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Characterization of Tn*2411* and Tn*2410*, Two Transposons Derived from R-Plasmid R1767 and Related to Tn*2603* and Tn*21*

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Two transposable elements, Tn*2410* and Tn*2411*, were isolated from *Salmonella typhimurium* R-factor R1767. They have sizes of 18.5 and 18.0 kilobases, respectively. Tn*2411* mediates resistance to streptomycin, sulfonamides, and mercury. In Tn*2410*, the streptomycin resistance gene was replaced by a gene coding for the production of the β-lactamase OXA-2, which is responsible for ampicillin resistance. Physical and functional maps of both transposons were compared with those of Tn*21*, Tn*4*, and Tn*2603*. From these data it appeared that Tn*21* could be an ancestral transposon from which Tn*2411*, Tn*2410*, Tn*2603*, and Tn*4* were evolved by the addition or deletion of small DNA segments.

Conjugally transferable antibiotic resistance plasmids have been found to be responsible for the rapid dissemination of antibiotic resistance (9). A great number of antibiotic resistance genes on such plasmids have been shown to be located on transposable elements, accounting for the transfer of these genes between replicative elements, such as plasmids, bacterial chromosomes, and bacteriophages (13).

Naturally occurring transposons can be divided into two groups. The elements of one group are flanked by functionally intact insertion sequences at both ends in direct or inverted repetition. The elements of the second group carry short inverted repeats (38 to 40 base pairs) at its ends and encode the transposition functions in the central region (13). The best-characterized element of the latter group is Tn*3*. The multiple-resistance transposons Tn*21* and Tn*2603* have recently been shown to belong to the second group (8, 31). Attempts to compare physical and functional maps of the multiple-resistance transposons Tn*2603*, Tn*21*, and Tn*4* showed that these structures are similar in most of the restriction sites and the location of the resistance genes (35). We isolated two multiple-resistance transposons coding for streptomycin, sulfonamide, and mercury resistance and for ampicillin, sulfonamide, and mercury resistance from plasmid R1767 and compared their physical and functional maps with those of Tn*21*, Tn*2603*, and Tn*4*. The data at hand suggest the formation of certain antibiotic resistance transposons by the integration or substitution of DNA segments carrying resistance genes into a common ancestral structure.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. The bacterial strains and plasmids used are listed in Table 1. Plasmid R1767 was originally isolated from a clinical strain of *Salmonella typhimurium* (29) and codes for resistance to chloramphenicol, tetracycline, ampicillin, sulfonamides, streptomycin, spectinomycin, mercury, and ColI production.

Media and drugs. L-broth and L-agar were used for the growth of the bacteria (20). L-broth and L-agar supplemented with 5 mM $CaCl_2$ were used in phage infection and transduction experiments. L-broth containing 10 mM $MgSO_4$ was used for phage propagation. Isosensitest broth and agar (Oxoid Ltd.) were used for the determination of sulfonamide resistance. Antibiotics were added at the following concentrations: sulfonamide, 300 μg/ml; kanamycin, 50 μg/ml; ampicillin, 100 μg/ml; tetracycline, 10 μg/ml; streptomycin, 10 μg/ml; spectinomycin, 40 μg/ml; chloramphenicol, 25 μg/ml; nalidixic acid, 100 μg/ml; rifampin, 50 μg/ml; $HgCl_2$, 20 μg/ml; and polymyxin B, 50 μg/ml.

Phage and genetic methods. The preparation of phage lysates by heat induction, phage titration, the test for phage production, plaque center tests, determination of phage immunity, and transduction were done as described by Iida and Arber (11). The curing of lysogenic cells was done as described by Rosner (25) with the addition of 5 mM citrate to prevent phage reinfection. Conjugation was carried out on membrane filters. For this procedure, a mixture of logarithmically growing cultures of donor and recipient cells was

TABLE 1. Bacterial strains, plasmids, and phages

Strain, plasmid, or phage	Relevant characteristics	Source or reference
Strains		
E. coli WA921 Nal^r	*lac thr leu met thi hsd*	Wood (38)
E. coli SK1592	*gal thi* T1^r *sbcB15 hsr-4 hsm*$^+$	Kushner (18)
E. coli JC2926 Rif^r	*recA thi thr arg his leu mal*	Lederberg
E. coli C600 Sm^r	*recA thi lac thr leu tonA*	Lebek
P. mirabilis PM13		Coetzee (6)
Plasmids		
R1767	Ap Su Tc Cm Sm Spec Hg ColI *tra*$^+$	Schmidt et al. (29)
R100	Cm Tc Sm Spec Su Hg *tra*$^+$	Rownd et al. (26)
pUB307	Km Tc *tra*$^+$	Bennett et al. (1)
pBR322	Ap Tc	Bolivar et al. (3)
pBP12	Ap Su	Kratz et al., in press
pBR322::Tn*2410*	Ap Tc Su Hg	This paper
pBR322::Tn*2411*	Ap Tc Sm Spec Su Hg	This paper
pBR322::Tn*21*	Ap Tc Sm Spec Su Hg	This paper
Phages		
P1Cm$_{tc}$	Heat inducible, Cm	Mise and Arbor (23)
P1Cm$_{ts}$::Tn*21*	Heat inducible, Cm Sm Spec Su Hg	This paper

filtered through membrane filters (pore size, 0.45 μm). The filters were placed on L-agar plates for 2 h at 37°C, and then the cells were washed from the filters with L-broth, diluted, and plated on selection plates. *Escherichia coli* SK1592 was transformed with plasmid DNA as described by Kushner (18).

Isolation of plasmid DNA. Minilysates were screened for plasmid content by the procedure of Klein et al. (14). Plasmid DNA from 500-ml cultures was prepared by the Triton X-100 cleared-lysate technique described by Kuperszock-Portnoy et al. (17) and centrifuged in ethidium bromide-CsCl gradients.

Electron microscopy. Heteroduplex molecules were prepared by the formamide technique of Davis et al. (7). For length calculations, double- and single-stranded DNAs of phage φX174 were used as internal standards (5,375 base pairs) (28). Contour lengths were measured with a Numonics digitizer.

Restriction enzyme analysis. The digestion of plasmid DNA and agarose gel electrophoresis in a horizontal gel apparatus (Bethesda Research Laboratories) were performed as described by Kratz et al. (J. Kratz, F. Schmidt, and B. Wiedemann, J. Gen. Microbiol., in press). pBR322::Tn*2411* and pBR322::Tn*21* plasmid DNA was completely digested with the restriction enzymes *Eco*RI, *Bam*HI, *Hin*dIII, *Hin*dII, *Sal*I, *Bgl*II, *Pst*I, and *Sma*I, and the resulting DNA digests were electrophoresed on agarose gels (0.7 to 1.2%). Fragments smaller than about 0.2 kilobases (kb) would not have been detected in the gels applied. The relative locations of restriction sites on both transposons were determined by a series of double-digestion experiments and by comparison of the cleavage patterns with that of pBR322 (30).

Isolation of plasmids containing Tn*2410* or Tn*2411*. *E. coli* JC2926 (*recA*) carrying R1767 and pUB307 (kanamycin, tetracycline) was mated with *Proteus mirabilis* PM13 as described above. (R1767 is not transmissible to *P. mirabilis*. pUB307 belongs to the incompatibility group P1 and thus transfers efficiently to *P. mirabilis*.) Transconjugants selected on sulfonamides were tested by a replica method for the resistance markers of R1767.

To isolate the pBR322::Tn*2410* and pBR322::Tn*2411* plasmids, we transformed *E. coli* SK1592 with plasmid DNA prepared from the *recA* strains JC2926, carrying pUB307::Tn*2410* and pBR322, and JC2926, carrying pUB307::Tn*2411* and pBR322. A high concentration of ampicillin (2 mg/ml) was used to select for the multicopy TEM-1 enzyme producing plasmid pBR322, and sulfonamide was used to select for Tn*2410* and Tn*2411*. The transformants were checked for sensitivity to kanamycin (indicating that the donor plasmid pUB307 was not present).

Isolation of pBR322::Tn*21* recombinants. Tn*21* was transposed from plasmid R100 with the aid of phage P1Cm$_{ts}$. *E. coli* JC2926 Rif^r(R100) was mated with *E. coli* C600 Sm^r *recA* cells that were lysogenic for phage P1Cm$_{ts}$. Phage lysates were prepared by heat induction (11) and used to infect *E. coli* WA921. Sm^r clones were selected on streptomycin-containing plates at 30°C and checked for sulfonamide resistance. To distinguish P1Cm$_{ts}$ Sm^r Su^r lysogenic cells from unwanted transductants mediated by generalized transduction, we checked Sm^r Su^r clones for tetracycline sensitivity and the production of bacteriophage (lysis of susceptible bacteria) after heat induction (11). Two strains were selected, and the phages were checked by plaque center testing (11) for chloramphenicol, streptomycin, and sulfonamide resistance. Two P1Cm$_{ts}$ Sm^r Su^r lysogenic strains were isolated and induced to prepare phage lysates, and 48 single plaques each were again tested by the plaque center test. Forty-two of forty-eight and forty of forty-eight plaques contained P1Cm$_{ts}$ Sm^r Su^r phages. *E. coli* JC2926 (*recA*) carrying pBR322 was infected with the P1Cm$_{ts}$ Sm^r Su^r phage at a multiplicity of 1 plaque-forming phage per cell, and lysogens were selected on ampicillin-streptomycin plates at 30°C and tested for ampicillin, tetracycline, chloramphenicol, streptomycin, and sulfonamide resistance and for bacteriophage production. Two strains were isolated and cured of bacteriophage by

the incubation of diluted overnight cultures on ampicillin-streptomycin plates at 42°C (25). After incubation for 24 h at 42°C, the survivors were further purified on ampicillin-streptomycin plates at 42°C and tested for chloramphenicol sensitivity, phage sensitivity, bacteriophage production, and resistance to ampicillin, tetracycline, streptomycin, and sulfonamide (11).

RESULTS

recA E. coli JC2926(R1767 and pUB307) was mated with *P. mirabilis* PM13. About 1,000 transconjugants were tested, and of these, 85% were Km^r Tc^r Ap^r Sm^r Su^r Hg^r Cm^s, 10% were Km^r Tc^r Ap^r Su^r Hg^r Cm^s, and 5% were Km^r Tc^r Sm^r Su^r Hg^r Ap^s Cm^s. Several clones of each resistance pattern were further tested by conjugation. The pUB307::Ap^r Su^r Hg^r clones and the pUB307::Sm^r Su^r Hg^r clones cotransferred all resistance markers that were used for selection. This is consistent with the transposition of genetic elements from R1767 to plasmid pUB307. The presumptive transposon carrying the resistance markers Sm^r, Su^r, Hg^r was designated Tn*2411*; the transposon carrying the resistance markers Ap^r, Su^r, Hg^r was Tn*2410* (Kratz et al., in press). The pUB307::Ap^r Sm^r Su^r Hg^r clones were found to be unstable. Transconjugants selected on ampicillin showed the resistance pattern Km^r Tc^r Ap^r Sm^r Su^r Hg^r or Ap^r Su^r Hg^r. Transconjugants selected on sulfonamides showed the resistance pattern Km^r Tc^r Ap^r Sm^r Su^r Hg^r or Km^r Tc^r Sm^r Su^r Hg^r. Further transposition experiments indicated that the resistance pattern Ap^r Sm^r Su^r Hg^r of these clones is due to plasmid pUB307 carrying Tn*2410* and Tn*2411*. Km^s transformants obtained by transforming *E. coli* SK1592 with plasmid DNA from *recA* strain JC2926(pUB307::ApSmSuHg, pBR322) carried the recombinant plasmids pBR322::Tn*2410* or pBR322::Tn*2411*.

Analysis of pBR322::Tn*2411* recombinants. DNA from recombinants of pBR322 and Tn*2411* showed, in agarose gel electrophoresis, only one plasmid band with a size of about 23 kb. Purified DNA from several clones was digested with *Hin*dII. All plasmids contained six fragments of 4.15, 3.46, 3.15, 3.0, 2.54, and 0.25 kb; the 3.26- or the 1.1-kb pBR322 *Hin*dII fragment; and two junction fragments of variable sizes carrying pBR322 and Tn*2411* sequences, indicating that Tn*2411* was inserted in different sites in plasmid pBR322. A comparison of the *Hin*dII fragments of Tn*2411* with the eight *Hin*dII fragments of Tn*2410* (Kratz et al., in press) showed that five of the six Tn*2411* fragments are common to both transposons (3.46, 3.15, 3.0, 2.54, and 0.25 kb) and that the 4.15-kb Tn*2411* fragment is missing in Tn*2410*. For the construction of a restriction map of Tn*2411*, pBR322::Tn*2411* DNA was completely digested by several restriction enzymes as described above. The resulting restriction map of Tn*2411* is represented in Fig. 1B. To look for inverted repeat sequences at the ends of Tn*2411* we formed heteroduplexes between pBR322 and pBR322::Tn*2411*, which were analyzed in the electron microscope. The single *Pst*I sites in pBR322 and Tn*2411* were used as internal reference points, and structures corresponding to Fig. 2 were measured. All of these structures demonstrated linear double-stranded DNA of the size of pBR322 and single-stranded tails corresponding to Tn*2411*. The single-stranded tails grow from a short double-stranded stalk, representing inverted repeat DNA segments. Comparative measurements of the duplications flanking Tn*2411* and Tn*3* (38 nucleotides) (24) revealed the same order of magnitude for both sequences.

Localization of the resistance determinants of Tn*2411*. The position of the Su and Ap genes on Tn*2410* has been mapped by heteroduplexing pBR322::Tn*2410* with pBP12, an Ap^r Su^r, but Hg^s derivative of R1767 (Kratz et al., in press). The region of homology between the two structures begins on Tn*2410*, next to its single *Sma*I site, and covers the following 0.22 and 4.1-kb *Bam*HI fragments. The resistance genes are located on the 4.1-kb fragment (Fig. 1A). The restriction maps of Tn*2411* and Tn*2410* share large regions of homology, but Tn*2411* carries, besides the 0.22-kb fragment, a 3.65-kb *Bam*HI fragment close to its single *Sma*I site (Fig. 1A and B). Both ends of this fragment show homology with the ends of the 4.1-kb Tn*2410* fragment. In heteroduplex formation between pBR322::Tn*2411* (linearized by *Sma*I) and pBP12, all structures showed two regions of double-stranded DNA interrupted by a small substitution loop. The first (2.1 ± 0.07 kb) region of homology begins on Tn*2411*, next to its single *Sma*I site, and is separated from the second (1.4 ± 0.08 kb) region of homology by two single-stranded DNA segments with sizes of 1.1 ± 0.09 and 0.8 ± 0.06 kb, respectively (data not shown). Within the limits of restriction and heteroduplex analyses, the left ends of the 3.65-kb Tn*2411* fragment and the 4.1-kb Tn*2410* fragment share a 1.9-kb segment, including restriction sites for *Bgl*II, *Pst*I, and *Hin*dIII. This site of the Tn*2410* fragment is known to carry the Su gene (Kratz et al., in press). At the right end, a 1.1-kb sequence is common to both fragments. The internal parts, 1.1 kb in the Tn*2410* fragment and 0.8 kb in the Tn*2411* fragment, are different. From these data we concluded that Tn*2410* and Tn*2411* carry the sulfonamide gene at the same position on their physical map.

To identify the location of the streptomycin gene in Tn*2411*, we analyzed the sequence homology between Tn*2411* and Tn*2410* by hetero-

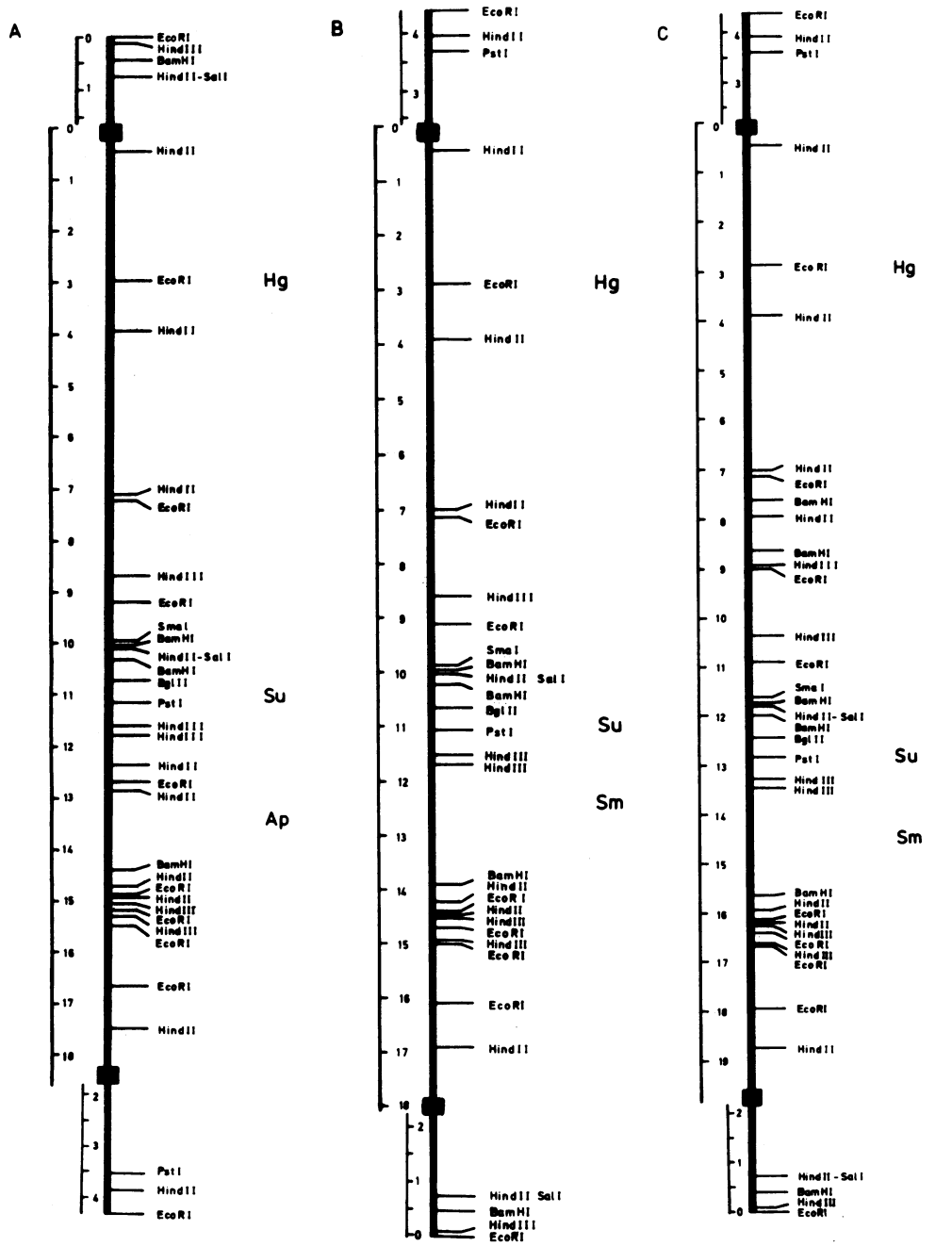

FIG. 1. Restriction maps of (A) Tn*2410*, (B) Tn*2411* and (C) Tn*21*. The black boxes indicate the inverted repeats flanking the transposable elements. The coordinates are given in kb relative to the single *Eco*RI site in the vector plasmid pBR322. The location of the resistance genes is indicated. (The Tn*2410* map is from Kratz et al., in press.)

duplex formation between pBP322::Tn*2411* and pBP322::Tn*2410*, both linearized by *Sma*I. All structures (Fig. 3) showed one large linear double-stranded DNA stretch interrupted by two substitution loops. The large single-stranded loop corresponds to pBP322 (with a size of 4.3 ± 0.2 kb), indicating different orientations of Tn*2410* and Tn*2411* relative to this plasmid.

Only one other region of nonhomology exists—2.0 ± 0.07 kb distant from the *Sma*I site.

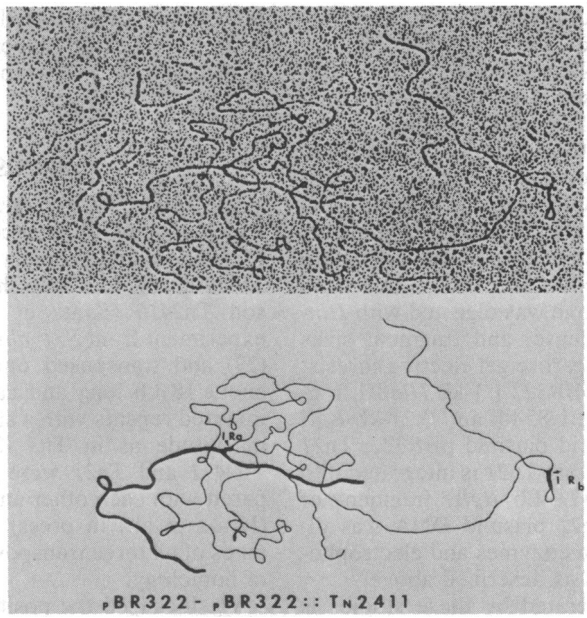

FIG. 2. Heteroduplex formation between pBR322::Tn*2411* and pBR322. Both plasmids were cleaved with *Pst*I before denaturation and renaturation. Inverted repeat sequences (IR_a and IR_b) are indicated with arrows. IR_a corresponds to the duplicated sequence flanking Tn*2411*.

In this region, the 4.1-kb *Bam*HI fragment on Tn*2410* with additional restriction sites for *Eco*RI and *Hin*dII corresponds to the 3.65-kb *Bam*HI fragment on Tn*2411* (Fig. 1A and B). These data are identical to those from the heteroduplex between pBR322::Tn*2411* and pBP12. As the position of the ampicillin gene in Tn*2410* has been identified, we concluded that the 1.1-kb single-stranded Tn*2410* segment codes for OXA-2 beta-lactamase production. This DNA segment is replaced in Tn*2411* by a 0.8-kb DNA sequence which codes for streptomycin resistance. The sulfonamide resistance gene is located on the 2.0 ± 0.07-kb sequence next to the single *Sma*I site on both transposons.

Analysis of pBR322::Tn*21* recombinants. Ko-

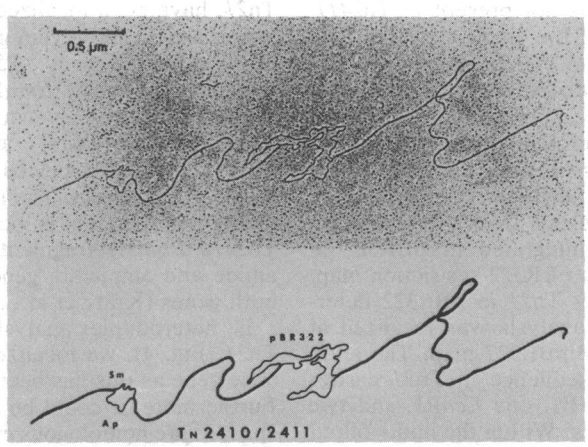

FIG. 3. Heteroduplex formation between pBR322::Tn*2411* and pBR322::Tn*2410*. Both plasmids were linearized by *Sma*I before denaturation and renaturation. The position of the streptomycin and ampicillin genes is indicated.

pecko et al. (16) described a Sm^r Su^r Hg^r transposon from plasmid R100 (25), which they designated Tn*21*. To compare Tn*21* with Tn*2411*, we transposed Tn*21* from R100 onto pBR322 with the aid of phage $P1Cm_{ts}$ as described above. DNA from 15 Ap^r, Tc^r, Sm^r, and Su^r clones which produced no bacteriophages (i.e., cured cells) showed only one plasmid band (24 kb) in agarose gel electrophoresis, indicating the transposition of Tn*21* onto plasmid pBR322. Purified DNA from plasmid pBR322 and from one pBR322::Tn*21* clone was digested with *Hin*dII or *Bgl*I endonuclease, and fragment sizes were determined by agarose gel electrophoresis. The presence of the pBR322 1.1-kb *Hin*dII fragment and the pBR322 1.85-kb and 0.21-kb *Bgl*I fragments in the hybrid plasmid pBR322::Tn*21* clearly demonstrated that Tn*21* is integrated into the 3.26-kb *Hin*dII (2.3-kb *Bgl*I) fragment of pBR322. pBR322::Tn*21* plasmid DNA was digested with restriction enzymes and electrophoresed on agarose gels as described above.

The final map generated by these studies is presented in Fig. 1C. A comparison of our restriction data with available *Eco*RI, *Hin*dIII, *Bam*HI, and *Sal*I maps of the corresponding R100 region (4, 10, 33) showed that our extended map is in good agreement with these results.

Comparison of the physical maps of Tn*2411* and Tn*21*. Tn*2410*, Tn*2411*, and Tn*21* seem to be identical in most of the restriction cleavage sites (Fig. 1). To determine the degree of homology between these transposons, we formed heteroduplexes between pBR322::Tn*2411* and pBR322::Tn*21*, both plasmids linearized by *Sma*I, and analyzed them. All of the heteroduplex structures examined demonstrated a linear double-stranded DNA with a size of pBR322::Tn*2411*. In addition, a single-stranded loop is apparent corresponding to a DNA segment of Tn*21* which is not present in Tn*2411*. The average length of the single-stranded loop was found to be 1.45 ± 0.1 kb, and it was located 2.4 ± 0.03 kb distant from the *Sma*I site, as calculated from measurements of six structures (Fig. 4). The heteroduplex analysis indicated that the integration sites of Tn*21* and Tn*2411* in pBR322 are nearly identical. This is in good agreement with the results from the restriction analysis. Tn*2411* is integrated in pBR322 at position 2.3 kb on the pBR322 restriction map. The integration site of Tn*21* in pBR322 determined by restriction analysis was localized at position 2.2 kb on the pBR322 map. The additional 1.45-kb DNA sequence in Tn*21* carries one *Hin*dII, one *Hin*dIII, one *Eco*RI, and two *Bam*HI restriction sites. Within the limits of our analysis, Tn*2411* and Tn*21* are identical except for this 1.45-kb DNA segment carried on Tn*21*. As Tn*2411* and Tn*2410* are homologous except for the substitution of the ampicillin resistance gene by the streptomycin resistance gene, the relatedness of Tn*2410* to Tn*21* is obvious.

DISCUSSION

We report here a new transposon, Tn*2411*, which encodes resistance to streptomycin, sulfonamides, and mercury. This transposon was isolated, together with the Ap^r Su^r Hg^r transposon Tn*2410* (Kratz et al., in press), in one experiment from *S. typhimurium* plasmid R1767 (29) and transposed onto pBR322. Tn*2411* is about 18 kb long and carries at its ends short inverted repeats with a size of the same order of magnitude as in Tn*3* (24). Physical maps of Tn*2411* and Tn*21* were constructed and compared with each other and with the Tn*2410* map (Kratz et al., in press) (Fig. 1). The physical maps of all three transposons show large regions of homology.

We localized the position of the sulfonamide and streptomycin genes on Tn*2411* by hybridization experiments with the Ap^r Su^r Hg^s R1767 derivative pBP12 and between Tn*2411* and Tn*2410* (Fig. 3) within the error of heteroduplex and restriction analyses. The 1.1-kb sequence in Tn*2410*, which coded for OXA-2 beta-lactamase, is substituted for by a 0.8-kb sequence in Tn*2411* coding for streptomycin resistance. The DNA sequence beside the ampicillin and streptomycin genes is common to both transposons on the basis of heteroduplex formation. It may be argued that Tn*2410* carries an inactivated streptomycin gene on its structure and that the 0.8-kb single-stranded segment is due to an unknown insertion sequence. This appears rather unlikely. The positions of the resistance genes on plasmids R100, R6, and R6-5, all carrying Tn*21*, have been localized (22, 34). The sulfonamide and streptomycin genes map together on these plasmids on a 5.25-kb *Eco*RI fragment, including the single *Sma*I site and the 3.65-kb *Bam*HI fragment known to carry the sulfonamide gene on Tn*2411* and Tn*21* (see below). This fragment is replaced on Tn*2410* by a 3.54-kb *Eco*RI fragment. The right *Eco*RI site terminating this fragment is located within the 4.1-kb Tn*2410* *Bam*HI fragment carrying the sulfonamide and ampicillin genes known to separate both genes (Kratz et al., in press).

By heteroduplex analysis between Tn*2411* and Tn*21* (Fig. 4), we localized the mercury resistance gene as this has been mapped on Tn*21* (8). Furthermore, it could be shown that both transposons are homologous except for an additional DNA segment carried on Tn*21* indicated by a 1.45-kb single-stranded loop. Since both structures encode for the same resistance genes and

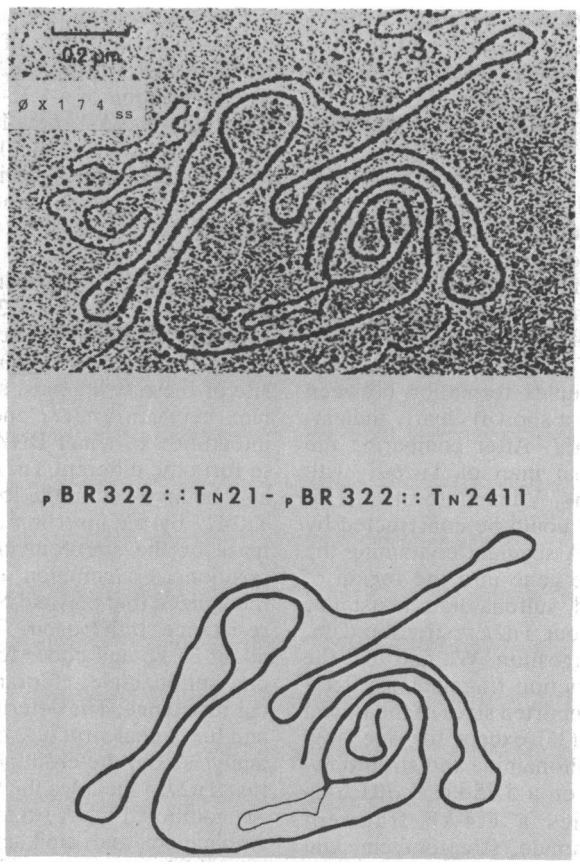

FIG. 4. Heteroduplex formation between pBR322::Tn*2411* and pBR322::Tn*21*. Both plasmids were linearized by *Sma*I before denaturation and renaturation.

both are transposable, until now no function could be assigned to this region.

R1767 was found to rearrange frequently its DNA sequence (37). The two closely related transposons carried by R1767 that were recently identified may be one explanation for the very unstable behavior of this molecule even in a *recA* background. Tn*2410* and Tn*2411* appear to transpose simultaneously. Of the transconjugants selected on sulfonamide in the transposition from R1767 to plasmid pUB307 (kanamycin, tetracycline) 85% carried pUB307::Tn*2410*::Tn*2411* recombinant molecules. These plasmids were found to be unstable after conjugation and transformation. Variously sized recombinant plasmids, carrying only parts of the sequence of both transposons and DNA segments of pUB307 coding for replication functions, were isolated after transformation with pUB307::Tn*2410*::Tn*2411* DNA in low frequency (unpublished data). Transposition functions similar to those encoded by the Tn*3* TnpA and TnpR genes have been recently shown to regulate the transposition of Tn*21* by the formation and resolution of cointegrate structures (8). The genes coding for transposition functions in Tn*2410* and Tn*2411* are homologous to those of Tn*21* (Fig. 4). The TnpR system promotes site-specific recombination between two direct repeated transposons in cointegrate structures (13). Therefore, any plasmid carrying Tn*2410* and Tn*2411* in direct orientation must be unstable, as shown by the pUB307::Tn*2410*::Tn*2411* recombinants after conjugation. TnpR may also stimulate DNA rearrangements by mediating deletions between the normal resolution site and similar sites (13). Molecular rearrangements in plasmids like R1767 or pUB307 carrying Tn*2410* and Tn*2411* may be stimulated by reciprocal recombination depending on *recA* functions between the two large homologous multiple-resistance transposons, together with "illegitimate" recombination promoted by the TnpR systems encoded on Tn*2410* and Tn*2411*.

A single-stranded loop formed by R100.1,

which is identical in its size, position, and restriction sites to the single-stranded loop formed by Tn*21* in the heteroduplex structure between Tn*2411* and Tn*21* (Fig. 4), was identified by Clerget et al. (5) in a heteroduplex structure between the plasmids R100.1 and R1*drd-19*. In addition, *Eco*RI and *Hin*dIII restriction fragments of R1*drd-19* and the resulting maps (2, 5) agree with our restriction data of Tn*2411* and the Tn*2411* map. Therefore, we conclude that plasmid R1*drd-19* carries Tn*2411* with Tn*3* inserted on its left side. A comparison of the reported restriction fragments of the closely related plasmid R6 (34) with our Tn*2411* and Tn*21* restriction data and heteroduplex formation between Tn*2411* and R6 (data not shown) clearly indicated that R6 carries Tn*21*. After comparing the physical and functional map of Tn*2603* with those of Tn*21* and Tn*4*, Yamamoto et al. (35) suggested that Tn*2603* could be constructed by the insertion of a DNA segment containing the OXA-1 beta-lactamase gene into the region of the streptomycin and sulfonamide resistance genes on Tn*21*. From our Tn*21* restriction data, we agree with this suggestion. We find that the *Eco*RI-generated restriction fragments of Tn*21* are identical with the reported sizes of the *Eco*RI fragments of Tn*2603* (35) except for one fragment. On Tn*21*, the sulfonamide and streptomycin genes are located on a 5.25-kb *Eco*RI fragment. Tn*2603* carries a 6.4-kb fragment containing the sulfonamide, streptomycin, and ampicillin genes. We conclude, therefore, that Tn*2603* is Tn*21*, carrying an additional 1.15-kb DNA segment containing the OXA-1 beta-lactamase gene.

From these data, we suggest that Tn*21* could be the basic structure of at least four different transposons: Tn*2410*, Tn*2411*, Tn*2603*, and Tn*4*. The integration of a 1.15-kb DNA segment carrying the OXA-1 beta-lactamase gene forms Tn*2603*, the deletion of a 1.45-kb DNA sequence forms Tn*2411*, the insertion of Tn*3* forms Tn*4*, and the replacement of the streptomycin gene in Tn*2411* by the OXA-2 beta-lactamase gene forms Tn*2410* (Fig. 5).

The postulated replacement of the streptomycin gene by the OXA-2 beta-lactamase gene could be explained by recombinational mechanisms involving the regions of homology at each site of these two genes, shown in the heteroduplex between Tn*2411* and pBP12 (Fig. 3). The integration of small DNA segments, necessary to form the different Tn*21*-like transposons, can also be involved in the formation of Tn*2410* and Tn*2411* by the insertion of the OXA beta-lactamase or the streptomycin gene into the same position as a common ancestor. Recently, we recognized that plasmid NR79 carries a multiple-resistance transposon. Tn*2424* has a size of about 25 kb and codes for mercury, streptomycin, sulfonamide, chloramphenicol, and amikacin resistance. The determination of the physical and functional structure of Tn*2424* by restriction analysis and heteroduplex formation showed that Tn*2424* includes the total sequence of Tn*21*. An additional DNA segment carrying the chloramphenicol and amikacin resistance genes is inserted between the sulfonamide and streptomycin genes of Tn*21* forming Tn*2424* (21). It is worth mentioning that all of the described integrations of DNA segments carrying additional

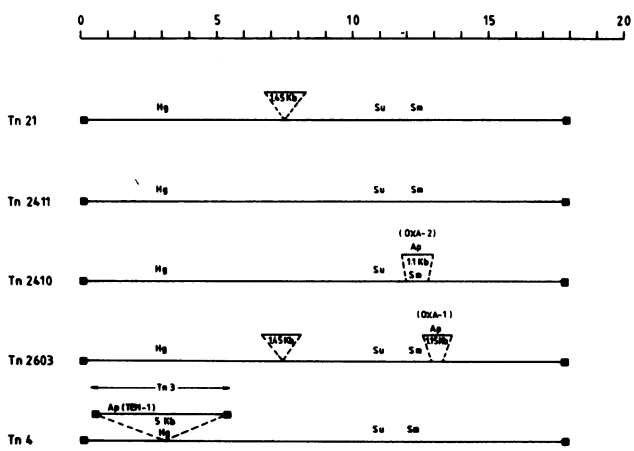

FIG. 5. Schematic representation of the structures of Tn*21* and related resistance transposons, as discussed in the text. The black boxes represent the flanking inverted repeats of the elements. The integration sites of DNA segments are indicated. (Heteroduplex analysis recently indicated that substitution between DNA segments is involved in the acquisition of the DNA sequence carrying the ampicillin gene by Tn*21*, forming Tn*2603* [31]. Abbreviations: OXA-1, beta-lactamase OXA-1; OXA-2, beta-lactamase OXA-2; TEM-1, beta-lactamase TEM-1.

resistance genes could be located within the 3.65-kb *Bam*HI fragment of Tn*21*. Furthermore, two new transposons carrying beta-lactamase genes have been reported. The 12.3-kb Tn*1401* (20) encodes for resistance to streptomycin and sulfonamide and produces a PSE-1 beta-lactamase, and Tn*2101* (12) has a size of 14.6 kb and encodes resistance to streptomycin, sulfonamide, mercury, and ampicillin (by a PSE-1 beta-lactamase). In addition, a 14-kb multiresistance transposon, Tn*1696*, mediating resistance to gentamicin, chloramphenicol, streptomycin, sulfonamides, and mercury, has been described (27). All three transposons are smaller than Tn*21*, but it would be interesting to look for a relationship with its structure by comparing physical data from these transposons with our results.

From the study of F-related plasmids, Kopecko (15), like others, stated that multiresistance plasmids evolved by the acquisition of different discrete transposable elements, each carrying genes conferring resistance to one or more antibiotics. Moreover, the finding that the transposons are located at the same position in these plasmids further suggested the evolution from a common ancestral plasmid. In addition to this reasonable explanation for the evolution of R-plasmids, we find that Tn*21*-related transposons cannot only transpose as intact units, but also pick up or even exchange single resistance determinants. These genes, however, are not able, in our present experimental schemes, to translocate themselves. The mechanisms possibly involved in these rearrangements are not yet known, but these data may indicate the existence of other naturally occurring mechanisms for spreading resistance genes. With more detailed knowledge of numerous transposons, these may perhaps be arranged in families (groups) of common basic structures, indicating evolutionary relations.

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