Mechanism of DNA-mediated transposition

DNA-mediated Transposition Mechanism

Some families of transposable elements that move via a DNA intermediate do so in a replicative manner. In this case, transposition generates a new copy of the transposable element at the target site, while leaving a copy behind at the original site. A cointegrate structure is formed by fusion of the donor and recipient replicons, which is then resolved (Figure 9.12). Other families use a nonreplicative mechanism. In this case, the original copy excises from the original site and move to a new target site, leaving the original site vacant.

![Diagram of DNA-mediated transposition](https://bio.libretexts.org/Bookshelves/Genetics/Book%3A_Working_with_Molecular_Genetics_(Hardison)/Unit_II%3A_Repli…)

**Figure 9.12.** Contrasts between replicative and nonreplicative transposition. The transposable element (TE) is shown as an open arrow. The thick line for each replicon represents double stranded DNA; the different shadings represent different sequences.
Studies of bacterial transposons have shown that replicative transposition and some types of nonreplicative transposition proceed through a **strand-transfer intermediate** (also known as a **crossover structure**), in which both the donor and recipient replicons are attached to the transposable element (Figure 9.13). For replicative transposition, DNA synthesis through the strand-transfer intermediate produces a transposable element at both the donor and target sites, forming the cointegrate intermediate. This is subsequently resolved to separate the replicons. DNA synthesis does not occur at the crossover structure in nonreplicative transposition, thus leaving a copy only at the new target site. In an alternative pathway for nonreplicative transposition, the transposon is excised by two double strand breaks, and is joined to the recipient at a staggered break (illustrated at the bottom of Figure 9.12).

In more detail, there are two steps in common for replicative and nonreplicative transposition, generating the strand-transfer intermediate (Figure 9.13).

1. **The transposase** encoded by a transposable element makes four nicks initially. Two nicks are made at the target site, one in each strand, to generate a staggered break with 5' extensions (3' recessed). The other two nicks flank the transposon; one nick is made in one DNA strand at one end of the transposon, and the other nick is made in the other DNA strand at the other end. Since the transposon has inverted repeats at each end, these two nicks that flank the transposon are cleavages in the same sequence. Thus the transposase has a sequence-specific nicking activity. For instance, the transposase from TnA binds to a sequence of about 25 bp located within the 38 bp of inverted terminal repeat (Figure 9.10). It nicks a single strand at each end of the transposon, as well as the target site (Figure 9.13). Note that although the target and transposon are shown apart in the two-dimensional drawing in Figure 9.13, they are juxtaposed during transposition.

2. At each end of the transposon, the 3' end of one strand of the transposon is joined to the 5' extension of one strand at the target site. This ligation is also catalyzed by transposase. ATP stimulates the reaction but it can occur in the absence of ATP if the substrate is supercoiled. Ligation of the ends of the transposon to the target site generates a strand-transfer intermediate, in which the donor and recipient replicons are now joined by the transposon.

After formation of the strand-transfer intermediate, two different pathways can be followed. For replicative transposition, the 3' ends of each strand of the staggered break (originally at the target site) serve as primers for repair synthesis (Figure 9.13). Replication followed by ligation leads to the formation of the cointegrate structure, which can then be resolved into the separate replicons, each with a copy of the transposon. The **resolvase** encoded by transposon TnA catalyzes the resolution of the cointegrate structure. The site for resolution (res) is located between the divergently transcribed genes for tnpA and tnpR (Figure 9.10). TnA resolvase also negatively regulates expression of both tnpA and tnpR (itself).

For nonreplicative transposition, the strand-transfer intermediate is released by nicking at the ends of the transposon not initially nicked. Repair synthesis is limited to the gap at the flanking direct repeats, and hence only one copy of the transposon is left. This copy is ligated to the new target site, leaving a vacant site in the donor molecule.
The enzyme transposase can recognize specific DNA sequences, cleave two duplex DNA molecules in four places, and ligate strands from the donor to the recipient. This enzyme has a remarkable ability to generate and manipulate the ends of DNA. A three-dimensional structure for the Tn5 transposase in complex with the ends of the Tn5 DNA has been solved by Rayment and colleagues. One static view of this protein DNA complex is in Figure 9.14.A. The transposase is a dimer, and each double-stranded DNA molecule (donor and target) is bound by both protein subunits. This orients the transposon ends into the active sites, as shown in the figure. Also, an image with just the DNA (Figure 9.14.B.) shows considerable distortion of the DNA helix at the ends. This recently determined structure is a good starting point to better understand the mechanism for strand cleavage and transfer.

A.
Figure 9.14. Three-dimensional structure of the Tn5 transposase in complex with Tn5 transposon DNA. A. The dimer of the Tn5 transposase is shown bound to a fragment of duplex DNA from the end of the transposon. Alpha helices are green cylinders, beta sheets are yellow-brown, flat arrows and protein loops are blue wires. The DNA is a duplex of two red wires, one for each strand. B. The DNA is shown without the protein and with the nucleotides labeled. The end of the DNA at the top of this panel is oriented into the active site in the middle of the protein in panel A. The structure was determined by Davies DR, Goryshin IY, Reznikoff WS, Rayment I. (2000) “Three-dimensional structure of the Tn5 synaptic complex transposition intermediate.” Science 289:77-85. These images was obtained by downloading the atomic coordinates from the Molecular Modeling Database at NCBI, viewing them with CN3D 3.0 and saving static views as screen shots.

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