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Tn7 Transposition In Vitro Proceeds through an Excised Transposon Intermediate Generated by Staggered Breaks in DNA

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Summary

We have developed a cell-free system in which the bacterial transposon Tn7 inserts at high frequency into its preferred target site in the Escherichia coli chromosome, *attTn7*; Tn7 transposition in vitro requires ATP and Tn7-encoded proteins. Tn7 transposes via a cut and paste mechanism in which the element is excised from the donor DNA by staggered double-strand breaks and then inserted into *attTn7* by the joining of 3′ transposon ends to 5′ target ends. Neither recombination intermediates nor products are observed in the absence of any protein component or DNA substrate. Thus, we suggest that Tn7 transposition occurs in a nucleoprotein complex containing several proteins and the substrate DNAs and that recognition of *attTn7* within this complex provokes strand cleavages at the Tn7 ends.

Introduction

Mobile DNA segments translocate by a variety of mechanisms. These mechanisms differ in the nature of the recombining sites, the type and order of strand breakage and joining reactions, and the degree of involvement of DNA replication. We are interested in understanding the molecular basis of transposition, a reaction in which a discrete DNA segment moves between nonhomologous DNA sites (Berg and Howe, 1989). This reaction is distinguished by the fact that it involves three distinct DNA segments, the two ends of the transposon and the target site; no sequence similarity between the transposon ends and the target site is required. Another hallmark of transposition is that element insertion is accompanied by the duplication of several base pairs of target sequence. This duplication results from the introduction and subsequent repair of a staggered break in the target DNA, the transposon being joined to the ends of the overhanging target strands at this break.

We are particularly interested in understanding the transposition of the bacterial transposon Tn7 (Barth et al., 1976; Craig, 1989), because this element displays an unusual degree of insertion specificity. Most transposable elements show relatively little insertion site selectivity when transposing to large DNA molecules (Berg and Howe, 1989). By contrast, Tn7 inserts at high frequency into a single site in the Escherichia coli chromosome called *attTn7* (Barth et al., 1976; Lichtenstein and Brenner, 1982; Craig, 1989). Tn7 is unrelated in nucleotide sequence to *attTn7* (Lichtenstein and Brenner, 1982; Gay et al., 1986; McKown et al., 1988); thus, no DNA sequence homology is involved in the selection of *attTn7* as a preferential target. This element's transposition machinery is elaborate. Tn7 encodes a surprising array of transposition genes, *tnsABCDE*. Insertion into *attTn7* in vivo requires four of these genes, *tnsABC* + *tnsD* (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). Large DNA sites at both ends of Tn7 (Arciszewska et al., 1989) and at *attTn7* (McKown et al., 1988; Gringauz et al., 1988; Qadri et al., 1989) are also required. When *attTn7* is unavailable, Tn7 resembles most other transposable elements, moving at low frequency and inserting into many different sites (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). This alternative transposition pathway requires a distinct, but overlapping, ensemble of four *tns* genes, *tnsABC* + *tnsE*.

Although many transposable elements have been characterized genetically, only a few have been examined biochemically (Mizuuchi, 1983; Brown et al., 1987; Morisato and Kleckner, 1987; Eichinger and Boeke, 1988). The high frequency of Tn7 insertion into *attTn7* suggested that this reaction would be amenable to biochemical dissection.

We report here the development of a cell-free system for Tn7 transposition. We find that ATP and four *tns* fractions, i.e., protein preparations derived from cells containing individual *tns* genes, are required for the formation of recombination intermediates or products; no recombination is detected when any fraction is omitted. Furthermore, the presence of the *attTn7* target site is required for the initiation of recombination, i.e., the production of recombination intermediates. These results suggest that Tn7 transposition is executed by a nucleoprotein assembly containing multiple proteins and the substrate DNAs and that the proper assembly of this entire recombination machine is required for the strand breakage reactions that initiate recombination.

We also demonstrate that Tn7 transposition in vitro is a nonreplicative reaction that proceeds by a series of double-strand breaks that disconnect the transposon from the flanking donor DNA to produce an excised transposon, which is then inserted into *attTn7*. The DNA breakage reactions at the transposon ends and at the target DNA both occur by staggered breaks that generate 5′ overhanging ends. Tn7 insertion occurs via single-strand joins between the 3′ transposon ends and the 5′ ends of target DNA.

Results

Establishment of a Cell-Free System for Tn7 Transposition

Tn7 transposition in vitro occurs efficiently when substrate DNAs, a donor plasmid containing a mini-Tn7 transposon,

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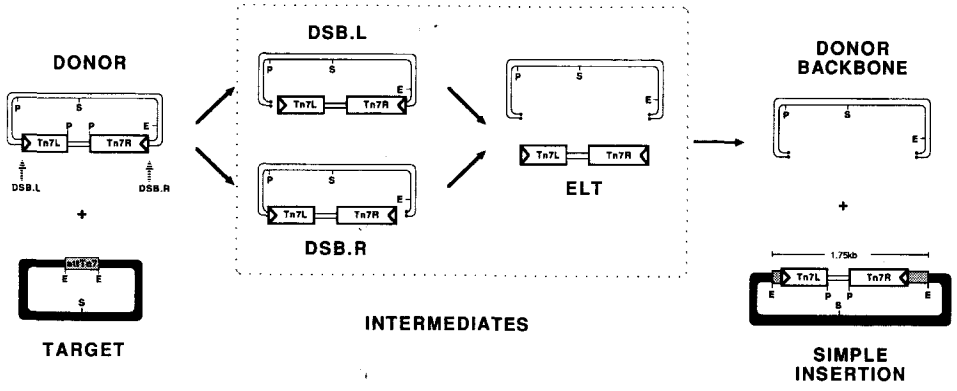

Figure 1. Tn7 Transposition Substrates, Intermediates, and Products

The 6.1 kb donor molecule contains a 1.6 kb mini-Tn7 element flanked by sequences unrelated to *attTn7*; the mini-Tn7 element contains segments from the left (Tn7L) and right (Tn7R) ends of Tn7 that provide the cis-acting transposition sequences. The 2.9 kb target molecule contains a 0.15 kb *attTn7* segment flanked by EcoRI sites. Double-strand breaks at the junction of either Tn7L or Tn7R with the donor backbone (arrows) produce the DSB.L and DSB.R species, which are transposition intermediates. An excised linear transposon (ELT) is generated by a second double-strand break in the DSBs. The excised mini-Tn7 element inserts site- and orientation-specifically into *attTn7*, producing a 4.5 kb simple insertion product and a 4.5 kb gapped donor backbone. EcoRI digestion of the simple insertion product releases a diagnostic 1.75 kb fragment. Restriction sites: E = EcoRI, P = PstI, and S = ScaI.

and a target plasmid containing *attTn7* (Figure 1), are incubated at 30°C with *tns* protein fractions, ATP, and MgAc. Recombination is detected by extraction of DNA from the reaction mixture, digestion with restriction enzymes, separation by gel electrophoresis, and hybridization with specific probes.

After incubation, several new species are detectable with a mini-Tn7-specific probe (Figure 2A): a simple insertion transposition product (mini-Tn7 in *attTn7*) and several recombination intermediates, including donor DNA molecules that are broken by a double-strand break at either the Tn7L–backbone junction (DSB.L) or the Tn7R–backbone junction (DSB.R) (see Figure 1). Insertion specificity was verified by DNA sequence analysis of cloned *attTn7*::mini-Tn7 segments. All insertions were orientation-specific, i.e., with Tn7R oriented towards the bacterial *glmS* gene, and were accompanied by a target duplication of 5 bp. More than 85% of the insertions occurred at either of two adjacent nucleotide positions in *attTn7*, and a few nearby insertions were also observed. This distribution closely resembles the pattern of Tn7 insertions into *attTn7* in vivo (Lichtenstein and Brenner, 1982; Gay et al., 1986; McKown et al., 1988; Gringauz et al., 1988). The efficiency of the cell-free reaction is striking, especially in view of the fact that it is conducted in crude extracts: translocation of about 25% of the Tn7 elements from donor molecules to target molecules is readily observed.

Tn7 transposition to *attTn7* in vivo requires four Tn7-

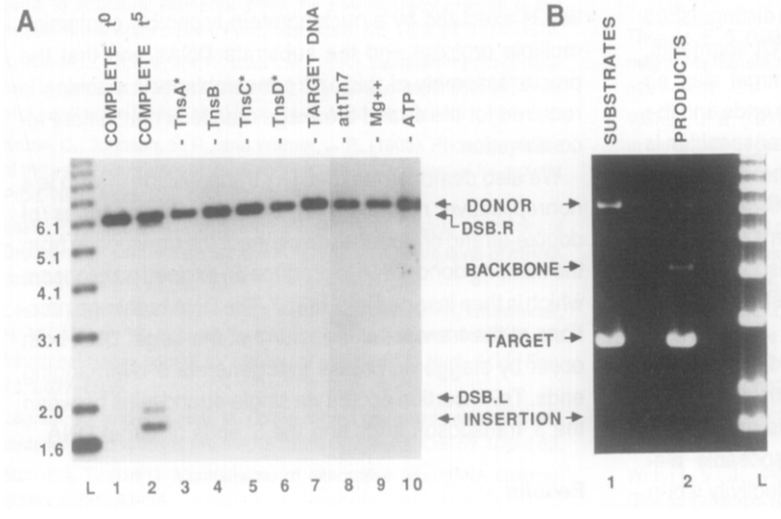

Figure 2. Tn7 Transposition In Vitro

(A) Requirements for Tn7 transposition. Shown is an autoradiogram that displays EcoRI-digested species detected with a mini-Tn7-specific probe from transposition reactions in which the indicated components were omitted. DNA molecules are labeled as in Figure 1; DSB.L and DSB.R appear very different in size because of the EcoRI digestion. Reactions were performed as described in the Experimental Procedures, except for lane 8, where plasmid lacking *attTn7* was used as a target instead of the usual *attTn7*-containing plasmid. Asterisks denote that the indicated Tns protein was supplied in a crude fraction that also contained host proteins. Following the preincubation step, reactions were incubated for 5 min at 30°C, except that shown in lane 1, which was stopped immediately after mixing of the reaction components. Lane L shows DNA size markers.

(B) A gapped donor backbone is a transposition product. An ethidium-stained agarose gel is shown, which displays the EcoRI-digested substrates (lane 1) and products (lane 2) of an in vitro Tn7 transposition reaction; DNA molecules are labeled as in Figure 1. After recombination, two new species are evident: an *attTn7* segment molecule containing a simple insertion of mini-Tn7 and a donor backbone from which the mini-Tn7 element has been excised. Lane L shows DNA size markers.

encoded genes, *tnsABC + tnsD* (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). Tn7 transposition in vitro can occur efficiently when four protein fractions, each derived from cells containing one of these *tns* genes, are mixed (Figure 2A). In this experiment, the *tnsA* fraction is a slightly fractionated extract containing TnsA, the *tnsB* fraction is substantially purified TnsB protein, the *tnsC* fraction is a slightly fractionated extract containing TnsC, and the *tnsD* fraction is partially purified TnsD protein. It is notable that no transposition intermediates or products are detected when any one of the *tns* fractions is omitted (Figure 2A, lanes 3–6).

We also find that no recombination intermediates or products are detected when *attTn7* is omitted (Figure 2A, lane 8). This result is particularly interesting, as it suggests that the transposon ends "see" the target site before recombination actually begins, i.e., before any double-strand breaks occur at the transposon ends. We have been unable to detect any changes in the target plasmid when any *tns* fraction or the mini-Tn7 donor plasmid is omitted from the reaction (data not shown).

These experiments show that the four *tns* fractions and the *attTn7* target are required for recombination. Notably, the production of both recombination intermediates and products requires the presence of all the reaction components, i.e., no partial reactions are detected when any single component is omitted. An attractive hypothesis that explains these results is that Tn7 transposition is a highly concerted reaction carried out by a nucleoprotein assembly (machine) containing the DNA substrates and multiple recombination proteins. Because transposition in vitro requires combining four different fractions, at least four different proteins are likely to participate directly in recombination.

Tn7 transposition in vitro has several other requirements. ATP (Figure 2A, lane 10) or dATP (data not shown) must be added. Efficient recombination also requires 10 mM MgAc (lane 9). If MgAc is included at the beginning of the incubation, little recombination is observed (data not shown); substantially more (perhaps 50-fold) recombination occurs when MgAc is added after a preincubation step. Transposition is most efficient with supercoiled substrates, but considerable transposition can be observed with relaxed DNAs (data not shown). Recombination is stimulated by the presence of polyvinyl alcohol or polyethylene glycol (data not shown).

Tn7 Transposition In Vitro Does Not Involve Replication of the Element

A key issue in understanding a transposition reaction is determining whether the transposon is copied by DNA replication or whether it is transferred from the donor molecule to the target molecule via a nonreplicative, cut and paste reaction. Several observations indicate that Tn7 transposition in vitro is nonreplicative. In addition to the simple insertion of mini-Tn7 into *attTn7*, another transposition product is a gapped donor molecule, i.e., a donor backbone from which mini-Tn7 has been excised (Figure 2B). The simple insertion product and gapped donor backbone appear with approximately the same time course and in equimolar amounts (data not shown). Production of this gapped donor backbone indicates that Tn7 translocation occurs by cutting the transposon away from the donor DNA and joining it to the target DNA without replication, a view supported by the demonstration that an excised transposon is a transposition intermediate (see below).

Another indication of the nonreplicative nature of Tn7 transposition is that recombination in vitro is not affected by the inclusion of DNA synthesis inhibitors such as dideoxynucleotides under conditions in which a strong inhibition of DNA polymerase activity is observed (data not shown).

Double-Strand Breaks at the Ends of Tn7 Produce Recombination Intermediates

Several types of evidence indicate that the DSBs, i.e., donor DNA molecules broken by a double-strand break at a transposon end, are indeed transposition intermediates. These species have the kinetic properties of intermediates: they are most abundant at early times and disappear at late times during the incubation (Figure 3). These kinetic properties are particularly evident when the overall rate of transposition is slowed by lowering the incubation temperature to 22°C. Here, the DSB species are detected earlier and in greater amounts than are the transposition products; it is also notable that a considerable fraction of the substrate donor DNA is converted to DSBs. Disappearance of the DSBs is not due to nonspecific nuclease degradation, because a control fragment was not degraded in these reactions. The view that the DSBs are transposition intermediates is also supported by the fact that they, like the simple insertion product, are generated by staggered breaks in DNA (see below). Similar levels of DSBs are observed when the reactions are stopped by a variety of

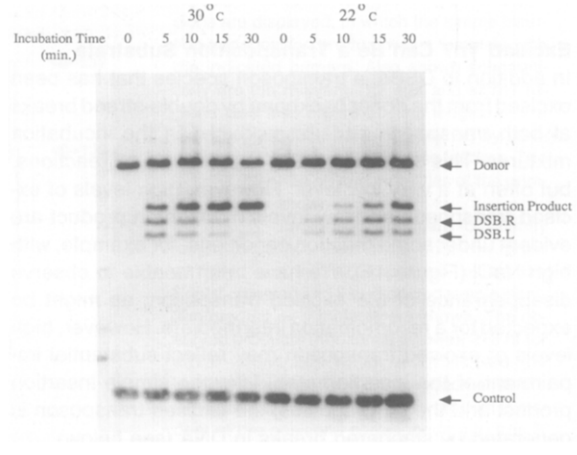

Figure 3. The Double-Strand Break Species Have the Kinetic Properties of Transposition Intermediates

Shown is an autoradiogram that displays ScaI-digested species detected by a mini-Tn7-specific probe produced in transposition reactions, after incubation for the indicated times at either 30°C or 22°C (preincubation also at 22°C). Reactions were performed as described in the Experimental Procedures, except that they also contained a 1200 bp linear fragment recognized by the probe ("Control") to evaluate nuclease degradation. DNAs are as described in Figure 1; the DSBs appear to be different in size because of the ScaI-digestion.

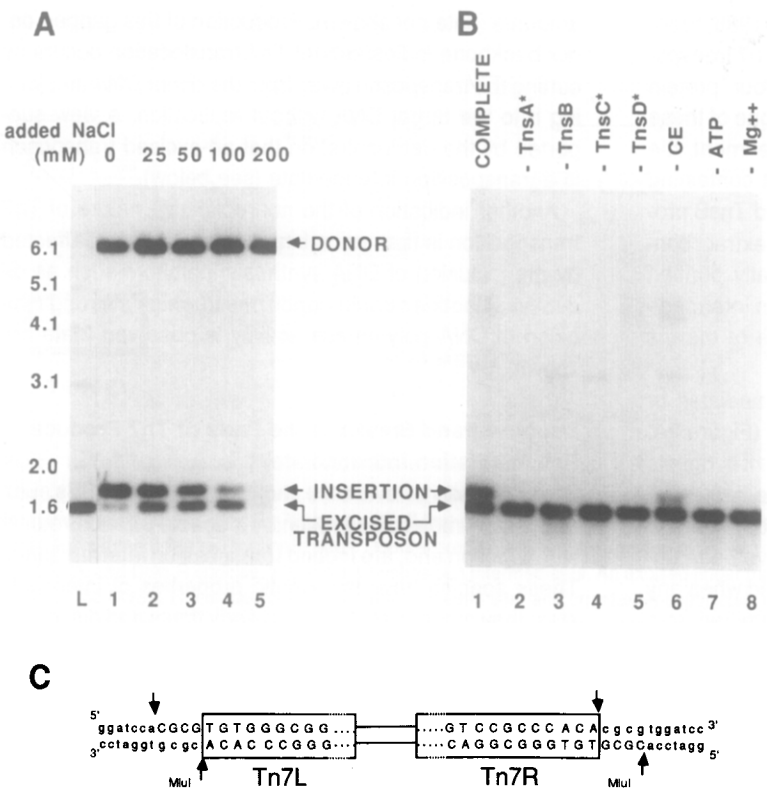

Figure 4. An Excised Transposon Is a Recombination Intermediate

(A) Production of excised transposon. Shown is an autoradiogram that displays EcoI-digested species detected with a mini-Tn7-specific probe from transposition reactions that contained various amounts of monovalent salt. Lanes 1–5: reactions supplemented with the indicated salt; the salt concentrations supplied by protein fractions were 30 mM KCl and 3 mM NaCl. Lane L: DNA size markers with a prominent 1.6 kb species. DNAs are as described in Figure 1.

(B) An "excised" transposon is an effective transposition substrate. Shown is an autoradiogram of the EcoRI-digested products detected with a mini-Tn7-specific probe of transposition reactions containing about 25 ng (0.25 nM) of MluI-generated "excised" transposon as the mini-Tn7 substrate, instead of the usual mini-Tn7-containing donor plasmid. Reactions were carried out as described in the Experimental Procedures, lacking particular components as indicated. Asterisks denote that the indicated Tns protein was supplied in a crude fraction that also contained host proteins. Addition of crude extract (CE) from cells lacking Tns proteins stimulates, but is not essential for, recombination. DNAs are labeled as in Figure 1, except that the "excised" transposon is generated by restriction instead of recombination (as in Figure 4A and elsewhere).

(C) MluI transposon. An MluI transposon–containing plasmid was produced as described in the Experimental Procedures. Digestion with MluI enzyme, which cuts at the indicated positions (arrows), releases a linear "excised" transposon, whose 3′ ends are cleanly exposed (as also with authentic excised transposon generated in recombination reactions) and whose 5′ ends have 4 nt extensions (as opposed to the 3 nt extensions of authentic excised transposon).

methods, including treatment with proteinase and phenol (data not shown); thus, the DSBs are not likely to be protein–DNA complexes, nor is DNA breakage likely to be provoked by our manipulations.

Excised Tn7 Can Be a Transposition Substrate

In addition to DSBs, a transposon species that has been excised from the donor backbone by double-strand breaks at both transposon ends is produced in the incubation mixtures. This excised species is present in all reactions, but often at a very low level. However, high levels of excised transposon and low levels of insertion product are evident under some reaction conditions, for example, with high NaCl (Figure 4A). We have been unable to observe disappearance of the excised transposon, as might be expected for a recombination intermediate. However, high levels of excised transposon may reflect substantial impairment of the insertion step. Like the simple insertion product and the DSB species, the excised transposon is generated by staggered breaks in DNA (see below).

To determine whether an excised mini-Tn7 can participate in recombination, we asked whether exogenously supplied, "excised" mini-Tn7 could insert into *attTn7* when present as the sole transposon substrate. We introduced restriction sites at the Tn7 termini such that restriction enzyme digestion generates a mini-Tn7 species with staggered ends (Figure 4C), the 3′ ends being cut adjacent to L1 and R1 and the 5′ ends cut within the flanking DNA to generate 4 nucleotide (nt) overhangs. (As described below, we believe that the excised mini-Tn7 species generated during transposition has exposed 3′ ends and 3 nt overhangs on its 5′ ends.) The exogenously supplied mini-Tn7 element also has two sequence changes in its Tn7L segment.

The restriction-generated "excised" mini-Tn7 inserts efficiently in site- and orientation-specific fashion into *attTn7* (Figure 4B), supporting the view that the excised transposon is indeed a transposition intermediate. As with a plasmid substrate, insertion of the "excised" transposon requires the presence of all of the *tns* fractions, ATP, and MgAc. In addition to demonstrating that a transposon species that is not connected to a donor backbone can participate in transposition, these results suggest that no *tns* function is involved exclusively in the excision stage of recombination.

We also note that we have been unable to detect a strand transfer intermediate under any condition, either in complete Tn7 transposition reactions or when any *tns* component is omitted. This species, in which the transposon, the donor backbone, and the target are covalently linked, is a key intermediate in bacteriophage Mu transposition (Shapiro, 1979; Craigie and Mizuuchi, 1985; Pato, 1989).

The Polarity of Strand Breakage and Joining

Tn7 insertion is accompanied by the duplication of 5 bp of target sequence (Lichtenstein and Brenner, 1982), pre-

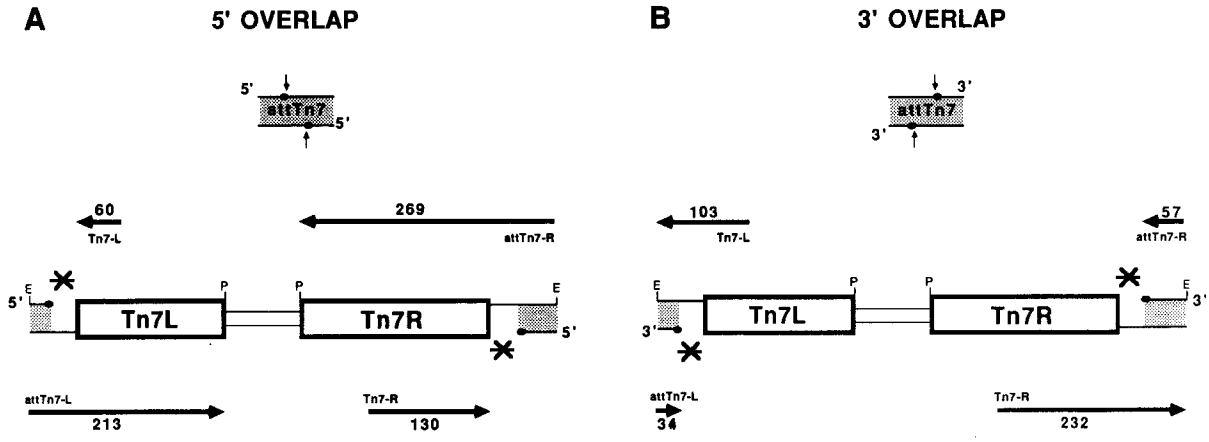

Figure 5. Transposition Can Occur via Two Different Polarities of Strand Breakage and Joining

The duplication of target sequences upon transposon insertion is presumed to result from cleavage of the target site by a staggered break and subsequent repair of the gap resulting from linkage of the transposon ends to the target site ends. Two polarities of target cleavage and transposon joining can be imagined: (A) the target is cleaved by a staggered break generating 5′ overhanging ends that join to the 3′ transposon ends, or (B) the target is cleaved by a staggered break generating 3′ overhanging ends that join to the 5′ transposon ends. The two polarities can be distinguished by identifying which target strands are covalently joined to which transposon strands in the simple insertion transposition product. Asterisks mark the specific, diagnostic gaps in the transposition product. The primers Tn7-L, attTn7-L, Tn7-R, and attTn7-R are complementary to particular strands in *attTn7* and the Tn7 ends. Extension from these primers yields products of different and defined lengths as indicated (thick arrows), depending on which polarity of target breakage and strand joining is used in Tn7 transposition. Restriction sites: E = EcoRI, P = PstI.

sumed to result from the cleavage and subsequent repair of a staggered break in the target DNA (Grindley and Sherratt, 1979; Shapiro, 1979). Two alternative types of target breaks can be imagined (Figure 5), one producing 5′ overhanging ends (Figure 5A) and the other producing 3′ overhanging ends (Figure 5B). These two types of target cleavage will result in two distinct types of joints between the transposon ends and the target site: either the 3′ transpo-

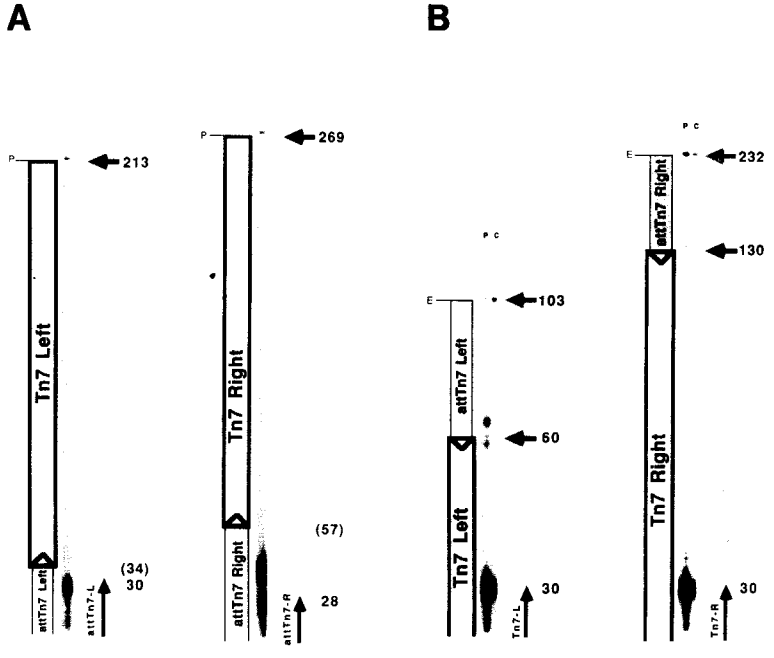

Figure 6. The 3′ Ends of Tn7 Join to the 5′ Target Ends

Shown are autoradiograms of DNA sequencing gels on which the products of primer extensions are displayed, in which the simple insertion transposition product was a template with the indicated primers. Also shown schematically are the relevant Tn7 end and *attTn7* regions (see also Figure 5). The lengths of the extension products were determined by comparison with dideoxy sequencing ladders. The lengths (28 or 30 nt) and mobilities of the primers are also shown.

(A) The 3′ transposon ends are covalently joined to *attTn7*. Extensions from the target attTn7-L and attTn7-R primers using the EcoRI-digested and PstI-digested simple insertion product as templates are shown. The observed products (horizontal arrows), 213 nt for attTn7-L and 269 nt for attTn7-R, are the lengths expected if the 3′ transposon ends are joined to *attTn7* (Figure 5A). No product is evident at the positions corresponding to free 3′ transposon ends, extension from attTn7-L is 34 nt, and that from attTn7-R is 57 nt.

(B) The 5′ transposon ends are not joined to *attTn7*. Extensions from the transposon end Tn7-L and Tn7-R primers using PstI-digested simple insertion product as template (P: left lane of pair) and, as a control, an intact *attTn7*::mini-Tn7 fragment as a template (C: right lane of pair). Two classes of products are observed from each primer with the simple insertion template. One class of products extends to about the 5′ transposon ends (black arrows), approximately 60 nt from Tn7-L and 130 nt from Tn7-R (the exact lengths of these products are considered in Figure 7). Thus, some 5′ transposon ends in the simple insertion product are not linked to *attTn7*, corresponding to a 5′ staggered break in the target (Figure 5A). The other class of products (upper, gray arrows) extend to the positions expected for linkage of the 5′ transposon ends to the target (103 nt from Tn7-L and 232 nt from Tn7-R), probably reflecting DNA repair.

son ends are joined to the 5′ target ends (Figure 5A), or the 5′ transposon ends are joined to the 3′ target ends (Figure 5B). In the first case (Figure 5A), gaps between the 5′ transposon ends and the 3′ target ends are expected; the second breakage and joining polarity leaves gaps adjacent to the 3′ transposon ends (Figure 5B).

To determine the polarity of Tn7 strand breakage and joining, we determined the positions of the intact and gapped strands in the simple insertion product, by using it as a template for extensions with end-labeled oligonucleotide primers specific to the transposon ends and the flanking *attTn7* DNA.

Our analysis has revealed that the target site is broken by staggered breaks with 5′ overhanging ends (Figure 5A). Primers attTn7-L and attTn7-R anneal with the strands of the target DNA that, following transposition using 5′ target breaks, will be joined with the 3′ ends of the inserted transposon. We find that the attTn7-L extension product is 213 nt and that the attTn7-R product is 269 nt (Figure 6A). These lengths correspond to the distance from the primers to restriction sites within the transposon ends (Figure 5A). Thus, in the simple insertion product, these template strands are intact, i.e., the 3′ transposon ends are covalently linked to the 5′ ends of the target. Similar primer extension analysis of the gapped donor backbone supports the view that Tn7 excision involves cleavages directly adjacent to the 3′ transposon ends, which are then joined to target DNA (data not shown).

If the 3′ ends of the transposon are joined to the target DNA, i.e., are intact, then it is expected that the other strands are gapped (Figure 5A). The state of the 5′ transposon ends was examined by extension from the Tn7-L and Tn7-R primers (Figure 6B). For both primers, two distinct classes of extension products are observed. From Tn7-L, there is a cluster of products at about 60 nt and another species at 103 nt from Tn7-R, there is a cluster of products at about 130 nt and another species at 232 nt. The shorter class of extension products from each primer (those at about 60 nt for Tn7-L and about 130 nt for Tn7-R) correspond closely to the distance to the 5′ transposon ends. (The exact lengths of these products are considered in detail below.) These results demonstrate that a considerable fraction of the 5′ transposon strands are broken and thus are not covalently linked to target DNA.

The longer extensions from the Tn7-L and Tn7-R primers do extend to lengths consistent with covalent linkage of the 5′ transposon end with the target DNA, i.e., for Tn7-L, 103 nt and for Tn7-R, 232 nt. We hypothesize that these species result from some repair in this crude system of the gap that initially flanks the 5′ ends. Repair of the right end gap appears to be more efficient than repair of the left end gap, i.e., more 232 nt Tn7-R product is observed than 103 nt Tn7-L product.

We interpret these results to indicate that during Tn7 transposition, the target DNA is cleaved by a staggered break that yields 5′ overhanging ends and that the 5′ ends of the target DNA are joined to the 3′ ends of the transposon (Figure 5A). We presume that a 5 bp gap flanks the 5′ transposon ends and is subsequently converted to duplex form through DNA repair.

Staggered Breaks at the Transposon Ends

What type of DNA strand cleavages release Tn7 from the donor molecule? Analysis of the simple insertion product suggests that Tn7 is cut away from the donor DNA via staggered breaks, in which one DNA strand is cleaved precisely at the 3′ transposon end, and the other strand is cleaved at a displaced position within the flanking donor DNA. We were led to this view through our characterization of the 5′ ends of the transposon in the simple insertion product by extension from the primers Tn7-L and Tn7-R (see Figure 5A). If the 5′ ends were cut flush with the 3′ ends, i.e., at exactly the end of the transposon, we would expect the Tn7-L extension product to be 60 nt and the Tn7-R product to be 130 nt. Rather, we find that the Tn7-L extension product (Figure 7A) and the Tn7-R extension product (Figure 7B) are both 3 nt longer than expected. This result would be obtained if cleavage of the 5′ strands occurs within the flanking donor DNA.

If Tn7 is excised from the donor backbone by staggered breaks involving strand cleavage within the flanking donor DNA, we would expect sequences from the flanking donor backbone to be attached to the 5′ transposon ends. Chemical sequence analysis of the 63 nt Tn7-L extension product reveals that the nucleotides attached to the 5′ terminus of the left end of Tn7 are those adjacent to this transposon end in the flanking donor DNA (data not shown). This result provides strong evidence that cleavage of the 5′ transposon strand actually occurs at a position displaced from the transposon end via a staggered break. We have also observed such staggered breaks at the transposon ends when Tn7 transposes in vitro from another unrelated donor site (data not shown); thus, these staggered breaks are not peculiar to a particular donor site.

We have performed similar primer extension analysis of the ends of the DSBs and the excised transposon generated in in vitro transposition reactions (Figure 7). In all cases, the structure of the 5′ transposon strands was identical, i.e., 3 nt of donor backbone are attached to the transposon. These results support the view that the DSBs and the excised transposon are authentic transposition intermediates and that the transposon is cut away from the donor backbone by staggered breaks in DNA (Figure 8).

The donor sequences attached to the excised transposon are not essential for transposition. An "excised" transposon species generated by the polymerase chain reaction (PCR) and primers whose 5′ strands end with the transposon termini (as in Figure 7, lane 1) inserts efficiently into *attTn7* (data not shown).

Tn7 Target Immunity Is Active in the Cell-Free Transposition System

Tn7 displays target immunity in vivo; that is, the presence of a copy of Tn7 in a target DNA molecule substantially reduces the frequency of a second Tn7 insertion into that target (Hauer and Shapiro, 1984; Arciszewska et al., 1989). Target immunity is fundamentally related to transposition, in that both events require the same transposon end sequences (Lee et al., 1983; Adzuma and Mizuuchi, 1988). The presence in a target molecule of the transposition sequences from the right end of Tn7 (R1–199) is suffi-

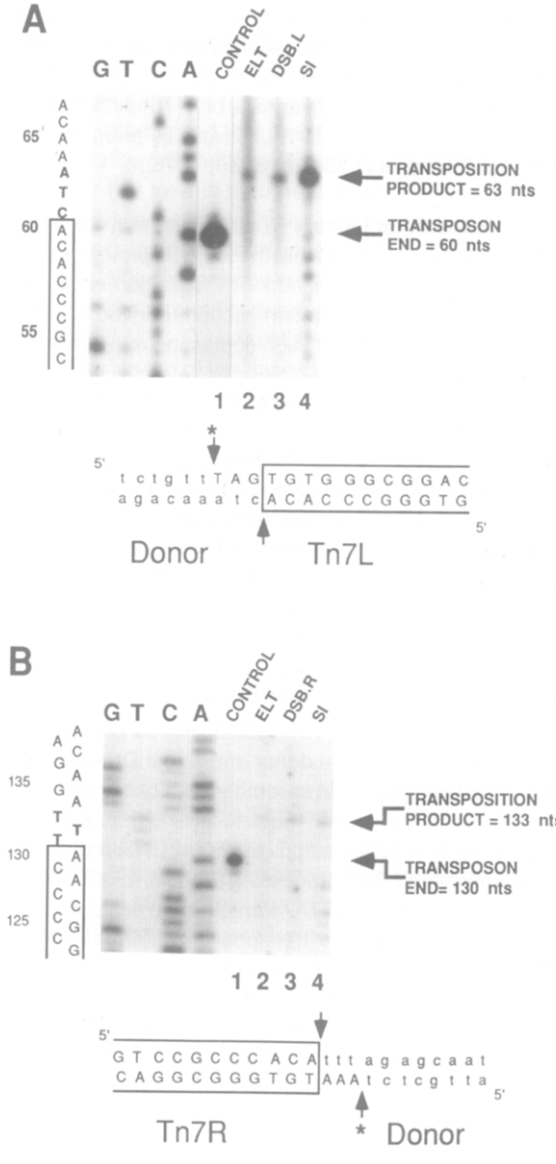

Figure 7. Donor Nucleotides Remain Attached to the 5′ Transposon Strands in Transposition Intermediates and the Simple Insertion Product

Shown are autoradiograms of DNA sequencing gels that display primer extensions from Tn7 end oligonucleotides (see Figure 5A), under conditions where nontemplated addition does not occur (see Experimental Procedures). The templates were: lane 1, as a control, a PCR-generated transposon that terminates exactly at the 5′ transposon ends; lane 2, excised linear transposon (ELT); lane 3, the DSB species; and lane 4, the simple insertion transposition product (SI). At the left is a dideoxynucleotide sequencing ladder generated with the same oligonucleotide on an intact *attTn7*::mini-Tn7 fragment. The nucleotide sequence of the corresponding 5′ transposon strand and the sequence of the flanking donor strand in pEM are shown, along with the distance in nucleotides to the oligonucleotide primer. At the bottom, the positions of strand cleavages at the transposon termini are shown schematically; the 5′ cleavage positions determined in this experiment are indicated with an asterisk.

(A) Extension from the Tn7-L primer. Extension using the simple insertion as template yields a product 3 nt longer than expected for extension to the 5′ transposon end.

(B) Extension from the Tn7-R primer. Extension using the simple insertion as template yields a product 3 nt longer than expected for extension to the 5′ transposon end.

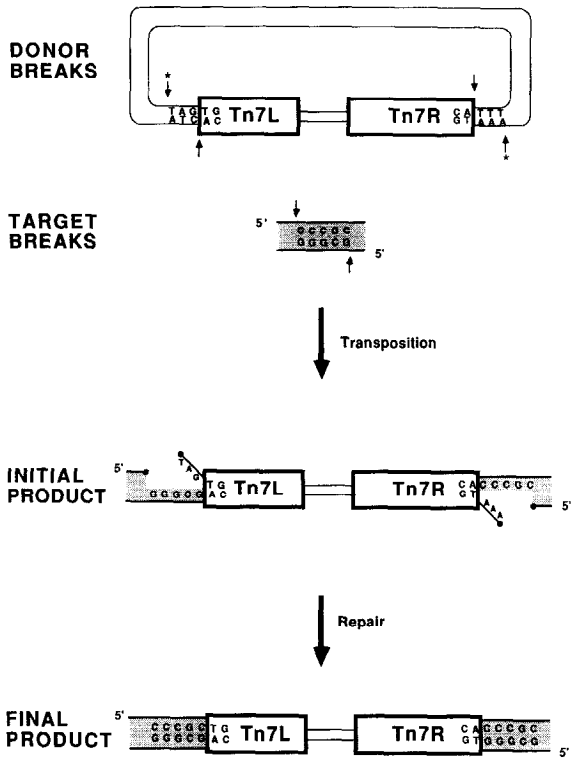

Figure 8. Strand Breakage and Joining during Tn7 Transposition

We suggest that both the ends of Tn7 and *attTn7* are broken by staggered breaks that generate 5′ overhanging ends. Breaks at the target site are proposed to be 5 bp in length, and those at the ends of Tn7 are proposed to be 3 bp in length. The 3′ Tn7 ends join to the 5′ target site ends, generating an initial transposition product whose 5′ transposon ends are attached to several nucleotides of donor DNA and are flanked by 5 bp gaps. DNA repair fills in the gaps and concomitantly removes the nonhomologous donor sequences from the 5′ transposon ends, generating an intact duplex flanked by 5 bp target duplications. The target sequence shown is the 5 bp in *attTn7* usually duplicated upon Tn7 insertion.

cient to provide a high level of immunity in vivo; by contrast, a truncated right end (R1–42) cannot provide immunity and is also defective in transposition (Arciszewska et al., 1989).

We find that Tn7 target immunity is operative in the cell-free transposition system. We evaluated immunity in vitro by examining the target activity of *attTn7* plasmids that also contain Tn7R segments (Figure 9). In the experiments shown in Figure 9, lanes 2 and 3, the reactions contained a mixture of two target DNAs, one an *attTn7* plasmid (target A) and the other a plasmid containing both an *attTn7* segment and a segment from Tn7R, either R1–199 or R1–42 (targets B and C, respectively), located at a distance of about 1 kb from *attTn7*. No mini-Tn7 insertion into the *attTn7* segment of target B is detectable (Figure 9, lane 2), although insertion into the control *attTn7* molecule target A is observed. This result suggests that the presence of R1–199 in target B inactivates this molecule. In contrast, the presence of R1–42 in target C does not block insertion (Figure 9, lane 3). We note that in the presence of target B, a modest decrease in insertion into the control target A

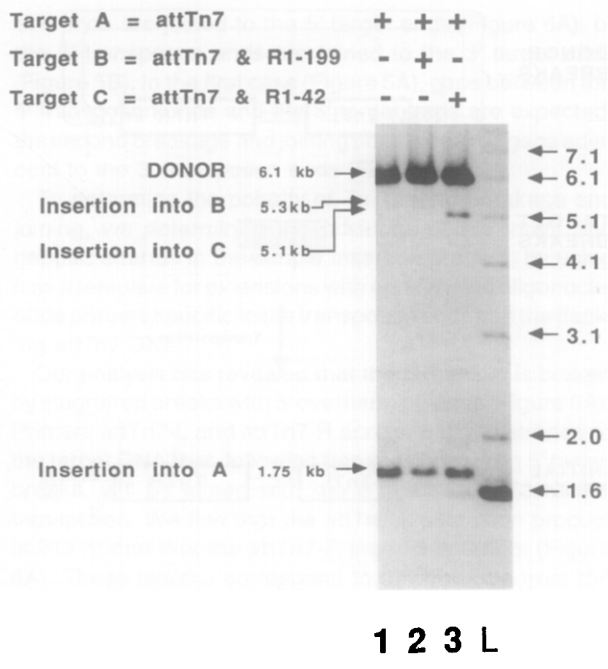

Figure 9. Tn7 Transposition In Vitro Displays Target Immunity

Shown is an autoradiogram that displays EcoRI-digested species detected with a mini-Tn7-specific probe from transposition reactions in which the *attTn7* target molecule also contained a Tn7R segment about 1 kb from *attTn7*. Target A is the *attTn7* target used in other experiments (pKAO4–3); Target B (pLA11) and Target C (pLA24) contain *attTn7* and the indicated Tn7R segment. The concentration of the target plasmids (3 nM each) is about 3-fold less than that of the standard conditions, to avoid a trans-inhibition by Tn7R-containing targets (see text). The position of a simple insertion product in each target plasmid is also shown. Lane 1, target A; lane 2: target A + target B; lane 3: target A + target C.

molecule is also observed (compare lanes 1 and 2). We suspect that this decrease results from a competition between the R1–199 segment in target B and the donor DNA for transposition proteins that interact with the transposon ends (the molar ratio of donor to target B is 1:7); such an effect has been observed in vivo (Arciszewska et al., 1989).

Discussion

We have developed a cell-free system that faithfully reproduces many aspects of Tn7 transposition in vivo. Tn7 inserts in vitro at high frequency in site- and orientation-specific fashion into its preferred target site, *attTn7*. Transposition occurs efficiently when exogenous DNA substrates, one a donor molecule containing a mini-Tn7 element and the other an *attTn7*-containing target molecule, are incubated with extracts derived from cells containing the Tn7-encoded transposition genes *tnsABC* + *tnsD* required for insertion into *attTn7* in vivo (Rogers et al., 1986; Waddell and Craig, 1988; Kubo and Craig, 1990). The in vitro system also displays the phenomenon of transposition immunity that Tn7 displays in vivo; the presence of Tn7 in a target molecule blocks subsequent insertion of another copy of Tn7 into that target molecule (Hauer and Shapiro, 1984; Arciszewska et al., 1989). Tn7 transposition in vitro also requires the presence of ATP, which is likely to be directly involved in recombination, because TnsC is an ATP-binding protein (P. Gamas and N. L. C., unpublished data). An auxiliary role of ATP may be to facilitate supercoiling of the DNA substrates by promoting the action of DNA gyrase (Gellert et al., 1976).

Tn7 Transposition Occurs in a Multiprotein Complex

An attractive hypothesis is that the four Tns proteins participate directly in recombination. Transposition in vitro requires the mixing of four protein fractions derived from four different bacterial strains, each containing one of the four *tns* genes, *tnsABC* + *tnsD*. Specific roles for some Tns proteins have emerged from other experiments (see below). It is likely that host proteins will also be directly involved; most transactions involving extrachromosomal DNA elements are mediated by a combination of element- and host-encoded proteins (Pato, 1989; Thompson and Landy, 1989; Kleckner, 1990). Mutations in several E. coli genes can affect Tn7 transposition (O. Hughes and N. L. C., unpublished data). Reconstitution of Tn7 transposition in vitro with purified proteins will be required to identify both the Tns- and host-encoded participants in recombination.

We propose that the Tn7 recombination proteins, together with the substrate donor and target DNAs, form a specialized nucleoprotein assembly in which transposition occurs. Elaborate protein–nucleic acid complexes also mediate other recombination reactions, DNA replication, transcription, and RNA processing (Echols, 1990). We are attracted to the view that Tn7 transposition occurs in such a complex, because no transposition intermediates or products are detected in the absence of any required protein or DNA component. Thus, we imagine that the complete transposition machinery must properly juxtapose the three DNA segments involved in recombination—the two transposon ends and the target DNA—and the multiple transposition proteins, prior to the initiation of the strand breakage and joining reactions that underlie recombination. In this scenario, *attTn7* is recognized by the rest of the recombination machinery before strand breakage at the transposon ends occurs. Thus, specific recognition of *attTn7* actually provokes transposition by stimulating the initiation of recombination.

What are the roles of the recombination proteins? Other work from this laboratory has established that two of the Tns proteins are sequence-specific DNA-binding proteins: TnsB binds to the ends of Tn7 (McKown et al., 1987; L. Arcisewska, R. McKown, and N. L. C., unpublished data), and TnsD binds to *attTn7* (Waddell and Craig, 1989; K. Kubo and N. L. C., unpublished data). Possible roles for TnsA and TnsC (and/or host proteins) include acting as linker proteins to mediate the specific juxtaposition of the TnsB-bound transposon ends and the TnsD-bound target site and interacting with the positions of strand breakage at the Tn7 termini and at *attTn7*.

Tn7 Transposition Is Nonreplicative

Two distinct pathways for the movement of transposable elements have been identified: nonreplicative transposi-

tion, in which the transposon is cut out of the donor site and pasted into the target site; and replicative transposition, in which a copy of the transposon is made by DNA synthesis and inserted into the target site (Berg and Howe, 1989). We have established that Tn7 translocates in vitro via a nonreplicative mechanism (Figure 1). The principal evidence for this view is that the transposition products we observe are a simple insertion of Tn7 into *attTn7* and a linear donor molecule from which Tn7 has been excised. In vivo studies also provide evidence that Tn7 transposes to *attTn7* via a nonreplicative mechanism (K. Orle, R. Gallagher, and N. L. C., unpublished data).

The fate in vivo of the donor backbone from which Tn7 is excised is unknown. Tn7 transposition in vivo provokes expression of the SOS system (A. Stellwagen and N. L. C., unpublished data), consistent with the production of a gapped donor backbone (Roberts and Kleckner, 1988). We do not observe simple rejoining of the backbone in vitro or in vivo (data not shown). Perhaps the broken donor backbone is lost when transposition occurs, i.e., donor suicide occurs. Another possibility is that the broken donor molecule is repaired by a double-strand-gap repair reaction using an unbroken Tn7-containing donor molecule as a template, as has been observed in Drosophila P-element transposition (Engels et al., 1990).

The Tn7 Transposition Pathway

Our analyses have revealed that Tn7 transposition occurs by a series of double-strand breaks and single-strand joins (Figures 1 and 8). Double-strand breaks at the transposon ends produce a linear excised transposon that is subsequently inserted by single-strand joins into an *attTn7* target site that is also broken by a double-strand break.

Double-strand cleavages of a donor molecule at both Tn7L and Tn7R generate an excised transposon intermediate. These double-strand breaks are staggered, generating 5′ overhanging ends such that the 3′ transposon termini are cleanly exposed, but several nucleotides of donor backbone remain attached to the 5′ transposon strands. We observe that three donor nucleotides are attached to the 5′ transposon ends, suggesting that these staggered cleavages are 3 bp in length. Because the species we have analyzed were generated in a crude system, however, it is difficult to exclude the possibility that a species with longer donor sequences is initially produced and then exonucleolytically reduced. Nonetheless, our studies have established that the transposon ends are released from the donor backbone via staggered breaks. The presence of donor sequences on the 5′ transposon strands is not essential for transposition.

The double-strand breaks at the termini of Tn7 need not occur simultaneously. Similar amounts of donor molecules broken at the junction of the donor backbone are observed with either Tn7L or Tn7R, which are both converted to insertion product. The ends of Tn7 are structurally and functionally distinct (Arciszewska et al., 1989). The orientation-specific insertion of Tn7 into *attTn7* also implies discrimination between the transposon ends. Our observations here suggest that discrimination between Tn7L and Tn7R occurs at a step other than formation and utilization of the double-strand break species.

The target site must also be broken by a staggered double-strand break; the joining of the ends of this break to the transposon ends and subsequent repair of the resulting gaps result in the hallmark target sequence duplication that accompanies transposon insertion (Grindley and Sherratt, 1979; Shapiro, 1979). Like the cleavages at the transposon ends, the staggered target cleavage generates 5′ overhangs; the target cleavage overhangs are 5 bp in length, as opposed to apparently 3 bp at the transposon ends. In contrast with the donor DNA, no cleavage of the target DNA is detected prior to its linkage to the transposon ends.

Upon Tn7 insertion, the 3′ transposon ends are ligated to the 5′ target ends (Figure 8). Formation of the new bonds between the target site and the transposon termini involves single-strand joins in which high energy phosphodiester bonds must be formed. The energy source for the covalent bonds between transposon ends and target strands is as yet undefined in Tn7 transposition. However, we have established that the energy of the transposon–donor bonds is not essential to the formation of the transposon–target bonds: a transposon lacking the transposon–donor bonds can join to the target site.

It will be important to identify the catalytic centers that execute strand cleavages at the transposon ends and at the target site. Because the two types of breaks have the same polarity, i.e., staggered with 5′ overlaps, both cleavages may be carried out by the same polypeptide with position specificity provided by other proteins that bind specifically to the transposon ends and to *attTn7*. Alternatively, the end and target cleavages may be carried out by different polypeptides with their own specificity determinants. Since conversion of the excised transposon to insertion product apparently requires the same *tns* fractions as a reaction using an intact donor molecule, no *tns* function appears to act exclusively in excision.

Comparison of Tn7 with Other Transposable Elements

Tn7 transposition resembles the movement of retrotransposons, particularly in forming an elaborate nucleoprotein recombination complex. The origins of these complexes are quite different: the Tn7 DNA in its complex is formed by excision, whereas retrotransposon DNA in its complex is formed by reverse transcription of an RNA intermediate (Bowerman et al., 1989; Varmus and Brown, 1989). However, the initial joints between the element ends and insertion sites are very similar. For both elements, the 3′ transposon strands are linked to the 5′ target ends, and several nucleotides that will not appear in the final transposition product are attached to the ends of the 5′ transposon strands (Fujiwara and Mizuuchi, 1988; Brown et al., 1989; Eichinger and Boeke, 1990). With Tn7, these extra nucleotides derive from staggered breaks at the transposon termini; with retrotransposons, these nucleotides derive from single-strand cleavages of the linear retroviral DNA. These extra nucleotides are presumably removed during repair of the gaps that flank the initial insertion product.

Prior to linkage with the insertion site, the phosphodiester bonds that link Tn7 to its donor site are broken. Tn7 is excised from the donor backbone by double-strand breaks, and formation of a strand transfer intermediate is not observed. The strand transfer intermediate, i.e., a joint molecule in which the transposon, donor, and target are linked, is a key intermediate in both nonreplicative and replicative bacteriophage Mu transposition (Shapiro, 1979; Craigie and Mizuuchi, 1985; Pato, 1989). Tn7, however, appears to move exclusively by a nonreplicative reaction; excision of the transposon prior to linkage to the target effectively precludes a replicative reaction. Although distinct in temporal order, the strand cleavages at the ends of Mu and Tn7 are chemically similar, as are their target cleavages. In both systems, the 3′ transposon strands are joined to 5′ overhanging target strands exposed by a staggered double-strand break at the target (Mizuuchi, 1984). However, Tn7 is cut away from the donor site by staggered double-strand breaks, whereas cleavage of the 3′ and 5′ Mu strands occurs at very different stages, recombination being initiated by single-strand breaks at 3′ transposon ends. The exact positions of the 5′ Mu strand cleavages remain to be established.

The movement of Tn7 is, in several respects, highly reminiscent of the movement of the bacterial transposon Tn10 (Kleckner, 1989). Tn10 also translocates via a nonreplicative mechanism (Bender and Kleckner, 1986) that involves double-strand breaks at the transposon termini (Morisato and Kleckner, 1987), an excised transposon is a key recombination intermediate (Haniford et al., 1991), and chemically similar joints are formed between the transposon ends and target DNA (Benjamin and Kleckner, 1989). The ends of Tn10, however, appear to be exposed by flush cleavages (Benjamin and Kleckner, 1989), whereas those that expose the Tn7 ends are staggered.

A striking feature of Tn7 transposition revealed by this work is the highly concerted nature of this reaction. It will be interesting to determine how recognition of a single site in a bacterial chromosome is achieved and is communicated through multiple recombination proteins to trigger strand cleavages at the transposon ends and thereby provoke transposition.

Experimental Procedures

DNA Substrates

The donor plasmid pEM-1 contains a 1.6 kb mini-Tn7 element flanked by E. coli chromosomal sequences unrelated to *attTn7* (Arciszewska et al., 1989). The mini-Tn7 element contains a 166 bp Tn7L segment and a 199 bp Tn7R segment flanking a kanamycin resistance gene; these end segments contain the Tn7 cis-acting transposition sequences. The terminal nucleotides of Tn7 are designated L1 and R1, the numbers increasing toward the middle of the element. The *attTn7* target plasmid pKAO4–3 (McKown et al., 1988) contains a 150 bp segment that includes the sequences necessary for *attTn7* target activity. Target DNA lacking *attTn7* is Bluescript pKS+ (Stratagene, San Diego, CA). pMIM-1 contains a mini-Tn7 element whose termini directly adjoin MluI restriction sites (see Figure 4). The mini-Tn7 element with flanking MluI and BamHI sites was made by PCR amplification (below). Following digestion with BamHI, the amplified transposon was ligated into BamHI-digested Bluescript pKS+. Prior to use, the transposon was released by MluI digestion and gel purified using Gene Clean. pLA11 and pLA24 are *attTn7* plasmids that also contain Tn7R segments. To make pLA11, the EcoRI DNA fragment containing R199 from pLA1 (McKown et al., 1987) was inserted by blunt ligation into DraII-digested pRM2 (McKown et al., 1987); position R199 is adjacent to the polylinker HindIII site. To make pLA24, the EcoRI fragment containing Tn7R42 from pCW5 (Arciszewska et al., 1989) was inserted by blunt ligation into DraII-digested pRM2; R42 is adjacent to the polylinker HindIII site.

tns Strains

Protein fractions were obtained from E. coli strains containing various *tns* plasmids. The bacterial strains used were: MC4100, F⁻ *araD139A* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR* (Casadaban, 1976); NLC51, MC4100 *valᴿ recA56* (McKown et al., 1987); and SY 903.1, *recA1 srl*::Tn10, Δ(*lac-pro*) *argE*am *araD* Rifᴿ Nalᴿ/ F′*lacIᵠ¹ lacZ*::Tn5 (Sauer et al., 1988). The *tnsA* strain was NLC51 carrying pKAO52 (Orle and Craig, 1990), the *tnsB* strain was SY 903.1 carrying ptac-*tnsB* (Arciszewska et al., 1989), the *tnsC* strain was NLC51 carrying pKAO53 (Orle and Craig, 1990), and the *tnsD* strain was MC4100 carrying pCW23 (Waddell and Craig, 1988).

Cell Growth and Preparation of *tns* Fractions

Cells were grown at 37°C in LB broth supplemented with 100 μg/ml carbenicillin. Cells were grown to an OD_{600} of approximately 0.6, harvested by centrifugation, washed in buffer A (25 mM HEPES [pH 7.5], 1 mM EDTA, 2 mM dithiothreitol [DTT], and 100 mM KCl) (Fuller et al., 1981) and frozen as cell paste. Crude cell lysates (stage I) were prepared by a freeze–thaw lysis method (Fuller et al., 1981) using buffer A. The protein concentration of all lysates was about 20 mg/ml except for that of NLC51 pKAO53, which was about 10 mg/ml. Stage II *tnsA* and *tnsC* fractions were prepared by treatment of the crude cell lysates with 1% polyethylenimine in buffer A at 500 mM KCl, collection of the resulting supernatant by centrifugation, protein precipitation with 60% saturation ammonium sulfate, and resuspension and dialysis of the resulting pellet with 25 mM HEPES (pH 7.5), 1 mM EDTA, 2 mM DTT, and 500 mM KCl. The *tnsB* fraction was fraction II, about 90% TnsB polypeptide (L. Arciszewska, R. McKown, and N. L. C., unpublished data). The *tnsD* fraction was stage III (about 1% TnsD, K. Kubo and N. L. C., unpublished data). The host extract fraction was prepared by boiling NLC51 crude cell lysate for 2 min and collecting the resulting supernatant after centrifugation in a microfuge for 2 min at room temperature.

In Vitro Transposition Reactions

Unless otherwise indicated, all reaction mixtures (100 μl) contained 0.1 μg (0.25 nM) of pEM donor plasmid, 2.0 μg (10.0 nM) of pKAO4–3 *attTn7* target plasmid, 2 mM ATP, 26 mM HEPES (pH 8.0), 1.3 mM Tris–HCl (pH 8.0), 2.5 mM KPO_4, 0.1 mM EDTA, 2.2 mM DTT, 15 mM MgAc, 100 μg/ml tRNA, 50 μg/ml BSA, and 5% polyvinyl alcohol MW 8000. Reactions in Figures 2B, 6, and 7 also contained approximately 10 μg of stage II *tnsA* extract, 100 ng of TnsB (fraction II), 2.5 μg of stage II *tnsC* extract, 4 μg of stage III *tnsD* fraction, 1 μg of stage II host extract, 128 mM KCl, 15 mM NaCl, and 0.8% glycerol (v/v). Reactions in Figures 2A, 3, and 4B contained 5 μg of stage II *tnsA* fraction, 75 ng of TnsB, 2.5 μg of stage II *tnsC* fraction, 4 μg of stage III *tnsD* fraction, and 1 μg of host fraction; the reactions in Figures 2A and 4B also contained 50 mM KCl and 6 mM NaCl, and those in Figure 3 also contained 136 mM KCl and 2.8 mM NaCl. The reactions in Figure 4A contained 80 μg of stage I *tnsA* fraction, 75 ng of TnsB, 15 μg of stage I *tnsC* fraction, 4 μg of stage III *tnsD* fraction, 30 mM KCl, and 3 mM NaCl, in addition to the NaCl indicated in the figure. The reactions in Figure 9 contained a lower concentration (3 nM) of target plasmid, 40 μg of stage I *tnsA* fraction, 100 ng of TnsB, 10 μg of stage I *tnsC* fraction, 4 μg of stage III *tnsD* fraction, 128 mM KCl, 15 mM NaCl, and 0.8% (v/v) glycerol.

The order of addition of the components is unimportant, except for MgAc, which must be added after preincubation of the other components for 7 min at 30°C. After precincubation, MgAc is added, and incubation is continued for 30 min at 30°C. $MgCl_2$ can be substituted for MgAc, but neither $CaCl_2$ nor spermidine is effective. Reactions are stopped and the DNAs recovered by a modification of the Hoopes protocol (Hoopes and McClure, 1981; G. Gloor and G. Chaconas, personal communication). Four hundred microliters of 10 mM Tris–HCl (pH 7.5), 100 mM KCl, 5 mM EDTA, and 55% (w/v) urea are added to the reactions, followed by 56 μl of 100 mM spermine and then

incubation for 15 min on ice. The resulting pellet is collected by centrifugation, resuspended in 200 μl of 10 mM MgAc and 300 mM NaAc, tRNA added (150 μg/ml), and the mixtures incubated for 10 min on ice. DNA is recovered by ethanol precipitation and resuspended in 25 μl of 10 mM Tris–HCl (pH 8.0), 1 mM EDTA, and 10 μg/ml RNAase.

Restriction and Gel Electrophoresis

Reaction DNA (5 μl) is digested with 30–40 U of EcoRI in 10 μl reactions for 30 min at 37°C. (These rapid digestion conditions were used because of nucleases in the extracted DNAs.) After digestion, the DNAs are electrophoresed in 0.7% agarose gels in 1 × Tris–borate–EDTA buffer.

Southern Hybridization

DNA was electrotransferred to Nytran membranes (0.45 micron, Schleicher and Schuell), and the DNA is covalently bound by ultraviolet irradiation in a Stratalinker (Stratagene). The membrane is probed with a radioactive DNA fragment specific for the mini-Tn7 element—the 1200 bp PstI fragment— generated by random priming labeling (Feinberg and Vogelstein, 1984) using [α-^{32}P]dATP and the Klenow fragment of DNA polymerase I. Hybridizations are carried out at 70°C in a Hybrid-Eaze chamber (Hoefer Scientific Instruments). A 1–3 hr prehybridization (1.0 M NaCl, 1.0% SDS) was followed by overnight hybridization (10% dextran sulfate, 1.0 M NaCl, 1.0% SDS, 100 μg/ml denatured salmon sperm DNA) with probe and followed by four washes in high stringency buffer (0.1 × SSPE, 1% SDS) over a 2 hr period.

Isolation of the Simple Insertion Transposition Product

The in vitro reaction product species were recovered and pooled from 20 individual reactions. After EcoRI digestion, the DNA was electrophoresed on a 0.7% agarose gel in 1 × Tris–acetate-EDTA buffer. The gel was stained with 0.5 μg/ml ethidium bromide, DNA was visualized with long wave-length ultraviolet light, gel slices containing the desired species were excised, and the DNA was purified with Gene Clean.

Oligonucleotide Primers

The primers are complementary to sequences at the junctions of the ends of Tn7 and *attTn7* (see Figure 5; sequences described in Craig, 1989). Tn7-L is complementary to L31–60 of the top strand at the left end of Tn7; Tn7-R is complementary to R101–130 of the bottom strand at the right end of Tn7. attTn7-L is complementary to *attTn7* −25 to −5 on the bottom strand of *attTn7* and also has at its 5′ end the 5 nt complementary to the flanking polylinker region in pKAO4–3, which flanks *attTn7* −25; attTn7-R is complementary to *attTn7* +32 to +56 on the top strand of *attTn7* and also has at its 5′end TTAA. Oligonucleotides were labeled at their 5′ ends using T4 polynucleotide kinase and [γ-^{32}P]ATP (6000 Ci/mmol; ICN Radionucleotides).

Primer Extensions

Extension reactions (10 μl) contained approximately 0.2 pmol of end-labeled oligonucleotide, approximately 10 ng of isolated simple insertion transposition product, 1 U of Taq polymerase (Cetus), dNTPs as indicated below, and PCR buffer (10 mM Tris–HCl [pH 8.3], 50 mM KCl, 2.0 mM $MgCl_2$, 0.01% gelatin). In some reactions (Figure 6), dNTPs were added to 0.2 mM. Under these conditions, Taq polymerase is highly processive, but 1–2 nt are added to the 3′ end of the extension product owing to a high level of nontemplated nucleotide addition (Clark, 1988). In other reactions (Figure 7), dNTPs were added to 2 mM. Under this condition, no template-independent addition is detected on the 3′ end of the extension product (see control in Figure 7), but the extensions are not highly processive. The extension products and DNA sequencing reactions, using the same primers as markers, were run on gels containing 6% polyacrylamide (19:1 acrylamide: bisacrylamide), 42% (w/v) urea, and 1 × Tris–borate–EDTA.

Production of the MluI Transposon by PCR Amplification

We synthesized an R-terminus oligonucleotide primer complementary to R1–19 of the top strand at the right end of Tn7 and to 17 of 19 positions in L1–19 of the bottom strand at the left end of Tn7 (positions L14 and L16 differ from R14 and R16), plus an additional MluI site, a BamHI site, and two extra nucleotides. Using this oligonucleotide and a mini-Tn7 plasmid as template (Mullis and Faloona, 1987), PCR amplification was used to generate a linear transposon whose ends were flanked by MluI and BamHI, which was used to produce pMIM-1 (see above). Reactions contained 100 pmol of primer, 1 fmol of EcoRI-cut pEM, 2 mM dNTPs, and PCR buffer.

Production of Control PCR Transposon

PCR amplification and a mini-Tn7 plasmid template were used to produce a control transposon whose 5′ ends terminate at the last transposon nucleotide (Figure 7). The primer was complementary to R1–19 of the top strand at the right end of Tn7 and to 17 of 19 positions in L1–19 of the bottom strand at the left end of Tn7 (positions L14 and L16 differ from R14 and R16). The 5′ ends of this PCR transposon are provided by the primer, and the 3′ ends will be generated by polymerization; thus, the 5′ ends have a known structure. The resulting PCR transposon was purifed through two cycles of agarose gel electrophoresis with Gene Clean extraction.

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References

Adzuma, K., and Mizuuchi, K. (1988). Target immunity of Mu transposition reflects a differential distribution of Mu B protein. Cell *53*, 257–266.

Arciszewska, L. K., Drake, D., and Craig, N. L. (1989). Transposon Tn7 cis-acting sequences in transposition and transposition immunity. J. Mol. Biol. *207*, 35–52.

Barth, P. T., Datta, N., Hedges, R. W., and Grinter, N. J. (1976). Transposition of a deoxyribonucleic acid sequence encoding trimethoprim and streptomycin resistances from R483 to other replicons. J. Bacteriol. *125*, 800–810.

Bender, J., and Kleckner, N. (1986). Genetic evidence that Tn10 transposes by a nonreplicative mechanism. Cell *45*, 801–815.

Benjamin, H. W., and Kleckner, N. (1989). Intramolecular transposition by Tn10. Cell *59*, 373–383.

Berg, D. E., and Howe, M. M., eds. (1989). Mobile DNA (Washington, DC: American Society for Microbiology).

Bowerman, B., Brown, P. O., Bishop, J. M., and Varmus, H. E. (1989). A nucleoprotein complex mediates the integration of retroviral DNA. Genes Dev. *3*, 469–478.

Brown, P. O., Bowerman, B., Varmus, H. E., and Bishop, J. M. (1987). Correct integration of retroviral DNA in vitro. Cell *49*, 347–356.

Brown, P. O., Bowerman, B., Varmus, H. E., and Bishop, J. M. (1989). Retroviral integration: structure of the initial covalent product and its precursor, and a role for the viral IN protein. Proc. Natl. Acad. Sci. USA *86*, 2525–2529.

Casadaban, M. J. (1976) Transposition and fusion of the *lac* genes to selected promoters in Escherichia coli using bacteriophage lambda and Mu. J. Mol. Biol. *104*, 541–555.

Clark, J. M. (1988). Novel non-templated nucleotide addition reactions

catalyzed by procaryotic and eucaryotic DNA polymerases. Nucl. Acids Res. *16*, 9677–9686.

Craig, N. L. (1989). Transposon Tn7. In Mobile DNA, D. E. Berg and M. M. Howe, eds. (Washington, DC: American Society for Microbiology), pp. 211–225.

Craigie, R., and Mizuuchi, K. (1985). Mechanism of transposition of bacteriophage Mu: structure of a transposition intermediate. Cell *41*, 867–876.

Echols, H. (1990). Nucleoprotein structures initiating DNA replication, transcription, and site-specific recombination. J. Biol. Chem. *265*, 14697–14700.

Eichinger, D. J., and Boeke, J. D. (1988). The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell *54*, 955–966.

Eichinger, D. J., and Boeke, J. D. (1990). A specific terminal structure is required for Ty transposition. Genes Dev. *4*, 324–330.

Engels, W. R., Johnson-Schlitz, D. M., Eggleston, W. B., and Sved, J. (1990). High-frequency P element loss in Drosophila is homolog dependent. Cell *62*, 515–525.

Feinberg, A. P., and Vogelstein, B. (1984). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. *137*, 266–267.

Fujiwara, T., and Mizuuchi, K. (1988). Retroviral DNA integration: structure of an integration intermediate. Cell *54*, 497–504.

Fuller, R. S., Kaguni, J. M., and Kornberg, A. (1981). Enzymatic replication of the origin of the Escherichia coli chromosome. Proc. Natl. Acad. Sci. USA *78*, 7370–7374.

Gay, N. J., Tubulewicz, V. L., and Walker, J. E. (1986). Insertion of transposon Tn7 into the Escherichia coli *glmS* transcriptional terminator. Biochem. J. *234*, 111–117.

Gellert, M. K., Mizuuchi, K., O'Dea, M. H., and Nash, H. A. (1976). DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. USA *73*, 3872–3876.

Grindley, N. D., and Sherratt, D. J. (1978). Sequence analysis of IS1 insertion sites: Models for transposition. Cold Spring Harbor Symp. Quant. Biol. *43*, 1257–1261.

Gringauz, E., Orle, K. A., Waddell, C. S., and Craig, N. L. (1988). Recognition of Escherichia coli *attTn7* by transposon Tn7: lack of specific sequence requirements at the point of Tn7 insertion. J. Bacteriol. *170*, 2832–2840.

Haniford, D. B., Benjamin, H. W., and Kleckner, N. (1991). Kinetic and structural analysis of a cleaved donor intermediate and a strand transfer intermediate in Tn10 transposition. Cell *64*, 171–179.

Hauer, B., and Shapiro, J. A. (1984). Control of Tn7 transposition. Mol. Gen. Genet. *194*, 149–158.

Hoopes, B. C., and McClure, W. R. (1981). Studies of the selectivity for DNA precipitation by spermine. Nucl. Acids Res. *9*, 5493–5504.

Kleckner, N. (1989). Transposon Tn10. In Mobile DNA, D. E. Berg and M. M. Howe, eds. (Washington, DC: American Society for Microbiology), pp. 227–268.

Kleckner, N. (1990). Regulation of transposition in bacteria. Annu. Rev. Cell Biol. *6*, 297–327.

Kubo, K., and Craig, N. L. (1990). Bacterial transposon Tn7 utilizes two different classes of target sites. J. Bacteriol. *182*, 2774–2778.

Lee, C. H., Bhagwat, A., and Heffron, F. (1983). Identification of a transposon Tn3 sequence required for transposition immunity. Proc. Natl. Acad. Sci. USA *80*, 6765–6759.

Lichtenstein, C., and Brenner, S. (1982). Unique insertion site of Tn7 in the E. coli chromosome. Nature *297*, 601–603.

McKown, R. L., Waddell, C. S., Arciszewska, L. A., and Craig, N. L. (1987). Identification of a transposon Tn7-dependent DNA-binding activity that recognizes the ends of Tn7. Proc. Natl. Acad. Sci. USA *84*, 7807–7811.

McKown, R. L., Orle, K. A., Chen, T., and Craig, N. L. (1988). Sequence requirements of Escherichia coli *attTn7*, a specific site of transposon Tn7 insertion. J. Bacteriol. *170*, 352–358.

Mizuuchi, K. (1983). In vitro transposition of bacteriophage Mu: a biochemical approach to a novel replication reaction. Cell *35*, 785–794.

Mizuuchi, K. (1984). Mechanism of transposition of bacteriophage Mu: polarity of the strand transfer reaction at the initiation of transposition. Cell *39*, 395–404.

Morisato, D., and Kleckner, N. (1987). Tn10 transposition and circle formation in vitro. Cell *51*, 101–111.

Mullis, K. B., and Faloona, F. A. (1987). Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Meth. Enzymol. *155*, 335–350.

Orle, K., and Craig, N. L. (1990). Identification of transposition proteins encoded by the bacterial transposon Tn7. Gene *96*, 1–7.

Pato, M. L. (1989). Bacteriophage Mu. In Mobile DNA, D. E. Berg and M. M. Howe, eds. (Washington, DC: American Society for Microbiology), pp. 23–52.

Qadri, M. I., Flores, C. C., Davis, A. J., and Lichtenstein, C. P. (1989). Genetic analysis of *attTn7*, the transposon Tn7 attachment site in Escherichia coli, using a novel M13-based transduction assay. J. Mol. Biol. *207*, 85–98.

Roberts, D., and Kleckner, N. (1988). Tn10 transposition promotes RecA-dependent induction of a λ prophage. Proc. Natl. Acad. Sci. USA *85*, 6037–6041.

Rogers, M., Ekaterinaki, N., Nimmo, E., and Sherratt, D. (1986). Analysis of Tn7 transposition. Mol. Gen. Genet. *205*, 550–556.

Sauer, R. T., Smith, D., and Johnson, A. D. (1988). Flexibility of the yeast alpha-2 repressor enables it to occupy the ends of its operator, leaving the center free. Genes Dev. *2*, 807–816.

Shapiro, J. A. (1979). Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci. USA *76*, 1933–1937.

Thompson, J. F., and Landy, A. (1989). Regulation of bacteriophage lambda site-specific recombination. In Mobile DNA, D. E. Berg and M. M. Howe, eds. (Washington, DC: American Society for Microbiology), pp. 1–22.

Varmus, H. E., and Brown, P. (1989). Retroviruses. In Mobile DNA, D. E. Berg and M. M. Howe, eds. (Washington, DC: American Society for Microbiology), pp. 53–109.

Waddell, C. S., and Craig, N. L. (1988). Tn7 transposition: two transposition pathways directed by five Tn7-encoded genes. Genes Dev. *2*, 137–149.

Waddell, C. S., and Craig, N. L. (1989). Tn7 transposition: recognition of the *attTn7* target sequence. Proc. Natl. Acad. Sci. USA *86*, 3958–3962.