**DNA transposition**

**1. Query**: In class, you said there are three different types of transposable elements: IS elements, Tn3 family, and complex. Does the grouping of Tn reflect anything about their mechanism of transposition?

The classification is somewhat arbitrary. Historically, the insertion sequences (IS elements) were the first to be discovered in bacteria. They are often relatively small, and carry relatively few genes. They can be part of a complex transposon by being present in two copies one at each end of the complex transposon. Tn refers to transposon. Tn3 family is characterized by short inverted repeats at the end s of the transposon. They follow a replicative mechanism for transposition. They also encode their own resolvases for converting the cointegrate intermediate into the simple integrant, the final product of transposition. Transposons designated by the Tn prefix can follow different mechanisms. Tn10 follows a cut- and-paste mechanism. As I pointed out already, Tn3 follows a replicative mechanism. Complex transposons carry IS elements at their ends, and these element themselves are capable of transposing independently.

In short, simple and complex transposons are not differentiated so much by mechanism as the different ways in which they move. Simple transposons have only one mode of movement. Complex transposons have three potential movement types. The IS element at the left can move on its own. The IS element at the right can move on its own. And the transposon as a whole can move (including the left and right IS elements).

2. Query: What is the chemistry of strand joining by transposase? I'm not sure what this is. (I thought that after that transposon attacked its target then the DNA was polymerized and then ligase sealed the ends.)

The chemistry of strand joining (joining the transposon end to the target DNA) is transesterification. The 3'-OH formed at the transposon end by the transposase attacks the phosphodiester bond on the target DNA. The result is the formation of a 3'-5' phosphodiester bond between the transposon end the target DNA. In addition, a 3'-OH is formed on the target DNA at the site of joining. Thus the old phosphodiester bond on the target DNA is broken; at the same time a new phosphodiester bond between the transposon and the target DNA is formed. Hence the reaction is transesterification.

By contrast to the strand joining reaction, the strand cleavage reaction by the transposase to form the 3'-hydroxyl group is a hydrolysis reaction. Here the phosphodiester bond is broken to generate the 3'-OH at the transposon end and the 5'-phosphate adjacent to it. No new phosphodiester bond is formed during the reaction, and the energy of the broken one is lost in the process.

After the joining reaction, the 3'-OH formed in the target DNA is used to prime DNA synthesis: either short repair synthesis for the 'cut-and-paste' type transposons or extensive synthesis for the 'replicative' transposons (resulting in co-integrate formation). Yes, it is the ligation reaction that completes the process by joining the 3'-OH formed at the end of the replicated DNA to the adjacent 5'-phosphate group.

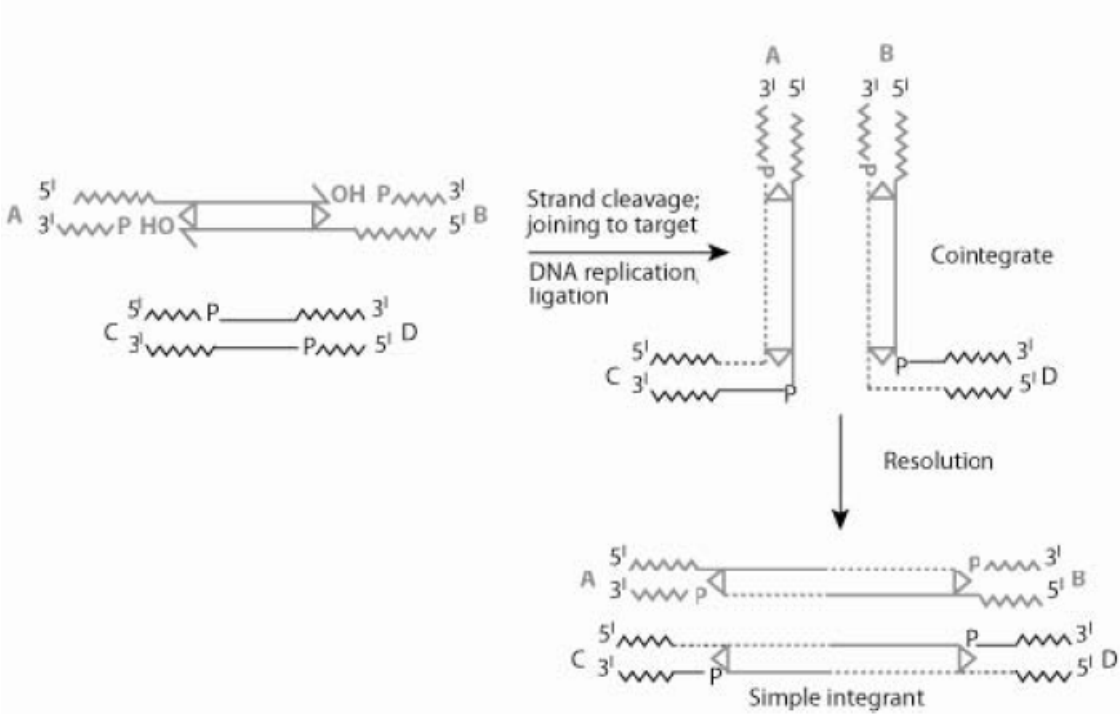
**4**. **Query**: What is a cointegrate intermediate formed during transposition? In my notes I have it written that a cointegrate has two copies of the transposon and two of the target.

In the cointegrate intermediate formed during replicative transposition, there are two copies of the transposon and two copies of the target site (the small number of base pairs duplicated during the replicative process). Recall that the donor DNA circle (containing the original copy of the transposon) and the recipient DNA circle containing no transposon initially are joined into one circle as a result of cointegrate formation. This intermediate contains two copies of the transposon and two copies of the target site (5 bp in the case of Mu transposition) in head-to-tail orientation. After the cointegrate is resolved (by recombination between the two head-to-tail copies of the transposon; this can be done by the resolvase enzyme coded for by the transposon, as in Tn3 or by the homologous recombination system of the host bacterium, as in the case of Tn5), the cointegrate circle is split into two. One of these is the recipient DNA circle containing one copy of the transposon with the duplicated target sequence flanking it in head-to-tail orientation. The other circle is the donor DNA with one copy of the transposon in its original location.

**5**. **Query**: Can you explain adjacent inversions and adjacent deletions during transposition?

**Simple rule**: If the cleaved strand attacks the target phosphodiester bond on the same strand, the result will be an adjacent deletion. If the attack occurs on the opposite strand, the result will be an adjacent inversion.

Now, let me explain in more detail using the basic features of the cointegrate intermediate shown in the diagram below.



This diagram is a simplified way of showing the steps of replicative transposition: formation of cointegrate and resolution of the cointegrate. I did not join the ends deliberately, so we can use this template to explain both intermolecular and intramolecular transposition.

If you join A and B ends and C and D ends in the left panel (to the left of the arrow), we are doing intermolecular transposition. So we have to join A and B ends and C and D ends on the right hand side panel (product of transposition) as well.

If you join A and C ends and B and D ends instead, we are doing intramolecualr transposition.

In the class, we drew transposition from a donor circle (closed the A and B ends to complete the circle) to the target site in the recipient circle (closed the C and D ends).

In the case of adjacent deletions and inversions, we are referring to intramolecular transposition, the transposon moving from one point in a circular molecule (E. coli chromosome) to another point in the same circle to form the cointegrate intermediate. Let us not worry about the resolution event.

So, we should start with a circle in which the A end is joined to C, and the B end is joined to D. In the case that we have illustrated, note that the top strand of the transposon is attacking the phosphodiester at the target site on the same strand (top strand to top strand attack). Similarly, the bottom strand of the transposon is attacking the phosphodiester on the same strand (bottom strand to bottom strand attack). You can see that an adjacent deletion event has occurred in the co-integrate. That is, the AC circle is split off from the BD circle. If the replication origin is on the AC circle, that is the only one that can replicate. All the genes B through D on the other circle will be deleted. The deletion covers a region starting adjacent to the B end of the transposon (hence adjacent deletion) and up to the target site of transposition.

If you do the cointegrate reaction from the A-B-D-C circle with the transposon ends attacking the phosphodiester targets on opposite strands, you will see adjacent inversion in the cointegrate formed. The configuration of the cointegrate circle will be A-D-B-C. That is the relative orientation of the genes B through D with respect to A-C has been inverted.

**Important note**: After you have joined the A-C ends and the B-D ends to form the A-B-D-C circle, the substrate circle for transposition, we have referred to the outer strand as the ‘top’ strand and inner strand as the ‘bottom’ strand. This is of course arbitrary.