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MicroReview

Tn*5* as a model for understanding DNA transposition

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Summary

Tn*5* is an excellent model system for understanding the molecular basis of DNA-mediated transposition. Mechanistic information has come from genetic and biochemical investigations of the transposase and its interactions with the recognition DNA sequences at the ends of the transposon. More recently, molecular structure analyses of catalytically active transposase; transposon DNA complexes have provided us with unprecedented insights into this transposition system. Transposase initiates transposition by forming a dimeric transposase, transposon DNA complex. In the context of this complex, the transposase then catalyses four phosphoryl transfer reactions (DNA nicking, DNA hairpin formation, hairpin resolution and strand transfer into target DNA) resulting in the integration of the transposon into its new DNA site. The studies that elucidated these steps also provided important insights into the integration of retroviral genomes into host DNA and the immune system V(D)J joining process. This review will describe the structures and steps involved in Tn*5* transposition and point out a biologically important although surprising characteristic of the wild-type Tn*5* transposase. Transposase is a very inactive protein. An inactive transposase protein ensures the survival of the host and thus the survival of Tn*5*.

Introduction

Transposition is an important mechanism in generating genetic diversity. Transposable elements are the causative agents of various insertion, deletion, inversion and chromosomal fusion mutations. These elements are found in almost all genomes and derivatives of transposable elements compose a large fraction of the human genome. In addition, as will be discussed in more detail later, some transposition processes are catalytically similar to HIV-1 integration (Craig, 1995) and the RAG-1 mediated cleavage reaction in the immune system V(D)J joining process (McBlane *et al*., 1995). Consistent with these catalytic similarities is the fact that transposase is structurally similar to retroviral integrases and probably to the RAG-1 protein (Davies *et al*., 1999; Gellert, 2002; Mizuuchi and Baker, 2002). Therefore, transposition is of general interest.

One class of transposable element moves via an excised DNA intermediate (so called 'cut and paste' transposition). Thanks to recent genetic, biochemical and structural studies, Tn*5* is an excellent model system for understanding 'cut and paste' transposition (Reznikoff, 2002). These studies have led to two key developments of general interest. Traditional molecular biology research has been visualized through cartoon models. These are satisfying but do not present the molecular reality. Current trends are to use true molecular models from, for instance, X-ray crystallography. The Tn*5* system is the first transposition system to make this transition fully. As a result of these structural studies and many biochemical and genetic investigations, an unprecedented detailed molecular picture of the transposition process is now coming into focus. During my graduate training, I was encouraged to think of proteins as products of evolution towards high levels of activity. The wild-type Tn*5* transposase has apparently done the reverse; transposase is a very inactive protein.

Specific macromolecular participants

It is surprising that a process as complex as Tn*5* DNA transposition requires but three macromolecules for all but the last step (Goryshin and Reznikoff, 1998). Two of these macromolecules are the transposon DNA, typically embedded in a donor DNA sequence, and the 476-amino-acid long transposase (Tnp) that catalyses transposition. The third macromolecule is the target DNA sequence that can be located on the same molecule as the transposon (even within the transposon) or on a second DNA molecule. There is little specificity in the target sequence.

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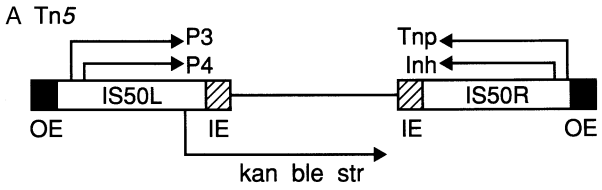
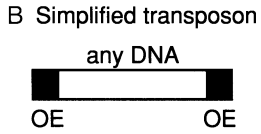
Transposon

Figure 1A presents a traditional view of the Tn*5* transposon as a composite structure containing two inverted versions of the transposable insertion sequence IS*50* bracketing three antibiotic resistance genes. The IS*50* elements are defined by two 19 bp sequences termed the outside end (OE) and inside end (IE). The OE and IE differ at seven positions (Fig. 1C) but, in the absence of Dam DNA methylation of the IE, are recognized similarly by Tnp. In fact, both OE and IE are suboptimal and a hyperactive mosaic version of the 19 bp end sequence (the mosaic end, ME) has been discovered (Fig. 1C). One of the IS50 elements (IS50R) encodes Tnp and an inhibitor of transposition (Inh), the other encodes two inactive proteins (for reviews, see Berg *et al.*, 1989; Reznikoff, 2002).

A simpler way to understand the transposon is to consider its critical components; these are as a source of Tnp (the Tnp can also be supplied in *trans*) and the 19 bp end sequences that can encompass any DNA sequence. The internal DNA need only be long enough to allow the two ends to come in close proximity so that Tnp bound to the end sequences can form a synaptic complex (see below)(Fig. 1B). The 19 bp end sequences can be two inverted OE sequences (for Tn*5* transposition), or inverted OE and IE sequences (for IS*50* transposition), or two inverted IE sequences, or two inverted ME sequences.





C End sequences

OE:	CTG**A**CTCTT**ATA**CA**C**A**AG**T
IE:	CTG**T**CTCTT**GAT**CA**G**A**TC**T
ME:	CTGTCTCTT**ATA**CA**C**ATCT

Fig. 1. Tn*5* structure.
A. Two nearly identical IS*50* elements bracket three antibiotic resistance genes. IS*50*R encodes the transposase (Tnp) and an N-terminal truncated version of Tnp the transposase inhibitor (Inh). IS*50*L encodes C-terminal truncated, inactive versions of Tnp and Inh. IS*50* elements are defined by two 19 bp sequences, OE and IE, which are critical Tnp binding sites.
B. A simplified transposon includes two specific 19 bp end sequences that bracket sufficient DNA to allow the DNA bending required for synaptic complex formation.
C. The non-transferred strand sequences of OE, IE and a hyperactive 19 related sequence, ME, are presented. Those positions that differ between OE and IE are presented in bold type. See Reznikoff (2002) for more details.

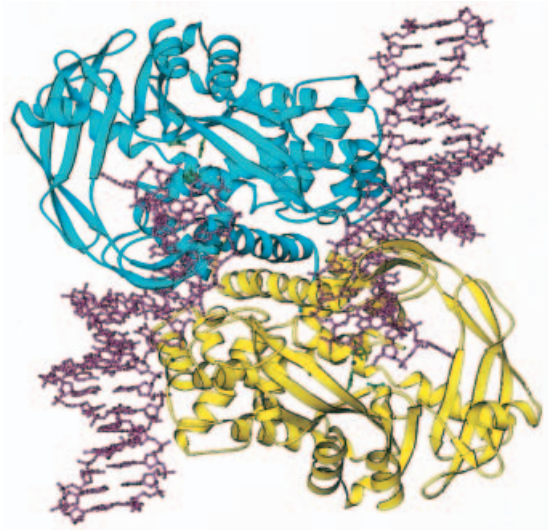


Fig. 2. Synaptic complex of hyperactive Tnp with OE DNA fragments. The figure is similar to that presented in Davies *et al.* (2000) and Reznikoff (2002). The Tnp used in this analysis contained the following mutations: E54K, M56A, and L372P. All of these mutations are described in the text, except for M56A, which has no effect on Tnp activity but prevents Inh synthesis. The structure is a *trans* dimeric structure with each subunit of Tnp binding one OE sequence and inserting it into the opposite subunit's active site.

Transposase

Tnp is 476 residues long. Key functional regions, such as the complex end DNA binding determinants, the dimerization contacts and the active site residues to be discussed in more detail later, have been mapped by genetic studies. Many of the mutations with interesting phenotypes resulted from random mutant hunts. Other genetic studies were guided by sequence comparisons to other transposases or by the X-ray crystallography results (see Fig. 2 for an over view of the Tnp structure as found in the synaptic complex). Like all other transposases (and retroviral integrases), Tn*5* Tnp contains critical DDE residues that chelate two Mg ions, which are critical for catalysis (Mizuuchi and Baker, 2002) (S. Lovell, I. Y. Goryshin, W. S. Reznikoff and I. Rayment, unpublished). Tn*5* Tnp is a member of the IS*4* family of transposases that contain a YREK motif around the E of the DDE residues (Rezsohazy *et al.*, 1993). The importance of the DDE and other residues in the active site for transposition catalysis have been confirmed by detailed genetic studies (Naumann and Reznikoff, 2002a; Peterson and Reznikoff, in press).

Structural comparisons indicate that, along with MuA (the only other transposase for which active site structural information is available), the catalytic core of Tn*5* Tnp is a member of the RnaseH superfamily of proteins. That is the overall architecture (but not the primary sequence) and the active site DDE are nearly identical to that of retroviral integrases, Mu transposase, RuvC and RnaseH (Bernstein *et al*., 1977; Bujacz *et al*., 1996; Goldgur *et al*., 1998; Davies *et al*., 1999; Mizuuchi and Baker, 2002).

Not surprisingly, the transposases and integrases also share common catalytic mechanisms resulting in transposon–donor backbone DNA nicking and strand transfer (Craig, 1995).

A key property of wild-type Tnp is that it is a very inactive protein. Purified wild-type Tnp shows no evidence of transposition activity *in vitro*, and *in vivo* the transposition frequency is on the order of 1 event in 10^5 cells, even though abundant protein is present. The biochemical basis for this inactivity has been studied through the isolation of four classes of hyperactive mutations that cumulatively can increase its activity at least four orders of magnitude.

The first class of mutation is typified by L372P (Weinreich *et al*., 1994). As will be discussed below, the N-terminus (containing the key DNA binding domain) and the C-terminus (containing the key dimerization domain) inhibit each other's activities most likely because they are located close together (York and Reznikoff, 1996; Braam *et al*., 1999). Tnp activity thus necessitates some sort of conformational change to separate these two domains. It is hypothesized that L372P facilitates this conformational change (Reznikoff, 2002). The introduction of the proline into an α-helix near the C-terminal breaks the helix and introduces a disordered region, thus tending to pull the C-terminal dimerization domain away from the DNA binding domain (Davies *et al*., 2000).

E54K is a representative of the second class of hyperactive mutation (Zhou and Reznikoff, 1997). E54K enhances the initial binding of Tnp to the 19 bp OE DNA (residue K54 is highlighted in green in Fig. 3A). Other mutations near residue 54 also enhance end DNA binding. Thus we can conclude that the end DNA binding domain has a suboptimal structure for the OE sequence. Other studies indicated that the N-terminal DNA binding domain is also suboptimal for IE binding (Zhou *et al*., 1998; Naumann and Reznikoff, 2002b).

The third class of hyperactive mutation includes E110K and E345K (Wiegand and Reznikoff, 1992). Residues E110 and E345 face each other through a salt bridge mediated by Mn^{++} (or Mg^{++}) (Davies *et al*., 2000). There are residues that contact the end DNA surrounding E345 (amino acids 342, 344 and 348 highlighted in green in Fig. 3A), suggesting that the residue 110 and 345 interaction may precisely arrange this region for these DNA contacts. Either of the above-mentioned genetic changes results in a direct E–K interaction, relieving the necessity for bridging through Mg^{++}. This direct E–K interaction might subtly improve the structure of this 345 region for DNA contacts or perhaps the intracellular availability of Mg^{++} is not sufficient to ensure its presence in this site for wild-type Tnp.

Finally, a P to A (or G) change at residue 242 results in hyperactivity (M. Steiniger-White, J. Metzler and W. S. Reznikoff, unpublished). The simplest way to explain this property is to postulate that these changes enhance the flexibility of the 242–247 sequence in forming a β-loop clamp (coloured in green in Fig. 3B) over the transposon end DNA as it is being inserted into the active site. Notice that proline 242, which is at the base of this loop,

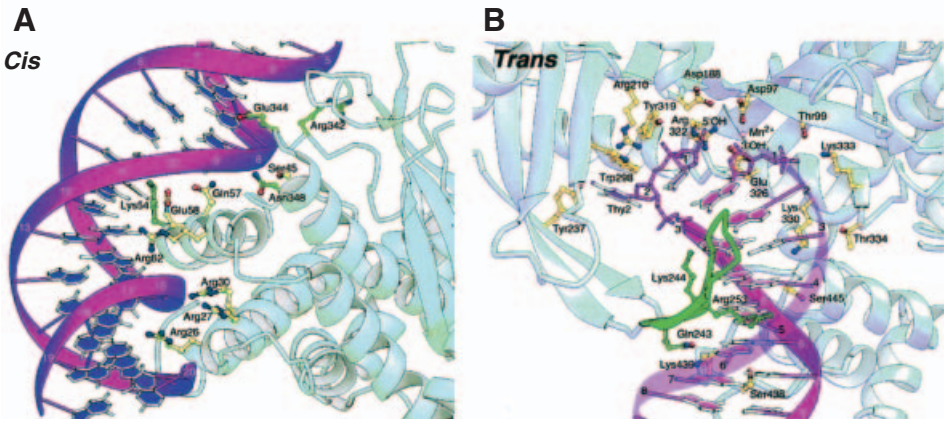


Fig. 3. Details of the Tnp–DNA contacts in the synaptic complex. The *cis* (A) and the *trans* (B) DNA contacts presented previously in Davies *et al*. (2000) are shown. The site of E54K hyperactive mutation and residues 342, 344 and 348 are emphasized in green in the *cis* contacts and the 252–259 β-loop clamp (also in green) is emphasized in the *trans* contacts.

will have a much more rigid backbone than either alanine or glycine.

The observation that Tn*5* Tnp is essentially inactive makes good biological sense. A highly active Tnp would lead to frequent transposition, genetic death for the host and loss of Tn*5*. Because of the critical importance of achieving a balance between transposition and host survival, there are other down regulatory mechanisms imposed on Tn*5* transposition. For instance, the Inh protein (an N-terminal deletion variant of Tnp) acts as a *trans* dominant negative inhibitor of Tnp activity by forming inactive Inh::Tnp dimers (reviewed in Reznikoff, 2002). In addition Tnp mRNA synthesis is downregulated by DNA Dam methylation and Tnp's expression of read-through transcripts is blocked by formation of an mRNA secondary structure (reviewed in Reznikoff, 2002). Other strategies have evolved that help other transposons address this balance between transposition and host survival. For instance, Tn*10*, in addition to having a similar DNA Dam methylation control, encodes an antisense RNA that inhibits translation of its transposase (reviewed in Kleckner, 1989; Haniford, 2002), and the high efficiency Tn*7* system transposases into a unique non-essential site (Craig, 2002).

Steps in Tn*5* transposition

Synapsis

The steps in Tn*5* transposition are outlined in the cartoon model shown in Fig. 4. The first steps result in the formation of the synaptic complex in which two molecules of Tnp are bound in a *cis*/*trans* relationship with two 19 bp end DNA sequences (Bhasin *et al*., 1999; Davies *et al*., 2000). From genetic and biochemical experiments and X-ray crystallographic analysis (see Figs 2 and 3) we know many of the detailed interactions resulting in synapsis but, surprisingly, we do not know whether synaptic complex formation occurs as pictured in Fig. 4 (monomeric Tnp binding to end DNA sequences followed by dimerization) or via Tnp dimerization first followed by DNA binding or by both routes. For this presentation we will assume that the first route is correct.

As mentioned above, the first step in synapsis must involve a conformational change in Tnp that moves the N-terminus away from the C-terminus. Although this conformational change is not well understood, we know that it is related to a key downregulatory mechanism in Tn*5* transposition.

Next, residues 26–65 form specific contacts with end DNA positions 6–17 (Zhou and Reznikoff, 1997; Davies *et al*., 2000; Reznikoff, 2002). These Tnp–end DNA contracts are presumed to form first in the transposition process and are many of the so-called 'cis' contacts. These contacts are visible in the crystal structure as shown in Fig. 3A. Additional *cis* contacts involve Tnp residues 342, 344 and 348 (Davies *et al*., 2000; Naumann and Reznikoff, 2002b; Reznikoff, 2002). It is not known whether the contacts with residues 342, 344 and 348 form during the initial end DNA binding event or subsequently during synapsis.

The following step involves Tnp–end DNA complex dimerization to form the synaptic complex. The two C-terminal α-helices cross and form a modest protein–protein contact (Davies *et al*., 1999; 2000; Steiniger-White and Reznikoff, 2000). The end DNA molecule positions 1–9 are inserted into the active site of the partner monomer forming multiple 'trans' Tnp–DNA contacts with residues near the active site in which the critical DDE residues are located (Fig. 3B) (Davies *et al*., 2000; Reznikoff, 2002). A conformational change occurs as an unstructured region of Tnp (residues 242–247 pictured in green in Fig. 3B) folds over the DNA as a β-loop clamp, making DNA contacts around position 4 (Davies *et al*., 2000; Reznikoff, 2002). The *trans* DNA contacts (and thus formation of the synaptic complex) are critical because all catalytic steps to be described subsequently are performed in *trans*, i.e. the Tnp that first contacts one end performs catalysis on the other end (Naumann and Reznikoff, 2000). Trans catalysis has also been shown for the bacteriophage Mu system (Aldaz *et al*., 1996; Savilahti and Mizuuchi, 1996; Namgoong and Harshey, 1998).

One major uncertainty is the disposition of the attached donor DNA within the synaptic complex as donor DNA was not present on the DNAs used for the crystallographic studies. Genetic and biochemical studies suggest that the active site must be quite crowded in the presence of the attached donor DNA. For instance, a D to E mutation (which adds a single carbon to the side chain) at either of the aspartate residues inhibits synaptic complex formation (Peterson and Reznikoff, in press). In addition, it is also known that the DNA is distorted at the transposon–donor DNA juncture (York and Reznikoff, 1997; Bhasin *et al*., 1999). This DNA distortion is likely to facilitate the catalytic steps described in the next section.

DNA cleavage

Extensive genetic, biochemical and structural information exists regarding the three catalytic steps involved in DNA cleavage; 3′-strand nicking, hairpin formation and hairpin cleavage (Bhasin *et al*., 1999; Reznikoff, 2002). The Tnp active site DDE residues co-ordinate two Mg^{++} ions (or for structural work, two Mn^{++} ions). The presence of two metals in the active site is consistent with previous structural work on ASV integrase (Wlodawer, 1999). The Mg^{++} ions play key roles in activating the oxygens that serve as nucleophiles and in contacting the oxygens in the P-O

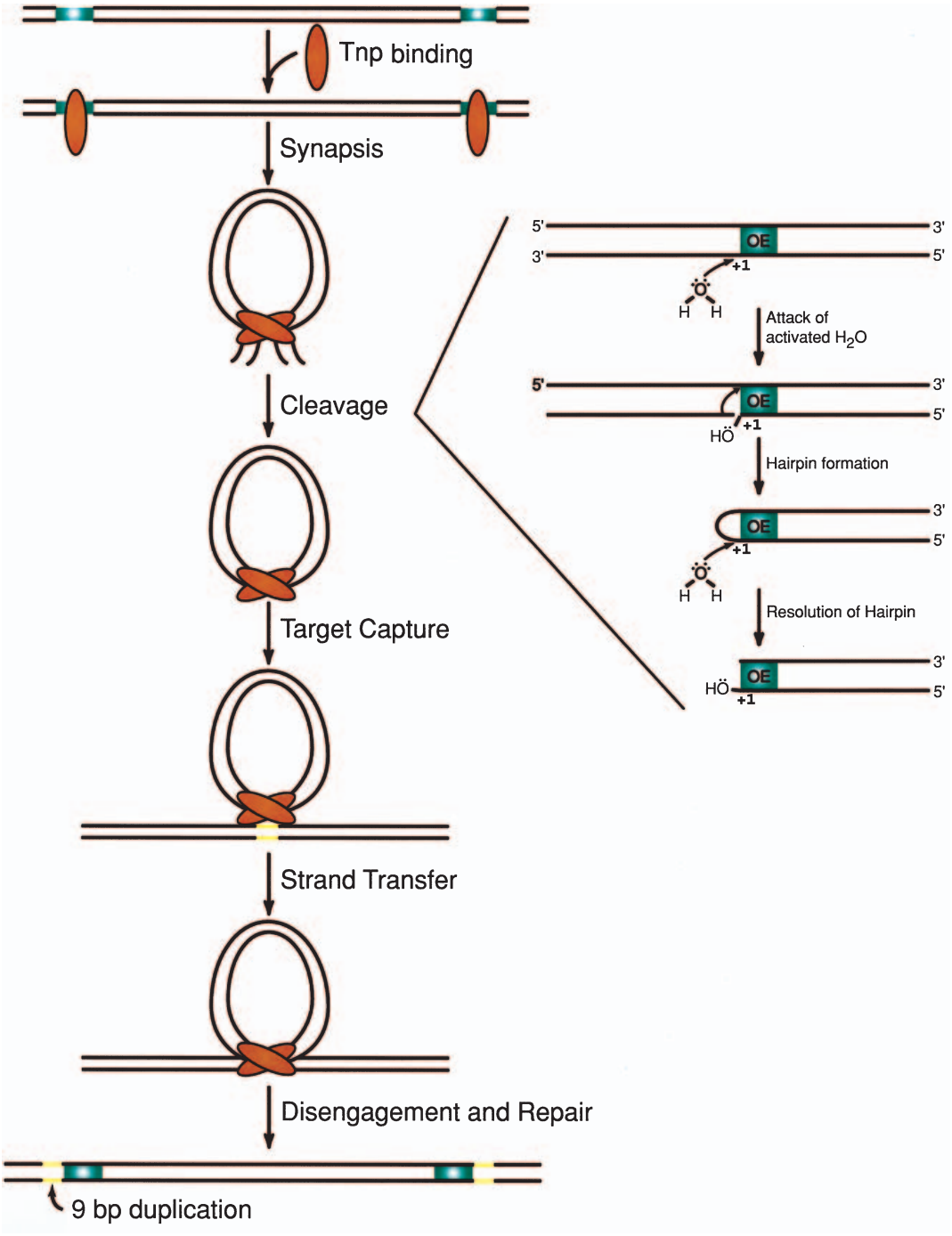


Fig. 4. Tn*5* transposition mechanism. Synaptic complexes are formed after Tnp binds to the ends and then dimerizes forming both Tnp–Tnp and *trans* Tnp–DNA contacts. In the presence of Mg^{++} or Mn^{++}, three catalytic steps occur resulting in cleavage of transposon–donor DNA bonds. The released synaptic complex then captures target DNA and strand transfer (that also requires the bound Mg^{++}) occurs. See Reznikoff (2002) for more details.

bonds that are cleaved (Lovell *et al*., 2002; Reznikoff, 2002). Unlike site specific recombination, no covalent protein–DNA bonds are formed during catalysis. Rather the reactions proceed through the following steps (see Fig. 4).

First oxygens from H_2O molecules are used as nucleophiles to nick the 3′(transferred)-strands on both ends. This first reaction is shared by all DNA transposases, by the retroviral integrases and by the long LTR retrotransposases (Craig, 1995). Remember, retroviral integrases

share structural features especially regarding the active site with Tn*5* Tnp (Davies *et al*., 1999).

The second step involves the nucleophilic attack of the 3′OH group from the transferred strand onto the 5′ (non-transferred)-strand generating a DNA hairpin (Bhasin *et al*., 1999). Hairpin generation frees the transposon DNA from the donor backbone DNA. Generation of a DNA hairpin is also the hallmark of the RAG-1-mediated DNA cleavage reaction (McBlane *et al*., 1995) and was previously found for the Tn*10* system (Kennedy *et al*., 1998). Although we do not know the structure of RAG-1, we do know that it has a critical triad of DDE residues (Kim *et al*., 1999; Landree *et al*., 1999; Fugman *et al*., 2000). Thus, we assume that RAG-1 is a member of the RNaseH super family of proteins and that the mechanism of hairpin formation resembles that catalysed by Tn*5* transposase.

Formation of the hairpin requires a denaturation of basepairs near the nicked site and reorientation of at least one of the phosphodiester backbones to bring the ends of the two strands close enough together to form the interstrand phosphodiester bond. Our studies have indicated that this terminal denaturation is likely facilitated by a flipping of the second base of the non-transferred (5′)-strand (thymine 2 in Figs 1C and 3B) out of the helix into a hydrophobic pocket of the Tnp where it stacks with a tryptophan 298 (Davies *et al*., 2000; Ason and Reznikoff, 2002).

Hairpin DNA containing the OE sequence will form synaptic complex like structures and these complexes have been used to generate crystals suitable for X-ray crystallography (S. Lovell, I. Y. Goryshin, W. S. Reznikoff and I. Rayment, unpublished). After incubation in the presence of Mn^{++} for 2 hours, the crystals were frozen and X-ray crystallography was performed. The overall complex structure is the same as that determined previously although two metal ions are present in the active site. The hairpin has been nicked, indicating that catalysis has occurred within the crystal and thus that the crystal structure is a *bona fide* representative of physiologically relevant synaptic complexes. The stereochemistry of the Mn^{++}-oxygen contacts is as predicted from biochemical studies with the related Tn*10* transposase (Kennedy *et al*., 2000).

Examination of the nicked hairpin structure suggests that some movement of the non-transferred (5′) strand must take place to make room for target DNA docking. In fact, when the structure of the Tnp–DNA complex containing the exact same DNA sequence structure as the DNA found in nicked hairpin was formed directly and analysed by X-ray crystallography, the non-transferred (5′) strand was found to have moved away from the active site (S. Lovell, I. Y. Goryshin, W. S. Reznikoff and I. Rayment, unpublished).

Target capture and strand transfer

We know very little about the target capture step. Judging from the specificity of Tn*5* insertions (Goryshin *et al*., 1998), there are some sequence biases for this step but we do not know how they are determined. From the synaptic complex structures solved to date, we can guess some features of target DNA docking (Davies *et al*., 2000). The two 3′OH groups are 41 Å apart, slightly further than desired for attacking the two target phosphodiester bonds the required 9 bp apart. These two 3′OH groups are embedded in a cleft the right width for holding a DNA helix and this cleft contains many positively charged residues. Thus we hypothesize that the target DNA is bound to the basic cleft awaiting strand transfer.

After target capture, the two 3′OH ends attack the target DNA phosphates 9 bp apart resulting in integration of the transposon into the target. Interestingly, even though strand transfer takes place within a synaptic complex, the strand transfer of the two ends is not always synchronized *in vitro* (Naumann and Reznikoff, 2002a).

Disengagement of the strand transfer complex

A key difference between the *in vitro* transposition process and the *in vivo* process involves the disengagement of the strand transfer complex. *In vitro* this is accomplished by the denaturation of Tnp through the use of a phenol or SDS heat treatment (Goryshin and Reznikoff, 1998). Clearly, some host function that is missing from our *in vitro* system is involved in this step. Similar phenomena have been found for other transposition systems. For instance a chaperone (ClpX) facilitates release of the Mu transposase (Levchenko *et al*., 1995). For Tn*5*, we hypothesize that a protease that cleaves the protein adjacent to residue R40 might be involved. An R to Q mutation at residue 40 results in a strong transposition defect *in vivo* but not *in vitro* (Twining *et al*., 2001). The primary difference between the *in vivo* and the *in vitro* system involves the disengagement mechanism.

After strand transfer complex disengagement, the host fills in the two 9 bp gaps at either end of the integrated gaps.

Conclusions

The Tn*5* system is helping to add molecular details to our understanding of ‘cut and paste’ transposition. Furthermore, as Tnp is a member of the RnaseH superfamily of proteins (which also includes retroviral integrases), these studies should also be of general importance. In regards to protein structure/function, these studies have demonstrated how one protein, Tnp, can be endowed with the ability to perform multiple activities and also how Tnp is

structured to be very inactive. These combined properties of Tn*5* transposase results in a system that rarely functions but, when it does so, is capable of generating a multistep process resulting in DNA transposition.

Acknowledgements

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