et al.*, 1996 Rearrangements of the bacterial chromosome—formation and applications, pp. 2256–2276 in *Escherichia coli and Salmonella typhimurium: Molecular and Cellular Biology*, edited by F. C. NEIDHARDT. American Society of Microbiology, Washington, DC.

RUDD, K. E., and R. MENZEL, 1987 *his* operons of *Escherichia coli* and *Salmonella typhimurium* are regulated by DNA supercoiling. Proc. Natl. Acad. Sci. USA **84:** 517–521.

SCHMIEGER, H., 1971 A method for detection of phage mutants with altered transducing ability. Mol. Gen. Genet. **110:** 378–381.

SCLAFANI, R. A., and J. A. WECHSLER, 1981 High frequency of genetic duplications in the *dnaB* region of the *Escherichia coli* K12 chromosome. Genetics **98:** 677–690.

SEGALL, A., 1987 Studies on genome rearrangements and intrachromosomal recombination in *Salmonella typhimurium*. PhD. Thesis, University of Utah, Salt Lake City, UT.

SMITH, G. R., 1988 Homologous recombination in procaryotes. Microbiol. Rev. **52:** 1–28.

STARK, M. J. R., 1987 Multicopy expression vectors carrying the *lac* repressor gene for regulated high-level expression of genes in *Escherichia coli*. Gene **51:** 255–267.

SYMINGTON, L., P. MORRISON and R. KOLODNER, 1985 Intramolecular recombination of linear DNA catalyzed by the *Escherichia coli* RecE recombination system. J. Mol. Biol. **186:** 515–525.

VOGEL, H. J., and D. M. BONNER, 1956 Acetylornithase of *Escherichia coli:* partial purification and some properties. J. Biol. Chem. **218:** 97–106.

WANG, T-C. V., and K. C. SMITH, 1983 Mechanisms for *recF*-dependent and *recB*-dependent pathways of postreplication repair in UV-irradiated *Escherichia coli uvrB*. J. Bacteriol. **156:** 1093–1098.

WING, J. P., M. LEVINE and H. O. SMITH, 1968 Recombination-deficient mutant of *Salmonella typhimurium*. J. Bacteriol. **95:** 1828–1834.

Communicating editor: N. L. CRAIG