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Mu Transpositional Recombination: Donor DNA Cleavage and Strand Transfer in *trans* by the Mu Transposase

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Summary

Central to the Mu transpositional recombination are the two chemical steps; donor DNA cleavage and strand transfer. These reactions occur within the Mu transpososome that contains two Mu DNA end segments bound to a tetramer of MuA, the transposase. To investigate which MuA monomer catalyzes which chemical reaction, we made transpososomes containing wild-type and active site mutant MuA. By preloading the MuA variants onto Mu end DNA fragments of different length prior to transpososome assembly, we could track the catalysis by MuA bound to each Mu end segment. The donor DNA end that underwent the chemical reaction was identified. Both the donor DNA cleavage and strand transfer were catalyzed in *trans* by the MuA monomers bound to the partner Mu end. This arrangement explains why the transpososome assembly is a prerequisite for the chemical steps.

Introduction

A large number of mobile DNA elements transpose from one location on their host chromosome to another by a fundamentally similar mechanism. This class of transposing DNA elements includes IS elements, both prokaryotic and eukaryotic transposons, and transposing bacteriophages such as Mu (Berg and Howe, 1989; Mizuuchi, 1992a; Craig, 1995). Retroviruses and LTR-containing retrotransposons also use essentially the same mechanism to integrate the DNA copy of their RNA genome into the host chromosome (Varmus and Brown, 1989; Brown, 1990).

Phage Mu is one of the best studied among this class of mobile genetic elements with respect to the mechanism by which it splices its DNA ends to a new target DNA site (Haniford and Chaconas, 1992; Mizuuchi, 1992a, 1992b). Central to this process are the two chemical steps, donor DNA cleavage and DNA strand transfer. First, a pair of endonucleolytic single-strand cleavages separates the 3′-OH termini of the Mu DNA from their original flanking strand. Next, the two strands of a target DNA are cut and joined to the 3′-OH termini of the Mu DNA by DNA strand transfer. In all, a set of four chemical reactions is required to generate a strand transfer product. The branched DNA at the Mu ends in this transposition intermediate can be converted into replication forks by recruiting host enzymes to complete replicative transposition (Craigie and Mizuuchi, 1985; Kruklitis and Nakai, 1994).

In contrast to Mu transpositional recombination, many transposons apparently transpose by a nonreplicative cut-and-paste mechanism (Bainton et al., 1991; Kaufman and Rio, 1992; van Luenen et al., 1994; Kleckner et al., 1995). Such elements cleave the 5′ ends of the element DNA in addition to the 3′ ends. Double-strand cuts liberate the element DNA from its initial flanking DNA. All of these elements studied so far use their 3′ ends for the strand transfer step (Bainton et al., 1991; van Luenen et al., 1994; Kleckner et al., 1995).

The donor DNA cleavage and DNA strand transfer steps, while clearly distinct, are similar as chemical reactions. The human immunodeficiency virus (HIV) donor DNA cleavage step can utilize a variety of nucleophiles for the reaction; a water molecule, certain alcohols, and the 3′-OH of the donor DNA itself can all be used (Engelman et al., 1991; Vink et al., 1991). The last reaction produces a new phosphodiester bond as does the authentic strand transfer reaction. The donor cleavage and strand transfer reactions are therefore chemically equivalent. The chirality of the scissile phosphorothioate bond has been shown to invert, supporting a one-step in-line mechanism for both the donor DNA cleavage and strand transfer reactions (Engelman et al., 1991; Mizuuchi and Adzuma, 1991). Thus, donor cleavage and strand transfer steps differ only in the location of the scissile phosphodiester and the choice of the attacking nucleophile.

Not surprisingly considering the above information, a single active site of transposase/integrase proteins apparently catalyzes both of these reactions. Three acidic amino acid residues were first identified for the integrase proteins of retroviruses and their prokaryotic relatives as highly conserved and critical for both donor DNA cleavage and strand transfer (Fayet et al., 1990; Rowland and Dyke, 1990; Engelman and Craigie, 1992; Kulkosky et al., 1992). These active site acidic residues that presumably coordinate the catalytic divalent metal ion have also been identified for MuA (Baker and Luo, 1994; Kim et al., 1995). Recently determined crystal structures of the core domains of retroviral integrase proteins and MuA protein further support this notion (Dyda et al., 1994; Bujacz et al., 1995, 1996; Rice and Mizuuchi, 1995).

Phage Mu transposase, MuA, is a multidomainal 663 amino acid polypeptide. The central parts of the protein that are essential for transpositional recombination include the Mu end DNA-binding domain (domain Ib, amino acids 77–247; Nakayama et al., 1987; Zou et al., 1991), the catalytic core (domain IIa, amino acids 248–490; Rice and Mizuuchi, 1995), the possible nonspecific DNA-binding domain (domain IIb, amino acids 491–574; Rice and Mizuuchi, 1995), and domain IIIa (amino acids 575–605), which is required for the active protein–DNA complex assembly (Baker et al., 1993) and has been shown to bind DNA and possesses endonuclease activity by itself (Wu and Chaconas, 1995). The endonuclease activity of the domain IIIa strongly suggests that this

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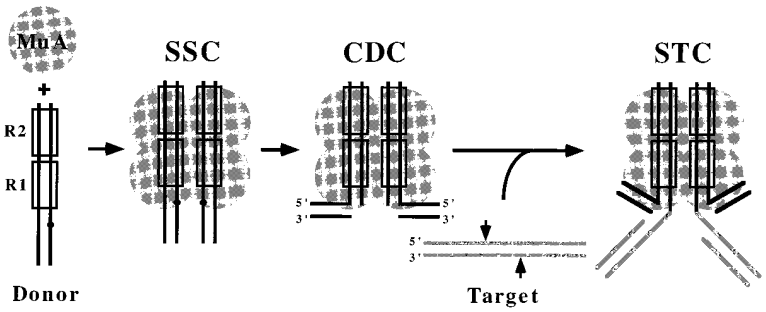

Figure 1. Steps in Mu Transpositional Recombination

Three types of transpososomes corresponding to the three stages of the reaction (SSC, stable synaptic complex; CDC, cleaved donor complex; STC, strand transfer complex) containing two Mu right end DNA segments, rather than the natural combination of a right end and a left end pair, are depicted. After generation of the SSC by the transpososome assembly step, the donor DNA cleavage step converts the SSC to the CDC, and the strand transfer step converts the CDC to the STC. In this study, short Mu right end DNA fragments of two different lengths and a short non-Mu target DNA fragment (see Figure 2) were used as substrates. The R1 and R2 MuA binding sites on the donor DNA are shown as rectangles. MuA is depicted as shaded circles. The small arrows on the target DNA indicate the 5 bp staggered locations for strand transfer on the two strands. The dots in the SSC indicate the Mu end cleavage sites.

domain is intimately involved in catalysis presumably in conjunction with the domain IIa. However, the structurally closely related core domain of the HIV integrase, which does not contain a domain that corresponds to the MuA domain IIIa, is capable of catalyzing a DNA strand transfer reaction with a special type of substrate DNA (Chow et al., 1992; Bushman et al., 1993). Therefore, in MuA also, domain IIa presumably plays the principal role in catalysis, and for this reason, in this paper we use the term "active site" as that composed of the active site acidic residues in domain IIa.

MuA free in solution exists predominantly as a monomer that appears to be catalytically inactive. For the chemical steps to take place, MuA and the two Mu DNA ends must be assembled into a higher order protein–DNA complex called a Mu transpososome (Craigie and Mizuuchi, 1987; Surette et al., 1987). Mu transpososomes contain a tetramer of MuA (Lavoie et al., 1991; Baker and Mizuuchi, 1992; Baker et al., 1993) that is stably bound to the two ends of the phage genome. Each genome end contains three copies of the end-type MuA binding sequence, which are named in order of their proximity to the ends: R1, R2, and R3 for the right end, and L1, L2, and L3 for the left end (Mizuuchi, 1992a). Assembly of Mu transpososomes with natural substrate DNA normally involves a number of regulatory protein and DNA sequence cofactors (Mizuuchi, 1992a; Haniford and Chaconas, 1992). However, under certain reaction conditions a Mu transpososome core can be assembled from only MuA and a short DNA fragment containing R1 and R2 sites (Savilahti et al., 1995). In this type of complex, four MuA monomers within the complex are stably bound to their binding sites on the DNA (Mizuuchi et al., 1991). Following assembly of a transpososome, the chemical steps of Mu transpositional recombination proceed while maintaining the general architecture of the transpososome (Lavoie et al., 1991; Mizuuchi et al., 1991; 1992). The transpososomes are named the stable synaptic complex (SSC, or type 0 complex) for the uncleaved initial complex, the cleaved donor complex (CDC, or type I complex) for the complex after donor DNA cleavage, and the strand transfer complex (STC, or type II complex) for the complex with covalently joined target DNA (Figure 1).

Why is assembly of the Mu transpososome a prerequisite for the chemical steps? Previously, we have discussed the physiological reasons for this requirement (Mizuuchi et al., 1992; Mizuuchi, 1992a). Here, we are interested in the architectural aspects of the Mu transpososome that underlie this phenomenon. The MuA core structure suggests its active site may be held in an inactive configuration prior to transpososome assembly (Rice and Mizuuchi, 1995). The domain IIIa of one monomer functions in *trans* with the domain IIa of another monomer (Yang et al., 1995; Aldaz et al., 1996 [this issue of *Cell*]), indicating that at least a dimer is required for a chemical reaction. Each MuA monomer within a tetramer has been shown to bear a unique responsibility for the completion of the four chemical reactions necessary in generating the STC (Baker et al., 1993, 1994). More specifically, each MuA monomer within the tetramer appears to have an assigned responsibility for one of the four chemical reactions involved (Baker et al., 1994). A question was left unanswered: Which MuA monomer within the tetramer contributes its active site for each of the four chemical reactions of Mu transpositional recombination?

Results

Experimental Outline

The chemical steps of Mu transposition take place within the context of a protein–DNA complex called Mu transpososome, which contains two Mu end DNA segments bound to a tetramer of MuA. In this study, we asked if a MuA monomer cleaves the Mu DNA end to which it is bound (cleavage in *cis*) or the partner Mu DNA end within the transpososome (cleavage in *trans*). Similarly, we examined whether strand transfer reactions take place in *cis* or in *trans*. We prepared transpososomes in which one Mu end DNA fragment was occupied by wild type MuA and the other Mu end DNA fragment by an active site mutant of MuA. As the active site mutant, we used MuA(E392Q), which is defective in both chemical steps of transpositional recombination but not in transpososome assembly (Baker and Luo, 1994; Kim et al., 1995). The two Mu end DNA fragments differed in length so that transpososomes containing a pair of long Mu end fragments, a pair of short Mu end fragments, or one long and one short fragment could be electrophoretically separated.

In the preloading step, each Mu end fragment was incubated with one of the MuA variants under conditions

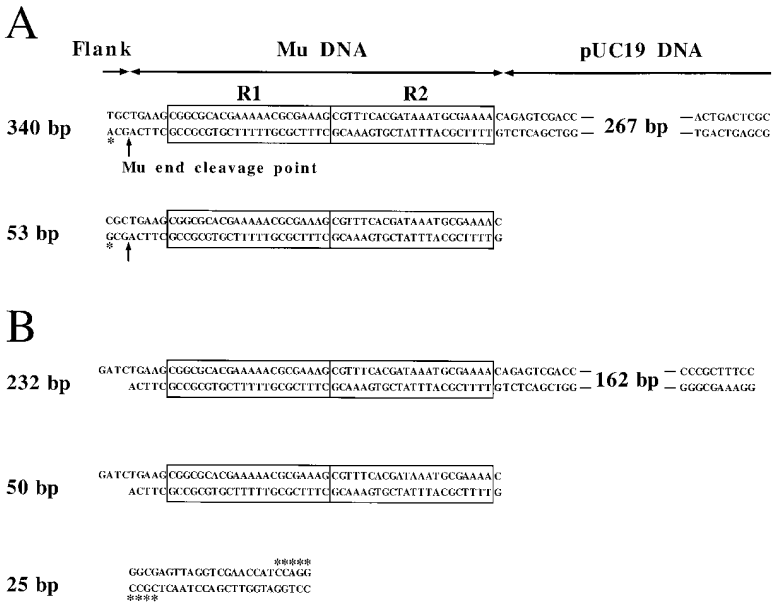

Figure 2. DNA Substrates Used for the Study of the Two Chemical Steps

MuA binding sites R1 and R2 are shown as rectangles. Positions of the radioactive label are shown by asterisks. The sequences of the 287 bp and 182 bp extensions in the longer donor DNA fragments of 340 bp and 232 bp are derived from pUC19 between nt 426 and 712 and nt 426 and 607, respectively (Yanisch-Perron et al., 1985). (A) Substrates for the donor DNA cleavage experiment. Note that the 3′-flanking trinucleotides differed between the two donor fragments to enable identification of the cleavage products by their electrophoretic mobility. (B) Substrates for the strand transfer reaction. Unlabeled donor DNA fragments (232 bp and 50 bp) were in the precut configuration with a four-nucleotide-overhang on the 5′-flanking strand. The target DNA fragment (25 bp) was labeled at both 3′ ends as depicted.

in which MuA binds to the DNA, but transpososome assembly does not take place. These presynaptic complexes were then mixed together under conditions that allow efficient transpososome assembly.

For the analysis of the donor cleavage step, MuA was preloaded onto Mu end DNA fragments that were ^{32}P-labeled at the 3′ end of the strand to be cleaved (Figure 2). Then the SSC assembly step was carried out under the conditions that do not allow donor DNA cleavage. Transpososomes were separated by agarose gel electrophoresis in the presence of heparin. The gel-purified uncleaved SSC's were incubated in the presence of Mg^{2+} and heparin to allow the donor cleavage reaction to proceed without reassembly of transpososomes from MuA and DNA dissociated during the purification. The donor cleavage products were analyzed by urea–polyacrylamide gel electrophoresis (urea–PAGE).

For the analysis of the strand transfer step, MuA was preloaded onto unlabeled precleaved Mu end fragments (Figure 2). A 3′-labeled short target DNA fragment was included during assembly of the CDC in the presence of Mg^{2+}, so that the strand transfer reaction could also take place. Reactions were stopped by adding heparin which efficiently blocked both strand transfer and further transpososome assembly by free MuA monomers and Mu end DNA. After separation of the three STC species by agarose gel electrophoresis, the DNA in the gel-purified complexes was analyzed by urea–PAGE to detect the strand transfer products.

Donor DNA Cleavage Occurs In *trans*

The donor DNA cleavage was studied using two different length DNA substrates both containing three bp of flanking DNA (Figure 2A). The flanking DNA length is one of the critical determinants for formation and stability of the transpososome (Savilahti et al., 1995). The length of three bp was chosen because it allowed efficient assembly of the SSC that withstands the electrophoresis conditions used. A single nucleotide difference in the flanking DNA sequence made it possible to electrophoretically distinguish between the trinucleotides that were released after donor cleavage and therefore to identify the DNA fragment from which they were derived.

The experimental details are depicted in Figure 3. The SSCs were formed by mixing two preloaded Mu end fragments and separated by agarose gel electrophoresis (Figure 4A). As can be seen, both mutant and wild-type proteins assembled transpososomes efficiently. The gel pieces containing the SSCs were excised and then soaked in buffer containing Mg^{2+} and heparin to allow donor DNA cleavage without allowing reassembly of new transpososomes. Then, the product DNA was isolated and analyzed by urea–PAGE (Figure 4B). The quantitation of the data is given in Table 1.

The complexes containing only the DNA fragment that was preloaded with MuA(E392Q) yielded essentially no cleavage products (Figure 4B, lanes 10–12). In contrast, complexes containing only the DNA fragment preloaded with wild-type MuA yielded a significant amount of cleavage products. As expected, the slowest migrating complex released only the trinucleotide that was derived from the long Mu end DNA fragment (Figure 4B, lane 7), while the fastest migrating complex released only the trinucleotide derived from the short fragment (lane 9). The complex with intermediate gel mobility released about equal amounts of trinucleotides from both of the fragments (Figure 4B, lane 8), confirming that this complex contained both fragments.

The complexes that contained both short and long Mu end fragments, each preloaded with different MuA variants, successfully carried out donor DNA cleavage. However, there was a clear bias in the cleavage products released. The fragment that was preloaded with the mutant protein was preferentially cleaved irrespective of which of the two fragments, long or short, was preloaded with which MuA protein (Figure 4B, lanes 2 and 5). As the mutant protein is catalytically inactive, this result shows that the cleavage step in Mu transposition must

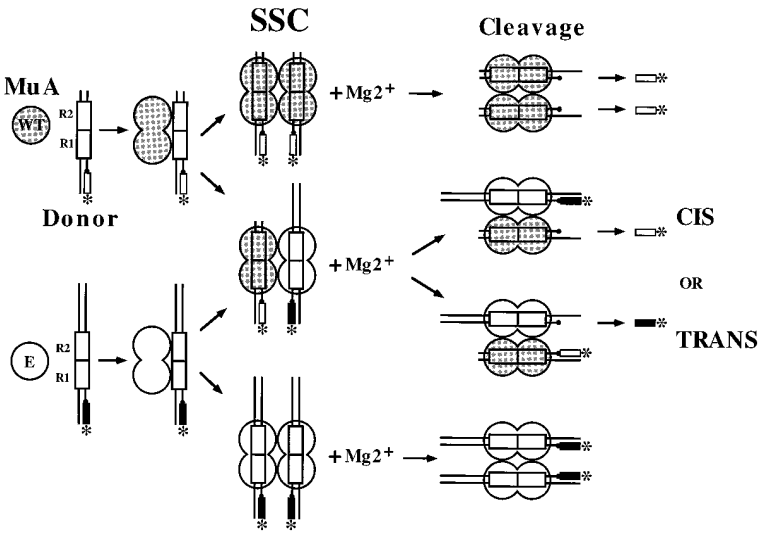

Figure 3. Design for the Experiment to Study the Donor DNA Cleavage Reaction

After preloading either the wild type (WT, shaded circles) or the active site mutant (E, open circles) MuA separately onto each donor DNA fragment, they were mixed together for SSC assembly. Three types of the SSC were gel purified and then allowed to carry out donor DNA cleavage. The SSC containing both wild-type and mutant proteins is expected to cleave only one of the two donor ends. Cleavage in *cis* mechanism would produce the trinucleotide depicted by the white box, while cleavage in *trans* mechanism would produce the trinucleotide depicted by the closed box. See the text for details.

occur in *trans*—i.e., the MuA monomer bound to one Mu end cleaves the other Mu end within the transpososome.

Strand Transfer Occurs In *trans*

The strand transfer step of Mu transposition was studied utilizing a 3′ end–labeled target DNA fragment and two unlabeled precleaved Mu end DNA fragments of different length (see Figure 2B). The strategy of the experiment is shown in Figure 5. Because in this experiment only the target DNA fragment was labeled, only the STCs were detected by autoradiography, as described earlier (Savilahti et al., 1995). Use of precleaved donor substrate allowed us to analyze the strand transfer step separately from the donor DNA cleavage step.

The preloaded donor DNA fragments were mixed together with simultaneous addition of Mg^{2+} and the labeled target DNA, and the temperature was shifted to 30°C to allow assembly of the CDC and strand transfer with the target DNA. The STCs formed after 1 or 4 min incubation were analyzed (Figure 6A). As expected, Mu(E392Q) could not generate strand transfer complexes by itself. In contrast, the wild-type MuA, by itself or in combination with MuA(E392Q), generated strand transfer complexes.

The amount of the STC containing two copies of the Mu end fragment that was preloaded with the mutant protein indicated how much background should be expected in the analysis of the STC containing one copy each of the longer and shorter Mu end fragments. When the longer Mu end DNA fragment was preloaded with wild type MuA and the shorter fragment with the mutant protein, the STC containing two copies of the longer Mu end fragment was more abundantly produced than the STC with two shorter fragments. When the preloading combination was reversed, the outcome was also reversed. The proportion of the STC containing two copies of the Mu end fragment that was preloaded with the mutant protein increased with longer reaction time after mixing the two preloaded fragments (Figure 6A, compare left and right gels). Since the CDC containing four mutant MuA monomers cannot carry out strand transfer, the above observations indicate that, while most of the preloaded DNA fragments retained their prebound protein, some rearrangements take place after mixing. Presumably, CDC assembly continued to take place from the DNA and protein molecules that remained unbound during the preloading step. Exchange of the preloaded protein prior to CDC assembly could also have taken place during this incubation.

The DNA in the isolated STC species was analyzed by urea–PAGE to separate chemically unreacted target DNA, target DNA that was joined to the longer Mu end DNA strand, and target DNA that was joined to the shorter donor strand (Figure 6B). As expected, the slowest migrating complex contained only the longer series of strand transfer products (Figure 6B, left three lanes), and the fastest migrating complex contained only the shorter series of strand transfer products (right three lanes). The complex with intermediate gel mobility contained both the longer and shorter series of strand transfer products (Figure 6B, middle three lanes). When both of the Mu end fragments were preloaded with the wild-type protein, this complex contained approximately equal amounts of the shorter and longer series of strand transfer products (Figure 6B, lane 6). When the longer Mu end fragment was preloaded with the wild-type MuA and mixed with the shorter Mu end fragment preloaded with the mutant MuA, the STC with intermediate gel mobility predominantly contained shorter series of strand transfer products (Figure 6B, lane 4). Conversely, when the shorter Mu end fragment was preloaded by the wild-type MuA and the longer fragment by the mutant MuA, strand transfer products were predominantly long (lane 5). These product biases were more pronounced after 1 min of reaction following the mixing of the two MuA-bound Mu end fragments than after 4 min of reaction (Table 2) for reasons discussed above. We conclude that within a transpososome, a cleaved Mu DNA end accomplishes strand transfer in *trans* by using the active site of the MuA monomer bound to its partner Mu end.

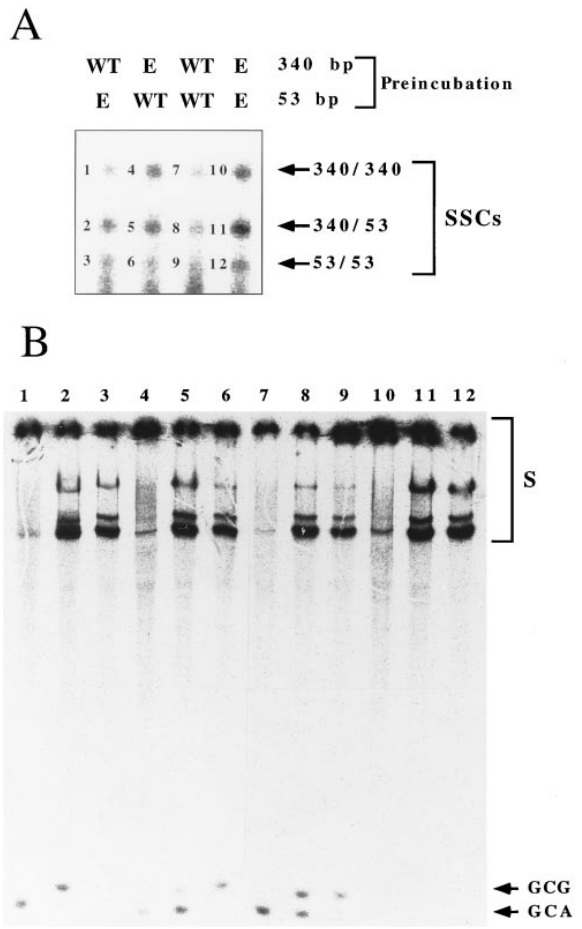

Figure 4. Separation of the Three Types of the SSC, and the Donor DNA Cleavage Products Released from Them

(A) Agarose gel electrophoresis of the SSC made with different preloading combinations of the donor DNA fragment and the MuA variant. The preloading combinations of the MuA variant, the wild type (WT) or the active site mutant (E), and the donor DNA fragment, 340 bp or 53 bp, are shown above the gel picture. The combinations of the two donor DNA fragments in the SSC are indicated on the right side. The radioactivity visible at the bottom is a part of smear of the unbound long donor DNA, which was mostly run out of the gel. The incubation time for SSC assembly was 35 s for the experiment shown. MuA(E392Q) was more efficient than the wild-type protein for transpososome assembly. This mutant protein is more efficient than the wild-type MuA in transpososome assembly in reactions with mini-Mu plasmid donor DNA as well (Baker and Luo, 1994; Mizuuchi et al., 1995). (B) A denaturing polyacrylamide gel electrophoresis of the donor DNA cleavage products after incubation of the gel purified SSCs in the presence of Mg^{2+}. The lane numbers correspond to the gel-purified complexes as indicated in the (A). The uncleaved donor DNA strands (S) migrated near the top of the gel, while the cleaved trinucleotide products, GCG and GCA, migrated near the bottom of the gel. We estimated the efficiency of donor DNA cleavage of the gel-purified SSC to be ~10% with a significant variability among samples presumably owing to inactivation of the SSC during the purification process.

Discussion

Active Sites Are Donated In *trans* within a Mu Transpososome

The chemical steps of Mu transpositional recombination take place only after the two ends of the Mu genome are synapsed by a MuA tetramer. The architecture of this synaptic complex must coordinate the multiple chemical steps required for generating strand transfer products. The findings reported here can explain how premature cleavage of the donor DNA prior to the synapsis of two Mu ends is prevented. The active sites of the MuA monomers bound at one Mu DNA end are not positioned to act at the cleavage site adjacent to the MuA-binding sites at this end. Instead, the scissile phosphodiester for cleavage at one Mu DNA end apparently can only be properly juxtaposed to the active site of the MuA monomer bound to the other Mu end. Therefore, the two ends of the Mu genome bound by MuA must be synapsed in order for the scissile bonds to stably occupy the active sites. This Mu end synapsis, coupled with MuA tetramer formation, may induce a structural transition that changes the MuA active site from the inactive to the active configuration as suggested by the crystal structure of the isolated core domain of MuA (Rice and Mizuuchi, 1995). The recent observations that the active site of one MuA monomer cooperates with the domain IIIa of another monomer for its activity (Yang et al., 1995; Aldaz et al., 1996), can be viewed as an additional way of avoiding premature reaction prior to transpososome assembly.

Previous studies have suggested that each monomer within the MuA tetramer is responsible for one specific chemical reaction among the four that are necessary for generating the strand transfer product (Baker et al., 1994). We should be able to test this model by determining which of the four MuA-binding sites on the two Mu ends is occupied by the particular MuA monomer that donated its active site for each of the four chemical reactions. The results presented here provide a partial answer to this question as we have not determined whether the MuA monomer that contributes its active site residues for a particular chemical step is bound to the R1 or R2 site. Recent results from other laboratories, however, complement our observations. The Baker laboratory has found that the MuA monomer within the tetramer that is bound to the R1 site at one Mu end contributes its active site residues for the strand transfer step at the partner Mu end (Aldaz et al., 1996). Results from the Harshey laboratory suggest that the donor DNA cleavage involves domain II of the MuA monomers bound to the R2 sites (J.-Y. Yang, M. Jayaram, and R. Harshey, personal communication). Because of the complex nature of these experiments, one should remain cautious in drawing a unified conclusion from the results of different experiments carried out in separate laboratories. Nevertheless, considered together, a coherent picture of the division of labor among MuA monomers within the tetramer is emerging from these results. It currently appears that the MuA monomers bound to the R2 sites cleave the donor DNA in *trans*, and those bound to the R1 sites carry out strand transfer in *trans* (Figure 7). The donor DNA cleavage site apparently is positioned at the active site in the domain II of the MuA monomer bound to the R2 site of the partner Mu end, and a water molecule must be in position to attack the scissile bond as a nucleophile. Following donor cleavage, the freed 3′-OH group repositions itself to act as the nucleophile attacking a target DNA phosphodiester.

Table 1. Quantitation of Donor Cleavage Products Released from the Complexes Containing One Long and One Short Donor DNA fragment

Time after Mixing	Cleavage Product Originated from Donor DNA Length[a]	Amount of Released Cleavage Products: as Percentage and in Arbitrary Units,[b] with MuA Variant Preloading Combinations Indicated		
		WT (340 bp) E (53 bp)	E (340 bp) WT (53 bp)	WT (340 bp) WT (53 bp)
35 s	GCA (340 bp)	18% (72)	79% (351)	48% (300)
35 s	GCG (53 bp)	82% (325)	21% (96)	52% (330)
60 s	GCA (340 bp)	24% (303)	71% (766)	43% (741)
60 s	GCG (53 bp)	76% (946)	29% (314)	57% (978)

[a] Numbers in parentheses indicate length.
[b] Numbers in parentheses are in arbitrary units.

The target phosphodiester must occupy the active site of the MuA monomer whose Mu end–binding domain is bound to the R1 site of the partner Mu end.

Active Site Organization in Other Transposition Reactions

Do other transposition reactions use similar assignment of labor for the transposase/integrase monomers within the higher order protein–DNA complex that carries out the chemical steps? While the active oligomeric state of HIV integrase for DNA integration has not been determined, formation of an integrase tetramer has been observed (Jenkins et al., 1996). Interdomainal complementation experiments have suggested some type of division of labor among the monomers within a functional integrase oligomer for the HIV DNA integration reaction (Engelman et al., 1993; van Gent et al., 1993). No direct experiments have been done to our knowledge. However the Mu-type division of labor is an attractive possibility for the HIV DNA integration reaction as well.

Recent results on the Tn10 transposition reaction suggest that for this element, the active sites of only two transposase monomers within the transpososome are responsible for all of the chemical steps (Bolland and Kleckner, 1996). In all, four donor DNA cleavages (both strands at the two transposon ends are cleaved in this system) and two strand transfers at the 3′ ends take place in this reaction. Thus, each catalytic monomer appears to carry out donor DNA cleavage of both strands and also the subsequent strand transfer. However, the oligomeric state of the transposase within the Tn10 transpososome has not been determined.

The Tn7 transposition reaction is more complex. Like Tn10, this element cleaves both donor DNA strands (Bainton et al., 1991). This element utilizes two different proteins for the three separate chemical steps of the reaction. TnsB binds to the repeated sequence elements at the two ends of the transposon (Arciszewska and Craig, 1991) and is responsible for the donor cleavage at the 3′ ends as well as for the strand transfer involving the cleaved 3′ ends, while the active site of TnsA is responsible for the donor cleavage outside of the 5′ ends (R. Sarnovsky, E. May and N. Craig, personal communication). An analogy to the Mu model of one transposase monomer per one chemical step would predict that four TnsB and two TnsA monomers divide their

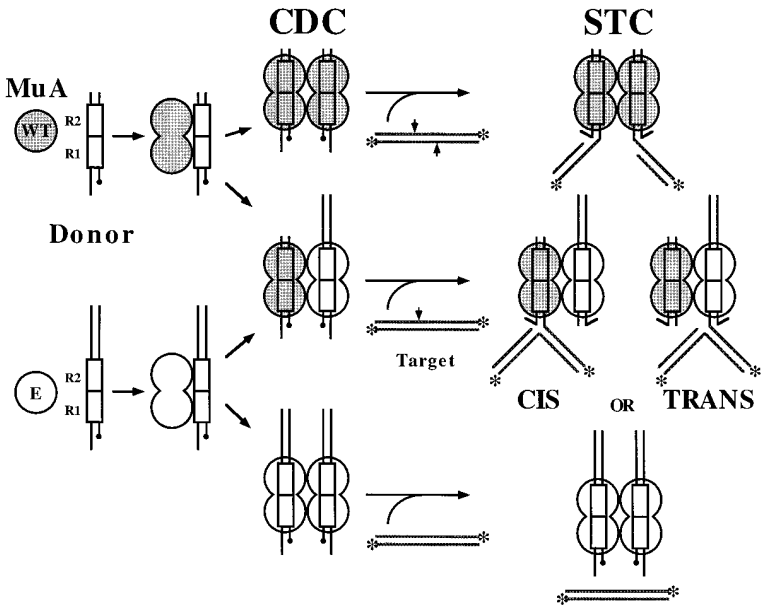

Figure 5. Design for the Experiment to Study the Strand Transfer Reaction

After preloading either the wild type (WT, shaded circles) or the active site mutant (E, open circles) MuA separately onto each precut donor DNA fragment, they were mixed together for CDC assembly and strand transfer with the labeled target DNA fragment. Three types of the resulting STC were gel purified. The STC containing both wild-type and mutant proteins is expected to contain only one of the two target DNA strands covalently joined to one of the two donor ends. Upon denaturation, strand transfer in *cis* mechanism would yield the shorter series of the strand transfer product, while strand transfer in *trans* mechanism would yield the longer series of the strand transfer product with the preloading combination depicted here. See the text for details.

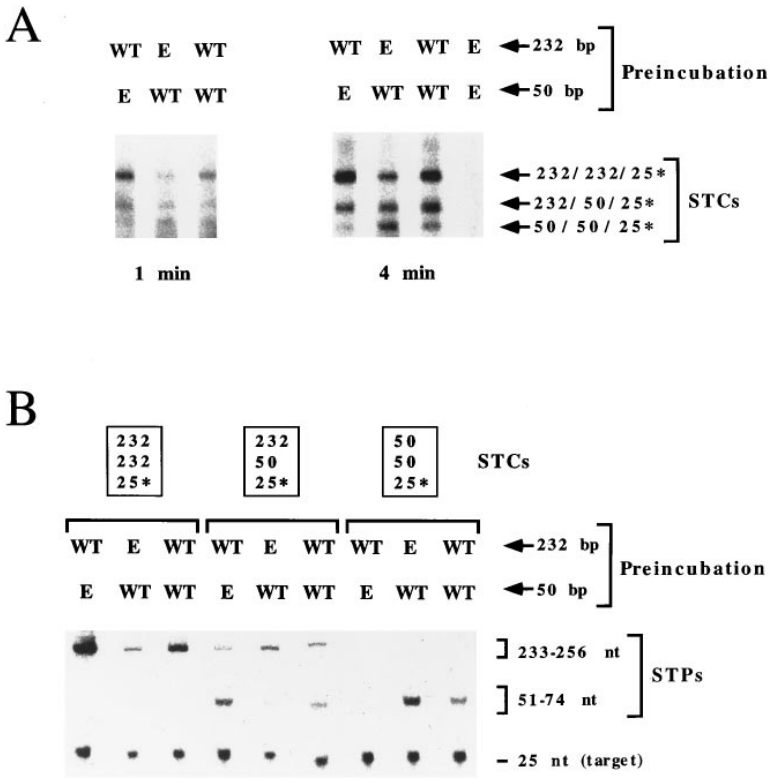

Figure 6. Separation of the Three Types of the STCs, and Analysis of the Strand Transfer Products Contained in Them

(A) Agarose gel electrophoresis of the STCs made with different preloading combinations of the donor DNA fragment and the MuA variant. The preloading combinations of the MuA variant, the wild type (WT) or the active site mutant (E), and the precut donor DNA fragment, 232 bp or 50 bp, are shown above the gel picture. The combinations of the two donor and the target DNA fragments in the STC are indicated on the right. The incubation for the CDC assembly and strand transfer was stopped after either 1 or 4 min as indicated below the gels. (B) A denaturing polyacrylamide gel electrophoresis of the strand transfer products after 1 min reaction. The combination of DNA fragments in each STC and the preloading MuA–Mu end combination are shown above the gel picture. The positions of the strand transfer products of the expected length and also the unreacted target DNA strands are indicated on the right.

responsibilities for the six chemical steps required for the formation of the transposition intermediate. However, the stoichiometry of each protein within the transpososome has not been determined for this element.

It is possible that among groups of transposons that share the two chemical step DNA splicing mechanism, we may find that evolution has invented a variety of ways for organizing the active sites of the oligomeric transposase proteins to generate physiologically viable systems. Evolutionary flexibility of the parasitic transposable genetic elements should provide a fertile ground for comparative studies among different elements and may teach us how similar physiological problems can be solved in widely different ways by changes in macromolecular interactions. In this regard, it is interesting to note that the early steps of the antigen receptor gene V(D)J recombination reaction have recently been found to share similar characteristics of the two chemical steps of the transpositional recombination reactions (van Gent et al., 1996). It would be interesting to compare the structural organization of the functional complex containing the RAG1 and RAG2 proteins that carry out this domesticated recombination reaction to that of the parasitic transposase/integrase oligomers.

Active Site Organization in Conservative Site-Specific Recombination Reactions

Like Mu transpositional recombination, conservative site-specific recombinations involve four sets of DNA cleavage and joining steps, and like in the Mu reaction, the responsibility for these steps is thought to be divided among four recombinase monomers within a recombination complex. Unlike in transposition, the functional demands for the four recombinase monomers are fundamentally similar; one recombinase monomer is thought to be responsible for one DNA cleavage and one DNA joining reaction, via a protein–DNA covalent intermediate (for review, see Stark et al., 1992). Thus, two sets of

Table 2. Quantitation of Strand Transfer Products in the STCs Containing One Long and One Short Donor DNA fragment

Time after Mixing	Strand Transfer Products, Donor DNA Length[a]	Amount of Strand Transfer Products: as Percentage and in arbitrary units,[b] with MuA Variant Preloading Combinations Indicated		
		WT (232 bp) E (50 bp)	E (232 bp) WT (50 bp)	WT (232 bp) WT (50 bp)
1 min	long (232 bp)	35% (261)	66% (388)	47% (281)
1 min	short (50 bp)	65% (487)	34% (201)	53% (313)
4 min	long (232 bp)	41% (1380)	63% (1630)	50% (2470)
4 min	short (50 bp)	59% (1953)	37% (976)	50% (2424)

[a] Numbers in parentheses indicate length.
[b] Numbers in parentheses are in arbitrary unit.

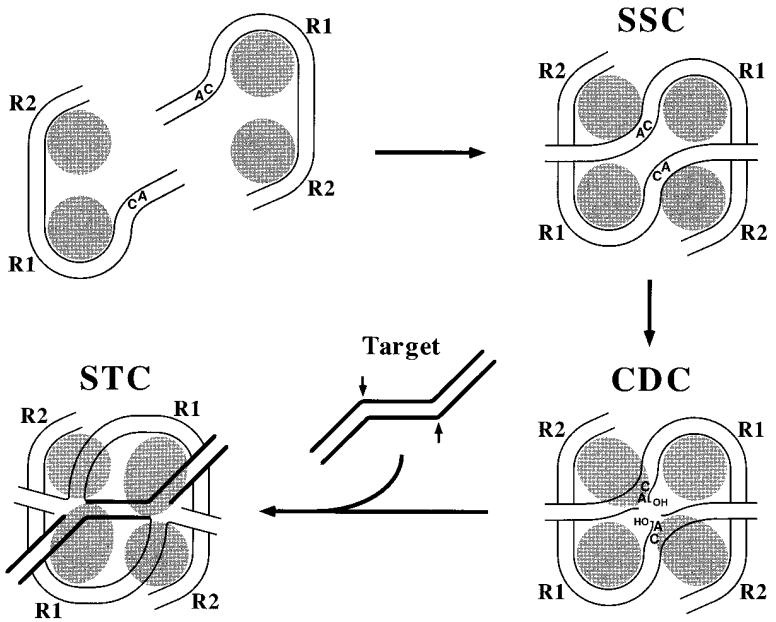

Figure 7. A Model of the Catalytic Role of Each MuA Monomer within a Mu Transpososome

After preloading of MuA monomers onto separate Mu end binding sites, the SSC is assembled in such a way that the active site of the monomer bound to the R2 site of one end is in position to cleave the partner end. After the donor cleavage, the active site of the monomer bound to the R1 site of one end is in position to catalyze the strand transfer with the CA-3′-OH of the partner end.

residues directly participate in the catalysis, a hydroxylated residue (tyrosine or serine) that forms the covalent bond with DNA in the intermediate and another set of residues that catalyze the reaction (for review, see Johnson, 1996). For these reactions, we might consider the hydroxylated residue, separately from other active site residues, as a built-in cosubstrate in the reaction. Which active site and which cosubstrate are involved in each DNA cleavage and each joining step? To make the matter even more complex, for a strand exchange to take place, the cut DNA ends must move relative to each other between the cleavage and joining steps. Thus, an additional question is, Which reaction components move together with each end of the cut DNA?

In the case of the γδ resolvase, both the active site and the serine cosubstrate function in *cis* (Boocock et al., 1995). In the crystal structure, the active site residues and the serine cosubstrate from the same resolvase monomer are located relatively close together (Sanderson et al., 1990; Rice and Steitz, 1994). In the structure of the resolvase–DNA complex, the active site residues of the monomer bound to one side of the substrate DNA are located relatively close to the scissile phosphodiester that will bridge this half of the DNA to this monomer (Yang and Steitz, 1995). Thus, the structural information agrees well with the results of biochemical experiments (Boocock et al., 1995).

Like the γδ resolvase, the active sites of the lambda Int family of recombinases also appear to function in *cis*; i.e., the active site belonging to the recombinase monomer bound to one half of one of the recombination partners catalyzes the reaction between the proximal cleavage site on this DNA and the tyrosine cosubstrate (for review, see Stark and Boocock, 1995). However, a unifying picture has not emerged as to which recombinase monomer donates the tyrosine cosubstrate among the systems studied. A variety of experiments on Flp recombination, Xer recombination, and lambda integration have yielded results indicating cleavage in *trans*, cleavage in *cis*, or even both possibilities (for review, see Stark and Boocock, 1995), again suggesting subtle organizational variations among closely related reactions.

Advances in our knowledge on the structural and functional organization of the higher order protein–DNA complexes within which DNA rearrangements take place are vital for our understanding of how macromolecular interactions can be organized to regulate and coordinate multiple reaction steps involving DNA substrates. In turn, it is hoped that better understanding of the mechanism of propagation of prokaryotic movable genetic elements will help further understanding of related pathogenic agents such as HIV.

Experimental Procedures

Proteins, Nucleotides, and Reagents

MuA and MuA(E392Q) were overexpressed and purified as described (Baker et al., 1993). Vent DNA polymerase, restriction enzyme BglII, and large fragment of Escherichia coli DNA polymerase I (Klenow fragment) were from New England Biolabs and bovine serum albumin (BSA) from Miles. [α-^{32}P]dATP (3000 Ci/mmol), [α-^{32}P]dCTP (3000 Ci/mmol) and [α-^{32}P]dGTP (3000 Ci/mmol) were purchased from New England Nuclear. Dimethylsulfoxide was from Aldrich, Triton X-100 from Boehringer Mannheim, Ficoll 400 from Pharmacia, heparin from Sigma, and NuSieve-GTG agarose from FMC Bioproducts.

DNA

DNA fragments used as substrates are shown in Figure 2. Oligonucleotides up to 54 nt in length were synthesized with a Millipore Expedite (Model 8909) DNA synthesizer and purified by urea–PAGE (Sambrook et al., 1989). The strands were annealed in TEN-buffer (10 mM Tris–HCl [pH 8], 1 mM EDTA, 50 mM NaCl) by boiling for 2 min and slowly cooling to room temperature. DNA fragments longer than 54 bp were generated first from appropriate DNA templates by overlapping polymerase chain reaction (Higuchi et al., 1988) and then amplified by standard polymerase chain reaction (Saiki et al., 1988), in both cases using Vent DNA polymerase according to the guidelines of the manufacturer. The 5′-overhang in the 232 bp substrate fragment was generated by digestion of the polymerase chain reaction product with BglII. The DNA fragments were purified

through agarose gel electrophoresis, sequential phenol and chloroform extractions, and passage through a spin column (P6 or P30, Bio-Rad). Alternatively, DNA fragments were purified by chromatography on a Gen-Pak anion exchange column (Waters). DNA fragments were ethanol precipitated and resuspended in TEN buffer. Double-stranded DNA fragments were labeled at their 3′ ends according to Sambrook et al. (1989) with Klenow fragment and an appropriate dNTP (or dNTPs). Protein and unincorporated nucleotides were then removed by sequential phenol and chloroform extractions, passage through a spin column (P6 or P30, Bio-Rad), and ethanol precipitation. The labeled DNA fragments were resuspended in TEN buffer. The specific radioactivities of the two donor fragments used for the study of the cleavage reaction were adjusted to match each other.

Formation and Purification of the SSCs

Preloading mixtures (25 μl) contained 100 nM ^{32}P-labeled donor DNA fragment, 232 nM MuA, 25 mM Tris–HCl (pH 8.0), 25 μg/ml BSA, 10% (w/v) glycerol, 15% (v/v) dimethylsulfoxide, 0.05% (w/v) Triton X-100 and 120 mM NaCl. After 30 min incubation at 0°C, the preloading mixtures (12 μl each) were combined with simultaneous addition of 10 mM $CaCl_2$ and the temperature was shifted to 30°C. The reaction was stopped after indicated time of incubation by the addition of 23% volume of Ficoll–heparin solution (17% Ficoll 400, 22 mg/ml heparin) and freezing in liquid nitrogen. After thawing, 5.5 μl of sample was loaded onto 4% NuSieve-GTG agarose gel containing 80 μg/ml BSA and 1 mg/ml heparin in 1× TAE buffer (40 mM Tris-acetate [pH 7.8], 8 mM sodium acetate, 1 mM EDTA). Transpososomes (SSCs) were separated by electrophoresis at 4°C for 4 hr at 5.3 V/cm in 1× TAE containing BSA (80 μg/ml) and heparin (1 mg/ml) with several buffer changes.

Formation and Purification of the STCs

Preloading conditions for the unlabeled precleaved donor DNA were essentially the same as for the uncleaved donor DNA. However, even preloading at 0°C without divalent metal ions permitted slow assembly of transpososomes with precleaved substrates (data not shown). Therefore, a relatively short incubation time, 9 min, was used for preloading to minimize transpososome assembly during this period. Two preloaded Mu ends (11.5 μl each) were combined together with simultaneous addition of 2 μl of 25 μM labeled target DNA containing 125 mM $MgCl_2$, and the temperature was shifted to 30°C. The reaction was stopped as described above, and 11 μl of the sample was loaded onto 4.5% NuSieve-GTG agarose gel containing 80 μg/ml BSA and 100 μg/ml heparin in 1× TAE. Transpososomes (STC) were separated by electrophoresis at 4°C for 7.5 hr at 5.3 V/cm in 1× TAE containing BSA (80 μg/ml) and heparin (100 μg/ml) with several buffer changes.

Donor DNA Cleavage and Analysis of the Products

The positions of the SSCs in the gel were located by autoradiography, gel pieces containing SSCs were excised, and 400 μl of the buffer containing 50 mM Tris (pH 8.0), 200 μg/ml BSA, 120 mM NaCl, 5 mg/ml heparin was added. After 15 min incubation on ice, the buffer was replaced with 200 μl of the same buffer and the gel piece was macerated. After an additional 20 min on ice, 4 μl of 1 M $MgCl_2$ was added to start the cleavage reaction at 30°C for 2.5 hr; the reaction was stopped by freezing the sample in liquid nitrogen. After thawing, the sample was boiled for 4 min and phenol extracted. The phenol phase was washed once with water; the aqueous phases were combined and extracted sequentially with 1-butanol and chloroform; and the sample was lyophilized and resuspended in 25 μl of sequencing dye (98% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol, 10 mM EDTA [pH 8.0]). Aliquots (4 μl) were analyzed on 1.6 mm thick 2 M urea–26% polyacrylamide gel (19:1, acrylamide:bis). The dried gel was subjected to autoradiography and quantitation by a Fujix BAS 2000 (Fuji Medical Systems).

Analysis of the Strand Transfer Reaction Products

The positions of the STCs in the gel were located by autoradiography and gel pieces containing STCs were excised. The gel piece was crushed with addition of 500 μl H_2O and extracted with buffer-saturated phenol, the phenol phase was washed once with water, and aqueous phases were combined and extracted sequentially with 1-butanol and chloroform. The sample was lyophilized and resuspended in 20 μl of sequencing dye, and an 8 μl aliquot was analyzed on 0.8 mm thick 8 M urea–12% polyacrylamide gel (19:1, acrylamide:bis). Autoradiography of the dried gel was carried out as above.

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References

Aldaz, H., Schuster, E., and Baker, T.A. (1996). The intertwined architecture of the Mu transposase couples DNA synapsis to catalysis. Cell, this issue.

Arciszewska, L.K., and Craig, N.L. (1991). Interaction of the Tn7-encoded transposition protein TnsB with the ends of the transposon. Nucleic Acids Res. *19*, 5021–5029.

Bainton, R., Gamas, P., and Craig, N.L. (1991). Tn7 transposition in vitro proceeds through an excised transposon intermediate generated by staggered breaks in DNA. Cell *65*, 805–816.

Baker, T.A., and Luo, L. (1994). Identification of residues in the Mu transposase essential for catalysis. Proc. Natl. Acad. Sci. USA *91*, 6654–6658.

Baker, T.A., and Mizuuchi, K. (1992). DNA-promoted assembly of the active tetramer of the Mu transposase. Genes Dev. *6*, 2221–2232.

Baker, T.A., Mizuuchi, M., Savilahti, H., and Mizuuchi, K. (1993). Division of labor among monomers within the Mu transposase tetramer. Cell *74*, 723–733.

Baker, T.A., Krementsova, E., and Luo, L. (1994). Complete transposition requires four active monomers in the mu transposase tetramer. Genes Dev. *8*, 2416–2428.

Berg, D.E., and Howe, M.M. (1989). Mobile DNA (Washington, D.C.: American Society for Microbiology).

Bolland, S., and Kleckner, N. (1996). The three chemical steps of Tn10/IS10 transposition involve repeated utilization of a single active site. Cell *84*, 223–233.

Boocock, M.R., Zhu, X., and Grindley, N.D.F. (1995). Catalytic residues of γδ resolvase act in *cis*. EMBO J. *14*, 5129–5140.

Brown, P.O. (1990). Integration of Retroviral DNA. Curr. Top. Microbiol. Immunol. *157*, 19–48.

Bujacz, G., Jaskolski, M., Alexandratos, J., Wlodawer, A., Merkel, G., Katz, R.A., and Skalka, A.M. (1995). High resolution structure of the catalytic domain of the avian sarcoma virus integrase. J. Mol. Biol. *253*, 333–346.

Bujacz, G., Jaskolski, M., Alexandratos, J., Wlodawer, A., Merkel, G., Katz, R.A., and Skalka, A.M. (1996). The catalytic domain of avian sarcoma virus integrase: conformation of the active-site residues in the presence of divalent cations. Structure *4*, 89–96.

Bushman, F.D., Engelman, A., Palmer, I., Wingfield, P., and Craigie, R. (1993). Domains of the integrase protein of human immunodeficiency virus type 1 responsible for polynucleotidyl transfer and zinc binding. Proc. Natl. Acad. Sci. USA *90*, 3428–3432.

Chow, S.A., Vincent, K.A., Ellison, V., and Brown, P.O. (1992). Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. Science *255*, 723–726.

Craig, N.L. (1995). Unity in transposition reactions. Science *270*, 253–254.

Craigie, R., and Mizuuchi, K. (1985). Mechanism of transposition of bacteriophage Mu: structure of a transposition intermediate. Cell *41*, 867–876.

Craigie, R., and Mizuuchi, K. (1987). Transposition of Mu DNA: joining

of Mu to target DNA can be uncoupled from cleavage at the ends of Mu. Cell *51*, 493–501.

Dyda, F., Hickman, A.B., Jenkins, T.M., Engelman, A., Craigie, R., and Davies, D.R. (1994). Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. Science *266*, 1981–1986.

Engelman, A., and Craigie, R. (1992). Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. J. Virol. *66*, 6361–6369.

Engelman, A., Mizuuchi, K., and Craigie, R. (1991). HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell *67*, 1211–1221.

Engelman, A., Bushman, F.D., and Craigie, R. (1993). Identification of discrete functional domains of HIV-1 integrase and their organization within an active multimeric complex. EMBO J. *12*, 3269–3275.

Fayet, O., Ramond, P., Polard, P., Prere, M.F., and Chandler, M. (1990). Functional similarities between the IS3 family of bacterial insertion elements? Mol. Microbiol. *4*, 1771–1777.

Haniford, D.B., and Chaconas, G. (1992). Mechanistic aspects of DNA transposition. Curr. Opin. Genet. Dev. *2*, 698–704.

Higuchi, R., Krummel, B., and Saiki, R.K. (1988). A general method of in vitro preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. Nucleic Acids Res. *16*, 7351–7367.

Jenkins, T.M., Engelman, A., Ghirlando, R., and Craigie, R. (1996). A soluble active mutant of HIV-1 integrase. J. Biol. Chem. *271*, 7712–7718.

Johnson, R. (1996). Site-specific recombinases and their interactions with DNA. In DNA-Protein Structural Interactions, D.M.J. Lilley, ed. (Oxford: IRL Press), pp. 141–176.

Kaufman, P.D., and Rio, D.C. (1992). P element transposition in vitro proceeds by a cut-and-paste mechanism and uses GTP as a cofactor. Cell *69*, 27–39.

Kim, K., Namgoong, S.-Y., Jayaram, M., and Harshey, R.M. (1995). Step-arrest mutants of phage Mu transposase: implications in DNA–protein assembly, Mu end cleavage, and strand transfer. J. Biol. Chem. *270*, 1472–1479.

Kleckner, N., Chalmers, R.M., Sakai, J., and Bolland, S. (1995). Tn10 and IS10 transposition and chromosome rearrangements: mechanism and regulation in vivo and in vitro. Curr. Top. Microbiol. Immunol. *204*, 49–82.

Kruklitis, R., and Nakai, H. (1994). Participation of the bacteriophage Mu A protein and host factors in the initiation of Mu DNA synthesis in vitro. J. Biol. Chem. *269*, 16469–16477.

Kulkosky, J., Jones, K.S., Katz, R.A., Mack, J.P., and Skalka, A.M. (1992). Residues critical for retroviral integrative recombination in a region that is highly conserved among retroviral/retrotransposon integrases and bacterial insertion sequence transposases. Mol. Cell. Biol. *12*, 2331–2338.

Lavoie, B.D., Chan, B.S., Allison, R.G., and Chaconas, G. (1991). Structural aspects of a higher order nucleoprotein complex: induction of an altered DNA structure at the Mu-host junction of the Mu type 1 transpososome. EMBO J. *10*, 3051–3059.

Mizuuchi, K. (1992a). Transpositional recombination: mechanistic insights from studies of mu and other elements. Annu. Rev. Biochem. *61*, 1011–1051.

Mizuuchi, K. (1992b). Polynucleotidyl transfer reactions in transpositional recombination. J. Biol. Chem. *267*, 21273–21276.

Mizuuchi, K., and Adzuma, K. (1991). Inversion of the phosphate chirality at the target site of Mu DNA strand transfer: evidence for a one-step transesterification mechanism. Cell *66*, 129–140.

Mizuuchi, M., Baker, T.A., and Mizuuchi, K. (1991). DNase protection analysis of the stable synaptic complexes involved in Mu transposition. Proc. Natl. Acad. Sci. USA *88*, 9031–9035.

Mizuuchi, M., Baker, T.A., and Mizuuchi, K. (1992). Assembly of the active form of the transposase–Mu DNA complex: a critical control point in Mu transposition. Cell *70*, 303–311.

Mizuuchi, M., Baker, T.A., and Mizuuchi, K. (1995). Assembly of phage Mu transpososomes: cooperative transitions assisted by protein and DNA scaffolds. Cell *83*, 375–385.

Nakayama, C., Teplow, D.B., and Harshey, R.M. (1987). Structural domains in phage Mu transposase: identification of the site-specific DNA-binding domain. Proc. Natl. Acad. Sci. USA *84*, 1809–1813.

Rice, P., and Mizuuchi, K. (1995). Structure of the bacteriophage Mu transposase core: a common motif for DNA transposition and retroviral integration. Cell *82*, 209–220.

Rice, P.A., and Steitz, T.A. (1994). Refinement of γδ resolvase reveals a strikingly flexible molecule. Structure *2*, 371–384.

Rowland, S.J., and Dyke, K.G. (1990). Tn552, a novel transposable element from *Staphylococcus aureus*. Mol. Microbiol. *4*, 961–975.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science *239*, 487–491.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Sanderson, M.R., Freemont, P.S., Rice, P.A., Goldman, A., Hatfull, G.F., Grindley, N.D.F., and Steitz, T.A. (1990). The crystal structure of the catalytic domain of the site-specific recombination enzyme γδ resolvase at 2.7 Å resolution. Cell *63*, 1323–1329.

Savilahti, H., Rice, P.A., and Mizuuchi, K. (1995). The phage Mu transpososome core: DNA requirements for assembly and function. EMBO J. *14*, 4893–4903.

Stark, W.M., and Boocock, M.R. (1995). Gatecrashers at the catalytic party. Trends Genet. *11*, 121–123.

Stark, W.M., Boocock, M.R., and Sherratt, D.J. (1992). Catalysis by site-specific recombinases. Trends Genet. *8*, 432–439.

Surette, M.G., Buch, S.J., and Chaconas, G. (1987). Transpososomes: stable protein–DNA complexes involved in the in vitro transposition of bacteriophage Mu DNA. Cell *49*, 253–262.

van Gent, D.C., Mizuuchi, K., and Gellert, M. (1996). Similarities between initiation of V(D)J recombination and retroviral integration. Science, in press.

van Gent, D.C., Vink, C., Oude Groeneger, A.A.M., and Plasterk, R.H.A. (1993). Complementation between HIV integrase proteins mutated in different domains. EMBO J. *12*, 3261–3267.

van Luenen, H.G.A.M., Colloms, S.D., and Plasterk, R.H.A. (1994). The mechanism of transposition of Tc3 in C. elegans. Cell *79*, 293–301.

Varmus, H., and Brown, P. (1989). Retroviruses. In Mobile DNA, D.E. Berg and M.M. Howe, eds. (Washington, D.C.: American Society for Microbiology), pp. 53–108.

Vink, C., Yeheskiely, E., van der Marel, G., van Boom, J., and Plasterk, R.H.A. (1991). Site-specific hydrolysis and alcoholysis of human immunodeficiency virus DNA termini mediated by the viral integrase protein. Nucl. Acids Res. *19*, 6691–6698.

Wu, Z., and Chaconas, G. (1995). A novel DNA binding and nuclease activity in domain III of Mu transposase: evidence for a catalytic region involved in donor cleavage. EMBO J. *14*, 3835–3843.

Yang, W., and Steitz, T.A. (1995). Crystal structure of the site-specific recombinase γδ resolvase complexed with a 34 bp cleavage site. Cell *82*, 193–207.

Yang, J.-Y., Kim, K., Jayaram, M., and Harshey, R.M. (1995). A domain sharing model for active site assembly within the Mu A tetramer during transposition: the enhancer may specify domain contributions. EMBO J. *14*, 2374–2384.

Yanisch-Perron, C., Vieira, J., and Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene *33*, 103–119.

Zou, A.H., Leung, P.C., and Harshey, R.M. (1991). Transposase contacts with mu DNA ends. J. Biol. Chem. *266*, 20476–20482.