Transposon Tn7 Directs Transposition into the Genome of Filamentous Bacteriophage M13 Using the Element-Encoded TnsE Protein

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The bacterial transposon Tn7 has a pathway of transposition that preferentially targets conjugal plasmids. We propose that this same transposition pathway recognizes a structure or complex found during filamentous bacteriophage replication, likely by targeting negative-strand synthesis. The ability to insert into both plasmid and bacteriophage DNAs that are capable of cell-to-cell transfer would help explain the wide distribution of Tn7 relatives.

Tn7 is a 14-kb bacterial transposon that only activates its transposition machinery when specific targets are found in the cell (reviewed in references 5 and 19). Tn7 can use two transposition pathways that recognize different types of target sites with distinct but overlapping sets of transposon-encoded proteins, TnsA, TnsB, TnsC, TnsD, and TnsE (TnsABCDE). The TnsABC proteins constitute the core transposition machinery that normally interacts with one of the two target site-selecting proteins, TnsD or TnsE, to carry out transposition. Transposition with the TnsABC+D proteins catalyzes Tn7 transposition into a single site found in Escherichia coli and a wide variety of other bacteria, called the attachment site (attTn7). TnsD is a sequence-specific DNA binding protein that recognizes a sequence in the C-terminal-encoding portion of glmS but directs transposition events into the attTn7 site in a single orientation with the TnsABC proteins (1). Presumably, the TnsE-mediated pathway would facilitate the dissemination of Tn7 to new hosts, while the TnsD-mediated pathway would provide a “safe haven” once in a new bacterial host.

The TnsABC+E transposition machinery recognizes a structure or complex found during active conjugal DNA replication. TnsE-mediated transposition events preferentially occur into the conjugal plasmid (>90%) in the cell, even though in these experiments the plasmid only comprises ~1% of the DNA in the cell (17, 27). Nonconjugal plasmids are not preferred targets, and mobilizable plasmids are only targets when actively mobilized (27). Genetic results show that TnsE-mediated transposition occurs into conjugal plasmids in recipient cells, likely by recognizing a component of lagging-strand DNA synthesis during rolling-circle DNA replication. In the donor cell, leading-strand DNA synthesis displaces the strand that is then transferred to the recipient bacterium (7, 26). Because single-stranded DNA is introduced into the recipient cell in the 5′-to-3′ direction, and because it is replicated as it enters the cell, DNA replication in the recipient cell must continually be reprimed in a discontinuous process. Tn7 displays an orientation bias in which the right end of the transposon is juxtaposed with the 3′ end of the nascent lagging strand of conjugal plasmid replication. The same orientation bias with regard to the lagging strand occurs when TnsE directs transposition to targets found in chromosomal replication (17). We presume that something is special about lagging-strand DNA synthesis found during conjugation that preferentially attracts TnsE-mediated transposition.

Tn7 is remarkably prevalent in a variety of environments (16). While conjugal plasmids are abundant in environmental settings, additional targets beyond actively conjugating plasmids may exist that could facilitate the dissemination of Tn7 and its relatives. Bacteriophage P1 replication was previously shown to not be a TnsE-mediated transposition target (27); however, it is unknown whether the filamentous bacteriophage are a productive target.

TnsD-mediated transposition can occur into the genome of an M13 derivative carrying attTn7. One potential complication in determining if M13 is a transposition target is that the majority of the M13 genome encodes functions essential for bacteriophage growth. Therefore, we inserted 2.3 kb of exogenous DNA that would not be required for bacteriophage growth. We chose to clone the attTn7 region of the E. coli chromosome into M13mp18 using PCR because it included the specific site normally utilized by TnsD-mediated transposition that could serve as a positive control in our experiments (subsequently called M13-attTn7). When the M13-attTn7 bacteriophage was grown on a Tn7 donor strain containing a mini-Tn7 element with the TnsABCD proteins provided in trans (JJ55 pOX-Gen pCW4minMu#76), bacteriophage conferring resistance to kanamycin (the transposon marker) were readily isolated (Tables 1 and 2). In this assay, bacteriophage were grown...
on the Tn7 donor strain for 2 hours to allow a 100-fold increase in bacteriophage. To determine the number of mini-Tn7-contain-
ing bacteriophage, 1.6 × 10^9 bacteriophages were used to infect 5 × 10^8 actively growing E. coli XL1-Blue cells as a reporter strain. M13 bacteriophage with mini-Tn7 was detected by the ability to confer kanamycin resistance. Transposition frequency was determined by dividing the number of CFU found on LB medium supplemented with kanamycin (50 μg/ml), tetracycline (20 μg/ml), and nalidixic acid (5 μg/ml) by the total number of infectious bacteriophage particles used to infect the host (tetracycline and nalidixic acid were used to counterselect against residual Tn7 donor cells in the bacteriophage lysate).

Sequencing confirmed that transposition events were occurring at the single predicted position and orientation within the fragment and made the expected 5-bp target site duplication (5). The frequency of kanamycin-resistant bacteriophages was 4.8 × 10^-7 (kanamycin resistance-conferring bacteriophages per total bacteriophages) after 2 hours of growth. The frequency of kanamycin-resistant bacteriophages modestly increased at 4 and 6 hours, to 6.2 × 10^-7 and 7.1 × 10^-7, respectively. No kanamycin-resistant bacteriophage was found with a vector-only control (i.e., lacking transposition cassettes). This experiment indicated that M13 could tolerate the insertion of a mini-Tn7 element and that the attTn7 site was functional. This was consistent with a previous result with full-length Tn7; however, in the experiment presented here we can rule out any contribution of TnsE or other known transpo-son-encoded proteins in targeting an attTn7 site in M13 (21).

**TnsE-mediated transposition occurs into the M13 genome.** When M13-attTn7 was grown for 2 hours on a host that expressed the TnsABC+E proteins, we were also able to identify kanamycin resistance-conferring bacteriophage (Fig. 1). The wild-type TnsE protein allowed transposition at a frequency of about 3 × 10^-10 when expressed from a lac promoter (Fig. 1). We also examined the ability of a series of high-activity mutants that were isolated previously in a genetic screen (17). The mutant proteins are toxic when expressed at anything but very low levels and are therefore introduced on a vector with a very low copy number and expressed from the native tnsE promoter (17). Despite the lower expression levels, the increased activity mutants directed transposition into M13 at levels higher than the wild-type protein. In fact, we found that transposition could not be detected when wild-type TnsE was expressed at this level; wild-type TnsE needed to be expressed at higher levels to give any kanamycin resistance-conferring bacteriophage in this assay (Fig. 1 and data not shown).

DNA sequencing revealed that TnsE-mediated transposition could occur throughout a large portion of the M13-attTn7 bacteriophage genome. This included 15 independent inser-
tion events in the M13mp18 backbone and 10 independent insertions into the cloned fragment from the E. coli chromo-

### TABLE 1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Construction or reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLC28</td>
<td>araD139 Δ(argF-lac)169 rpsL150 relA ilvD5301 Δ(fimB-fimE)632:IS1 (fimK-yeiT725 Val)</td>
<td>3, 12, 20</td>
</tr>
<tr>
<td>JF39</td>
<td>NLC28 attTn7::mini-Tn7 Kan^a</td>
<td>P1(JF400) × JF31^a</td>
</tr>
<tr>
<td>JF55</td>
<td>NLC28 recA56 attTn7::mini-Tn7 Kan^a</td>
<td>This work^b</td>
</tr>
<tr>
<td>JF31</td>
<td>NLC28 attD-500::Tn10</td>
<td>P1 (CAG18431) X NLC28</td>
</tr>
<tr>
<td>JF400</td>
<td>NLC28 pCW4 mini-Mu#76 pEMΔ</td>
<td>This work</td>
</tr>
<tr>
<td>CAG18431</td>
<td>MG1655 attD-500::Tn10</td>
<td>JF31, NLC28</td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdRI71 glnV44 relA1 [F' proAB lacF (lacZ)ΔM15 Tn10]</td>
<td>Strategene</td>
</tr>
</tbody>
</table>

^a P1 transduction was via linkage between attD and the chromosomal attTn7 site. Donor phage was grown on strain JF400, which allows transposition into the chromosomal attTn7 site from plasmid pEMΔ (this table and Table 2) (1). The mini-Tn7 Kan^a transposition events in the donor cell attTn7 site could be cotransduced to the recipient strain using the linked wild-type attD gene.

^b The recA56 derivative was constructed using strD-3131::Tn10 (25) linked to recA56 as described previously (18).

### TABLE 2. Plasmids and bacteriophages used in this study

<table>
<thead>
<tr>
<th>Plasmid or bacteriophage</th>
<th>Relevant information^c (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCW4 miniMu#76</td>
<td>Tetracycline-resistant (Tet') pACYC184 derivative expressing TnsABC+D (25)</td>
</tr>
<tr>
<td>pCW15</td>
<td>Chloramphenicol-resistant (Cam') pACYC184 derivative encoding TnsABC (25)</td>
</tr>
<tr>
<td>pJP103</td>
<td>pTA106 (pSC101) derivative encoding TnsE; low-level TnsE expression from the native tnsE promoter; ampicillin resistant (Amp') (17)</td>
</tr>
<tr>
<td>pJP104</td>
<td>pTA106 (pSC101) derivative encoding TnsE; high TnsE expression from a lac promoter; Amp' (17)</td>
</tr>
<tr>
<td>pGEM-attTn7</td>
<td>3,372-bp attTn7-containing fragment amplified using PCR primers JEP17 (5'-ACA TGG GAT GAG GAG ATA ACA TAA TCT CCC-3') and JEP18 (5'-TGT TAA TGC CGG ATG CGG CTA AAA ACA CCG-3') and cloned using the Promega pGEM-T Easy Vector system 1 kit</td>
</tr>
<tr>
<td>M13-attTn7</td>
<td>Contains a mini-Tn7 element, a Kan' cassette flanked by left and right Tn7 ends (1)</td>
</tr>
<tr>
<td>pACYC184</td>
<td>Contains a mini-Tn7 element, a Kan' cassette flanked by left and right Tn7 ends (1)</td>
</tr>
<tr>
<td>pTA100</td>
<td>pSC101 replicon; Tet' Cam' (4)</td>
</tr>
<tr>
<td>M13mp18</td>
<td>Contains a mini-Tn7 element, a Kan' cassette flanked by left and right Tn7 ends (1)</td>
</tr>
<tr>
<td>pOX-Gen</td>
<td>F plasmid derivative; Gen' (9)</td>
</tr>
</tbody>
</table>

^c Plasmids used in this study were made using standard methods (13, 22).

^d Restriction analysis of the replicative (double-stranded) form of the bacteriophage and DNA sequencing of the ends of the new fusion joints confirmed that the fragment was cloned as expected.
some (Fig. 2). We assume that transposition events in the remainder of the M13 genome could not be detected because they would result in bacteriophage that could not be further propagated, a requirement for this assay. As expected, we were never able to isolate kanamycin-resistant cells (−10−11) was found in the assay where the F−Tn10 had mated into residual host strains from the XL1-Blue strain. These were easily identifiable because they contained all of the JF55 chromosomal and plasmid makers (i.e., resistance to rifampin, chloramphenicol, and ampicillin but sensitivity to gentamicin via loss of the pOX-Gen by replicon exclusion). Restriction analysis and DNA sequencing absolutely confirmed that the mini-Tn7 element was not contained on the M13-attTn7 plasmid. Error bars show the standard errors of the means (n = 9).

The TnsE-mediated transposition events occurred at many different positions in M13, consistent with previous results with the TnsE pathway of transposition; TnsE-mediated transposition into the M13 genome occurred with a specificity that was reminiscent of a bias of TnsD-mediated insertion events (14). Negative-strand DNA synthesis is required to produce the replicative (double-stranded) form of the bacteriophage and sequencing from the left end of the element using a primer complementary to this end. To confirm that the process of transposition was responsible for relocating the element into the bacteriophage, we sequenced a subset of the insertions from both ends and in all cases identified a 5-bp duplication that is indicative of Tn7 transposition. The asterisk indicates where identical insertions were found in this experiment that are likely siblings.

FIG. 1. Frequency of Tn7 transposition into the M13-attTn7 genome in E. coli in various genetic backgrounds. Wild-type TnsABC was expressed from pCW15 (wt) or a pCW15 derivative with the TnsCA225V mutation (mut) (24). pTA106 was included as a vector control (vector only). Wild-type or mutant derivatives of TnsE, TnsEA453V (A453V), TnsED523N (D523N), or the double mutant TnsE-A453V-D523N (A453V D523N) were expressed from pJP103. Wild-type TnsE was expressed with pJP104 (wt*). M13-attTn7 bacteriophage containing mini-Tn7 in strains expressing wild-type TnsABC without TnsE, in the mutant TnsABC A225V, or with TnsABC with wild-type TnsE expressed from pCW15 (wt) or a pCW15 derivative with the TnsCA225V mutation (mut) (24). pTA106 was included as a vector control (vector only). Wild-type or mutant derivatives of TnsE, TnsEA453V (A453V), TnsED523N (D523N), or the double mutant TnsE-A453V-D523N (A453V D523N) were expressed from pJP103. Wild-type TnsE was expressed with pJP104 (wt*). M13-attTn7 bacteriophage containing mini-Tn7 in strains expressing wild-type TnsABC without TnsE, in the mutant TnsABC A225V, or with TnsABC with wild-type TnsE expressed from pJP103 was never identified (<3 × 10−11). A very low background of spontaneous nalidixic acid-resistant cells (−10−11) was found in the assay where the F−Tn10 had mated into residual host strains from the XL1-Blue strain. These were easily identifiable because they contained all of the JF55 chromosomal and plasmid makers (i.e., resistance to rifampin, chloramphenicol, and ampicillin but sensitivity to gentamicin via loss of the pOX-Gen by replicon exclusion). Restriction analysis and DNA sequencing absolutely confirmed that the mini-Tn7 element was not contained on the M13-attTn7 plasmid. Error bars show the standard errors of the means (n = 9).

FIG. 2. Representation of the 9,505-bp M13-attTn7 bacteriophage genome and the position of the TnsE-mediated mini-Tn7 insertion events. Numbering follows the previously established convention for M13mp18 (28). The M13mp18 sequence extends from bp 8529 to 9505/1 to 6266. The M13 origin (ori; bp 5487 to 5867), ‘glmS’ (bp 6267 to 7371), ‘pstS’ (bp 7708 to 8529), and the position of TnsD-mediated insertion events (attTn7) are indicated. Independent transposition events are indicated by arrows either inside (left-to-right insertion events) or outside (right-to-left insertion events) the circle. The positions of mini-Tn7 insertion events are indicated in parentheses along with the strain background according to the following letter code: A, pCW15 (TnsABC) pJP104 (TnsEwt); B, pCW15 pJP103 (TnsEwtD523N); C, pCW15 pJP103 (TnsEwtD523N). The position and orientation of individual transposition events were determined by isolating the replicative (double-stranded) form of the bacteriophage and sequencing from the left end of the element using a primer complementary to this end. To confirm that the process of transposition was responsible for relocating the element into the bacteriophage, we sequenced a subset of the insertions from both ends and in all cases identified a 5-bp duplication that is indicative of Tn7 transposition. The asterisk indicates where identical insertions were found in this experiment that are likely siblings.

Frequency and targeting of transposition. High-frequency transposition with a random transposition pathway catalyzed by the mutant TnsABC-A225V machinery did not give detectable transposition into the M13-attTn7 vector (Fig. 1). Transposition with the core machinery with this TnsCA225V mutant...
allows transposition into the chromosome that is approximately 10-fold higher than levels found with TnsABC+E (24). The lack of observable transposition with the TnsABC-A225V machinery into the M13-attTn7 vector, under conditions in which we do see transposition with TnsABC+E, suggests that the ability to target the M13 genome is specific to the TnsE pathway. This result is consistent with previous results with conjugal DNA replication (24, 27).

The low frequency of transposition into the bacteriophage genome is likely due to the limited availability of duplex DNA during the M13 life cycle; duplex DNA is required for a DNA molecule to act as a transposition target, because only the 3' ends of the element are joined to a target DNA (6). The short amount of time that the filamentous bacteriophage normally spends as a double-strand breaks and into regions where chromosomal DNA replication terminates. Mol. Cell Biol. 20:3492–3501.

Tn7 has two pathways of transposition that facilitate the dissemination and establishment of the element within a bacterial genome while minimizing the impact on the host bacterium. Here we have described how Tn7 targets a filamentous bacteriophage genome, suggesting an expanded repertoire of vectors capable of transferring Tn7 from host to host.

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REFERENCES


