The Chromosome of *Salmonella paratyphi* A Is Inverted by Recombination between *rrnH* and *rrnG*

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Salmonella paratyphi A, a human-adapted bacterial pathogen, causes paratyphoid enteric fever. We established the genome map of strain ATCC 9150 by the use of four endonucleases, *Xba*I, *I-Ceu*I, *Avr*II (= *Bln*I), and *Spe*I, which generated 27, 7, 19, and 38 fragments, respectively; the sum of the fragments in each case indicates a genome size of ca. 4,600 kb. With phage P22, we transduced Tn10 insertions in known genes from *Salmonella typhimurium* LT2 to *S. paratyphi* A ATCC 9150 and located these insertions on the *S. paratyphi* A chromosome through the *Xba*I and *Avr*II sites in Tn10 and through the increased size of the *Spe*I fragment bearing a Tn10. Compared with the maps of other *Salmonella* species, the *S. paratyphi* A genomic map showed two major differences: (i) an insertion of about 100 kb of DNA between *rrnH/G* and *proB* and (ii) an inversion of half the genome between *rrnH* and *rrnG*, postulated to be due to homologous recombination between the *rrn* genes. We propose that during the evolution of *S. paratyphi* A, the first rearrangement event was the 100-kb insertion, which disrupted the chromosomal balance between *oriC* and the termination of replication, forcing the *rrnH/G* inversion to restore the balance. The insertion and the inversion are both present in all 10 independent wild-type *S. paratyphi* A strains tested.

There are over 2,300 serovars of *Salmonella*, which are distinguished on the basis of their somatic and flagellar antigens in the Kauffmann-White scheme of classification. Only a few serovars are exclusively or primarily restricted to growth in humans. One of these is *Salmonella paratyphi* A, which causes paratyphoid enteric fever, a disease also caused by *Salmonella paratyphi* B and C and *Salmonella sendai*. *Salmonella typhi* causes typhoid fever, the most-studied enteric fever, which is a severe, prolonged disease with a high rate of complications. The clinical features of paratyphoid fever resemble typhoid fever, but it is a milder illness (12). In this report we construct and analyze a genomic cleavage map of the chromosome of *S. paratyphi* A.

S. paratyphi A has the same flagellar antigens as *S. sendai* (though the phase 2 antigens are frequently not expressed in *S. paratyphi* A), but it is a member of somatic O group A while *S. sendai* is a member of O group D1. Analysis by multilocus enzyme electrophoresis shows that most electrophoretic types of *S. paratyphi* A are closely related to *S. sendai* and not closely related to other species which cause enteric fevers (34).

The division of the genus *Salmonella* into many serovars is based on the somatic and flagellar antigens; these serovars have been called species (13), e.g., *Salmonella typhimurium*. Molecular genetic evidence indicates close relationships of all these serovars (6), and some investigators now use a single species designation (15); in this report we retain the use of different species names.

The general structure of the chromosome and the order of genes on the chromosome of enteric bacteria are usually strongly conserved. The genetic maps of *S. typhimurium* LT2 (17, 20), *Escherichia coli* K-12 (30), *Salmonella enteritidis* (19), and *S. paratyphi* B (16) are very similar; they sometimes differ by an inversion in the termination of replication (TER) region,

by insertions of nonhomologous DNA (loops), and by the presence or absence of a plasmid. In addition, about 20 independent wild-type strains of *S. typhimurium* have very similar chromosomes (21). Although the chromosomes of these species have been stable during the evolution of these species, their chromosomes frequently rearrange in culture: spontaneous duplications produced by recombination between *rrn* operons occur at frequencies of 10^{-4} to 10^{-3} (1, 7), deletions occur frequently (10), and inversions with endpoints in the seven copies of the *rrn* (rRNA) operons are common in *S. typhimurium* (1) and *E. coli* (11) while inversions at other sites vary from low frequency to undetectable (24). The occurrence of rearrangements in culture, yet the observed stability of the chromosome during evolution of species, indicates that cells with rearrangements are strongly selected against during evolution and do not survive to form species. In contrast, we recently reported that *S. typhi* does not display this conservative chromosome structure, because inversions and transpositions of the chromosome segments between the *rrn* genes are common; none of the 20 wild-type strains examined had the same order of these segments as that found in *S. typhimurium* and *E. coli* (22, 23). The order of genes on the individual chromosome segments between the *rrn* genes is very similar in *S. typhi* and *S. typhimurium*, but these segments are postulated to be rearranged because of homologous recombinations between the *rrn* genes.

We have constructed a genomic cleavage map of the chromosome of *S. paratyphi* A for the endonucleases *Xba*I, *Avr*II (= *Bln*I), *I-Ceu*I, and *Spe*I. This map reveals that the chromosome of this strain has an inversion of about 50% of the chromosome compared with that of *S. typhimurium*, postulated to be due to a homologous recombination between the *rrnH* and *rrnG* genes. All 10 *S. paratyphi* A strains which were studied have the same inversion.

MATERIALS AND METHODS

Bacterial strains, phage, and cultivation conditions. The wild-type strain of *S. paratyphi* A used for detailed genomic analysis was strain ATCC 9150, ob-

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tained from Robert Selander as RKS4993, also designated strain SARB42 of the set salmonella reference B (3); this strain belongs to the electrophoretic type Pa1. Other strains of *S. paratyphi* A studied were RKS2907 (electrophoretic type Pa3), from Robert Selander, and the following strains from the Alberta Provincial Laboratory, obtained through Chandar Anand and stocked in the *Salmonella* Genetic Stock Centre: SA4249, SA4249, SA4861, R737, R120, R76, R291, R2213, and R2083. Strains of *S. typhimurium* LT2 with Tn10 insertions were part of a kit maintained at the *Salmonella* Genetic Stock Centre (31). Tn10 was transduced from *S. typhimurium* LT2 to *S. paratyphi* A ATCC 9150 by phage P22HT105/int followed by selection for tetracycline resistance. When the Tn10 insertion in *S. typhimurium* produced a detectable phenotype, the transductant in *S. paratyphi* A showed the same phenotype, indicating that insertion was due to homologous recombination.

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 contained 1.5% agar. The minimal medium used was a modified Davis medium, which has been described previously (32). Tetracycline was used at 20 µg/ml. Strains were maintained in 15% glycerol at -70°C, and a single colony was isolated prior to use.

Enzymes and chemicals. Endonucleases were obtained from New England Biolabs (*AvrII* [= *BlnI*], *I-CeuI*, and *SpeI*) and Boehringer Mannheim (*XbaI*). Agarose was obtained from Bethesda Research Laboratories, and most other chemicals were from Sigma Chemical Co.

PFGE methods. The preparation of high-molecular-weight genomic DNA, endonuclease cleaving of DNA in agarose blocks, separation of the DNA fragments by pulsed-field gel electrophoresis (PFGE), and double digestion techniques used were performed as reported previously (17, 20, 21); analysis with *I-CeuI* was done as described previously (21). The analysis by PFGE was done with the Bio-Rad DR11, the Bio-Rad contour-clamped homogeneous electric field mapper, or the Hoefer Hula electrophoresis system.

RESULTS

Genomic cleavage map of *S. paratyphi* A ATCC 9150 for *XbaI*, *I-CeuI*, *AvrII*, and *SpeI*. Digestion of the DNA of *S. paratyphi* A ATCC 9150 by the endonucleases *XbaI*, *I-CeuI*, *AvrII*, and *SpeI* yields 27, 7, 19, and 38 fragments, respectively (Table 1); all but the smallest fragments can be seen in Fig. 1A. These fragments were located on a circular chromosome, and the positions of the genes were located on that chromosome by the following methods. Firstly, Tn10 elements in specific genes in *S. typhimurium* were transduced by phage P22 into *S. paratyphi* A ATCC 9150 followed by selection for tetracycline resistance. Tn10 contains sites for *XbaI* and *AvrII*; thus, digestion with these enzymes results in cleavage of the fragment containing the Tn10 into two fragments. For example, digestion of DNA of a strain with *pyrC::Tn10* by *XbaI* results in two fragments, *XbaI*-I' (513 kb) and *XbaI*-I' (182 kb) (Fig. 1B, lane 1); digestion by *AvrII* results in *AvrII*-F' (24 kb) and *AvrII*-F' (38 kb) (Fig. 1B, lane 2). *XbaI* and *AvrII* digestion of the wild-type strain, lacking Tn10, is shown in Fig. 1A, lanes 1 and 2. Digestion of the same DNA by *SpeI* resulted in fragment *SpeI*-T (Fig. 1B, lane 3) being slightly larger (about 9 kb larger, the size of Tn10) than the same fragment in the parent strain (Fig. 1A, lane 4).

These data show that the *pyrC* gene is 182 kb from one end of *XbaI*-I and 24 kb from one end of *AvrII*-F and is located in *SpeI*-T; these three fragments are thus shown to share a common region, and the gene *pyrC* is thus located at about 1,700 kb on the genome map (Fig. 2). Therefore, the analysis of strains with Tn10 insertions gives the same data as probing with cloned genes but also locates the specific position of the gene within the fragment. A total of 74 strains carrying transduced Tn10s in specific genes and 4 strains with Tn10s in known regions of the chromosome but not in known genes (designated as z with an allele number) were analyzed in the same way (data not shown), and the resulting genes were placed on the genome map (Fig. 2 to 4).

Secondly, the order of fragments around the chromosome and the locations of cleavage sites of the different enzymes with respect to each other were determined by double digestion techniques, following the methods previously reported for *S.*

TABLE 1. Cleavage fragments of the *S. paratyphi* A genomic DNA by *XbaI*, *AvrII*, *I-CeuI*, and *SpeI*

Fragment	<i>XbaI</i>		<i>I-CeuI</i>		<i>AvrII</i>		<i>SpeI</i>	
	Size ^a	Site ^b	Size	Site	Size	Site	Size	Site
A	655	549	2,342	2,642	585	440	189	185
B	5	554	750	3,392	735	1,175	(78)	
C	131	685	508	3,900	255	1,430	(7.1)	270
D	189	874	92	3,992	271	1,701	70	340
E	106	980	146	4,138	30	1,731	133	473
F	347	1,327	44	4,182	60	1,791	153	626
G	235	1,562	713	300	718	2,509	269	895
H	32	1,594			13	2,522	22	917
I	686	2,280			14	2,536	112	1,029
J	238	2,518			64	2,600	44	1,073
K	17	2,535			44	2,644	165	1,238
L	60	2,595			124	2,768	54	1,292
M	49	2,644			154	2,922	7	1,299
N	82	2,726			24	2,946	92	1,391
O	136	2,862			338	3,284	32	1,423
P	22	2,884			110	3,394	158	1,581
Q	230	3,114			504	3,898	76	1,657
R	280	3,394			282	4,180	(21)	
S	6.4	3,400			270	4,450	(1)	1,679
T	20	3,420					495	2,174
U	275	3,695					107	2,281
V	78	3,773					170	2,451
W	20	3,793					58	2,509
X	105	3,898					149	2,658
Y	282	4,180					10	2,668
Z	306	4,486					85	2,753
AA	3	4,489					(11)	
BB							(3.5)	2,768
CC							18	2,786
DD							151	2,937
EE							328	3,265
FF							90	3,355
GG							242	3,597
HH							324	3,921
II							250	4,171
JJ							303	4,474
KK							19	4,493
LL							98	4,591

^a The sizes are given in kilobases.

^b The map location, in kilobases, starts arbitrarily from *thr* (0 kb) clockwise. When the map order of two fragments was not determined, the fragment location is given in parentheses.

typhimurium (17), *S. enteritidis* (19), and *S. typhi* (22, 23). Briefly, DNA fragments obtained by PFGE separation following digestion of DNA with the first enzyme were excised in an agarose block, and this block was redigested with the second enzyme; the fragments were end labelled after the second digestion, electrophoresed, and autoradiographed. For example, digestion of the fragment *AvrII*-A by *XbaI* produced three fragments, inferred to be *XbaI*-I'Z (36 kb, the counterclockwise [CCW] end of *AvrII*-A), *XbaI*-AA (3 kb, a fragment internal to *AvrII*-A), and *XbaI*-A' (550 kb clockwise from the end of *AvrII*-A). These data indicate that the *AvrII*-A fragment has two *XbaI* sites which are 36 and 39 kb counterclockwise from the end. All *XbaI*, *AvrII*, *SpeI*, and *I-CeuI* fragments larger than 40 kb were digested with the three other enzymes (data not shown). By using these two methods, the map of the chromosome was constructed (Fig. 2A). The fragments from the digestion with all four enzymes were named in alphabetical order, starting at the top of the chromosome (*thr*, at 0 kb).

I-CeuI, an intron-encoded endonuclease, digests a site in each of the seven *rm* operons (25) (at bp 3909 in the *rm* gene

FIG. 1. (A) PFGE patterns of genomic DNA of *S. paratyphi* A ATCC 9150 cleaved with endonucleases. Lanes: 1, *Xba*I; 2, *I-Ceu*I; 3, *Avr*II; 4, *Spe*I. The designations and sizes (in kilobases) of the bands are given on the left for *Xba*I and *I-Ceu*I and on the right for *Avr*II and *Spe*I. (B) PFGE patterns of *S. paratyphi* A ATCC 9150 with a *Tn10* insertion in the *pyrC* gene, showing the way the cleavage fragments (Frag) are identified and genes located. Lanes: 1, *Xba*I; 2, *Avr*II; 3, *Spe*I. The fragments with the *Tn10* insertion are indicated on the left.

for 23S rRNA [Fig. 2B]), producing seven fragments (18) (Fig. 1A). These seven fragments are inferred to be homologous to the seven fragments with similar sizes in *S. typhimurium* and in other species (18, 21) and are located in the same order around the chromosome, starting at A. *rrnD*, *rrnC*, *rrnA*, *rrnB*, and *rrnE* are given the same names as the *rrn* genes at the equivalent positions in *S. typhimurium*. However, the orientation of the *I-Ceu*I-A fragment is reversed compared with its orientation in *S. typhimurium* and most other enteric bacteria; the order of genes within this fragment is identical in *S. paratyphi* A and *S. typhimurium* (Fig. 2 to 4), but *proB* is located near kb 2400, close to *pheA*, while *purG* at kb 400 is close to *pan*. Several types of data indicate that *I-Ceu*I-A is inverted, but the strongest evidence is that genes such as *serB*, *thr*, *leu*, and *pan* are located on the same fragments (*Xba*I-A and *Avr*II-A) as genes such as *purG* and *glyA* which are expected to be at the opposite side of the chromosome, but they are not in the same fragment as *proB*, which is close to *pan* in *S. typhimurium*.

The *rrn* operons are shown outside the main circle (Fig. 2A). The positions of cleavage sites for *Xba*I, *Avr*II, and *I-Ceu*I were determined by double digestion of fragments bearing the *rrn* operons. Thus, digestion of *I-Ceu*I-F (which has *rrnE* on its clockwise end) by *Xba*I yields a large fragment (about 42 kb) and a small fragment (about 2.3 kb), while digestion by *Avr*II yields similarly sized fragments though the small fragment is a bit smaller (2.2 kb). These data indicate that *rrnE* has *Xba*I and *Avr*II sites which are about 2.2 kb away from the *I-Ceu*I site. These sites were previously shown, by nucleotide sequence data, to be 2.2 kb apart in *rrnB* of *E. coli* (5), which has the glutamyl-tRNA gene; *rrn* operons with the alanyl tRNA gene

do not have *Xba*I and *Avr*II sites. Thus the genes identified by map position as *rrnD*, *rrnC*, and *rrnE* as well as *rrnG/H* are of the glutamyl-tRNA type.

The positions of genes in *S. typhimurium* and *S. paratyphi* A were compared in a linear map which lists the positions (in kilobases) from *thr* (Fig. 4). To facilitate comparisons, the *I-Ceu*I-A fragment of *S. paratyphi* A is shown inverted compared with the actual chromosome. Gene order and map positions are strongly conserved, though there are some differences in the lengths of map intervals. The *rrn-proB* interval is 175 kb in *S. paratyphi* A but much shorter in *S. typhimurium* (only 75 kb) and in *S. enteritidis* (19) and other *Salmonella* species (65 kb); this spacing indicates the presence of an insertion of 100 kb between *rrn* and *proB* in *S. paratyphi* A.

Analysis of a group of wild-type strains. Inversions due to homologous recombination between the *rrn* operons have been detected as sporadic mutation events in *E. coli* K-12; for example, strain W3110 has an inversion resulting from an exchange between *rrnD* and *rrnE* (9). *S. paratyphi* A ATCC 9150 might be a sporadic event of this type, due to an exchange between *rrnH* and *rrnG*, and the majority of *S. paratyphi* A strains might have the gene order which is common in the enteric bacteria. We therefore examined nine other wild-type strains of *S. paratyphi* A to see whether the *I-Ceu*I-A fragment is normally inverted. The *Xba*I-A fragment is 655 kb, with 406 kb counterclockwise from the *rrn* operon (called A') and with 249 kb clockwise from the operon (called 'A). If the *I-Ceu*I-A fragment were in the orientation that is common for enteric bacteria and the *Xba*I restriction sites were unaltered in different strains, the *Xba*I-A' and *Xba*I-'A fragments would be

FIG. 2. (A) Genomic cleavage map of chromosome of *S. paratyphi* A ATCC 9150 for *Xba*I, *I-Ceu*I, *Bln*I, and *Spe*I. The size of the chromosome is 4595 kb, determined by summing the sizes of individual fragments. The *thr* (threonine) gene is placed at 0 kb, as in *S. typhimurium*. The fragments are named in alphabetical order, beginning with A at 0 kb or just clockwise to it. The enzyme *I-Ceu*I digests the seven *rm* operons in the *rrl* genes for 23S rRNA (25). The structure of the *rm* operons is shown in detail outside the circle; the positions of the cleavage sites in the *rm* genes are from double digestion data. The seven *I-Ceu*I fragments are in the same order as the homologous seven fragments in *S. typhimurium*, but the orientation of the *I-Ceu*I-A fragment is reversed; therefore, the *rm* genes are designated *rmH*/G and *rmG*/H to indicate that they are inferred to be hybrid genes resulting from homologous recombination between *rm* genes. The four *rm* genes which have *Xba*I and *Bln*I sites are inferred to have the tRNA gene for glutamate-tRNA, and the other three are inferred to have the gene for alanyl-tRNA, by analogy with *E. coli* (27). The arrow indicates the direction of transcription originally determined for *E. coli* in *rmB* (27). The relative locations of endonuclease cleavage sites (Table 1) were determined by double digestion data (see the text). The genes are located through mapping of the *Xba*I and *Bln*I sites in *Tn10* insertions in the genes (Fig. 1B). (B) Structure of *rmB* operon of *E. coli* (27); endonuclease cleavage sites were determined from the nucleotide sequence, and the order of the genes is *rrs* (16S rRNA)-*t* (tRNA)-*rrl* (23S rRNA)-*f* (5S rRNA). The nucleotide numbers of the cleavage sites and of the ends of genes are shown.

linked to *Xba*I-M and *Xba*I-N, respectively. Since M and N are quite small, both of these combinations would be much shorter than *Xba*I-A. The PFGE patterns obtained following *Xba*I digestion of the DNA of 10 independently isolated strains of *S.*

paratyphi A, electrophoresed for 24 h (Fig. 5A) or 48 h (Fig. 5B), show that all these strains have an *Xba*I-A fragment which is indistinguishable from that of strain ATCC 9150 (lane 3), indicating that the gene order in that strain is typical of the

FIG. 3. Comparison of chromosomes of *S. typhimurium* and *S. paratyphi* A, showing the I-CeuI fragments. The open arrowhead indicates the location of an insertion of ca. 100 kb (see open arrowhead at the same location in Fig. 4). The shaded fragment, I-CeuI-A of *S. paratyphi* A, is inverted compared with that of *S. typhimurium*.

species and that the inversion is present in all the strains that we have tested. The same set of strains was digested with *AvrII*; the finding that all but one of these strains retain the *AvrII*-A fragment found in ATCC 9150 (Fig. 6A) also supports the idea that the *rrnH/G* inversion is present in all *S. paratyphi* A strains.

The fact that the *XbaI*-A and *AvrII*-A fragments are, in almost all cases, indistinguishable in *S. paratyphi* A strains indicates that the 100-kb insertion inferred between the *rrn* operon and *proB* is present in all these strains, not just in ATCC 9150.

Analysis by I-CeuI digestion. The 19-bp I-CeuI digestion site is conserved in the seven rRNA genes (25) but has not yet been observed at any other site in the chromosomes of enteric bacteria (16–19, 21–23). In most cases, the identities of segments of the chromosome can be determined from the sizes of the I-CeuI fragments alone (18). Thus, analysis using this enzyme is a powerful tool for detecting insertions or deletions in specific segments of the chromosome. I-CeuI digestion of the 10 wild-type strains reveals that the sizes of I-CeuI-F, -D, and -C fragments in all strains are indistinguishable (Fig. 6B), indicating near identity of DNA segment length. However, the I-CeuI-E fragment (146 kb) is missing from two strains (R76 in lane 7 and R2213 in lane 9), and a larger fragment of 185 kb is present; we propose that the larger fragment, E', is the E fragment containing a 39-kb insertion of unknown DNA. There are some apparent changes in the I-CeuI-B and -G fragments as well; separation of the A fragment is not adequate for analysis.

Partial digestion with I-CeuI enables some of the fragments to be put into order on the chromosome. The presence of the DE, EF, and DEF bands, but no DF band (Fig. 6B), shows that the order I-CeuI-DEF, already proven by other methods for ATCC 9150 (Fig. 2 and 3), is present in all 10 strains. The CD band indicates that the order is I-CeuI-CDEF. The order of other fragments cannot be determined from these data.

DISCUSSION

Inversions and transpositions due to homologous recombination between the *rrn* genes occur frequently in cultured cells, at a rate of about 10^{-3} to 10^{-4} (1, 7), yet the order of the seven segments between *rrn* genes has remained unaltered in *E. coli* K-12, *S. typhimurium* LT2, *S. enteritidis*, and *S. paratyphi* B, indicating that all of the rearranged combinations have been

selected against during the process by which these species have evolved; *Escherichia* and *Salmonella* spp. are proposed to have diverged over 100 million years ago (29). Three possible explanations for conservation of gene order are that (i) inversions which displace *oriC* may change gene expression because of changes in the dosage of genes, outside the inversion segment, relative to *oriC* (33); (ii) these inversions will move the *oriC* relative to the TER site, affecting DNA replication (1, 24); and (iii) genes transcribed at high rates are usually replicated in the same direction as transcription (4). Conservation of gene order is also seen in higher organisms. Cytological analysis of G-banding patterns of over 80 primate species shows extensive conservation of chromosome banding patterns and gene locations among groups, some of which diverged over 100 million years ago (28). The same conservation is also seen when comparing humans, cattle, and mice (28). Further work is needed to determine if the basis for this evolutionary conservation is the same in bacteria and higher organisms.

The genome map of *S. paratyphi* A (Fig. 2) resembles that of *S. typhimurium* (17), *S. enteritidis* (19), *S. paratyphi* B (16), *S. typhi* (22, 23), and *E. coli* K-12 (2) in several respects. The order of genes on chromosome segments is usually identical; chromosome sizes are between 4,600 and 4,800 kb; all have seven *rrn* operons, four of which have *XbaI* and *AvrII* sites, indicating the presence of the gene for glutamyl-tRNA.

S. paratyphi A differs from the other enteric species in two aspects. Firstly, there are significant differences in some of the intervals between genes. Specific pairs of genes in *S. paratyphi* A and *S. typhimurium* and the lengths of the intervals between them with the differences in brackets are as follows (Fig. 4): *rrnH-proB*, 175 and 75 [100]; *proB-purE*, 172 and 232 [60]; *rrnG-pheA*, 10 and 43 [33]. The longer segments between gene pairs constitute loops of DNA, postulated to be longer because of an insertion of novel DNA (14). These unique blocks of DNA, called pathogenicity islands when they contain genes required for virulence, have been found in *S. typhimurium* (26), in *E. coli* uropathogenic strains (14), and in *S. typhi* (22, 23) and are frequently observed in *Salmonella* species (17, 19, 22).

Secondly, the order of the seven I-CeuI fragments is rearranged in *S. paratyphi* A compared with that in *S. typhimurium*. These seven fragments represent the DNA segments between the seven *rrn* genes, and rearrangements of this type may result from homologous recombination between the *rrn* genes. The order of genes on the chromosome of *S. paratyphi* A ATCC

FIG. 4. Comparison of order of genes in genomes of *S. typhimurium* (S. tm) and *S. paratyphi* A (S. pA). *rrn* loci are shown in bold. The open arrowhead indicates the 100-kb insertion. The location of each gene (in kilobases) from *thr* is shown to the left of the gene for *S. typhimurium* and to the right for *S. paratyphi* A. For convenience, the inverted part in *S. paratyphi* A relative to that of *S. typhimurium* has been put in the same orientation as the homologous part of the *S. typhimurium* genome; genes in the inverted segment are joined by dashed lines.

FIG. 5. (A) PFGE following *Xba*I digestion of DNA from 10 wild-type strains of *S. paratyphi* A. Lanes: 1, λ ladder; 2, RKS2907; 3, ATCC 9150; 4, SA4861; 5, R737; 6, R120; 7, R76; 8, R291; 9, R2213; 10, R2083; 11, SA4249. This gel was electrophoresed for 24 h. (B) The gel in panel A was electrophoresed for an additional 24 h.

9150 indicates recombination between *rrnH* and *rrnG*, resulting in inversion of the I-*Ceu*I-A fragment. These genes are at 300 and 2,642 kb, respectively, thus 1,342 kb apart, dividing the ca. 4,600-kb chromosome into almost equal halves.

S. typhi was the first enteric bacterium in which the chromosomes of the wild-type cell are known to be rearranged due to homologous recombination between *rrn* genes. The order of I-*Ceu*I fragments (which indicates the order of segments between the *rrn* genes) is ABCDEFG in most enteric bacteria but AGCEFDB in *S. typhi* Ty2, and other combinations are found in other *S. typhi* strains (22, 23). We report here that *S. paratyphi* A ATCC 9150 also shows a rearrangement, an inverted I-*Ceu*IA fragment due to an *rrnH*/*G* exchange. Nine other wild-type strains of *S. paratyphi* A have the same inversion. In *E. coli* K-12, inversions due to *rrn* operon exchanges which displace *oriC* (site of initiation of DNA replication) from its site on the chromosome (180° from the TER) grow at a slightly reduced rate (where displacement is 6%) or greatly reduced rate (where displacement is 19%); revertants with faster growth are selected (8). However, inversions involving *rrnG* and *rrnH*, which do not significantly displace the positions of *oriC* and the TER, do not result in a measurable reduction in growth rate (11). The wild-type strains of *S. paratyphi* A that we are studying grow rapidly on agar plates and in broth, but we cannot test whether the inversion has affected the growth rate unless we find a reversion to the orientation usually found in *S. typhimurium* LT2 and *E. coli* K-12 in one of these strains.

We postulate that a large insertion of DNA (such as the 100-kb insertion between *rrnH* and *pro*) displaces *oriC* with respect to the TER site and that the rearrangement which results from the recombination between the *rrnH* and *rrnG* operons may be a compensation mechanism to maintain the genomic balance, keeping the *oriC* and the TER site separated by 180°.

FIG. 6. (A) PFGE following *AvrII* (A) and *I-CeuI* (B) digestion of DNA from 10 wild-type strains of *S. paratyphi* A. The lanes are described in the legend to Fig. 5A. Fragments and their sizes (in kilobases) are designated on the right. Two strains, R76 and R2213, have a larger fragment E (designated as E').

Some strains of *S. paratyphi* A have an added segment of about 39 kb of DNA in the *I-CeuI*-F fragment (Fig. 6B). The genes carried on this segment are not yet known.

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