Recombination Between Chromosomal IS*200* Elements Supports Frequent Duplication Formation in *Salmonella typhimurium*

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ABSTRACT

Spontaneous tandem chromosomal duplications are common in populations of *Escherichia coli* and *Salmonella typhimurium*. They range in frequency for a given locus from 10^{-2} to 10^{-4} and probably form by RecA-dependent unequal sister strand exchanges between repetitive sequences in direct order. Certain duplications have been observed previously to confer a growth advantage under specific selective conditions. Tandem chromosomal duplications are unstable and are lost at high frequencies, representing a readily reversible source of genomic variation. Six copies of a small mobile genetic element IS*200* are evenly distributed around the chromosome of *S. typhimurium* strain LT2. A survey of 120 independent chromosomal duplications (20 for each of six loci) revealed that recombination between IS*200* elements accounted for the majority of the duplications isolated for three of the loci tested. Duplications of the *his* operon were almost exclusively due to recombination between repeated IS*200* elements. These data add further support to the idea that mobile genetic elements provide sequence repeats that play an important role in recombinational chromosome rearrangements, which may contribute to adaptation of bacteria to stressful conditions.

BACTERIAL genomes are constantly in flux. Chromosomal duplications are common and are frequently selected in the laboratory and presumably in nature. Direct order repeated chromosomal sequences can serve as substrates for the recombinational machinery, and unequal exchanges between such repeats can result in formation of amplifications and deletions (DEONIER 1987; ROTH *et al.* 1996). RecA-dependent recombination between the repeated ribosomal RNA genes has been shown to result in formation of tandem duplications (HILL *et al.* 1977; ANDERSON and ROTH 1981), inversions (HILL and HARNISH 1981) and transpositions (HILL and HARNISH 1982). The repeated sequence, *rhs,* is found in multiple copies in *Escherichia coli* but is absent from *Salmonella typhimurium;* this sequence provides the homology required for the frequent tandem duplication of the *glyS* region (HILL and CAPAGE 1979; LIN *et al.* 1984). The *S. typhimurium* and *E. coli* genomes both contain REP sequences, small imperfect inverted repeats, scattered throughout the chromosome (HIGGINS *et al.* 1982). These REP sequences are generally found between genes, often within transcribed regions. Recombination between separated REP elements can result in rare tandem chromosomal duplications that fuse genes to foreign promoters at the duplication join point and thus provide novel regulatory behavior (ANDERSON and ROTH 1978a; SHYAMALA *et al.* 1990).

Such rearrangements generate diversity in bacterial populations. The ability to amplify chromosomal DNA sequences by tandem duplication has been shown to convey a selective advantage in chemostats. Growth of *S. typhimurium* on limiting malate or arabinose selects for duplication of a specific region of the chromosome (SONTI and ROTH 1989). This region encodes the *crp* locus and permease genes for several carbon sources. Duplication of this region confers improved growth on limiting levels of several carbon sources (R. SONTI and J. R. ROTH, unpublished results). These results suggest that the ability to amplify specific sets of genes can provide a reversible mechanism for bacterial adaptation.

IS*200* is a 708-bp (GIBERT *et al.* 1991) insertion sequence found predominately within species of Salmonella (LAM and ROTH 1983a) and sporadically in other enteric bacteria (GIBERT *et al.* 1990; BISERICIC and OCHMAN 1993b). Wild-type *S. typhimurium* LT2 has six chromosomal copies of IS*200* (LAM and ROTH 1983a) evenly distributed around the chromosome (LAM and ROTH 1983b; SANDERSON *et al.* 1993). ANDERSON and ROTH described large spontaneous tandem chromosomal duplications in *S. typhimurium* LT2. Some of these duplications arose by unequal recombination between *rrn* loci, while others had endpoints that corresponded to the known map positions of IS*200* elements (ANDERSON and ROTH 1978b, 1981). We have investigated the possibility that IS*200* sequences could be used as sequence

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TABLE 1

Bacterial strain list

Strain[a]	Genotype	Source[b]
LT2	Wild-type	Lab collection
TT11330	*nadA504*::Mu*dJ*	Lab collection
TT12903	*pyrC2688*::Mu*dJ*	Lab collection
TT16759	*hisC10174*::Mu*dF*	Lab collection
TT12307	*purG2148*::Mu*dJ*	Lab collection
TT13041	*cysG1573*::Mu*dJ*	Lab collection
TT12910	*pyrE2678*::Mu*dJ*	Lab collection
TT398	*nadA213*::Tn*10*	Lab collection
TT459	*pyrC691*::Tn*10*	Lab collection
TT13	*trp-1041*::Tn*10*	Lab collection
TT28	*hisC8579*::Tn*10*	Lab collection
TT2090	*cysA1539*::Tn*10*	Lab collection
TT281	*purG876*::Tn*10*	Lab collection
TT215	*lysA565*::Tn*10*	Lab collection
TT172	*cysG1510*::Tn*10*	Lab collection
TT9521	*pyrE2678*::Mu*d*1-8	Lab collection
TT5	*metE1974*::Tn*10*	Lab collection
TT273	*purA874*::Tn*10*	Lab collection
TT15008	*serA977*::Tn*10*	Lab collection
TT18371	DUP 1906 [(IS*200* IV *hisC10174*::Mu*dF*)*IS*200*(*his*$^+$IS*200* V)]	
TT18372	DUP 1907 [(IS*200* V *purG2148*::Mu*dJ*)*IS*200*(*pur*$^+$IS*200* I)]	
TT18373	DUP 1908 [(IS*200* V *purG2148*::Mu*dJ*)*IS*200*(*pur*$^+$IS*200* II)]	
TT18374	DUP 1909 [(IS*200* I *cysG1573*::Mu*dJ*)*IS*200*(*cys*$^+$IS*200* II)]	
TT18375	DUP 1910 [(IS*200* I *cys*$^+$)*IS*200*(*cysG1573*::Mu*dJ* IS*200* II)]	
TT18376	DUP 1911 [(IS*200* II *cysG1573*::Mu*dJ*)*IS*200*(*cys*$^+$ IS*200* V)]	
TT18377	*his*$^+$	haploid segregant of TT18371
TT18378	*hisC10174*::Mu*dF*	haploid segregant of TT18371
TT18379	*pur*$^+$	haploid segregant of TT18372
TT18380	*purG2148*::Mu*dJ*	haploid segregant of TT18372
TT18381	*pur*$^+$	haploid segregant of TT18373
TT18382	*purG2148*::Mu*dJ*	haploid segregant of TT18373
TT18383	*cys*$^+$	haploid segregant of TT18374
TT18384	*cysG1573*::Mu*dJ*	haploid segregant of TT18374
TT18385	*cys*$^+$	haploid segregant of TT18375
TT18386	*cysG1573*::Mu*dJ*	haploid segregant of TT18375
TT18387	*cys*$^+$	haploid segregant of TT18376
TT18388	*cysG1573*::Mu*dJ*	haploid segregant of TT18376

[a] All TT strains are derivatives of LT2.
[b] All strains were isolated during the course of this study unless otherwise noted.

repeats for formation of duplications by homologous recombination.

MATERIALS AND METHODS

Bacteria, phage and genetic methods: All strains used in this study are derivatives of *S. typhimurium* LT2 (Table 1). All phage used are derivatives of P22. Methods for manipulating phage and bacteria were described (DAVIS *et al.* 1980).

Media: Luria-Bertani (LB) and E media were used as rich and minimal media, respectively (VOGEL and BONNER 1956; DAVIS *et al.* 1980). E medium was supplemented with 0.2% glucose and, when necessary, with various nutritional supplements (Sigma Chemical Co.) at final concentrations as suggested (DAVIS *et al.* 1980). Kanamycin(monosulfate) and tetracycline(HCl) were obtained from Sigma Chemical Co. Final concentrations of tetracycline(HCl) for all media were 20 μg/ml; kanamycin(monosulfate) was used in rich medium at 50 μg/ml and in minimal medium at 125 μg/ml.

Transductional methods: The high frequency, generalized transducing phage P22 HT105/1 *int-201* (SCHMIEGER 1972) was used for all transductional crosses. Transductional crosses were achieved by adding 0.1 ml of an overnight broth culture of the recipient strain to 0.1 ml of a generalized transducing lysate. This mixture was incubated with shaking at 37° for 1 hr before spreading on selective medium.

Isolation of independent duplication-containing strains: Twenty independent overnight cultures of wild-type *S. typhimurium* LT2 were transduced with a series of transducing lysates generated from strains containing an auxotrophic insertion of one of the transposition-defective Mu*d* elements, Mu*dJ* (HUGHES and ROTH 1988) or Mu*dF* (R. SONTI and J. R. ROTH, unpublished results). These elements encode kanamycin-resistance determinants. We determined the frequency of duplications that include each donor locus by transducing the unselected recipient culture of strain LT2 to kanamycin resistance and screening for prototrophic transductants. A kanamycin-resistant prototrophic recombinant arises when the do-

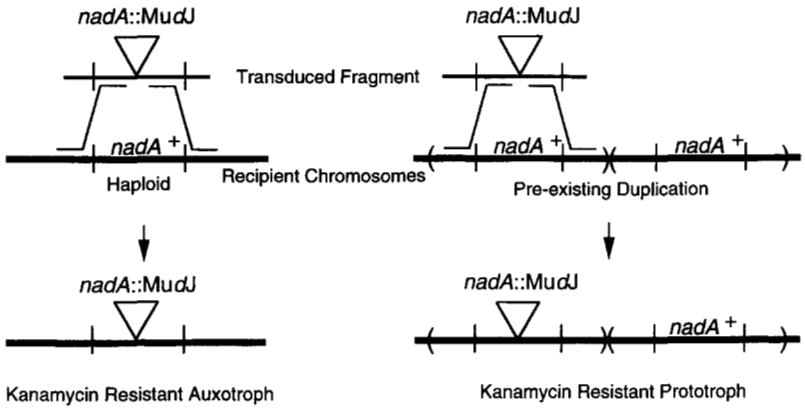

FIGURE 1.—Isolation of spontaneous duplications. Independent overnight cultures were transduced with lysates generated from strains containing auxotrophic insertions of Mu*d* elements that encode resistance to kanamycin. The *nadA*::Mu*d* insertion depicted above causes a requirement for nicotinamide. Haploid recipient cells that inherit the Mu*d* insertion by homologous recombination are kanamycin-resistant auxotrophs. Recipient cells that contain a preexisting duplication of the relevant region can inherit the insertion by homologous recombination in either copy of the locus and remain prototrophic due to the duplicate copy. Duplications identified in this way can be maintained by selection for both alleles of the locus (kanamycin resistance and prototrophy).

nor auxotrophic Mu*d* element is inherited by a recipient cell carrying a preexisting duplication of the particular chromosomal locus. The transductant inherits the donor insertion by homologous recombination with one copy of the duplicated region. The second copy remains functional and confers prototrophy (Figure 1).

Mapping of duplication endpoints: Duplication endpoints were genetically mapped by transducing a duplication strain with a series of transducing lysates generated from strains that each contain an auxotrophic Tn*10* insertion of known map position. Inheritance of a Tn*10* insertion whose locus is included in the recipient duplication will yield prototrophic tetracycline-resistant transductants; inheritance of a Tn*10* insertion whose gene is not included in the recipient duplication will cause the recipient to acquire the particular auxotrophic requirement conferred by the Tn*10* insertion.

Detection of IS*200*-bearing restriction fragments: Genomic DNA was isolated and cut with restriction enzymes obtained from New England Biolabs. DNA restriction fragments were separated by agarose gel electrophoresis for 18 hr at 40 V in 0.7% agarose. DNA size standards were obtained from GIBCO BRL (1 kb ladder). The gel itself was probed as described (TSAO *et al.* 1983), using hybridization buffers and wash solutions as described (CHURCH and GILBERT 1984). The internal 300-bp *Eco*RI-*Hin*dIII fragment of the IS*200* element was used as the probe to detect specific IS*200*-bearing genomic restriction fragments. The probe DNA was radioactively labeled using the Random Primed DNA Labeling Kit obtained from Boehringer Mannheim and (α-^{32}P)dATP obtained from ICN. Autoradiographs were achieved by the exposure of Kodak Scientific Imaging Film to the radioactive gel.

Duplication instability assay: Six strains containing each characterized duplication type (TT18371–18376) were assayed for their ability to lose the duplication and generate haploid segregants. A single colony of each duplication-containing strain was used to inoculate a tube containing 2 ml of E medium with 0.2% glucose and kanamycin to select for maintenance of the duplication. After overnight growth, these cultures were diluted 100-fold into LB (nonselective) to allow the loss of the duplication. This culture was incubated overnight at 37° with shaking, diluted 10^6-fold and plated for single colonies on LB plates. Each plate was then printed to LB kanamycin and E glucose plates to score loss of kanamycin resistance and the appearance of the auxotrophy conferred by the Mu*d* insertion. The two classes, Kn^R auxotroph and Kn^S prototroph, represent the phenotypes expected for the two haploid segregant types (Figure 3). The segregant frequency is the sum of the kanamycin-sensitive prototrophs and the auxotrophic kanamycin-resistant clones divided by the total number of colonies scored.

RESULTS

Isolation of spontaneous chromosomal duplications: Spontaneous duplications were selectively "trapped" by transducing a wild-type recipient strain with phage P22 lysates grown on a series of auxotrophic drug-resistant insertion mutants. Drug-resistant transductants were subsequently screened for prototrophy; only recipient cells with a preexisting duplication of the region corresponding to the donor marker can give rise to drug-resistant prototrophic transductants (Figure 1). The duplication-containing transductant can be selectively maintained on minimal medium including the relevant drug. Duplications trapped in this way are subject to loss when the selection is relaxed. A segregation event generates clones of the two haploid genotypes (auxotrophic drug-resistant and prototrophic drug-sensitive).

In isolating duplications, six auxotrophic Mu*d* insertions in the following loci were used as donor markers: *nadA, pyrC, hisC, purG, cysG* and *pyrE* (Figure 2). These were chosen such that one insertion lies between each pair of IS*200* elements and none lies within a region bounded by direct order repeats of the ribosomal RNA cistrons (*rrn*). This was done to allow the detection of several types of IS*200*-mediated duplications without having to screen through the large number of duplications formed between *rrn* repeats. For each donor Mu*d* insertion used, 20 independent duplications were isolated and further characterized.

Genetic mapping of the duplication endpoints: Duplication endpoints were genetically mapped by transduction using donor lysates generated from strains containing auxotrophic Tn*10* insertions near known IS*200* positions in the chromosome. The duplications in the 120 strains (described above) were genetically mapped by transducing each strain to tetracycline resistance using a series of auxotrophic Tn*10* insertion mutants as trans-

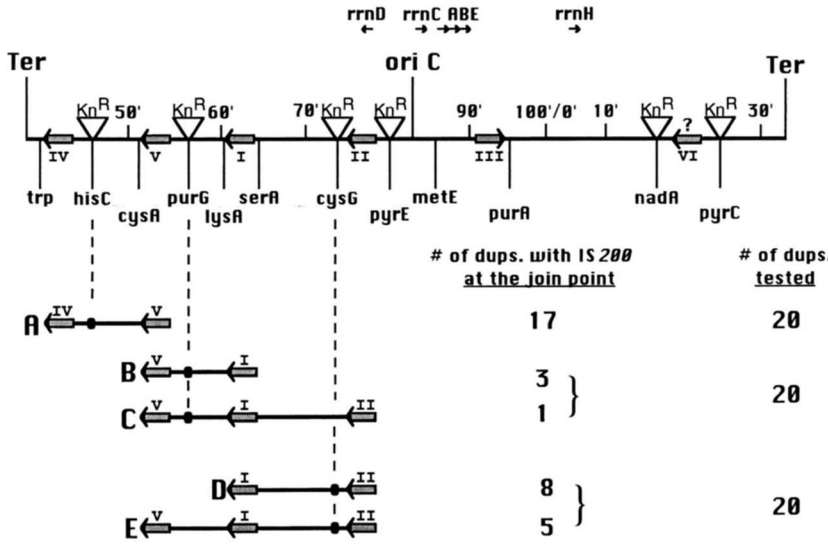

FIGURE 2.—Chromosomal duplication types with IS*200* endpoints. In the above map of the Salmonella chromosome, the filled boxes with the arrowheads indicate the position and orientation of the six IS*200* elements in the *S. typhimurium* LT2 chromosome. The roman numerals indicate a previous designation for each IS*200* element based on *Pvu*II restriction fragment mobility (LAM and ROTH 1983b). Auxotrophic insertions of Mu*d* elements, used to isolate the duplications, are indicated by the triangles with Kn^R. All other loci shown indicate auxotrophic Tn*10* insertions used to map the extent of the duplication intervals (merodiploidy analysis). The letters A–E indicate various types of tandem chromosomal duplications that have IS*200* at the join point. The filled dot within each interval indicates the position of the locus used to isolate that duplication strain.

ductional donors. If a duplication strain yielded prototrophic recombinants, the duplication was inferred to include the site of the donor Tn*10* insertion (Figure 2). This gives a very approximate position of duplication endpoints; resolution depends on the distance between the flanking markers used. For each IS*200* copy with a nearby duplication endpoint, the mapping places that endpoint within a region of the following sizes: copy I, 1 min; copy II, 7 min; copy IV, 8 min; copy V, 4.5 min. Of the 120 independent duplications mapped by this method, 39 (32.5%) have both endpoints near a known chromosomal IS*200* element (Table 2).

Characterization of join point restriction fragments: Duplication-containing strains with endpoints near IS*200* positions were further characterized by restriction fragment analysis to test whether they had formed by exchanges between copies of IS*200*. Such duplication strains are expected to have a novel IS*200*-containing restriction fragment at the duplication join point (fragment C-B, Figure 3). This join point fragment should be present in addition to all six of the standard parental IS*200*-bearing restriction fragments. DNA was isolated from each strain having a duplication with endpoints near IS*200* elements; this DNA was digested with *Pvu*II or *Hinc*II, restriction endonucleases that do not cut within the IS*200* element. The fragments were separated by agarose gel electrophoresis, and the gel was probed using a 300-bp internal fragment of IS*200* as described in MATERIALS AND METHODS. Novel IS*200*-bearing restriction fragments were observed for 34 of the 39 duplication-containing strains with endpoints near known IS*200* positions (data not shown). These results suggest that 34 of the duplications tested have an IS*200* element at the join point. For

TABLE 2

Genetic characterization of spontaneous duplications

Duplicated locus	Map location (min)	Kn^R transductants per pfu ($\times 10^7$)	Duplication frequency ($\times 10^4$)	Fraction with endpoints mapping near IS*200*	IS*200* Duplication types[a] (number)	Segregant frequency[b]
nadA::Mu*d*J	17′	1.6	2.5	2/20	None	
pyrC::Mu*d*J	23′	5.4	2.7	0/20	None	
hisC::Mu*d*F	42′	630	1.5	17/20	A (17)	3%
purG::Mu*d*J	54′	290	3.1	5/20	B (3)	14%
					C (1)	25%
cysG::Mu*d*J	73′	77	13	13/20	D (8)	15%
					E (5)	56%
pyrE::Mu*d*J	83′	11	2.8	2/20	None	

[a] See Figure 2 for endpoints of each duplication type that had IS*200* at the join point. Note that several duplications whose endpoints mapped genetically to positions corresponding to IS*200* copies turned out not to have IS*200* at the join point.

[b] Each strain was grown to full density (2×10^9 cells/ml) in E glucose plus kanamycin that selects for maintenence of the duplication. The culture was diluted 100-fold into LB broth lacking kanamycin and grown to full density to allow duplication segregation. The final population was diluted and plated for single colonies. The number presented is the percentage of total colonies that had lost either kanamycin resistance or prototrophy by segregation of the duplication.

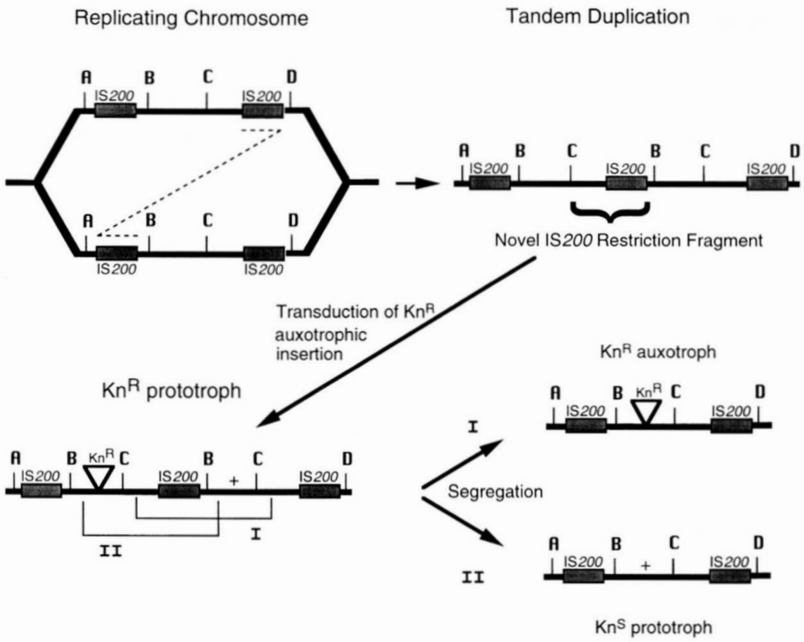

FIGURE 3.—Formation and loss of duplications with an IS*200* at the join point. ▨, IS*200* elements present in the Salmonella chromosome; ▬, represent the bacterial chromosome. Restriction sites are indicated by A, B, C and D. Unequal sister strand exchange between ectopic copies of IS*200*, in direct orientation, is represented by - - - and results in the formation of a tandem chromosomal duplication that contains a novel copy of IS*200* at the join point. The duplication join point will generate a novel IS*200*-bearing restriction fragment not found in haploid strains. The duplication is unstable but can be selectively maintained by culturing cells in minimal medium with kanamycin. When this selection is relaxed, the duplication can segregate by recombination events between the extensive repeated sequence, to yield either of the two haploid types (kanamycin-resistant auxotrophs and kanamycin-sensitive prototrophs).

the *Hin*cII restriction digests, the novel IS*200*-bearing restriction fragments are between 1.6 and 4 kb in length, indicating that in each case IS*200* is at or very near the join point.

Duplication segregation, frequency and structure: Tandem duplications generated by unequal recombination between copies of IS*200* would be expected to segregate, returning the strain to the haploid state. All such segregants would lose the join point and the associated novel copy of IS*200*. The haploid segregant is expected to be either a kanamycin-resistant auxotroph or a kanamycin-sensitive prototroph, depending on the position

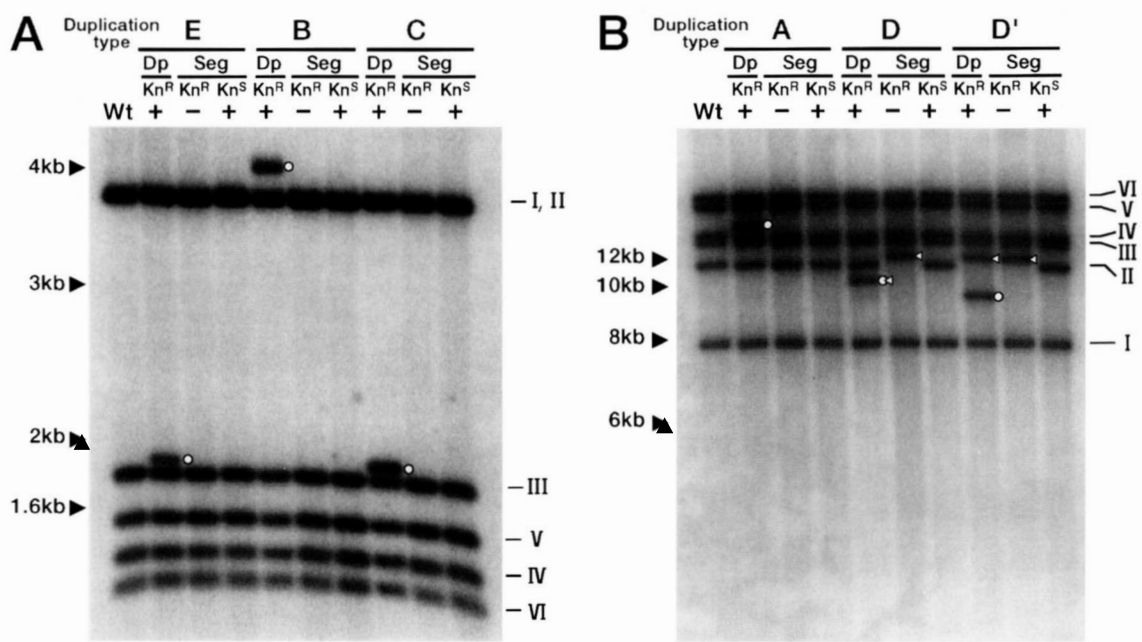

FIGURE 4.—Novel IS*200*-bearing join point fragments. Genomic DNA was isolated and digested, and fragments were separated by agarose gel electrophoresis. DNA for A was cut with *Hin*cII; DNA for B was cut with *Pvu*II. A radioactively labeled, internal fragment of IS*200* (300 bp) was used to probe the gel directly. The six copies of IS*200* native to *S. typhimurium* are indicated by the roman numerals I–VI (Figure 2). Each novel IS*200*-bearing restriction fragment (inferred to be at the join point) is indicated by the white dot outlined in black. For both panels wild-type *S. typhimurium* LT2 is indicated by a Wt. Each set of three lanes represents DNA from the duplication-containing strain (Dp), a kanamycin-resistant auxotrophic haploid segregant and a kanamycin-sensitive prototrophic haploid segregant, respectively. The duplication type is indicated by the letters A–E (Figure 2). +, prototrophy; −, auxotrophy; ▷, IS*200*-bearing restriction fragments that exhibit an altered mobility due to the *cysG1573*::Mu*dJ* insertion.

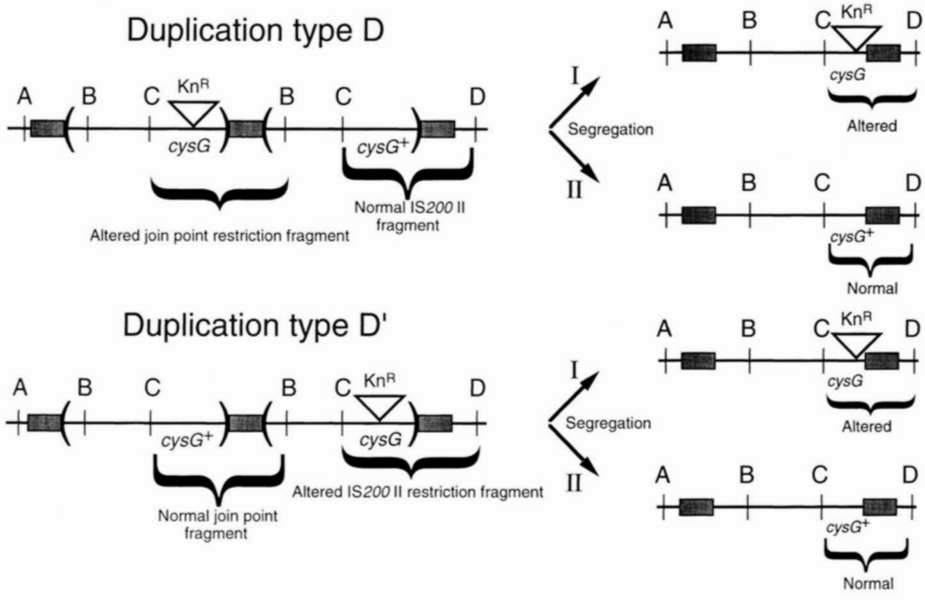

FIGURE 5.—Insertion of a Mu*d* element alters restriction fragments that include IS*200* II. The letters A–D indicate *Pvu*II restriction sites. The triangle with Kn^R indicates the *cysG1573*::Mu*dJ* insertion. For the duplication type D, the Mu*d* element is inherited near the join point of the duplication and therefore alters the IS*200*-bearing join point fragment. For the duplication type D′ the Mu*d* element is inherited in the other copy of the duplication where the *cysG* locus is far from the join point and therefore alters the mobility of the IS*200* II-bearing restriction fragment. Both duplication types segregate haploid clones that are either kanamycin-sensitive prototrophs or kanamycin-resistant auxotrophs. The kanamycin-resistant auxotrophs from both duplication types exhibit altered IS*200* II-bearing restriction fragments.

of the recombination event with respect to the Mu*d* insertion used to trap the duplication (Figure 3). Both classes of segregants were isolated from six strains containing duplications of each type (A–E, Figure 2). To determine if the novel IS*200*-bearing restriction fragment is lost in each segregant, genomic DNA was isolated from each segregant type and compared with DNA from the haploid wild-type LT2 and the duplication-containing parent. Upon segregation of the duplication, every tested duplication type that exhibited a novel IS*200*-bearing restriction fragment lost that fragment and exhibited a hybridization pattern like the wild-type parent (Figure 4). This suggests that the novel IS*200* copy is at the join point and does not represent a transposition event of any one of the six resident copies of IS*200* present in the parent. The appearance of a novel IS*200*-bearing restriction fragment and the appearance of both classes of haploid segregants supports the conclusion that the duplications (type A–E) are tandem in structure.

Location of IS*200* II: The location of IS*200* II in the Salmonella chromosome was previously placed near the *cysG* locus (LAM and ROTH 1983b; SANDERSON *et al.* 1993). Results presented here refine that position and place this copy within 12 kb clockwise of the *cysG* locus. This was inferred by the observation that the *cysG1573*::Mu*dJ* insertion (used in trapping the duplication) affects the mobility of a 12-kb *Pvu*II restriction fragment bearing the IS*200* II element (Figure 4). The *cysG*::Mu*d* element can be inherited within either of the two copies of a tandem duplication of *cysG* (Figure 5). If the Mu*d* element is inherited near the join point, the novel IS*200*-bearing restriction fragment at the join point will exhibit a mobility distinct from that of the standard join point fragment. When the Mu*d* element is inherited in the copy of the *cysG* locus far from the join point of the duplication, the restriction fragment corresponding to the IS*200* II element should exhibit an altered mobility. Both classes of transductants were observed for duplications including the *cysG* locus.

The two haploid segregants isolated from each of these strains, Kn^R auxotroph and Kn^S prototroph, both lost the novel IS*200*-bearing restriction fragments. In each kanamycin-resistant haploid segregant (which retained the Mu*d* element), the restriction fragment corresponding to the IS*200* II element exhibited an altered mobility, while the haploid prototrophic kanamycin-sensitive segregants in each case exhibited the wild-type parental pattern (Figure 4). These data demonstrate that the *cysG* locus is within 12 kb of IS*200* II and within the type D duplication. Therefore, IS*200* II must lie just clockwise of the *cysG* locus (Figure 2).

IS*200* copies involved in duplication formation: All IS*200* elements except III and VI were involved in forming duplications. Of the 120 independent duplications characterized, no duplications were formed using either of the two copies of IS*200* present in the chromosomal arc from the origin clockwise to the terminus (IS*200* III or IS*200* VI, Figure 2). Four duplications had endpoints that mapped to the general region of these two elements, but in each case the restriction fragment hybridization analysis suggested that the duplications were not due to recombination between IS*200* elements (*i.e.,* no novel IS*200* restriction fragments were observed). Explanations of this behavior are suggested below.

DISCUSSION

Recombination between repetitive sequences has been implicated in rearrangements of both prokaryotic

and eukaryotic chromosomes. We present evidence here that recombination between IS*200* elements is a major contributor to formation of tandem duplications in Salmonella. The frequencies of these duplications are surprisingly high considering the small size of the IS*200* element (708 bp). Thus the relatively small mobile genetic elements are efficiently recognized and acted upon by the homologous recombinational machinery of the host. Of the spontaneous duplications of the histidine operon, 85% are due to recombination between IS*200* elements. A somewhat smaller fraction of the duplications of other loci are due to recombination between IS*200* elements (20 and 65% for *purG* and *cysG*, respectively). These data suggest that the four copies of IS*200* that are located counter-clockwise of the origin of replication are all in the same orientation and are similar enough in sequence to support frequent homologous recombination. Analysis of IS*200* elements from many Salmonella isolates shows that these sequences are invariant, while the same elements in *E. coli* isolates show ~3% divergence from each other and ~7% divergence from the Salmonella version (BISERICIC and OCHMAN 1993a).

Transductional recombination data suggest that IS*200* I and IS*200* V are in the same orientation as the *hisD984*::IS*200* insertion (C. CONNER and J. R. ROTH, unpublished results). The orientation of the *hisD984*::IS*200* insertion is known from the sequence data of the insertion site in *hisD* (LAM and ROTH 1986). From these data we infer that the four IS*200* elements counter-clockwise from the origin of replication (IS*200* II, I, V and IV) are all oriented with their open reading frame (ORF) pointing toward the terminus.

The absence of IS*200*-mediated duplications for the intervals that correspond to the two copies of IS*200* clockwise of the origin of replication, IS*200* III and IS*200* VI, could be due to sequence divergence or to improper orientation of these elements. We think sequence divergence is an unlikely explanation since all six chromosomal IS*200* elements recombine equally well in transductional crosses, with a drug resistance marker flanked by direct copies of IS*200* (K. R. HAACK and J. R. ROTH, unpublished results). The failure to detect IS*200*-mediated duplications between IS*200* III and the four elements counter-clockwise of its position is due to the orientation of the IS*200* III element. The orientation of IS*200* III has been determined by sequence analysis of the corresponding region of the *S. typhimurium* LT2 chromosome (K. K. WONG, personal communication). Its orientation is opposite that of the four IS*200* elements counter-clockwise of Ori C. The orientation of IS*200* VI is unknown, but the failure to detect duplications involving this element can be explained if its orientation were opposite to that of IS*200* III. This would explain the absence of IS*200*-mediated duplications between elements III and VI. The absence of IS*200*-mediated duplications clockwise of element VI could be explained by the apparent lethality of duplications including the replication terminus. All previous attempts to isolate or construct duplications that include the terminus region have failed, suggesting that these duplications are either lethal or are destabilized by chromosome partitioning functions (D. R. HILLYARD and J. R. ROTH, unpublished results). DAVID SHERRATT (personal communication) has suggested the possibility that the *dif*/*xer* system might act to remove any duplications that include the *dif* site. The inferred orientation of all IS*200* elements present in the *S. typhimurium* LT2 chromosome is indicated in Figure 2.

In the course of these studies we have determined the orientation of several of the chromosomal IS elements (Figure 2) and have refined the mapping of two elements with respect to known chromosomal markers. A *cysG* insertion alters the mobility of a 12-kb *Pvu*II restriction fragment that includes IS*200* II. Our data show that IS*200* II lies within 12 kb of the *cysG* gene. Since the *cysG* insertion is within the duplication type D, IS*200* II must lie clockwise of the *cysG* locus, within 12 kb of the *cysG* gene. A kanamycin-resistance determinant placed by recombination within the IS*200* V, described above, exhibits 24% cotransduction with an insertion in the *eutA* gene (C. A. RAPPLEYE and J. R. ROTH, unpublished results), suggesting that IS*200* V lies within 13–15 kb of the *eutA* gene. This refines previous mapping of IS*200* elements (LAM and ROTH 1983b; SANDERSON, *et al.* 1993).

Spontaneous duplications are detected with high frequency under laboratory selection conditions and presumably are also selected in nature. Several duplication types are advantageous under laboratory conditions that would appear to resemble natural conditions. The plasmid NR1 confers resistance to elevated levels of antibiotics when the resistance determinant is amplified by unequal recombination between flanking copies of IS*1* elements (ROWND 1982). Duplication of a specific region of the *S. typhimurium* chromosome provides a dramatic increase in growth rate on limiting levels of several carbon sources (SONTI and ROTH 1989). The duplication includes the permease genes for several different carbon sources and the *crp* locus (R. SONTI and J. R. ROTH, unpublished results). Perhaps the best example is in *Vibrio cholerae* whose toxin genes are amplified during intestinal growth in rabbits; this occurs by RecA-dependent unequal exchanges between small (2.7 kb) repeats flanking the toxin genes (MEKALANOS 1983; GOLDBERG and MEKALANOS 1986).

Duplication may represent a reversible adaptive mutation that alters gene dosage without permanently altering genetic information. After the selective challenge has been removed, the chromosome can return to a haploid state. The positions of repetitive sequences within the genome may dictate the ability to duplicate

discrete segments of the chromosome whose amplification confers a selective advantage under certain conditions. The mobility of transposable elements allows an almost unlimited variety of duplication endpoints. These duplications will be valuable whenever regions flanked by direct repeats include genes that individually or synergistically enhance growth when amplified.

Transcription of an IS*200*-encoded ORF not only stimulates transposition but also results in a >32-fold increase in the frequency of homologous recombination between directly repeated chromosomal IS*200* elements (K. R. HAACK and J. R. ROTH, unpublished results). Perhaps transposase can stimulate homologous recombination between IS*200* copies by introducing specific cuts at the ends of the element. We suggest that IS*200* may be maintained and conserved in Salmonella because the element promotes beneficial genome rearrangements and supports frequent recombinational interactions between elements.

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