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Structure/function insights into Tn*5* transposition

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Prokaryotic transposon *5* (Tn*5*) serves as a model system for studying the molecular mechanism of DNA transposition. Elucidation of the X-ray co-crystal structure of Tn*5* transposase complexed with a DNA recognition end sequence provided the first three-dimensional picture of an intermediate in a transposition/retroviral integration pathway. The many Tn*5* transposase–DNA co-crystal structures now available complement biochemical and genetic studies, allowing a comprehensive and detailed understanding of transposition mechanisms. Specifically, the structures reveal two different types of protein–DNA contacts: *cis* contacts, required for initial DNA recognition, and *trans* contacts, required for catalysis. Protein–protein contacts required for synapsis are also seen. Finally, the two divalent metals in the active site of the transposase support a 'two-metal-ion' mechanism for Tn*5* transposition.

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Current Opinion in Structural Biology 2004, **14**:50–57

This review comes from a themed issue on
Protein–nucleic acid interactions
Edited by Gregory D Van Duyne and Wei Yang

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2004.01.008

Abbreviations

bp	base pairs
IE	inside end
NTS	non-transferred strand
OE	outside end
p-csc	post-cleavage synaptic complex
Tn*5*	transposon *5*
Tnp	transposase
TS	transferred strand

Introduction — overview of Tn*5* transposition

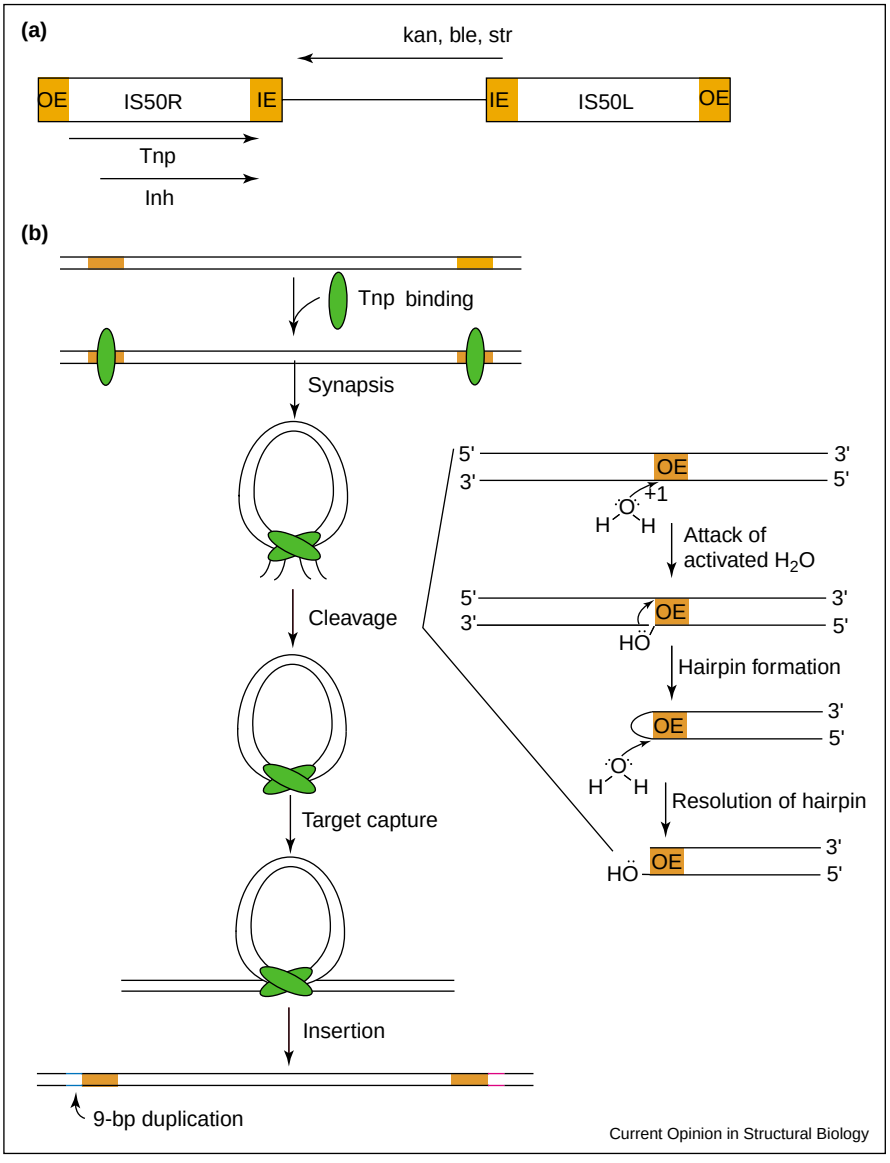
Transposition is a ubiquitous process that causes genomic evolution through the insertion, deletion and duplication of DNA sequences. Although many variations of transposition occur in nature, this review will focus only on the mechanism of prokaryotic 'cut-and-paste' transposition, as exemplified by transposon *5* (Tn*5*). Because Tn*5* is one of the most studied transposons and because crystal structures are available of a protein–DNA complex required for Tn*5* transposition, the relationship between protein structure and function is well established for this system.

Generally, transposons are defined as segments of DNA capable of moving from one location in a genome to another location in the same or a different genome. Specifically, Tn*5* is a prokaryotic composite transposon consisting of two insertion sequences, IS50R and IS50L, flanking a region of DNA containing three antibiotic resistance genes (see Figure 1a). Tn*5* is delineated by 19 base pair (bp) inverted repeat recognition sequences termed outside ends (OEs), whereas each IS element is flanked by an OE and an inside end (IE) [1,2]. These two wild-type end sequences are semi-perfect repeats differing at seven of the nineteen base pairs. Movement of Tn*5* requires two OEs, whereas movement of an individual IS element requires an OE and an IE. Tn*5* transposition requires the 476 amino acid transposase (Tnp) translated from IS50R and DNA flanked by the 19 bp inverted repeats [3–5]. Tn*5* transposition is partially regulated by the inhibitor protein Inh, which is identical to Tnp, but is missing the N-terminal 55 amino acids [4–6]. Inh inhibits transposition by forming nonproductive multimers with Tnp ([7,8]; for a review, see [9••]). Tn*5* transposes by the cut-and-paste mechanism detailed in Figure 1b [10].

Tn*5* transposition is inefficient *in vivo*, as careless movement of transposons in a genome would be hazardous to cells. This inefficiency partly arises from the suboptimal activity of Tnp. We hypothesize that the C-terminal domain inhibits the N-terminal DNA-binding domain via interaction of residues 40 and 450 (R Gradman, S Lovell, personal communication), effectively reducing the activity of Tnp following complete translation of the protein [11]. Consequently, wild-type Tnp is completely inactive *in vitro*. Luckily, a mutation in Tnp that negates this type of inhibition has been isolated [12]. LP372 introduces a break into an α helix, allowing the N-terminus to move away from the C-terminus and bind DNA [13••]. All Tnp discussed in this review contains the LP372 mutation.

Several X-ray crystal structures of Tn*5* transposition proteins have been elucidated [13••,14–16]. The first was an Inh dimer [14]. The remaining structures are co-crystals of Tnp complexed with end sequence DNA. Although these structures differ slightly in end sequence [13••,16], Tnp mutation [13••,15], number of base pairs in the DNA substrate [13••,15] and metal ions present in the Tnp active site [13••], all represent post-cleavage synaptic complexes (p-csc). These structures

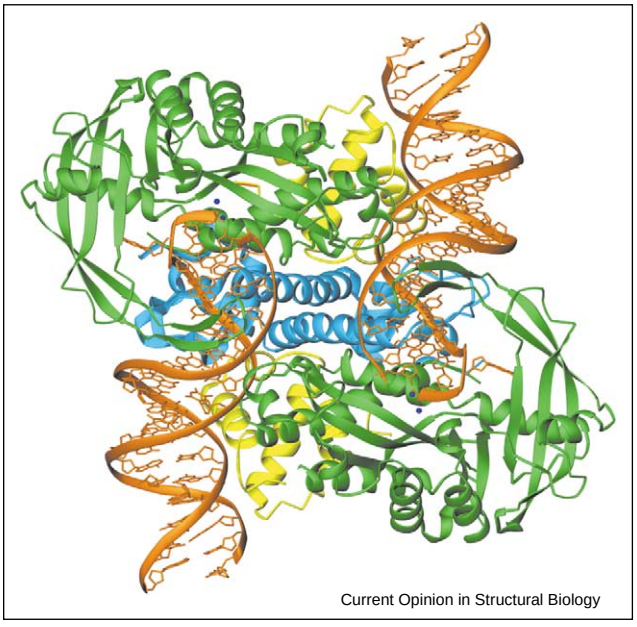
Figure 1



The mechanism of Tn*5* transposition. **(a)** A schematic of the Tn*5* transposon is shown in this figure. The transposon consists of two insertion sequences (IS50R and IS50L) flanking a region of DNA encoding kanamycin (kan), bleomycin (ble) and streptomycin (str). Tn*5* is delineated by 19 bp inverted repeat recognition sequences termed OEs; each IS element is flanked by an OE and an IE, shown in orange [1,2]. More details about the end sequences are given in the text. IS50R encodes two proteins: the 476 amino acid Tnp protein and an inhibitor protein (Inh) [3–5]. **(b)** First, monomers of Tnp (shown in green) are postulated to bind each OE sequence (shown in orange) [22]. Homodimerization of these bound Tnp monomers via their C-termini forms a synaptic complex [21,25]. All chemical steps of Tn*5* transposition occur within this complex [36]. Following synaptic complex formation, nicking of one DNA strand, the TS, occurs via nucleophilic attack of a water molecule (activated by a Tnp-coordinated Mg^{2+}) on the phosphodiester backbone between the +1 position of the OE and the −1 position of the flanking DNA, resulting in the generation of a 3′-OH. This 3′-OH then attacks the opposite DNA strand, the NTS, creating a hairpin intermediate [37] and releasing the flanking DNA from the blunt-ended transposon [38]. A second activated water molecule then resolves the hairpin intermediate. The resulting p-csc then captures a target DNA molecule and strand transfer occurs via a transesterification reaction in which the 3′-OH groups of the transposon attack phosphodiester bonds of the target in a staggered fashion. Formation of a covalent bond between the 3′-OH groups of the transposon ends and the 5′-phosphate groups of the target integrates the transposon [39]. Integration results in a 9 bp duplication of target DNA flanking the transposon ends, shown in blue [9••,40].

show the complex following complete cleavage of the transposon from the flanking DNA. Because of space limitations, not all the structures can be discussed. Instead, we examine what the structures of Tn*5* p-csc, when considered in conjunction with recent biochemical experiments, can tell us about Tn*5* transposition.

Figure 2



Structure of a Tn*5* p-csc. The X-ray crystal structure of Tn*5* Tnp complexed with OE DNA is shown in this figure. In this p-csc, two Tn*5* Tnps are complexed with two OE sequences. The N-terminal DNA-binding domain is shown in yellow, the catalytic domain containing the active site is shown in green and the C-terminal domain, which is primarily responsible for protein–protein interactions, is shown in cyan. The OE DNA is shown in orange and the two Mn^{2+} in each Tnp active site are shown as blue spheres.

General features of the Tn*5* p-csc co-crystal structure

The structure of the Tn*5* p-csc is shown in Figure 2. Tn*5* Tnp consists of three distinct domains: N-terminal, catalytic and C-terminal. The N-terminal domain consists of the first 70 amino acids, and is composed of exclusively α helices and turns used for DNA binding. The Tnp active site is found within the 300 amino acid catalytic domain. This domain contains a 'ribonuclease H like motif', an α/β/α fold with a mixed β sheet of five strands in which strand 2 is antiparallel to the other four strands [17]. This fold defines the catalytic domain of all transposases and retroviral integrases for which an X-ray crystal structure is currently available [14,18–20]. The C-terminal domain is comprised entirely of α helices and turns, and is primarily responsible for protein–protein interactions.

The p-csc is a homodimer, with each monomer containing one Tnp and one recognition end sequence [21]. These monomer complexes are related to each other via a crystallographic twofold axis of symmetry. Each DNA duplex contacts both protein subunits, forming two independent sets of Tnp–DNA end sequence contacts. Initial contacts made between Tnp and the recognition end sequence before synaptic complex formation are termed *cis* contacts. These contacts primarily occur between the N-terminal domain of Tnp and bases 6–15 of the end sequence. Tnp contacts to the opposite inverted repeat in the p-csc are termed *trans* contacts. These contacts occur between the catalytic domain of Tnp and bases 1–6 of the end sequence. The *cis* and *trans* protein–DNA contacts account for two-thirds of the buried solvent-accessible surface area between symmetry-related subunits. This indicates that the p-csc is maintained primarily by protein–DNA contacts rather than by protein–protein contacts [13••] (see Figure 2).

Two Mn^{2+} are present in the active site of each Tnp. One Mn^{2+} is coordinated by a water molecule, $O^{\varepsilon 1}$ and $O^{\varepsilon 2}$ of Glu326, $O^{\delta 1}$ of Asp97 and the deoxyribose 3′-OH of transferred strand base 1 (TS1; see Figure 1 for a more detailed description of the transferred and non-transferred strands [TS and NTS]). This 3′-OH would attack the target DNA to integrate the transposon at the next step of transposition. The second Mn^{2+} is coordinated by two water molecules, O1P of the NTS 5′-phosphate, $O^{\delta 2}$ of Asp97 and $O^{\delta 1}$ of Asp188. Interestingly, both Mn^{2+} are bridged by O2P of the NTS 5′-phosphate. This coordination is shown in Figure 3. The catalytic triad residues (Asp97, Asp188 and Glu326) are collectively referred to as the DDE motif and are conserved among transposases and retroviral integrases.

Structure/function relationships revealed by the Tn*5* p-csc

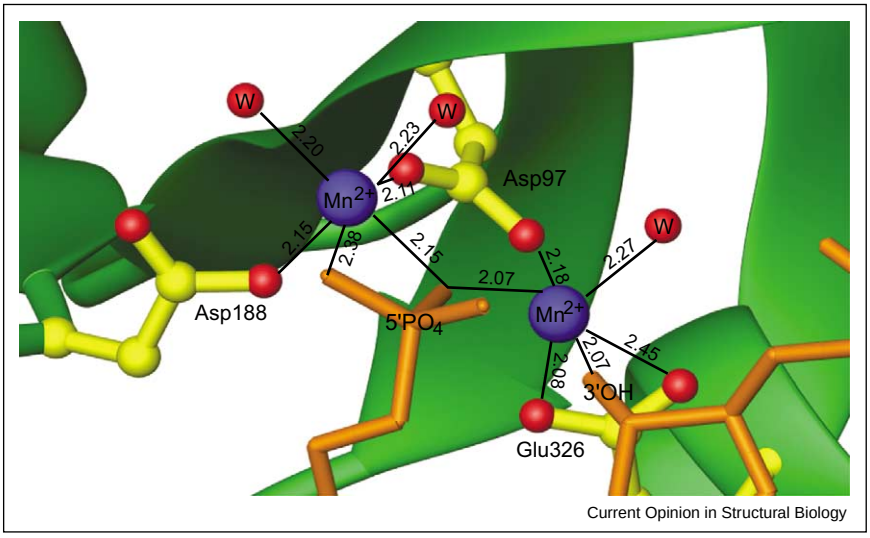
The Tn*5* p-csc co-crystal structure, when considered in conjunction with biochemical and genetic data, helps to define the Tnp–DNA end sequence and protein–protein interactions necessary for Tn*5* transposition, and to discern the relationship between Tnp structure and function. Below, the mechanism of transposition will be discussed sequentially to review this relationship.

Cis protein–DNA contacts

Cis contacts are believed to occur before synaptic complex formation and may be involved in initial end sequence recognition by Tnp. The p-csc co-crystal structure reveals both base-specific and phosphate backbone contacts between the N-terminal domain of Tnp and bases 6–15 of the end sequence (Figure 4); mutation of N-terminal domain residues reveals that both types of contacts are required for transposition.

In wild-type Tnp, amino acid 54 is a glutamate. Previously, a hyperactive Tnp mutant was isolated with a lysine at this position [22]. This mutation, EK54, together with LP372, defines the hyperactive EK/LP Tnp used for all crystallography studies. The co-crystal structure reveals that a favorable base-specific contact between N^{ζ} of Lys54 and O4 of TS10 confers this hyperactivity. The interaction between Glu54 of the wild-type protein and TS10 might even inhibit binding during early steps of transposition.

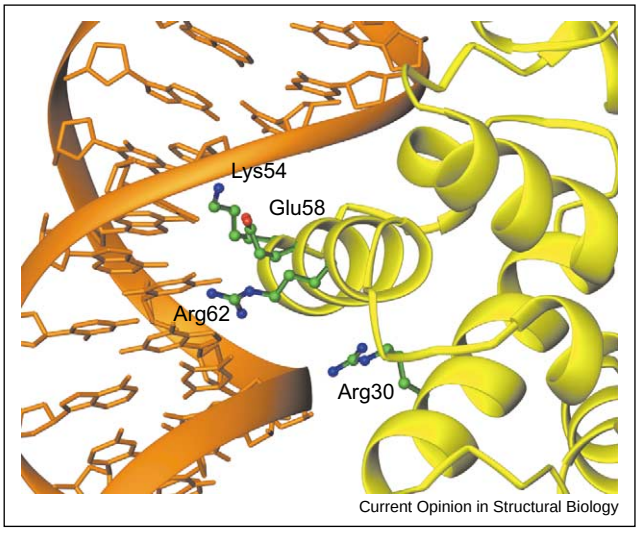
Figure 3



The 'catalytic triad' DDE residues in the Tnp active site coordinate two Mn^{2+}. Each Mn^{2+} has six ligands, including the DDE catalytic triad residues, several water molecules (represented by red balls labeled with a 'W'), the 5′-PO_4 of the NTS DNA and the 3′-OH of the TS DNA (DNA is shown in orange). The distance between the Mn^{2+} and each ligand is shown in angstroms. The distance between the two Mn^{2+} is 3.80 Å.

The co-crystal structure also shows base-specific contacts between O^{ε} of Glu58 and NTS10 and NTS11. Interestingly, when valine is substituted at this position, the mutant Tnp has stronger affinity for the IE, which is methylated at TS11 *in vivo* [23]. Wild-type Tnp is blocked from binding methylated IE because of steric hindrance between Glu58 and the added methyl group. Mutation of residue 58 to valine relieves this hindrance, introduces a beneficial hydrophobic interaction and causes this mutant to efficiently bind the methylated IE.

Figure 4



Cis contacts between Tnp and the OE in the p-csc are shown in this figure. *Cis* contacts occur before synaptic complex formation, and involve the N-terminal domain of Tnp (yellow) and bases 6–15 of the end sequence (orange). Some specific Tnp residues known to be important for this interaction are represented as green ball-and-stick models (see text for further discussion).

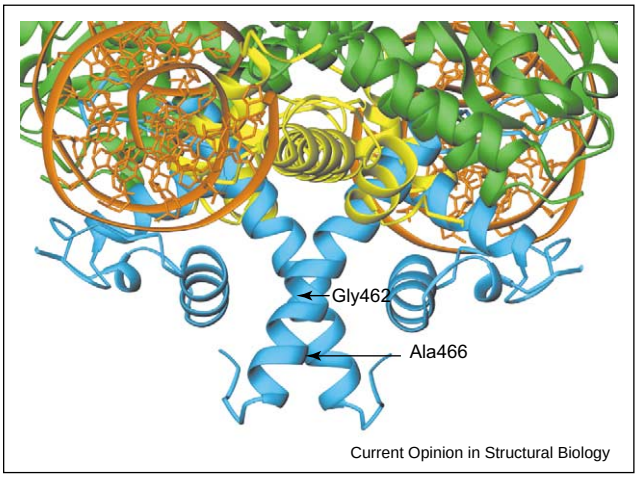
The amide group of Arg62 makes base-specific contacts with NTS12, TS12 and TS13, whereas the amide group of Arg30 interacts with the backbone phosphates of TS13 and TS14. When these residues are changed to glutamine, removing the positive charge, the mutant Tnps can no longer efficiently interact with DNA [24].

Protein–protein interactions

Few Tnp–Tnp interactions are seen in the p-csc co-crystal structure. The most important of these contacts create a scissor-like dimerization interface between residues 452–476 of the C-terminal α helix from each Tnp monomer (Figure 5). We hypothesize that, following the formation of *cis* contacts, the C-terminal α helices of two Tnps bound to end sequence DNA homodimerize to begin synaptic complex formation. Mutation and deletion of residues from this helix confirm the importance of these interactions [25].

The progressive deletion of residues from the C-terminus reveals that the removal of more than seven amino acids abolishes *in vivo* and *in vitro* transposition due to lack of synaptic complex formation by these mutants. Examination of the p-csc co-crystal structure reveals that the dimer interface interactions are primarily hydrophobic; Gly462 and Ala466 from one Tnp monomer interact with the equivalent residues from the other monomer. Also,

Figure 5



The dimerization of two Tnp C-terminal domains is shown in this figure. In the p-csc, protein–protein interactions occur primarily between two Tnp C-terminal domains. The two domains form a scissor-like dimerization interface (shown in turquoise), with Gly462 from each monomer at the cross-over point. Ala466 from each monomer also interact 1.5 α-helical turns beyond the Gly462 cross-over point. Only seven amino acids can be deleted from the C-terminus before this interaction is disrupted, abolishing synaptic complex formation [25].

Ser458 interacts with Lys459 from the other Tnp monomer. Mutation of Gly462 and Ala466 to aspartates completely disrupts synaptic complex formation, resulting in loss of transposition activity. The larger, charged aspartate residue might cause repulsion between the Tnp monomers. Mutation of Ser458 to alanine only partially affects synaptic complex formation, indicating that this interaction is less important than the hydrophobic interactions.

Trans protein–DNA contacts: formation of a catalytically competent synaptic complex

Following C-terminal domain dimerization, the cleavage site of end sequence DNA bound to one Tnp monomer must be inserted into the active site of the other Tnp monomer. Many *trans* protein–DNA contacts are involved in the formation of a synaptic complex that is competent for the first catalytic step (Figure 6). When the DNA is inserted into the Tnp active site, specific contacts occur between active site residues and the DNA to ensure proper orientation for TS nicking. Also, proper complex formation requires contacts between a Tnp β-loop (residues 240–260) and the end sequence that are not present in the final catalytically competent synaptic complex.

The p-csc co-crystal structure reveals that the conserved DDE residues coordinate the two metal ions required for all catalytic steps of transposition (see Figure 3) [15,26••]. When these residues are mutated to alanines, no effect on synaptic complex formation is observed [27]. More surprisingly, when Asp97 and Asp188 are individually mutated to glutamate, synaptic complex formation is greatly reduced. When Glu326 is changed to aspartate, synaptic complex formation becomes more efficient [28]. These data indicate that the overall architecture of the DDE residues is important, not only for metal binding but also for proper positioning of the flanking DNA in the Tnp active site.

Mutation of other conserved active site residues further emphasizes the importance of active site architecture. The p-csc co-crystal structure shows that Arg322 contacts Glu326 and the phosphate of NTS2, and Lys333 contacts the phosphate of TS1. Mutation of Arg322 to alanine, lysine or glutamine, or mutation of Lys333 to either alanine or arginine reduces synaptic complex formation [27]. At both positions, conservation-of-charge mutants form synaptic complexes most efficiently. These data indicate that positively charged residues might be required at these positions to properly orient end sequence DNA in the Tnp active site.

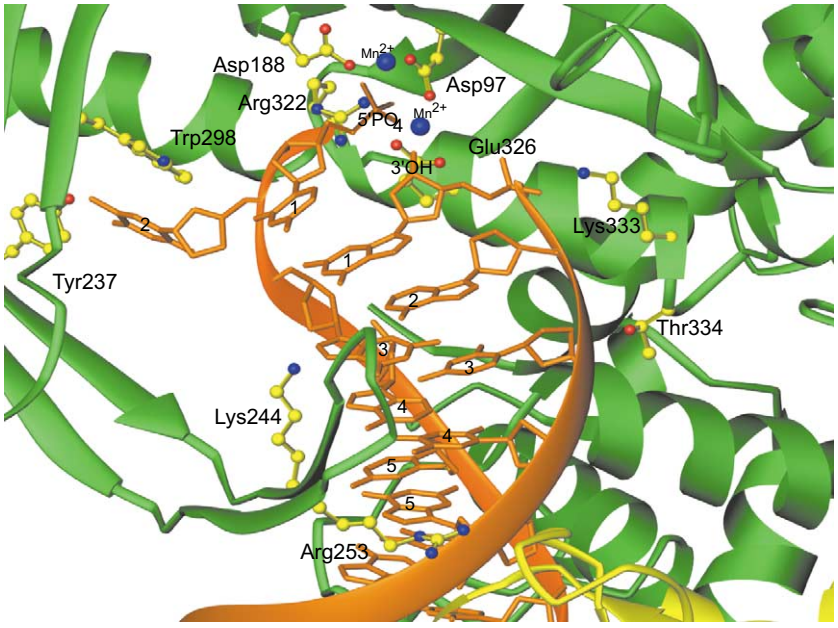
Finally, examination of the p-csc co-crystal structure shows some interaction between Tnp residues 240–260 (a β-loop) and bases 3–7 of end sequence DNA. The limited number of contacts seen in the crystal structure is deceptive with regard to the importance of these *trans* contacts. Mutation of Arg253 and Arg256 reduces synaptic complex formation *in vitro*, but contacts to these residues are not seen in the p-csc co-crystal structure (M Steiniger-White, unpublished). These (and other) data indicate that specific, transient contacts are made between the β-loop and the major groove of the DNA as Tnp changes conformation during synaptic complex formation.

Trans protein–DNA contacts: catalysis

Each catalytic step of transposition requires specific protein–DNA contacts to properly orient the DNA substrate in the Tnp active site for nucleophilic attack by a Mg^{2+}-activated water or hydroxyl group. All current p-csc co-crystal structures reflect the Tnp conformation and DNA orientation at the same point in the mechanism, but interesting observations can still be made about other catalytic steps by examining these structures. A wealth of biochemical data substantiates these observations.

Although DNA base flipping has been proposed to be a general feature of many enzymes, this phenomenon was not revealed in the Tn*5* system (or other transposase or retroviral integrase systems) until the elucidation of the p-csc co-crystal structure [29–32]. In the p-csc structure, NTS2 (thymine) is rotated out of the DNA helix into a pocket in Tnp. O^4 of NTS2 forms a hydrogen bond with the hydroxyl of the Tyr237 R-group, the aromatic R-group of Trp298 stacks with the pyrimidine base of NTS2 and the guanidinium group of Arg322 contacts the backbone phosphate of NTS2 (Figure 7). Unlike the

Figure 6



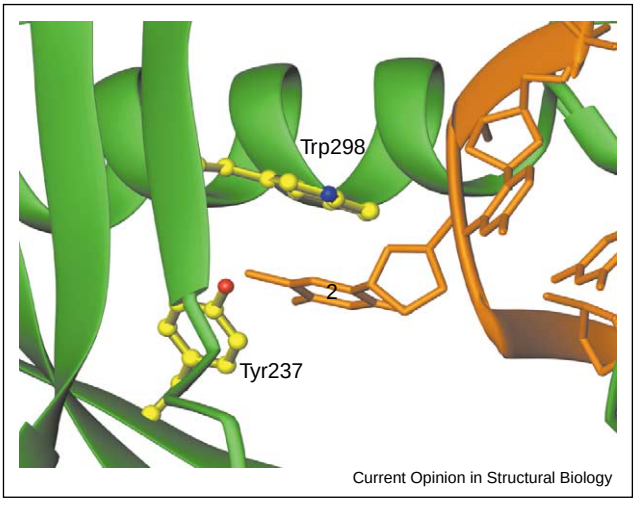
Trans contacts between Tnp and the OE in the p-csc are shown in this figure. Many important contacts between Tnp and the OE are required for synapsis and proper orientation of the DNA for catalysis. Some of these contacts are shown here, with amino acids represented as yellow ball-and-stick models and DNA shown in orange. A β-loop, including Lys244 and Arg253, contacts bases 3–6 of the end sequence DNA, Tyr237 and Trp298 interact with a 'flipped-out base' (NTS2) and many active site residues contact the first two nucleotides of OE DNA.

Arg322 mutants discussed earlier, when Tyr237 is changed to phenylalanine and Trp298 is mutated to alanine or phenylalanine, synaptic complex formation is largely unaffected [33]. These data indicate that contacts between DNA and these residues are not necessary for proper synaptic complex formation. Further biochemical experiments show that stacking interactions between Trp298 and NTS2 are required for transposition *in vitro* and aid the last three catalytic steps: hairpin formation, hairpin resolution and strand transfer [33]. Thus, contacts between Trp298 and NTS2 help orient the DNA for the intermediate steps of transposition.

Although Arg322 mutants form synaptic complexes inefficiently, additional experiments indicate that TS nicking is unaffected [27]. Furthermore, when a positive charge is retained at this position, the mutant can form hairpins, indicating that the contact to the backbone phosphate of NTS2 may be important for steps preceding the formation of the p-csc.

The hydrogen bond between Tyr237 and NTS2 is not required for any chemical step of transposition. Interestingly, the mutation of all residues lining the flipped base binding pocket affects strand transfer [33]. It is difficult to

Figure 7



Tnp–DNA end sequence contacts involving NTS2, the flipped-out base (2), are shown in this figure. A close-up view of interactions between Tyr237 and Trp298 and NTS2 are shown. Stacking interactions between Trp298 and NTS2 are required for *in vitro* transposition and aid hairpin formation, hairpin resolution and strand transfer. Hydrogen bonding between Tyr237 and NTS2 aids the strand transfer step of transposition [33].

form a hypothesis for this phenotype with the crystal structures currently available.

Two metal ions in the Tnp active site are responsible for proper orientation of the nucleophile for catalysis. In the p-csc co-crystal structure, the nucleophile for strand transfer (the next catalytic step) is the 3′-OH of TS1, which is coordinated to Mn^{2+} in the Tnp active site. It is this hydroxyl that would attack a phosphate on the target to integrate the transposon. As mentioned above, the conserved DDE motif coordinates the two Mn^{2+} in the Tnp active site. When these residues are mutated to alanines, or Asp97 and Asp188 are changed to glutamates and Glu326 is changed to aspartate, all catalytic steps are affected [27,28]. These data indicate that two metal ions may be required for all steps of transposition, supporting a 'two-metal-ion' mechanism similar to that proposed for the Klenow fragment of DNA polymerase [34,35].

Conclusions

Study of the Tn*5* system has vastly aided our understanding of 'cut-and-paste' transposition. Recent crystallographic studies have provided the most complete co-crystal structures of any Tnp, integrase or related protein; the use of these structures to interpret biochemical data has been invaluable. The integration of biochemical and structural data allows us to better understand synaptic complex formation and catalysis within this complex. More importantly, we appreciate the many specific Tnp and DNA conformational changes required to allow completion of each individual catalytic step.

Although several structures of the Tn*5* p-csc have been solved, Tnp–DNA end sequence co-crystal structures that represent other steps of the transposition mechanism have been more difficult to obtain. Nevertheless, through ongoing structural work, we hope to gain further insights into the molecular mechanism of Tn*5* transposition.

Acknowledgements

We would like to thank Eric Wise for aid in preparation of the figures, and Robert White and Brandon Ason for helpful discussions.

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- • of special interest
- •• of outstanding interest

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The increased resolution of this structure compared to the originally published Tn*5* Tnp p-csc structure [13••] (1.9 Å versus 2.3 Å) reveals further details about Tn*5* Tnp–DNA end sequence interactions. Of specific interest are protein–DNA interactions in the Tnp active site.

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