Recombination Between Homologies in Direct and Inverse Orientation in the Chromosome of Salmonella: Intervals Which Are Nonpermissive for Inversion Formation

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ABSTRACT

Sequences placed in inverse order at particular chromosome sites (permissive) recombine to generate an inversion; the same sequences, placed at other sites (nonpermissive) interact recombinationally but do not form the expected inversion recombinants. We have investigated the events that occur between sequences at nonpermissive sites. Genetically marked *lac* operons in inverse order were placed at nonpermissive sites in a single chromosome and Lac^+ recombinants were selected. No inversions were formed. The Lac^+ recombinants recovered include double-recombinant types in which information appears to be reciprocally exchanged between the two *lac* operons. A second frequent recombinant type appears to have undergone a nonreciprocal information exchange; one mutant copy is repaired with no alteration of the other copy. Recombination within the *lac* operon is stimulated more than 100-fold by the presence of extensive homology (antenna sequences) outside of the region for which recombination is selected. Sequences placed in direct order at the ends of the same noninvertible chromosome segment recombine to form all the expected recombinant types *including* those in which a reciprocal exchange has generated a duplication. All the detected recombinant types can be accounted for by recombination between sister chromosomes. These results are discussed in terms of two alternative models. One explanation of the failure to detect inversion of some intervals is that particular inversions are lethal, despite the fact that no essential sequences are disrupted. Another explanation is that chromosome topology prevents sequences at nonpermissive sites in a single chromosome from engaging in the direct interaction required for inversion formation, but allows the sister strand exchanges that can generate the recombinant observed.

WE have previously studied the recombinational interactions between homologous sequences placed, in inverse order, at separated sites in the bacterial chromosome (SEGALL, MAHAN and ROTH 1988). A single reciprocal recombination event between such inverse repeats is expected to generate an inversion of the intervening chromosomal segment. For some pairs of sites (permissive), the expected inversions are found among the products of recombination. For other pairs of sites (nonpermissive), the same homologous sequences interact but do not generate the expected inversion recombinants.

In order to better understand the cause of nonpermissive intervals and the nature of recombination events within a single genome, we have characterized the recombination events that occur between genetically marked *lac* operons placed in inverse order at the ends of two nonpermissive intervals. Here we present evidence that these intervals are, in fact, nonpermissive for inversion formation and describe the recombinant products that are recovered. We also describe the recombination products found when the same sequences are placed at these chromosomal sites in *direct order*.

MATERIALS AND METHODS

Bacterial strains: All strains used in this study are derivatives of *Salmonella typhimurium* strain LT2. Their genotypes and laboratory of origin are listed in Table 1. **Media:** The minimal medium used was the E medium of VOGEL and BONNER (1956), with 0.2% glucose added. Rich medium (NB) consisted of Difco nutrient broth (NB, 8 g/liter) with NaCl added (5 g/liter). To make solid medium, Difco agar was added at a final concentration of 1.5%. Medium was supplemented with amino acid solutions as necessary, at final concentrations specified by DAVIS, BOTSTEIN and ROTH (1980). Ampicillin (Ap) was added at final concentrations of 30 μg/ml in NB and 15 μg/ml in E medium. Tetracycline HCl (Tc) was added at final concentrations of 20 μg/ml in NB and 10 μg/ml in E medium. Kanamycin SO_4 (Km) was added at final concentrations of 50 μg/ml in NB and 125 μg/ml in E medium. The indicator dye 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal) was added at a final concentration of 25 μg/ml. Following hydrolysis by a functional *lacZ* product, the colorless Xgal releases the blue dye 5-bromo-4-chloro-indigo. A derivative of E medium lacking

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TABLE 1

Strain list

Strain	Genotype	Source
S. typhimurium		
LT2	Wild type	Lab collection
TR2246	*srl* + *recA1*	Lab collection
TR6948	*srl-201*	
SL1657	*hspLT6 hspS29* (r_{LT} − m_{LT} + r_S − m_S+) H1-b *nml-* H2-e, n, × Fels2- *galE550 ilv-452 metA22 metE551 trpB2 strA120 xyl-404 flaA66*	B. A. D. STOCKER
JK66	*leuD798 ara- fol-101*	J. KEMPER
TT1977	*pyrC7 supQ1238 proAB47 leuD798 ara- fol-101*/F′ *proB* + *lacZ*::Tn*5*	D. STETTLER
TT7692	*hisD9953*::MudA (b)	K. HUGHES
TT7946	*trpE.D2484*::MudA (b) *hisO1242 hisD6404-C6 leuA414am supD10*	K. HUGHES
TT9531	*pyrC2688*::MudA (b)	K. HUGHES
TT9782	*trpE.D2490*::MudA (w)	K. HUGHES
TT11183	*srl-203*::Tn*10d*Cam *recA*+	T. ELLIOTT
TT12239	SL1657/F′ *lacZ951*::Tn*10* [del6-7]	
TT12240	SL1657/F′ *lacZ950*::Tn*10* [del26]	
TT12241	*hisD9953*::MudA (*lacZ951*::Tn*10*)	
TT12242	*hisD9953*::MudA (*lacZ950*::Tn*10*)	
TT12243	*hisD9953*::MudA (*lacZ*::Tn*5*)	
TT12244	*pyrC2688*::MudA (*lacZ951*::Tn*10*)	
TT12245	*pyrC2688*::MudA (*lacZ950*::Tn*10*)	
TT12246	*trpC.B.A2485*::MudA (b)	K. HUGHES
TT12247	*trpC.B.A2485*::MudA (*lacZ950*::Tn*10*)	
TT12248	*trpE.D2490*::MudA (*lacZ950*::Tn*10*)	
TT12249	*srl-201 hisD9953*::MudA	
TT12250	*srl-201 trpC.B.A2485*::MudA	
TT12251	*pyrC2688*::MudA (*lacZ*::Tn*5*)	
TT12252	*srl-201 hisD9953*::MudA (*lacZ*::Tn*5*)	
TT12253	*srl-201 trpE.D2490*::MudA	
TT12254	*srl-201 trpC.B.A2485*::MudA (*lacZ*:Tn*5*)	
TT12255	*trpE.D2490*::MudA (*lacZ*:Tn*5*)	
TT12256	*srl-201 trpC.B.A2485*::MudA (*lacZ*::Tn*5*) *pyrC2688*::MudA (*lacZ951*::Tn*10*)	
TT12257	*srl-201 trpC.B.A2485*::MudA (*lacZ*::Tn*5*) *pyrC2688*::MudA (*lacZ950*::Tn*10*)	
TT12258	*srl-201 hisD9953*::MudA (*lacZ*::Tn*5*) *trpE.D2490*::MudA (*lacZ950*::Tn*10*)	
TT12259	*srl-201 trpE.D2490*:MudA (*lacZ*::Tn*5*)	
TT12260	*srl-201 pyrC2688*::MudA (*lacZ*::Tn*5*)	
TT12261	*srl-201 pyrC2688*::MudA (*lacZ*::Tn*5*) *trpC.B.A2485*::MudA (*lacZ950*::Tn*10*)	
TT12262	*srl-201 trpE.D2490*::MudA (*lacZ*::Tn*5*) *hisD9953*:MudA (*lacZ950*::Tn*10*)	
TT12263	*srl-201 trpE.D2484*::MudA	
TT12264	*srl-201 trpE.D2484*::MudA (*lacZ*::Tn*5*)	
TT12265	*srl-201 trpE.D2484*::MudA (*lacZ*::Tn*5*) hisD9953::MudA (*lacZ950*::Tn*10*)	
TT10286	*hisD9953*::MudJ	T. ELLIOTT
TT12921	TT12253 *hisD9953*::MudJ	
TT12922	TT12253 *hisD9953*::MudJ(*lacZ951*::Tn*10*)	
TT12923	TT12253 *hisD9953*::MudJ(*lacZ950*::Tn*10*)	
E. coli		
DU2900	K12 *ara-* del(*lac-pro*) *thi-rif*^{R}/F′ *lacZ950*::Tn*10* [del26]	T. FOSTER
DU2901	K12 *ara-* del(*lac-pro*) *thi-rif*^{R}/F′ *lacZ951*::Tn*10* [del6-7]	T. FOSTER
14/1163	K12 *trp-* str^R *su-* del(*proB-lac*)/F′ *proB*+ *lacZ*::Tn*5*	D. BERG

All strains were constructed in this study unless otherwise specified.

citrate (BERKOWITZ *et al.* 1968) was supplemented with lactose or sorbitol at 0.2% w/v when testing growth on these alternative carbon sources. All nutritional supplements were obtained from Sigma Chemicals. Xgal was obtained from Bachem.

Transduction methods: We used the generalized transducing phage P22 (HT105/1 *int-201*), a high frequency transducing derivative of wild type P22 (SANDERSON and ROTH 1988). Transduction crosses were performed at a multiplicity of infection between 1 and 10 and the infected cells were plated directly on selective medium. Transductants were single-colony-isolated on nonselective green indicator plates (CHAN *et al.* 1972), on which phage-free colonies can be distinguished from phage-infected colonies by their light green color. Phage sensitivity was tested by cross-streaking phage-free clones against phage P22 H5, a clear plaque phage mutant. P22 lysates were made as described by DAVIS, BOTSTEIN and ROTH (1980).

MudA phage: The phage MudA, used to provide sequences for recombination, is a derivative of the original Mu *d*1(Ap, *lac*) CASADABAN and COHEN 1979); the derivative carries several mutations that leave it conditionally transposition-defective. It was originally called Mu *d*1–8(Ap, *lac*) by HUGHES and ROTH (1984) and is here referred to as MudA. This phage only transposes in hosts carrying an amber suppressor (Su$^+$); in suppressor-free (Su^0) hosts it is stable and is inherited only by recombination. Like its parent phage, the MudA genome encodes ampicillin resistance (Ap^R) and carries the promoter-less *lacZYA* operon with *trp* translational start signals (CASADABAN and COHEN 1979). Insertions of the MudA prophage in the appropriate orientation within a gene results in fusion of the *lac* operon of

the prophage to the transcript initiated at the promoter of the target gene. Such fusion strains form blue colonies on Xgal indicator plates, and may or may not be Lac$^+$ on plates containing lactose as a sole carbon source, depending on the strength and induction level of the promoter in question. Due to the large size of the MudA element (39 kb), two P22 transduced fragments are required for inheritance by recombination (HUGHES and ROTH 1985). Therefore, a high m.o.i. (>10) was used in transductions when it was desired to transfer the entire insertion for inheritance by recombination through flanking homologies; a low m.o.i. (<1) was used to transduce markers internal to the MudA, *e.g.*, *lacZ*::Tn*10*.

Tn*5* and Tn*10* insertions: Tn*10* insertions in different deletion intervals of the *lacZ* gene of *Escherichia coli* were kindly provided by T.FOSTER. Their isolation and mapping within *lacZ* is described by FOSTER (1977). The 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. From this mapping we conclude that the Tn*5* is located between the Tn*10* insertion in deletion interval 6 (early in *lacZ*) and the Tn*10* insertion in interval 26 (late in *lacZ*). (The intervals of the *lacZ* gene we refer to here were defined by ZIPSER *et al.* 1970.) The Tn*10* insertions were located in the *lac* operon of F-prime plasmids in *E. coli*. The F-prime plasmids were transferred from *E. coli* into Salmonella strain SL1657, a host with a defective restriction system but a wild-type modification system. The *lacZ*::Tn*5* insertion was similarly moved into Salmonella by D.STETTLER. The Tn*10* and Tn*5* insertions were then moved by P22-mediated transduction from SL1657 into the lactose operons of various MudA insertions in the chromosome of *Salmonella typhimurium*.

Construction of experimental strains: The general scheme for constructing strains in which recombination between inverse and direct order repeats could be tested is as follows. (A flowchart of the following steps is shown in Figure 1). The *trp*(*E,D*)::MudA insertion from TT9782 was transduced into a *srl* recipient (TR6948) selecting Ap^R resistance (carried by MudA). The *lacZ*::Tn*5* insertion was then transduced into the MudA prophage (generating strain TT12253). Another strain (TT12242) was similarly constructed, containing a *hisD*::MudA with an added *lacZ*::Tn*10* insertion. (Unless specified, the Tn*10* insertion used is late in the *lacZ* sequences, in deletion interval 26.) The latter strain was used as a donor of Tc^R into TT12259 *trpE,D*::MudA(*lacZ*::Tn*5*)]. Two alternatives were possible: (1) the Tn*10* insertion alone could recombine into the recipient's MudA prophage, using *lac* homology and frequently repairing the Tn*5* insertion; or (2) the entire donor MudA prophage could be inserted, using its flanking chromosomal homology. This second event was detected by screening Tc^R transductants for those that acquired the auxotrophy conferred by the incoming MudA; such transductants were kept for further use. Strains containing either direct-order or inverse-order MudA insertions were constructed in the same way, using MudA prophages whose location and orientation had been previously characterized (HUGHES and ROTH 1984). The MudA insertions, which define the endpoints of the duplications or inversions, were verified by transducing each one to prototrophy and testing the concomitant disappearance of either Tn*5* or Tn*10*. Strains were stored frozen in DMSO at −70° and single-colony-isolated before use.

Isolation of Lac$^+$ recombinants: The screen for Lac$^+$ clones arising from Lac$^-$ parents was carried out on appropriately supplemented minimal medium containing the indicator dye Xgal, a substrate for β-galactosidase. Positive

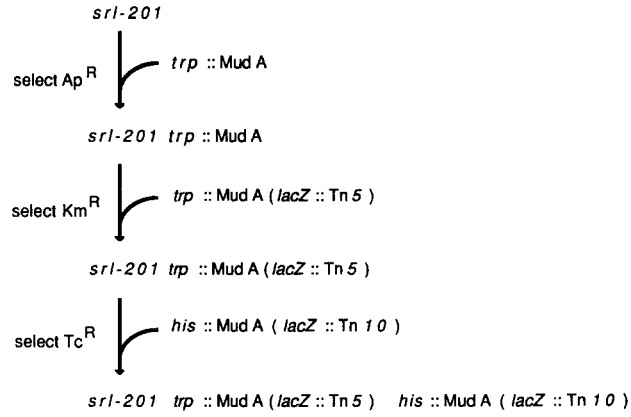

FIGURE 1.—Flowchart of experimental strain construction. The starting genotype is shown at the top. Each subsequent modification to this genotype is shown, along with the selection used to introduce the new genetic marker.

selection for Lac$^+$ clones was carried out on minimal medium containing lactose as the sole carbon source and supplemented with the necessary amino acids. Lac$^+$ clones obtained by either of the above methods were placed into categories (gene convertants, double recombinants, inversions, or duplications) by the genetic criteria described below.

Genetic tests for inversions: Inversion rearrangements disrupt the order of the chromosomal sequences which flank the recombining sites (Figure 2). The inversion event brings to each recombinant MudA element sequences which were originally far apart in the chromosome. Thus the inversion recombinant causes a disruption of genetic linkage across the site of each of the involved MudA prophages. This disruption (assayed as described in MATERIALS AND METHODS) causes a large (>25-fold) reduction in the efficiency of transduction to prototrophy for each of the nutritional requirements generated by the parental MudA insertion, compared to the parent (noniversion) strain. Strains without an inversion are transduced to prototrophy for either requirement at a frequency indistinguishable from that of the parent strain.

Genetic tests for retention of parental *lacZ* alleles: Each of the parental *lacZ* mutations has an associated drug resistance. The *lacZ*::Tn*10* insertion has a tetracycline resistance (Tc^R) phenotype and the *lacZ*::Tn*5* insertion a kanamycin resistance (Km^R) phenotype. We can check each of the Lac$^+$ recombinants for retention of the parental mutations by simply scoring drug resistance. The chromosomal position (copy 1 or copy 2) of the mutant allele can be assessed by determining which drug resistance is lost when the auxotrophic requirement of each of the parental Mud insertions is repaired by transduction. This is difficult to assess if an inversion occurs since the parental insertions in an inversion recombinant cannot be individually transduced to prototrophy. This is not a problem in the experiments reported here since the recombining sequences are located at nonpermissive sites and no inversions are recovered.

If, for example, His$^+$ transductants of a Lac$^+$, Tc^R, Km^R recombinant become Lac$^-$, it is inferred that the Lac$^+$ allele must be located at the *his* locus. Similarly if Trp$^+$ transductants of the same recombinant remain Lac$^+$ but lose both drug resistances, it is inferred that both parental mutations are now carried by the *lac* operon copy at the *trp* locus of the recombinant.

Clones that have lost one or both transposons (Tn*5* or Tn*10*) become Km^S and/or Tc^S; these are referred to here

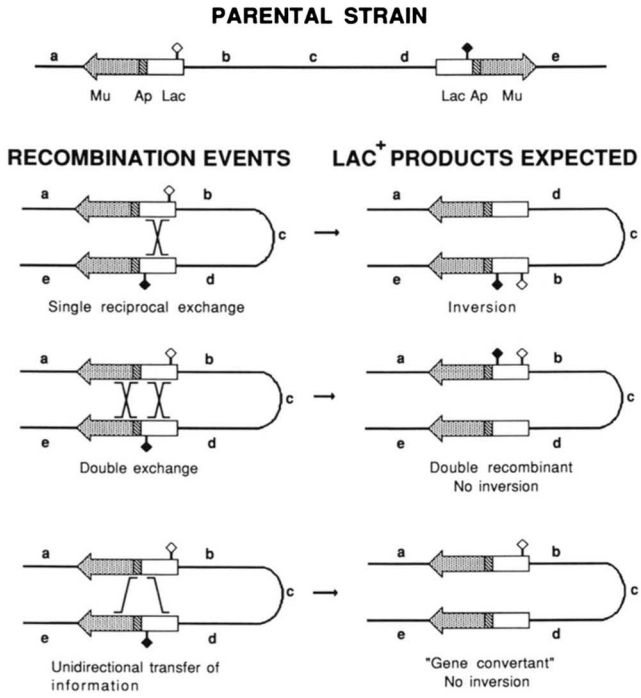

FIGURE 2.—Recombination between inverted repeats: expected products. The large arrows denote MudA sequences. The open and filled diamonds denote different transposon insertions (Tn*5* or Tn*10*) in the *lacZ* gene of MudA. These symbols are used throughout the remaining figures.

as showing "gene conversion," since some information is lost. This is a reasonable classification only if all the recombination events that generate Lac$^+$ clones occur between sequences on the same chromosome. However it should be kept in mind that this recombinant class can also arise by sister chromosome exchange. In this case the Lac$^+$ clone is selected and the other recombination product segregates as part of the genome of a different cell and is lost. This will be discussed in more detail in the RESULTS and DISCUSSION sections.

Characterization of recombinants between sequences in direct order: In testing recombinants arising in strains with *lac* sequences in direct order, it was necessary to distinguish duplications from double recombinants. To do this, Lac$^+$ Km^R Tc^R recombinant clones were tested for the ability to segregate Lac$^-$ colonies, since duplications are expected to be unstable. Overnight cultures of Lac$^+$ recombinant cells were grown in nonselective (NB) medium, diluted 10^6-fold and plated on Xgal indicator plates. When grown under these conditions, a parent strain with a duplication will give rise to cultures in which 20–50% of the cells have lost the duplication join point with its expressed (Lac$^+$) copy of the *lac* operon. Such segregants therefore form white (Lac$^-$) colonies on Xgal plates. This test gives a minimum estimate of the frequency of duplications, since some may segregate during purification of the recombinant clone.

RESULTS

The general method used to select directed rearrangements: We have tested recombination between *lac* sequences placed at separated sites in the bacterial chromosome using a variation of the method of KONRAD (1969, 1977). The strategy is to provide the cell with homologous, nonfunctional sequences at two chromosomal locations and to select Lac$^+$ clones in which recombination has occurred between the mutant alleles. Depending on the orientation of the homologies with respect to each other, either duplications/deletions (recombination between direct repeats) or inversions (recombination between inverse repeats) can be formed.

The *lac* sequences used to select recombinants are part of two transposition-defective MudA prophages. These prophages are inserted within genes that are far apart in the bacterial chromosome, and each insertion generates a distinct auxotrophic requirement. Each *lac* operon carries a different transposon inserted in its *lacZ* gene (Tn*5* or Tn*10*); therefore the parental strain is phenotypically Lac$^-$. Recombination between the two mutant chromosomal *lacZ* genes can generate a Lac$^+$ recombinant. These recombinants can be tested for chromosome rearrangements; the fate of the parental *lac* mutations can be determined by checking for the presence and position of the Tn*10*(Tc^R) and Tn*5*(Km^R) elements. Methods for assaying recombinant genotypes are described in MATERIALS AND METHODS.

Recombination events between inverse order repeats: In strains with MudA repeats in inverse orientation, three alternative recombination events can generate a Lac$^+$ clone (illustrated in Figure 2). A single reciprocal crossover between the Tn*5* and Tn*10* insertions in the same chromosome will generate one Lac$^+$ operon and one operon containing both transposons. This event will also invert the chromosomal segment bounded by the two Mud prophages. Alternatively, two reciprocal exchanges can transfer one of the *lacZ* mutations to the other *lac* operon, replacing it with wild-type material. This event would generate a Lac$^+$ operon, leaving both tranposons in the other *lac* operon, but does not cause inversion of the intervening chromosome segment. Yet a third possibility is a nonreciprocal transfer of information, which we will designate as gene conversion (discussed later); the latter event may repair either the Tn*5* or the Tn*10* mutation and result in a net loss of one of the transposons. Some of these events can occur by sister chromosome exchange as well as intrachromosomal recombination; this will be discussed later.

Recombination between inverse-order *lac* regions flanking the *his-trp* interval: The first interval tested involves an insertion in the *his* operon (minute 42) and in the *trp* operon (minute 34). These operons are transcribed in the same direction in Salmonella; thus, in order to place the MudA(*lac*) prophages in inverse orientation, one of the MudA elements must be in an orientation that does not allow fusion of its *lac* operon to the host transcript. The strain used (TT12262) has

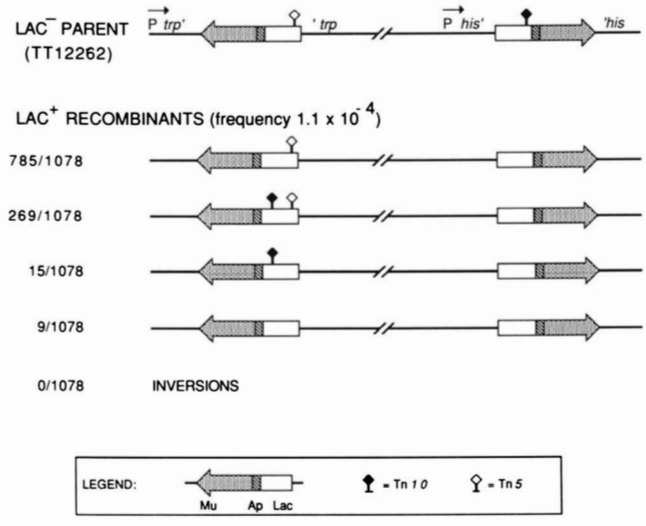

FIGURE 3.— Frequency and types of products of recombination between inversely repeated sequences. Double recombinant products have both Tn insertions. "Gene conversion" products have lost one or both of the Tn elements.

a *trp*::MudA prophage in the unfused orientation and a *his*::MudA prophage in the fused orientation. Recombinants can become Lac$^+$ only if the recombinant *lac*$^+$ allele arises in the prophage adjacent to the *his* promoter.

The above strain (TT12262) yielded Lac$^+$ recombinants at an average frequency of $1/10^4$ cells, as determined by screening colonies of the parental strain on Xgal medium for rare blue colonies. All of these Lac$^+$ clones appear to have arisen by recombination events as judged by two criteria. First, the frequent Lac$^+$ clones depend on the presence of both mutant lac alleles; no Lac$^+$ clones were detected among 10^9 cells carrying either the *his* or the *trp* insertion alone. Second, the formation of Lac$^+$ clones depends on homologous recombination; introducing the *recA1* mutation into such strains severely decreases the formation of Lac$^+$ clones (data not shown).

None of the Lac$^+$ recombinants tested has an inversion. All Lac$^+$ clones fall into one of the four classes diagrammed in Figure 3; as expected, all carry their Lac$^+$ allele at the *his* locus. One class (72.8%) has lost the Tc^R marker from the *his* locus and may have been generated by gene conversion events (to be discussed later). We are confident that this class is not due to simple reversion since it arises 10^5-fold more frequently than reversion events scored in a strain with only one MudA insertion; furthermore, its occurrence in the strain with two MudA insertions depends on the presence of a *recA*$^+$ allele. About 25% of the Lac$^+$ recombinants retain both Tc^R and Km^R phenotypes, consistent with double recombination events; this class of recombinants carries both resistance determinants in the second (Lac$^-$) copy of the Mud prophage, present at the *trp* locus. Two minor classes were also observed: 1.4% of the Lac$^+$ clones carry a Tn10

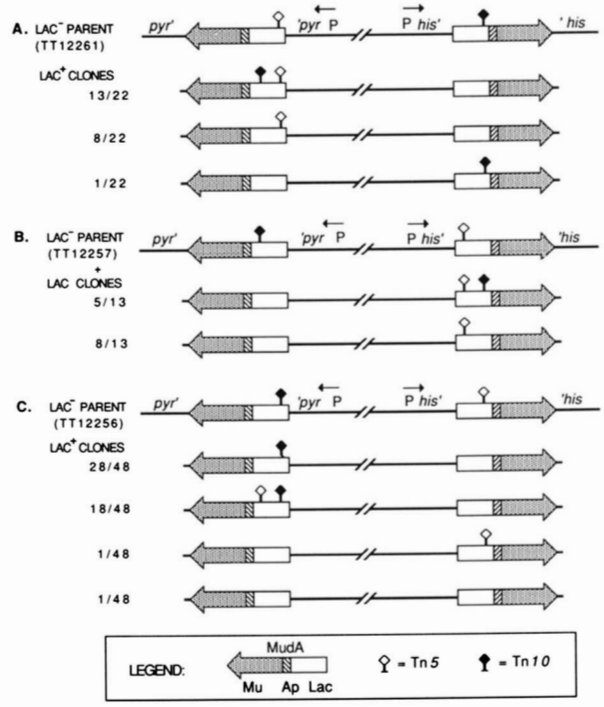

FIGURE 4.— Bias in gene conversion between inverted repeats.

element that had been transferred from its MudA of origin (at *his*) into the second MudA (at *trp*) replacing the Tn*5* originally present there; 0.8% of the clones have lost both Tn*5* and Tn*10*.

To test further the conclusion that no inversion recombinants appear, 872 additional Lac$^+$ clones were selected for growth on lactose as sole carbon source (using strain TT12262). These recombinants were screened only for linkage disruption; none appears to carry an inversion.

Recombination between *lac* regions flanking the *pyrC-trp* interval: The second nonpermissive interval tested, between the *pyrC* locus (minute 23) and *trp* locus (minute 34), includes the replication terminus. The transcription of the *trp* and *pyrC* loci is in opposite directions, so both the MudA prophages in the strains used (TT12256, TT12257 and TT12261 in Figure 4) could be placed with their *lac* operon fused to a promoter near the insertion site. Thus, a screen for blue colonies on Xgal indicator plates may yield clones in which the *lacZ* gene of either MudA insertion has been repaired. However, since the repressed *trp* promoter is weak, growth on lactose as sole carbon source occurs only when the *lac*$^+$ allele is adjacent to the stronger *pyrC* promoter. We analyzed 83 Lac$^+$ clones, obtained as blue colonies on Xgal; all were tested both for linkage disruption and for retention and location of drug resistance markers. Results are diagrammed in Figure 4. When the data for the three strains are pooled, one can see that the Lac$^+$ recombinants fall into the same general classes seen for the *his-trp* interval. Again, none of the Lac$^+$ recombinants carries an inversion. Apparent gene conversions accounted for

55.4% of the products; 43.4% were "double recombinant" types. Only one clone lost both Tn*5* and Tn*10* elements. Other aspects of these data will be described later.Since the number of recombinants scored here is small, an additional 1112 Lac$^+$ clones were obtained by positive selection (using strain TT12257). These Lac$^+$ clones were tested only for linkage disruption. None of these recombinants showed the linkage disruption characteristic of an inversion.

Apparent bias in gene conversion: In Figure 4A there is an apparent bias in the nature of the distribution of recombinant types. In 21 of 22 recombinants the Tn*5* element remained in place at the *pyr* site, while the Tn*10* sequences "moved" or were removed from *trp* the site by the recombination event. This was true even though the experiment could detect the *lac*$^+$ allele at either the *trp* or the *pyr* site. This sort of asymmetry was seen in many experiments of this type. To test the basis of this asymmetry, the other strains in Figure 4, B and C, were constructed and tested.

If position of the *lac* operons with respect to chromosomal markers determines conversion bias, switching the positions of the *lac* mutations (Tn*10* and Tn*5*) between the two Mud phages should result in a different transposon being preferentially retained. In Figure 4B we present a strain (TT12257) which is identical in genotype to the strain in part A of Figure 4, except that the *lacZ*::Tn*5* and the *lacZ*::Tn*10* have been switched in position. Of 13 Lac$^+$ clones tested, 8 kept the Tn*5* insertion and lost the Tn*10* insertion, and in the remaining 5, the Tn*10* insertion changed locations while the Tn*5* did not "move" (Figure 4B). Therefore, regardless of the chromosomal location of the MudA prophage, the Tn*10* insertion (located farthest from the promoter end of the Mud element) was preferentially lost or moved to the other copy of the *lac* region.

If the position of transposons within the *lac* sequences of the Mud prophage is important, switching the relative position of the elements within the *lacZ* gene would result in preferential retention of a different transposon. If the sequence of the element itself determines the tendency to be retained, the same element would be preferentially converted to the wild-type allele regardless of its position in the chromosome or within the Mud insertion. In Figure 4C, we present a strain in which the positions of Tn*5* and Tn*10* within the Mud prophage were reversed; that is, a Tn*10* insertion located at the 5' end of the *lacZ* sequence was tested *vis à vis* a Tn*5* insertion located further downstream in *lacZ* (the Tn*10* insertion in interval 6 was used—see MATERIALS AND METHODS). The positions of the elements are diagrammed in Figure 4C. Among 48 Lac$^+$ clones analyzed from this strain, 28 had lost the Tn*5* element, and in another 18, although both elements were retained, the Tn*5* element was the one to change location (Figure 4C). Thus the element closest to the promoter-fusion end of the Mud insertion (the extreme end of the shared homology) preferentially retains its position, while the element located closer to the middle of the shared homology is preferentially lost or moved. This suggests that there are more opportunities to involve the central insertion in heteroduplexes, or more opportunities for repair and recombination to remove this element. Neither the sequence of the particular transposon nor the position of MudA prophages in relation to chromosomal landmarks appear to dictate the bias of recombinant types.

Homologous "antenna" sequences outside the *lac* operon stimulate recombination: To form a Lac$^+$ recombinant in all the situations described above, recombination must occur within a region of about 1 kb between the Tn*5* and Tn*10* insertion sites in the *lacZ* genes. However, the two MudA elements which include the *lac* sequence also share more than 30 kb of uninterrupted homology, including the rest of the *lac* operon, the ampicillin resistance determinant and Mu phage sequences. We considered that secondary recombination events in this extensive homology may mask inversions generated by recombination events within the *lacZ* genes. To determine whether this shared outside homology contributes to the recombination events seen, we reduced its extent by using a MudJ (Mu *d11734*, miniMud) element in place of one of the MudA elements. The MudJ element is another derivative of the Mu *d1* transposon (CASTILHO, OLFSON and CASADABAN 1984), and is identical to MudA from the fusion end through the lactose operon. Most of the Mu sequences and the Ap^R marker present in MudA are replaced in MudJ by a smaller fragment containing the Km^R gene. Only about 1 kb of homology between MudA and MudJ remains at the fusion-distal ends of the two elements (heads of the arrows in Figure 5).Parental strains were built exactly as before, except that the smaller MudJ(Km) element is used in place of one of the large MudA(Ap) prophages used previously. The large *trp*::MudA insertion is in the unfused orientation and is thus Lac$^-$. The smaller *hisD*::MudJ insertion is in the fused orientation but is made Lac$^-$ by a *lacZ*::Tn*10* insertion. (Strains TT12292 and TT12293 in Figure 5 are identical except that in the first the *hisD*::MudJ element carries the insertion *lacZ951* ::Tn*10*, near the promoter end of the gene, and in the second strain it carries the more distal *lacZ950*::Tn*10* insertion.)

The recombinants observed in these strains are shown in Figure 5. The overall frequency of Lac$^+$ recombinants is $4/10^7$; this frequency is more than two orders of magnitude *lower* than that found in parental strains for which both endpoints were speci-

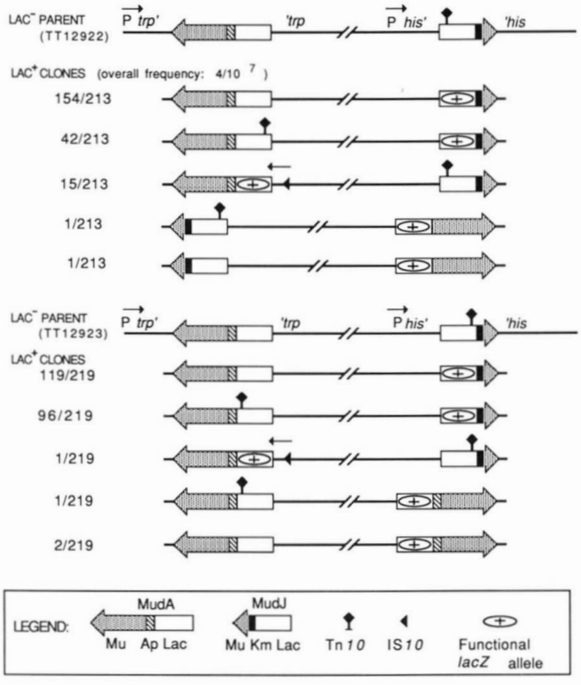

FIGURE 5.— Frequency and types of products of recombination between one MudA and one MudJ. The arrow above the IS*10* symbol denotes the outwardly pointing promoter of the insertion sequence. This promoter may be responsible for the Lac$^+$+ phenotype of the *trp*::MudA.

fied by MudA insertions (Figure 3). This suggests that the extensive shared homology *outside* of the *lac* region in previous experiments serves to stimulate the frequency of recombination events between the two *lac* insertion mutations.

Of 431 Lac$^+$ clones analyzed, none showed any evidence of linkage disruption; thus even in the absence of the extensive shared homology, inversion recombinants are not recovered. Parent strain TT12292 gave rise to 72.3% gene convertants (Tc^S) and 20.7% "double recombinant" clones (Tc^R). The other parent (TT12293) gave rise to 55.5% gene convertants and 43.6% "double recombinants." Five of the 431 clones (pooling data from both parents) showed evidence of exchange in the fusion-distal end of the Mud sequences. In two of these clones, the drug resistances of Mud phages were exchanged between the *his* and *trp* sites, such that the MudA sequence (Ap^R), although originally at the *trp* locus of the parent, was found at the *his* locus of the Lac$^+$ recombinant; the MudJ sequences (Km^R) showed the reverse shift. In three clones, MudA sequences had entirely replaced MudJ sequences; the Km^R marker of MudJ was lost, and both *trp* and *his* prophages were of the MudA(Ap) type (Figure 5). Both sorts of events are expected at a low frequency since the MudJ and MudA elements have in common a short sequence at the end of the element farthest from the *lac* operon. It should be kept in mind that none of these recombinants have an inversion; therefore all of these recombinants must have been generated by double exchanges.

Another class of Lac$^+$ clones was found in these experiments that had not been detected in previous experiments, using two large Mud prophages. In 16 of the 431 Lac$^+$ clones, the functional *lac* allele was found at the *trp* locus; this was unexpected since the *trp*::MudA insertion is in the "wrong" (nontranscribed) orientation. No linkage disruption was seen in any of these Lac$^+$ clones and the *his*::MudJ prophage in all these strains still carries the original *lacZ*::Tn*10*. We have considered two likely explanations; either a *trp* sequence downstream of the Mud insertion could mutate to create a new promoter able to transcribe the fusion, or the promoter initiating transcription of the *trp*::MudA sequence might be contributed by a transposed IS*10* element (CIAMPI, SCHMID and ROTH 1982; HALLING *et al.* 1982; CIAMPI and ROTH 1988; WANG and ROTH 1988).

If the first possibility were true, Lac$^+$ clones should arise with the same frequency in a strain carrying only the unfused *trp*::MudA prophage in an otherwise wild-type background. The frequency of Lac$^+$ revertants in such a Tn*10*-free strain (TT12253) is $2/10^9$, an order of magnitude lower than the frequency of the unusual clones seen in the double Mud insertion parent.

If an IS*10*-carried promoter is responsible for the Lac$^+$ phenotype of these clones, the promoter sequence should frequently be outside the MudA prophage and separable from the Mud element. We used the His$^+$ derivatives of the unusual Lac$^+$ clones as donors of Ap^R to LT2. All Ap^R transductants became Trp$^-$, as predicted. In the case of two of the exceptional donors, Ap^R transductants included both Lac$^+$ and Lac$^-$ clones, showing that the *lacZ* gene of the *trp*::MudA prophage is expressed due to a mutation that is separable from the MudA insertion (data not shown). We think it is likely that this class of Lac$^+$ clone arose by insertion of a promoter-bearing IS*10* element near the *trp*::MudJ fusion. Detection of these mutations in this experiment was probably facilitated by the reduced frequency of Lac$^+$ recombinants caused by the reduction in "antenna" sequences.

Recombination events between direct order repeats: A single reciprocal recombination event between sequences in the same orientation in different sister chromosomes is expected to yield a Lac$^+$ duplication recombinant carrying the recombinant *lac* region at the duplication join point (Figure 6). If this exchange is fully reciprocal, one would expect a corresponding large deletion. Deletions can also form by exchanges between sequences in the same chromosome; since the sequences used here are far apart, these deletion recombinants would not be viable. In addition to duplications, we expect the same classes of

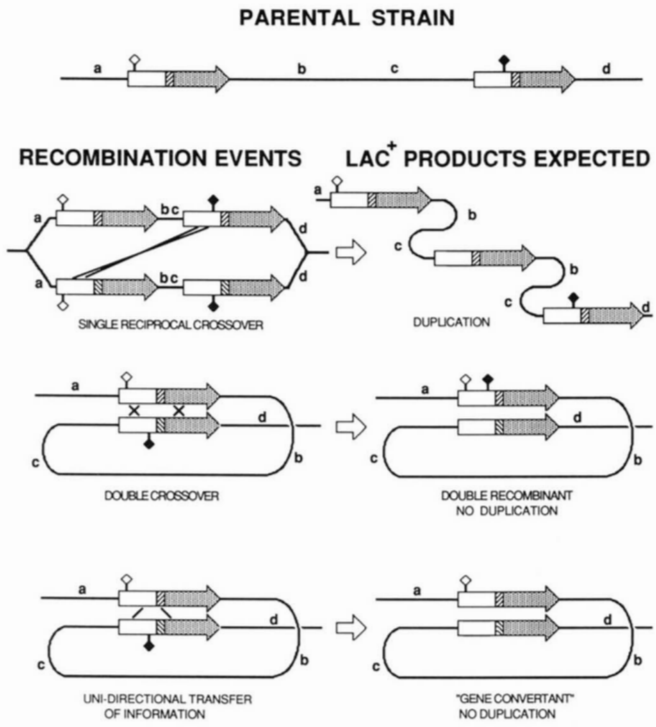

FIGURE 6.— Recombination between direct repeats: expected products.

recombination events seen in previous experiments: double recombinants and gene conversions, both having one *lac*$^+$ allele but no duplication. The general nature of these recombinants are diagrammed in Figure 6. The results of the actual experiments are presented below.

Recombination between direct-order *lac* regions flanking the *his-trp* interval: We tested the types and frequency of Lac$^+$ products arising in a strain (TT12265) that carries MudA elements inserted in the *same* orientation at the *his* and *trp* loci (Figure 7). The frequency of Lac$^+$ clones from strain TT12265 is 1/1200. Duplications, the expected single crossover product, accounted for 11% of the total number of clones (96 independent clones were analyzed); 16% of the Lac$^+$ clones were double recombinant types which do not have a duplication of the *his-trp* segment, but carry a *lac*$^+$ allele in one of the original Mud prophages and both Tn*10* and Tn*5* transposons in the other. The remaining 73% of the Lac$^+$ clones were classified as gene convertants since they have lost one of the *lac* insertion mutations.

The Lac$^+$ clones scored are almost certainly recombinants, since strains with either of the two MudA prophages alone show no Lac$^+$ revertants among 10^9 cells tested. Thus, virtually all the Lac$^+$ colonies tested are produced by interactions between the two direct-order prophages. Since the duplication class is detected, we conclude that sequences at these sites (when in direct order) can interact and exchange flanking sequences. This is in contrast to the behavior of the

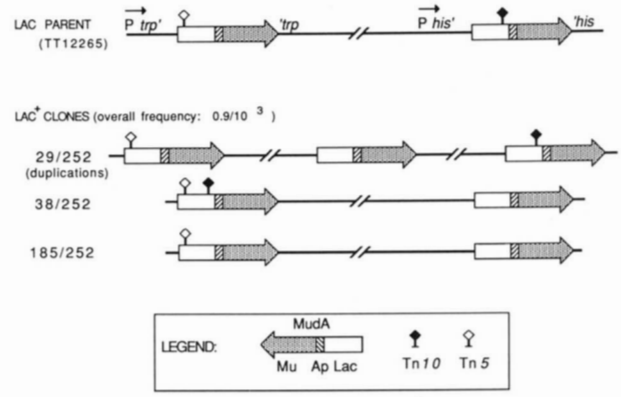

FIGURE 7.— Frequency and types of products of recombination between direct repeats.

same sequences when placed at the same sites in inverse orientation; under these conditions, no exchanges of flanking sequences (inversions) were detected.

"Gene conversion" events: For convenience in describing all the preceding results, we have used "gene conversion" to describe the class of recombinants that have lost one of the drug resistance markers initially inserted within one of the *lac* operons. Superficially these recombinants resemble products of gene conversion because some information (part of the normal *lac* operon) is duplicated while other information (one mutant site with its drug resistance) is lost. While some of these recombinants may, in fact, have been generated by such a nonreciprocal information exchange, other explanations are possible.

The *lac*$^+$ allele in the "gene conversion" class may have been generated by sister-chromosome exchange, such that the *lac*$^+$ recombinant allele and the second product of the exchange are left in different sister chromosomes which segregate from each other at the next cell division. This event, which can occur between inverse or direct order sequences, generates a *lac*$^+$ allele which is not associated with the other product of *lac*$^+$ recombination; the recombinant that is recovered carries one repaired (*lac*$^+$) copy of the *lac* operon and one copy which was not involved in any exchange. If this occurs, we cannot determine whether or not information is lost in the course of the actual recombination event. These points will be discussed below.

DISCUSSION

Although strains carrying inversion rearrangements are known, they are rare. Attempts to select spontaneous inversions formed by recombination between naturally repeated chromosomal sequences (REP) have yielded inversions at very low frequency (SCHMID and ROTH 1983a,b); the same selection scheme permits recovery of duplications (ANDERSON and ROTH

1978). When more extensive repeated sequences are deliberately placed in the chromosome, one finds that some endpoints permit inversion while others do not (SEGALL, MAHAN and ROTH 1988; REBOLLO, FRANCOIS and LOUARN 1988). In view of this, we presume that the recombination system used earlier by KONRAD (1969, 1977) and by ZIEG and KUSHNER (1977) involved a nonpermissive interval, since no inversions were recovered. In contrast, the 45-kb long homologies that include the *rrn* operons used by HILL and HARNISH (1981) must flank a permissive interval, since inversions were found.

In this study, we have placed mutant *lac* operons at sites flanking two intervals *his*(42′) to *trp*(34′) and *trp*(34′) to *pyrC*(23′). The second interval includes the terminus of replication; the first does not. None of the Lac^+ recombinants arising in these strains carries an inversion; thus both intervals are classified as nonpermissive. We have examined the nature of the noninversion recombinants that are recovered. The fact that observation of frequent Lac^+ clones (10^{-4}) requires both parental alleles and a functional *recA* gene suggests that recombination between alleles is occurring; this conclusion is reinforced by the finding of recombinant types in which genetic information has moved reciprocally between the two parental sequences. However, the recombinational interactions between these alleles do not yield inversions, which should be generated by a single reciprocal exchange between sequences in the same chromosome. We are confident that the methods used here to select and detect inversion are effective, because the same methods reveal inversions of permissive chromosomal segments (SEGALL, MAHAN and ROTH 1988). While the sites used here are nonpermissive for inversion, sequences placed in *direct* order at these sites interact and are able to complete the joining of at least one pair of flanking sequences required for generation of a duplication. Thus the exchange of flanking markers occurs for sequences at sites (*e.g.*, *his* to *trp*) known to be nonpermissive for the fully reciprocal exchange required to form an inversion. If sequences at these sites do interact and can exchange flanking sequences when in direct order, why are reciprocal exchanges between inverse order repeats (inversions) not detected? We will discuss two general possibilities: (1) lethal inversions, and (2) mechanistically impossible inversions. We will argue against lethal effects and present a model to explain a means of mechanistically preventing inversions of particular chromosomal segments.

The failure to find inversion recombinants for the nonpermissive intervals described here could be explained if these recombinants are formed but are not viable. The basis for this lethality is not obvious since the formation of these inversions would not disrupt any essential sequence. Several mechanisms have been suggested by which large inversion mutations might have lethal consequences. Inversions could cause lethal changes in gene dosage as a result of altering the position of many genes *vis à vis* the origin and terminus of replication (SCHMID and ROTH 1987). Lethal effects could also be caused by disturbing the mechanisms of replication termination. It has been suggested that the terminus of replication includes sequences which can only be replicated in one direction; if an inversion placed such sequences in the wrong orientation, replication would be impossible (REBOLLO, FRANCOIS and LOUARN 1988). In wild-type *E. coli*, most transcription proceeds in the same direction as replication (BREWER 1988). Some inversions could reorient transcribed sequences such that their direction of transcription opposes the direction of replication; evidence has been presented that heavy transcription can inhibit replication in some systems (BREWER 1988). Inversions might alter the spacing of sequences critical to chromosome folding or chromosome distribution (SEGALL, MAHAN and ROTH 1988). These are just a few hypothetical mechanisms which could explain why some inversions might be lethal; any or all of these factors *could* be involved. However it has yet to be demonstrated that *any* inversion is lethal and it is clear that many inversions are not lethal. Several lines of reasoning lead us to believe that functional difficulties such as those listed above are not the major cause of nonpermissive intervals.

One of the nonpermissive intervals described here (*trp-pyr*) is symmetrically placed *vis à vis* the terminus of replication. This inversion does not change the dosage of any genes and does not change the orientation of any transcript with respect to the direction of replication. If some inversions had serious consequences for cell function, we would expect that many other inversions would have sublethal deleterious effects on growth. Only one of the inversions we have studied previously shows a reduced growth rate. The main basis of our belief that lethality is not the underlying explanation for nonpermissive intervals is the fact that we have been able to construct an inversion of the nonpermissive *his-trp* region of the chromosome. This inversion was constructed by a transduction cross in which both inversion join points are simultaneously inherited. The resulting inversion mutant shows no impairment of growth. Although only one such case has been tested, it seems clear that lethality can not explain all nonpermissive intervals. For this reason we have pursued possible mechanistic explanations for nonpermissive intervals.

One point has been noted. All of the recombinant types that are described here, double recombinants and "gene convertants," can all arise by sister chromosome exchanges. Inversions, which are not ob-

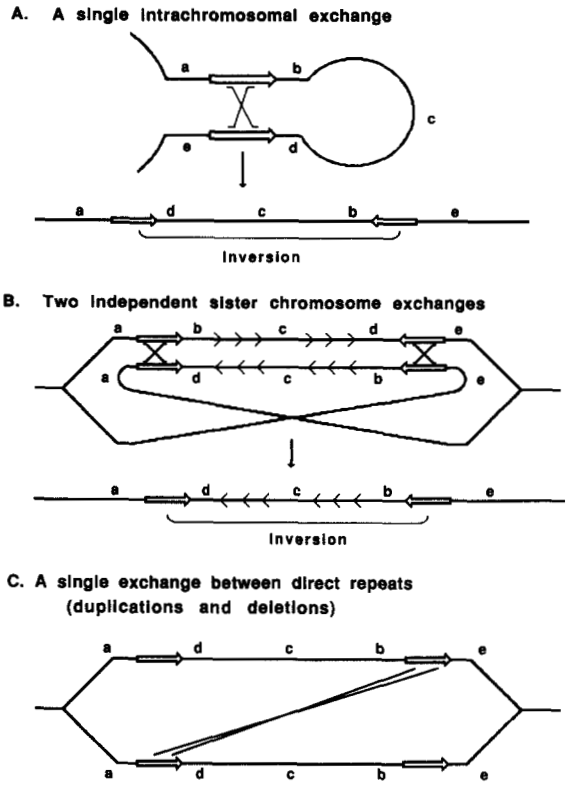

FIGURE 8.— Recombination events which can give rise to chromosomal rearrangements.

served for the intervals studied here, require a reciprocal exchange between sequences in the *same* chromosome. Inversion formation is diagrammed in Figure 8. A single, fully reciprocal exchange between two sequences in the same chromosome leads to an inversion (Figure 8A). In principle, an inversion can also occur by sister chromosome exchanges; however, this requires two independent events (Figure 8B). In contrast to inversions, duplications and deletions require only one exchange between sister chromsomes (Figure 8C). Extrapolating from the frequency of duplications (10^{-4}), we would expect that the two coincident exchanges required for generation of an inversion by sister chromosome exchange would be extremely infrequent (10^{-8}). We would be unable to detect such events in the experiments described here since it would require screening more than 10^4 Lac^+ recombinants to observe inversions above the background of the noninversion Lac^+ recombinants. Thus we suspect that inversion formation, observable in our experiments, cannot occur by sister chromosome exchange; inversion requires an intrachromosomal exchange (Figure 8A).All recombinant types that were found in the experiments described here can, in principle, be generated by recombination between sister chromosomes. This is most difficult to visualize for the double recombinant types and for the other types in which recombination leads to alteration of both copies of the repeated sequences. These types may

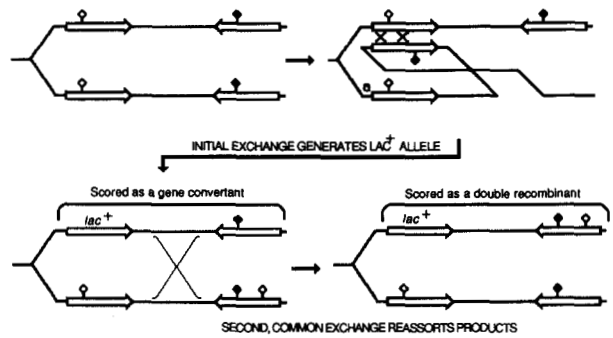

FIGURE 9.— The generation of apparent gene convertants and of double recombinant products by recombination events between sister chromosomes.

arise as shown in Figure 9. We have diagrammed a double recombination event occurring between dissimilar alleles present on different sister chromosomes. A single exchange between such sequences breaks the chromosome, and, if replication continues, will lead to a palindromic double chromosome which we presume would be lethal. If a double exchange occurs, the reciprocal products are left on different sister chromosomes as shown in Figure 9. A secondary single sister-strand exchange anywhere in the large region between the sites of the two repeated homologies will reassort the alleles and bring the two products of the initial exchange onto the same chromosome; this chromosome can then segregate to yield a clone scored as a double recombinant. Any lac^+ sister strand recombinant in which this secondary exchange does not occur (or any one with an even number of exchanges) will carry a lac^+ allele at one site and an uninvolved parental allele at the second site; these recombinants will be classified as "gene conversion" types regardless of the nature of the initial exchange. We believe that these secondary events must be extremely likely since they can occur at any point in the large chromosomal region between the inverse homologous sequences. If essentially unlimited sister chromosome exchanges occurred in this large region, then the Lac^+ recombinants would be evenly distributed between the double recombinant class and the apparent gene conversion class (*i.e.*, 50% recombination). Our data support this possibility, in that both of these classes are large. For the larger *trp-pyr* interval (11 min) it is nearly 1:1, and for the *his-trp* interval (8 min) the ratio of classes is 3:1. The difference in these ratios could reflect differences in the frequency of the secondary reassorting exchanges within these two intervals.Thus we find recombinants that can all be explained by sister chromosome exchange; we fail to find recombinants which require an exchange between two sequences in the same chromosome. The failure to detect inversion of nonpermissive intervals could be explained if sequences at certain pairs of sites are barred from intrachromosomal exchanges. We

propose that the folded chromosome of Salmonella is so structured that some pairs of sites (permissive) can contact each other while others (nonpermissive) cannot contact each other *when prospective partners are present in the same circular molecule.* Since sequences at all sites interact to give noninversion recombinants, we propose that recombination between sister chromosomes is not subject to these constraints of chromosome packaging. That is, pairs of homologies located in different sister chromosomes can contact each other regardless of whether they flank a permissive or nonpermissive interval. If these proposals are correct, then all the recombinant types described here (which involve sequences at nonpermissive sites) must have formed by interactions between sister chromosomes. Sequences at permissive sites would, according to this proposal, be able to contact each other in a single chromosome or by sister-strand exchanges; the inversion class would result only from intrachromosomal exchanges. If our proposal is correct, then use of inverse-order sequences at nonpermissive sites allows one to specifically observe sister-strand exchanges; by scoring inversion formation for permissive intervals, one specifically observes intrachromosomal exchanges.

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