Approaches to Half-Tetrad Analysis in Bacteria: Recombination Between Repeated, Inverse-Order Chromosomal Sequences

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ABSTRACT

In standard bacterial recombination assays, a linear fragment of DNA is transferred to a recipient cell and, at most, a single selected recombinant type is recovered from each merozygote. This contrasts with fungal systems, for which tetrads allow recovery of all meiotic products, including both ultimate recombinant products of an apparent single act of recombination. We have developed a bacterial recombination system in which two recombining sequences are placed in inverse order at widely separated sites in the circular chromosome of *Salmonella typhimurium*. Recombination can reassort markers between these repeated sequences (double recombination and apparent gene conversion), or can exchange flanking sequences, leading to inversion of the chromosome segment between the recombining sequences. Since two recombinant products remain in the chromosome of a recombinant with an inversion, one can, in principle, approach the capability of tetrad analysis. Using this system, the following observations have been made. (a) When long sequences (40 kb) recombine, conversion frequently accompanies exchange of flanking sequences. (b) When short sequences (5 kb) recombine, conversion rarely accompanies exchange of flanks. (c) Both *recA* and *recB* mutations eliminate inversion formation. (d) The frequency of exchanges between short repeats is more sensitive to the distance separating the recombining sequences in the chromosome. The results are presented with the assumption that inversions occur by simple interaction of two sequences in the same circular chromosome. In an appendix we discuss mechanistically more complex possibilities, some of which could also apply to standard fungal systems.

THE study of homologous recombination in fungi has benefited from the recovery and characterization of both products of a single meiotic recombination event associated as haploid spores in an ascus (reviewed by STAHL 1979; PETES, MALONE and SYMINGTON 1991). In bacteria, the haploid nature of the genome makes it difficult to recover both products of an exchange. In most bacterial recombination systems, a single selected recombinant type is recovered; the second, unselected, product of the same exchange is lost. Exceptions to this rule are phage crosses, in which both recombination products may be represented among the progeny of a single burst (SARTHY and MESELSON 1976). However, since multiple rounds of phage recombination occur, the origin of complementary recombinant types remains uncertain. Much information on bacterial recombination has been gathered from conjugation and transduction experiments, both of which involve interaction between a linear donated molecule (with recombinogenic double-stranded ends) and the uninterrupted circular bacterial chromosome. Double-stranded ends are also present in the infecting lambda phage genome prior to circularization and in the replicated lambda concatamer following cutting at *cos*. Thus, in these standard recombination systems, substrates are provided with features that are unlikely to be present in "normal" uninfected (or unmated) cells but are likely to influence the recombination process.

Transfer of chromosome fragments (mating, transduction, transformation) appears to be rare for enteric bacteria in nature (OCHMAN and SELANDER, 1984; MILKMAN and BRIDGES, 1993). In view of this, it seems likely that the recombination systems of these bacteria evolved, not for mating, but to perform the internal sister-strand exchanges needed for repair and duplication formation, processes for which the initiating substrates (nicks or breaks) must be generated endogenously. This endogenous recombination is like that in eukaryotic cells, which also uses internal substrates and does not normally deal with injected fragments. The sexual recombination systems used to study bacterial recombination may focus on events that differ in important respects from the events for which the recombination system of bacteria evolved.

If the bacterial recombination system normally acts primarily on sequences within a single chromosome, it has the potential to cause chromosome rearrangements. Selective forces acting to conserve the genetic map may have secondarily shaped the bacterial

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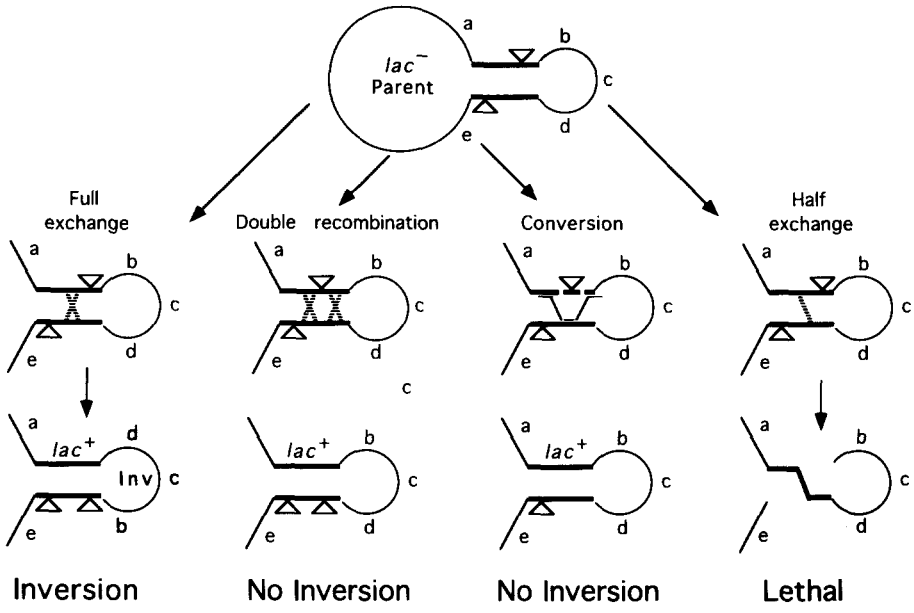


FIGURE 1.—Recombination events between inversely oriented homologous sequences in the same circular bacterial chromosome. Thick lines represent homologous sequences. Triangles represent insertions of drug resistance elements within the homologous sequences. Note that inversion requires a full exchange, defined as an event in which both pairs of flanking sequences are rejoined.

recombination mechanisms so as to minimize inversion formation (which destabilizes the map), while permitting the selectively valuable repair and duplication processes (ANDERSON and ROTH 1977; TLSTY, ALBERTINI and MILLER 1984; SONTI and ROTH 1989).

We have previously described a recombination detection system in which all the recombining sequences are located within the circular bacterial chromosome (SEGALL, MAHAN and ROTH 1988). In this system, initiating substrates for recombination (nicks or breaks) must be generated endogenously. Two homologous sequences, containing distinct genetic markers, are placed in inverse orientation at separated sites in the chromosome (adapted from KONRAD 1969, 1977). Recombination between these inverse-order sequences can have either of two outcomes (see Figure 1). Some recombinants carry an inversion of the chromosomal segment between the sites of the recombining sequences. These could be caused by a single full crossover event. Other recombinants show reassortment of genetic markers between the repeated sequences but have not acquired an inversion. Some of these retain all genetic markers and may have been caused by double crossovers; others have lost one of the genetic markers and appear to have resulted from gene conversion events.

Only a subset of the many chromosomal intervals tested are permissive for inversion. Inverse repeats placed at nonpermissive sites can exchange information (apparent double exchanges and conversions), but do not generate a recoverable inversion (KONRAD 1969, 1977; ZIEG and KUSHNER 1978; REBOLLO, FRANCOIS and LOUARN 1988; SEGALL, MAHAN and ROTH 1988; SEGALL and ROTH 1989). The nonpermissivity of recombination at some sites is not due to lethality of the final inversion product, but could reflect a paucity of sites for initiating or resolving the required full exchange (SEGALL, MAHAN and ROTH 1988; L. MIESEL, A. M. SEGALL and J. R. ROTH, manuscript submitted).

Direct order repeats can recombine to form duplications regardless of the chromosomal position of the recombining sequences (ANDERSON and ROTH 1981). These internal recombinants can arise by sister strand exchanges in which either one pair of flanking sequences is rejoined (half exchange) or by exchange of both flanks (MAHAN and ROTH 1988, 1989) (see Figure 4). The unrestricted formation of duplications and the fact that they can form by a half exchange suggests that they might form by a different mechanism from that leading to inversion.

We have tested this prediction by observing the dependence of various internal recombination events on the RecBCD enzyme, which is required for the major, reciprocal recombination pathway in *Escherichia coli* (CLARK 1973; THALER and STAHL 1988; KING and RICHARDSON 1986; TAKAHASHI *et al.* 1992). We show here that mutations in the *recB* gene eliminate inversion formation, but have little effect on the frequency of duplications. We suggest that this difference reflects the fact that inversion formation formally requires a full exchange (both flanks rejoined), which can be provided only by the RecBCD pathway. In contrast, duplications can be generated either by a full exchange (using RecBCD) or by a half-exchange event (RecBCD-independent), in which only one pair of flanking sequences is exchanged and two broken ends are generated.

Furthermore, we examine the products of recombination between inversely oriented sequences located at sites that permit inversion of the intervening chromosomal segment (permissive sites). We have assessed

TABLE 1

Strain list

Strain	Genotype
TT12262	*trp(ED)2490*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *srl-201* (inverse order MudA insertions)
TT12265	*trp(E,D)2484*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *srl-201* (direct order MudA insertions)
TT13420	*thr-469*::MudA(*lacZ478*::Tn*5*) *pyrB2691*::MudA(*lacZ950*::Tn*10*) *srl-201* (inverse order MudA insertions)
TT13422	*pyrC2688*::MudA(*lacZ478*::Tn*5*) *hisD9953*MudA(*lacZ950*::Tn*10*) *srl-201* (inverse order MudA insertions)
TT13425	*pncB220*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *srl-201* (inverse order MudA insertions)
TT13427	*trp(ED)2490*::MudA *hisD9953*::MudJ(*lacZ951*:Tn*10*) (inverse order MudA and MudJ insertions)
TT13428	*trp(ED)2490*::MudA *hisD9953*MudJ(*lacZ950*::Tn*10*) (inverse order MudA and MudJ insertions)
TT13430	*araC661*::MudJ(*lacZ950*::Tn*10*) *thr-458*::MudA (inverse order MudA and MudJ insertions)
TT13435	*araC661*::MudJ(*lacZ951*::Tn*10*) *his(BH)1261*::MudA *trpB164* (inverse order MudA and MudJ insertions)
TT13437	*araC661*::MudJ(*lacZ950*::Tn*10*) *nadA216*::MudA (inverse order MudA and MudJ insertions)
TT13477	*araC661*::MudJ(*lacZ950*::Tn*10*) *thr-458*::MudA *leuA414*(am) *recB556*(del) *zbg-53*::Tn*10*dCm (inverse order MudA and MudJ insertions)
TT13478	*araC661*::MudJ(*lacZ950*::Tn*10*) *thr-458*::MudA *leuA414*(am) *recB*$^+$ *zbg-53*::Tn*10*dCm (inverse order MudA and MudJ insertions)
TT13481	*araC661*::MudJ(*lacZ950*::Tn*10*) *thr-458*::MudA *leuA414*(am) *recA1 srl-203*::Tn*10*dCm (inverse order MudA and MudJ insertions)
TT13482	*araC661*::MudJ (*lacZ950*::Tn*10*) *thr-458*::MudA *leuA414*(am) *rec*$^+$ *srl-203*::Tn*10*dCm (inverse order MudA and MudJ insertions)
TT17402	*trp(E,D)2484*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *srl-203*::Tn*10d*Cm, *recA1* (direct order MudA insertions)
TT17403	*trp(E,D)2484*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ*950::Tn*10*) *srl-203*::Tn*10d*Cm, *recA*$^+$ (direct order MudA insertions)
TT17404	*trp(E,D)2484*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *zbg-53*::Tn*10d*Cm, *recB556*(del) (direct order MudA insertions)
TT17405	*trp(E,D)2484*::MudA(*lacZ478*::Tn*5*) *hisD9953*::MudA(*lacZ950*::Tn*10*) *zbg-53*::Tn*10d*Cm, *recB*$^+$ (direct order MudA insertions)
TT17432	*araC661*::MudJ(*lacZ951*::Tn*10*) *trp(ED)2490*::MudA *srl-201* (inverse order MudA and MudJ insertions)

the effect of chromosomal position and length of recombining sequences on the frequency of inversions and on the association of gene conversion with exchange of flanking sequences.

The results are described as if inversions were generated by single full exchanges between inverse-order sequences in the same circular chromosome. While we think it is likely that inversions arise in this way, we wish to make it clear that alternative explanations exist. These more complicated alternatives are described and discussed in the APPENDIX.

MATERIALS AND METHODS

Bacterial strains: All the strains used in this study are derived from *Salmonella typhimurium* strain LT2. Their genotypes are listed in Table 1. Both Mud elements used are transposition-defective and are not subject to movement in the course of the experiments presented. Genotypes of parental strains were tested fully before each experiment, since the strains are complicated and interpretation of results depends on the correctness of these strains.

Media: We used standard media for Salmonella (VOGEL and BONNER 1956; DAVIS, BOTSTEIN and ROTH 1980). The indicator dye 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal; Bachem Fine Chemicals) was added at a final concentration of 25 mg/ml. E medium lacking citrate (BERKOWITZ *et al.* 1968) was supplemented with lactose or sorbitol at 0.2% w/v when testing growth on these carbon sources. All nutritional supplements were obtained from Sigma.

Transduction methods: We used a high frequency transducing derivative of phage P22 (HT105/1 *int-201*) (SANDERSON and ROTH 1983). Transductants were purified non-selectively on green indicator plates (CHAN *et al.* 1972), to distinguish phage-free colonies from phage-infected colonies. Phage sensitivity was tested by cross-streaking phage-free clones with P22 H5, a clear plaque mutant. P22 lysates were made as described (DAVIS, BOTSTEIN and ROTH 1980).

MudA and MudJ phage: The MudA phage (originally Mu *d*1-8(Ap, *lac*); HUGHES and ROTH 1984) is a transposition-defective, full-sized (39 kb) derivative of Mu *d1* (Ap, *lac*) (CASADABAN and COHEN 1979). This phage transposes only in hosts carrying an amber suppressor; in Su^{wt} (non-suppressor) hosts the prophage can be inherited in crosses only by recombination and, once acquired, is not subject to transposition. The MudA variant encodes ampicillin resistance (Ap^R) and carries the promoter-less *lacZYA* operon with *trp* translational start signals. The smaller MudJ element (11 kb) is the transposition defective MudII1683(Kn, *lac*) mini-Mud element of CASTILHO, OLFSON and CASADABAN (1984). In MudJ, a Kn^R determinant (derived from Tn*5*) replaces most of the Mu genes. The MudA and MudJ phages share a promoterless *lac* operon at one end and about 1 kb of Mu sequence at the opposite end. When inserted within a gene in the proper orientation, both Mud transposons form operon fusions that permit *lac* operon expression using the promoter of the target gene. Such fusion strains form blue colonies on Xgal indicator plates, and may or may not grow on plates containing lactose as a sole carbon source, depending on the strength of the promoter in question. Transductional inheritance of the *ca.* 40-kb MudA element requires two transduced fragments (HUGHES and ROTH 1985). Therefore, a high m.o.i. (>10) was used in all P22 transduction crosses in which we selected for inheritance of the entire insertion by recombination through flanking homologies; a low m.o.i. (<1) was used to transduce MudJ insertions and to transfer markers (*e.g.*, *lacZ*::Tn*10*) internal to either of the two Mud elements.

Tn*5* and Tn*10* insertions: Insertions of Tn*10* in different

deletion intervals of *lacZ* (FOSTER 1977) were kindly provided by T. FOSTER. The *lacZ478*::Tn*5* insertion in *lacZ* was generously donated by D. BERG. Its location in *lacZ* was roughly mapped by cotransduction tests with the above Tn*10* insertions to a region between the Tn*10* insertion in deletion interval 6 (*lacZ*; *lacZ951*::Tn*10*) and the Tn*10* insertion in deletion interval 26 (*lacZ*; *lacZ950*::Tn*10*). These intervals of the *lacZ* gene were defined by ZIPSER *et al.* (1970). Recently, many Tn*10* insertion sites within *lacZ* have been sequenced (BENDER and KLECKNER 1992). The *lacZ950*::Tn*10* is located at the major Tn*10* hotspot in the *lacZ* gene, at base pair 3026. The *lacZ951*::Tn*10* insertion is most likely located between base pair 178 and base pair 286, the site of a less prominent Tn*10* hotspot.

Construction of experimental strains: The construction of strains used in the recombination screens has been described in detail (SEGALL and ROTH 1989). Briefly, the first MudA prophage was transduced into the desired background selecting Ap^R. The *lacZ* gene of this first Mud prophage was then inactivated by transducing in a *lacZ*::Tn*5* insertion. The *lacZ* gene of the second MudA element was independently inactivated with a *lacZ*::Tn*10* in a separate background, and this element MudA(*lacZ*::Tn*10*) was transduced as a unit into the strain with the MudA(*lacZ*::Tn*5*), selecting Tc^R. Tetracycline-resistant transductants were screened for the presence of Tn*5* (kanamycin resistance) and for the auxotrophic requirements conferred by both Mud insertions.

The construction of strains with one MudA element and one MudJ element was similar. A non-transcribed MudA insertion (Lac^-) was transduced into the background of choice selecting Ap^R. The MudJ(*lacZ*::Tn*10*) element was transduced into this strain selecting Tc^R. Since the MudJ element is small (11 kb), it was frequently inherited by recombination through flanking homology. Tetracycline resistant transductants were tested for antibiotic resistance (Ap^R, Kn^R) and for the presence of auxotrophic requirements conferred by the two Mud insertions. The final product of these constructions was a phenotypically Lac^- strain with two unexpressed *lac* regions that could recombine to yield a Lac^+ derivative.

Linkage disruption tests: Linkage disruption by an inversion endpoint was diagnosed by a decrease in the efficiency of repair of the auxotrophic requirements caused by the parental Mud insertions (SCHMID and ROTH 1983). Lac^+ clones were transduced with P22 lysates grown on wild-type LT2, and transductants prototrophic for one or the other endpoint were selected. In strains carrying an inversion of the interval between the Mud sequences, prototrophic transductants were recovered >10-fold less frequently than in strains without an inversion. The rare prototrophic transductants recovered from inversion strains arise in recipient clones in which inverted material had re-flipped to the parental orientation.

To test large numbers of independent Lac^+ clones, 25 transductions were performed on a single plate spread with approximately 10^9 phage particles. Single colonies of the recipients were transferred to individual patches on the phage plate. The parental noninverted strain was included on the same plate, allowing comparison of the number of Lac^+ transductants. If a transduction patch showed evidence of linkage disruption (a reduced number of prototrophic colonies), the Lac^+ clone was retested individually on an entire plate, using an LT2 donor phage at a m.o.i. of 0.1. To verify that the reduction in the number of prototrophic recombinants was specific for the boundary of the rearrangement (i.e., linkage disruption), putative inversion clones were also transduced with a marker unlinked to the inversion endpoints, often an unlinked Tn*10d*Cm element, to show that such markers were inherited normally.

Construction of recombination-deficient strains: A *recB* deletion mutation (*recB556*) was constructed by excision of a *recB*::Tn*10* insertion (K. HUGHES, unpublished results). This *recB* deletion was introduced into strains with two Mud elements (direct or inverse order) by cotransduction with a nearby Tn*10d*Cm element (*zbg-53*::Tn*10d*Cm); the donor strain in this cross (TT12272) was constructed by Michael Mahan. Transductants were screened for sensitivity to UV to identify those inheriting the linked *recB* deletion. An isogenic UV^S and UV^R pair of transductants was used to study the effect of a *recB* mutation on formation of inversions.

The *recA1* mutation was moved into appropriate backgrounds by linkage to the *srl-203*::Tn*10d*Cm element (ELLIOTT and ROTH 1988). Transductants (Cm^R) were tested for UV sensitivity, and isogenic Rec^+ and Rec^- transductants were compared in the recombination screen.

Linkage disruption tests in *rec*$^-$ strains: The presence of inversions in the *recB* strain was tested initially by transducing the Lac^+ clones (derived from strain TT13477) to Thr^+ in small "patch" transductions as described above. (This was possible despite the reduction in total recombinants caused by the *rec* mutation). Recombinant (Lac^+) clones showing even a slight reduction in transduction frequency compared to the parent strain (*recB*) were retested as donors. P22 lysates were grown on the questionable recombinant Lac^+ clones and the MudJ element was transduced into a wild-type recipient. All the Lac^+ clones were efficient donors of Kn^R, demonstrating that the strains are all competent donors and show no linkage disruption. Since the parental MudJ element can be inherited with high efficiency, without any evidence of linkage disruption, we conclude that no inversions occurred in the *recB* strain.

P22 lysates were grown on all Lac^+ recombinants arising in the *recA* strain and used to transduce the MudJ element into wild-type LT2 as a test of linkage disruption. For testing Lac^+ clones that arose in a *recA* background, the initial patch tests (above) could not be done. Clones were used as donors in transduction crosses to test transmission of the parental Mud insertions to a wild-type recipient. Only one of the few Lac^+ clones formed in a *recA* strain carries an inversion.

RESULTS

The recombination system: By observing recombination between inverse sequences in the bacterial chromosome, one can recover both the selected and unselected products of a single recombinational event. This system detects events in which both pairs of sequences flanking the recombining site are exchanged (full exchanges, leading to an inversion) and events in which no flanking sequences are exchanged (apparent conversions and double recombinants). If only one pair of flanks is exchanged (half exchange), the chromosome is broken and no recombinant can be recovered (see Figure 1).

This recombination system has been described in detail previously (SEGALL and ROTH 1989). It employs strains carrying two Mud(*lac*) sequences inserted either in inverse or direct orientation at separated sites in the bacterial chromosome. Each Mud element is inserted within a gene whose disruption causes an

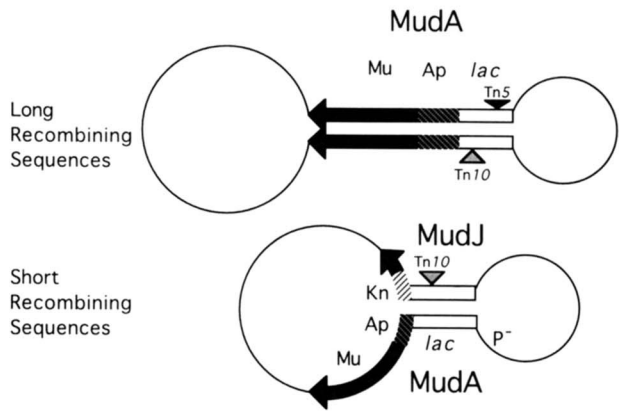
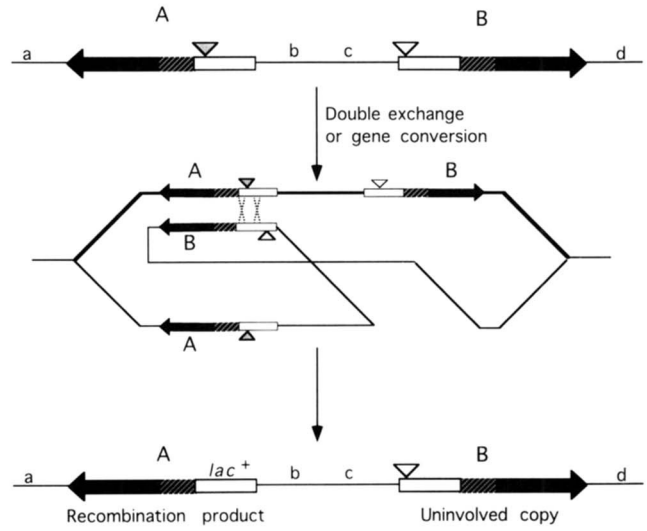


FIGURE 2.—Structure of chromosomes in which recombination between inverse repeats is scored. Top diagram represents strains with two MudA elements, which share roughly 40 kb of homologous sequence. The lower diagram represents strains with one MudA element and one MudJ element; these elements share about 5 kb of homologous sequence in the *lac* region and about 1 kb of Mu sequence at the opposite end of the elements. In the figure, filled boxes represent Mu sequences, hatched boxes represent drug resistance markers and open boxes represent *lac* operon sequences.



Apparent Gene Conversion Without Inversion

FIGURE 3.—Sister strand recombination events between inversely oriented homologous sequences. Note that a single exchange between sisters cannot generate an inversion, but a variety of recombination events generate chromosomes that appear to have undergone a gene conversion event. In fact, these recombinant chromosomes retain only one product of recombination, so true gene conversion cannot be scored.

auxotrophic phenotype. The Mud(*lac*) elements are of two varieties, called MudA and MudJ. The MudA element is a transposition-defective derivative of CASADABAN and COHEN's original MudI(Ap, *lac*) (1979) described by HUGHES and ROTH (1984); the element is nearly 40 kb long. The MudJ element is the MudII1683(Kn, *lac*) of CASTILHO, OLFSON and CASADABAN (1984). This mini-Mud element is roughly 11 kb long and carries the neomycin phosphotransferase gene, conferring resistance to kanamycin (Kn^R). The two elements, MudA and MudJ, share about 5 kb of homology, including the lactose operon at one end and about 1 kb of Mu sequence at the opposite end. (These elements are diagrammed in Figure 2).

A Lac^+ phenotype for either Mud element depends on fusing an intact *lacZ* gene to a functional host promoter that can provide expression. In the parent strains used here for recombination tests, the *lac* sequences of both elements are inactive either because of a transposon insertion in the *lacZ* gene or because the Mud element is inserted at a chromosomal site that does not provide a promoter. In each parent strain, the two Mud elements are constructed so that recombination between them can restore a functional *lacZ* sequence fused to an active promoter.

When two MudA elements were used, one *lacZ* gene carried a Tn*10* insertion and the other a Tn*5* insertion. Recombination in the *lacZ* gene between the sites of these transposons can restore a functional *lacZ* sequence. To select recombination between a MudA and a MudJ element, strains were used in which the *lacZ* gene of MudJ has a functional promoter but is inactivated by a Tn*10* insertion. The *lacZ* gene of the MudA element is intact but has no chromosomal promoter. Thus, both elements are individually Lac^-, but the silent *lacZ* gene of MudA can provide sequences for repair of the mutant *lacZ* gene of MudJ (see Figure 2).

In this system, we can score three kinds of recombination events: single crossovers with and without conversion (inversions), double crossovers, and "apparent gene conversions" (Figure 1). A simple single crossover (full exchange) between the sites of the inserted Tn elements generates a Lac^+ recombinant and a doubly mutant second *lacZ* allele; this single exchange inverts the chromosomal region between the insertion sites. The same inversion event can be accompanied by loss of one of the insertion elements; these are true conversion events associated with the full exchange.

Two crossovers within the Mud elements can simply exchange the transposons reciprocally between the two *lac* sequences such that one recombinant allele is Lac^+ and the other carries both insertions. A double recombinant of this type has no inversion. The third class of recombinants (apparent gene conversions) are those that have no inversion but acquire one lac^+ allele associated with loss of the transposon from the repaired site. These recombinants appear to reflect intrachromosomal gene conversion events. However, this sort of Lac^+ recombinant can also be generated by sister strand exchanges as diagrammed in Figure 3; the lac^+ allele can form either by gene conversion or double recombination between different parental alleles on sister chromosomes. Since the origin of this class is unclear, we have termed it "apparent gene

conversion" to emphasize that we cannot be sure how the recombinant gene arose.

In yeast, conversion and exchange of flanking sequences are frequently associated (reviewed by PETES, MALONE and SYMINGTON 1991). We can score this association for the full exchange class (carrying an inversion), since this class maintains both recombination products. This association between recombination and conversion is seen in some but not all of the bacterial situations described here.

Inversions are identified by scoring recombinants for disrupted association of the sequences that flank the insertion sites of the parental Mud elements (SCHMID and ROTH 1983). In the parent strain, the normal association of flanking sequences is demonstrated by transductional repair of each insertion by a donated wild-type allele of the insertion site (*i.e.*, by transducing a *trp*::Mud allele to *trp*$^+$). In a strain with an inversion endpoint at this locus, one of the sequences that previously flanked the Mud element will be moved away by the inversion. This reassociation of flanking sequences will occur at both of the Mud elements, making it impossible to repair either of the parental auxotrophies by a single transduced fragment. (In practice, the auxotrophy of an inversion strain can be repaired at a low frequency due to the presence of rare recipient cells in which recombination has reversed the inversion and restored the parental combination of flanking sequences.)

Recombination between long inverse-order sequences: We have tested recombination between pairs of MudA elements placed in inverse order at the ends of four different chromosomal segments. Three of the intervals tested were permissive for inversion (P); the fourth is nonpermissive (NP). The Lac$^+$ recombinant products recovered are described in Table 2. For all the strains in Table 2, the recombining sequences are MudA elements, about 40 kb in length. To generate a Lac$^+$ recombinant, an exchange must occur within the *lacZ* gene between the sites of the two transposon insertions, a 1–2 kb region.

It should be noted that for all of the first three strains described in Table 2, full exchange events (inversions) are frequently associated with gene conversion. The inversion class is the only class for which true gene conversion can be scored. The non-inversion recombinant class includes double recombinants and "apparent gene conversion events," which may arise in several ways.

In the *pyrB-thr* interval, Lac$^+$ colonies were detected by their blue color on X-gal indicator plates. Since the color assay requires very little β-galactosidase activity, the difference in the strength of the two promoters does not bias which of the two *lac* alleles is repaired. In this interval, the Tn*10* element was removed more frequently than the Tn*5* by the apparent gene conversion event (93% of gene conversion products). We have observed this phenomenon previously (SEGALL and ROTH 1989); the rarer removal of the Tn*5* is almost certainly due to the close proximity of the Tn*5* to the end of the homology shared by the Mud elements. When a Tn*10* insertion nearer to the end of the MudA element is used, the opposite bias (preferential loss of the Tn*5*) was seen (SEGALL and ROTH 1989).

In the *pncB-hisD* interval, Lac$^+$ recombinants were selected by their ability to grow using lactose as the sole carbon source. While both of these MudA elements are placed so as to be expressed by a chromosomal promoter strong enough to permit growth on lactose, the *pncB* promoter is much stronger than the *his* promoter (A. SEGALL, unpublished data). The majority of Lac$^+$ gene convertants (95%) lost kanamycin resistance. This bias for conversion of the element located early in the *lacZ* sequence may reflect a selective advantage of higher β-galactosidase expression when growth on lactose as a carbon source is selected. Alternatively, the high level of transcription from the *pncB* promoter may influence the recombination event itself by affecting the structure of the recombination substrate. Both negative and positive effects of transcription on recombination frequency have been reported (HERMAN 1968; DUL and DREXLER 1988a,b; STEWART and ROEDER 1989; THOMAS and ROTHSTEIN 1989; ROCCO, DE MASSY and NICOLAS 1992). Firm predictions for the outcome of a crossover cannot yet be made based on its position with respect to a promoter.

The *trp-his* interval is nonpermissive for inversions and has been described previously (SEGALL and ROTH 1989). Although the frequency of Lac$^+$ clones, 1.1×10^{-4}, is similar to the frequency of Lac$^+$ clones in permissive intervals, we found no inversions among the Lac$^+$ recombinants. The Lac$^+$ clones were selected for growth on lactose as sole carbon source. In this strain, the selection conditions are met only if the Lac$^+$ recombinant gene is fused to the *his* promoter. In 75% of the recombinants, a Lac$^+$ phenotype was achieved by apparent gene conversion, with the Tn*10* element being lost from the *his*::MudA element in 98% of the recombinants.

Recombination between short inverse-order homologies: Five chromosomal intervals were tested using a MudA element in combination with a MudJ element as regions of homology; four of the tested intervals are permissive for inversion. In all these strains, only the MudJ elements are fused to active promoters; thus, a selective bias is imposed for the repair of the MudJ(*lacZ*) copy. The MudA and MudJ elements share about 5 kb of sequence homology (including the *lac* region), about 8-fold less than the homology shared by two MudA elements. Reducing

TABLE 2

Recombination between two inversely oriented MudA sequences

Interval (strain no.)[a]	Interval size (min)	Total Lac$^+$ clones/10^5	Frequency of various Lac$^+$ products ($\times 10^5$)[b]			
			Inversions		Non-inversions	
			Non-conversions (all Tc^R Kn^R)	Conversions	Apparent gene conversions	Double recombinants (all Tc^R Kn^R)
pyrB-thr (P) (TT13420)	2	**34.0** (63)	**4.3** (8)	**2.7** (5 Tc^S Kn^R)	**12.4** (21 Tc^S Kn^R) (2 Tc^R Kn^S)	**14.6** (27)
pncB-hisD (P) (TT13425)	11	**9.4** (193)	**0.2** (5)	**1.9** (33 Tc^R Kn^S) (2 Tc^S Kn^R) (5 Tc^S Kn^S)	**6.3** (111 Tc^R Kn^S) (7 Tc^S Kn^R) (12 Tc^S Kn^S)	**0.9** (18)
pyrC-hisD (P) (TT13422)	24	**7.1** (120)	**0.8** (13)	**2.9** (41 Tc^S Kn^R) (7 Tc^R Kn^S) (1 Tc^S Kn^S)	**1.0** (12 Tc^S Kn^R) (1 Tc^R Kn^S) (4 Tc^S Kn^S)	**2.4** (41)
trp-his (NP) (TT12262)	10	**11.0** (1078)	**0**	**0**	**8.2** (785 Tc^S Kn^R) (15 Tc^R Kn^S) (9 Tc^S Kn^S)	**2.7** (269)

[a] P designates intervals permissive for inversions; NP designates intervals nonpermissive for inversions.
[b] Numbers in bold denote the frequency of clones in the particular recombinant class. In parentheses are the number and phenotype of Lac$^+$ clones represented in this class.

the length of shared sequence did not alter the frequency of inversions; however, it did influence the types of recombination products recovered.

Of inversions between long sequences (MudA-MudA recombination; Table 2), 38–88% showed conversion. Of inversions generated by exchanges between short homologous sequences, only 1–4% showed associated gene conversion (Table 3). The relative frequency of apparent conversion events seen among non-inversion recombinants is also reduced.

Role of RecA and RecBCD functions in recombination between short inverse repeats: Using a MudJ element (at the *ara* locus) and a MudA element (at the *thr* locus), we tested the effects of *recA* and *recB* mutations on the frequency of Lac$^+$ recombinants. The data are shown in Table 4. In the Rec$^+$ strain, the frequency of Lac$^+$ clones was 3.4×10^{-3}. Most of the recombinants carry inversions, and only two of 129 inversions were accompanied by gene conversion. This confirms the rarity of conversion among recombinants generated between short sequences.

In the RecA$^-$ background, the frequency of Lac$^+$ clones is reduced 10^5-fold. Only one of 11 Lac$^+$ clones recovered carried an inversion; 7 others were double recombinants. The remaining 3 Lac$^+$ products were clones in which the MudA element (originally at the *thr* locus) acquired an active promoter, either by transposition to another site or by transposition of an IS*10* sequence such that its promoter expressed the previously non-transcribed MudA. We have described such rare events previously (SEGALL and ROTH 1989).

The overall frequency of Lac$^+$ recombinants in the

TABLE 3

Recombination between inversely oriented MudA and MudJ sequences

Interval (strain no.)[a]	Interval size (min)	Total Lac$^+$ clones/10^5	Frequency of Lac$^+$ products ($\times 10^5$)[b]			
			Inversions		Non-inversions	
			Non-conversions (Tc^R)	Conversions (Tc^S)	Apparent conversions (Tc^S)	Double recombinants (Tc^R)
thr-araC (P) (TT13430)	2	**320.0** (221)	**243.2** (168)	**4.3** (3)	**15.9** (11)	**56.5** (39)
araC-nadA (P) (TT13437)	17	**22.0** (398)	**16.4** (296)	**0.3** (5)	**0.8** (14)	**4.6** (83)
araC-trpE (P) (TT17432)	32	**5.9** (99)	**3.6** (60)	**0.06** (1)	**0.06** (1)	**2.2** (37)
araC-hisH (P) (TT13435)	42	**1.4** (105)	**0.3** (26)	**0.02** (1)	**0.1** (10)	**0.9** (68)
trp(ED)-hisD (NP) (TT13428)	10	**0.02** (198)	**0**	**0** (0)	**0.015** (154)	**0.005** (42)
trp(ED)-hisD[c] (NP) (TT13427)	10	**0.02** (218)	**0**	**0** (0)	**0.011** (119)	**0.009** (96)

[a] P designates intervals permissive for inversions; NP designates intervals nonpermissive for inversions.
[b] Numbers in bold type denote the frequency of a particular recombinant class. Numbers in parentheses denote the number of Lac$^+$ clones tested for each class.
[c] This Tn*10* element is located early in the *lacZ* gene.

TABLE 4

Effect of Rec⁻ mutations on recombination between inverse repeats of MudJ (at *ara*) and MudA (at *thr*)

Genotype	Lac⁺ clones/10^6 cells	Frequency of various Lac⁺ products (× 10^6)[a]			
		Inversions	Apparent conversions	Double recombinants	Transpositions
Wild type[b] (TT13482) (TT13478)	**3350** (156)	**2770** (129)	**64.4** (3)	**515.4** (24)	**0** (0)
recA (TT13481)	**0.053** (11)	**0.005** (1)	**0** (0)	**0.034** (7)	**0.014** (3)
recB (TT13477)	**44** (188)	**0** (0)	**34.9** (149)	**9.1** (39)	**0** (0)

[a] Numbers in parentheses represent the number of Lac⁺ clones scored in each class.
[b] The two strains listed are constructed to be isogenic with the *recA* and *recB* strains listed below. Since they gave essentially identical results, data for the two *rec*⁺ strains was combined.

RecB⁻ strain (4.4×10^{-5}) is about 100-fold lower than in the RecB⁺ strain. None of 188 independent Lac⁺ recombinants in the *recB* strain has an inversion; for the isogenic *recB*⁺ strain, 80% of Lac⁺ recombinants had an inversion. The majority (79%) of the Lac⁺ recombinants in the *recB* background had lost the Tn*10* element and are scored as "apparent gene conversions." Although the *recB* mutation eliminated inversions, it had very little effect on the frequency of apparent gene conversion, 64 compared to 35 (per 10^6 cells). Double recombinant frequency was strongly reduced by the *recB* mutation, from 515 to 9 (per 10^6 cells).

Role of RecA and RecBCD functions in recombination between direct repeats: When the recombining repeated sequences are in the same orientation in the chromosome, three distinguishable outcomes can be detected. These are duplications, apparent gene conversions and double recombinants; all are diagrammed in Figure 4. A duplication is generated by a recombination event between sister strands that exchanges one or both pairs of sequences flanking the recombining sequences. When a Lac⁺ recombinant is formed by this sort of event, the Lac⁺ recombinant allele is left at the duplication join point and the other product of the exchange is lost. The second product is segregated to a non-viable daughter cell with a broken chromosome (in the case of a half exchange) or to a daughter with a large lethal deletion (in the case of a full exchange). Because only one recombination product is recovered, it is impossible to score gene conversion among recombinants that acquire a duplication.

Non-duplication recombinants (*lac*⁺) can arise by sister strand exchange (double recombination or gene conversion). Double recombination or gene conversion between sister strands generates a Lac⁺ recombinant allele at one site, while the second product of the exchange is lost. Since only one recombination product is recovered, and the other *lac* region in the final recombinant was uninvolved in the exchange, one cannot score gene conversion. Regardless of the nature of the event, the recombinant clone has one Lac⁺ allele and one unaffected allele; we call these apparent gene convertants (Table 5; second event in Figure 4).

When a Lac⁺ recombinant arises by intrachromosomal exchange between direct repeats, the double recombinant class can be identified because both of the two *lac* regions are altered; one becomes *lac*⁺ and the other inherits both of the parental drug resistance markers (fourth event in Figure 4). Intrachromosomal gene conversion (the third event in Figure 4) yields one (Lac⁺) recombinant allele. This event cannot be distinguished from the non-duplication class generated by sister strand exchanges (described above); all are scored as "apparent gene conversion" events (see Figure 4).

The various recombinant types can be easily distinguished. Since duplications generate a Lac⁺ allele at the join point between two tandem copies of a large chromosome segment, the join point element (Lac⁺) is frequently lost by recombination between the two copies. This segregation event returns the chromosome to its parental haploid state (Lac⁻; one drug resistance at each locus) and can be easily detected by screening for loss of the Lac⁺ phenotype. The presence of the Tn*10* or Tn*5* in the *lac* sequences is signaled by the antibiotic-resistance phenotype; the position of the *lac*⁺ allele and the remaining drug resistance markers are determined by observing what aspects of phenotype are lost when the auxotrophies of the parental Mud insertions are repaired by transduction.

The recombinant types were classified for isogenic Rec⁺, RecA⁻, or RecB⁻ derivatives of the parent strain; all have MudA(*lac*⁻) elements in direct orientation at the *his* and *trp* loci. In the Rec⁺ strain, Lac⁺ recombinants are found at a frequency of 1×10^{-4} (Table 5). About 87% of these clones were non-

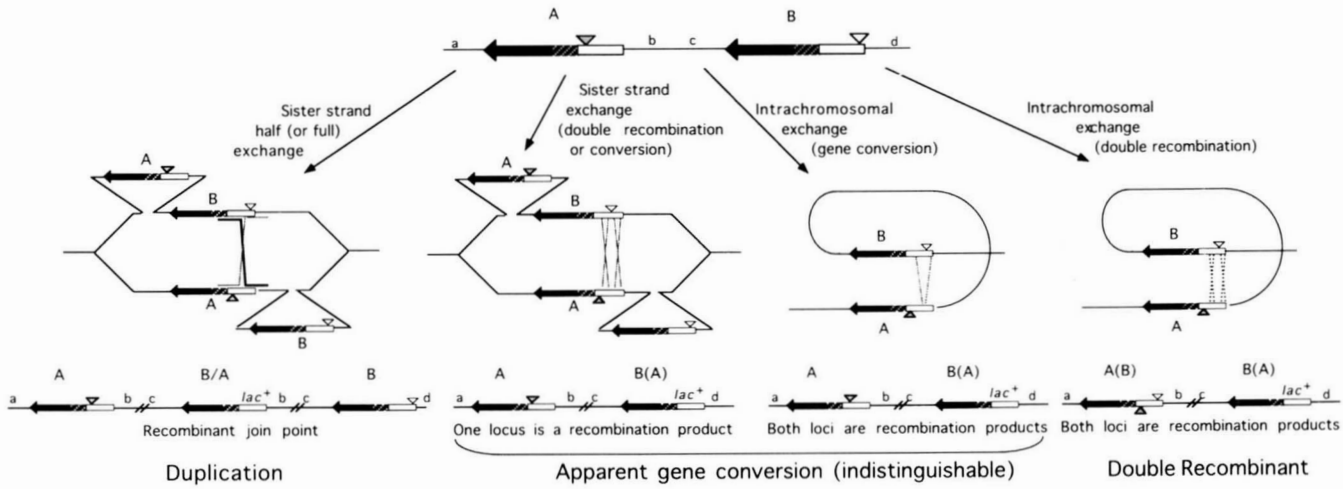


FIGURE 4.—Recombination events between direct order homologous sequences. Note that exchanges between sister chromosomes generate products in which only the allele at the join point was involved in the recombination event. For the diagrams of sister strand exchanges, it is assumed that the top sister is recovered as a recombinant. Exchanges between elements in the same chromosome can lead to products in which both alleles were involved in the exchange event, but this can only be identified if information in both copies was altered by the event (double recombination); true gene conversion events can not be scored for exchanges between direct order sequences.

TABLE 5

Effect of Rec mutations on recombination between direct repeats of MudA elements at the *his* and *trp* loci

Genotype	Lac$^+$ clones/ 10^6 cells	Frequency of various Lac$^+$ products ($\times 10^6$)[a]		
		Duplications	Apparent conversions	Double recombinants
Wild type[b] (TT17403) (TT17405)	**130** (71)	**1.8** (1)	**114** (62)	**14.2** (8)
recA (TT17402)	**0.028** (45)	**0.0006** (1)	**0** (0)	**0.027** (44)
recB (TT17404)	**12** (104)	**8.9** (77)	**2.4** (21)	**0.7** (6)

[a] Numbers in parentheses represent the actual number of Lac$^+$ clones scored in each class.

[b] The two strains listed are constructed to be isogenic with the *recA* and *recB* strains listed below. Since the two *rec*$^+$ strains gave essentially identical results, data for the two strains were combined.

duplication, "apparent gene conversion" types (see above) and 11% were double recombinants. Only one duplication was seen among 71 independent Lac$^+$ clones. In other experiments, up to 10% of the Lac$^+$ clones carried duplications (SEGALL and ROTH 1989).

The frequency of Lac$^+$ recombinants in the RecA$^-$ background was reduced 10^4-fold. Most (92%) of the recovered Lac$^+$ clones were double recombinants, 6% were apparent gene conversions, and one clone segregated Lac$^-$ colonies (when made *recA*$^+$) as expected for a duplication. (True gene conversion cannot be scored among recombinants between direct repeats.)

The Lac$^+$ clones arising in the *recB* deletion background grew slowly (4 days) but the overall frequency of Lac$^+$ clones was reduced only 10-fold. (All Lac$^+$ clones maintained a UV-sensitive phenotype and a lower frequency of transduction, and thus did not reflect reversion of the *recB* mutation.) The major effect of the *recB* mutation was to reduce the frequency of the "apparent gene conversion" class and the "double recombinant" class. Remarkably, the *recB* mutation did not significantly affect the frequency of duplications, confirming earlier observations (MAHAN and ROTH 1988, 1989).

Since detection of a duplication depends on scoring segregation, it is important that this event can occur in the parent strain used. Since the *recA* mutation prevents duplication segregation, recombinants arising in a *recA* background were made RecA$^+$ before being tested for duplication segregation. The segregation of duplications is independent of the RecBCD protein. We have tested this by introducing a *recB*::Tn*10* insertion into a strain bearing a pre-existing duplication of the *trp-his* segment with a Mud(Lac$^+$) element at the join point between the two copies. We detected recombination between the duplicated material by scoring the formation of white, Lac$^-$ colonies on Xgal medium. The Mud element was lost with equal frequency in RecB$^+$ and RecB$^-$ backgrounds (between 45 and 60% of the cells in an overnight culture started from a single colony). Similar data have been reported by SCLAFANI and WECHSLER (1981) and MAHAN and ROTH (1989). In contrast, introducing a *recA* mutation into a pre-existing duplication reduces the frequency of segregants to less than 2%.

DISCUSSION

We have assayed recombination using a system that permits recovery of both products arising from an individual exchange event. This was made possible through use of strains in which the recombining se-

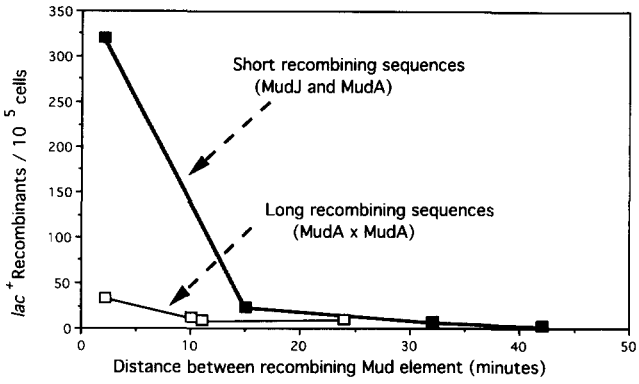


FIGURE 5.—Effect of distance between recombining inverse-order sequences on the frequency of recombinants. Note that proximity stimulates recombination most for the short recombining sequences.

quences are placed in inverse order in the same chromosome. In this situation, the selected and the unselected products of recombination remain in the same chromosome and are available for analysis. As for half-tetrad analysis, this assay allows recovery of both products of a recombination event. Since we must select one recombinant phenotype (Lac$^+$), we cannot recover products of exchanges in which both *lac* sequences remain inactive. It should be understood that situations that reduce apparent recombination frequency could merely bias the nature of the product in favor of undetected events. (This problem is not peculiar to this recombination system.)

Recombination products were classified with respect to exchange of flanking sequences (inversions) and loss of information (gene conversion) associated with the crossover event. Gene conversion leads to loss of one of the drug resistance markers placed within the recombining repeats.

Below is a summary of our observations:

When long repeats (39 kb) recombine, full exchanges frequently show concomitant conversion: All of the expected products arise from exchanges between long homologies. Results generally resemble those obtained in fungi. Recombinant (Lac$^+$) clones arise with and without exchange of flanking sequences and exchanges of flanks are frequently associated with gene conversion. In the case of long repeats, the majority of initiating events may occur (in Mu sequences) far from the eventual site of resolution (in *lac* sequences). Since the heteroduplex region must extend from the initiation site to the resolution site, the intervening marker allele has a high likelihood of being converted.

When short repeats recombine, conversion is rare: Gene conversion occurs rarely in exchanges between Mud elements that share only 5 kb of homologous sequence. Most inversions occur without an associated conversion event. The small number of gene conversion events may reflect shorter regions of heteroduplex DNA. In the case of short repeats, the distance between the scored markers is a large fraction of the total recombining sequence. Under these conditions conversion tracts are likely to fall entirely between the markers and affect neither of the markers.

A *recB* mutation eliminates inversion formation: The absence of inversions in a *recB* mutant strongly supports a model in which the full exchange (rejoining both flanking sequences), required to form an inversion, can only be accomplished by the RecBCD pathway (MAHAN and ROTH 1988). Using very short recombining sequences, SCHOFIELD, AGBUNAG and MILLER (1992) have also found that inversion formation depends heavily on RecBC function. The frequency of the double recombinant class (not associated with inversions) is also depressed significantly in the *recB* mutant, but is not eliminated. We infer that noninversion double recombinants can be generated both by the RecBCD and also by some alternative recombination pathway (perhaps the *recF* pathway).

The frequency of Lac$^+$ recombinants without an inversion (apparent conversions) is unaffected by a *recB* mutation, and the majority of these recombinants may occur by a different pathway (perhaps *recF*). We suspect that many of these apparent conversion recombinant types may arise by sister strand exchange as diagrammed in Figure 3. This sort of exchange leads to correction of one *lac* locus (apparent conversion). We cannot score true gene conversions in this situation because the final recombinant (*lac*$^+$) carries only one of the two *lac* loci known to have been involved in the exchange.

For direct repeats, a *recB* mutation reduces total recombinants but has no effect on duplication frequency: The frequency of duplications, in contrast to the frequency of inversions, is unaffected by a *recB* mutation. We interpret this as evidence that duplications, which can form by a half exchange (one pair of flanks rejoined), can form by an alternative pathway that does not involve the RecBCD function. Since this alternative can not accomplish inversions, we interpret this result as indicating that the major alternative recombination pathway is unable to perform a full exchange (rejoining both flanks).

Double recombinants between direct repeats are assumed to reflect exchanges between two sequences in the same chromosome since information is moved reciprocally between two *lac* sites; these products are reduced by the *recB* mutation. The "apparent gene conversion" class of recombinants between direct repeats can arise from several different events (see Figure 4).

Effect of distance between sites on recombination frequency: In considering this and the next point (below), it is important to note that there is an uncontrolled variable in these experiments. The chromo-

somal intervals tested with short homologies are different from the ones tested with long homologies. Therefore it is possible that differences between short and long homologies could reflect idiosyncrasies of the particular sites used in each case.

Frequency of recombination between short inverse-order repeats appears to be more sensitive to the separation between the repeats than is recombination between long repeats (Figure 5; data presented is from Tables 2 and 3). We have varied the distance between the repeated sequences from 2 to 44% of the chromosome. Proximity of the recombining sequences seems to stimulate recombination between short sequences (MudA-MudJ) more than that between long sequences (MudA-MudA). This suggests that the long sequences provide sufficient initiating substrate (DNA strand ends) to saturate the recombination process regardless of the chromosomal distance separating the interacting sequences; for short homologies, the number of initiating ends may be limiting and therefore recombination is stimulated by bringing the rarer structures closer together.

Recombination is very frequent between the most closely placed Mud elements, which are separated by only 2% of the chromosome (*ara-thr*, 3.2×10^{-3}; *thr-pyrB*, 3.4×10^{-4}). Despite the high frequency of recombination events, these intervals show a distribution of products similar to that seen for more widely separated Mud elements; thus, recombination does not appear to be qualitatively affected by the distance separating the interacting sequences. The high recombination frequency may reflect a nearby hotspot for initiation of recombination, or an underlying feature of chromosome structure that favors the juxtaposition of homologies separated by short stretches of DNA.

Effect of sequence length on recombination frequency: For all but the two shortest intervals, the length of total homology shared between the two repeats has a minimal effect on recombination frequency. Repeated elements that share either 5 or 40 kb of identical sequence yield *lac*$^+$ recombinants at about the same frequency. Excluding the *ara-thr* and *pyrB-thr* intervals, the average frequency of recombination between short repeats (MudA-MudJ) is 9.8×10^{-5} as opposed to 8.2×10^{-5} between long repeats (MudA-MudA). This suggests that 5 kb of homology is longer than the minimal length required for efficient pairing. As mentioned above, the two short intervals recombine more frequently than expected (Figure 5).

A more striking exception to the general observation is the large reduction in the recombination frequency seen for short sequences (MudA-MudJ) flanking an interval that is nonpermissive for inversion. The nonpermissive *trpA-hisD* interval showed a 1000-fold lower recombinant frequency than the *araC-nadA* interval (data in Table 3). In both situations the elements are separated by about 17% of the chromosome. This difference between a permissive and a nonpermissive interval is not seen when long homologous sequences recombine (MudA-MudA). It is possible that longer homologous sequences provide sufficient initiating substrates within the recombining sequences to support frequent gene conversion. Initiation of gene conversion between short sequences may have to rely on nearby sites in the chromosome (outside the recombining sequences). For nonpermissive intervals, we propose that regions adjacent to the recombining sequences are deficient in providing initiating structures; this results in a much lower frequency of recombination between sequences at these sites.

Summary: We describe a recombination system that is useful for studying the nature of intrachromosomal exchange events and for testing the effect of known recombination functions on the various product classes. We consider two of our observations particularly significant: (1) Inversion formation depends completely on the *recBCD* function while duplications occur in the absence of the *recBCD* function. (2) Gene conversion is often seen among recombinants with a full exchange between long sequences; conversion is rarely associated with recombination between short sequences.

Overall, these results suggest that exchanges initiated by different DNA substrates may lead to distinct outcomes. The frequency with which various initiating substrates (nicks, breaks) and various critical secondary sequences (*chi* sites) arise in particular sequences may determine the distribution of products seen in each situation. It should be possible to use the system described here to learn how the several bacterial recombination functions act on various chromosomal substrates and how initiating substrates are generated.

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APPENDIX

Recombinational exchanges of flanking sequences and gene conversion (gain and loss of information in an exchange) can be observed without regard to the exact mechanism of the recombinational process. We have described these events with the assumption that inversions occur by a single reciprocal exchange between two sequences within one circular chromosome as diagrammed at the left side of Figure 1 above. We

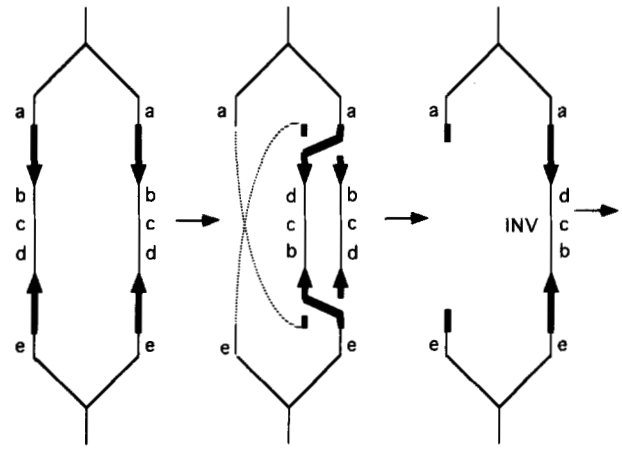

FIGURE 6.—Inversion formation by coinciding half-exchanges. The sister strands of a replicating chromosome are presented. If the inverse-order repeats line up properly, two independent half-exchanges can generate an inversion.

think it likely that the events described here occur in this way. However other mechanisms can also generate an inversion. Below we present two possibilities.

Coincidental half-exchanges between sister chromosomes: This mechanism (Figure 6) for inversion formation has been discussed previously (SEGALL and ROTH 1989). By two half-exchanges with a sister chromosome, an inversion is made in one sister chromosome and the other sister chromosome is destroyed. A single half-exchange of this type can occur between direct repeats to generate a duplication. Monitored in this way, the frequency of each event is about 10^{-4}. We have argued that the two coincidental events needed to generate an inversion would be expected at a frequency of 10^{-8} and could not be detected in our system. Counter-arguments are: (a) The first event might cause a general increase in recombination rate, making the second more likely and (b) All such events might occur in recombinationally "hot" cells in which multiple events are more likely. We have tested these counter-arguments by constructing cells with two independent duplications and observing the segregation frequency of each duplication (L. MIESEL and J. R. ROTH, unpublished results); the two duplications segregate independently and show no evidence of recombinationally "hot" cells. We think it highly unlikely that inversions form by two coincident, unrelated sister strand exchanges. More likely are scenarios in which the "hot" end generated by the first half exchange is involved in a cascade of events that ultimately results in inversion.

Half-reciprocal exchanges followed by double-strand break repair: Formation of an inversion can be accomplished without a reciprocal exchange. The process could be initiated by a half-exchange between inverse-order sequences in the same chromosome (see Figure 7). This leads to an inversion with an associated double-strand break that must be repaired. If the two ends are restricted to the region of shared homology (top right), the break can be repaired using any copy of the repeated sequence as template. If one such end is degraded beyond the region of inverse homology, the gap can be repaired only by using the indicated copy of the repeat in the sister chromosome. This mechanism involves two copies of the inverse-order sequence in one chromosome and a copy of the repeated sequence in a sister chromosome. If inversions occur by a process of this sort, they could arise without a standard reciprocal exchange, and the interpretation of gene conversion is made more complicated.

The problem of how apparently reciprocal exchanges are formed is not unique to the recombination system described here; even in tetrads for which all ultimate recombination products are recovered, the observed reciprocality could result from a half-exchange followed by repair of the produced double strand breaks, perhaps using a sister chromosome as template.

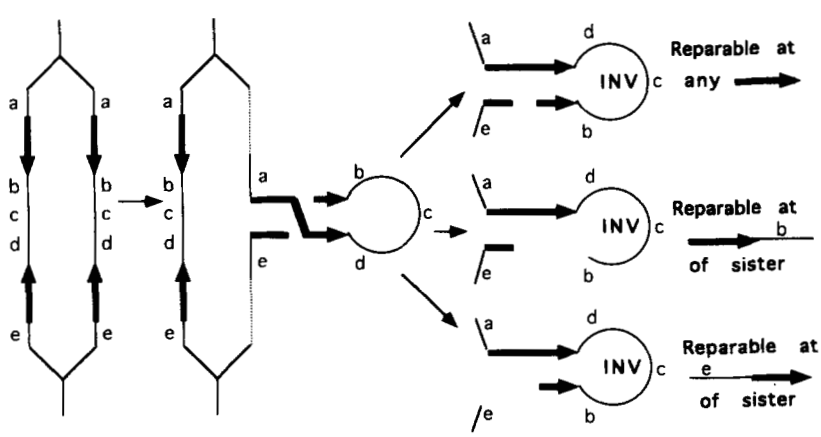

FIGURE 7.—Inversion formation by a half-exchange followed by double strand break repair. An initial half-exchange can generate an inversion and an associated double strand break; the ends may be highly recombinogenic and stimulate the subsequent events needed to repair the break. This general process can also be initiated by a half exchange between sisters.