GENETIC MAPPING OF IS*200* COPIES IN *SALMONELLA TYPHIMURIM* STRAIN LT2

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ABSTRACT

The wild-type *Salmonella typhimurium* strain LT2 contains six copies of the insertion sequence element IS*200* which is unique to Salmonella. We have determined the chromosomal locations of all six copies of IS*200* in strain LT2. This was done by mapping the positions of Tn*10* elements inserted near each copy of IS*200*. Such Tn*10* insertions were detected by Southern hybridization as IS*200*-containing restriction fragments with altered electrophoretic mobility. The copies are located at quite evenly spaced sites in the chromosome. Some are found in regions with many known genes; others are in regions with few known functions. There is no indication of a possible function for IS*200*. The method described here should be applicable to the mapping of IS elements in general.

INSERTION sequences (IS) are genetic elements that can insert copies of themselves into different sites in a genome. These elements can also mediate various chromosomal rearrangements (including inversion, deletion and fusion of DNA segments) and alter the expression of adjacent genes (STARLINGER 1980; CALOS and MILLER 1980). Although IS elements appear to be present in most bacterial chromosomes, no functional role has been found for them.

A new insertion sequence element, IS*200*, has recently been identified in *Salmonella typhimurium* (LAM and ROTH 1983). The wild-type *S. typhimurium* strain LT2 contains six copies of IS*200*. A survey of the distribution of IS*200* in enteric bacteria suggests that the element may be limited to the genus Salmonella. Similar surveys carried out for IS*1*, IS*2*, IS*4* and IS*5* (NISEN, PURUCKER and SHAPIRO 1979; NYMAN *et al.* 1981; LAM and ROTH 1983) show that these elements may also be confined to relatively few host bacteria. These findings raise the possibility that IS elements may serve some functional role in their respective hosts such that they are selectively maintained in a particular group of organisms and do not persist in other hosts.

In hopes of finding some indication of possible function, we have determined the chromosomal locations of all six copies of IS*200* in LT2. In this report we describe a method that permits mapping of these copies despite the fact that no mutants are available and no known phenotype is associated with IS*200*.

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MATERIALS AND METHODS

Bacterial strains and nomenclature: The strains used in this study are listed in Table 1. The nomenclature for Tn*10* insertions outside of known genes has been described by CHUMLEY, MENZEL and ROTH (1979). Tn*10* insertions for which cotransducible markers were found were designated by a three-letter symbol indicating the map positions of the cotransduced markers. For the two Tn*10* insertions for which no cotransducible markers could be found, the last letter of the three-letter symbol was designated *z* to indicate the uncertainty of the map position within the 10-min segment.

Media: Difco nutrient broth containing 0.5% NaCl was used as rich medium. Minimal medium was the E medium of VOGEL and BONNER (1956). Solid medium contained 1.5% Difco agar. To select for growth on various sugars as sole carbon sources, the NCE medium of BERKOWITZ *et al.* (1968) was used, supplemented with 0.2% final concentration of the appropriate sugar. When required, tetracycline was added to a final concentration of 25 μg/ml in rich medium or 10 μg/ml in minimal medium. Kanamycin sulfate was added to a final concentration of 50 μg/ml in rich medium or 100 μg/ml in minimal medium. Histidine was used at a final concentration of 0.1 mM, and histidinol was used at a final concentration of 1 mM in minimal medium.

Phage growth and transductional methods: Phage P22 (HT 105/2) which transduces with high frequency (SCHMIEGER 1971) and carries an *int*⁻ mutation (isolated by G. ROBERTS) was used. Phage was grown according to HOPPE *et al.* (1979). In transductional crosses, phage and bacteria were mixed directly on selective media. When selection involved tetracycline, phage and bacteria were mixed and incubated for 20 min in nonselective liquid medium before plating. When selection involved kanamycin, phage and bacteria were plated on nonselective rich medium, allowed to incubate overnight and then printed onto selective medium. Transductants were purified and made free of phage by streaking alternately on selective and rich media.

Hfr construction and conjugational crosses: Methods for the construction of Hfr's using homology provided by Tn*10* sequences have been described by CHUMLEY, MENZEL and ROTH (1979). In conjugational crosses, Hfr's constructed using Tn*10* homology were used as donors, and various strains carrying single auxotrophic markers with known map positions were used as recipients. The selection was for prototrophic recombinants. In crosses in which the Hfr donor carried the *his-644* deletion as a counterselective marker, the selective medium used was the minimal (E) medium of VOGEL and BONNER (1956). In crosses in which the Hfr donor was prototrophic, the selection medium used was E medium containing 2 mg/ml of streptomycin to select against the donor; all auxotrophic recipient strains used in these crosses are streptomycin resistant. For conjugation, donor and recipient strains (0.1 ml of each) were mixed and plated directly on selective medium without prior incubation. Prototrophic recombinants were counted after incubation at 37° for 2 days.

Restriction fragment hybridization: The probe used in the Southern hybridization experiments was radioactively labeled replicative-form DNA of the M13 phage derivative M13Ho176-*hisD984*. This recombinant phage contains an IS*200* element inserted into *S. typhimurium his* operon sequences. The construction of M13Ho176-*his984* will be described elsewhere (S. LAM and J. R. ROTH, unpublished results). The probe DNA was radioactively labeled by nick translation as described by DAVIS, BOTSTEIN and ROTH (1980). [α-^{32}P]dATP was obtained from Amersham. DNA polymerase I was from New England Biolabs, Inc.

DNA was isolated from 2-ml cultures by the method described by DAVIS, BOTSTEIN and ROTH (1980). All restriction enzymes were obtained from New England Biolabs, Inc. and used according to the supplier's instructions. DNA fragments were separated by horizontal gel electrophoresis in 0.7–1.2% agarose (MCB Chemicals) gels as described by DAVIS, BOTSTEIN and ROTH (1980). Procedures for the transfer of DNA from agarose gel onto nitrocellulose filter (SCHLEICHER and SCHUELL BA85) and hybridization to radioactive probe were as discussed by DAVIS, BOTSTEIN and ROTH (1980). Filters were routinely prehybridized for 30 min in hybridization buffer before addition of radioactive probe. Hybridization buffer was 5×SSPE, 0.3% SDS, 100 μg/ml of denatured sonicated salmon sperm DNA; 1×SSPE was 0.18 M NaCl, 10 mM $(Na_{1.5})PO_4$, 1 mM Na_2EDTA, pH 7.0 (see DAVIS, BOTSTEIN and ROTH 1980). Hybridization was at 65° without formamide for 12–18 hr. After hybridization, blots were washed four times for approximately 15

TABLE 1

Bacterial strain list

Strain	Genotype[a]	Source[b]
TR6239	*his-644*	
NK337	*leu-414 SuII*$^+$ (P22 *c2t29 12amN11 13amH101 int-3* TC10)	N. KLECKNER
TT627	*strA1 pyrC7*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* (A)	F. CHUMLEY
TT628	*strA1 pyrC7*/F'*ts114 lac*$^+$ *zzf-21*::Tn*10* (B)	F. CHUMLEY
TT2870	*his-644 leu-1110 zej-636*::Tn*5*	L. BOSSI
TT6929	*zgc-1010*::Tn*10 his-644*	
TT6930	*zgc-1011*::Tn*10 his-644*	
TT6931	*zgc-1012*::Tn*10 his-644*	
TT6932	*zhz-1013*::Tn*10 his-644*	
TT6933	*zhz-1014*::Tn*10 his-644*	
TT6934	*zhz-1015*::Tn*10 his-644*	
TT6935	*zjc-1016*::Tn*10 his-644*	
TT6936	*zdz-1017*::Tn*10 his-644*	
TT6937	*zdz-1018*::Tn*10 his-644*	
TT6938	*zfd-1019*::Tn*10 his-644*	
TT6939	*zfd-1020*::Tn*10 his-644*	
TT6940	*zbj-1021*::Tn*10 his-644*	
TT6941	*zdz-1017*::Tn*10*	
TT6942	*zdz-1018*::Tn*10*	
TT6952	*zhz-1014*::Tn*10 his-644*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* (A)	
TT6953	*zhz-1014*::Tn*10 his-644*/F/*ts114 lac*$^+$ *zzf-21*::Tn*10* (B)	
TT6955	*zdz-1017*::Tn*10*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* (A)	
TT6956	*zdz-1017*::Tn*10*/F'*ts114 lac*$^+$ *zzf-21*::Tn*10* (B)	

[a] Nomenclature for Tn*10* insertions is described in MATERIALS AND METHODS.
[b] Unless otherwise noted, all strains were constructed for this study.

min each in 250 ml of 2×SSPE with 0.2% SDS at 55° with agitation. They were then exposed to Kodak XR-5 X-ray film at −70° with a DuPont Cronex Lightning Plus XL intensifying screen.

RESULTS

Mapping: The mapping of genetic elements with no selectable phenotype has become feasible with the recent development of genetic techniques using transposons (KLECKNER, ROTH and BOTSTEIN 1977). The transposon Tn*10*, which encodes tetracycline resistance, is particularly useful in this respect because of the methodology for Hfr formation developed by CHUMLEY, MENZEL and ROTH (1979): a chromosomal Tn*10* element can be used as a region of homology to direct the insertion of an F′ which also contains a Tn*10* element into the chromosome. An Hfr formed in this manner has an origin of transfer determined by the location of the chromosomal Tn*10* element. The origin of transfer and, therefore, the location of the Tn*10* element can be mapped by using such an Hfr in conjugational crosses. Thus, the approximate location of any genetic element can be determined if a Tn*10* insertion near it can be obtained.

The IS*200* elements in *S. typhimurium* have no selectable phenotype. How-

ever, they can be detected by hybridization with the appropriate probe DNA, as restriction fragments of particular sizes in Southern hybridization experiments (SOUTHERN 1975). The insertion of Tn*10* into a restriction fragment containing a copy of IS*200* increases the size of the fragment and is detected as a fragment with altered electrophoretic mobility.

The strategy was, therefore, to construct a large collection of strains containing random Tn*10* insertions. These strains were screened for possession of an IS*200*-containing fragment with increased size due to Tn*10* insertion. Finally, the Tn*10* insertions affecting each of the IS*200* sites are mapped genetically.

*Construction of a collection of strains containing random Tn*10 *insertions*: The detection of Tn*10* insertions near IS*200* copies depends on hybridization experiments using radioactively labeled replicative-form DNA of the M13 phage derivative M13Ho176-*hisD984* (S. LAM and J. R. ROTH, unpublished results) as probe. This recombinant phage contains an IS*200* element inserted into *S. typhimurium his* operon sequences. To unambiguously identify fragments containing IS*200*, it was important to eliminate interference from hybridized *his* sequences. This was done by using a strain carrying deletion mutation *his-644* (TR6239), which removes all *his* sequences hybridizable to the probe. Tn*10* was inserted randomly into the chromosome of TR6239 by the method of KLECKNER *et al.* (1975), and 500 independent tetracycline-resistant (*i.e.*, Tn*10* containing) derivatives were isolated.

*Identification of Tn*10 *insertions near IS*200 *copies*: DNA was isolated from each of the 500 strains containing Tn*10* insertions and digested with the restriction enzyme *Pvu*II. *Pvu*II was chosen for the following reasons: (1) it produces clearly separated fragments carrying IS*200* sequences; and (2) it does not cut within Tn*10*. The latter fact simplifies interpretation of the SOUTHERN data since one can predict the mobility of the altered fragments; each altered fragment should be increased in size by 10 kb (the size of Tn*10*). The fragments generated by *Pvu*II were separated by electrophoresis in agarose gels, transferred to nitrocellulose filters and hybridized to the radioactively labeled IS*200* probe. The locations of fragments hybridized to the probe were visualized by autoradiography.

Representative data are shown in Figure 1. Because of the large number of strains involved, two series of digests were run on each gel. One row of slots was formed across the top of the gel and another across the middle of the gel (see Figure 1). It can be seen that one strain in each series of 24 has a Tn*10* insertion in a fragment containing an IS*200* copy. From the 500 strains tested, Tn*10* insertions near all six IS*200* copies were obtained. Selected strains containing such insertions are shown in Figure 2. Lane 1 contains DNA from the tetracycline-sensitive parent (TR6239). Lanes 2–7 contain DNA from strains with Tn*10* insertions near the different IS*200* copies. The six IS*200* copies have been designated IS*200*(I) through IS*200*(VI) according to the sizes of the *Pvu*II fragments that carry them, IS*200*(I) being the copy on the smallest fragment. In each case the size increase is approximately 10 kb (the size of Tn*10*) as judged by size standards run in the same gel. More than one Tn*10*

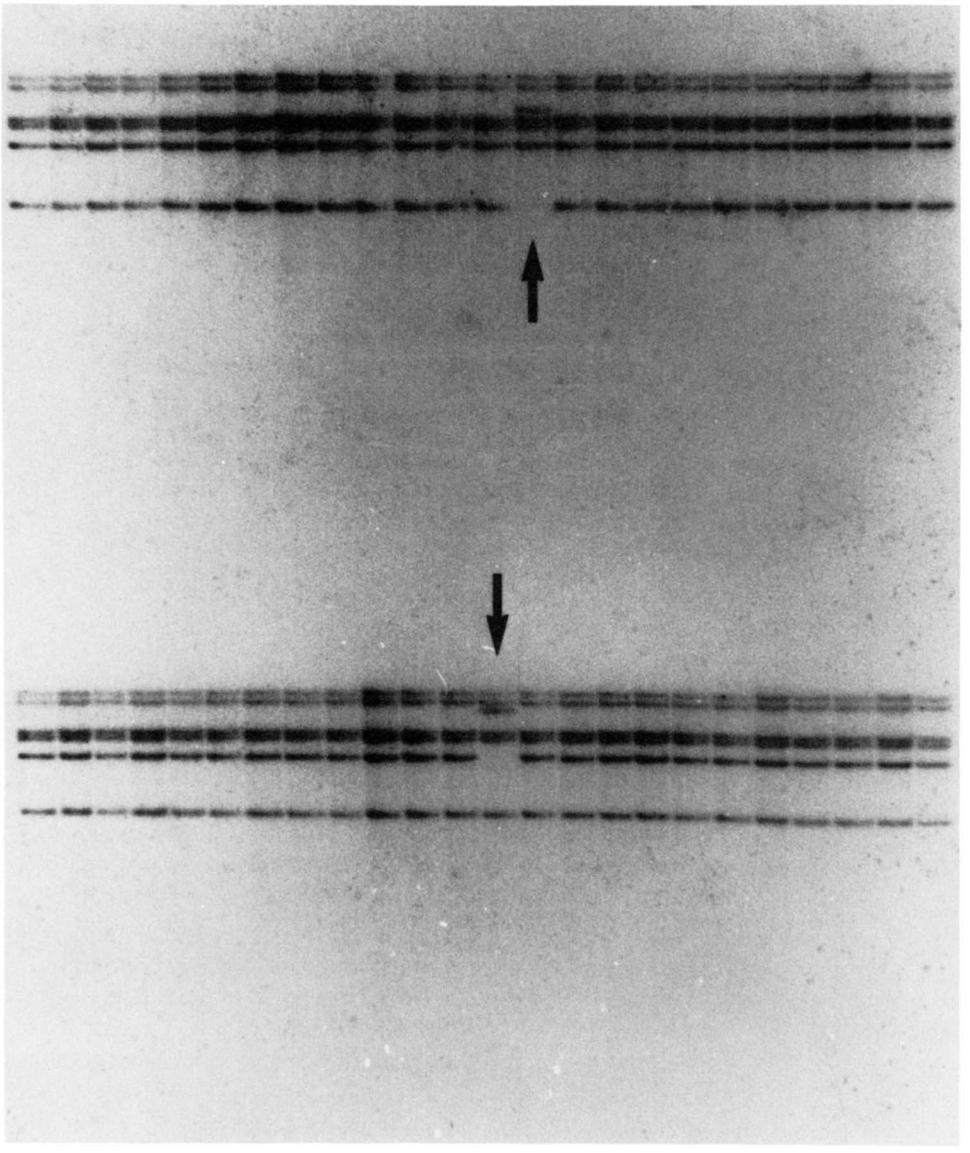

FIGURE 1.—Identification of Tn*10* insertions near IS*200* copies. Arrows indicate strains that have a Tn*10* inserted near one copy of IS*200*.

insertion has been obtained near four of the six copies. The distribution is as follows: near IS*200*(I), three strains; near IS*200*(II), three strains; near IS*200*(III), one strain; near IS*200*(IV), two strains; near IS*200*(V), two strains; and near IS*200*(VI), one strain. From the 500 strains tested, 12 strains (TT6929–TT6940) were identified that have a Tn*10* located within the same *Pvu*II fragment as a copy of IS*200*.

The altered fragments in these strains were shown to be the results of Tn*10*

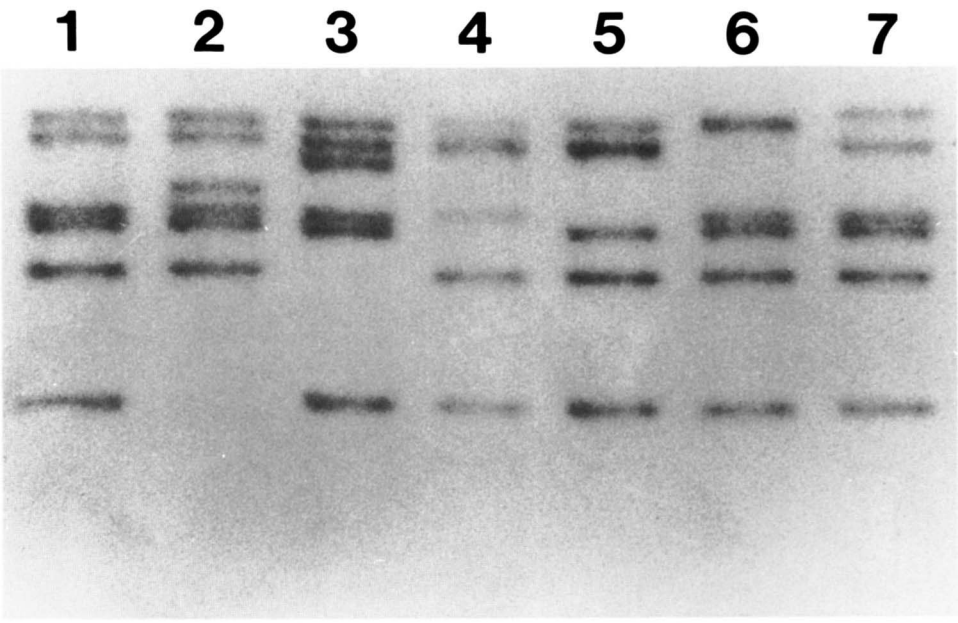

FIGURE 2.—Tn*10* insertions near all six copies of IS*200* in *S. typhimurium* strain LT2. Lane 1 contains DNA from the tetracycline-sensitive parent TR6239. Lanes 2–7 contain DNA from strain carrying Tn*10* insertions near IS*200* copies of I–VI.

insertions as follows. P22 phage grown on these strains were used to transduce the tetracycline-resistant (Tet^R) phenotype into a strain (TT2870) carrying the same *his* deletion (*his-644*) as the donor strains and a Tn*5* insertion elsewhere in the chromosome. The Tet^R transductants were shown to be recombinants by virtue of their resistance to both tetracycline and kanamycin. Seven transductants from each cross were then examined by Southern hybridization experiments for their contents of IS*200*. Representative data are presented in Figure 3. All transductants were shown to have the altered restriction patterns of the donor parents. Thus, the introduction of each Tn*10* into a new genetic background invariably led to alteration of the mobility of one fragment carrying IS*200*.

*Mapping of the Tn*10 *insertions*: The chromosomal location of each Tn*10* insertion was determined genetically. The results are presented in Figure 4. The insertions are identified by the IS*200* copies (I–VI) that they are associated with.

The approximate location of the Tn*10* insertions was initially determined by conjugational crosses (see MATERIALS AND METHODS). Donors were Hfr strains formed by inserting an F′*lac* plasmid at the site of a Tn*10* element in the chromosome (CHUMLEY, MENZEL and ROTH 1979). Each of these donors was crossed with a series of recipient strains each carrying a single auxotrophic marker of known map position. Recipient strains carrying auxotrophic markers located farther from the origin of transfer are expected to yield fewer proto-

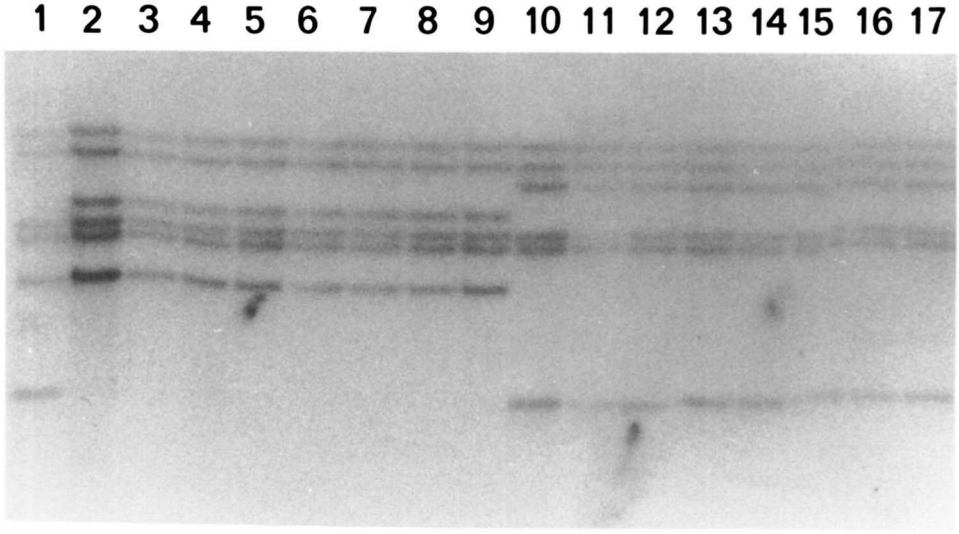

FIGURE 3.—Representative data showing correlation between the inheritance of Tn*10* insertions and changes in hybridization patterns. Autoradiogram of Southern blot showing hybridization patterns of Tet^R transductants that have inherited Tn*10* insertions from donor strains carrying such insertions (presumably) near IS*200* sites. For details of experiments see text. Data from two crosses are shown. Lane 1 contains DNA from the Tet^S-recipient strain TT2870. Lane 2 contains DNA from a donor strain carrying the Tn*10* insertion *zgc-1010*::Tn*10* (TT6929). Lanes 3–9 contain DNA from Tet^R transductants that have inherited *zgc-1010*::Tn*10*. Lane 10 contains DNA from another donor strain carrying the Tn*10* insertion *zhz-1013*::Tn*10* (TT6932). Lanes 11–17 contain DNA from Tet^R transductants that have inherited *zhz-1013*::Tn*10*.

trophic recombinants than those carrying markers located close to the origin of transfer. When the data obtained with Hfr's constructed from the same chromosomal Tn*10* insertion but having opposite directions of transfer are compared, the origin of transfer (and, therefore, the position of the Tn*10* insertion) can be located unambiguously between two markers where a drastic decrease in the number of recombinants obtained with one Hfr is accompanied by a drastic increase in the number of recombinants obtained with the other. Representative conjugation data are shown in Table 2. Data obtained from two sets of crosses are presented. The donors used in the crosses were Hfr's with opposite directions of transfer constructed from strains carrying the Tn*10* insertions *zhz-1014*::Tn*10* (TT6952 and TT6953) and *zdz-1017*::Tn*10* (TT6955 and TT6956), respectively. It can be seen that *zdz-1014*::Tn*10*, inserted near IS*200*(II), is located between *asd* and *cysE*, and *zdz-1017*::Tn*10*, inserted near IS*200*(IV), is located between *aroD* and *his*. The approximate locations of Tn*10* insertions near the other IS*200* copies were similarly determined. The insertions near IS*200*(I) were located between *cysC* and *serA*, the insertion near IS*200*(III) was located between *metA* and *purA*, the insertions near IS*200*(V) were located between *cysA* and *purC*, and the insertion near IS*200*(VI) was located between *nadA* and *aroA* (see Figure 4).

More precise locations of the Tn*10* insertions were then determined by P22

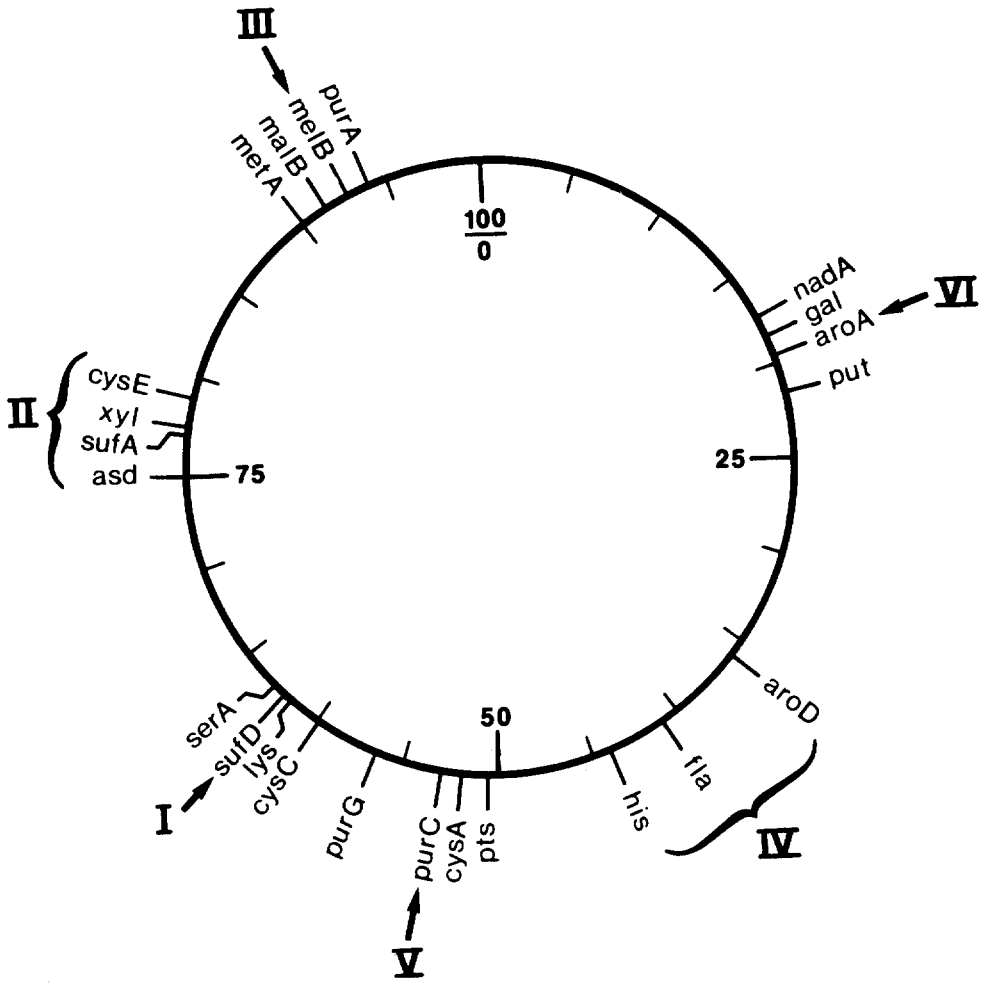

FIGURE 4.—Map locations of Tn*10* insertions near IS*200* copies. The insertions are identified by the IS*200* copies (I–VI) with which they are associated. Arrows link those mapped by transduction to the markers with which they are cotransduced. The approximate locations of the other two are shown by the braces.

transductional crosses. P22 transducing phages grown on the insertion-containing strains were used as donors, and strains containing selectable markers near the origins of transfer, determined in the conjugational crosses, were used as recipients. The selection was for prototrophy or sugar utilization, and the transductants were then scored for coinheritance of Tet^R. Representative cotransduction data are presented in Table 3. Tn*10* insertions near four of the six IS*200* copies are cotransducible with at least one of the markers tested. The other two copies of IS*200* (II and IV) are located in regions of the chromosome where few markers are available, and no selectable marker was

TABLE 2

Conjugation mapping data

Recipient		No. of prototrophic recombinants with Hfr constructed from:			
Strain	Genotype	TT6952[a]	TT6953[a]	TT6955[a]	TT6956[a]
TR5654	*strA1 thrA9*	1500	1000	558	279
TR5655	*strA1 leu-485*	1500	1500	634	342
TR5656	*strA1 proA36*	1000	1000	503	73
TR5657	*strA1 purE8*	522	483	388	28
TR5658	*strA1 purC7*	205	337	314	21
TR5660	*strA1 pyrF146*	800	1000	736	35
TR5686	*strA1 aroD140*	173	1500	511	26
TR5662	*strA1 his2236*			9	856
TR5663	*strA1 purF145*	54	800	5	780
TR5661	*strA1 aroC5*	28	1500	23	804
TR5664	*strA1 cysA533*	12	1000	18	552
TR5665	*strA1 cysC519*	16	1500	65	628
TR5666	*strA1 serA13*	17	2000	134	486
TR6297	*argG10*	68	2000		
TR5667	*strA1 cysG439*	8	1000	222	369
TT1395	*asd-174*::Tn*10*	30	2000		
TR5668	*strA1 cysE396*	2000	18	294	420
TR5669	*strA1 ilv-508*	2000	11	419	373
TR5670	*strA1 metA53*	2000	35	356	258
TR5688	*strA1 purA155*	1000	20	216	187
TR5671	*strA1 pyrB64*	2000	565	477	283

[a] The genotype of these strains before Hfr formation are: TT6952, *zhz-1014*::Tn*10 his-644*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* (A); TT6953, *zhz-1014*::Tn*10 his-644*/F'*ts114 lac*$^+$ *zzf-21*::Tn*10* (B); TT6955, *zdz-1017*::Tn*10*/F'*ts114 lac*$^+$ *zzf-20*::Tn*10* (A); TT6956, *zdz-1017*::Tn*10*/F'*ts114 lac*$^+$ *zzf-21*::Tn*10* (B).

found that showed cotransduction with Tn*10* insertions near them. For the insertions near IS*200*(II), markers tested include *asd*, *sufA*, *xyl* and *cysE*; for those near IS*200*(IV), markers tested include *aroD* and *his*. The map locations of these Tn*10* insertions have been determined only by conjugational crosses (see Table 2).

DISCUSSION

We have determined the chromosomal locations of the six copies of IS*200* present in the wild-type *S. typhimurium* strain LT2. It was hoped that their locations might give some indication of interactions among the copies or between them and their host. For example, they might be clustered near the origin or terminus of replication, in which case one might want to examine the possibility of a functional role of IS*200* in cellular replication. Clustering of the copies in any region of the chromosome could also be the result of intrinsic limitation of the mechanism of transposition (*e.g.*, transposition can only occur within a certain distance on the chromosome), or of limitation imposed by chromosomal organization such that only a portion of the chro-

TABLE 3

Cotransduction frequencies of Tn10 near IS200 with various genetic markers

Strain[a]	Near IS*200*	% Cotransduction of Tn*10* near IS*200* with various genetic markers[b,c]									
		lys	*sufD*[d]	*serA*	*asd*	*sufA*[d]	*xyl*	*cysE*	*malB*	*melB*	*purA*
TT6929	I	3 (1563)	47 (698)	0 (856)							
TT6930	I	5 (1223)	31 (609)	0 (764)							
TT6931	I	3 (1678)	62 (802)	0 (832)							
TT6932	II				0 (627)	0 (375)	0 (1248)	0 (758)			
TT6933	II				0 (437)	0 (446)	0 (1032)	0 (694)			
TT6934	II				0 (344)	0 (547)	0 (1164)	0 (826)			
TT6935	III								0 (843)	7 (1675)	0 (540)
		aroD	*his*	*cysA*	*purC*	*nadA*	*gal*	*aroA*	*pyrD*		
TT6941	IV	0 (872)	0 (1086)								
TT6942	IV	0 (698)	0 (1122)								
TT6938	V			0 (896)	35 (1004)						
TT6939	V			0 (1048)	53 (1648)						
TT6940	VI					0 (662)	0 (1244)	19 (998)	0 (813)		

[a] Each of these strains contains a Tn*10* insertion near a copy of IS*200*. The genotypes of these strains are listed in Table 1.

[b] Except when *sufA* or *sufD* was in the recipient, recombinants were selected that had received the wild-type allele of the indicated marker. These combinants were then scored for coinheritance of Tet^R.

[c] The number of transductants tested is given in parentheses.

[d] In these cases, each recipient contained a suppressor and a suppressible *his* mutation and was phenotypically His^+. Tet^R was selected, and the transductants were scored for their His phenotype. Transductants that became phenotypically His^- had coinherited the wild-type allele of the suppressor.

mosome is accessible as target for transposition. However, the mapping data show that the copies of IS*200* are quite evenly spaced in the chromosome.

Some are found in regions with many known genes; others are in regions with few known functions. These findings are consistent with the possibility that the copies have arisen by random transposition from a maternal copy, with the entire chromosome being accessible as target for transposition. There is no indication of possible functions. The mapping results also show definitely that all of the IS*200* copies are located in the chromosome, and none are in the cryptic plasmid that strain LT2 carries. This conclusion had been reached earlier from the observation that a strain thought to lack the cryptic plasmid retained all six copies of IS*200* (LAM and ROTH 1983).

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