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Organization of the Tn*6*-Related Kanamycin Resistance Transposon Tn*2680* Carrying Two Copies of IS*26* and an IS*903* Variant, IS*903*.B

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The kanamycin resistance transposon Tn*2680*, which originates from the R plasmid Rts1, is homologous to Tn*6* and carries two directly repeated copies of IS*26*, one at each end. The kanamycin resistance gene codes for type I aminoglycoside-3′-phosphotransferase. Tn*2680* also contains, in the middle of the transposon, an additonal IS element homologous to IS*903*. This element, designated IS*903*.B, is flanked by a 9-base-pair direct target duplication. A novel kanamycin resistance transposon, Tn*2681*, can be generated from Tn*2680* by IS*903*.B-mediated cointegration and subsequent reciprocal recombination between the directly repeated IS*26* sequences. Tn*2681* carries a single IS*26* element in the middle of the transposon and is flanked by two directly repeated copies of IS*903*.B. Possible evolutionary relationships between Tn*2680* and other kanamycin resistance transposons such as Tn*903* and Tn*2350* are discussed, based on the gene organization and DNA sequences.

One group of the R plasmid-specified enzymes, which inactivate kanamycin, an aminoglycoside antibiotic, is aminoglycoside-3′-phosphotransferase [APH(3′)]. Enzymes belonging to this group have been divided into four subtypes, primarily on the basis of differences in substrate profile (12). Comparison of DNA sequences of several genes for APH(3′) suggest that the different subtype enzymes are related evolutionally (38, 39). These genes for kanamycin resistance (Km^r) are often flanked by insertion sequences (IS elements) and thus form part of a transposon (Tn). IS elements can flank the Km^r gene either in direct orientation, like IS*1* in Tn*2350* (9), IS*15* in Tn*1525* (24), and IS*26* in Tn*2680* (21, 27), or inverted orientation, like IS*50* in Tn*5* (3, 4, 23, 33) and IS*903* in Tn*903* (Tn*601*) (11, 13, 29, 30) (see also Fig. 1). The Km^r genes in Tn*6*, Tn*903*, and Tn*1525* are homologous and code for a type I APH(3′) enzyme (11, 13, 24, 30). The flanking element IS*26* is known to be closely related to IS*15* (21, 22, 24, 27, 40).

In this paper, we show that Tn*2680*, isolated from plasmid Rts1 (21, 37), is homologous to Tn*6* from pJR72 (3), and that their Km^r genes are also homologous to those of Tn*903* originating from R6 (13, 29, 30) and of Tn*2350* from R1 (9). Experiments with Tn*2680* revealed the presence of a third IS element in the middle of the transposon. This IS element, designated IS*903*.B, is homologous to IS*903*. The possible evolutionary relationships between these Km^r transposons encoding a type I APH(3′) enzyme are discussed on the basis of a comparison of sequences and gene organization.

MATERIALS AND METHODS

Bacteria, phages, and plasmids. Table 1 lists the bacterial strains, phages, and plasmids used. Phage P1-15Δ*mod*(P15), which is a P1-15 hybrid 2 derivative with the *Bgl*II-7 and -12 fragments in the *mod*(P15) gene region deleted (2, 19), was constructed by Sabine Burckhardt. Plasmid pLC850 was constructed by cloning the *Bgl*II fragment of the P1Km genome, which contains the invertible C segment and Tn*2680* (21), into the *Bam*HI site of pBR322 (6). The sites of integration of Tn*2680* in plasmids pSHI97::Tn*2680* M2 and pOX38::Tn*2680* A21 have been previously described (22). Unless otherwise stated, C600 and WA921 were employed to prepare phages and plasmids.

Media. LB and LBMg media and LB agar have been previously described (9, 21, 22). Antibiotics were added to LB agar, containing neither glucose nor $CaCl_2$, at the following concentrations: ampicillin (200 μg/ml), kanamycin (25 μg/ml), rifampin (100 μg/ml), and streptomycin (200 μg/ml).

Isolation of IS*903*.B-mediated cointegrates between pOX38::Tn*2680* and pSHI99. Isolation of cointegrates was performed in the same way as the isolation of IS*26*-mediated cointegrates (22). Strain WA3782(pOX38::Tn*2680*, pSHI99) was mated with strain HB101 for 1 h at 37°C, and Cm^r Km^r Str^r transconjugants were selected. Cointegrates were subsequently identified by their ability to efficiently transfer the Cm^r marker from HB101 to BzB1139 (22). IS*903*.B-mediated cointegrates were distinguished from IS*26*-mediated cointegrates (22) by restriction analysis with the enzymes *Ava*I, *Hin*dIII, *Mlu*I, *Pvu*II, *Sal*I, and *Xho*I.

Isolation of pSHI99::IS*903*.B and pSHI99::Tn*2681* from the IS*903*.B-mediated cointegrates between pOX38::Tn*2680* and pSHI99. pSHI99::IS*903*.B and pSHI99::Tn*2681* were obtained by Rec-dependent recombination in vivo between the directly repeated IS*903*.B and IS*26*, respectively. The cointegrates were first transferred into CH102 rec^+ by conjugation. Plasmids prepared from this rec^+ strain were then used to transform WA921. Cm^r Km^s (pSHI99::IS*903*.B) and Cm^r Km^r (pSHI99::Tn*2681*) transformants were isolated. Plasmids were prepared from the individual transformants and were screened by restriction analysis with *Hin*dIII, *Mlu*I, *Pst*I, and *Sal*I.

Isolation of P1-15 phage derivatives carrying Tn*2681*. P1-15Δ*mod*(P15)::Tn*2681* phages were obtained in essentially the same way as P1-15::Tn*9* phages (15). WA921[P1-15Δ*mod*(P15), pSHI99::Tn*2681*] was heat induced, and the resulting lysates were used to infect WA921 at a multiplicity of about 0.02 plaque-forming phages per cell. Km^r transductants appeared with a frequency of about 4×10^{-6} per plaque-forming phage. In each experiment, 50 Km^r transductants were screened for Cm^s and Km^r phenotypes and for the ability to produce high-frequency Km^r transducing lysates.

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TABLE 1. Bacterial strains, phages, and plasmids

Designation	Relevant characters	Drug resistance phenotype	Reference or source
Escherichia coli K-12			
C600	*thr leu thi lac supE*		(1)
WA921	*thr leu met thi lac supE hsdS*		(41)
WA3782	*met thi lac supE hsdS recA*		(22)
HB101	*pro leu thi lac str hsdS recA*	Str^r	(7)
BzB1139	*met hsdR str recA srl*::Tn*10*	Str^r Tet^r	(22)
CH102	*str rpo* $P1^r$	Str^r Rif^r	(14)
Phages			
λ *kan-3*	λ *b515 b519* c1857 *S7* Tn*6*	Km^r	(3)
λ::Tn*2350*	λ *b515 b519 nin5 xis6* c1857 *S7* Tn*2350*	Km^r	(9)
P1-15Δ*mod*(P15)	P1-15 hybrid2 Δ*mod*(P15) *c1ts225*		S. Burckhardt
P1-15Δ*mod*(P15)::Tn*2681*		Km^r	This study
Plasmids			
pLC850	pBR322 derivative carrying Tn*2680*	Ap^r Km^r	This study
pSHI248	pBR322 derivative carrying Tn903	Cm^r Km^r	(20)
pSHI97	pBR322 deletion derivative	Ap^r	(27)
pSHI97::Tn*2680* M2		Ap^r Km^r	(22)
pOX38::Tn*2680* A21	F deletion derivative carrying Tn*2680*	Km^r	(22)
pSHI99	pBR325 deletion derivative	Ap^r Cm^r	(22)
pSHI99::IS*903*.B P3		Cm^r	This study
pSHI99::IS*903*.B P11		Cm^r	This study
pSHI99::IS*903*.B W1		Ap^r Cm^r	This study
pSHI99::IS*903*.B W10		Ap^r Cm^r	This study
pSHI99::Tn*2681* P3		Cm^r Km^r	This study
pSHI99::Tn*2681* P11		Cm^r Km^r	This study

About 6 to 10% of the Km^r transductants fell into this class. The genome structure of P1-15Δ*mod*(P15)::Tn*2681* was confirmed by restriction analysis with the enzymes *Bam*HI, *Bgl*II, *Eco*RI, and *Mlu*I (15, 16).

Nucleic acid procedures. Restriction enzymes, the Klenow fragment of DNA polymerase I, and T4 ligase were obtained from New England Biolabs or from Boehringer Mannheim Biochemicals. They were generally used as recommended by the suppliers.

Isolation of phage DNAs and plasmids, restriction cleavage analyses, and electron microscopic heteroduplex studies were performed as previously described (9, 20, 21). DNA sequencing was carried out by both the chemical degradation method (25) and the chain termination method (26, 34).

RESULTS

Tn*2680* and Tn*6* are homologous. The 4.95-kilobase (kb) Km^r transposon Tn*6*, first identified in 1975 (3), is known to carry the Km^r gene for the type I APH(3′) enzyme (11). Its structure had not been analyzed extensively. Another Km^r transposon, Tn*2680*, which contains the 820-base-pair (bp)-long IS*26* as terminal direct repeats, is also 4.95 kb in length (21, 22, 27). Restriction cleavage patterns obtained with *Hin*dIII, *Pst*I, *Sal*I, and *Xho*I indicate that Tn*6* is similar to Tn*2680* (Fig. 1). For a direct sequence comparison between Tn*2680* and Tn*6*, heteroduplex molecules between the linearized plasmid pLC850, which carries Tn*2680*, and λ::Tn*6* DNA were studied in the electron microscope (Fig. 2). A homologous segment of 4.95 ± 0.13 kb (n = 11) was found in the region carrying the transposons. Thus, at the level of resolution of the electron microscope, Tn*6* and Tn*2680* are identical; therefore Tn*6* is likely to carry direct repeats of IS*26* at its ends.

Tn*2680* carries a third functional IS element. During studies on IS*26*-mediated cointegrations between a conjugative F plasmid derivative, pOX38::Tn*2680* A21, and a pBR325 derivative, pSHI99, in the *Escherichia coli* K-12 *recA* strains WA3782 and BzB1139 (22), we isolated two (out of six cointegrates) that were not mediated by IS*26*. Restriction cleavage analysis of these cointegrates, pSHI99ΩpOX38::Tn*2680* P3 and P11, revealed that pSHI99 had been fused to pOX38::Tn*2680* within the Tn*2680* sequence. A DNA element, approximately 1 kb in length, from within Tn*2680* had been duplicated and now brackets

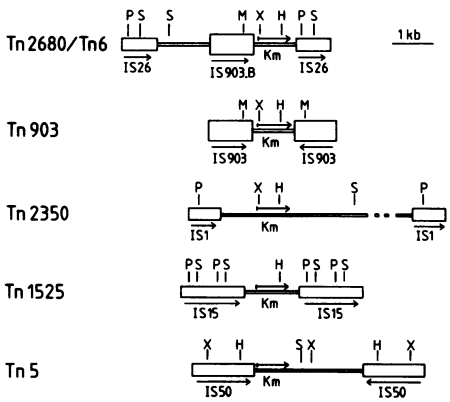

FIG. 1. Structure of relevant Km^r transposons. Maps are redrawn from the literature (9, 21, 24, 28, 33) and are complemented by results from this paper. The arrows with Km indicate the location and the orientation of the *aph* gene. Restriction cleavage sites: S, *Sal*I; P, *Pst*I; H, *Hin*dIII; X, *Xho*I; R, *Eco*RI; M, *Mlu*I. The arrows under the boxes representing IS elements identify the orientation of the elements.

pSHI99 as direct repeats in these cointegrates (Fig. 3). To characterize this element further, we isolated Cm^r Km^s segregant plasmids from the cointegrates through Rec-dependent recombination between the directly repeated elements. These plasmids are pSHI99 derivatives that carry a single copy of the duplicated element at the site at which pOX38::Tn*2680* had been integrated in the parental pSHI99ΩpOX38::Tn*2680* cointegrates (Fig. 3). The integration sites of the element on pSHI99 were mapped more precisely with *Hpa*II and *Hae*III enzymes and were found to differ in pSHI99::IS*903*.B P3 and P11 (Fig. 4A). Duplication of the element during cointegration and its insertion at different sites strongly indicate that this 1-kb element is an active IS element. Figure 4A also includes two additional integration sites of the element in pSHI99 derivatives obtained as pSHI99ΩpOX38:Tn*2680* cointegrates W1 and W10.

Characterization of IS*903*.B, the third IS element on Tn*2680*. The presence of terminal inverted repeats and the generation of direct repeats at a target sequence upon insertion are characteristic features of procaryotic IS elements (18, 32). We determined the DNA sequence of both ends of the element in pSHI99::IS*903*.B P3 and found that they were identical to the corresponding ends of IS*903* and IS*102* (5, 13, 28–30). Like IS*903* and IS*102*, this element also generated a 9-bp target duplication (Fig. 4B). To examine whether this element is homologous to IS*903*, we analyzed heteroduplex molecules between appropriate restriction fragments of pSHI97::Tn*2680* M2 and the Tn*903*-containing plasmid pSHI248 under the electron microscope (Fig. 5A, B, and C). On both plasmids the DNA sequence that derived from pBR322 provide homology for a duplex segment of 2.68 kb, interrupted by a 1.70-kb loop representing the Cm^r segment. A second homologous region would be indicative of DNA sequence relations between IS*903* and the element. Indeed, all of the heteroduplex molecules had the expected two duplex segments and thus document homology between IS*903* and the new element over their entire length of 1.05 ± 0.06 kb (n = 21) (Fig. 5D and E). This view was also supported by the patterns of restriction fragments produced

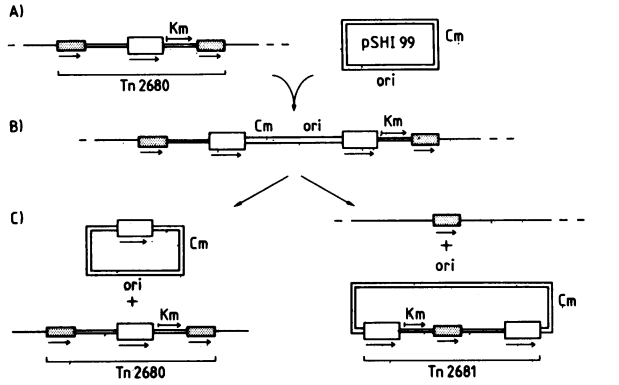

FIG. 3. Schematic representation of IS*903*.B-mediated cointegration and subsequent Rec-mediated resolution of the cointegrate. (A) Tn*2680* located on pOX38::Tn*2680* and the pBR325-derived plasmid pSHI99. (B) Cointegrate mediated by IS*903*.B, which has been duplicated in the process. (C) Rec-dependent homologous recombination between the two IS*903*.B elements or between the two IS*26* sequences in the cointegrate gives rise to segregant plasmids carrying the transposons Tn*2680* or Tn*2681*, respectively. The shadowed boxes and the open boxes represent the IS*26* and the IS*903*. B, respectively, and the arrows under the boxes define the orientation of the IS elements. Km with an arrow, Cm, and ori indicate the *aph* gene, the *cat* gene, and the origin of replication of the plasmid pSHI99, respectively.

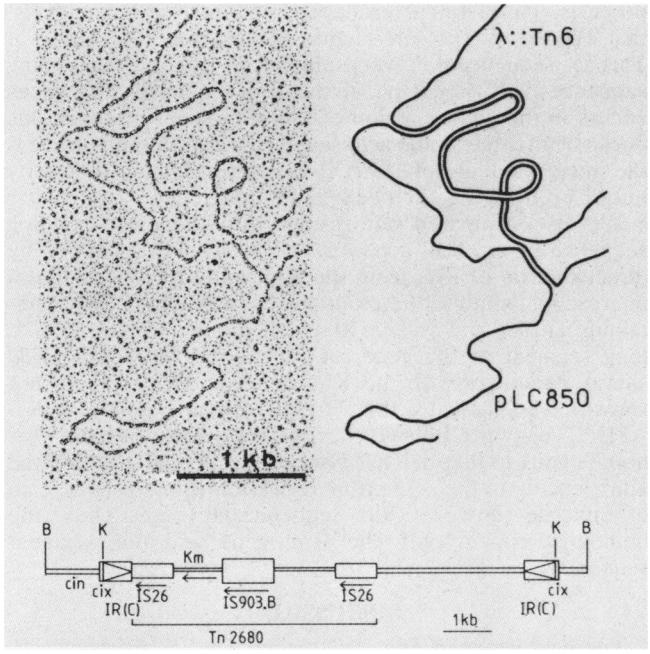

FIG. 2. Heteroduplex between pLC850 carrying Tn*2680* and λ::Tn*6* DNA. Before hybridization, pLC850 was linearized by cleaving with *Eco*RI. The scheme under the electron micrograph shows the *Bgl*II fragment of pLC850, which derives from P1Km and was cloned into the *Bam*HI site of pBR322 to yield pLC850. In this *Bgl*II fragment, Tn*2680* is inserted into the C segment near the internal end of one of the inverted repeats (16, 21). The boxes containing the triangle with IR(C) indicate the 0.62-kb inverted repeats of the C segment in the P1 genome. These IR(C) segments form the snap back in pLC850 seen in the electron micrograph. Km, cix, and cin indicate the kanamycin resistance gene, the crossover sites for C inversion, and the *cin* gene, respectively. Restriction cleavage sites: B, *Bgl*II; K, *Kpn*I.

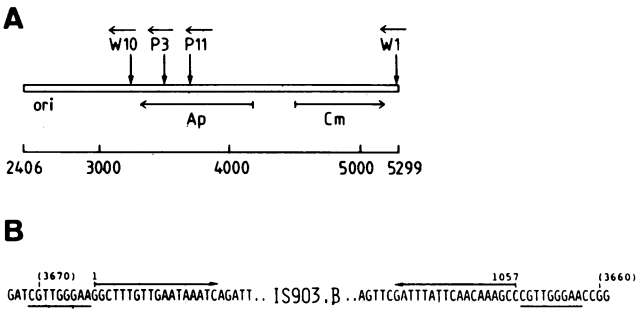

FIG. 4. Integration sites of IS*903*.B on pSHI99::IS*903*.B. (A) Plasmid pSHI99, a deletion derivative of pBR325, linearized at the junction site of the deletion at positions 2406 and 5299 of pBR325 (22, 31). Insertion sites and the orientation of IS*903*.B on pSHI99::IS*903*.B P3, P11, W1, and W10 are indicated by arrows. The arrows labeled with Ap and Cm under the map represent the *bla* and the *cat* gene, respectively. The origin of pSHI99 replication is indicated by ori. (B) DNA sequence of the junctions at the integration site of IS*903*.B on pSHI99::IS*903*.B P3. The numerical coordinates for IS*903*.B and pSHI99 are indicated without and with parentheses, respectively. The 9-bp target duplication is underlined, and the terminal inverted repeats of IS*903*.B are indicated by horizontal arrows.

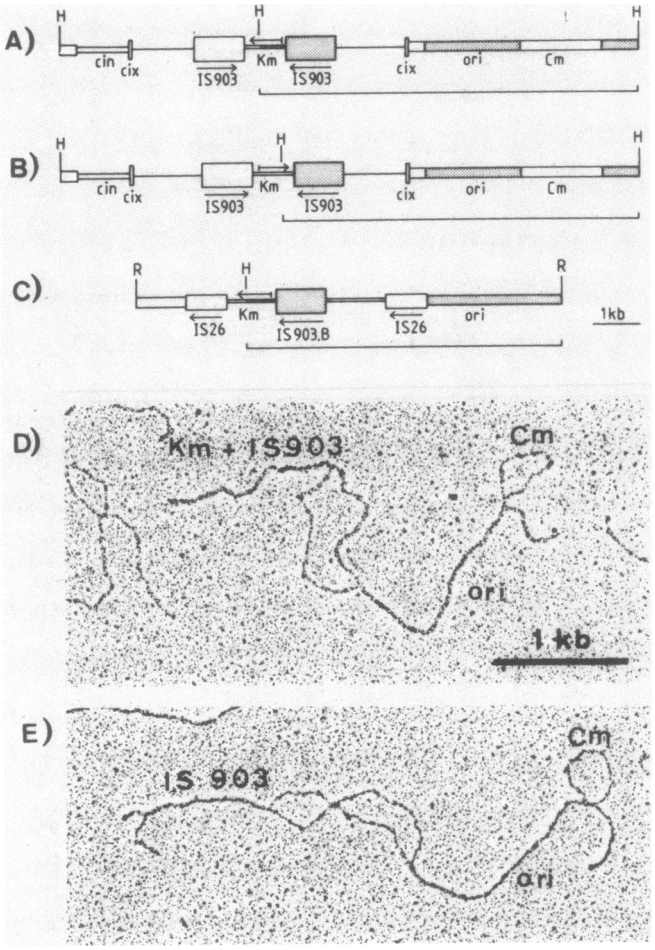

FIG. 5. Homology between IS*903* on Tn*903* and IS*903*.B on Tn*2680*. pSHI248 is a pBR322 derivative carrying the 1.70-kb Cm^r segment at the *Pst*I site of pBR322, the pBR322 segment between positions 380 and 1360 is substituted by a 7.0-kb segment (6, 20, 35). This 7.0-kb segment contains the *Kpn*I fragment, which carries Tn*903* and is derived from pCR1 (10), and the phage P1-encoded DNA invertase gene *cin* and its crossover *cix* sites (20). Since the segment containing Tn*903* is flanked by the *cix* sites, *cin*-mediated inversion results in two isomeric forms (A and B). Cleavage of pSHI248 with *Hin*dIII produces two different fragments, each containing the origin of pBR322 replication (ori) and one copy of IS*903* as indicated by the brackets under the maps. A *Hin*dIII-*Eco*RI fragment of pSHI97::Tn*2680* M2 (22) also contains the *ori* of pBR322 and IS*903*.B as indicated by the bracket under map C. A heteroduplex molecule between these partly homologous fragments from A and C is shown in D, and a representative between B and C is shown in E. Symbols are as in Fig. 1 through 3. Restriction sites: E, *Eco*RI; H, *Hin*dIII. The shaded segments in the maps are homologous regions in the heteroduplex molecules. The location of Tn*903* within the invertible *Kpn*I fragment is based on the published map (10) and our own data.

by *Hpa*II and *Hae*III. A subsequent DNA sequence study has revealed that this element is identical to neither IS*903* nor IS*102* (B. Mollet, S. Iida, and W. Arber, Mol. Gen. Genet., in press). Since it is more closely related to IS*903* than to IS*102*, we consider this element as a variant of IS*903*; we call it IS903.B.

Since IS*903*.B originally derives from the transposon Tn*2680*, we have also sequenced both ends of IS*903*.B situated within Tn*2680*. The result indicates that IS*903*.B lies in front of the Km^r gene and that it is flanked by a 9-bp target duplication (Fig. 6).

Like IS*903*, IS*903*.B can also serve as an active component of a transposon. This was shown in the following experiment. From the P3 and P11 cointegrates between pOX38::Tn*2680* and pSHI99, we first obtained Km^r Cm^r segregant plasmids pSHI99::Tn*2681* P3 and P11, respectively (Fig. 3). These plasmids were formed by reciprocal recombination between the two directly repeated IS*26* sequences in the cointegrates. To demonstrate that the IS*903*.B-flanked Km^r segment, designated as Tn*2681*, is able to transpose as a unit, plaque-forming P1-15Δ*mod*(P15) phages carrying the Km^r marker were isolated from WA921[P1-15Δ*mod*(P15), pSHI99::Tn*2681*]. Such phages were obtained with a frequency of about 3×10^{-7} per plaque-forming P1-15Δ*mod*(P15) phage from cells harboring either pSHI99::Tn*2681* P3 or P11. Restriction analysis of the genomes of four different phages from two independent experiments showed that they had all acquired Tn*2681* as a unit at four different sites (data not shown). The results confirmed the notion that Tn*2681*, containing directly repeated IS*903*.B at its ends, is a transposon.

DNA sequence of the control region of the Km^r genes for APH(3′) on Tn*2680* and its relatives. We have determined the DNA sequences of the control regions of the APH(3′) genes, upstream from the *Xho*I site, on the transposons Tn*2680* and Tn*6*, and we compared them with the corresponding sequence of Tn*903* (13, 30). The sequence on Tn*6* is identical to the sequence on Tn*2680* (Fig. 6). This supports the hypothesis, based on heteroduplex and restriction analysis, that Tn*2680* and Tn*6* are identical. Moreover, the region of Tn*2680* sequenced is very similar to the corresponding sequence of Tn*903*. One of the few sequence differences occurs in the coding region of the Km^r gene; however this does not alter the amino acid sequence. Another difference is the integration site of IS*903*.B in Tn*2680*, which differs by only 1 bp from the site of IS*903* in Tn*903*.

We also compared our results with the corresponding sequence of the Km^r gene on Tn*2350*, which is flanked by direct repeats of IS*1*, from the R plasmid R1 (9). Electron microscopic study of heteroduplexes between pLC850 containing Tn*2680* and λ::Tn*2350* revealed that a 0.94 ± 0.06-kb long segment of the gene for type I APH(3′) on Tn*2680* shares homology with the Km^r gene on Tn*2350* (data not shown). This indicates that Tn*2350* also encodes the type I APH(3′) enzyme. Indeed, the DNA sequence of Tn*2350* is homologous to that of Tn*2680* from the *Xho*I site within the Km^r gene up to the −35 promoter region (Fig. 6). Upstream of this site, however, the sequences diverge. Thus, the homology covers only the Km^r gene and not adjacent sequences.

DISCUSSION

The Km^r genes in Tn*6*, Tn*903*, and Tn*1525* are known to encode a type I APH(3′) enzyme (11, 13, 24, 30). We have shown here that two Km^r transposons, Tn*2350* and Tn*2680*, flanked by direct repeats of IS*1* and IS*26*, respectively, also carry the genes for type I APH(3′) protein by electron microscopic study of heteroduplexes and by sequencing the promoter proximal regions of their genes. We have also shown that Tn*6*, which originates from pJR72 (3), and Tn*2680*, which originates from Rts1 (21), are homologous throughout their length. Sequences of the promoter regions of these Km^r genes are identical between Tn*2680* and Tn*6* and differ in a few positions from those of Tn*903* and Tn*2350*.

Tn*2680* is flanked by direct repeats of IS*26* and contains

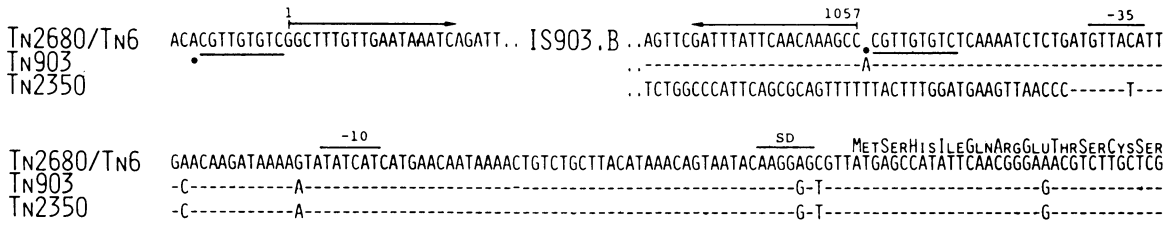

FIG. 6. DNA sequence of part of Tn*2680* showing the integration site of IS*903*.B and a comparison of it with Tn*6*, Tn*903*, and Tn*2350*. The sequence at the right end of IS*903*.B on the transposons Tn*2680* and Tn*6* was found to be identical. The numerical coordinate for IS*903*.B is indicated. The 9-bp target duplication is underlined, and the terminal inverted repeats are indicated by horizontal arrows. The sequence at the right end of IS*903*.B on Tn*2680* and Tn*6* is compared with the corresponding sequences of Tn*903* (13, 30) and Tn*2350* (8, 9). A hyphen represents a nucleotide identical to Tn*2680* or Tn*6*. The dot under the sequence of Tn*2680*/Tn*6* indicates the nucleotide A, which is present on Tn*903*, but is absent on Tn*2680*/Tn*6* at the right end of IS*903*.B, and which appears on Tn*2680* at the left end of the IS*903*.B next to the 9-bp target duplication. Overlines with −35 and −10 indicate the promoter for the Km^r gene and that with SD points to the sequence complementary to the 3′ end of the 16S rRNA, respectively. The beginning of the Km^r gene is indicated by the amino acid sequence.

one copy of an IS*903* variant, IS*903*.B, at the promoter-proximal region of its Km^r gene in the middle of the transposon. Cointegration between pOX38::Tn*2680* and pSHI99 occurs in *rec*$^+$ and *recA* strains with similar frequency; we found that the cointegrates mediated by IS*26* and those mediated by IS*903*.B appeared with similar frequencies (22) (this study). Thus, not only IS*26*, but also IS*903*.B, can promote cointegration independent of the host *recA* function. Upon cointegration, IS*903*.B is duplicated and generates 9-bp target duplications. IS*903*.B in Tn*2680* is also flanked by 9-bp direct repeats, indicating that simple transposition of IS*903*.B had occurred during generation of Tn*2680*. Since the two inversely repeated IS*903* sequences in Tn*903* are identical (13, 30), it is likely that transposition of IS*903* into the vicinity of a Km^r gene and subsequent IS*903*-mediated DNA rearrangement(s) have occurred to generate Tn*903* (18, 30). Interestingly, the target site used for transposition of IS*903* to form Tn*903* is different by 1 bp from that used for integration of IS*903*.B in Tn*2680*. The sequences studied are considered "molecular fossils" and allow us to speculate that these two transposition events had occurred independently. That the sequences of IS*903*.B and IS*903* are not identical is consistent with this notion.

An IS*903*.B-mediated cointegrate between a plasmid carrying Tn*2680* and another plasmid contains direct repeats of IS*26* and IS*903*.B. Recombination between directly repeated IS*26* sequences yields a new Km^r transposon, Tn*2681*, which is flanked by direct repeats of IS*903*.B. Another Km^r transposon, Tn*602*, flanked by directly repeated IS*903*-like elements, has recently been characterized (J. Davies, personal communication). Since Tn*2681* contains one copy of IS*26* in the middle of the transposon, IS*26*-mediated cointegration and the subsequent recombination between direct repeats of IS*903*.B would regenerate Tn*2680* flanked by IS*26*. Plasmid pTW20, a derivative of Rts1, was reported to carry an about 1.7-kb Km^r segment flanked by 1.05-kb inverted repeats (36). It is likely that these 1.05-kb inverted repeats in pTW20 are IS*903*.B, described here.

Genesis of IS-mediated transposons and subsequent IS-promoted rearrangements, including integration of a transposable element into another element, have been shown experimentally (14, 17, 18, 32). Integration of IS*26* near the Km^r gene and subsequent IS*26*-mediated DNA rearrangements together with integration of IS*903*.B must have been involved in generation of Tn*2680* and Tn*6*, because they are flanked by IS*26*. IS*1*-flanked Tn*2350* and IS*15*-flanked Tn*1525* are also likely to have been formed in similar processes. The observations described here support the idea that IS-promoted processes such as those demonstrated experimentally are indeed involved in the natural evolution of transposons.

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LITERATURE CITED

1. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *E. coli* K12. Genetics **39**:440–452.
2. **Arber, W., M. Hümbelin, P. Caspers, H. J. Reif, S. Iida, and J. Meyer.** 1981. Spontaneous mutations in the *Escherichia coli* prophage P1 and IS-mediated processes. Cold Spring Harbor Symp. Quant. Biol. **45**:38–40.
3. **Berg, D. E., J. Davies, B. Allet, and J.-D. Rochaix.** 1975. Transposition of R factor genes to bacteriophage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:3628–3632.
4. **Berg, D. E., C. Egner, B. J. Hirschel, J. Howard, L. Johnsrud, R. A. Jorgensen, and T. D. Tlsty.** 1981. Insertion, excision and inversion of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45**:115–123.
5. **Bernardi, A., and F. Bernardi.** 1981. Complete sequence of an IS element present in pSC101. Nucleic Acids Res. **9**:2905–2911.
6. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterisation of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
7. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
8. **Clerget, M.** 1984. A 140 base-pair DNA segment from the kanamycin resistance region of plasmid R1 acts as an origin of replication and promotes site-specific recombination. J. Mol. Biol. **178**:35–46.
9. **Clerget, M., M. Chandler, and L. Caro.** 1980. Isolation of an IS*1*-flanked kanamycin resistance transposon from R1*drd*19. Mol. Gen. Genet. **180**:123–127.
10. **Coggins, L. W., and E. McCluskey.** 1979. Characterization of the ColE1-Km plasmids pCR1 and pCR11 by electron microscope and restriction endonuclease mapping. Plasmid **2**:446–453.
11. **Courvalin, P., M. Fiandt, and J. Davies.** 1978. DNA relationships between genes coding for aminoglycoside-modifying enzymes from antibiotic-producing bacteria and R plasmids, p. 262–266. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.

12. **Foster, T. J.** 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. **47**:361–409.
13. **Grindley, N. D. F., and C. M. Joyce.** 1980. Genetic and DNA sequence analysis of the kanamycin resistance transposon Tn*903*. Proc. Natl. Acad. Sci. U.S.A. **77**:7176–7180.
14. **Hänni, C., J. Meyer, S. Iida, and W. Arber.** 1982. Occurrence and properties of the composite transposon Tn*2672*: evolution of multiple drug resistance transposons. J. Bacteriol. **150**:1266–1273.
15. **Iida, S.** 1983. On the origin of the chloramphenicol resistance transposon Tn*9*. J. Gen. Microbiol. **129**:1217–1225.
16. **Iida, S.** 1984. Bacteriophage P1 carries two related sets of genes determining its host range in the invertible C segment of its genome. Virology **134**:421–434.
17. **Iida, S., J. Meyer, and W. Arber.** 1981. Genesis and natural history of IS-mediated transposons. Cold Spring Harbor Symp. Quant. Biol. **45**:27–43.
18. **Iida, S., J. Meyer, and W. Arber.** 1983. Prokaryotic IS elements, p. 159–221. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
19. **Iida, S., J. Meyer, B. Bächi, M. Stålhammar-Carlemalm, S. Schrickel, T. A. Bickle, and W. Arber.** 1983. DNA restriction-modification genes of phage P1 and plasmid p15B. Structure and *in vitro* transcription. J. Mol. Biol. **165**:1–18.
20. **Iida, S., J. Meyer, K. E. Kennedy, and W. Arber.** 1982. A site-specific, conservative recombination system carried by bacteriophage P1. Mapping the recombinase gene *cin* and the cross-over sites *cix* for the inversion of the C segment. EMBO J. **1**:1445–1453.
21. **Iida, S., J. Meyer, P. Linder, N. Goto, R. Nakaya, H.-J. Reif, and W. Arber.** 1982. The kanamycin resistance transposon Tn*2680* derived from the R plasmid Rts1 and carried by phage P1Km has flanking 0.8 kb-long direct repeats. Plasmid **8**:187–198.
22. **Iida, S., B. Mollet, J. Meyer, and W. Arber.** 1984. Functional characterization of the prokaryotic mobile genetic element IS*26*. Mol. Gen. Genet. **197**:84–89.
23. **Isberg, R. R., and M. Syvanen.** 1981. Replication fusions promoted by the inverted repeats of Tn*5*. The right repeat is an insertion sequence. J. Mol. Biol. **150**:15–32.
24. **Labigne-Roussel, A., S. Briaux-Gerbaud, and P. Courvalin.** 1983. Tn*1525*, a kanamycin R determinant flanked by two direct copies of IS*15*. Mol. Gen. Genet. **189**:90–101.
25. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499—560.
26. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19**:269–276.
27. **Mollet, B., S. Iida, J. Shepherd, and W. Arber.** 1983. Nucleotide sequence of IS*26*, a new prokaryotic mobile genetic element. Nucleic Acids Res. **11**:6319—6330.
28. **Ohtsubo, H., M. Zenilman, and E. Ohtsubo.** 1980. Insertion element IS*102* resides in plasmid pSC101. J. Bacteriol. **144**:131–140.
29. **Oka, A., N. Nomura, K. Sugimoto, H. Sugisaki, and M. Takanami.** 1978. Nucleotide sequence at the insertion sites of a kanamycin transposon. Nature (London) **276**:845–847.
30. **Oka, A., H. Sugisaki, and M. Takanami.** 1981. Nucleotide sequence of the kanamycin resistance transposon Tn*903*. J. Mol. Biol. **147**:217–226.
31. **Prentki, P., F. Karch, S. Iida, and J. Meyer.** 1981. The plasmid cloning vector pBR325 contains a 482 base-pair-long inverted duplication. Gene **14**:289–299.
32. **Reznikoff, W. S.** 1983. Some bacterial transposable elements: their organization, mechanisms of transposition, and roles in genome evolution, p. 229–252. *In* J. Beckwith, J. Davies, and J. A. Gallant (ed.), Gene function in prokaryotes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Rothstein, S. J., R. A. Jorgensen, J. C.-P. Yun, Z. Yong-Di, R. C. Johnson, and W. S. Reznikoff.** 1981. Genetic organisation of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45**:99–105.
34. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
35. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *E. coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
36. **Terawaki, Y., Y. Kobayashi, H. Matsumoto, and Y. Kamio.** 1981. Molecular cloning and mapping of a deletion derivative of the plasmid Rts1. Plasmid **6**:222–234.
37. **Terawaki, Y., H. Takayasu, and T. Akiba.** 1967. Thermosensitive replication of a kanamycin resistance factor. J. Bacteriol. **94**:687–690.
38. **Thompson, C. J., and G. S. Gray.** 1983. Nucleotide sequence of a streptomycete aminoglycoside phosphotransferase gene and its relationship to phosphotransferases encoded by resistance plasmids. Proc. Natl. Acad. Sci. U.S.A. **80**:5190–5194.
39. **Trieu-Cuot, P., and P. Courvalin.** 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′5″-aminoglycoside phosphotransferase type III. Gene **23**:331–341.
40. **Trieu-Cuot, P., A. Labigne-Roussel, and P. Courvalin.** 1983. An IS*15* insertion generates an eight-base-pair duplication of the target DNA. Gene **24**:125–129.
41. **Wood, W. B.** 1966. Host specificity of DNA produced by *Escherichia coli*: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. **16**:118–133.