GENETIC AND BIOCHEMICAL CHARACTERIZATION OF A STREPTOCOCCAL CONJUGATIVE TRANSPOSON

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STATEMENT OF THE PROBLEM

Tn*5253*, formerly called the Ω(*cat tet*) element, was originally detected in the chromosome of the plasmid-free clinical isolate, *Streptococcus pneumoniae* BM6001 (6,12). The transposon encodes functions for its transfer en bloc among and within several streptococcal species via a process requiring cell-to-cell contact (31). A variety of conjugative transposons have been identified among streptococci so far. Besides transfer functions, each transposon may also carry one or more antibiotic resistance determinants, and the common feature among most, if not all, of these is the presence of a homologous tetracycline resistance determinant (33) of the type *tet*M (5). Other antibiotic resistance determinants include chloramphenicol (Cm^r), kanamycin (Km^r), erythromycin (Em^r), and streptomycin (Sm^r). Some of the other conjugative transposons include the Tn*916* (Tc) from *E. faecalis* DS16 (7,13), Tn*1545* (Tc Em Km) from *S. pneumoniae* BM4200 (9,10,15), Tn*3701* (Tc) from *S. pyogenes* A454 (2), and Tn*3951* (Tc Em Cm) from *S. agalactiae* B109 (19,20). Based on the size, these conjugative elements could be grouped into two types, one ranging from 16 to 25 kb, and the other around 60 kb.

The structural and genetic organization of Tn*5253*, as carried by the pneumococcal laboratory strain DP1322 (30), was studied. By inserting an *E. coli* vector plasmid, pVA891 (22), at many sites specifically within Tn*5253*, it was possible to clone and to recover parts of the element in *E. coli* (37). The

physical analysis of the passenger DNAs from these plasmids made it possible to construct a detailed restriction map of this 65.5 kb element, to localize the drug resistance determinants, and to identify its junction and target regions in the pneumococcal chromosome (Fig. 1) (36).

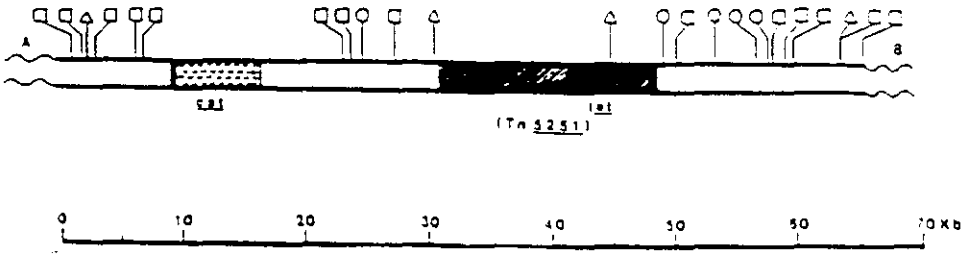

FIG. 1. Physical structure of Tn5253. Straight lines, Tn5253 DNA; wavy lines, pneumococcal DNA; A and B refer the cat and tet ends of the element respectively; □, cat region; □, direct repeats flanking the cat segment; □, tet region that transposes when removed from Tn5253; □, XbaI; ▵, KpnI; O, BamHI.

From an arbitrarily chosen left end of the element, the cat gene is 14 kb inside and flanked by direct repeats of about 3 kb, a copy of which is also present in the wild type Rx1 chromosome. These are thought to be possibly related to the frequent spontaneous curing of cat (26,30). Another internal region of 2.5 kb is also present in Rx1 in two copies. It remains to be seen whether these represent IS elements or their remains. The tet gene is located about 44 kb from the left end. In Rx1, Tn5253 prefers to insert at a specific site (36), and this is the same spot Tn3951 transfers to when Rx1 is mated with S. agalactiae B109 (16). However, unlike these larger conjugative

transposons, Tn916 inserts at several sites in the chromosome when introduced into Rx1 (26). With the cloned fragments derived from Tn5253, this study sought to undertake a comparative analysis of related conjugative transposons. Reported are some of the results that indicate that Tn5253 is a composite element of two independent conjugative transposons, Tn5251 and Tn5252.

METHODOLOGY

Bacterial strains, transformation, and conjugation.

Streptococcus pneumoniae Rx1 is our standard laboratory strain (30), and DP1322 is Rx1 carrying Tn5253 (30). DP1324 is DP1322 carrying the str-1 chromosomal point mutation conferring resistance to streptomycin (str^r). S. pyogenes ATCC21547 was obtained from the Stillwater Medical Center. S. agalactiae ATCC12386 and recombination deficient, rifampin resistant (rif^r), and fusidic acid resistant (fus^r) Enterococcus faecalis UV202 (38) were used as recipients in some of the conjugation experiments. Growth of pneumococcal cultures, conjugation, competence regimen, and plating techniques have been described (30,31). All the streptococcal strains were grown without aeration at 37°C in CAT medium, a rich broth containing casein hydrolysate and tryptone (USBC and Difco). Conjugal transfer was seen to be enhanced when both donors and recipients were grown in Difco casitone. Filter-matings between pneumococci were limited to four hours, whereas matings between pneumococcus and E. faecalis were allowed 18 hours at 37°C. Recombinant plasmids

were generated in recombination deficient E. coli strains DH1 or HB101 by transformation according to Hanahan (17).

DNA manipulation and analyses.

Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories and Promega. Enzyme reactions were carried out according to suppliers' recommendations.

Chromosomal DNA from S. pneumoniae was prepared by the method of Marmur (24). Lysis and DNA isolation from E. faecalis have been described (16). Similar methods were employed to isolate the chromosomal DNAs from S. agalactiae and S. pyogenes. E. coli isolates were screened for recombinant plasmids by agarose gel electrophoresis of rapid alkaline lysates (1). For other purposes, plasmid DNA was isolated from E. coli by using standard methods of cell lysis involving lysozyme treatment followed by sedimentation on cesium chloride-ethidium bromide density gradients.

Agarose gel electrophoresis and DNA purifications from agarose gels by electroelution were done essentially as described by Maniatis, et al. (23). DNA hybridizations were done by the method of Southern (34) using the GeneScreen Plus membrane (New England Nuclear Corp.) as support. Radioactive probe DNA was prepared by nick translation (23) of appropriate plasmids with ^{32}P-dCTP (New England Nuclear Corp.).

FINDINGS

The tet determinant in Tn5253 resides on an independent conjugative transposon, Tn5251.

The prevalence of a tet determinant of the type M and extensive homology between regions of DNA surrounding this gene among most of the conjugative transposons suggested that a smaller element such as the tet carrying Tn916 (16 kb) could have served as a progenitor in the evolution of the larger elements (8,16). Autoaccumulation of other heterologous elements was speculated to have resulted in the observed increase in size. If so, the termini of all the conjugative transposons would be expected to carry some degree of homology. However, two experimental observations were not consistent with this speculation. First, it was observed that the plasmid, pAM118 (14), containing the entire Tn916, failed to hybridize to either of the termini of Tn5253 when used as a probe in blot hybridization experiments. The homology within Tn5253 to Tn916 was confined only to the region containing tet in one contiguous segment (data not shown).

Further, when a 23 kb XbaI fragment containing the tet region from Tn5253 was cloned into pVA891 to create pVJ403, the plasmid was stable in E. coli if tetracycline selection was maintained. In the absence of selective pressure, an 18 kb segment containing the tet gene was excised and lost. The restriction maps of several of the deletion plasmids (Fig. 2B) were all similar, suggesting the excision of a defined segment of

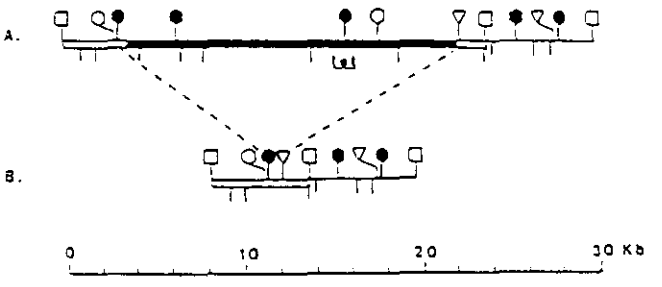

FIG. 2. Restriction endonuclease map of the E. coli plasmid, pVJ403, and the deletion derivative, pVJ403 tet. A, pVJ403; B, pVJ403 ▲ tet; □, XbaI; ●, KpnI; ▲, BamHI; ●, HindIII; O, EcoRI; T, HincII. The shaded region shows the 18 kb Tn5251 that excises in E. coli in the absence of tetracycline selection. The ends of Tn5251 were determined from DNA sequencing studies (not given). The location of the tet determinant is indicated.

DNA from pVJ403; and one of the deletion derivatives, pVJ403 ▲ tet, was kept for further study. Under similar conditions, the excision of a related 25 kb conjugative transposon, Tn1545, from a plasmid replicon and transposition into the E. coli chromosome had been shown to occur (11), suggesting that the excision of the tet from pVJ403 also could have been related to its transposition. To determine whether the tet resided on an independent transposon, even though it was recovered from a part of the larger Tn5253, pVJ403 was introduced into competent pneumococcal Rx1 cells. As the entry of donor DNA during pneumococcal transformation is in single stranded form (21), plasmid establishment would require entry of two overlapping complementary molecules and subsequent generation of an intact circle (27). Since the vector portion, pVA891, was incapable of autonomous replication in streptococci (22), Em^r transformants

were not expected, and none were found. As the wild-type Rx1 genome did not carry homology to any portion of the passenger DNA in pVJ403, genetic recombination by the normal pathway was not expected. However, about 50 Tc^r transformants per 2 x 10^7 cfu per 10 μg plasmid DNA resulted. All were sensitive to erythromycin, indicating that the vector was lost, and that the insertion of the heterologous tet marker did not involve the homology dependent insertion-duplication pathway (35). To determine whether the transposition of Tn5251 during transformation involved unique or multiple target sites, chromosomal DNAs from several Tc^r transformants were analyzed in blot hybridization experiments using pVJ403 and pVJ403 Δ tet as probes. Transposition did not involve any sequences beyond the 18 kb tet segment in pVJ403. This was evident as pVJ403 Δ tet did not react with any of the samples (data not shown). On the other hand, pVJ403 strongly hybridized to at least two fragments representing chromosome-tet element junction regions in each case (not shown). The differences in sizes of the junction fragments in different clones indicated random insertion of the element and ruled out the possibility of any plasmid forms. Further, three Tc^r clones were used as donors in filter-mating experiments with Rx1 recipients to test whether tet could be conjugally transferred from these transformants. Two were able to transfer tet at a frequency of 3 x 10^{-5} per donor under conditions where transfer of the chromosomal marker, str, could not be detected. From these results, it was clear that the tet determinant was

within a conjugative transposon, now termed Tn5251. The restriction map of this 18 kb transposon showed significant similarities to the 16 kb transposon, Tn916, suggesting possible common ancestry.

Tn5253 is a composite structure of two independent conjugative transposons, Tn5251 and Tn5252.

Since the properties of Tn5251, such as the target selection following conjugal transfer, were different from those of Tn5253, the study sought to determine the role of Tn5251 in the transfer of the larger element. If the transfer of the entire Tn5253 during conjugation was due to the presence of Tn5251, then Tn5253 devoid of Tn5251 would have been transfer deficient. To induce the deletion of Tn5251 from within Tn5253, using the plasmid pVJ403 Δ tet, the strategy shown in Fig. 3 was employed. As previously mentioned, Tn5251 was excised and lost from pVJ403 when propagated in E. coli without tetracycline selection. This gave rise to the deletion derivative, pVJ403 Δ tet. The derivative, pVJ403 Δ tet, was digested with XbaI, the 5 kb fusion fragment, isolated, and fed to competent DP1324 cells carrying the entire Tn5253. Due to the homology provided by the flanking regions in the fusion fragment, Tn5251 was expected to be deleted during transformation. In pneumococcal transformation a single stranded donor DNA molecule could have introduced either deletion or insertion of a heterologous DNA segment into the chromosome, as long as the region of alteration was flanked on either side by

a sufficient length of homologous DNA to permit efficient register. After allowing two hours for phenotypic expression in the liquid broth, the transformants were plated on a nonselective medium. The following day, four thousand colonies were replica-plated prior to screening for Tc^s and Cm^r transformants. Thirty Tc^s clones were found.

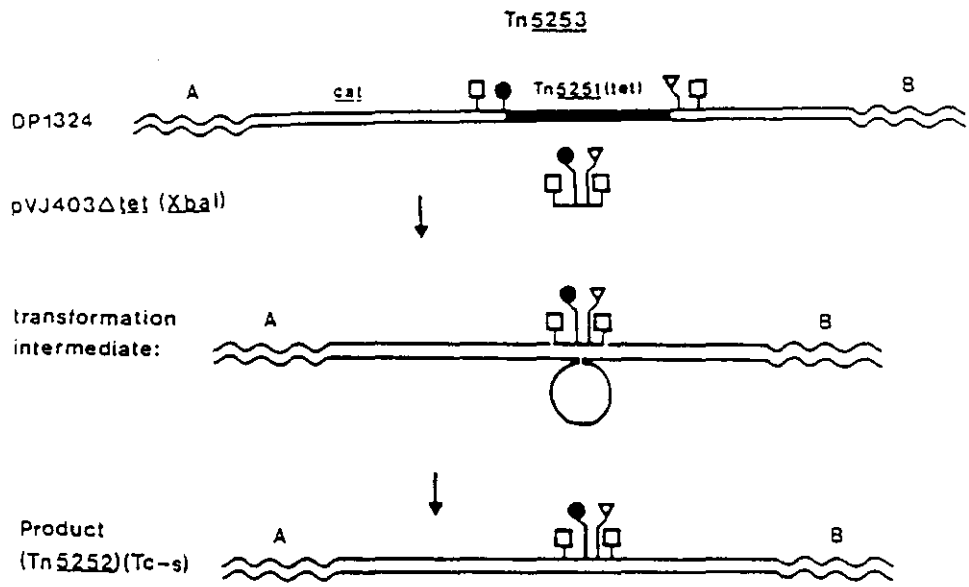

FIG. 3. Strategy for deleting Tn5251 from Tn5253. The relevant restriction sites are shown: □, XbaI; ●, HindIII; ▼, BamHI. The passenger DNA, the 5 kb fusion fragment carrying sequences flanking Tn5251 in Tn5253, from pVJ403 Δ tet was used as donor DNA. The donor molecule taken up as a single strand was expected to displace the resident strand and pair with the complementary strand, inducing the intervening segment containing Tn5251 to loop out. After one round of replication and segregation of markers, Tc^s transformants arose.

Physical analysis of the chromosomal DNAs from four of these clones in blot hybridization experiments using pVJ403 as a probe

confirmed the deletion of the 18kb Tn5251 from within Tn5253 in each case. One of these Cm^r Tc^s clones was designated SP1000. To determine whether SP1000 was capable of conjugal transfer of the sequences beyond Tn5251 within Tn5253, SP1000 cells were used as donors in filter-mating experiments with Rx1 recipients. Interestingly, Cm transferred at a frequency of 10^{-6} to 10^{-7} per donor, which was comparable to that of the intact parental Tn5253. As the DNA beyond Tn5251 within Tn5253, as in SP1000, was found to be capable of conjugal transfer by itself, this segment of DNA was termed Tn5252.

The results presented in this report showed that the streptococcal conjugative transposon, Tn5253, was a composite of at least two mobile elements. In parallel to our findings, Le Bouguenec, et al. have localized the transposon, Tn3703, within the >50 kb conjugative transposon, Tn3701, carried by S. pyogenes A454 (2-4). Based on hybridization studies done in other laboratories (3) and this one, Tn5251 appeared to be closely related to the Tn916, Tn1545, and Tn3703 class of transposons and could be distinct from the Tn5252 class of elements. The observed differences in the target selection following conjugation between the two types of elements may be due to different modes of transfer (26,36). Convincing evidence of a circular intermediate in the transposition of Tn916 has been presented (28), and it is likely that the other transposons of this type function similarly. However, while simultaneously

entering plasmids were being restricted, the lack of restriction of Tn5253 during conjugal transfer into recipients carrying DpnII system suggests an alternate transfer pathway for the larger conjugative transposons (16). Even though Tn5251 and Tn5252 are both capable of independent conjugal transfer, the separation of these elements has not been observed when both were associated together as Tn5253.

The origin and composition of the larger conjugative transposons may turn out to be somewhat complicated as indicated by the presence of Tn5251 and the two IS-like elements within Tn5253. The cat region flanked by direct repeats in Tn5253 has been shown to be homologous to the staphylococcal plasmid, pC194 (25). Further work may reveal whether a propensity exists for the autoaccumulation of various genetic units into prototype elements such as Tn5252 and Tn3701 to form larger conjugative structures. Without the 18 kb Tn5251 and the 7.5 kb cat segment, the remaining portion of DNA of Tn5253 constituting Tn5252 is about 40 kb. Localization of transfer-related and other genes within Tn5252 may provide further insight into the nature of this interesting element.

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