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Location of IS*200* on the Genomic Cleavage Map of *Salmonella typhimurium* LT2

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Locations of six Tn*10*s, closely linked to each of the six IS*200*s on the genomic cleavage map of *Salmonella typhimurium* LT2, were determined by digestion with *Xba*I and *Bln*I and separation of the fragments by pulsed-field gel electrophoresis; the locations were then further defined by P22-mediated joint transduction. The orientation of each IS*200* with respect to its linked Tn*10* was determined by Southern blotting. The locations of IS*200*-I, IS*200*-III, and IS*200*-V were confirmed to be close to *sufD*, *melB*, and *purC*, as previously indicated. IS*200*-II is jointly transduced with *cysG*. IS*200*-IV is near *fliA*; the linked Tn*10* is inserted in *fli*, making the strain nonmotile. IS*200*-VI is jointly transduced with *aspC* but not with *aroA*. IS*200* is transposed to a seventh site in some strains, while remaining in the other six locations described above. These data indicate that genome analysis by pulsed-field gel electrophoresis can locate the positions of Tn*10*s with accuracy sufficient to predict P22-mediated joint transduction.

Salmonella typhimurium LT2 contains six copies of the insertion sequence IS*200* (9, 10). IS*200* is 708 bp in length and is thus the smallest known bacterial insertion sequence (4–6, 11).

The structure of the chromosome and the order of genes in genera in the family *Enterobacteriaceae*, especially *Escherichia coli* and *S. typhimurium*, reveal strong conservation (8, 16, 19, 21, 22). However, it was proposed that there is divergence for insertion sequences, because IS*200* was found in almost all serovars of *Salmonella* but was rarely found in other enteric bacteria (6, 9, 10). Bisercic and Ochman (2) detected IS*200* in 20% of *E. coli* strains. IS*200* appears to be genetically homogeneous among serovars of *Salmonella*, but it is polymorphic within *E. coli* (2). The distribution of IS*200* has been used as part of a molecular typing scheme to determine the phylogenies of *Salmonella enteritidis* (28), *Salmonella berta* (27), *Salmonella heidelberg* (26), and *S. typhimurium* (25).

Spontaneous mutations are rarely due to insertion sequences in *S. typhimurium* (3); only two mutations are reported to be due to IS*200* insertions, *hisD984*::IS*200* (9, 10) and *gpt-83*::IS*200* (18). By contrast, spontaneous mutations in *E. coli* are frequently due to insertion sequences and were the basis for the original detection of these elements (7, 24).

Tn*10* insertions which are close to each of the six IS*200*s in *S. typhimurium* LT2 were isolated and mapped by conjugation and transduction (9, 10). Recently, genomic cleavage maps of the *S. typhimurium* chromosome have been developed by using pulsed-field gel electrophoresis (PFGE) with the enzymes *Xba*I, *Bln*I, and I-*Ceu*I (12–14, 29). The objective of this study was to further define the physical positions of the six IS*200*s on the genomic cleavage map. We also detected transposition of IS*200* to a seventh site in some strains.

MATERIALS AND METHODS

Bacterial strains, plasmids, and cultivation. Some of the bacterial strains used are listed in Table 1. The strain carrying pIZ45, which has an *Eco*RI-*Hin*dIII insert with IS*200* sequences (6), was obtained from J. R. Roth. The following plasmids carrying the indicated genes were used for probing the various *Xba*I fragments listed: pRSM15 (*cysJIH*), *Xba*I-M, obtained from N. M. Kredich; pJH6 (*pgtABCD*), *Xba*I-G, obtained from J. S. Hong; pRSM3 (*cysB*), *Xba*I-D, obtained from N. M. Kredich; pST280 (*crp*), *Xba*I-N, obtained from P. Cossart; pRSM (*purA*), *Xba*I-W, obtained from N. M. Kredich; and pHH11 (*galT*), *Xba*I-A, obtained from H. S. Houng. All strains were stored in 15% glycerol at −70°C. Strains were cultivated in Luria-Bertani medium with the appropriate antibiotics added at the following concentrations (in micrograms per milliliter): tetracycline, 20; and ampicillin, 100.

Chemicals. Restriction enzymes and buffers were from Boehringer Mannheim (*Xba*I), Takara Biochemicals (*Bln*I), and Pharmacia (*Eco*RI and *Hin*dIII); a T7 QuickPrime kit was from Pharmacia, and agarose was SeaKem from FMC or from Sigma Chemical Co. All other analytical-grade chemicals were from Sigma Chemical Co. Radioisotope [^{32}P]dCTP was from New England Nuclear Co.

Transduction. P22 HT105/*int* was grown on the Tn*10*-containing donor strain as described earlier (23). The lysate was spread on cells of the recipient strain on Luria-Bertani agar with tetracycline. The resulting transductants were replica plated to test for joint transduction of genes linked to the Tn*10*.

Genomic DNA isolation and digestion. Genomic DNA was isolated in agarose blocks as described by Liu and Sanderson (14). DNA samples were digested in 30 μl of digestion mixture containing 1× Restriction Buffer H (Boehringer Mannheim), *Xba*I restriction enzyme (12 U), and double-distilled H_2O. The samples were incubated at 37°C for 3 to 3.5 h.

Electrophoresis of digested genomic DNA. Digested genomic DNA samples were separated with either a Bio-Rad DRII or a Bio-Rad Chef Mapper PFGE unit by using methods described earlier (13).

DNA probe construction, labelling, and Southern blotting. Plasmid DNA to be used as a probe was isolated by the rapid alkaline method (20), digested with *Eco*RI and *Hin*dIII, and electrophoresed in low-melting-point agarose, and then the 300-bp *Eco*RI-*Hin*dIII fragment was isolated. DNAs were radiolabelled with [^{32}P]dCTP by using a Pharmacia T7 QuickPrime kit according to the instructions of the manufacturer. Electrophoresed genomic DNA samples were trans-

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TABLE 1. Genetic and physical analysis of the locations of Tn*10* insertions linked to IS*200*s on the chromosome of *S. typhimurium* LT2

IS*200*[a]	Strain[b]	Tn*10* allele	Genetic data and % P22 joint transduction[c]	Physical data for:					
				*Xba*I fragment[d]			*Bln*I fragment[e]		
				Intact	a	b	Intact	a	b
I	TT6929	*zge-1010*	*sufD*, 47; *lys*, 3[f]	M (675)	270	370	E (590)	180	410
II	TT6932	*zhh-1013*	*cysG458*, 65 (112)[g]; *cysG439*, 44 (87)[g]	N (233)	36	200			
III	TT6935	*zjc-1016*	*melA*, 7[f]	W (365)	165	210	A (1580)	160	1500
IV	TT6941	*zdz-1017* (*fli-8017*)[h]	*fli*$^+$, 100 (3)[g]	D (457)	328	138	C (830)	100	730
V	TT6938	*zfd-1019*	*purC*, 35[f]	G (225)	80	155	C (830)	645	185
VI	TT6940	*zbj-1021*	*aroA*, 19[f]; *aroA64*, 0 (108)[g]; *aspC*, 100 (3)[g]	A (800)	450	360	A (1580)	1505	75

[a] The designations I to VI were made by Lam and Roth (10) and refer to the six IS*200* elements on the chromosome.
[b] The six strains listed each have a Tn*10* linked to the indicated IS*200*. These strains were obtained from J. R. Roth. All of the strains except TT6941 also have a *his-644* allele; thus, the genotype of TT6929 is *his-644 zye-1010*::Tn*10*.
[c] The genetic data are based on P22-mediated joint transduction, with the Tn*10* strain acting as the donor and the mutants with the genes listed in this column as recipients. The numbers are the percent joint transductions between the Tn*10* (tetracycline resistant) and the gene.
[d] The intact fragment indicated by an uppercase letter is the *Xba*I fragment which is present following *Xba*I digestion of the wild type but which is missing following digestion of the strain with the Tn*10* insertion; the number is the size (in kilobases) of that fragment, reported earlier (10). Fragments a and b are the two new fragments which are detected; fragment a is inferred to be CCW on the chromosome, and fragment b is inferred to be CW. Numbers indicate sizes (in kilobases).
[e] Fragments are as described above in footnote *d*, except that *Bln*I is used for digestion.
[f] Transduction data are from Lam and Roth (10).
[g] Transduction data are from this study. The following recipient strains were used: SA2585 (*cysG458*), SA2584 (*cysG439*), and SA2009 (*aroA64*). The strain with the indicated Tn*10* was the donor; tetracycline-resistant transductants were replica plated to determine joint transduction in auxotrophs or stabbed into semisolid medium to test motility. The number in parentheses is the total number of transductants tested.
[h] *zdz-1019*::Tn*10*, isolated and named by Lam and Roth (10), is now known to be the result of an insertion into the *fli* genes, so it is renamed *fli-8017*::Tn*10*.

ferred onto Millipore Immobilon P transfer membranes by Southern transfer and probed as described earlier (14).

RESULTS AND DISCUSSION

Localization of Tn*10*s linked to IS*200*. DNA from the six strains of *S. typhimurium*, each of which has a Tn*10* insertion close to IS*200*, was digested with *Xba*I and separated by PFGE (Fig. 1). Since Tn*10* has an *Xba*I site, one *Xba*I fragment which is present in Tn*10*-deficient strains was missing from each strain, and two new fragments whose sizes add up to the size of the missing band were detected (Table 1). The position of each *Xba*I fragment on the chromosome is known (13), and the fragment into which each of the six Tn*10*s is inserted was identified (Table 1 and Fig. 2). The distances of the Tn*10*s from the ends of the fragments were shown by the sizes of the two fragments. However, the Tn*10* might be closer to either the counterclockwise (CCW) or the clockwise (CW) end of the fragment. The orientation was determined as follows. First, a gene probe known to be at one end of the *Xba*I fragment (14) was used to probe the *Xba*I digest. These probes are indicated at one end of each of the six fragments by a gene shown in boldface type with an asterisk (Fig. 2); the source of the probe is given in Materials and Methods. For example, plasmid pRSM15 carrying the genes *cysJIH* hybridized with the 270-kb fragment of *Xba*I-M (data not shown); these genes are at the CCW end of *Xba*I-M (14), indicating that *zge-1010*::Tn*10* is 270 kb CW of the site *Xba*I 13 (sX13) and 370 kb CCW of sX14. Orientations of the other five *Xba*I fragments with Tn*10* insertions were determined in the same way (Fig. 2). Second, orientation of the *Xba*I fragments was tested by *Bln*I digestion, which yielded two new fragments; the size in kilobases of the shorter one is shown at the arrow on the *Bln*I map in Fig. 2. In all cases the fragment orientation determined by probe data was confirmed by *Bln*I analysis.

Localization of IS*200*s on *Xba*I fragments. The *Xba*I digests were probed with the 300-bp *Eco*RI-*Hin*dIII fragment from pIZ45, which contains sequences from IS*200* (Fig. 2). The six fragments shown to have a Tn*10* insertion closely linked to IS*200*, *Xba*I-A, -D, -G, -M, -N, and -W, were all detected by this probe. In each strain with a Tn*10* close to IS*200* one of the two new fragments, a or b (Table 1), was detected by the probe. For example, in DNA from TT6941 the IS*200* probe detected the 328-kb fragment but not the 138-kb fragment, indicating that the IS*200* is CCW from *zdz-1017*::Tn*10*. The orientations of all six Tn*10*s and IS*200*s are indicated by arrowheads and squares, respectively, in Fig. 2.

Figure 2, incorporating data from Liu et al. (13), compares the positions of Tn*10*s linked to IS*200*-I, -IV, and -VI with the positions of other genes. For IS*200*-II, -III, and -V, DNA from strains with linked Tn*10* insertions was digested with *Xba*I, and then the fragments were separated by PFGE and probed with IS*200*. The shaded fragments shown in Fig. 2 were detected by the IS*200* probe, indicating the presence of IS*200* in that fragment. When probing was done, only one of the two new fragments hybridized; when neither fragment is shaded in Fig. 2, probing was not done. These data are consistent with the location of the IS*200* determined earlier.

Correlation of Tn*10* position deduced from linkage data with position on the genomic cleavage map. Lam and Roth (10) mapped the IS*200*-linked Tn*10*s by F-factor-mediated conjugation; in addition, in four of the six cases they detected P22-mediated joint transduction with known genes (Table 1). The locations of the six IS*200*-linked Tn*10*s are discussed below. (i) Since *zge-1010*::Tn*10* (linked with IS*200*-I) is in *Xba*I-M, 270 kb from sX13, while *lysA*::Tn*10* is 260 kb from the same site (Fig. 2), *zge-1010* should be 10 kb CW of *lysA*. According to the formula of Wu (30), calculated by Sanderson and Roth (22) for strains with Tn*10* insertions, a 10-kb separation of these genes should lead to 35% joint transduc-

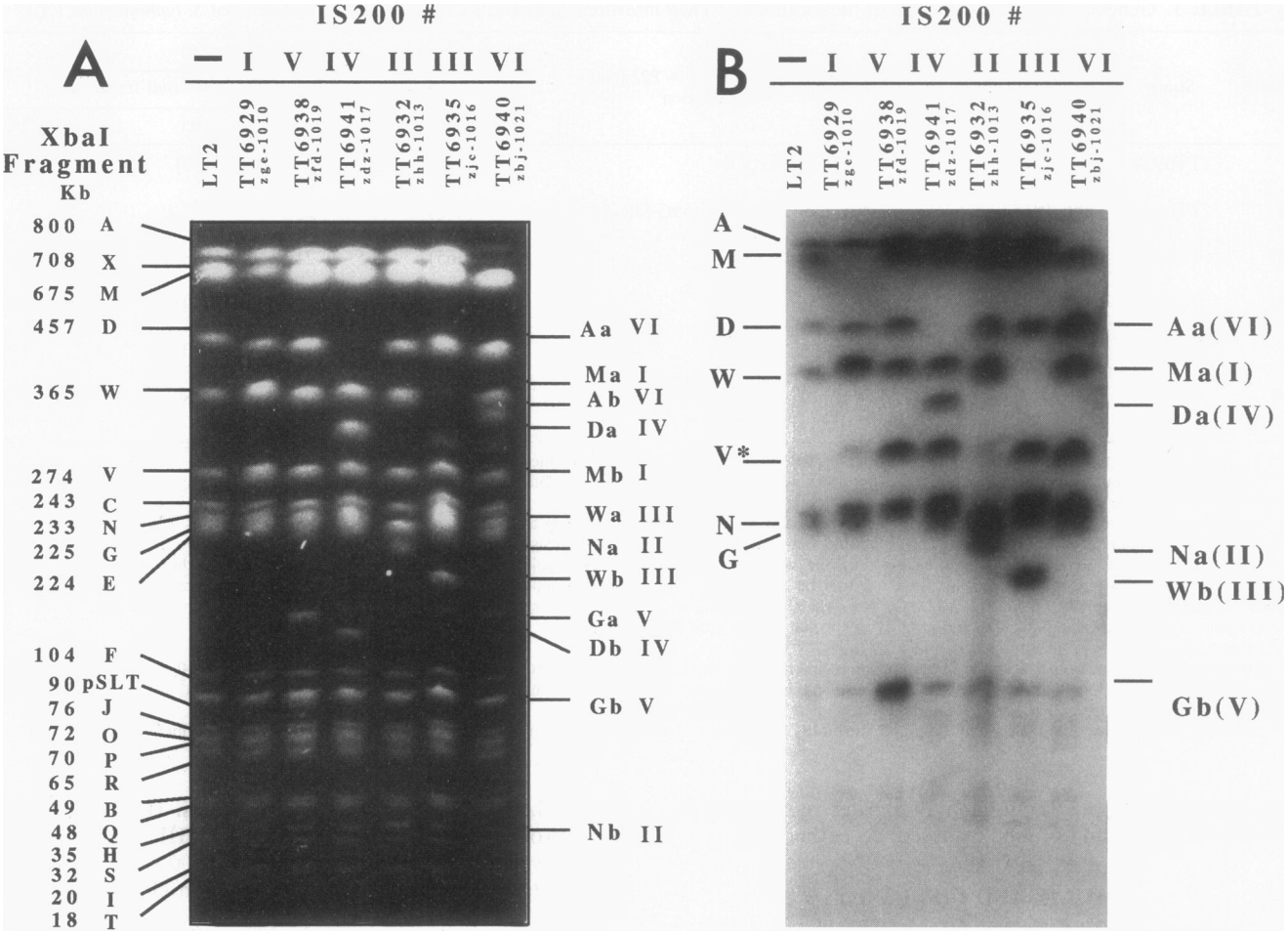

FIG. 1. Analysis of positions of Tn*10*s in the chromosome of *S. typhimurium*. (A) *Xba*I digests of DNA from strains of *S. typhimurium* with Tn*10* insertions, separated by PFGE and stained by ethidium bromide. The strain number and the allele number of the Tn*10* insertion for the source of the DNA is given at the top, along with the number of the IS*200* near which the Tn*10* is located. The *Xba*I fragment and its size in kilobases are indicated on the left; these sizes were determined earlier (13). On the right are indicated the new fragments which originate from digestion of strains with Tn*10* insertions. The first letter indicates the fragment; the second indicates whether it is the larger fragment (a) or the smaller one (b); the number indicates the IS*200* near which the Tn*10* is located. (B) The DNA in the gel shown in panel A was blotted to a membrane and probed with a fragment from pIZ45 carrying IS*200* sequences. Six of the fragments indicated on the left have insertions in normal strains; V* indicates that only some of the strains have IS*200* in *Xba*I-V. On the right are indicated the fragments detected by the probe in the strains with Tn*10* insertions. For example, Da(IV) indicates that the labelled fragment is from the strain with Tn*10* linked to IS*200*-IV and that *Xba*I-D disappears but *Xba*I-Da (the CCW fragment, 320 kb in size) is detected by the probe. The labelling on LT2 is weak; other gels show the expected pattern of labelling of the six fragments (data not shown).

tion by P22; *lysA* joint transduction was detectable but at a lower level than expected (3%) (Table 1). Since *sufD* is between *lysA* and *serA* on the basis of linkage data (22), the high frequency of joint transduction which is observed (47%) (Table 1) is predicted by the genomic cleavage map. (ii) *zhh-1013*::Tn*10*, linked to IS*200*-II, was mapped by conjugation near the site where we found it, but joint transduction was not detected (Table 1) (10). Our data predicted close linkage with *cysG*; we detected 44 and 65% joint transduction to two separate *cysG* alleles (Table 1). *Xba*I digestion indicates that *zhh-1013* is 3 kb CW of *cysG* (Fig. 2 and Table 1). (iii) *zjc-1065*::Tn*10*, linked to IS*200*-III, is 28 kb CW of *melA* on the basis of *Xba*I digestion data (Fig. 2); the observed joint transduction of 7% (Table 1) predicts a 20-kb separation between the genes, close to the 28 kb observed. In addition, IS*200* probing of the *Xba*I digests from several strains with Tn*10* insertions locates IS*200*-III in the 28-kb *melA-zjc-1065* interval (Fig. 2). (iv) *zdz-1017*::Tn*10*, linked to IS*200*-IV, was located by conjugation, but not by transduction (10). *Xba*I digestion data locate the insertion near *fliC* (Fig. 2). A BLAST search (1) of GenBank shows IS*200* sequences near flagellar genes (17) upstream of *fliA*, which is in flagellar gene region IIIa (15). We found the strain with *zdz-1017*::Tn*10* to be nonmotile, and transduction of the Tn*10* into LT2 yielded nonmotile transductants, indicating that Tn*10* had inserted in the *fli* genes; we have therefore renamed this allele *fli-8017*::Tn*10*. (v) *zfd-1019*::Tn*10*, linked to IS*200*-V, is 80 kb CW of sX7, while *purC* is 98 kb CW of the same site (Fig. 2). The joint transduction observed (35%) (Table 1) predicts a 10-kb separation between the genes (22); the 18-kb distance observed is a fairly good fit. (vi) *zbj-1021*::Tn*10*, linked to IS*200*-VI, is 450 kb CW of sX1 by our data (Table 1 and Fig. 2) and 115 kb from *aroA*. According to Lam and Roth (10), this

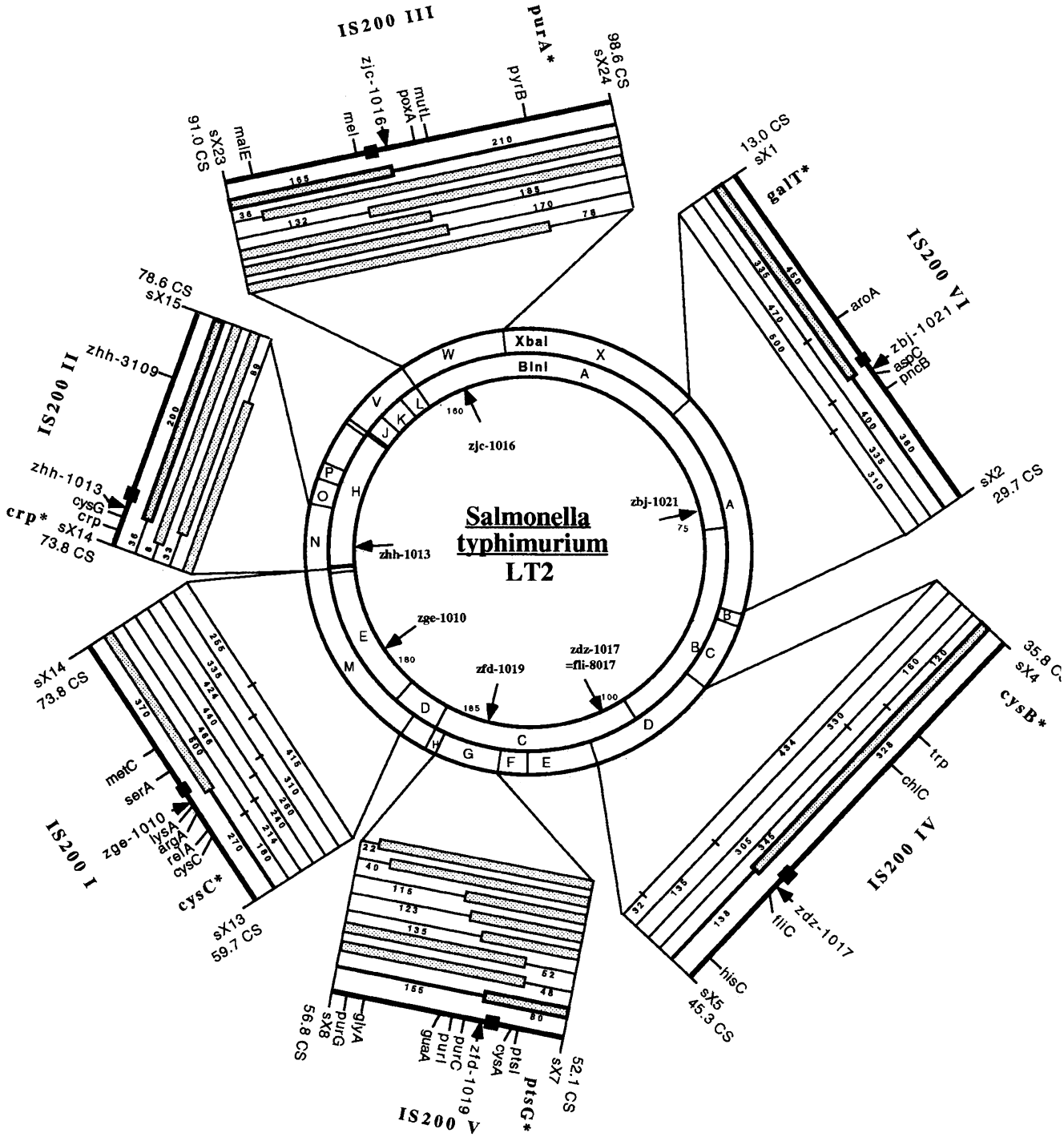

FIG. 2. Genomic cleavage map of *S. typhimurium* LT2, showing *Xba*I and *Bln*I sites and the positions of IS*200*s (solid squares). The *Xba*I and *Bln*I maps are from Liu et al. (13). The Tn*10* insertions marked inside the inner circle (e.g., *zbj-1021*::Tn*10*) were mapped by *Bln*I and *Xba*I digestion of DNA from a strain with the insertion. The *Xba*I fragment which is cut into two fragments is diagrammed. The sizes of the resulting fragments are given in Table 1. The position of the Tn*10* is marked by an arrowhead. An IS*200* probe (see Materials and Methods) was used to probe the *Xba*I digests; the shaded fragments were detected, revealing the orientations of IS*200* and Tn*10* in each case. The genes shown in boldface type with an asterisk indicate probes known to be at one end of the fragment (14) and were used to orient the fragment on the chromosome. The site number for *Xba*I (shown as sX1, etc.) and the location of the *Xba*I fragment in centisomes (CS) are from Liu et al. (13).

gene is jointly transduced with *aroA*; we failed to confirm this, but we detected joint transduction with *aspC* (Table 1).

Detection of an IS*200* at a new site. Probing strains with IS*200*-linked Tn*10* insertions revealed six bands (Fig. 1B) as predicted by Lam and Roth (10), but four of the six strains showed an extra band in fragment *Xba*I-V. Hybridization with this fragment was not observed in any other strains probed, including the wild type (LT2), and about 15 other strains with Tn*10* insertions. There was no detectable phenotypic difference between these four strains and any others and thus no evidence that this insertion is into an active gene. We presume that transposition into this site occurred in an early stage of replication of a culture of TR6239 (*his-644*), the strain in which the IS*200*-linked Tn*10*s were constructed (10).

In summary, we have determined the locations of the six IS*200*s on the genomic cleavage map of *S. typhimurium* and shown their locations relative to the IS*200*-linked Tn*10*s and to other genes. We have detected transposition to a seventh site. This work shows that analysis of the positions of Tn*10*s by *Xba*I and *Bln*I digestion and separation of the fragments by PFGE can successfully predict the P22-mediated joint transduction anticipated for that Tn*10* insertion.

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