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The Mu Transpososome Through a Topological Lens

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ABSTRACT Phage Mu is the most efficient transposable element known, its high efficiency being conferred by an enhancer DNA element. Transposition is the end result of a series of well choreographed steps that juxtapose the enhancer and the two Mu ends within a nucleoprotein complex called the 'transpososome.' The particular arrangement of DNA and protein components lends extraordinary stability to the transpososome and regulates the frequency, precision, directionality, and mechanism of transposition. The structure of the transpososome, therefore, holds the key to understanding all of these attributes, and ultimately to explaining the runaway genetic success of transposable elements throughout the biological world. This review focuses on the path of the DNA within the Mu transpososome, as uncovered by recent topological analyses. It discusses why Mu topology cannot be analyzed by standard methods, and how knowledge of the geometry of site alignment during Flp and Cre site-specific recombination was harnessed to design a new methodology called 'difference topology.' This methodology has also revealed the order and dynamics of association of the three interacting DNA sites, as well as the role of the enhancer in assembly of the Mu transpososome.

KEYWORDS phage Mu, DNA transposition, transpososomes, enhancer, DNA topology, topological filter, site-specific recombination

INTRODUCTION

Genomes are in a state of flux. They expand, contract, and rearrange primarily by the activities of transposons and retrotransposons that carry out either DNA- or RNA-mediated transposition (Craig *et al*., 2002). The original discovery of these elements by Barbara McClintock, who demonstrated their role in changing the structure of the maize chromosome, was likened by Jim Shapiro to the observation of spontaneous atomic decay (Shapiro, 1983). The most stunning evidence of the extent to which these elements have colonized and shaped genomes has come from recent sequencing projects, which have shown that nearly half of the human genome and up to 90% of some plant genomes are constituted from these elements (Kazazian, 2004). Although constantly changing, genomes are stable enough to have allowed genetic maps to be drawn. Their restructuring by the fixation of transposition events within a population is a slow process, occurring over an evolutionary time scale. Transposition frequencies must be kept obligatorily low so that the spread of elements and their

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genetic load do not pose impediments to genome integrity. In a balancing act that is apparently beneficial to both, a variety of host- as well as element-encoded mechanisms regulate the frequency of movement.

Phage Mu is a paradigm of a transposable element with a binary switch for regulating transposition. Mu can either shut down all its transposition functions and maintain a prophage state, or enter the lytic state to amplify its genome over 100-fold by replicative transposition (Symonds *et al*., 1987). The same *cis*-acting segment of DNA plays a dual role of silencer/enhancer in the respective states (Chaconas & Harshey, 2002). The high frequency of Mu transposition was critical to the reconstitution of the first *in vitro* system to study transposition (Mizuuchi, 1983). Indeed, the Mu *in vitro* system served as a basis for the development of *in vitro* systems for other DNA elements (Tn*10*, Tn*5*, Tn*7*) as well as retroviral elements (see Craig *et al*., 2002). A crystal structure of a Tn*5* transpososome is now available (Davies *et al*., 2000), as is a cryo-EM structure of a Mu transpososome (Yuan *et al*., 2005). A recent review spotlights insights gained from studies with several of these transpososomes (Gueguen *et al*., 2005). Here, we focus on the structure and regulation of the Mu transpososome as determined by topological studies, which have been particularly helpful in understanding the contribution of the enhancer to the assembly process. For earlier reviews on Mu transposition (see Chaconas & Harshey, 2002; Mizuuchi, 1992; Pato, 1989).

MU TRANSPOSITION: THE BASICS

Cis and *trans* Requirements of Transposition

Figure 1 summarizes the DNA sites and proteins involved in transposition (for more detailed references, see Chaconas & Harshey, 2002). The left (attL) and right (attR) ends of the 36,717 base pair (bp) Mu genome (Morgan *et al*., 2002) have three binding sites each for the transposase MuA: L1–L3 and R1–R3 (Craigie *et al*., 1984; Zou *et al*., 1991) (Figure 1A). These sites share a 22 bp consensus. Optimal transposition requires all of these sites to be present on supercoiled DNA. MuA binds as a monomer to each site, introducing an 80° to 90° bend (Kuo *et al*., 1991). L2 is the weakest binding site, showing protection over only the distal half of the consensus, and a smaller bend. L1 and L2 are separated

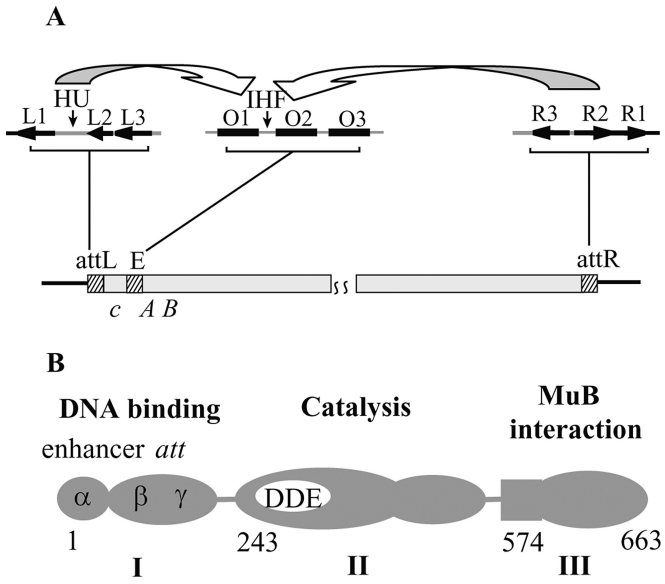

Figure 1 ***Cis* and *trans* requirements for Mu transposition. A. Relative arrangement of subsites within attL, attR and enhancer (E) sites on the 37 Kbp Mu genome. Genes regulating transposition (*c, A, B*), and *E. coli* proteins HU and IHF that bind to attL and enhancer, respectively, during transposition are indicated. The Mu transposase has a higher affinity for the att sites, from where it is thought to make cross-bridging interactions with the weak-affinity enhancer sites (*curved arrows*). *B*. Domain organization of the transposase MuA. Based on limited proteolysis, three domains (I–III) have been assigned to MuA. Amino acid numbers corresponding to the boundary of each major domain are shown beneath the structure. The function of each domain is indicated at the top. See text for details.**

by ~80 bp, a gap that is closed by binding of the *E. coli* protein HU in this region (Lavoie *et al*., 1996).

The enhancer (E; O1-O3) is located ~1 Kbp from attL. The 'O' designation originally came from identification of this region as the operator sequence at which the lysogenic repressor (c protein or Rep) binds to repress or silence the transcription of early phage functions, which include the transposition proteins A and B (Figure 1A). The alternate 'enhancer' label arose from the discovery that this sequence also stimulates *in vitro* transposition at least 100-fold (Leung *et al*., 1989; Mizuuchi & Mizuuchi, 1989; Surette *et al*., 1989). Like transcription enhancers, it acts in a distance-independent, albeit not orientation-independent, manner (Arnosti & Kulkarni, 2005). The Hin and Gin site-specific recombination systems employ enhancers that act in an orientation-independent manner (Heichman & Johnson, 1990; Kanaar *et al*., 1990). Only Mu and related phages employ transposition enhancers. O1-O2 sites constitute the 'minimal enhancer' since their *in vitro* activity is not significantly compromised in the absence of O3. There is no shared consensus

sequence between the three O sites, which are defined by protection patterns of the Rep and MuA proteins; these range from 30 to 60 bp at each site. The O1-O2 region, which includes an intervening IHF binding site, is ~150 bp. IHF, shown to introduce hairpin bends at other sites (Rice, 1997), is absolutely required for transposition when the superhelical density of plasmid DNA is low (Surette & Chaconas, 1989).

The transposase MuA is a 663 amino acids long tri-domainal protein (Nakayama *et al*., 1987), partial X-ray and NMR structures for which are available (Figure 1B). The N-terminal domain I contacts the end and enhancer sites through separate regions. Domain Iα, which shares extensive homology with Rep, interacts with the enhancer through a winged-helix DNA-binding motif, while domain I$\beta\gamma$ interacts with each end-binding site over two major and an intervening minor groove using separate helix-turn-helix motifs (see Chaconas & Harshey, 2002). MuA has a higher affinity for the end-binding sites, from where it is thought to make cross-bridging interactions with the weak-affinity enhancer sites (Figure 1A). DNA bending at the enhancer is expected to assist end-enhancer interactions. The central domain II of MuA encodes the signature DDE (Asp, Asp, Glu) residues involved in catalysis (Baker & Luo, 1994; Kim *et al*., 1995) (Figure 1B). The C-terminal domain III is responsible for interaction with the MuB protein, which modulates the activity of MuA as well as delivers target DNA to the transpososome (see Chaconas & Harshey, 2002).

Mechanism of Catalysis

The cleavage and strand transfer steps of catalysis during transposition from supercoiled substrates are summarized in Figure 2. DNA supercoiling, HU, and divalent metal ions are essential for initial assembly of the transpososome, which first promotes a helix-opening event near the termini (see Chaconas & Harshey, 2002). Within the transpososome, the successive steps of catalysis are carried out by the same two subunits positioned at L1 and R1 (Namgoong & Harshey, 1998). These subunits act in *trans* (*i.e.*, the subunit bound to L1 catalyzes chemistry at the R1 end and vice versa) to first nick the DNA at 3′ end of the terminal CA dinucleotide, using water as the nucleophile (Mizuuchi, 1984). The liberated 3′ OH ends serve as nucleophiles for the subsequent joining or strand transfer step, attacking phosphodiester bonds spaced 5 bp apart on target DNA in a one-step transesterification reaction (Mizuuchi & Adzuma, 1991; the stereochemistry experiments were performed on oligonucleotide substrates). Both nucleophiles are likely activated by divalent cations bound by the DDE residues within the MuA active site. The structure of the Tn*5* transposase synaptic complex, where (as for Mu)

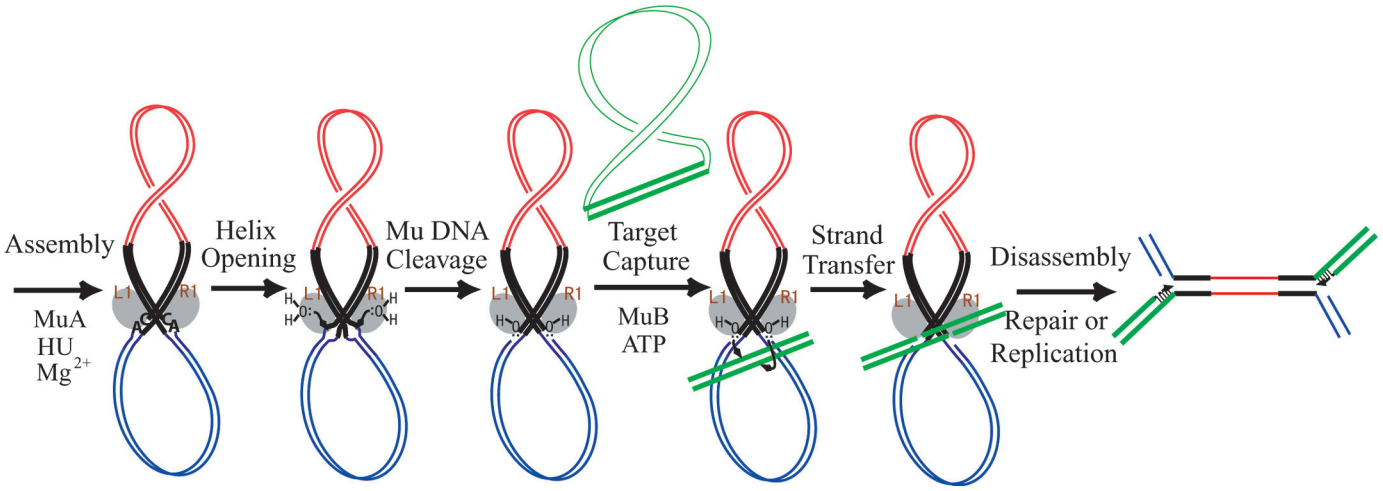

Figure 2 **Mechanism of Mu DNA transposition on supercoiled substrates. Only elements essential for catalysis are indicated. MuA subunits (*grey oval*) bound to L1 and R1 subsites act in *trans* to first cleave and then join the cleaved ends to target DNA. DNA supercoiling, HU and divalent metal ions are essential for initial assembly of the transpososome, which first promotes a helix opening event near the CA termini. The metal ions also activate water molecules to cleave adjacent to the terminal A residues, generating 3′OHs. MuB captures and delivers target DNA to the complex, wherein the newly created 3′OH groups now serve as nucleophiles for the joining or strand transfer step, attacking phosphodiester bonds spaced 5 base pair apart on target DNA in a one-step transesterification reaction. The strand transfer intermediate is resolved either by repair or replication using host proteins to extend the 3′OHs left on the target. See text for details.**

two transposase subunits also catalyze transposition in *trans*, provides a snapshot of how cleaved DNA is positioned within the active site (Davies *et al*., 1999; Rice & Baker, 2001; Lovell *et al*., 2002). In this structure, where the transposase was assembled on oligonucleotide substrates, DDE residues coordinate the two metal ions required for all catalytic steps of transposition. The distance between the two 3′OH ends is larger than the expected distance between the two scissile phosphates on target DNA, a situation similar to that observed within the cryo-EM structure of the cleaved Mu complex discussed below (Yuan *et al*., 2005). It is surmised that conformational changes induced upon target binding align the cleaved ends at the right distance for strand transfer. The MuB protein plays an essential role in binding target DNA and delivering it to the Mu transpososome through its interaction with the C-terminus of MuA (see Chaconas & Harshey, 2002). After completion of strand transfer, an ordered series of steps result in disassembly of the transpososome and recruitment of the replication apparatus to either replicate the entire Mu genome, or repair the single-stranded 5 bp gaps at the Mu-target junction (Nakai *et al*., 2001).

Nucleoprotein Complexes

Nucleoprotein complexes at several stages of transposition were first identified using gel electrophoresis/electron microscopy techniques (see Figure 9 later). LER was the earliest 3-site complex containing the L and R ends and the enhancer E (Watson & Chaconas, 1996). This complex is unstable, and was trapped only after glutaraldehyde cross-linking. Interaction of MuA subunits within this complex leads to formation of a stable MuA tetramer, which is the structural and functional core of subsequent complexes (Lavoie *et al*., 1991). Electron microscopy of all complexes following LER failed to find an association of the enhancer with the L and R ends, suggesting that the enhancer exits the transpososome during or upon its transition to the tetrameric complex (Watson & Chaconas, 1996). However, subsequent topological, gel electrophoresis and cross-linking methods have shown the enhancer to be clearly associated with the ends throughout transposition (see below), indicating that the earlier results were likely due to use of procedures that destabilized enhancer-end interactions.

The first tetrameric complex following LER is type 0, which can be trapped prior to catalysis by omitting divalent metal ions or using catalytic residue (DDE) mutants of MuA (Mizuuchi *et al*., 1992). A helix-opening event or 'open termini formation' is associated with formation of this complex, which is the rate determining step of the overall cleavage reaction (Wang & Harshey, 1994; Wang *et al*., 1996) (Figure 2; for simplicity, enhancer association with the ends is not shown in this drawing). The MuA footprint is extended ~15 bp into the Mu-host junction in this complex (Mizuuchi *et al*., 1992), with a likely kinking of DNA in the host region (Lavoie *et al*., 1991). The type 0 conformation promotes engagement of the terminal 5′T residues within the active site (Lee & Harshey, 2003) and leads to cleavage of the opposite 3′A residues at each end to produce the type 1 complex, in which supercoils in the non-Mu portion of the donor DNA molecule are relaxed (Surette *et al*., 1987). This complex is more stable than type 0, but less stable than the strand transfer complex type 2 where the cleaved Mu ends are joined to target DNA, assisted by MuB protein (Figure 2; see also Figure 9). Unlike in other transposons such as Tn*7* (Craig, 2002) or Tn*10* (Haniford, 2002), DNA cleavage is not a prerequisite for target capture, which can occur even at the LER stage (Naigamwalla & Chaconas, 1997). All three complexes–type 0, type 1, and type 2–can be identified by their distinct mobility on agarose gels.

The progressive stability of the nucleoprotein complexes as the reaction proceeds is an effective strategy for driving the transposition reaction forward (Mizuuchi, 1997). Strand transfer, or integration of Mu by attack of the 3′OHs at cleaved ends on target DNA, is a transesterification reaction. It should be isoenergetic with the reverse reaction, namely, 'disintegration' by the attack of 3′OHs left on the target DNA to cleave the newly formed Mu-target joint. Hence, there must exist mechanisms to block reversal of strand transfer. Conditions that cause reversal of integration involve incubation of the type 2 strand transfer complex at 75°C (Au *et al*., 2004), and suggest an altered active site configuration upon strand transfer, in which the reactive groups are misaligned to prevent reversal.

The multiplicity of DNA-protein interactions between the end and enhancer sites required for assembly implies that the DNA within the Mu transpososome is likely restricted to a fixed geometric path. While deciphering the precise topology of the DNA within

the complex has to await the DNA-protein co-crystal structure, the overall topology can be deduced from topological methods such as those developed for analysis of site-specific recombination. However, as described below, these methods are not directly applicable to the Mu transpososome. We have therefore developed a new methodology we call 'difference topology,' which may be generally useful for probing the topology of other complex systems as well.

TOPOLOGICAL ANALYSIS

Standard Topological Analyses of Site-Specific Recombination: DNA Transposition Does Not Easily Lend Itself to Such Analyses

The practice of analyzing DNA recombination by virtue of its topological consequences was pioneered by Cozzarelli and colleagues (Cozzarelli *et al*., 1990; Cozzarelli *et al*., 1984; Wasserman & Cozzarelli, 1986). More recently, the application of tangle calculus by Sumners, Cozzarelli and their associates has further enhanced the power of topology as a tool in deriving recombination mechanisms (Sumners *et al*., 1995). The rationale is first to determine experimentally the topology of the DNA knots or links (catenanes) produced by a recombinase enzyme as it carries out inversion reactions or deletion and rejoining reactions. The product topology is then subjected to mathematical analyses to deduce the nature of DNA crossings present within the recombination synapse as well as those introduced by the recombinase enzyme in the process of strand exchange. The precise strand cleavage and strand joining reactions mediated by conservative site-specific recombinases make them readily amenable to topological interpretations. They carry out these chemical steps via transesterification, conserving the energy of the phosphodiester bond, without net gain or loss of DNA and without the need for repair synthesis. By contrast, homologous recombination proceeds via hydrolytic cleavage, requires energy input to restore the phosphodiester bonds, and may involve extensive DNA synthesis. The topological insignia of the original reaction complex is often blurred or lost in the subsequent steps that are required to fill gaps, and tie loose ends.

Topology of Tn*3* or $\gamma\delta$ Resolvase Reaction

We shall first focus on the topology of the closely related resolvase enzymes of Tn*3* or $\gamma\delta$ transposons, serine family site-specific recombinases that reduce cointegrate intermediates formed during movement of these elements to simple integrants (Grindley, 2002; Stark et al., 1989). We do so because the topological treatment of Mu DNA transposition synapse discussed further on is developed on the assumption that the resolvase reaction occurs within a DNA-protein assembly of unique topology. The resolvase synapse is designated as -3 because the number of negative supercoils sequestered within it is three.

The wild-type resolvase requires negatively supercoiled substrates, is selective with respect to the relative orientation of recombination sites, and acts only on sites located on the same DNA molecule (reviewed in Grindley, 2002). The complete resolvase recombination site is composed of three subsites: *res* I, *res* II and *res* III (Figure 3). Two such sites, oriented head-to-tail, are the targets for the resolvase reaction. Each of the three subsites is bound by a resolvase dimer; however, only the dimers bound at *res* I are competent to carry out strand cutting and exchange. Interactions of resolvase dimers present at the accessory sites (*res* II and *res* III) are responsible for arranging a functional synapse at the *res* I sites. The topological analysis of the resolvase reaction is aided by the fact it does yield processive recombination (more than one round of reaction from the same synapse), so that a series of sequential recombination products are available for characterization.

The product of the first round of recombination by resolvase is a two-noded catenane (2^-): a pair of linked DNA circles with two negative crossings (the Hopf link*; Figure 3). The subsequent rounds of recombination yield a four-noded knot ($2^+, 2^-$), a figure-eight catenane ($3^+, 2^-$) and a six-noded knot ($4^+, 2^-$), respectively. These product configurations are most easily accommodated by a synapse in which three negative crossings are trapped by the resolvase dimers bound at *res* II and *res* III, the *res* I sites are arranged in a parallel orientation, and each DNA exchange step results in one positive crossing (Grindley, 2002). Although the

*The two-noded catenane, the simplest two component link, is also called the Hopf link after the German mathematician Heinz Hopf.

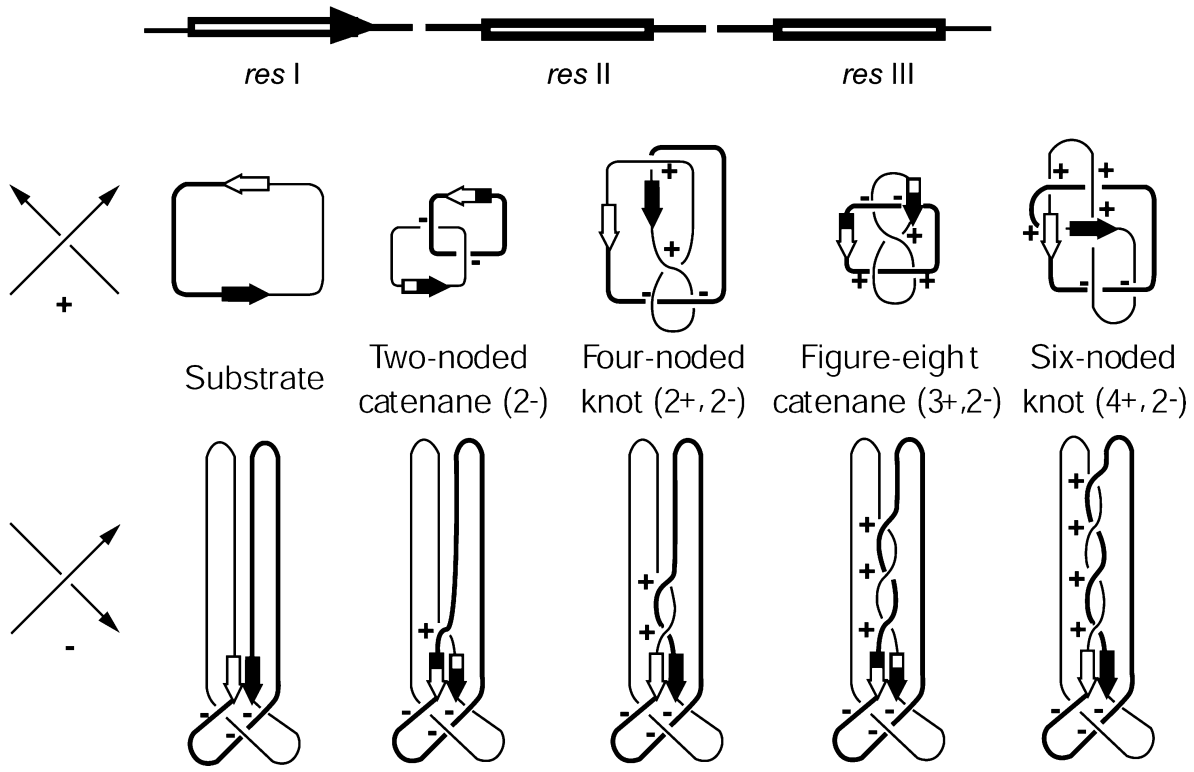

Figure 3 **The resolvase synapse and the products of processive recombination. The organization of the *res* site is schematically diagrammed at the top. The topology of the resolvase recombination conforms to a three-noded synapse (−3), with a right-handed DNA swap during each exchange event. The *res* I sites are shown by the unfilled and filled arrows in the substrate, so that an odd number of recombinations results in the 'hybrid' arrows. In order to assign signs to the DNA crossings, the DNA axis in the substrate circle is assigned an arbitrary, fixed direction. The conventions for the + and − signs are shown at the left. The continuous and broken arrows correspond to the 'overlying' and 'underlying' axes segments, respectively. During the experimental procedures, supercoils are removed by topoisomerase I, leaving behind only the knot and catenane nodes in the recombination products. The figure is adapted from (Grindley, 2002).**

available crystal structures of the $\gamma\delta$ resolvase (Li *et al*., 2005; Rice & Steitz, 1994; Sarkis *et al*., 2001; Yang & Steitz, 1995) do not suggest a definitive architecture for the synaptic complex, there is strong support for a parallel arrangement of the *res* I sites within the synapse and exchange of the cut strands by a rotary mechanism, as depicted in Figure 3. We are therefore comfortable in accepting the -3 resolvase synapse as the standard for deriving topological features of other DNA transactions.

Topology of the Hin or Gin Invertase Reaction

The unique topology of the resolvase reaction derives further support from similar topological characterization of two mechanistically related enzymes, the Hin and Gin invertases of Salmonella and phage Mu, respectively (Johnson, 2002; Kanaar *et al*., 1990). Like resolvase, Hin and Gin are serine recombinases that demand negative supercoiling of substrates as well as orientation specificity and intramolecular configuration of the target sites. Unlike resolvase, the invertases act only on sites arranged in a head-to-head fashion. Hin and Gin bind as dimers to their target site *hix* and *gix*, respectively, and mediate DNA inversion with the assistance of the *E. coli* Fis protein as an accessory factor. Interaction of a Fis dimer bound at its cognate enhancer site, with the *hix*-bound Hin dimers (or the *gix*-bound Gin dimers) is responsible for organizing the active recombination complex. The sequential products for the Hin/Gin reaction are: an unknotted inversion circle, a three-noded knot with all negative crossings (3^-), a four-noded knot with two positive and two negative crossings (2^+, 2^-) and a five-noded knot with all negative crossings (5^-) (Figure 4). The synaptic configuration that most easily

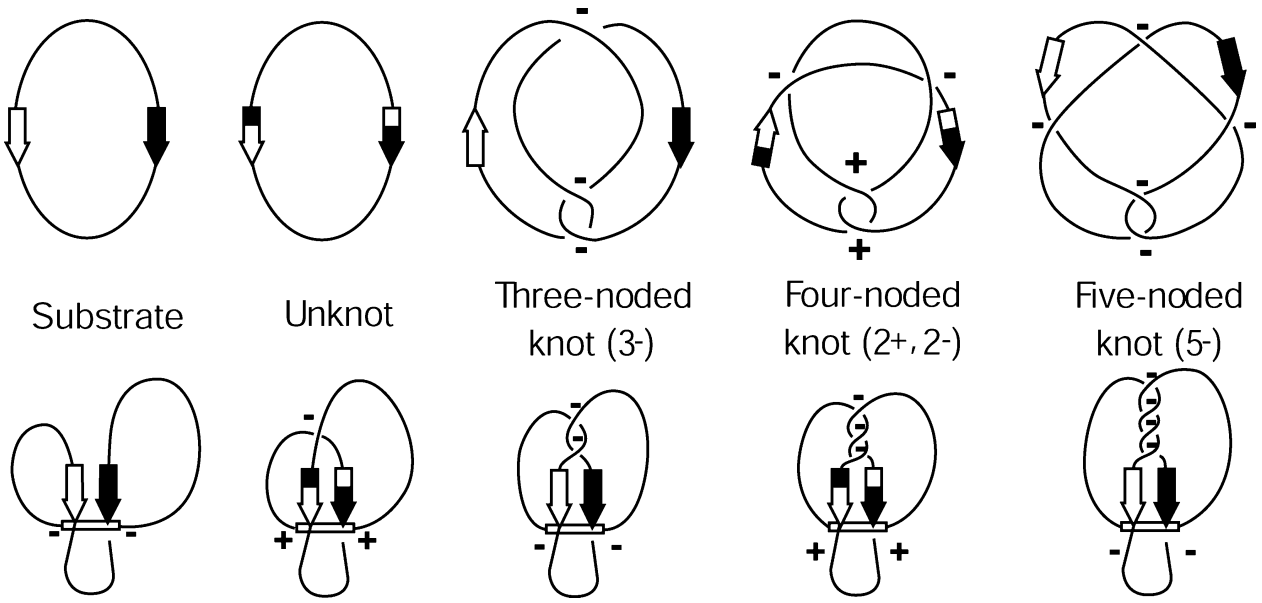

Figure 4 The Gin inversion synapse and the products of processive recombination. The synapse topology consists of two DNA crossings, and strand exchange follows a right-handed rotation as in the resolvase reaction. Because each recombination event inverts the DNA segment between the *gix* sites (changing the direction of the DNA axis), the synaptic nodes change in sign with each recombination event. For the same reason, the DNA crossings resulting from strand exchange have −signs as opposed to the + signs in the resolvase reaction (Figure 3). The figure is adapted from (Johnson, 2002) and (Kanaar *et al*., 1990).

explains the above product topologies contains two trapped DNA crossings with a parallel orientation of the recombination sites, or a −2 synapse (Johnson, 2002; Kanaar *et al*., 1990). Since each round of recombination causes relative inversion of the DNA segment between the *hix* or *gix* sites, the sign of the synaptic nodes alternates between − and +, as illustrated in Fig. 4. Each act of strand exchange introduces a right-handed crossing by DNA rotation, as in the resolvase case, but with a negative sign in the product. Strong biochemical evidence supports the DNA rotation mechanism (Dhar *et al*., 2004).

To generalize, the synapses arranged by the serine recombinases have a characteristic topological signature. These enzymes impose a parallel geometry on the recombination partners and carry out recombination with DNA rotation in the right-handed sense. Note from Figures 3 and 4 that the target sites divide the circular DNA into two distinct domains, and it takes two and three interdomainal crossings (or nodes) to arrange the head-to-head (*hix* or *gix*) sites and head-to-tail (*res* I) sites, respectively, in parallel fashion. In other words, in inversion substrates, zero or even number of interdomainal crossings will give rise to a parallel synapse; odd number of such crossings will yield an anti-parallel synapse. The reverse is true for deletion substrates. It is useful to keep this simple rule in mind, as the topological features of the Mu transpososome in its different forms (to be discussed later) have been deciphered based on them.

Phage Mu Transposition Complex is Refractory to Direct Topological Analysis

As pointed out earlier, the strand cleavage step of Mu transposition proceeds via hydrolysis, the strand joining step via transesterification. The transposition complex is then disassembled actively to make way for repair of the broken Mu DNA ends by replication and ligation (Figure 2). Since the initial chemistry of the transposition reaction, unlike site-specific recombination, leaves behind loose DNA ends, standard analytical methods fail to preserve the topological features of the reaction. To overcome this impediment, one might first arrange the transposition synapse with its native topological features, but not permit the transposase to trigger the chemistry of strand cleavage. One could then permanently trap this topology by cutting and joining DNA strands outside the synapse using a topologically well characterized conservative site-specific recombinase. The total number of DNA crossings trapped in the recombination product will

convey the sum of the topologies of the transposition and recombination reactions. If one subtracts the recombination topology from the product topology, one can derive the transposition topology. This is the rationale of 'difference topology,' and we shall describe its applications in further detail later.

Harnessing Site-Specific Recombinases Cre and Flp to Reveal the Topology of the Mu Transpososome

Cre and Flp, members of the tyrosine family recombinases, can carry out DNA inversion and deletion equally well, depending on the relative orientation of their target sites: *loxP* and *FRT*, respectively (Jayaram *et al*., 2002; Van Duyne, 2002). They do not require accessory protein factors, and are indifferent to the topology of the substrates on which they act. In principle, they are ideally suited for sealing off, through the act of recombination, the DNA crossings confined within an external synapse. However, to derive the topology of the 'unknown synapse,' one has to know what the contribution of the recombination reaction itself is to the topology of the final product. What is the local geometry of the *loxP* or *FRT* sites during recombination? Will Cre (or Flp) introduce DNA crossings during strand exchange or not?

In principle, simple relaxation of supercoils by toposiomerase I can be utilized to analyze DNA crossings trapped by a recombination synapse. Alternatively, provided the assembly of the synapse is not restricted to supercoiled DNA, ligase-mediated joining of a DNA nick can also be used for the same purpose. Since topoisomerase I changes linking number in steps of 1, it cannot remove double stranded DNA crossings that are protein-anchored within a two-site synapse. Ligation produces a distribution of topoisomers due to thermal fluctuations that introduce different amounts of twist/writhe into DNA. The DNA nodes introduced by synapsis would be evident as a shift in the distribution of topoisomers. For example, ligation and relaxation assays have been utilized to analyze the simple synapse formed by Flp and the more complex synapse formed by XerC/XerD and its accessory proteins PepA and ArgR (Grainge *et al*., 2000; Alen *et al*., 1997). However, as elaborated in detail below under 'Difference Topology,' recombination by Flp or Cre is more fool-proof as it permits each synapse to be tested in two ways, by an inversion reaction and a deletion reaction.

The Geometry of Site Alignment During Flp and Cre Recombination

In principle, the *loxP* (or *FRT*) sites may be arranged in a parallel or antiparallel orientation during recombination, provided the reaction is carried out within a planar DNA-protein complex. Recombination from sites arranged in the parallel geometry will result in a DNA crossing between the exchanged sites (Figure 5). It may be denoted by +1 or −1, depending on whether the DNA rotation is right-handed or left-handed during the exchange event. By contrast, recombination from the antiparallel geometry of the recombining sites will not introduce a DNA node (zero crossing). As briefly outlined below, this predicted difference, combined with the known topology of the −3 resolvase synapse, has been exploited to reveal the mode of alignment of the *FRT* and *loxP* sites in their respective synapses (Grainge *et al*., 2000; Grainge *et al*., 2002; Kilbride *et al*., 1999). The underlying assumption that the sites are essentially planar during recombination has been justified by the crystal structures of the Cre and Flp synapses (Chen *et al*., 2000; Guo *et al*., 1997). We describe here the experiments conducted with Flp, but the results hold true for Cre as well.

In the assays performed by Grainge and colleagues (2000, 2002), a hybrid *res-FRT* site was constructed by replacing the *res* I subsite with *FRT* but retaining *res* II and *res* III in their native positions (Figure 6). In a supercoiled plasmid containing two such sites with the normal orientations of *res* II-*res* III, the characteristic synapse with the three negative crossings could be assembled by the addition of resolvase. Subsequently, recombination was performed at the *FRT* sites using Flp, and the product topology was examined. When the *FRT* sites were in head-to-head orientation, the inversion reaction from the hybrid synapse yielded a three-noded knot with + crossings. When the *FRT* sites were in head-to-tail orientation, the deletion reaction gave a four-noded catenane.

The outcomes from the *res-FRT* hybrid site recombination reactions are consistent with an antiparallel geometry for the *FRT* sites within the Flp synapse, with no DNA crossing being added during recombination (Figure 7). The three external DNA crossings will synapse the head-to-head *FRT* sites in the antiparallel

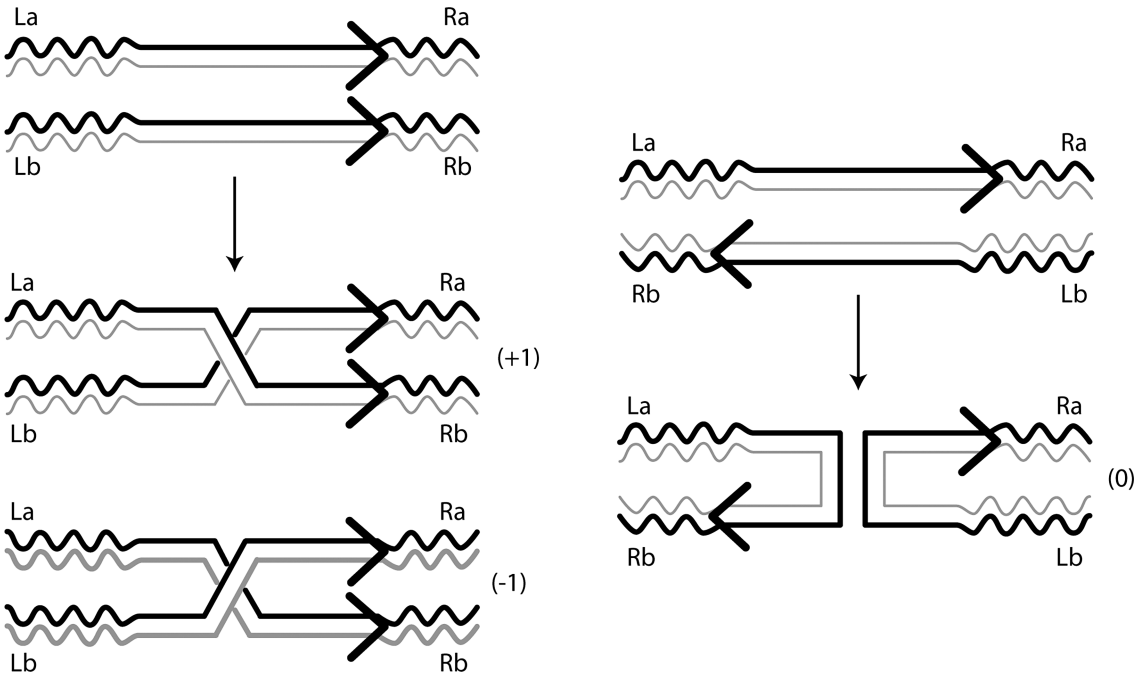

Figure 5 **Geometry of site alignment during recombination by Flp or Cre. The two partner sites are named La-Ra and Lb-Rb to orient them left to right. In the reaction shown at the left, the two sites are arranged in a parallel fashion (left to right in both cases) in the plane of the paper. If strand exchange occurs with a right-handed rotation of the DNA, the recombinant products (La-Rb and Lb-Ra) cross each other to introduce a +1 node. If the sense of rotation is left-handed during exchange, the crossing between La-Rb and Lb-Ra will be −1. In the reaction shown at the right, the partner sites are arranged in the antiparallel orientation (left to right for La-Ra and right to left for Rb-Lb). The act of recombination does not introduce a crossing between La-Rb and Lb-Ra.**

mode. Recombination preserves these three nodes in the inversion knot. For the head-to-tail *FRT* sites, a fourth node from the negatively supercoiled DNA must be trapped to orient them in the antiparallel fashion. Hence recombination results in the four-noded catenane. The antiparallel geometry of the *FRT* and *loxP* sites deduced by the topology method agrees with the arrangements of these sites in the Flp and Cre crystal structures, respectively. Furthermore, as illustrated in Table 1, recombination in the parallel mode with a +1 crossing or a −1 crossing can be ruled out.

The top panel of the Table 1 refers to right-handed DNA rotation during strand exchange (+1 crossing), and the bottom panel refers to left-handed rotation (−1 crossing). Note that the 'additional' crossing represents the extra negative supercoil from the plasmid substrate that is trapped to bring the *FRT* sites in parallel geometry. Note also that the signs of all the crossings change during inversion (rows 1 and 3), and hence the knot nodes are positive. For the +1 mode of strand exchange from the parallel sites (top panel in Table 1), the predicted products are a three-noded knot

TABLE 1 **Parallel geometry of *FRT* sites: topological predictions**

	Resolvase Synapse	Additional crossing	Crossing during recombination	Product topology
Inversion	−3	−1	+1	+3 Knot
Deletion	−3	0	+1	−2 Catenane
Inversion	−3	−1	−1	+5 Knot
Deletion	−3	0	−1	−4 Catenane

Predicted topologies of inversion knots and deletion catenanes during resolvase-assisted Flp recombination if the *FRT* sites have a parallel geometry. In the top panel, strand rotation during Flp-mediated cross-over is assumed to be right-handed (+1); in the bottom panel, the rotation is assumed to be left-handed. For the inversion and deletion reactions, the external resolvase synapse has a fixed topology (−3 crossings). An additional negative supercoil (−1) is trapped in the inversion substrate (rows 1 and 3) for parallel alignment of the *FRT* sites. The signs of the DNA crossings in the substrate and product are reversed when recombination inverts DNA (+3 knot in row 1 and +5 knot in row 3). Flp recombination with *FRT* sites arranged in parallel fashion predicts either the +3 knot/−2 catenane (top panel) or the +5 knot/−4 catenane (bottom panel) pair of inversion and deletion products. The experimental results, yielding the +3 knot/-4 catenane combination, contradict these predictions.

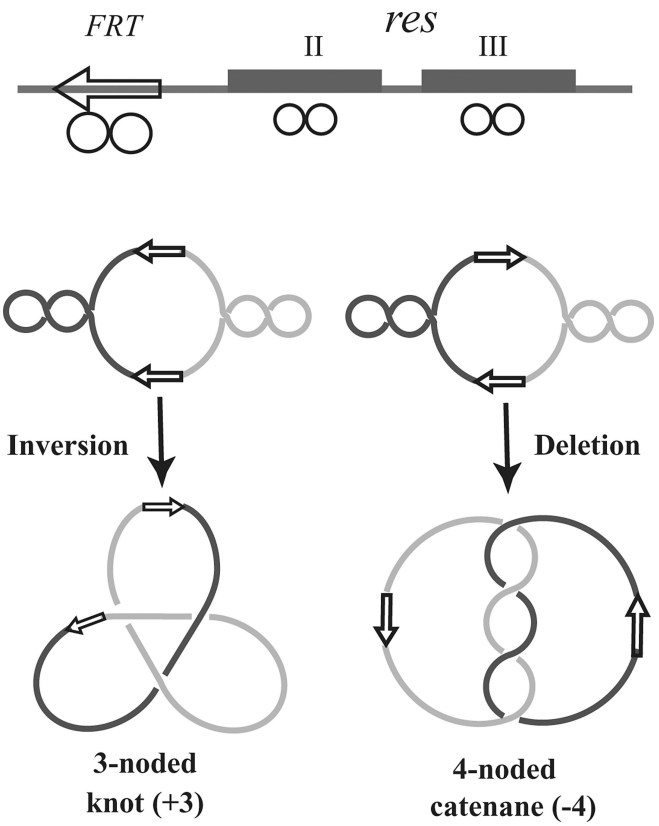

Figure 6 **Flp-mediated recombination after the assembly of the resolvase synapse. The general organization of a hybrid *FRT*/*res* site is diagrammed schematically at the top. The *res* II and *res* III accessory sites together with the resolvase protein will establish a well characterized −3 synapse (see Fig. 3). The *res* I site is replaced by an *FRT* site. In the two hybrid *FRT*/*res* sites present on a plasmid, the *res* II/III site are arranged in their native orientation. The *FRT* sites are present in a head-to-head orientation in the inversion substrate (*left*) and a head-to-tail orientation in the deletion substrate (*right*). Recombination after preincubation with resolvase (to establish the three-noded synapse) enriches the three-noded knot for inversion and the four-noded catenane for deletion.**

for inversion and a two-noded catenane for deletion. For the −1 mode of strand exchange (bottom panel), the corresponding products are a five-noded knot and a four-noded catenane. The experimentally observed pair of products, a three-noded knot and a four-noded catenane (see Figure 6), disagree with the topological predictions for parallel site alignment, and strongly support antiparallel synapsis of the *FRT* sites.

Difference Topology

The fact that Flp and Cre perform recombination without introducing a DNA crossing offers a simple method of deriving the number of DNA crossings trapped in an 'unknown' synapse, formed by, say, a recombinase or a transposase. In principle, the analysis is applicable to any high-order DNA-protein complex in which two DNA sites make a fixed number of plectonemic wraps with each other. As described with resolvase, the unknown synapse is first assembled in two matched plasmid substrates that differ only in the relative orientation of the recombination sites (head-to-head in one case and head-to-tail in the other), after which the deletion and inversion reactions are carried out. The prediction is that the number of crossings in the inversion knot and those in the deletion catenane will differ by one. The smaller of the two numbers then gives the DNA crossings within the unknown synapse. The additional crossing in one of the products reflects the need to keep the sites antiparallel for recombination. The conditions for this analysis to work are that the extraneous synapse must be stable in the context of the Cre/Flp recombination synapse, and the recombination sites are placed sufficiently close to a DNA region of interest to avoid random entrapment of interdomainal nodes.

The success of the difference topology method in unveiling the path of DNA within the phage Mu DNA transposition system is described below (Pathania *et al*., 2002). The analysis has taken us further in illuminating the sequence and dynamics of the DNA-protein interactions that dictate the stepwise assembly of the transpososome (Pathania *et al*., 2003; Yin & Harshey, 2005; Yin *et al*., 2005).

A Three-Site, Five-Noded Mu Transposition Synapse Revealed by Difference Topology

As described previously, transposition of phage Mu requires negative supercoiling of the DNA substrate plus the interaction of three separate DNA sites, the left and right ends of Mu (attL and attR, refered to simply as L and R, respectively) and the enhancer element (E), mediated by the transposase protein MuA. Do these interactions sequester a fixed number of supercoils within the transpososome?

Difference topology can only tell the number of crossings between two DNA domains: those separated by the *loxP* sites, or more precisely, the crossover points in Cre recombination. Hence the larger question regarding the total number of L × E × R nodes has to be subdivided into three smaller ones (Figure 8, right panel): (a) how many times does E cross L and R (E × [L, R]); (b) how many times does L cross R and

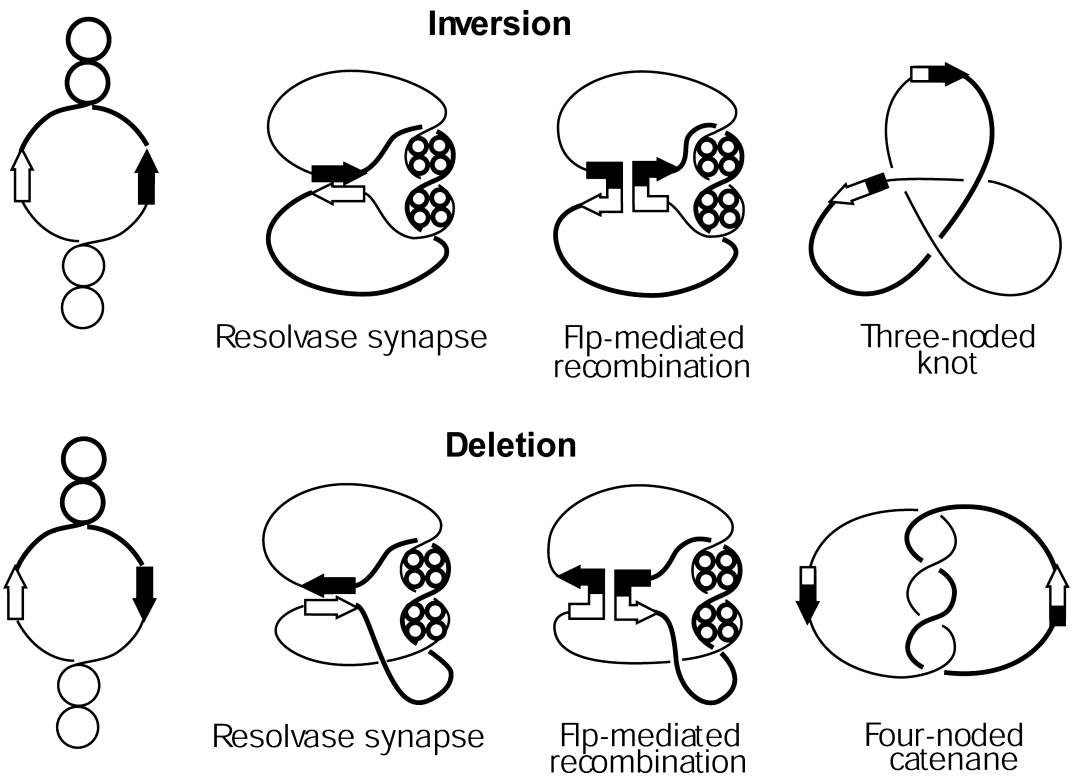

Figure 7 **The product topologies during Flp-mediated recombination performed from a prearranged resolvase synapse are consistent with antiparallel alignment of the *FRT* sites. The three negative supercoils (odd number of DNA crossings) sequestered by the resolvase synapse will arrange the *FRT* sites in an antiparallel manner in the Flp synapse during the inversion reaction (*top*). The product, in which these DNA crossings are trapped, will be a three-noded knot. With one more negative supercoil from the substrate (or a total of four crossings), the *FRT* sites can establish antiparallel synapse during the deletion reaction (*bottom*). The catenane product will contain four links between the two deletion circles. The signs of the DNA crossings in the knot and catenane products are opposite because the direction of the DNA axis is changed in the inverted DNA segment (see Figure 6).**

E (L × [R, E]); and (c) how many times does R cross E and L (R × [E, L])? To answer a, the recombination sites must be placed flanking E, isolating this site into one DNA domain while sequestering L and R into the second domain. To answer b and c, the recombination sites must flank L in the first case and R in the second. Furthermore, as explained in the case of the resolvase-assisted Flp reaction, a matched pair of inversion and deletion substrates must be constructed in each case. The experimental protocol involves the arrangement of the Mu synapse first in the presence of a catalytically defective MuA variant MuA(E392A), followed by the execution of recombination using Cre, and finally the analysis of the knot and catenane products after all supercoils have been removed by DNase I nicking. The experimental results with the Mu transposition synapse and their interpretations are given below.

For (a), E × [L, R], the products were found to be a three-noded knot for inversion and a four-noded catenane for deletion (Figure 8B, 1). Hence, there must be three crossings between E and [L, R]. For (b), L × [R, E], the products were, again, a three-noded knot and a four-noded catenane (Figure 8B, 4). So there must be also three crossings between L and [R, E]. For (c), R × [E, L], the products were a four-noded catenane and a five-noded knot (Figure 8B, 5). Or, there must be four crossings between R and [E, L].

The only mutually consistent DNA path that satisfies the results from a, b and c contains one crossing between E and L, two between L and R and two between R and E. In other words, the transposition synapse has the three-branched five-noded configuration shown in Figure 8 (left panel). The number of crossings between L and R were further verified by an alternative strategy. Here, the enhancer was deleted from the substrate and provided as a linear DNA fragment in *trans*, thus topologically dissociating it from the synapse (Figure 8B, 2 & 3). In agreement with the two DNA crossings

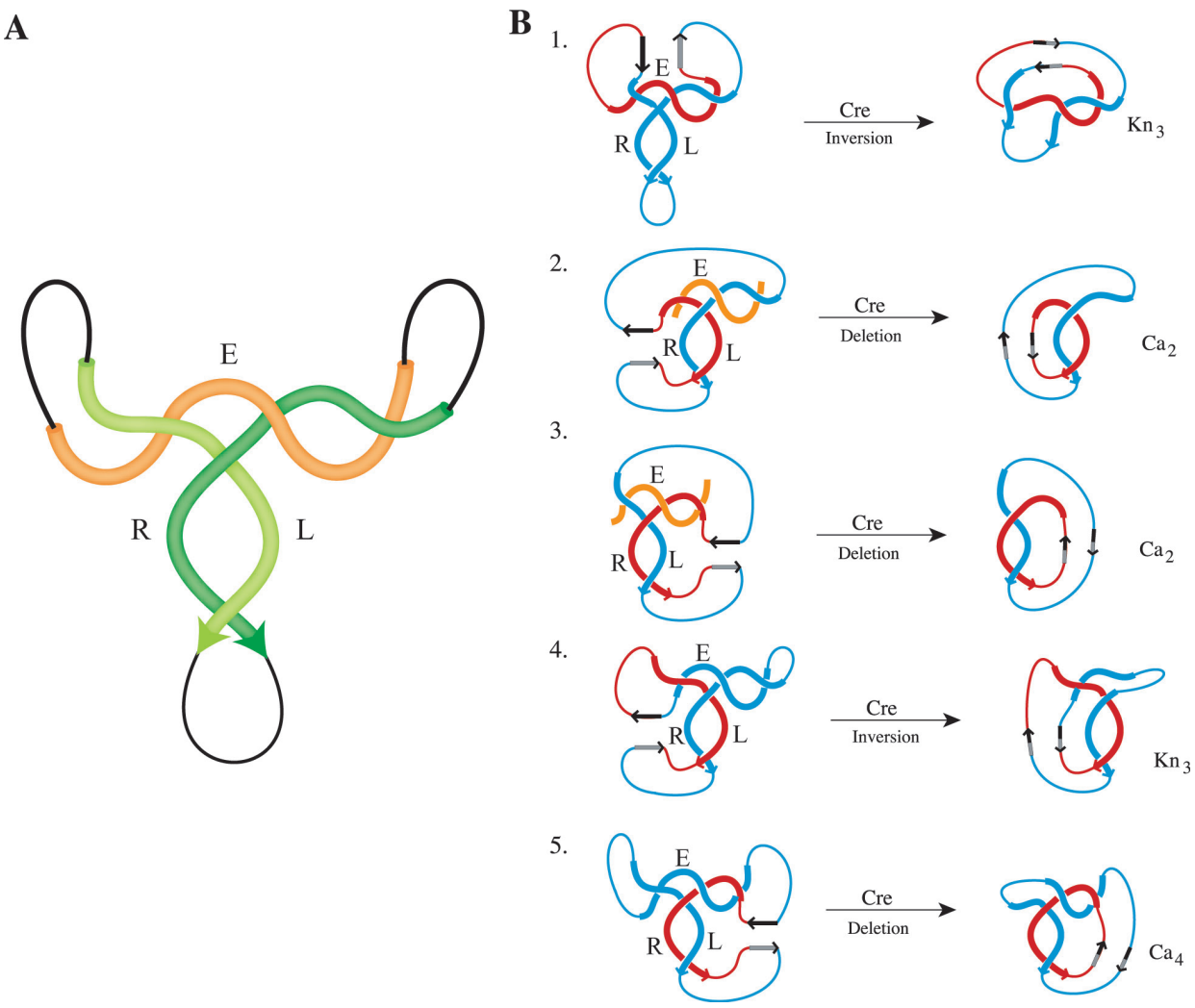

Figure 8 **The topology of the three-site synapse during the phage Mu transposition reaction. *A*. The five DNA crossings trapped by the left (*L*) and right (*R*) ends of Mu and the transposition enhancer (*E*), when the transposase protein bridges these three sites, are schematically depicted. The crossing numbers between each of the three sites and its two partners were deduced by the difference topology method. *B*. Some of the key experimental results supporting the topology of the transposition synapse are shown. For simplicity, in each reaction depicted in 1 to 5, only the recombination product with the smaller crossing number (corresponding to the number of plectonemic DNA wraps within the Mu synapse) is shown. The matched deletion substrates in 1 and 4 yield a four-noded catenane as the recombination product. Similarly, the matched inversion substrate in 5 gives a five-noded knot; those in 2 and 3 give a three-noded knot. The figure is adapted from (Pathania *et al*., 2002). See text for details.**

between L and R, the inversion product in this case was a three-noded knot, and the deletion product was a two-noded catenane. Similarly, the number of crossings between E and R could also be confirmed as two by deleting L from the substrate. The products in this case were a two-noded catenane and a three-noded knot (not shown; see Pathania *et al*., 2003). A similar analysis of the crossings between E and L was not possible, because a stable E-L MuA complex cannot be assembled in the absence of R.

It is satisfying to note that the two-site topologies derived from independent assays are mutually concordant with a unique three-site topology for the Mu synapse. This internal consistency suggests that the DNA configuration within the Mu transpososome, as delineated by the application of difference topology, is authentic.

Potential Wider Applications of Difference Topology

In principle, the difference topology analysis can be extended to complex interactions involving multiple DNA sites (for example, replication, transcription, or repair complexes). By the strategy discussed for the transpososome, the number of crossings made by each site with the rest of the sites can be determined after isolating it into one DNA domain with the recombination target sites placed on either side of it.

The subtopologies obtained by iterating this procedure for all of the sites can then be integrated to obtain the final composite topology of the entire DNA path.

DYNAMICS OF MU TRANSPOSOSOME ASSEMBLY AND ROLE OF THE ENHANCER

Ordered Pathway of Interactions

Difference topology was used to address whether there is a specified order with which the three Mu sites come together (Figure 9). The only stable two-site interactions reported by this methodology were those between E and R, or between L and R with the enhancer supplied in *trans* (Pathania *et al*., 2003). The E-R interaction was seen in the complete absence of L and was independent of the HU protein. Even in the presence of all three sites, upon omission of HU, only E-R interactions were topologically identified. A stable E-L crossing was not formed in the absence of R even when HU was present, and L-R crossings were not established unless the enhancer was present. It appears therefore that ER is the first two-site intermediate that nucleates the initiation of transpososome assembly (Figure 9). Biochemical experiments using a transposase protein from a Mu-related phage D108, which shares the same end-binding specificity but has a distinct enhancer specificity, suggest that the critical ER interactions are between R1 and O1 (Jiang *et al*., 1999), even when the enhancer is provided in trans (Jiang & Harshey, 2001).

ER progresses into LER in the presence of HU. Glutaraldehyde crosslinking experiments showed that a 3-site complex could be trapped in the absence of L1; provision of a cleaved L1 fragment to this complex promoted stable transpososome assembly and strand transfer in an HU-independent manner (Kobryn *et al*., 2002). It is surmised that in the native configuration, HU promoted DNA-bending at the L end serves to deliver L1 to its partner R1 end.

Analysis of each of the complexes from LER through type 2 showed the same overall topology, with the enhancer associated with each of the complexes (Pathania *et al*., 2002; Pathania *et al*., 2003) (Figure 9). Since earlier electron microscopy and footprinting experiments had failed to observe the enhancer in post-LER complexes (Mizuuchi *et al*., 1992; Watson & Chaconas, 1996), it is apparent that the noninvasive nature of the Cre recombination assay employed in the difference topology experiments preserved the fragile end-enhancer interactions. Indeed, more recent experiments employing glutaraldehyde crosslinking have observed a footprint at the enhancer in type 0 and type 1 complexes (Kobryn *et al*., 2002). These experiments suggest that the enhancer is more strained in the LER complex than it is in type 0 or type 1. Thus, conversion of MuA to a stable tetramer within type 0 is associated with a more relaxed enhancer conformation. As determined by difference topology, the enhancer remains associated with the MuA tetramer until completion of strand transfer.

Enhancer-Independent Mu Transposition from two Topologically Distinct Synapses

The phage Mu transposase, and the resolvase and invertase systems discussed above are examples of recombination systems that are highly selective with

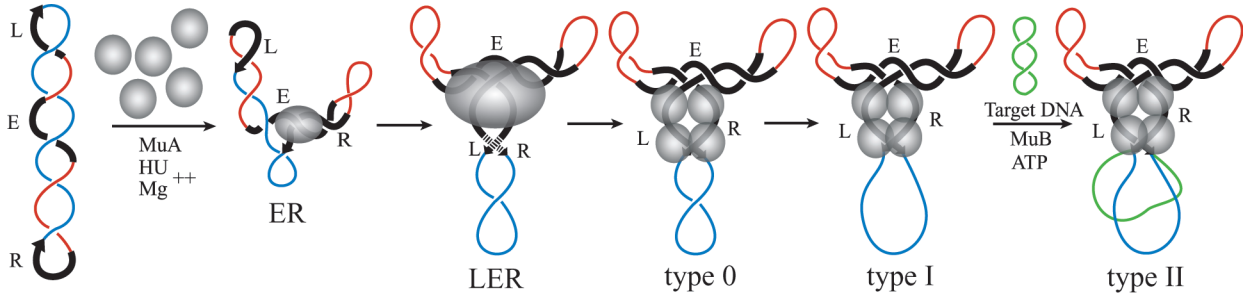

Figure 9 **Order and dynamics of Mu transpososome assembly. Assembly of the Mu transpososome is initiated by the interaction of E and R segments. HU promotes capture of L by ER to form LER which traps a total of five negative supercoils (see Figure 8). The five-noded DNA topology is maintained through cleavage and likely through strand transfer. The tetrameric form of MuA is first detected in type 0, where the MuA footprint extends outside the Mu ends and the DNA near the ends has a single-stranded feature (see Figure 2). Although only a tetramer is shown, two other MuA subunits are expected to be loosely associated in the complex. The complexes get progressively more stable, with LER being the least stable and type 2 the most stable. The enhancer remains associated with the ends throughout transposition. See text for details.**

respect to orientation of the interacting sites, and recombine through a very specific synapse topology. A hallmark of these systems is the participation of three sites in the productive synapse. On a supercoiled substrate, a three-site synapse is thought to arise by DNA slithering or branching rather than by random collision. Recombinases such as Flp/Cre and the phage λ integrase on the other hand, have two sites that participate in recombination; these interact by random collision and recombine through a spectrum of DNA topologies (Beatty *et al*., 1986; Crisona *et al*., 1999). If a three-site system could be converted to a two-site system, would its synapse topology be randomized? Indeed, there exist mutant invertases and resolvases that do not require complex synapse architecture or substrate circularity, and give random collision recombination products. These proteins have acquired independence from the enhancer or accessory binding sites (Arnold *et al*., 1999; Crisona *et al*., 1994; Haffter & Bickle, 1988; Klippel *et al*., 1988).

For the Mu transposase (MuA), two different enhancer-independent situations have been described. One involves an enhancer-independent transposase that, unlike the invertase and resolvase systems, does not relieve the dependence on DNA supercoiling or on the correct orientation of Mu ends (Jiang & Harshey, 2001; Yang *et al*., 1995). The other involves addition of Me_2SO (di-methyl sulfoxide) to the reaction; this situation does provide independence from constraints of substrate topology or site orientation (Mizuuchi & Mizuuchi, 1989). To determine if the Mu synapse topology is randomized under both of these enhancer-independent conditions, difference topology was employed (Yin & Harshey, 2005). Indeed, the Mu transpososome displayed a relaxed topological specificity on supercoiled substrates under enhancer-independent Me_2SO conditions: when the L and R ends were in their normal orientation and the enhancer was absent, a random series of synapses were detected, including the interwrapped synapse where L and R cross each other twice (IW and RC complexes; Figure 10). Presence of the enhancer under these enhancer-independent conditions channeled synapsis exclusively through the interwrapped pathway, showing that the enhancer imposes topological specificity on the synapse (Figure 10). When the L and R ends were in their wrong relative orientation, however, they synapsed only by random collision regardless of the presence of the enhancer. This result is consistent with an earlier proposal that

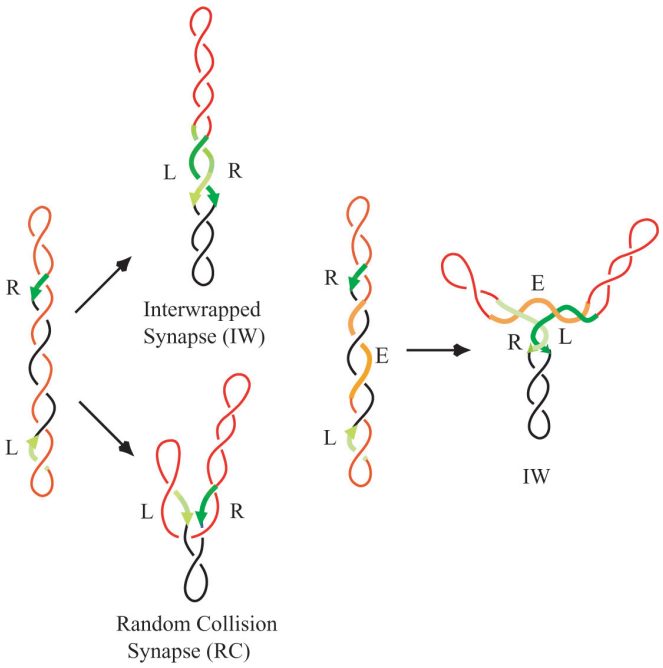

Figure 10 **Enhancer-independent Mu transposition from two topologically distinct synapses: the enhancer is a topological filter. L and R ends within Mu (*arrows*) can approach each other either by slithering to form an IW synapse, or by random collision to form an RC synapse. The sites are plectonemically interwrapped in the IW synapse, but not in the RC synapse. A parallel alignment of sites is assumed in both synapses. Under enhancer-independent Me_2SO conditions in the absence of enhancer, both complexes are recovered. Presence of the enhancer (*E*) under these conditions channels synapsis exclusively through the IW pathway. See text for details.**

orientation specificity of reacting sites is dictated by their ability to form an interwrapped 'plectosome;' incorrectly oriented ends would experience difficulty doing so (Craigie & Mizuuchi, 1986). Thus, when correctly oriented, Mu ends can interact either by interwrapping or by random collision in enhancer-independent Me_2SO conditions.

The Mu transpososome assembled with the enhancer-independent transposase provides additional insights into the topological attributes of the synapse (Yin & Harshey, 2005). In the presence of only two interacting sites–the L and R ends–this transposase could not synapse the sites by random collision, and only the interwrapped synapse was recovered. This finding implies that the enhancer is not the only determinant of topological selectivity; the interaction of MuA with the Mu ends is also important. The mutant transposase has acquired independence from enhancer requirement, but not from orientation specificity of the ends. Thus, studies with this enhancer-independent Mu transposase have revealed that systems

involving two-site interactions, and not necessarily three-site interactions, can also be subject to strict topological restrictions. Me_2SO conditions promote not only enhancer-independence, but independence from end orientation as well.

A Three-Site Topological Filter

How do the three Mu sites contribute to topological specificity of the synapse? Why is the Mu synapse normally limited to the IW variety, when it is clear that an RC synapse can be made? How is the topology of a recombination synapse determined?

An earlier 'topological filter' model proposes that selection of a particular synapse occurs after the initial encounter of sites by any mechanism, such that the chemical steps of the reaction can proceed only if the required local DNA geometry can be formed (Boocock *et al*., 1986; Craigie & Mizuuchi, 1986). According to this model, collision complexes that cannot readily be converted to the productive synapse are unstable, implying that the observed topology must support the most stable synapse. We interpret the Mu topology data within the framework of this model to suggest that during normal transpososome assembly, the sequential establishment of plectonemic DNA crossings between the enhancer and the Mu ends, and between the ends themselves, leads progressively to a more stable synapse. We hypothesize that the end-to-enhancer and end-to-end cross-bridging communications bring about ordered conformational changes in the transposase that give rise to a functional tetramer. In this scenario, the enhancer-independent MuA mutant still requires intertwining of L and R to achieve the active synaptic conformation. By contrast, in Me_2SO, the assembly of the transpososome occurs without the enhancer and without interwrapping of ends, and in the absence of supercoiling.

If either synapse can be made under Me_2SO conditions, why is the IW synapse favored when the enhancer is introduced? It is likely that differences in the initial stability of the nascent complexes are responsible. Structural differences between the two synapses were evident from their differential response to a nick introduced into the Mu domain; the nick was contained within the Mu domain in the IW synapse, but apparently dissipated to the non-Mu domain in the RC synapse (Yin & Harshey, 2005). The basis for the differential effect of the nick is not known, but it is possible that the DNA crossings within the IW synapse lend it more stability. These considerations support the topological filter model, and show that the correct geometry of all three sites generates the most stable synapse. Thus, all three sites can be viewed as DNA chaperones that filter the transposase along a specific assembly pathway to yield a 'properly folded' high-order reaction complex.

Why Use an Enhancer?

If transposition can be supported by enhancer-independent pairing of Mu ends through an RC synapse, there must be advantages to designing an IW synapse whose formation is dependent on a third enhancer element. Several can be enumerated, each providing regulatory control both in gene expression as well as in the mechanistic aspects of transposition. First, the N-terminal region of MuA shares extensive homology with the Mu repressor or Rep protein, and both proteins bind the enhancer through this homologous region (Craigie *et al*., 1984; Harshey *et al*., 1985; Leung *et al*., 1989). When bound by Rep, the enhancer serves as a 'silencer' to block the transcription of the early transposition functions. Thus, recruitment of this same DNA segment as an integral part of the transposition synapse must prevent access to the repressor, and regulate the lysis-lysogeny decision by signaling a commitment to transposition, and hence to lytic growth. Second, the enhancer binding region of MuA is at least partly responsible for keeping the MuA monomer catalytically silent, since enhancer-independent mutants have deletions removing this region from the amino-terminus of MuA (Yang *et al*., 1995). We have argued above that the ordered pathway of transpososome assembly (Figure 9) is designed to unfold gradually the catalytic potential of the transposase. Third, the first productive encounter of the enhancer with the R end, despite its proximity to the L end, may be designed to shorten the distance between L and R and to facilitate synapsis. A centrally located strong gyrase binding site or SGS is also implicated in such a function (Pato *et al*., 1990); plectonemic supercoiling mediated by gyrase is thought to organize the Mu DNA into a supercoiled loop with SGS and Mu ends at its apex and base, respectively (Pato & Banerjee, 1996). Fourth, if presence of the enhancer lends more stability to the synapse, then this will be advantageous during the lytic cycle of phage growth

where transposition is coupled to replication, and the transpososome aids in the transition between these two phases (Nakai *et al*., 2001). Fifth, as multiple copies of Mu start to accumulate in the host genome, assembly of an IW three-site synapse would ensure that the Mu ends present on the same Mu genome are paired for transposition, rather than ends from neighboring genomes. A similar three-site design in invertase and resolvase systems ensures specificity of site selection during recombination (Bednarz *et al*., 1990; Colloms *et al*., 1997; Kanaar *et al*., 1990; Moskowitz *et al*., 1991; Murley & Grindley, 1998). Finally, since the enhancer is not required for the chemical steps of strand cleavage and strand transfer (Surette & Chaconas, 1992), its continued association with the transpososome through strand transfer suggests that it may influence some as yet unknown step(s). Inefficient strand transfer associated with removal of excess MuA from the type 1 complex by procedures that likely strip the enhancer from the complex (Wu & Chaconas, 1997), suggest a potential involvement of the enhancer at this stage. Given that only Mu and related phages employ enhancers, the contribution of these elements to regulation of the many unique aspects of the phage lifestyle is clearly apparent.

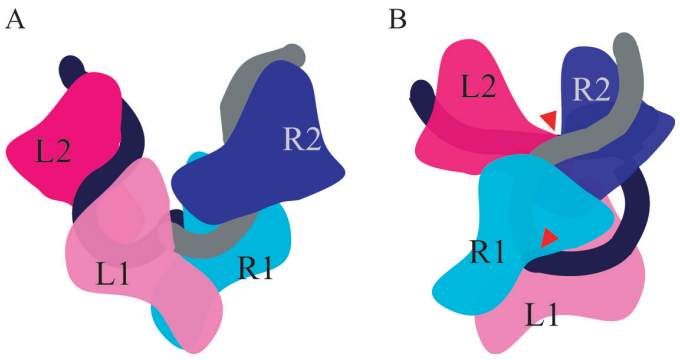

Figure 11 **Model for an interwrapped Mu transpososome. *A*. Schematic arrangement of MuA subunits within a tetrameric V-shaped complex assembled on R1-R2 DNA segments under enhancer-independent Me_2SO conditions, as imaged by scanning transmission electron microscopy (Yuan *et al*., 2005). The individual subunits are labeled L1, L2, *etc*., and the DNA shown as tubular segments. *B*. Alternative IW arrangement in which the two DNA end segments cross twice (*arrowheads*), as determined by difference topology. One DNA crossing is near the bottom L1-R1 subunits where catalysis occurs in *trans* (this is similar to that in the V complex). The second DNA crossing is between the L2-R2 subunits. We suggest that the HU-induced bend within the DNA normally present between the L1 and L2 (not shown; see Figure 1A) allows this DNA segment sufficient flexibility to cross over. The latter crossing changes the disposition of the top two subunits with respect to the bottom two subunits, when compared with the arrangement in *A*. For convenience of viewing, the structure in *B* is presented with the R1 subunit on the left, because the disposition of the R1-R2 subunits is not changed between *A* and *B*. See text for details.**

TRANSPOSOSOME STRUCTURE

Cryo-EM Structure

Discovery of the Me_2SO reaction conditions have allowed the development of transposition assays using short oligonucleotide substrates spanning the R1-R2 sites (Savilahti *et al*., 1995). These substrates faithfully reproduce the chemistry of the transposition reaction established on supercoiled substrates. A tetrameric MuA is involved in pairing the two R-end sequences (Baker & Mizuuchi, 1992), and catalyzes the reaction in *trans* (Aldaz *et al*., 1996; Savilahti & Mizuuchi, 1996). Complexes assembled on these substrates, however, are not as stable as those reported for supercoiled substrates (Surette *et al*., 1987).

Scanning transmission electron microscopy has been recently used to produce a 3D reconstruction of a tetrameric Mu transpososome complex assembled under Me_2SO conditions on 50 bp R-end substrates in the absence of the enhancer (Yuan *et al*., 2005) (Figure 11A). This complex resembles a large V, and appears to be held together largely by protein-DNA contacts. The two subunits at the top of the V do not interact with each other, but show limited interaction with the bottom subunits which are positioned appropriately for *trans* catalysis. The 3′-OH ends of Mu appear to be located ~20 Å away from the *trans* catalytic DDE motif, a distance too large for catalysis. Target DNA, proposed to enter through the large central cleft within the V to access the catalytic residues located at the bottom of the cleft, may induce conformational changes in the cleaved complex that correctly align the 3′-OH ends within the active sites for strand transfer (Yuan *et al*., 2005).

We believe the V complex, assembled under enhancer-independent Me_2SO conditions, may be akin to the RC synapse identified in topological experiments (Yin & Harshey, 2005) (Figure 10). We propose that rearranging this complex by introducing an additional DNA crossing within the tetramer would be more compatible with the difference topology data (Figure 11B). Of the two L-R crossings seen in an IW synapse, we propose that one arises from the act of *trans* catalysis within the L1 and R1 subunits, and the other is formed between the L2 and R2 subunits, facilitated by HU-promoted DNA bending at the L end

(Kobryn *et al*., 2002). The latter DNA crossing results in swapping of the top two subunits of the V structure such that the R2 subunit is now on top of L1 and the L2 subunit on top of R1. The IW arrangement allows more extensive protein-protein contacts within all the subunits in the tetramer, better accounting for the extraordinary stability of the transpososomes such as resistance to 4 M urea, or 6 M NaCl or 65°C temperatures (Surette *et al*., 1987). This arrangement would not allow target access to the catalytic subunits from top of the V cleft as proposed (Yuan *et al*., 2005). A better target approach may be made from the opposite side, at the bottom of the V. Clearly, a crystal structure of the five-noded three-site complex would be most helpful.

FUTURE DIRECTIONS

The future clearly lies in elucidation of the structure of transpososomes arrested at various stages of transposition. These will provide an understanding of the mechanistic details of catalysis and of target site selection, and also of structural transitions that regulate assembly and control the directionality of transposition.

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