The Mu Transposase Interwraps Distant DNA Sites within a Functional Transpososome in the Absence of DNA Supercoiling*

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A Mu transpososome assembled on negatively supercoiled DNA traps five supercoils by intertwining the left (L) and right (R) ends of Mu with an enhancer element (E). To investigate the contribution of DNA supercoiling to this elaborate synapse in which E and L cross once, E and R twice, and L and R twice, we have analyzed DNA crossings in a transpososome assembled on nicked substrates under conditions that bypass the supercoiling requirement for transposition. We find that the transposase MuA can recreate an essentially similar topology on nicked substrates, interwrapping both E-R and L-R twice but being unable to generate the single E-L crossing. In addition, we deduce that the functional MuA tetramer must contribute to three of the four observed crossings and, thus, to restraining the enhancer within the complex. We discuss the contribution of both MuA and DNA supercoiling to the 5-noded Mu synapse built at the 3-way junction.

Many recombinases, including the phage Mu transposase, are strictly dependent on DNA supercoiling for activity. These recombinases and their accessory factors take advantage of both the conformational and thermodynamic properties of supercoiled DNA, which include DNA unwinding, formation of single-stranded regions, extrusion of cruciform structures, sequence-dependent induction of Z-DNA conformation, and DNA bending as well as an increase in the local concentration of DNA sites (1). The present study was undertaken to examine the role of DNA supercoiling in establishing the five-noded topology observed in Mu transposition complexes (2, 3).

The Mu transposition pathway is shown in Fig. 1 (4). Two families of DNA sites (high affinity attL and attR sites and lower affinity enhancer (E)† sites (Fig. LA)) are recognized by the transposase MuA through separate DNA binding domains. On the linear phage genome, attL and attR are 37 kilobases apart, whereas the enhancer is 1 kilobase from attL (5). MuA monomers likely first bind the att ends and then interact with the enhancer through bridging contacts. A criss-crossed network of att-enhancer interactions has been deduced (6, 7), an ordered progression of which is thought to result in the series of complexes observed during transposition (Fig. 1B).

Mu transposition is strictly dependent on DNA supercoiling of the donor substrate under normal reaction conditions. Several roles for supercoiling have been deciphered. Supercoiling increases the binding affinity of MuA for the L and R ends (8) and of the accessory host factor HU for the L end (9), favors bending at the enhancer (10), facilitates interwrapping of the L, E, and R segments within the Mu synapse (11, 2), and assists “open termini” formation, a rate-limiting step in the assembly of the transpososome (12–16). Supercoiling is not required for the chemical steps of transposition per se (4).

During assembly of the five-noded Mu synapse, the enhancer first uniquely interacts with R and interwraps with it twice to form an ER complex (Fig. 1B) (2, 3). In the absence of Escherichia coli HU protein, L is freely mobile. Occupancy by HU restricts this mobility and guides L to the ER complex, where it becomes assimilated into the final synapse by establishing one DNA crossing with E and two with R. Intuition would suggest that establishment of this intricate topology must depend critically on DNA supercoiling. Does it? We have addressed this question by examining the topology of the synapse assembled on nicked substrates. This is possible to do by the addition of 10–15% Me2SO to the reaction, when Mu transposition is no longer dependent on DNA supercoiling or the enhancer or HU protein (17).

The Mu synapse on nicked substrates was not expected to be elaborate or to include the enhancer, since Mu ends present on short oligonucleotides will form a productive complex in Me2SO (18). Surprisingly, however, we find that under the “permissive” conditions, MuA can still interact with the enhancer and generate both E-R and L-R crossings. Thus, through multiple protein-DNA interactions, MuA itself can induce the necessary conformational changes that generate a unique DNA path during the assembly of a functional reaction synapse. We also show here that treatments known to strip loosely associated MuA from stable transpososomes, leaving a functional tetrameric complex at the synapse (4), disrupt only one of the two E-R crossings. Thus, subunits in the MuA tetramer contribute not only to the L-R crossings as expected but also to a crossing with the enhancer.

MATERIALS AND METHODS

Plasmids—Construction of pSP(R)Dir, pSP(R)In, pSP(L)Dir, pSP-(L)In, pSP(L/R)Dir, and pSP(L/R)In is described in Refs. 2 and 3. pZYAE(R)Dir, pZYAE(R)In, pZYAR(L)Dir, and pZYAR(L)In are variants of the pSP(R)Dir, pSP(R)In, pSP(L)Dir, and pSP(L)In, respectively, and were constructed by replacement of an E- or R-containing fragment with the same length of a nonspecific DNA fragment. All of these plasmids were originally derived from mini-Mu pMK21 (19) and carry two loxP sites at suitable positions. In pZYE(Dir there are 125 bp

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The abbreviations used are: E, enhancer element; L and R, left and right ends of Mu, respectively; IHF, integration host factor; HMG1, high mobility group protein; Dir, direct; In, inverted; HJ, Holliday junction.

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between O1 and loxP and 108 bp between O3 and loxO. These distances are 125 and 116 bp in pZY(E)In.

Proteins—MuA, MuA(E392A), and HU proteins were purified as described (20). Cre protein was isolated as described (21). HIF was a gift from Steve Goodman (USC), and HMG1 was a gift from Reid Johnson (University of California, Los Angeles).

Analysis of Nicked Substrates—Supercoils were removed from DNA by limited nicking with DNase I. 100–μl reactions containing 10 μg of DNA, 5 units of DNase I, 20 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 50 mM NaCl, and 0.3 mg/ml ethidium bromide were incubated at 30 °C for 30 min. Reactions were quenched by EDTA (final concentration 10 mM), extracted with phenol-chloroform and chloroform, and DNA was precipitated by ethanol.

Assembly of Mu Transposition Complexes and Cre Recombination Reactions—Mu transpososomes on nicked substrates were assembled with 30 μg/ml mini-Mu plasmid DNA, 10 μg/ml HU, and 7 μg/ml MuA in 20 μl of 20 mM HEPES-KOH (pH 7.6), 140 mM NaCl, and 15% Me₂SO with the addition of 10 mM CaCl₂ for type 0 complex or 10 mM MgCl₂ for type I complex for 30 min at 30 °C (22). When these complexes needed to be subject to gel-filtration columns, the concentration of DNA and proteins were doubled. Cre recombination was initiated by the addition of 0.1 μg of Cre followed by further incubation at 30 °C for 30 min. Reactions were stopped by SDS addition to a final concentration 0.5% and heat inactivation of the protein at 75 °C for 10 min. Samples were then processed as described (23).

Loosely bound MuA was removed from Mu DNA by the addition of a final concentration of 0.4 μg/μl heparin (8) as well as 0.5 mM NaCl (24). Removal of free protein or exchange of reaction buffers was achieved by spin-gel filtration through Sepharose 6B columns (25) pre-equilibrated with the desired reaction buffers. The original reaction buffer was, thus, restored before Cre reaction.

Ligation Assays—To detect changes in supercoiling on nicked substrates, the type 0 complex was assembled as described above except with MuA(E392A) and 10 mM MgCl₂. Then, 10 μl of the same buffer containing 3 mM ATP, 75 μg/ml bovine serum albumin, and 2000 units of T4 DNA ligase was added, and the reaction was incubated at 20 °C for 4 h. Reactions were stopped by the addition of SDS to final concentration 0.7% followed by phenol-chloroform extraction. Samples were electrophoresed in 0.8% agarose gels containing 1.0 μg/ml chloroquine phosphophate (Fig. 2B) when ligation of the nicked substrate is performed in the absence of transposase, the distribution of topoisomers seen is the result of thermal fluctuations in DNA conformation (Fig. 2B, lane 1) (26). In the presence of MuA(E392A), an upward shift in this distribution was observed (Fig. 2A, lanes 3 and 4). The complexes migrate at different positions because although cleaved, a type I complex on a supercoiled substrate still retains supercoils in the Mu domain (see Fig. 1B). In the absence of Me₂SO, no complex was observed on the nicked substrate (Fig. 2A, lane 5). Although all the topological experiments on nicked substrates reported in this study (Figs. 3–5) were performed with type 0 complexes assembled in the presence of Ca²⁺ ions to avoid Mu transposition, these complexes do not survive gel electrophoresis and, hence, are not shown here (see Fig. 5).

To determine whether MuA introduces DNA crossings in nicked substrates, the catalytically inactive variant MuA(E392A) was used to assemble the type 0 complex in the presence of Mg²⁺ (19). The topology of this complex on negatively supercoiled plasmids is indistinguishable from that of a similar uncleaved complex formed by wild-type MuA in Ca²⁺ (2, 9). After assembly, T4 DNA ligase was added to seal the nicks in the substrate to trap any supercoils introduced by MuA. These can be observed as a change in linking number when the substrate is electrophoresed in agarose gels in the presence of the DNA-intercalating agent chloroquine phosphophate (Fig. 2B). When ligation of the nicked substrate is performed in the absence of transposase, the distribution of topoisomers seen is the result of thermal fluctuations in DNA conformation (Fig. 2B, lane 1) (26). In the presence of MuA(E392A), an upward shift in this distribution was observed (Fig. 2B, lane 2). Chloroquine unwinds DNA, and introduces compensatory positive supercoils in closed circles. A shift up, therefore, denotes a deficit in linking (or presence of negative supercoils) in the ligated DNA before chloroquine treatment; DNA band intensities in lanes 1 and 2 were quantitated and are shown in Fig. 2C. The centers of topoisomer distributions (larger arrowheads in Fig. 2, B and C), were consistent
with roughly four extra negative supercoils being trapped in the sample when the ligation reaction contained MuA(E392A) (compare lanes 1 and 2 in Fig. 2, B and C). This can also be seen by comparing the upper boundaries (smaller arrowheads) of the topoisomer distributions in the two lanes. The broader distribution of the topoisomer in lane 2 is likely due to background from uncomplexed substrate. Because writhe and twist are interconvertible, the linking difference caused by MuA(E392A) under conditions where synapsis of Mu ends does not occur (Fig. 2B compare lanes 3 and 4; see also Fig. 2A, lane 5). Thus, simple binding of MuA to DNA does not cause the linkage change. In summary, MuA can apparently introduce four negative supercoils upon assembling a transpososome on nicked substrates in the presence of Me$_2$SO.}

In the absence of Me$_2$SO, the distributions were similar for ligations carried out with or without MuA(E392A) (lanes 3 and 4, Fig. 2B). Between MuA(E392A)-free ligations carried out in the presence and absence of Me$_2$SO, the centers of distributions were offset by one (lanes 1 and 4, Fig. 2B). This difference was likely due to some underwinding of DNA by Me$_2$SO. Note also that there is no change in linkage in the presence of MuA(E392A) under conditions where synopsis of Mu ends does not occur (Fig. 2B compare lanes 3 and 4; see also Fig. 2A, lane 5). Thus, simple binding of MuA to DNA does not cause the linkage change. In summary, MuA can apparently introduce four negative supercoils upon assembling a transpososome on nicked substrates in the presence of Me$_2$SO.

**R Crosses E-L Four Times within a Mu Synapese Assembled on Nicked Substrates, Similar to That on Supercoiled Substrates**—To address the distribution of the four trapped supercoils among L, E, and R, we resorted to the use of “difference topology.” In this assay the intersections between two DNA domains were counted by sealing them off via Cre recombination, which makes with E-L, the loxP sites were positioned on either side of MuA in the presence of HU and Ca$^{2+}$ to assemble the type 0 complex. In pSP(R), the reporter for the number of crossings R makes with E-L, the loxP sites were positioned on either side of R (Fig. 3). The orientation of the loxP sites will be referred to from here on as Dir (direct) or In (inverted) repeats for all substrates. For pSP(R)/Dir, the Cre-alone reactions yielded two unlinked deletion circles, the smaller D1 of which had migrated off the gel (Fig. 3A, lane 2). An equivalent reaction on pSP(R)/In produced the unknotted inversion product, whose migration was indistinguishable from that of the relaxed substrate (Fig. 3B, lane 2). Generation of the inversion product was verified by digestion with appropriate restriction enzymes (not shown). Products labeled HJ (indicated by arrowheads) are Holliday junction intermediates, as demonstrated by the experiments described in Fig. 3C. Separation of the HJ band from the “oc” position depends on substrate configuration and gel running (buffer) conditions. This band is indicated where observed (Figs. 3–5). In Cre-alone reactions ~40–60% of the nicked substrate was recombined, with the efficiencies for deletion and inversion being similar (data not shown). Upon MuA assembly, Cre recombination efficiency was somewhat lower (30–50%), depending on the substrate; see Figs. 3–5).

The Cre reaction subsequent to assembly of the type 0 complex gave the 4-noded catenane for deletion (Fig. 3A, lane 3) and the 5-noded knot for inversion (Fig. 3B, lane 3) as the predominant products. As explained earlier and as illustrated in the schematics in Fig. 3, a 4-Cat/5-Knot configuration shows that R crosses E-L 4 times within the type 0 synapese assembled on nicked substrates. This is similar to that seen in the type 0 complex assembled on supercoiled substrates (Fig. 3, A and B, lane 4).
A small amount of 2-Cat and 3-Knot product seen in the nicked substrates (Fig. 3, A and B, lane 3) could result from a partially assembled synapse with two E-R crossings (3). The band below the 4-Cat in lane 3 (Fig. 3A) was likely a 5-noded knot, generated from a second round of Cre recombination (29). Similarly, formation of the 6-Cat and 7-Knot products from the supercoiled substrates (Fig. 3, A and B, lane 4) was likely due to the fortuitous trapping of an extra supercoil. In summary, the data in Fig. 3, A and B, show that R × E-L = 4 on both nicked and supercoiled substrates.

The HJ Band Is a Holliday junction—The Cre recombinase exchanges DNA strands via a Holliday junction intermediate.
We suspected that products labeled HJ in Fig. 3 (indicated by arrowheads) were stalled at this intermediate stage (as illustrated in Fig. 3B). To verify their identity, the HJ band (lane 2, Dir, Fig. 3A) was excised from the gel and cut with AseI (Fig. 3C, lane 1), which cuts once within Mu next to attR and once outside Mu. As a result, a figure-eight junction would be converted to a four-way linear junction. In some of these molecules the top strand of the parental substrates P1 and P2 would be exchanged (HJ2); others would contain exchanged bottom strands (HJ1). Heat denaturation of the two junctions followed by renaturation can re-assert the eight DNA strands to give the parent duplexes, P1 and P2, as well as the recombinants RC1 and RC2. This was indeed the case (Fig. 3C, lane 2), as confirmed by digesting the whole Cre reaction mixture shown in Fig. 3A, Dir, lane 2, with AseI (Fig. 3C, lane 3). The smallest of these duplexes (RC1) had migrated out of the gel. Bands labeled H1 and H2 are likely the result of hybridization between a parental and a recombinant strand to form an incomplete duplex with single-stranded tails.

Under standard reaction conditions, Cre tends to exchange strands in an ordered fashion (31). In this case, HJ1 and HJ2 (see Fig. 3C) would have been highly unequal in their representation in the HJ population. And contrary to the results in Fig. 3C, denaturation and renaturation of the purified HJ should not have yielded parentals and recombinants. It is likely that the reaction conditions used in the present assays (including the presence of Me2SO) can reduce or eliminate this bias. Accumulation of the HJ intermediates was not seen in earlier experiments using supercoiled substrates (Refs. 2 and 3; see also the supercoiled reactions in Figs. 3 and 4) and appears to
be a property of the nicked substrates/Me\textsubscript{2}SO conditions. Holliday junction intermediates have been reported to accumulate when Cre reaction is performed on supercoiled substrates in the presence of ethidium bromide (28). Indeed, an HJ product was observed for every knotted recombinant formed when type 0 complexes were treated with Cre in the presence of ethidium bromide (Fig. 3B, lane 5; compare with lane 4).

L Crosses R-E Twice on Nicked Substrates and Not Three Times as on Supercoiled Substrates—Reactions analogous to those described above were performed on substrates with loxP sites bordering L. Cre deletion and inversion reactions carried out after assembling type 0 complexes produced predominantly 2-noded catenanes and 3-noded knots, respectively (Fig. 4A, Dir/In, lane 3). Thus, mainly two DNA crossings are contributed by L within a type 0 synapse assembled on nicked substrates (L × E-R = 2). This finding is different from that observed earlier for the type 0 synapse assembled on supercoiled substrates (2, 3) and shown here again (Fig. 4A, Dir/In, lane 4), where the 4-Cat/3-Knot combination shows that L × E-R = 3. Thus, either the single E-L crossing or one of the two L-R crossings seen on supercoiled substrates is not formed on the nicked substrates.

The E-L Crossing Is the Only One Missing on Nicked Substrates—To dissect the L-E-R crossings further, plasmid pZY(E), where loxP sites flank the enhancer (Fig. 4B), was analyzed as described above. A 2-Cat/3-Knot result with the Dir and In substrates, respectively (lanes 3), shows that E × L-R = 2. Taken together, data from Figs. 2, 3, and 4 yield the following mutually consistent solution for the transpososome assembled on nicked substrates: E × R = 2, L × R = 2, and E × L = 0. This inference was further confirmed by analysis of L and In derivatives of three mutant substrates summarized in Fig. 4C. The 2-Cat/3-Knot result from [pSP\textsubscript{DL}(R)] and [pZY\textsubscript{AE}(R)] and 0-Cat/0-Knot from [pZY\textsubscript{AR}(L)] is consistent with the deduced composite topology. Thus, E and L do not cross on nicked substrates as they do on supercoiled ones.

There could be two reasons why the E-L crossing is not observed on nicked substrates. This crossing may require a bend at the enhancer normally promoted by supercoiling (10, 14). Alternatively or in addition, it may require binding of HU at L, which is promoted by DNA supercoiling (9). We tested the first proposition by adding IHF in the reaction with the pSP(L)Dir substrate shown in Fig. 4A. IHF is a sequence-specific DNA-binding protein that introduces sharp bends in DNA (32, 33). It is known to bind stoichiometrically at the enhancer and lower the DNA supercoiling density normally required for Mu transposition (10). The addition of IHF, however, did not restore the E-L crossing (data not shown). Thus, a bend at the enhancer is not sufficient to promote an E-L crossing. To test if the supercoiling-dependent site-specific binding of HU between L1 and L2 subsites was responsible, we introduced an IHF binding site in two different orientations between L1 and L2; IHF did not restore the E-L crossing on these substrates either (data not shown). Inclusion of HMG1, a eukaryotic protein (34) that can bind to DNA independent of supercoiling and can functionally replace HU in Mu transpososome assembly (35), also did not regenerate the E-L crossing (data not shown). Thus, it appears that the DNA-bending effects of supercoiling are likely not responsible for the absence of the E-L crossing on nicked substrates. We conclude that some other property of DNA supercoiling contributes to this crossing (see “Discussion”).

A Functional MuA Tetramer Holds One E-R and Two L-R Crossings—Five of the six att subsites are essential for assembly of the transpososome (24). Treatment of the assembled transpososome with high salt or heparin is reported to remove MuA from the “accessory” sites L2, L3, and R3 (see Fig. 1A), leaving behind a tetramer that can be footprinted on the core sites L1, R1, and R2 (4). To determine the contribution of tetrameric MuA to the DNA crossings, the topological analysis shown in Fig. 5 was carried out.

Cre deletion and inversion reactions carried out after treatment of the type 0 complexes with high salt and heparin gave a 4-Cat/3-Knot product set on pSP(R) (Fig. 5A, Dir/In, lane 3), indicating that R makes three crossings with E-L. In the absence of such treatment, the 4-Cat/5-Knot product set was formed (Fig. 5A, Dir/In, lane 4; see also Fig. 3); thus, one crossing is lost under the high salt/heparin conditions. To determine whether this lost crossing belongs to the R-E or R-L synapse, pSP(L) was similarly analyzed. Treatment of pSP(L) complexes with high salt and heparin, however, gave the same 2-Cat/3-Knot topology as seen in the absence of such treatment (data not shown). Thus, the two L-R crossings are still intact, and one E-R crossing must, therefore, be lost upon depletion of MuA from the accessory site R3.

To ascertain that the type 0 complexes are still functional after treatment with high salt and heparin, Ca\textsuperscript{2+} was exchanged for Mg\textsuperscript{2+} on gel-filtration columns. The mobilities of complexes assembled on nicked pMK21 in Ca\textsuperscript{2+} or Mg\textsuperscript{2+} with and without high salt plus heparin treatment are shown in Fig. 5B. Type 0 complexes assembled in Ca\textsuperscript{2+} with wild-type MuA did not show a distinct mobility when compared with the control, likely because of their instability during electrophoresis (Fig. 5B, lanes 1 and 2); type 0 complexes assembled on supercoiled substrates have been reported to be unstable after nicking with nuclease (36, 13). Cleavage at the ends of Mu, however, is known to stabilize the transpososome; indeed, type I complexes have a distinct mobility on nicked substrates (Fig. 5B, lane 4). However, the formation of Ca\textsuperscript{2+}-promoted complexes on nicked substrates is supported by the topological experiments shown in Figs. 3 and 4. Furthermore, when Ca\textsuperscript{2+} was exchanged with Mg\textsuperscript{2+} on gel-filtration columns, promoting Mu end cleavage, the stabilized cleaved type I complex was readily observed (Fig. 5B, lane 5). The type 0 complexes treated with high salt plus heparin to remove loosely bound MuA were also converted to type I complexes in the presence of Mg\textsuperscript{2+}. However, the complexes migrated with a slightly lower mobility than those in the absence of this treatment (Fig. 5B, lane 3; compare with lanes 4 and 5). We suggest that the resultant synapse topology missing one E-R crossing is not as stable with respect to the enhancer, causing its release from the complex during electrophoresis and accounting for the slower migration of the type I complexes (Fig. 5B, compare lane 3 versus 4). This conclusion also implies that the enhancer remains associated with the ends in wild-type type I complexes during gel electrophoresis. Indeed, we have ascertained that this is so in other experiments.\textsuperscript{2} In summary, the data in Fig. 5 show that the MuA tetramer contributes to two L-R and one E-R crossing and, thus, is involved in interacting with the enhancer and maintaining it within the transpososome.

DISCUSSION

In this study we examined the contribution of supercoiling to the five-noded Mu DNA synapse by difference topology (2, 3). The basis of this assay is that recombinases such as Cre or Flp carry out recombination from a planar, antiparallel synapse (37, 38), and do not cross the recombination sites during DNA exchange (23, 29). Hence, the DNA crossings established by an external synapse can be read off indirectly from the knot and catenane nodes of recombination products. The product topologies obtained by this method using hybrid resolvase-Cre and

\textsuperscript{2} Z. Yin and R. M. Harshey, unpublished data.
hybrid Flp-Cre reactions are in agreement with the previously established −3 resolvase synapse (28, 29). Furthermore, the composite topology of the three-site Mu transposition synapse derived by difference topology is mutually consistent with the subtopologies of the different two-site combinations (2). The method has general applicability with the caveat that, after assembling the external synapse, the substrate geometry still has to accommodate the Flp recombination target/loxP sites in one plane and in antiparallel orientation. This may require the addition of an extra node. This is usually not a serious problem provided the inversion knot and the deletion catenane each show a strong enrichment for a given topology, and the two differ by one crossing number. Combining difference topology with reaction conditions that bypass the need for substrate supercoiling, we made the unexpected observation that the MuA protein itself is able to set up four of the five crossings normally observed within the transpososome. We also deduce that three of these crossings must be maintained by the functional tetrameric unit of the transposase. These findings are summarized in Fig. 6.

In the normal transposition pathway the ER synapse appears to be the first to be established and is a prerequisite for the HU-assisted capturing of the L end to form LER (3). HU binds between the L1 and L2 subsites in a supercoiling-dependent manner (9, 39), assimilating L2-L3 into the complex before the stable entry of L1 (14). On nicked substrates under Me2SO conditions, the L end has joined the synapse, establishing two crossings with R but without crossing with E (Fig. 4). We were unable to restore the E-L crossing by introducing IHF or HMG1-induced bends at either the enhancer or the L end. It is possible that differences between supercoiled and nicked substrates with respect to attL-attR-enhancer interactions mediated by multiple MuA monomers may give rise to two types of transpososome assemblies that differ by one DNA crossing. Alternatively, the architecture of the transposome may be similar in both cases, and the additional crossing may be required to overcome the steric constraints imposed by supercoiling.

The functional form of MuA is a tetramer as revealed by cross-linking experiments (24). When transpososomes are treated with 0.5 mM NaCl before cross-linking, the tetramer is seen to footprint only on L1, R1, and R2. Experiments using heparin or competitor DNA have similarly revealed that MuA is loosely associated with the accessory sites L2, L3, and R3 and can be removed by these treatments (8, 40). We find that such treatments retain the L-R crossings and one of the two E-R crossings (Fig. 5). This suggests that the MuA subunit bound to R3 must play a role in maintaining the crossing that was lost (likely the one designated ER-2 in Fig. 6B, since it is more distal to L-R). These data also show that the MuA tetramer must maintain both L-R as well as the ER-1 crossings. On supercoiled substrates it is likely that the tetramer also maintains the E-L crossing. Although we have arbitrarily placed the tetramer between the L-R nodes in Fig. 1, the actual distribution of the subunits between the three crossings is uncertain.

How might the MuA protein generate four DNA crossings in the absence of supercoiling? It is reasonable to suppose that the dual DNA binding specificity of MuA for the att sites and the enhancer is responsible for cross-bridging E and R in nicked substrates and E and L and E and R in supercoiled substrates. The situation is rather different for L-R interactions. It is known that the MuA subunit at the L1 subsite directs its active site toward and cleaves R1 in trans and vice versa (22, 41, 42). Thus, MuA-att interactions, in conjunction with MuA-MuA interactions, likely set up the L-R crossings.

This study has shown that MuA has the intrinsic capacity to interwrap E, L, and R into a topologically defined synapse. What is the normal role of supercoiling in establishing this interwrapped synapse, and why is the supercoiling requirement overcome in Me2SO? There must be finite enthalpic and entropic costs associated with interwrapping of the three sites. We believe that Me2SO induces conformational changes in both protein and DNA, facilitating transpososome organization by lowering the energy cost of the assembly process normally promoted by DNA supercoiling. That MuA can still interact with the enhancer and recreate 4/5 crossings normally seen on supercoiled DNA suggests that in the process Me2SO has not altered essential protein-DNA or protein-protein interactions.

Site-specific recombinases of the tyrosine family (phage λ Int and E. coli XerC/XerD) and the serine family (Tn3 or γδ resolvase and phage Mu Gin or Salmonella Hin) are known to assemble topologically complex synaptic arrangements to trigger the chemical steps of recombination (43). The Int recombinase utilizes accessory proteins IHF, Fis, and Xis to carry out integrative/exclusive recombination between the phage site attP and the bacterial site attB. Furthermore, Int has hi-specificity in DNA binding, one for the “arm-type” sequence and the other for the “core-type” sequence (44). The XerC/XerD recombinase relies on PopA and ArgR proteins bound to their target sites to set up a topologically unique recombination synapse on plasmid target sites (45). Along the same lines, the Hin and Gin

**FIG. 6. Dissection of the Mu synapse.** A, five-noded topology of the Mu synapse on supercoiled substrates (2, 3). L, R, and E segments are shown in light green, dark green, and orange, respectively. Individual nodes have been named ER-1, ER-2, etc. B, in the absence of supercoiling, MuA assembles a four-noded synapse missing the E-L crossing. C, conditions to strip MuA from L2, L3, and R3, leaving an intact tetramer, result in the loss of one E-R crossing.
proteins organize their synapses with assistance from the Fis protein associated with its binding site (46). By contrast, in the resolvase system the same binding specificity of the resolvase protein comes into play at the target site res I, where the recombination reaction occurs, and the accessory sites res II and res III (47). MuA shares features of the Int and resolvase proteins in having dual DNA binding capacity on the one hand and being self-sufficient for both catalytic and accessory functions on the other. The unique spatial architecture of the Mu synapse must not only assist in critical events in Mu transposition such as trans catalysis, where MuA bound to one end catalyzes reaction chemistry at the opposite end, thus coordinating reaction at both ends (22, 41, 42), but must also contribute to the high stability of the Mu transpososome, which must play an important role during amplification of Mu DNA during the phage life cycle (4). The observation that enhancer is sequestered within the synapse throughout transposition (3) suggests that the enhancer may signal a commitment to transposition in vivo by blocking binding of the Mu repressor to this region (see Fig. IA). Our finding that subunits in the tetramer contribute to holding the enhancer in place implicates a continued role for the enhancer during transposition. It is possible that the specific architecture of the synapse influences capture of target DNA, which unlike other transposons can occur before initiation of transposition chemistry (43, 48).

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