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The *Xba*I-*Bln*I-*Ceu*I Genomic Cleavage Map of *Salmonella typhimurium* LT2 Determined by Double Digestion, End Labelling, and Pulsed-Field Gel Electrophoresis

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Endonuclease digestion of the 4,800-kb chromosome of *Salmonella typhimurium* LT2 yielded 24 *Xba*I fragments, 12 *Bln*I fragments, and 7 *Ceu*I fragments, which were separated by pulsed-field gel electrophoresis. The 90-kb plasmid pSLT has one *Xba*I site and one *Bln*I site. The locations of the fragments around the circular chromosome and of the digestion sites of the different endonucleases with respect to each other were determined by excision of agarose blocks containing fragments from single digestion, redigestion with a second enzyme, end labelling with ^{32}P by using T7 DNA polymerase, reelectrophoresis, and autoradiography. Forty-three cleavage sites were established on the chromosome, and the fragments and cleavage sites were designated in alphabetical order and numerical order, respectively, around the chromosome. One hundred nine independent Tn*10* insertions previously mapped by genetic means were located by pulsed-field gel electrophoresis on the basis of the presence of *Xba*I and *Bln*I sites in Tn*10*. The genomic cleavage map was divided into 100 units called centisomes; the endonuclease cleavage sites and the genes defined by the positions of Tn*10* insertions were located by centisome around the map. There is very good agreement between the genomic cleavage map, defined in centisomes, and the linkage map, defined in minutes. All seven rRNA genes were located on the map; all have the *Ceu*I digestion site, all four with the tRNA gene for glutamate have the *Xba*I and the *Bln*I sites, but only four of the seven have the *Bln*I site in the 16S rRNA (*rrs*) gene. Their inferred orientation of transcription is the same as in *Escherichia coli*. A rearrangement of the *rrnB* and *rrnD* genes with respect to the arrangement in *E. coli*, observed earlier by others, has been confirmed. The sites for all three enzymes in the *rrn* genes are strongly conserved compared with those in *E. coli*, but the *Xba*I and *Bln*I sites outside the *rrn* genes show very little conservation.

The introduction of pulsed-field gel electrophoresis (PFGE) techniques for separating large DNA molecules has had a large impact on the study of genetics of both prokaryotic and eukaryotic organisms. PFGE overcomes the size limitations of conventional electrophoresis by forcing DNA molecules to reorient periodically from one electric field direction to another (51). Physical maps have been established for the chromosomes of several bacteria, and the methods are also being used in studying eukaryotic chromosomes. In studies of the covalently closed circular genomes of bacteria, genomic DNA embedded in agarose is digested with an endonuclease which cuts the chromosome into a limited number of fragments, and then these fragments are separated by PFGE.

Several methods have been used to arrange these fragments into a physical map. For *Escherichia coli* K-12, for which an extensive linkage map was already known (1), the restriction map was based on probing with known cloned genes, analysis of strains with prophages or transposons at known locations, partial digestion and DNA probing, and analyses of strains with chromosomal rearrangements (52). When a linkage map was not available, other methods were used. Two-dimensional pulsed-field gels were used for *Mycoplasma mobile* (2) and *Pseudomonas aeruginosa* (39). Probing fragments from digestion with one enzyme with fragments from another enzyme, isolated following PFGE, was used to derive the maps of *Haemophilus influenzae* (20) and of *Myxococcus xanthus* (9).

The genus *Salmonella* belongs to the large eubacterial family *Enterobacteriaceae*. It was originally classified into many serotypes by use of somatic and flagellar antigens to develope the Kauffmann-White classification, but more recently all strains have been proven to belong to the same DNA reassociation group, representing one or a very small number of species (10, 24). The salmonellae have recently been separated by electrophoretic typing into numerous clones (47). Studies on the cellular and molecular biology of *Salmonella typhimurium* have been summarized in volumes edited by Neidhardt et al. (33). The use of the name *Salmonella enterica* serovar typhimurium rather than the name *S. typhimurium* is gaining wide acceptance and is a better indicator of relationships within this group, but we use the traditional name *S. typhimurium* in this report. Linkage maps of *S. typhimurium* have reported 680 genes on a circular linkage map (46). Physical maps of the *S. typhimurium* LT2 chromosome have been determined for *Xba*I (27, 28), for *Bln*I (=*Avr*II) (57), and for I-*Ceu*I (26). The fragments were arranged on a circular map by using cloned genes as probes, using transposon Tn*10* inserted at known sites to add a restriction site, and using "linking probes" derived from induction of lysogens for Mu*d*-P22 (3, 59). Further locations on the genome have been determined with Tn*5* derivatives (58).

An important task in construction of the physical map of a genome for more than one enzyme is to determine the exact locations of restriction sites for a second enzyme on the

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restriction fragments of the first enzyme. This can be done through double digestion with the two enzymes, but the resulting double digest may yield too many fragments for analysis by standard methods. The fragments may be separated by two-dimensional electrophoresis, with two different enzymes used in the two dimensions, as was accomplished by Bautsch (2) with the small (780-kb) genome of *M. mobile* and by Römling and Tümmler (39) with the much larger (5,900-kb) genome of *P. aeruginosa*. However, obtaining accurate data on two-dimensional gels with larger genomes such as that of *P. aeruginosa* is a technically daunting task. In this paper, we report a technically simpler method to get data for two enzymes. We excised agarose slices containing the fragments obtained following digestion with the first enzyme and separation by PFGE, digested with the second enzyme, and then reseparated in another round of PFGE. The amount of DNA was often too small to show up clearly in PFGE, so we end labelled the fragments and autoradiographed following PFGE separation. In this way, even small fragments could be reproducibly and accurately detected. Using these methods, we located the *Xba*I sites on *Bln*I fragments and vice versa.

Detailed analysis of the genome by PFGE is dependent on the availability of further rarely cutting restriction enzymes. Most restriction endonucleases cut the genome of *S. typhimurium* into more fragments than can be readily resolved by PFGE; in this report, we describe use of the recently available intron-encoded enzyme I-*Ceu*I (hereafter called *Ceu*I). *Ceu*I is an endonuclease, derived from a group I intron in *Chlamydomonas eugametos*, which digests a 26-bp sequence in the gene for the large-subunit rRNA (12, 30). We previously reported that *Ceu*I cuts the genome of *S. typhimurium* into only seven fragments through cleavage of the rRNA (*rrn*) genes (26). Using the method of fragment excision and end labelling, we determined the locations of *Ceu*I sites on the *Xba*I-*Bln*I map. These procedures allow the chromosome to be divided into 43 separate fragments. By using a combination of digestion and electrophoresis, DNA representing these fragments can be isolated and used for cloning or for further restriction analysis.

There are seven *rrn* genes for synthesis of rRNA and tRNA in *E. coli* (1, 6, 34). The structures and map locations of seven homologous *rrn* genes in *S. typhimurium* are partially known (16, 21–23). Analysis of the genomic cleavage map confirms the location and orientation of the seven *rrn* genes of *S. typhimurium* to be the same as in *E. coli* and confirms an apparent rearrangement between the two genera involving *rrnB* and *rrnD* which was reported by Lehner et al (21).

The total size of the chromosome of *S. typhimurium* is about 4,800 kb, on the basis of analysis by each of the three enzymes used here. We report the sizes of the fragments, but we describe the size of the chromosome in centisomes (CS) on the basis of the earlier use by Rudd and colleagues (5, 40–42), in which the physical chromosome is divided into 100 equal units. In *S. typhimurium* each CS is equivalent to about 48 kb. We use CS rather than kilobase pairs at this time to avoid overstating the accuracy of the conclusions; although some parts of the genomic cleavage map are determined to 1 kb or even less, in other cases, involving large fragments, the accuracy is much lower. When more sequence data are available, or when a high-resolution restriction map of the type determined for *E. coli* by Kohara et al. (18) is available for *S. typhimurium*, the chromosome size can be stated in kilobase pairs.

MATERIALS AND METHODS

Bacterial strains, phages, plasmids, and cultivation conditions. The strains of *S. typhimurium* LT2 and the plasmids used in this study were obtained from numerous sources and are maintained at the Salmonella Genetic Stock Centre (SGSC). The reference wild-type strain LT2 was obtained from Joshua Lederberg as part of the set of the 22 *S. typhimurium* strains isolated by Lilleengen (25) and used by Zinder and Lederberg (60). The strains with insertions of the transposon Tn*10* at different points around the linkage map are part of a "kit" composed of several hundred strains of *S. typhimurium* LT2 collected over several years from many laboratories and maintained in the SGSC; the kit was reported earlier (46) and has been expanded since that time. Any of these strains are available on request.

Luria-Bertani medium (10 g of tryptone, 5 g of yeast extract, 10 g of NaCl, 3.5 ml of 1 M NaOH) was used for cultivation of all strains; solid medium also contained 1.5% agar. Tetracycline, ampicillin, and chloramphenicol were used at 20, 100, and 40 μg/ml, respectively. Strains were maintained in 15% glycerol at −70°C, and a single colony was isolated prior to use.

Enzymes and chemicals. Endonucleases were from New England Biolabs (*Xba*I and *Ceu*I), Takara Biochemicals (*Bln*I), Pharmacia, and Boeringer-Mannheim. [^{32}P]dCTP was from New England Nuclear. Most other chemicals, including agarose, were from Sigma Chemical Co.

Preparation and digestion of high-molecular-weight genomic DNA. Cells were grown overnight at 37°C in Luria-Bertani broth with antibiotics when appropriate, diluted 10-fold in fresh Luria-Bertani broth, and incubated for 3 h with vigorous shaking. The cells were then embedded in low-melting-point agarose by using the New England Biolabs ImBed Kit according to the manufacturer's instructions, with any further modifications as described earlier (27). The resulting agarose blocks containing lysozyme and proteinase K-treated cells could be held in storage buffer at 4°C.

For restriction digestion, agarose pieces were immersed in a 2× concentration of the buffer supplied by the manufacturer and incubated for 15 min at room temperature. The 2× buffer then was replaced with fresh 1× buffer containing 100 μg of bovine serum albumin per ml (for *Xba*I) and 0.4 U of enzyme per μl. Five agarose pieces were digested in a volume of 100 μl at 37°C for 2 h.

Preparation of probe DNA. Plasmid DNA was isolated by the rapid alkaline lysis method described by Sambrook et al. (43). P22 DNA was prepared from lysogens with Mu*d*-P22 following induction of the lysogens as described by Liu and Sanderson (27). DNA was ^{32}P labelled for Southern blotting by using the Quick-Prime kit and procedures of Pharmacia.

PFGE and Southern hybridization. DNA digests were separated electrophoretically by using a Bio-Rad DRII contour-clamped homogeneous electric field apparatus or a Bio-Rad Mapper. Typically, for fragments of 600 to 800, 200 to 500, and <200 kb, pulse times of 150, 40, and 8 s, respectively, were used. The total running time was usually 24 h. The gels contained 0.5× TBE buffer (1× buffer contains 90 mM Tris, 90 mM boric acid, 2 mM EDTA [pH 8.0]) with 1.5 μg of ethidium bromide per ml and 0.7% agarose. Electrophoresis was in the same buffer without ethidium bromide. The gels were photographed, and the sizes of the fragments were estimated by using lambda concatamers as the molecular weight standards.

DNA fragments were transferred onto Immobilon-P Transfer Membrane (Millipore) by Southern blotting and

hybridized according to methods described previously (27), and the resulting membranes were washed, air dried, and autoradiographed.

Isolation, redigestion, and end labelling of DNA fragments. Genomic DNA fragments from digestion by the endonuclease were excised from agarose gels under long-wavelength UV light, and the block of agarose containing the DNA was trimmed to a 1-mm (or smaller) slice. This fragment in the agarose block was then digested with a second endonuclease by the methods described above. The agarose block, containing double-digested DNA, was loaded into a well, and the resulting fragments were separated by PFGE as described above. However, in many cases the concentration of the resulting fragments was too low to be detectable by ethidium bromide staining (because many of the fragments were short and/or because of losses in the isolation process); we therefore end labelled the DNA as follows. When the digested DNA had 5′ overhangs containing G as one of the nucleotides, we incubated the agarose block with T7 DNA polymerase in buffer containing [^{32}P]dCTP plus the other three deoxynucleoside triphosphates in unlabelled form. Following this, the DNA fragments in the agarose block were electrophoresed and detected by autoradiography. Any double-stranded DNA fragment, regardless of the ends, could be end labelled by the use of the Klenow fragment of DNA polymerase I. The agarose block was incubated in 20 μl of buffer containing 1 U of Klenow fragment plus [^{32}P]dCTP. As expected, the 3′,5′-exonuclease activity combined with the DNA polymerase activity of the Klenow fragment resulted in an exchange (replacement) reaction which added an end label of radioactive dCTP. The resulting agarose block was electrophoresed and autoradiographed. For separation of double digests, we constructed and usually used a bigger gel (16 cm wide and 23 cm long) rather than the smaller gel (14 cm wide and 12.8 cm long) provided by the manufacturer.

RESULTS

***Xba*I physical map.** Digestion of the genome of *S. typhimurium* with *Xba*I and separation of the fragments with PFGE was previously reported to yield 24 fragments, 23 of which were from the chromosome and one from the plasmid pSLT; all but two fragments, of 6.6 and 6.4 kb, were located on a circular restriction map (27). We report here further analysis by the same methods and some modifications which have confirmed most of the earlier locations, revealed the locations of the above two fragments, and revealed a fragment of <1 kb which has been approximately located. We determined a CS map, composed of 100 physical units, on which most of these fragments were located. In addition, we located the positions of 109 different Tn*10* insertions, many of which are in known genes.

The *Xba*I fragments larger than 18 kb are seen in Fig. 1A, lane 3; smaller fragments are not always visible on ethidium bromide-stained gels but are observed following end labelling with ^{32}P (see below). The fragments are listed along with their sizes in Table 1. The order of most of the fragments on the chromosome had been previously reported (27), but all the fragments have been redesignated to reflect their alphabetical order around the chromosome; the new designations are used in Fig. 1A, while both the new and the former designations are listed in Table 1. The size of each fragment, in kilobase pairs, was determined by comparison with the lambda concatamer and by the examination of the sizes of the two fragments resulting when strains with Tn*10* insertions (which have an added *Xba*I site from the Tn*10*) were digested with the enzyme (see Table 2 and Fig. 1B). The *Xba*I restriction map of the chromosome of *S. typhimurium* is displayed in a circular form in Fig. 2. This map is divided into 100 units called CS according to the suggestion of Rudd (40). Since the whole chromosome is about 4,800 kb in size (26, 27, 57; this report), a CS is equivalent to 48 kb. The sizes of individual *Xba*I fragments in CS and the inferred locations of *Xba*I sites on the chromosome are listed in Table 1 and illustrated in Fig. 2. The *Xba*I digestion sites are labelled sX1, sX2, etc.; they start at the counterclockwise (CCW) end of the *Xba*I-A fragment and then proceed clockwise (CW) around the map. All of the *Xba*I fragments are located with certainty, at least to region, except for the 32-kb fragment *Xba*I-S; its assignment next to fragments Q, R, and T is provisional.

End labelling of the *Xba*I fragments of *S. typhimurium* genomic DNA followed by electrophoresis and autoradiography reveals, in addition to the larger bands seen following ethidium bromide staining, a fragment at about 6 kb composed of two bands, at 6.6 kb (*Xba*I-K) and 6.4 kb (*Xba*I-U) (data not shown) and a very small fragment at the bottom of the gel (*Xba*I-L) which is less than 1 kb but must be >0.56 kb because the lambda standard of this size has run off the gel (Fig. 3B, lane 7). These fragments are located on the map, in some cases only approximately, as shown in Fig. 2.

Transposon Tn*10* has a restriction site for *Xba*I located in the gene for tetracycline resistance. Thus, any strain with a Tn*10* insertion will add a new *Xba*I site, and following *Xba*I digestion the *Xba*I fragment in which the Tn*10* is located is expected to disappear and two new fragments, summing to the size of the missing fragment, are expected to appear. This identifies the fragment in which the Tn*10* is located and gives information on its location within the fragment. This type of analysis has been reported earlier for *S. typhimurium* for *Xba*I (27) and *Bln*I (57). *Xba*I digests of DNA of five strains with Tn*10* insertions in known genes are shown in Fig. 1B; a listing of the genotypes of these five strains, along with others to give a total of 109 strains, and analysis of the results of the work is reported in Table 2. For example, digestion of DNA of strain TT315, known to have a Tn*10* insertion at min 54.0 on the linkage map (46), results in the loss of band G and the appearance of two new bands, G′ (estimated to be 212 kb) and ′G (estimated to be 22 kb) (Fig. 1B, lane 1; Table 2 at min 54.0). Data for four other strains, involving insertions in the genes *nadB*, *tctA*, *proU*, and *argR*, are shown in Fig. 1B; analysis of these data indicates that these insertions are in fragments *Xba*I-H (for *nadB*) and -M (for the others). Table 2 summarizes data for a total of 109 strains with Tn*10* insertions, including the above five strains; some of these data were reported earlier (27), although there are some quantitative corrections reported here. The Tn*10* insertions in all of these strains were located to an *Xba*I fragment. The results for approximately 20 other strains with Tn*10* insertions at known locations are not reported here; these results, which were difficult to analyze because of indications of major chromosomal rearrangements, will be reported at a later time.

In addition, the positions of individual Tn*10* insertions within each fragment were determined. The sum of the two new fragments should equal the length of the missing fragment plus either 9.3 kb (the length of Tn*10*) or 4.0 kb (the length of Tn*10*dTet, derived from the mutant element Tn*10* del-16 del-17 [55], which was used to construct many of the Tn*10* insertion strains in the collection at the SGSC).

*Xba*I digestion of DNA of a strain with mutation

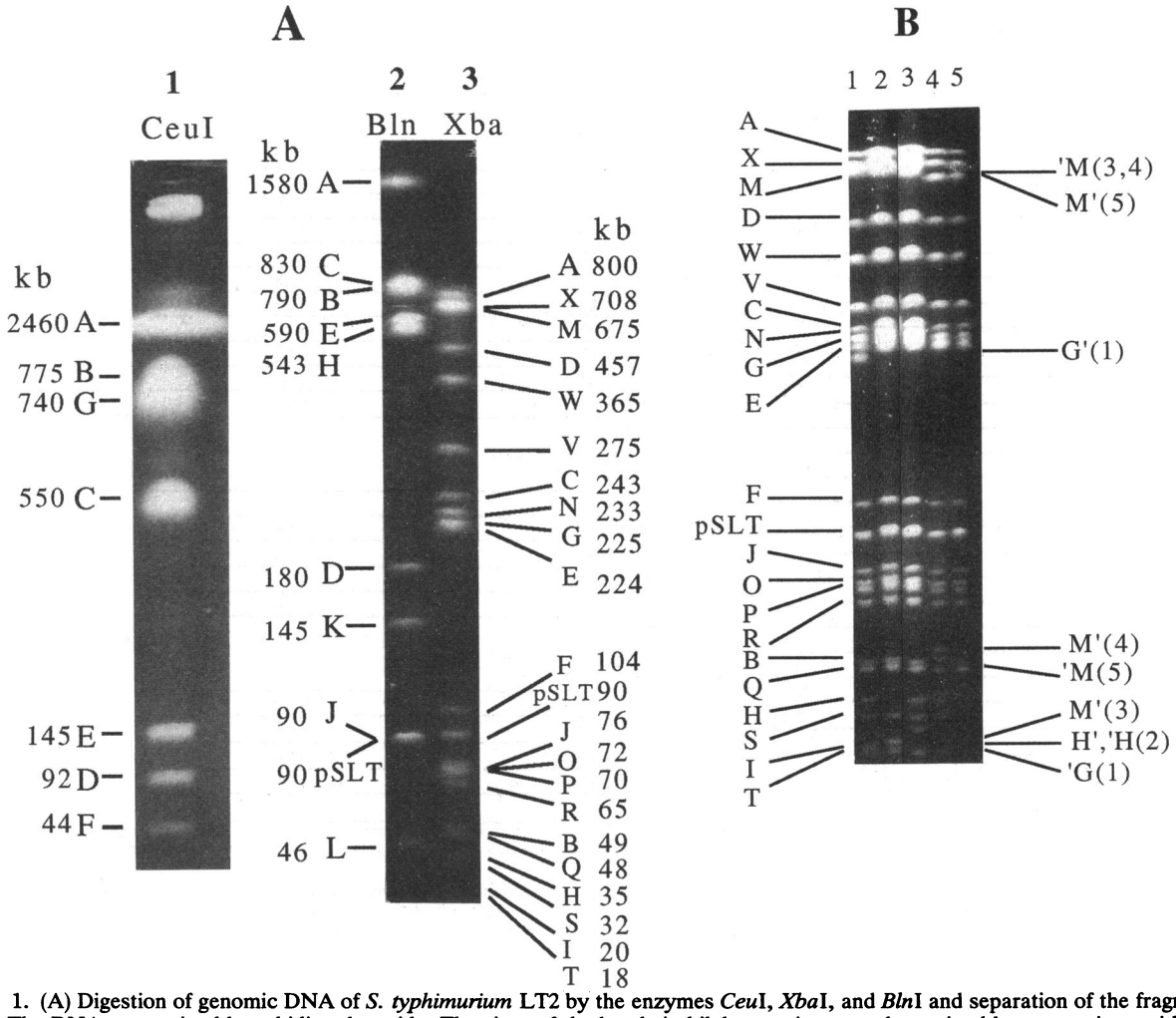

FIG. 1. (A) Digestion of genomic DNA of *S. typhimurium* LT2 by the enzymes *Ceu*I, *Xba*I, and *Bln*I and separation of the fragments by PFGE. The DNA was stained by ethidium bromide. The sizes of the bands in kilobase pairs were determined by comparison with lambda concatamers or, for the larger fragments, by summing the fragments obtained with DNA from strains with an insertion of the transposon Tn*10*, which adds restriction sites for *Xba*I and *Bln*I, or to determine the size the fragment was excised from the gel and redigested. The details of the sizes of the fragments for *Xba*I, *Ceu*I, and *Bln*I are given in Tables 1, 3, and 4, respectively. The sizes of the *Xba*I, *Ceu*I, and *Bln*I fragments were reported earlier (26, 27, 57), but some of the sizes have been corrected, and the fragments have been renamed so that they are in alphabetical order around the chromosome starting near the *thr* genes. The new and the former fragment designations are in Tables 1 and 4. (B) PFGE of *Xba*I digests of genomic DNA of strains of *S. typhimurium* with Tn*10* insertions at known locations on the chromosome. A detailed description of each strain is shown in Table 2; this description is found at the linkage map location corresponding to the gene with the Tn*10* insertion. The normal fragments are labelled on the left side. On the right side the new fragments are shown, along with a number showing the lane in which they appear. Lane 1, DNA of strain TT315 (*purG1739*::Tn*10*). The insertion is in the linkage map at 54.0 min, fragment G is lost, and fragments G' and 'G appear. Lane 2, TT399 (*nadB214*::Tn*10*). The insertion is at 55.0 min, fragment H is lost, and fragments H' and 'H appear. Lane 3, KS204 (*tctA1511*::Tn*10*). The insertion is at 56.9 min, fragment M is lost, and fragments M' and 'M appear. Lane 4, TL188 (*proU1655*::Tn*10*). The insertion is at 57.4 min, fragment M is lost, and fragments M' and 'M appear. Lane 5, KR1400 (*argR372*::Tn*10*). The insertion is at 68.3 min, fragment M is lost, and fragments M' and 'M appear.

tctA1511::Tn*10* causes the loss of fragment M and the appearance of two new bands, of 30 and 655 kb. Digestion of DNA of a strain with mutation *proU165*::Tn*10* also causes the loss of fragment M and new bands of 55 and 630 kb. Thus, the genes *tct* and *proU* are indicated to be 30 and 55 kb from one end of *Xba*I fragment M, but these data do not prove that they are at the same end, nor do they indicate if they are at the CW or CCW end of the fragment as located on the chromosome. The linkage map, which is based on quite independent data (46), indicates that these genes are close to each other, at 56.9 and 57.4 min; separation by 0.5 min indicates that they are about 24 kb apart. In addition, the locations of these genes relative to others and relative to *Xba*I sites indicate that they are both expected to be at the CCW end of *Xba*I fragment M; they are therefore thus shown in Table 2 and Fig. 2. From the conclusion that they are both at the same end of the fragment, *tctA* is 30 kb from sX13 at 60.3 CS, and *proU* is 55 kb from sX13 at 60.9 CS, 25 kb apart. The very close agreement with the distance inferred from the linkage map in this randomly selected example is partly fortuitous, since other gene intervals do not always agree so well. The position of each Tn*10* insertion site is indicated in Table 2 and illustrated in Fig. 2.

The positions of all the genes in which Tn*10* insertions

TABLE 1. *Xba*I fragments from the *S. typhimurium* genome[a]

Fragment[b] Designation		Size of fragment		XbaI site[e]	rrn Gene at XbaI site[f]	Position on chromosome[g] (in CS)
New	Former	kb[c]	CS[d]			
				sX24		98.6
X	B	708	14.7	sX1		13.0
A	A	800	16.7	sX2		29.7
B	Q	49	1.00	sX3		30.7
C	G	243	5.0	sX4		35.8
D	D	457	9.50	sX5		45.3
E	J	224	4.7	sX6		50.0
F	K	104	2.1	sX7		52.1
G	I	225	4.7	sX8		56.8
H	S	35	.8	sX9	gltW	57.5
I	U	20	.4			
J	M	76	1.60	sX10, 11, 12		
K	W	6.6	0.1			
L	Y	<1	--			
M	C	675	14.0	sX13		59.7
N	H	233	4.8	sX14		73.8
O	N	72	1.5	sX15	gltT	78.6
P	O	70	1.5	sX16		80.1
Q	R	48	1.00	sX17		81.6
R	P	65	1.4			
S	T	32	0.6	sX18, 19, 20		
T	V	18	0.4			
U	X	6.4	0.1	sX21		85.0
V	F	275	5.8	sX22	gltU	85.1
W	E	365	7.6	sX23	gltV	91.0
				sX24		98.6
pSLT	L	90				

[a] The genome of *S. typhimurium* is composed of the chromosome and the virulence plasmid, pSLT, which is ca. 90 kb in size. It has one *Xba*I site; the resulting fragment was formerly designated *Xba*I-L (27), and it is now listed as pSLT.

[b] The fragments are as in reference 27 except for the addition of the very small fragmentL. The fragments are in order of their location on the circular chromosome, starting at *Xba*I-X, which contains the gene *thr*, and proceeding A, B, etc. They have been renamed from the former designation (27) for the convenience of alphabetical order.

[c] The size in kilobase pairs is based on PFGE of the entire fragment or on the sum of two fragments when the fragment is cut because it carries a Tn*10* (which has an *Xba*I site).

[d] The chromosome is divided into 100 physical units called CS, as proposed by Rudd et al. (42). Each CS is equivalent, in *S. typhimurium*, to about 48 kb because the entire chromosome is estimated to be about 4,800 kb (27). CS are shown to one decimal place, indicating that the accuracy of the size estimate is not greater than 4.8 kb; occasionally the accuracy is greater, but for most fragments it is less.

[e] The *Xba*I sites are numbered in order around the chromosome, beginning at site *Xba*I-1 (sX1) on the CCW side of fragment *Xba*I-A at 13.1 CS.

[f] The *rrn* genes associated with specific *Xba*I sites are indicated.

[g] The positions of the *Xba*I sites on the chromosome are illustrated in Fig. 2. The position of the *thr* gene is estimated from analysis of DNA from a strain with Tn*10* inserted in *thr* (see Table 2 and text).

allowed physical mapping had previously been located on the linkage map, which was constructed mostly by classical genetic exchange methods (F-mediated conjugation and phage-mediated transduction) (46). These positions on the linkage map are listed in Table 2 and are illustrated in Fig. 2 as a concentric circle outside the *Xba*I restriction map. A line joining equivalent gene loci in the physical map and the linkage map shows the degree of correspondence between the maps. Overall the agreement is very good, with only a limited number of cases of disagreement.

***Ceu*I cleavage map.** Digestion of the genome of *S. typhimurium* LT2 with *Ceu*I produces seven fragments due to digestion in the seven *rrl* genes for large-subunit (23S) rRNA (26); these seven fragments are seen in Fig. 1A, lane 1. Bands B and G were not separated in this electrophoresis. The sizes in kilobase pairs of the larger bands are determined by excision of the band and redigestion with other enzymes (see below). The locations of these seven *rrl* genes on the physical map in terms of CS were determined as follows. *Ceu*I fragments were excised in agarose following PFGE and were then redigested with *Xba*I, end labelled with ^{32}P, reelectrophoresed, and autoradiographed. The sizes of the fragments resulting from the digestion of each *Ceu*I fragment are recorded in Table 3. Some of the *Ceu*I fragments are small, and *Xba*I digestion produced no change in the fragment size; for example, digestion of *Ceu*I-D and -E produced unaltered fragments with sizes of 92 and 145 kb, respectively (Table 3), inferred to be part of *Xba*I-V (see Fig. 4). However, digestion of larger fragments such as *Ceu*I-A with *Xba*I produced the following *Xba*I fragments: 'X, A, B, C, D, E, F, G, and H' (Table 3; Fig. 3B, lane 4). 'X is 325 kb and is inferred to be from the CW end of *Xba*I-X, and H' is 33 kb and is inferred to be from the CCW end of *Xba*I-H; the other fragments correspond to unaltered *Xba*I fragments (Fig. 3B, lane 7). The *Ceu*I site at the CCW end of *Ceu*I-A, designated site *Ceu*I-1 (sC1) and inferred to be in *rrlH*, is 325 kb CCW from sX1, at the CCW end of the *Xba*I-A fragment, previously located at 13.0 CS on the physical map (Table 1; Fig. 2 and 4). Since 48 kb equals one CS, sC1 is at 13.0 − (325/48) = 6.3 CS. The *Ceu*I site at the CW end of *Ceu*I-A, sC2, is

TABLE 2. Strains of *S. typhimurium* LT2 with Tn*10* insertions and their physical locations on the genome

*Xba*I fragment	Strain no.[a]	Mutation (Tn*10* insertion[b])	Linkage data (% point transduction)[c]	Physical data (kb)[d]			Location of Tn*10* insert (CS)[e]	Linkage map location (min)[f]
				Intact fragment	Fragment a	Fragment b		
X	TT21	*serB965*		708	58	660	99.7	99.7
	TT6736	*zaa-1004*	*pnuA*, 66; *thr*, 15; *serB*, 50	708	57	660	99.7	99.6
	TT191	*thr-557*		708	82	633	0	0
	TT1198	*pyrA234*		708	160	555	1.6	1.8
	TT206	*leu-1151*		708	220	495	2.8	2.8
	TT421	*pan-540*		708	300	415	4.5	4.7
	AK3149	*zae-3149*	*pepM*, 42	708	330	360	5.3	3.0
	AK3262	*zag-3262*	*dnaE*, 54	708	370	345	6.0	6.0
	JL2690	*proA1656*		708	450	260	7.6	7.0
	AK3150	*zah-3150*	*proAB*, 82	708	475	240	8.1	7.0
	AK3030	*zai-3030*	*proC*, 28	708	515	200	8.9	8.5
	TN789	*zai-808*	*proC*, 30	708	534	166	9.5	8.9
	TN1781	*zbb-876*	*apeA*, 75	708	670	55	12.0	11.5
	TT289	*purE884*	*clmG*, 68	708	695	22	12.6	11.8
	TN966	*apeE2*		708	700	16	12.8	12.3
A	TA4190	*ahp-11*		800	57	750	14.1	13.0
	TT7247	*zbe-1023*	*lip*, 90	800	85	720	14.8	13.7
	TT2342	*zbf-99*	*supE*, 74	800	135	670	15.9	15.1
	TT398	*nadA213*		800	205	600	17.3	16.4
	TN1117	*zbi-812*	*galE*, 90	800	235	570	17.8	18.0
	AK3020	*zbi-3020*	*oxd*, 6; *galE*, 10	800	240	565	18.0	18.7
	TT403	*bio-102*		800	250	560	18.2	18.3
	SL1346	*aroA554*		800	335	400	20.1	19.3
	SMS409	*aspC409*		800	470	335	22.8	19.5
	TT6197	*pncB150*		800	500	310	23.5	20.0
	TA262	*nanH*		800	520	390	23.9	
	AK3232	*zcb-3232*	*pyrD*, 32; *pepN*, 20	800	530	280	24.0	21.0
	TT468	*pyrD2266*		800	545	290	24.3	21.0
	TT770	*pepN88*		800	555	260	24.4	20.8
	TT946	*putA810*		800	607	200	25.6	22.0
	KK2087	*flgL*		800	657	150	26.7	23.2
	TT6850	*zce-850*	*purB*, 50	800	680	126	27.1	25.0
	TT6195	*pncA148*		800	765	42	28.9	27.0
	TT10281	*zcg-1819*	*pepT*, 41	800	780	30	29.1	25.0
	TT282	*purB877*		800	800	7	29.6	25.0
C	CH695	*tppB16*		243	72	180	32.2	26.5
	AK3314	*zci-3314*	*dcp*, 6	243	92	160	32.6	28.5
	SH7235	*ompD159*		243	190	59	34.6	32.5
D	TN2336	*oxrA2*		457	48	420	36.7	30.2
	TT464	*pyrF696*		457	95	370	37.7	33.4
	TT15115	*cysB3305*		457	97	365	37.8	33.6
	TT1333	*trp-2451*		457	120	345	38.2	34.0
	CH50	*oppB255*		457	125	355	38.3	34.5
	AK3211	*zdf-3211*	*divF*, 50; *chlC*, 48	457	150	315	38.8	34.8
	TT10269	*chlC1130*		457	160	305	39.0	35.0
	KK2503	*fliC*		457	330	135	42.5	40.0
	TT1127	*hisC8667*		457	434	32	44.6	42.0
	NK114	*gnd-161*		457	442	16	44.9	42.2
E	AK3061	*zee-3061*	*metG*, 6	224	93	142	47.2	44.2
	SH7241	*ompC396*		224	200	35	49.3	45.8
F	SMS408	*ack-408*		104	53	60	51.1	46.4
	AK3138	*zeh-3138*	*purF*, 14	104	60	53	51.2	46.6
	TA3178	*hisJ8908*		104	66	46	51.4	46.7
	TT317	*purF1714*		104	90	15	51.8	47.0
G	PP1228	*ptsI421*		225	48	187	53.0	49.3
	PP994	*crr-307*		225	49	186	53.1	49.4
	NK186	*cysA1367*		225	52	182	53.2	50.0
	AK3271	*zej-3271*	*cysA*, 99	225	52	180	53.2	50.0
	TT11567	*zfa-3644*	*eut*, 95	225	70	140	53.6	50.5
	TT287	*purC882*		225	98	135	54.1	51.3

Continued on following page

TABLE 2—*Continued*

*Xba*I fragment	Strain no.[a]	Mutation (Tn*10* insertion[b])	Linkage data (% point transduction)[c]	Physical data (kb)[d]			Location of Tn*10* insert (CS)[e]	Linkage map location (min)[f]
				Intact fragment	Fragment a	Fragment b		
	TT11	*purI1757*		225	112	123	54.4	51.7
	TT278	*guaA554*		225	120	115	54.6	52.0
	TN858	*zfd-801*	*pepB*, 65; *glyA*, 10	225	180	58	55.7	53.3
	TT418	*glyA540*		225	195	40	56.0	53.5
	TT315	*purG1739*		225	212	22	56.4	54.0
H	TT399	*nadB214*		35	22	22	57.1	55.0
J	TT1339	*pheA534*		76	11	74		55.4
	TT126	*tyrA555*		76	16	70		55.6
M	KS204	*tctA1511*		675	30	655	60.3	56.9
	TL188	*proU1655*		675	55	630	60.9	57.4
	TT14835	*srl-203*		675	130	600	62.2	58.8
	TT173	*cysC1511*		675	180	500	63.4	60.0
	TT7542	*relA21*		675	214	466	64.1	60.8
	TT146	*argA1832*		675	240	440	64.6	61.3
	AK3122	*zgc-3122*	*thyA*, 15	675	245	440	64.7	61.3
	TT215	*lysA565*		675	260	424	65.0	61.8
	AK3085	*zgd-3085*	*pepP*, 78; *serA*, 36	675	306	366	66.0	62.5
	TT169	*serA977*		675	310	335	66.0	62.7
	AK3076	*zge-3076*	*oxd*, 10	675	410	275	68.1	64.5
	TT14	*metC1975*		675	415	255	68.4	65.0
	TT142	*argG1882*		675	570	115	71.3	68.0
	AK3163	*zgi-3163*	*dna*, 85; *argG*, 49	675	575	110	71.4	68.0
	KR1400	*argR372*		675	635	48	72.9	68.3
N	PP1037	*crp-773*		233	8	227	73.9	72.3
	TT172	*cysG1510*		233	33	209	74.4	72.7
	AK3081	*zhd-3081*	*aroB*, 70	233	50	190	74.7	73.2
	AK3109	*zhh-3109*	*opt-10*, 78	233	174	68	77.2	76.0
	AK3108	*zhh-3108*	*opt-10*, 50	233	180	60	77.3	76.2
O	SA1982	*xyl-183*		72	17	62	78.8	78.0
P	TT7433	*zia-1036*	*spoT*, 90	70	26	53	80.5	79.8
	AK3205	*zia-3205*	*mgtB*, 36	70	53	26	81.0	81.5
Q	TT1039	*uncA*		48	31	26		82.0
	TT1044	*unc*		48	31	26		82.0
	JF753	*psiR1*		48	42	15		82.0
V	TT66	*ilvG1007*		275	15	270	85.3	83.0
	TT58	*ilvA595*		275	17	267	85.4	83.1
	AK3145	*zie-3145*	*pepQ*, 89; *ilv*, 33	275	115	167	87.6	84.2
	TT225	*metB879*		275	206	76	89.5	87.5
	TT137	*argH1823*		275	227	55	89.9	88.2
	TT501	*thiA541*		275	267	20	90.6	88.8
	TT311	*purD1735*		275	275	9	90.9	89.2
W	TS616	*malE616*		365	36	338	91.6	91.0
	TT1662	*mel-351*		365	132	240	93.6	93.0
	SMS401	*poxA401*		365	185	187	94.7	95.4
	GW1714	*mutL111*		365	200	170	95.1	95.7
	TT273	*purA874*		365	210	165	95.2	96.1
	AK3196	*zji-3196*	*argI*, 72; *pepA*, 38; *pyrB*, 22	365	285	60	96.8	97.5
	TT460	*pyrB692*		365	296	78	97.0	98.0
	TT147	*argI1833*		365	304	70	97.2	97.0

[a] Each strain has a Tn*10* insertion, usually wild-type Tn*10* or Tn*10*dTet (Tn*10* Δ16 Δ17). The strains and linkage data have been provided by many investigators. The strains are maintained at the SGSC and are available for distribution.

[b] Gene into which Tn*10* is inserted and allele number of the insertion; e.g., *thr-557*::Tn*10* is an insertion into the *thr* gene. Mutations designated *z*.. are in a region of unknown function. Some of the strains carry other mutations.

[c] The linkage data are the percent joint transduction by phage P22 of the Tn*10* insertion with the gene indicated. These data were determined by other workers and were provided as personal communications to the SGSC.

[d] The physical data were from this study; some of these data were reported previously (27). The fragment into which the Tn*10* is inferred to be inserted is in the left column, and the intact fragment size is listed here. The fragments observed, inferred to result from cleavage of the missing band in the *Xba*I site of the inserted *Tn*10, are indicated. Fragment a is inferred to be the CCW fragment, and fragment b is inferred to be the CW fragment.

[e] A CS is 1/100 of the physical length of the chromosome, numbered starting at *thr*. The positions of the ends of the *Xba*I fragment (in CS) are shown in Table 1; the positions of Tn*10* insertions (in CS) are in this column. One CS = 48 kb. For some strains the CS value is not shown because the exact location of the fragment into which Tn*10* is inserted is not known.

[f] The position of the gene into which the Tn*10* is inserted is shown in minutes on the linkage map, as shown by Sanderson and Roth (46). When the insertion is into a region of unknown function, the allele is shown as *z*.., and the map position is derived from these genes to which the insertion is linked by joint transduction.

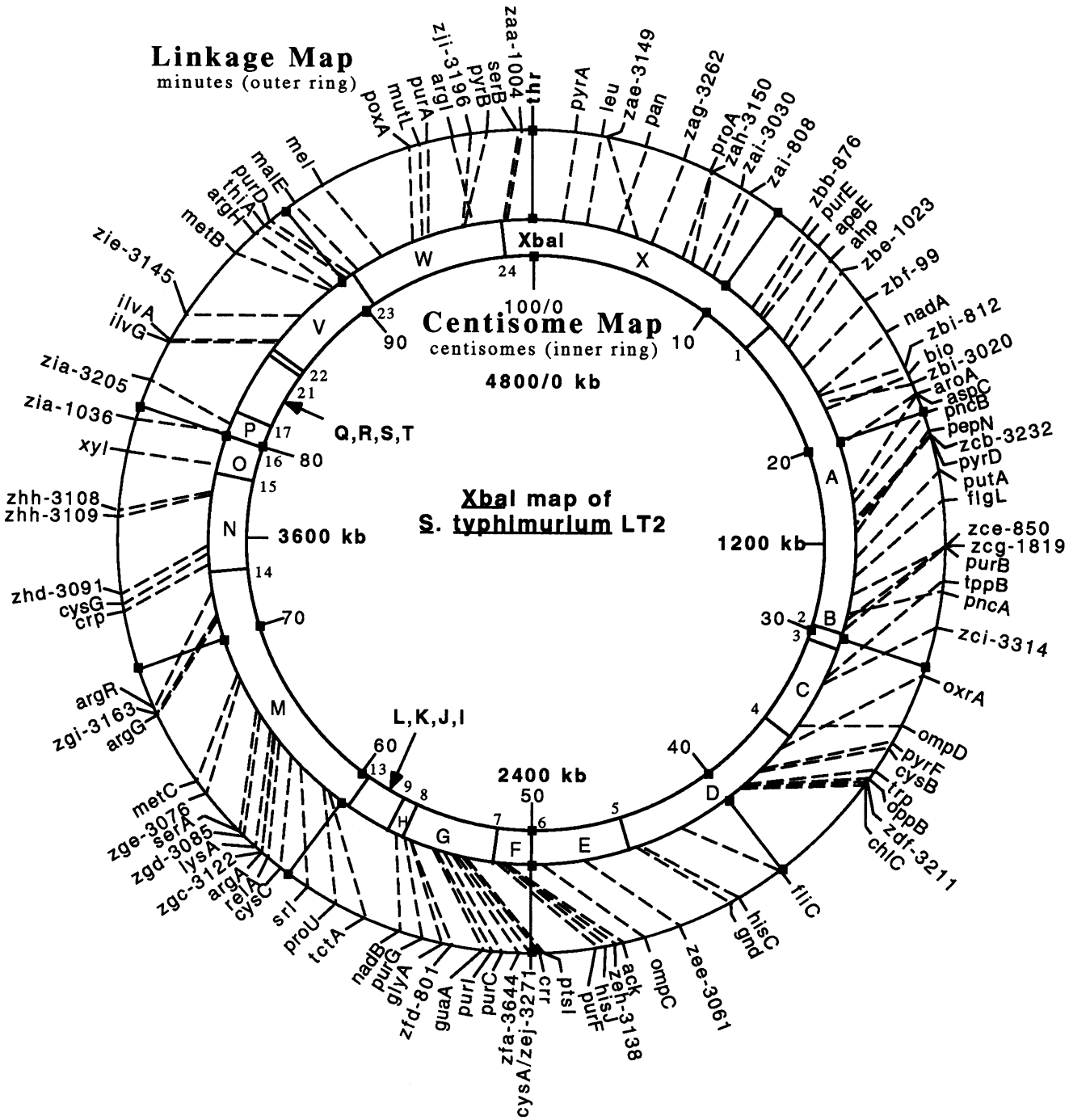

FIG. 2. *Xba*I genomic cleavage map of the chromosome of *S. typhimurium* LT2, shown in comparison with the linkage map. The *Xba*I fragments of the genomic cleavage map are on the inner circle; the letters representing each fragment in alphabetical order around the chromosome are new, changed from the former designations which were published earlier (27), but both new and former designations are shown in Table 1. The sizes of the fragments are in proportion to their size in CS, as calculated in Table 1. The total size of the restriction map sums to 4,808 kb, but here it is rounded to 4,800 kb. The *Xba*I sites are numbered in order CW around the chromosome beginning at the CCW end of fragment *Xba*I-A; these sites are abbreviated sX1, sX2, etc., in the text. The exact order of *Xba*I sites is not known at two locations: at fragments I, J, K, and L and at fragments Q, R, S, and T. The positions of the Tn*10* insertion in 109 strains with independent insertions have been calculated in CS (Table 2), and the positions of most of these genes are shown on the inner circles (the *Xba*I genomic cleavage map). The positions of these same genes are indicated and the genes are labelled on the outer circle, which represents the linkage map based on classical genetic methods such as F-mediated conjugation and phage-mediated transduction (46). The position for each gene on the linkage map in minutes is listed in Table 2. Those genes beginning with *z*, e.g., *zae-3149* at 3 min on the linkage map, represent the locations of Tn*10* insertions into a region which produces no detectable change in phenotype.

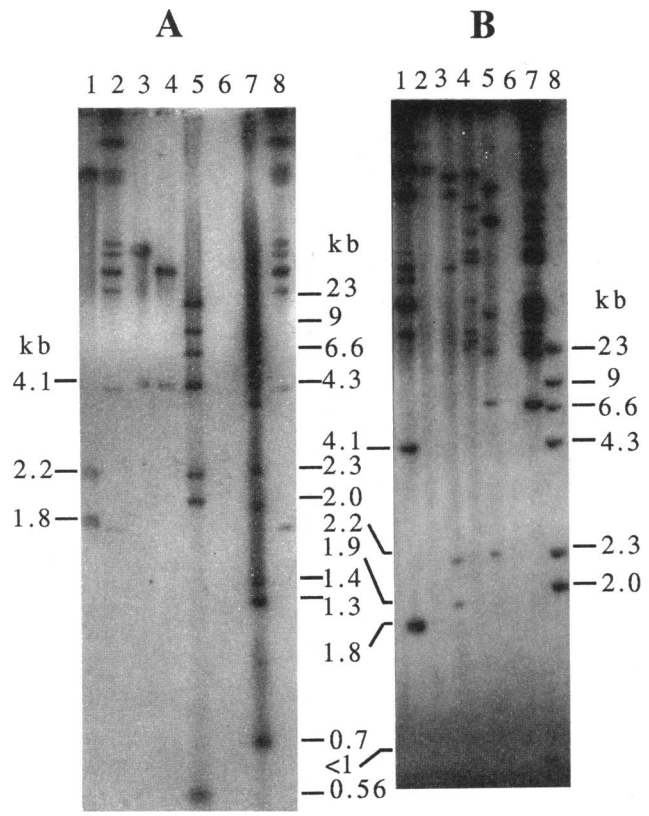

FIG. 3. (A) Autoradiograph of ^{32}P-end-labelled fragments of single- or double-digested DNA of *S. typhimurium*. After digestion and end labelling, the samples were electrophoresed and then autoradiographed. DNA in some of the lanes was digested only once. In other lanes the DNA was digested and electrophoresed, and then fragments were excised from the gel in an agarose block, redigested in the block with a second enzyme, end labelled, and then reelectrophoresed and autoradiographed. Lanes: 1, *Ceu*I fragment C, redigested by *Bln*I; 3, *Ceu*I-E, redigested by *Bln*I; 4, *Ceu*I-D, redigested by *Bln*I; 2 and 8, genomic DNA digested by *Bln*I only; 5, lambda *Hin*dIII standard; 7, lambda *Bst*EII standard; 6, lambda concatamer (not end labelled). The sizes of some of the fragments of the standards are shown on the right side; the sizes of some of the small fragments from the digests are on the left. (B) Autoradiograph, produced in the same manner as in panel A. Lanes: 2, *Ceu*I-A, redigested by *Bln*I; 3, *Ceu*I-B and -G (these fragments do not fully separate in the first electrophoresis), redigested by *Bln*I; 4, *Ceu*I-A, redigested by *Xba*I; 5, *Ceu*I-B and -G, redigested by *Bln*I; 1 and 7, genomic DNA singly digested by *Bln*I and *Xba*I, respectively; 8, lambda *Hin*dIII standard; 6, lambda concatamer (not end labelled). The sizes of some of the fragments of the standards are on the right side; the sizes of some of the small fragments from the digests are on the left.

inferred to be in *rrlG*; this site is very close to the site at the CW end of *Xba*I-H (sX9) and is designated 57.5 CS. The locations of the five other *Ceu*I sites, which correspond to the five other *rrl* genes, were determined in the same way.

Analysis of the *rrn* genes. In *E. coli* K-12 the four *rrn* genes *rrnG*, *-C*, *-B*, and *-E* contain tRNA genes for glutamate (Glt); the others have tRNA genes for alanine (Ala) and isoleucine (Ile). Lehner et al (21) concluded that in *S. typhimurium*, the *rrn* gene at the *rrnD* location, rather than the *rrnB* gene, contained the tRNA-Glt gene. Our data confirm these conclusions. The tRNA gene for Glt, but not those for Ala and Ile, has an *Xba*I site in *E. coli*; we find *Xba*I sites associated with the four *rrn* genes *rrnG*, *-D*, *-C*, and *-E* but not with the other *rrn* genes, suggesting a gene exchange involving *rrnB* and *rrnD* during the course of evolution of these genera.

Analysis of double-digested fragments which are end labelled with ^{32}P permits recognition of small fragments which yield detailed information about the *rrn* genes. For example, redigestion of the *Ceu*I-B and -G bands by *Xba*I (the two are of very similar size, so they were not separated in this case) yields bands of 670, 375 and 363 (seen as a single band), 76, 20, 6.6, 2.3, and <1 kb (Fig. 3B, lane 5). We infer that the 2.3-kb band results from the *Ceu*I-B fragment and represents *Xba*I-'H, digested from the middle of the *rrnG* gene. The *Ceu*I site of the *rrlB* gene of *E. coli* K-12 is at bp 3909 (see Fig. 4B), and the *Xba*I site in the tRNA gene *gltU* is at bp 1723, indicating a distance of about 2.19 kb; we infer that the 2.3-kb fragment 'H represents the equivalent of this fragment, digested from *rrnG*.

The structure and orientation of transcription of *rrn* genes in *E. coli* K-12 are as shown in Fig. 4B; the locations of these genes in *S. typhimurium* are the same (21), but the detailed structure and orientation of the genes are not known. Our data with *Ceu*I-*Xba*I digestion confirm this inferred identity of orientation and similarity of structure, at least for the *rrn* genes which contain tRNA-Glt genes. From the structure of the *rrnB* gene of *E. coli* (Fig. 4B), the *Xba*I site is upstream and the *Ceu*I site is downstream in the *rrl* gene. *Ceu*I-A does not yield a 2.3-kb *Xba*I fragment, but *Xba*I-B does; this fits the orientation of the *rrnG* and *rrnD* genes in Fig. 4A. *Ceu*I-C spans the interval between *rrnD* and *rrnC*; both have tRNA-Glt genes, and they are in reverse orientation in *E. coli* K-12. The 2.3-kb fragment observed following *Xba*I digestion is double the normal intensity of other equivalent bands, indicating that there are 2.3-kb bands produced at both ends due to *Xba*I digestion; this confirms the orientation shown. Similarly, digestion of *Ceu*I-F with *Xba*I yields a 2.3-kb band, indicating that *rrnE* is oriented clockwise, as in Fig. 4A.

***Bln*I restriction map.** Genomic DNA was digested with *Bln*I (=*Avr*II); 10 bands were visible when these digests were separated by PFGE and stained by ethidium bromide (Fig. 1A, lane 2). Wong and McClelland (57) observed these same 10 bands, and 9 of them were arranged around the chromosome by a variety of methods. We have confirmed the locations of these nine fragments, determined the locations of *Bln*I sites with respect to the *Xba*I and *Ceu*I maps, and renamed the *Bln*I fragments in alphabetical order around the chromosome, starting with band A, which overlaps the *thr* locus; the new and the former fragment designations are in Table 4, and the positions of the fragments are displayed in Fig. 4A. The sizes which we record for the fragments are similar but not always identical to those recorded by Wong and McClelland (57). They identify a 10th fragment (which they call G2) which is the virulence plasmid pSLT of *S. typhimurium* with a single *Bln*I site. We confirm their observations, but we record the plasmid as pSLT (Fig. 1A, lane 2). Using end-labelling methods, Wong and McClelland (57) found two small fragments which they recorded as 7 and 4 kb. We have detected a 4.1-kb band, which we call fragment F, and a band at 1.8 kb which represents two fragments, G and I, inferred to be cut from *rrn* genes (see below).

The nine larger *Bln*I fragments were excised from the gel, redigested with *Xba*I, end labelled, electrophoresed, and autoradiographed; the sizes of bands detected are recorded in Table 4. For example, *Xba*I digestion of *Bln*I-A gives fragments of 365 kb (*Xba*I-'W), 708 kb (XbaI-X), and 520 kb (the CCW part of *Xba*I-A). Thus, the sites for *Bln*I digestion

TABLE 3. *Ceu*I fragments redigested by *Xba*I

CeuI fragment	Size (Kb)[a]	Fragments from redigestion by XbaI[b]	CeuI site[c]	rrn Gene at CeuI site[d]	Centisome (CS)[e]
			sC1	rrlH	6.3
A	2460	325('X), 800(A), 49(B), 243(C), 457(D), 224(E), 104(F), 225(G), 33(H')			
			sC2	rrlG	57.5
B	775	2.3('H), 20(I), 76(J), 6.6(K), <1(L), 670(M')			
			sC3	rrlD	73.7
C	550	2.3('M), 233(N), 72(O), 70(P), 48(Q), 65(R), 18(T), 6.4(U), 2.3(V')			
			sC4	rrlC	85.2
D	92	92('V')			
			sC5	rrlA	87.1
E	145	145('V')			
			sC6	rrlB	90.1
F	44	42('V), 2.3(W')			
			sC7	rrlE	91.0
G	740	363('W), 375(X')			
			sC1	rrlH	6.3

[a] The *Ceu*I fragments of *S. typhimurium* LT2 were reported earlier (26).

[b] The *Ceu*I fragments were separated by PFGE, excised in the agarose block, redigested with *Xba*I, end labelled with ^{32}P, electrophoresed, and autoradiographed. The sizes were correlated with *Xba*I digests of whole genomic DNA (as in Table 1). Fragments marked 'X are inferred to be at the CCW end of the *Ceu*I fragment; those marked X' are inferred to be at the CW end. For example, *Xba*I digestion of *Ceu*I-A produces a 325-kb fragment, designated 'X, inferred to be the CCW end of *Ceu*I-A (and at the CW end of *Xba*I-X).

[c] The *Ceu*I digestion sites are designated in numerical order.

[d] the *rrn* gene corresponding to each *Ceu*I site is designated in correspondence with *E. coli* K-12 (6) and with *S. typhimurium* (21).

[e] The location of each *Ceu*I site in CS is calculated by determining the distance from the location of *Xba*I sites (see Table 1). For example, sC1 is 325 kb CCW from sX1, which is at 13.0 CS. Since 1.0 CS = 48 kb, *rrnH* is placed at 6.3 CS. The positions of other *Ceu*I sites are located in the same way.

are sB1 at 91.0 CS (very close to sX23; Table 1) and sB2 at 23.9 CS (derived from calculation of the location of sB2 inside the *Xba*I-A fragment). The positions of the other *Bln*I sites were determined in the same way (Table 4).

Fragments obtained from *Ceu*I digestion were excised and redigested with *Bln*I (Table 5). These data confirmed the locations of *Bln*I sites on the chromosome and in some cases allowed location of *Bln*I sites within *rrn* genes. For example, digestion of *Ceu*I-C with *Bln*I produced three visible bands (Fig. 3A, lane 1); a band of 543 kb is inferred to be *Bln*I-H, while the two bands of 2.2 and 1.8 kb, both of which are double the intensity normally seen for this molecular weight, are considered to represent fragments cut from *rrnD* and *rrnC*. The 1.8-kb band represents the distance from the *Bln*I site in the *glt* gene to the *Bln*I site in the *rrs* gene (1.50 kb in *E. coli rrnB*; Fig. 4B). The 2.2-kb band is the interval from the *Ceu*I site to the *Bln*I site in the *glt* gene, which is 2.13 kb in the *E. coli rrnB* gene (Fig. 4B). These data support the idea that these *rrn* genes are reversed in *S. typhimurium* relative to *E. coli*. The 4-kb bands observed when *Ceu*I-D and -E are recut by *Bln*I come from *rrnA* and *-B*, respectively, and represent the interval from the *Bln*I site of the *Ceu*I site; this is 3.64 kb in *rrnB* of *E. coli* (Fig. 4B). The fact that *Bln*I digestion of *Ceu*I-F produces a 2.3-kb band but no 1.8- or 4.0-kb band indicates that there is a *Bln*I site in *gltV* but not in *rrsE*. *Bln*I digestion of *Ceu*I-B and -G (not separated from one another) yielded three large fragments of 740, 590, and 180 kb and two small fragments of 2.2 and 1.9 kb (Fig. 3B, lane 3). The 2.2-kb fragment is *Bln*I-'C from *Ceu*I-B, while the 1.9-kb fragment is *Bln*I-F'; it is clear that F' is different from any of the 1.8-kb fragments from *rrn* genes (compare lanes 1 and 2 in Fig. 3B), confirming the position of the 4.1-kb band *Bln*I-F near *rrnD*.

DISCUSSION

For many years there has been a recognition that the chromosomal structure and gene order in the linkage maps of *S. typhimurium* and *E. coli* K-12 are strongly conserved (19, 37, 38, 44); we have now confirmed this conservation by analysis of genomic cleavage maps of these two organisms. A genomic cleavage map of *E. coli* K-12 was developed initially for *Not*I (52), and since then similar maps have been developed for *Avr*II (=*Bln*I) (11, 36), *Not*I (14), *Sfi*I (35), *Xba*I (36), and *Ceu*I (26); much but not all of the latter work has been with the K-12 strain MG1655. Low-resolution genomic cleavage maps of *S. typhimurium* LT2 for *Xba*I (27), *Bln*I (57), and *Ceu*I (26) have been coordinated and mostly completed in the present report.

The conservation of genome size and gene order in the two genera observed in earlier work is confirmed. The chromosome of *S. typhimurium* is similar in size to that of *E. coli*, which is 4,600 kb (18, 40, 52), but it appears to be a little larger (4,800 kb). The order of genes on the linkage maps of

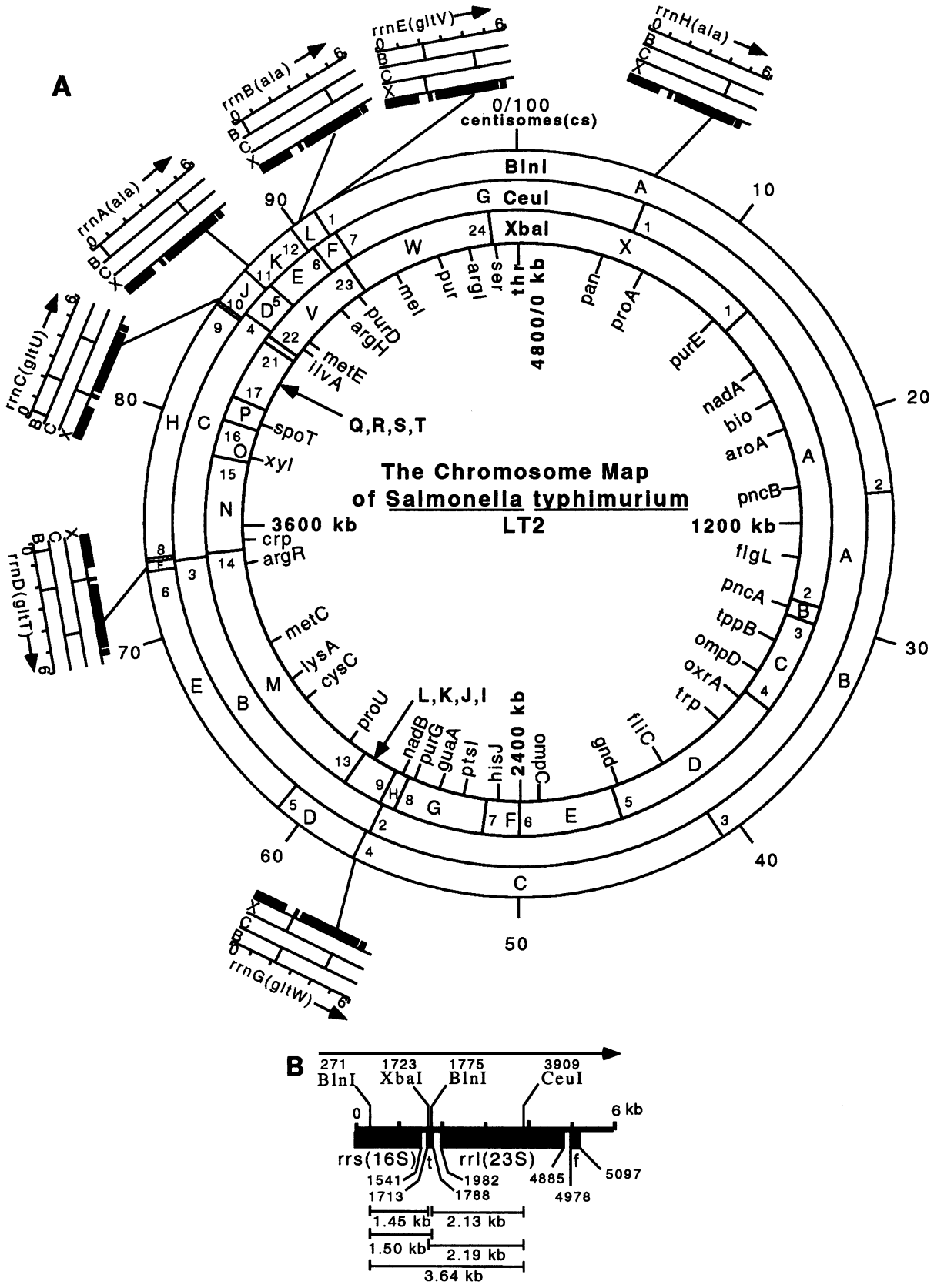
A
The Chromosome Map
of Salmonella typhimurium
LT2
0/100
centisomes(cs)
BlnI
CeuI
XbaI
4800/0 kb
1200 kb
2400 kb
3600 kb
rrnE(gltV)
rrnB(ala)
rrnH(ala)
rrnA(ala)
rrnC(gltU)
rrnD(gltT)
rrnG(gltW)
Q,R,S,T
L,K,J,I
thr
ser
argI
pur
mel
purD
argH
metE
ilvA
spoT
xyl
crp
argR
metC
lysA
cysC
proU
nadB
purG
guaA
ptsI
hisJ
ompC
gnd
fliC
trp
ompD
tppB
pncA
flgL
pncB
aroA
bio
nadA
purE
proA
pan
B
271 BlnI
1723 XbaI
1775 BlnI
3909 CeuI
6 kb
rrs(16S)
rrl(23S)
1541
1713
1982
1788
4885
4978
5097
1.45 kb
2.13 kb
1.50 kb
2.19 kb
3.64 kb

FIG. 4. (A) Genomic cleavage map of *S. typhimurium* LT2. The cleavage sites for the enzymes *Xba*I, *Ceu*I, and *Bln*I are indicated in CS around the chromosome; the CS scale is shown on the outside of the circles. The structure for *Xba*I is based on data in Tables 1 and 2 and Fig. 1 and is the same as in Fig. 2; the locations of some of the genes on the CS map in Fig. 2, which are based on analysis of strains with Tn*10* insertions, are shown on the inside of the circles. The *Ceu*I map was determined by isolation of *Ceu*I fragments and digestion with *Xba*I (Table 3); all the *Ceu*I sites are postulated to be in 23S rRNA genes (*rrl*). The *Ceu*I fragments are in alphabetical order, and the *Ceu*I sites (sC1 to sC7) are labelled. The locations of *Bln*I sites are determined by isolation of *Bln*I fragments from the gel and redigestion by *Xba*I (Table 4); the designations for the fragments (57) have been altered to put them in alphabetical order (Table 4). The seven *rrn* genes are indicated in detail outside the circle at the locations determined by redigestion of *Ceu*I fragments by *Xba*I (Table 3) or by *Bln*I (Table 5). The structure of the *rrn* genes and their location and orientation on the chromosome were originally determined for *E. coli* (1, 34), and the locations were confirmed for *S. typhimurium* (21). The arrow indicates the direction of transcription inferred for *E. coli*. The order of the genes in each *rrn* group is indicated by the solid bar under the gene and is *rrs* (16S rRNA)-tRNA-*rrl* (23S rRNA)-(5S rRNA) as in panel B. The tRNA genes in the intervening regions are shown as in *E. coli*, except that *gltT* is in *rrnD* at 73 CS rather than in *rrnB* at 90 CS. The orientation of transcription indicated by the arrow on each gene in from *E. coli* (34). The positions of cleavage sites in the *rrn* genes are from data in this report (see Results); these data confirm the orientations determined originally for *E. coli*. (B) Structure of the *rrnB* gene of *E. coli* (6, 34). The *rrsB* (16S rRNA), *rrlB* (23S rRNA), and *rrfB* (5S rRNA) genes are indicated by rrs, rrl, and f, respectively; t stands for the tRNA region, which in the *rrnB* gene of *E. coli* is the *gltT* gene for glutamyl-tRNA. The nucleotide coordinates (34) of the genes and the positions of the *Bln*I, *Xba*I, and *Ceu*I sites are shown, as well as the distances between the sites in kilobase pairs.

the two organisms is highly conserved, with a few exceptions. For example, the order of the genes in Fig. 2 determined from physical analysis confirms the inversion of about 10% of the chromosome, observed earlier by classical linkage analysis, around the 24- to 36-min region of the linkage map (8, 37, 45). The genome of *S. typhimurium* normally includes the 90-kb virulence plasmid, pSLT, which is present except in those rare cases when it is intentionally cured (32, 54).

Comparison of nucleotide sequences of *S. typhimurium* and *E. coli* shows a broad range of relatedness from almost complete identity in the rRNA and tRNA genes to low levels of identity between noncoding sequences outside genes. Analysis of sequences in coding regions shows that all nucleotide changes are subject to selective pressure, even the synonymous substitutions (48, 49). The degrees of similarity range from 77 to 100% for amino acid sequences and from 75 to 99% for nucleotide sequences. Conservation between the two genera in nucleotide sequences in the genes for rRNA and tRNA and significant changes in the se-

TABLE 4. *Bln*I fragments redigested by *Xba*I

BlnI fragment Designation New	BlnI fragment Designation Former [a]	BlnI fragment Size (kb)	Fragments from redigestion by XbaI [b]	BlnI site	rrn Gene at Site [c]	Centisome (CS) [d]
				sB1	gltV	91.0
A	A	1580	365('W),708(X),520(A')	sB2		23.9
B	C	790	280('A),49(B),243(G),215(D')	sB3		40.3
C	B	830	242('D),224(E),104(F),225(G),35(H')	sB4	gltW	57.5
D	F1	180	20(I),76(J),6.6(K),<1(L),78(M')	sB5		61.3
E	D	590	590('M')	sB6		73.7
F		4.1	N.T.	sB7	gltT	73.8
G		1.8	N.T.	sB8	rrsD	73.8
H	E	543	233(N),72(O),70(P),48(Q),65(R),18(T),4.6(U')	sB9	rrnC	85.1
I		1.8	N.T.	sB10	gltU	85.1
J	G1	90	90 ('V')	sB11	rrsA	87.0
K	F2	145	145('V')	sB12	rrsB	90.0
L	H	46	46('V)	sB1	gltV	91.0

[a] These fragments were formerly designated by Wong and McClelland (57). The new designations are in alphabetical order around the chromosome.
[b] Fragments were excised and redigested as described in footnote *b* of Table 3.
[c] The *Bln*I site is in the 16S rRNA (*rrs*) gene or tRNA-*glt* gene in several cases.
[d] The position in CS of the *Bln*I site is calculated as described in footnote *e* of Table 3.

TABLE 5. *Ceu*I fragments of *S. typhimurium* LT2 redigested by *Bln*I

*Ceu*I fragment	Size (kb)	Fragments (kb) from redigestion by *Bln*I[a]
A	2,460	845 ('A), 790 (B), 830 (C')
B	775	2.2 ('C), 180 (D), 590 (E), 1.9 (F')
C	550	2.2 ('F), 1.8 (G), 543 (H), 1.8 (I), 2.2 (J')
D	92	88 ('J), 4 (K')
E	145	141 ('K), 4 (L')
F	44	42 ('L), 2.3 (A')
G	740	740 ('A')

[a] *Ceu*I fragments were excised, redigested, and end labelled as described in footnote *b* of Table 3.

quences outside these genes have had a predictable effect on the comparative genomic cleavage maps; cleavage sites in the genes for rRNA and tRNA are usually conserved, while sites in other regions are usually not conserved. The *Ceu*I sites in the genomic cleavage maps of *S. typhimurium* LT2 and *E. coli* K-12 appear to be completely conserved. This is as expected, because the cleavage site of the intron-encoded *Ceu*I enzyme is a 26-bp site in the chloroplast DNA encoding the large-subunit rRNA of *C. eugamatos*, and this 26-bp site is completely conserved in other chloroplast and mitochondrial DNAs and also in the *rrlB* gene of *E. coli* K-12 (12), as well as in many other ribosomal sequences from prokaryotes as determined by a BLAST search of GenBank sequences. This high degree of conservation of sequence is common in ribosomal sequences (56). Since the site was conserved over such a long time frame, it is not surprising that it is conserved in all seven *rrl* genes of *S. typhimurium* (26; this report), *E. coli* (26), and several other *Salmonella* serovars (26). Since the conservation requires 26 bp, it is also not surprising that it is not found at any site other than the *rrl* genes.

The *Bln*I and *Xba*I enzymes show a high level of conservation for sites in tRNA genes, but not, surprisingly, in the gene for 16S RNA (*rrs*); they show very little conservation in other regions of the chromosome. There are 12 *Bln*I sites in *S. typhimurium* LT2 but 17 in *E. coli* K-12 MG1655 (Table 6). All four *Bln*I sites in *glt* tRNA genes are conserved in both genera. The *rrnB* and *rrnD* genes were postulated to be exchanged between the genera (21). We confirm this because we find a *Bln*I site and an *Xba*I site in *rrnD* but not in *rrnB*; these sites are characteristic of the *glt* tRNA gene, which is in *rrnB* in *E. coli*. Thus, sB7 at 73.8 CS in *S. typhimurium*, in the *rrnD* gene, is designated *gltT*, like the gene at sB14 in the *rrnB* gene of *E. coli*. (There may be nomenclatural inconsistency in maintaining the *rrn* designations according to locus [position] on the chromosome while at the same time moving the *gltT* designation from *rrnB* to *rrnD*, but it seems the best solution until sequencing reveals whether the *rrn* genes should be renamed as well.) The *Bln*I sites in the *rrs* genes show little conservation; in *S. typhimurium* they are found in *rrsD*, *-C*, *-A*, and *-B*, while in *E. coli* K-12 they are in *rrsH*, *-G*, *-C*, *-A*, *-B*, and *-E*. However, the conclusion that the sequences underlying these *Bln*I sites are not conserved is not certain; Perkins et al. (36) observed that there are four *Bln*I sites, one of which is in *rrsD*, which have been detected in nucleotide sequencing and recorded in the sequence compiled by Kenn Rudd at the National Library of Medicine

TABLE 6. Comparison of *Bln*I sites in *S. typhimurium* LT2 and *E. coli* K-12 MG1655

S. typhimurium LT2[a]			*E. coli* K-12[b]			
*Bln*I site	Location on physical map (CS)	Gene at site	*Bln*I site	Linkage map position (min)	Location on physical map (kb)[c]	Gene at site
			sB1	3.7	169	*fhuA*
			sB2	5.1	232	*rrsH*
			sB3	6.5	300	—[d]
sB2	23.9	—[e]	sB4	25.5	1207	—[e]
			sB5	30.7	1439	—
			sB6	34.6	1643	—
sB3	40.3	—				
sB4	57.5	*gltW*	sB7	56.2	2738	*gltW*
			sB8	56.2	2740	*rrsG*
sB5	61.3	—				
sB6	73.7	—				
sB7	73.8	*gltT*				
sB8	73.8	*rrsD*				
			sB9	81.3	3819	*rfa*
sB9	85.1	*rrsC*	sB10	84.7	3968	*rrsC*
sB10	85.1	*gltU*	sB11	84.7	3969	*gltU*
sB11	87.0	*rrsA*	sB12	87.3	4063	*rrsA*
sB12	90.0	*rrsB*	sB13	89.7	4194	*rrsB*
			sB14	89.7	4196	*gltT*
			sB15	90.5	4237	*rrsE*
sB1	91.0	*gltV*	sB16	90.5	4238	*gltV*
			sB17	98.1	4587	—

[a] The data for *S. typhimurium* are from Table 4.
[b] The data for *E. coli* are from Table 6 of reference 36.
[c] The physical map location is derived from EcoSeq6.0.
[d] —, gene at the *Bln*I site is not known.
[e] In some cases the sites of the two genera are aligned because the sites are at similar locations, but there is no proof that these are actually at homologous sites.

TABLE 7. Comparison of *Xba*I sites in *S. typhimurium* LT2 and *E. coli* K-12 MG1655

S. typhimurium LT2[a]			*E. coli* K-12[b]			
*Xba*I site	Location on physical map (CS)	Gene at site	*Xba*I site	Linkage map position (min)	Location on physical map (kb)[c]	Gene at site
			sX1	0.4	26	*lspA*
			sX2	2.9	132	—[d]
			sX3	5.2	243	—
			sX4	6.1	278	IS*30*
sX1	13.0	—[e]	sX5	12.9	600	—[e]
			sX6	18.1	859	—
			sX7	19.0	930	*clpA*
			sX8	25.7	1190	—
sX2	29.7	—	sX9	25.7	1203	*lit*
sX3	30.7	—				—
			sX10	31.0	1478	IS*30*
			sX11	33.4	1508	—
sX4	35.8	—	sX12	36.4	1751	—
			sX13	36.8	1765	*lpp*
			sX14	41.5	1983	*tar*
			sX15	42.6	2052	—
			sX16	44.1	2118	—
			sX17	45.1	2164	—
			sX18	45.2	2191	—
sX5	45.3	rfb				
			sX19	46.7	2231	—
sX6	50.0	—				
sX7	52.1	—	sX20	52.5	2566	—
sX8	56.8	—	sX21	55.7	2724	—
sX9	57.5	*gltW*	sX22	56.2	2738	*gltW*
sX10	ND[f]	—	sX23	56.7	2769	—
sX11	NK	—	sX24	57.1	2805	—
sX12	NK	—				
sX13	59.7	—	sX25	61.5	3010	—
			sX26	72.1	3451	*fms*
sX14	73.8	*gltT*				
sX15	78.6	—	sX27	76.0	3603	—
			sX28	79.4	3723	—
sX16	80.1	*rfaZ*				
			sX29	81.7	3827	*rfaG*
sX17	81.6	—				
sX18	NK	—				
sX19	NK	—				
sX20	NK	—				
sX21	85.0	—				
sX22	85.1	*gltU*	sX30	84.7	3969	*gltU*
			sX31	89.7	4196	*gltT*
sX23	91.0	*gltV*	sX32	90.5	4238	*gltV*
			sX33	97.0	4536	IS30
sX24	98.6	—	sX34	98.4	4602	—
			sX35	98.5	4611	*hsdS*

[a] The data for *S. typhimurium* are from Table 1.
[b] The data for *E. coli* are from Table 3 of reference 36.
[c] The physical map location is derived from EcoSeq6.0.
[d] —, gene at the *Xba*I site is not known.
[e] In some cases the sites of the two genera are aligned because they have similar locations, but there is no proof that they are homologous sites.
[f] NK, not known; i.e., the order of *Xba*I fragments in this region is unknown.

(EcoSeq6) but which are not cleaved in any of six different K-12 strains they examined. This strongly suggests that *Bln*I sites are sometimes not cleaved, perhaps because of nucleotide modifications. Of the four *Bln*I sites in *S. typhimurium* and seven sites in *E. coli* outside the *rrn* genes, most are not conserved as judged by location, although sB2 in *S. typhimurium* and sB4 in *E. coli* might be in the same (unknown) gene.

There are 24 *Xba*I sites in *S. typhimurium* but 35 in *E. coli* K-12 (Table 7). The four sites in the *glt*-tRNA genes are all conserved in both species, although the *gltT* gene is rearranged (see the above discussion on *Bln*I sites). Most of the other sites in the two genera are not homologous, judging by locations of sites. There are a few cases in which sites are at similar locations, but even in these cases (sX1 in *S. typhimurium* and sX5 in *E. coli*) there is no proof that they are in homologous genes. Even the sites in the *rfa* genes are not homologous; sX16 at 80.0 CS in *S. typhimurium* is in *rfaZ* (29), while sX29 in *E. coli* at 81.7 min is in *rfaG* (36).

DNA which includes the sequence CTAG occurs rarely in enteric bacteria (31); both *Xba*I (with the restriction site TCTAGA) and *Bln*I (CCTAGG) have this rare sequence, and

their cleavage sites occur much less frequently than expected for enzymes with 6-bp specificity and than observed for other enzymes of this type (18, 40). It is postulated that these sequences were removed by the action of the *vsr* gene product, which repairs away the sequence CTAG when it is involved in a DNA mismatch (15). Bhagwat and McClelland (4) report that the targets of the Vsr enzyme, including CTAG, are underrepresented, while the tetramers resulting from the Vsr repair are overrepresented. Why then have the sequences not been removed in all cases, leaving no sites for *Xba*I and *Bln*I? We assume that the cleavage sites have been retained in the *rrn* genes because of the need for specific sequences to maintain functionality in tRNA and rRNA (34, 56). We can speculate that many of the other *Bln*I and *Xba*I sites, which are conserved very little between *E. coli* and *S. typhimurium*, have entered these genomes in recent evolutionary times by lateral transfer and have not yet had time for the CTAG sequence to be removed by the Vsr system. For *S. typhimurium* some indications support this. The only two *Xba*I sites identified by sequence are in the *rfb* gene (17) and in the *rfaZ* gene (29); both of these sites are part of a block of DNA of low GC content and were previously postulated to be the result of lateral transfer. In *E. coli* (Table 7) *Xba*I sites are identified in the following genes (36) (in addition to *rrs* genes): *lspA*, *clpA*, *lit*, *lpp*, *tar*, *fms*, *rfaG*, *hsdS*, and IS*30* (three copies). Of these, the IS*30* and *rfaG* genes and perhaps some others may be new arrivals by lateral transfer. Only *lit* (on prophage e14 and therefore presumably a product of lateral transfer) and *hsdS* have a low GC content; more data are required to discover if the hypothesis of lateral transfer as a source of *Xba*I sites is correct.

There is good agreement between the order and location of genes on the linkage map (outer circle of Fig. 2) based on genetic analysis (46) and the order of the genomic cleavage map, where the genes are placed on the *Xba*I map on the basis of the positions of Tn*10* insertions in genes (inner circle of Fig. 2). Most lines joining the two circles do not cross over restriction fragment boundaries, although there are differences in the lengths of intervals in the two maps. Some of the apparent conflicts between the two maps reflect the positions of insertions which are not into known genes but into regions between genes where the location is determined by joint transduction with a known gene; these are listed in Fig. 2 and Table 2 as *z*.. genes with an allele number. The following are some of the genes whose positions were known only approximately from linkage analysis and whose positions have been refined from the genomic cleavage map. *tppB* was placed at approximately 27 min on the linkage map on the basis of conjugation analysis with Hfr strains (13); our analysis shows it to be on fragment *Xba*I-C at 32.2 CS (Table 2). Conjugation analysis showed *oxrA* to be linked to *trp* in conjugation and to an unmapped Tn*10* insertion by transduction (53), and it was placed at 30.2 min on the linkage map, but on the genomic cleavage map it is at 36.7 CS (Table 2; Fig. 2). On the linkage map *argI* was placed at 97 min and *pyrB* was placed at 98 min on the basis of transduction analysis (46); according to the genomic map they are closer together and in reverse order, with *pyrB* at 97.0 CS and *argI* at 97.2 CS. The locations in the genomic map fit with data for *E. coli* (1) and some data for *S. typhimurium* (17a), so we think that the genomic map locations are correct.

We report here on a modified method of construction of genomic cleavage maps. This method involves separation of digested DNA by PFGE, excision of fragments from the gel in an agarose block, redigestion in the block by a second enzyme, end labelling, reelectrophoresis, and autoradiography. The data from the first digestion, followed by ethidium bromide staining, are in Fig. 1A. Representative data from double digestion and end labelling are illustrated in Fig. 3, and the data from this type of analysis are in Tables 3, 4, and 5. Large fragments can be recognized in this way, and fragments as small as 1.8 and 2.2 kb (Fig. 3A, lane 1) or <1 kb (Fig. 3B, lanes 5 and 7) are also detectable, even following the second digestion. This procedure allows us to determine which sites for the second enzyme are present on a specific fragment resulting from digestion with the first enzyme and to determine the physical distances of these sites from the ends of the fragment and thus from the digestion sites for the first enzyme. We believe that the method is technically simpler than double digestion in two dimensions (2, 39), and it is more economical for the second enzyme, large amounts of which are needed in the second dimension in two-dimensional electrophoresis, when a large block of agarose must be treated.

The structure of the *rrn* genes for rRNA in *S. typhimurium* is very similar to that in *E. coli* K-12. There is the same number of genes (seven), they have the same distribution around the origin of chromosome replication (*oriC*), and they have the same orientation of *rrs* and *rrl* genes and hence presumably the same orientation of direction of transcription. There are, however, some differences. The *gltT* gene, which is in *rrnB* at 90 min in *E. coli*, is at 73.8 CS in *S. typhimurium*, from the data of Lehner et al. (21) and the restriction sites we observed. The distances between *Bln*I, *Xba*I, and *Ceu*I restriction sites in *rrn* genes are close to but not exactly those predicted from the *rrnB* gene of *E. coli* (Fig. 4B), which shows the *Bln*I-*Bln*I distance to be 1.5 kb (we observe 1.8 kb; e.g., Fig. 3A, lane 1) and the *Bln*I-*Ceu*I distance to be 3.64 kb (we observe 4.1 kb; e.g., Fig. 3B, lane 1). Part of the basis for these differences may be the intervening sequences in the *rrl* gene for 23S RNA which result in cleavage of that RNA into two segments in the mature ribosome (7, 50), but these intervening sequences are less than 100 bp and not enough to explain the observed differences.

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