NOTES

Transposon Tn4556 of *Streptomyces fradiae*: Nucleotide Sequence of the Ends and the Target Sites

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A transposon, Tn4556, has recently been isolated from *Streptomyces fradiae* (S.-T. Chung, J. Bacteriol. 169:4436-4441, 1987). The ends of Tn4556 were found to contain inverted repeats of 38 base pairs with 70% sequence identity with the ends of Tn3. Insertion of Tn4556 into a *Streptomyces* plasmid resulted in a 5-base-pair duplication of the target site.

Transposable elements and their engineered derivatives are important tools in the study of bacterial gene expression. The recent description of a transposon isolated from the neomycin-producing bacterium *Streptomyces fradiae* (4), therefore, is of considerable interest. This transposon, Tn4556, is a 6.8-kilobase element that has been shown to transpose from chromosome to plasmids and vice versa. This paper describes the cloning and the sequencing of the ends of Tn4556 and the apparent relatedness of the transposon to members of the Tn3 family of transposons. This information will be useful in developing additional genetic tools with which to study *Streptomyces* genetics and in understanding the evolution of and functional differences between bacterial transposons.

**Subcloning the ends of Tn4556 into pBR322.** Plasmids pUC1210 and pUC1211 are thiostrepton-resistant deletion derivatives of the *S. fradiae* phage, SP1, that acquires Tn4556 upon transformation into *S. fradiae* UC8592 (4). One of these pUC1210::Tn4556 derivatives, pUC1214 (Fig. 1), contains a BamHI fragment not found in pUC1210 that is approximately the same size as Tn4556 (6.8 kilobases). It appears that Tn4556 has either inserted between two BamHI sites very near each other in pUC1210 or has created a new BamHI site upon insertion (data not shown). Since the ends of the transposon are probably very close to the BamHI site, analysis of the nucleotide sequence from the BamHI sites flanking the transposon should allow us to determine the sequence of the ends of Tn4556. The fragments containing the ends of Tn4556 were subcloned into pBR322 for sequencing (Fig. 1). Plasmid pUC1214 was cut with BamHI and ClaI, and the 1.5-kilobase fragment corresponding to the right end of the element was isolated and ligated into BamHI-ClaI-cut pBR322. All ligations were done in 15 μl (final volume) of 1× ligation buffer (11) at 15°C for 16 h. *Escherichia coli* DH1 (8) was transformed with the ligation mix, and the resulting ampicillin-resistant colonies were screened for tetracycline sensitivity. Plasmid DNA was isolated (1) from some of the tetracycline-sensitive colonies and analyzed by restriction endonuclease digestion and agarose gel electrophoresis (data not shown). Plasmid pUC1233, containing the BamHI-ClaI fragment corresponding to the right end of Tn4556, was identified by its restriction pattern and by its ability to hybridize to the purified right-end fragment. In a similar manner, the 1.2-kilobase BamHI-ClaI fragment containing the left end of Tn4556 was cloned into the BamHI site of pBR322. One of the plasmids from this construction, pUC1232, was used for further analysis (Fig. 1).

**Sequencing the ends of Tn4556.** The junctions between the BamHI site in pBR322 and the inserts in pUC1232 and pUC1233 were sequenced by the dideoxy method (14) as adapted to double-stranded templates (17). Plasmid pUC123 was sequenced with BamHI counterclockwise primer (New England Biolabs, Inc.), and pUC1232 was sequenced with BamHI clockwise primer. The sequence revealed the presence of two inverted repeats (IRs) adjacent to the BamHI sites (Fig. 2). The nucleotide sequence of the other strand for each IR was determined, and these sequences confirmed the sequence shown in Fig. 1. Figure 2a shows a comparison of the repeats with those from Tn3 (10, 13), Tn501 (2, 16), Tn917 (15), and Tn2501 (12). It appears from this analysis that Tn4556 is a member of the Tn3 family of transposons.

**Tn4556 insertion and 5-bp duplication of target site.** The sequences of the two junctions between Tn4556 and pUC1210 in pUC1214, as well as the site on pUC1210 into which the transposon had inserted, were determined with two oligonucleotides that are homologous to unique sequences in the ends of Tn4556 and one that is homologous to a site in pUC1210 that is 25 to 48 base pairs (bp) from the left end of Tn4556 (PL-1, Pharmacia PL Inc.; EO-17 and EO-20, N. Theriault, The Upjohn Co.). The DNAs used as templates for determining these sequences and the sequences of all other pUC1210::Tn4556 and pUC1211::Tn4556 derivatives were double-stranded plasmids purified through CsCl-ethidium bromide gradients as described previously (3). In pUC1214, the BamHI site was found to be directly adjacent to the terminus of the IRs, and at least part of the site was duplicated upon insertion of the transposon (Fig. 3). To precisely define the boundaries of the transposon and the size of the target site duplication, we sequenced three more left- and right-end junction fragments (Fig. 2b). The only model consistent with the sequencing data is one in which the left end of Tn4556 begins with 5'CGGGGT, the right end ends with 5'ACCCCC, and 5 bp of the target site are

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FIG. 1. (a) Maps of pUC1214 (4), used as a source of Tn4556 DNA and of pBR322 derivatives containing the ends of Tn4556. BamHI, EcoRI, BclI, and CiaI restriction sites are represented by Ba, Ec, Bc, and Cl, respectively. The heavy lines represent Tn4556 DNA. Ts', Tc', and Ap' refer to thiostrepton, tetracycline, and ampicillin resistance, respectively. Sequencing primers are represented by arrows and accompanying numbers; 1 is the BamHI clockwise primer, 2 is E0-17, 3 is the BamHI counterclockwise primer, and 4 is PL-1. (b) Nucleotide sequences of the IRs (uppercase letters) and flanking pBR322 DNA (lowercase letters). The BamHI site is underlined, Kb, Kilobases.

- Tn4556 IR-L: CGGGTTGAGAACATCGGAAACCGGGCCTAAG
- Tn4556 IR-R: **********************
- Tn501: GGGGAAACCGCGAAATCGGAAACCGGGCCTAAG
- Tn3: GGGGCTAGCTAGCTAAGCTAAG
- Tns01: GGGGTTGAGAACATCGGAAACCGGGCCTAAG
- Tn917 IR-L: GGGGTTGAGAACATCGGAAACCGGGCCTAAG
- Tn917 IR-R: GGGGTTGAGAACATCGGAAACCGGGCCTAAG
- Tn2501 IR-L: GGGGTTGAGAACATCGGAAACCGGGCCTAAG
- Tn2501 IR-R: GGGGTTGAGAACATCGGAAACCGGGCCTAAG

The underlined bases correspond to bases duplicated upon insertion. The analysis of left-end junctions for four more insertions (pUC1124, pUC1220, pUC1221, and pUC1222) supports our model. Analyses of other Tn3-like transposons (for a review, see reference 9) have shown that they also duplicate 5 bp of the target site.

Class II transposons that have homology to the ends of Tn3 have been divided into two groups (6). One group is represented by Tn3 (7, 9, 10, 13); examples of the second group include Tn21 (5, 18) and Tn501 (2, 16). The bases of this division are the organization of the two transposon-encoded genes known to be involved in transposition and the ability of these functions to complement each other. Two other class II transposons, Tn917 and Tn2501, that do not fit into the Tn3 or Tn501 subclass have been isolated. Tn4556 shares with these other members the property of duplicating 5 bp of the target site during transposition (9). A comparison of the IRs from Tn501, Tn3, Tn917, and Tn2501 with Tn4556 is shown in Fig. 2a. Lining up the IRs base for base shows two regions of sequence identity among other members. First, except for the left end of the Tn4556 IR, they begin

FIG. 2. (a) Comparison of the Tn4556 IR with those from Tn501, Tn3, Tn917, and Tn2501 (9, 12, 15). Nucleotides that are conserved are marked by asterisks. L and R indicate left and right ends, respectively. (b) Nucleotide sequences of several junction fragments between Tn4556 IRs and their insertion sites. The plasmids analyzed have been described previously (4). Lowercase letters refer to flanking sequences, and uppercase letters refer to the IR termini. The underlined bases correspond to bases duplicated upon insertion.
with a run of four Gs at their outside ends (the left end of the Tn4556 IR starts with pCGGG). The second area conserved is from positions 32 to 38 and consists of 5' CGN TAAG. In all class II transposons but Tn2501, this sequence corresponds to the internal end of the IR. Additional identities can be observed if one allows for 1-bp bulges at different locations. The strongest sequence identity observed is between the Tn4556 IR and the Tn3 IR, with 26 (70%) of 38 bp identical. Since the ends of these elements are thought to function as the target for the transposase as well as to interact with the DNA at the insertion site, the mechanism of transposition of Tn4556 probably shares some features with that of the other class II transposons. Additional physical and biological characterizations of Tn4556 and the transposase and resolvase likely to be encoded by it will not only aid in understanding the molecular events involved in transposition but also help in understanding the evolution of these elements in bacteria.

*S. fradiae* is a gram-positive soil bacterium used in the production of the antibiotic neomycin. Although Tn4556 was isolated from *S. fradiae* UC8592, it has been demonstrated to transpose both from a plasmid to a chromosome and vice versa in other strains of streptomycetes (4; S. T. Chung, unpublished results). By adding other genetic elements to Tn4556 (e.g., other resistance elements, bacteriophage-packaging sites, plasmid-transfer sites, or biosynthetic antibiotic genes), derivatives of Tn4556 could be constructed and used for manipulating and increasing understanding of the genes involved in antibiotic production by and cellular differentiation of streptomycetes.

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**LITERATURE CITED**