Domain III function of Mu transposase analysed by directed placement of subunits within the transpososome

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Assembly of the functional tetrameric form of Mu transposase (MuA protein) at the two att ends of Mu depends on interaction of MuA with multiple att and enhancer sites on supercoiled DNA, and is stimulated by MuB protein. The N-terminal domain I of MuA harbours distinct regions for interaction with the att ends and enhancer; the C-terminal domain III contains separate regions essential for tetramer assembly and interaction with MuB protein (III*a* and III*b*, respectively). Although the central domain II (the 'DDE' domain) of MuA harbours the known catalytic DDE residues, a 26 amino acid peptide within IIIα also has a non-specific DNA binding and nuclease activity which has been implicated in catalysis. One model proposes that active sites for Mu transposition are assembled by sharing structural/catalytic residues between domains II and III present on separate MuA monomers within the MuA tetramer. We have used substrates with altered att sites and mixtures of MuA proteins with either wild-type or altered att DNA binding specificities, to create tetrameric arrangements wherein specific MuA subunits are nonfunctional in II, III*a* or III*b* domains. From the ability of these oriented tetramers to carry out DNA cleavage and strand transfer we conclude that domain III*a* or III*b* function is not unique to a specific subunit within the tetramer, indicative of a structural rather than a catalytic function for domain III in Mu transposition.

1. Introduction

The DNA cleavage and joining reactions of transposition are carried out within a tetrameric unit of the Mu transposase (MuA protein) (figure 1A; reviewed in Mizuuchi, 1992; Lavoie and Chaconas 1995). While MuA can bind six att sites (L1-L3 at the left or attL end, and R1-R3 at the right or attR end), and three internal enhancer sites (O1-O3) on Mu DNA, the MuA tetramer footprints on only three of the att sites (L1, R1 and R2; figure 1B), and only two specific phosphodiester bonds (adjacent to L1 and R1) are cleaved on opposite DNA strands. The resulting 3′ OH groups are joined or strand transferred to two phosphodiesters placed 5 bp apart on two strands of target DNA. The enhancer interacts with the L and R ends early in the Mu transposition reaction to form a nucleoprotein complex LER (Watson and Chaconas 1996; see figure 1A), interactions within which lead to formation of a stable type 0 complex in which the Mu transposase (MuA protein) assumes its active tetrameric form, catalyzing the subsequent cleavage (type I complex) and joining (type II complex) reactions (see Chaconas *et al* 1996). The MuA tetramer is eventually dislodged by the action of a host protein ClpX, followed by replication of Mu (Kruklitis *et al* 1996; Levchenko *et al* 1997).

The MuA monomer (its normal form in solution; Kuo *et al* 1991) is a multi-domain protein (Nakayama *et al* 1987; figure 1C). The amino-terminal domain I contains the recognition motifs for two types of DNA sites – the enhancer and att sites (see Chaconas *et al* 1996). The central domain II contains a triad of 'DDE' residues present within II*a* (refered to here as the DDE domain), that are

essential for the strand cleavage and strand transfer steps of transposition (Baker and Luo 1994; Kim *et al* 1995; Rice and Mizuuchi 995). Subdomain (II*b*) has a large positive charge potential (Rice and Mizuuchi 1995) and has been implicated in metal-assisted assembly of the MuA tetramer and in intramolecular DNA strand transfer (Namgoong *et al* 1998a). This domain apparently belongs to the same catalytic complementation group as the amino-proximal portion of carboxy-terminal domain III (III*a*), which is also required for assembly of the MuA tetramer (Namgoong *et al* 1998a; Krementsova *et al* 1998). Basic residues (RRRKQ) within a 26 residue peptide in III*a* have been implicated in contributing to a non-specific DNA binding and nuclease activity (Wu and Chaconas 1995). The distal region of domain III (III*b*) is required for interactions with the accessory transposition factor, the MuB protein (Harshey and Cuneo 1986; Leung and Harshey 1991; Wu and Chaconas 1994; Levchenko *et al* 1997). MuB is not only required for capturing target DNA during intermolecular strand transfer (Craigie and

Mizuuchi 1987; Maxwell *et al* 1987; Nagaimwalla and Chaconas 1997; Yamauchi and Baker 1998), but also modulates the activity of MuA at several stages of the transposition reaction (Baker *et al* 1991; Surette *et al* 1991; Mizuuchi *et al* 1995). MuB also competes with ClpX for binding the C-terminus of MuA during the transition of the transpososome from strand transfer to replication (Levchenko *et al* 1997).

A mutant MuA [MuA (R146V)] was isolated that can bind an altered att site but cannot recognize the wild-type att site (Namgoong *et al* 1998b). This allowed directed placement of MuA (R146V) and its variants at specific att sites. These studies found only two subunits within the tetramer, those located on L1 and R1, to contribute DDE residues to catalysis on supercoiled substrates (figure 2A; Namgoong and Harshey 1998). The catalytic 'DDE' residues of the active subunits work in *trans* (Aldaz *et al* 1996; Savilahti and Mizuuchi 1996) i.e. DDE$^+$ subunit at L1 cleaves and strand transfers the opposite R end, while the DDE$^+$ subunit at R1 carries out similar chemistry at

Figure 1. (**A**) Nucleoprotein complexes in Mu transposition. Monomeric MuA protein binds to the two Mu ends L and R (each composed of three att sites), as well as interacts with an enhancer element E (also composed of three separate sites) on a negatively supercoiled plasmid, to promote rapid formation of the LER complex in presence of divalent metal ions and *E. coli* HU protein. Conversion of LER to type 0, in which MuA has tetramerized and the enhancer is no longer associated with the ends, is a slow step. Type 0 can be trapped in the presence of Ca^{2+} or catalytically inactive MuA mutants. Mg^{2+} or Mn^{2+} ions promote cleavage of Mu ends to produce the type I complex. MuB protein modulates the activity of MuA at each stage of the reaction, and captures target DNA in the presence of ATP to generate the type II strand transfer complex. (**B**) Arrangement of attL (L1-L3), attR (R1-R3) and enhancer (O1-O3) sites on Mu DNA. The MuA tetramer footprints on only three att sites (L1, R1, R2). Non-Mu DNA is indicated by broken lines. (**C**) Domainal organization of MuA. Functions attributed to various domains are indicated (see text). Amino acid numbers corresponding to the amino terminus of each major domain/subdomain are shown beneath the structure.

the L end. The specific function of the other two subunits (see figure 1B) is not known.

The studies described above showed that the DDE domain (II) was clearly involved in catalysis. A catalytic function for domain III*a* has seemed attractive because of a non-specific DNA-binding and nuclease activity associated with an isolated peptide within this domain (Wu and Chaconas 1995). A model proposed by Yang *et al* (1995) has invoked that an active site might be built by sharing structural/catalytic residues between the DDE and III*a* domains from separate MuA monomers, similar to the shared active site proposed for the Flp recombinase tetramer (Chen *et al* 1992). While DDE residues have been postulated to co-ordinate metal ions that activate the nucleophile during cleavage and strand transfer (see Grindley and Leschziner 1995), a possible role for orienting the scissile phosphodiesters was envisioned for domain III*a* (figure 2B; Yang *et al* 1995; Wu and Chaconas 1995).

The domain sharing model proposed by Yang *et al* (1995) was based on the observation that when a tetramer was assembled by two MuA variants, one lacking III*a* and the other lacking a functional DDE domain, either strand cleavage within a supercoiled substrate or strand transfer of a pre-cleaved substrate, but not strand cleavage plus strand transfer, could be performed. The 'reciprocal domain sharing' model accommodated this observation by proposing that the DDE and III*a* domains were contributed by different pairs of subunits during strand cleavage and strand transfer. Thus, the tetramer assembled from the mutant pair was capable of assembling either the active site for strand cleavage or the active site for strand transfer, but not both active sites at the same time. This model was seemingly challenged by Namgoong and Harshey (1998) whose experiments showed that the cleavage event promoted by a MuA tetramer containing a single DDE donor could be channelled into strand transfer by the same complex. However, in the latter experiments both MuA partners (the DDE$^+$ and DDE$^-$) contained an intact III*a* domain (Namgoong and Harshey 1998) while in the Yang *et al* (1995) experiments, this domain was deleted from one partner. It is possible, therefore, that the III*a* domains (and not the DDE domains), were provided by separate MuA monomers for the cleavage and transfer reactions. Alternatively, the same active site could carry out cleavage and target joining, but the transition from one mode to the other may require participation of monomers that do not directly contribute to the chemical steps.

To investigate whether the contribution of domain III to active site assembly is catalytic or structural, we have directed a MuA variant deleted in domain III*a* to specific att sites on supercoiled DNA, and assessed its activity in both cleavage of Mu ends, and in MuB-assisted strand transfer. We have also assessed the contribution of domain III*b* in similar experiments. Our results show that

Figure 2. (**A**) A model for the arrangement of DDE-contributing MuA subunits during transposition from supercoiled Mu DNA. Subunits bound through their DNA-binding domains to L1 and R1 donate their DDE domains in *trans* to cleave (white dot) and subsequently strand transfer (not shown) specific phosphodiester bonds at the two Mu ends. Specific structural/catalytic functions have not yet been assigned to the two other MuA subunits in the tetramer (see figure 1B). (**B**) Domain-sharing model for transposition proposes that an active site is built by sharing catalytic residues from domain II of one subunit with domain III residues from another subunit (Yang *et al* 1995). In the diagram, DDE residues in domain II*a* are shown co-ordinating Mg^{2+} ions for generating the hydroxide ion nucleophile, while the basic RRRKQ residues in domain III*a* are shown activating the scissile phosphodiester bond (circle). Alternatively, domain III*a* residues may provide a structural function.

unlike the DDE contribution, where two unique subunits of MuA (see figure 2A) provide DDE residues in *trans* for both cleavage and strand transfer, no unique position could be identified for the III*a* contribution. These results could be extended to include domain III*b* function (MuB interaction domain) as well. Our studies also shed light on the original observation by Yang *et al* (1995) which prompted the 'reciprocal domain sharing' model (see above).

2. Materials and methods

2.1 *DNA substrates and proteins*

pL1**, pR2** and pJMM plasmids have been described (Namgoong and Harshey 1998; Jiang *et al* 1999). Plasmid pL1**-R2** was constructed by appropriate exchange of restriction fragments between pL1** and pR2**.

MuA (E392A), MuA (R146V) and MuA (Δ560-663) have been described (Kim *et al* 1995; Namgoong *et al* 1998a, b). MuA (Δ609-663) was constructed by PCR mutagenesis procedures similar to those described for MuA (Δ560-663) (Namgoong *et al* 1998a). The R146V mutation was moved into these variants by appropriate restriction fragment exchange. Heart muscle kinase (HMK) recognition tags were engineered at the N-termini of MuA and its derivatives by addition of the sequence MGSRRASV (Li *et al* 1989; the underlined sequence is the kinase recognition pentapeptide) before the MuA start codon (at the NdeI site on the pET vector) by PCR methodology.

Wild-type and variant forms of MuA, as well as MuB and HU proteins were purified as described by Yang *et al* (1995). The kinase-tagged proteins required a slight modification in the purification procedure: SP-sepharose fast-flow column was substituted for phosphocellulose and the pH at this step was maintained at 7·0.

2.2 *Radiolabelling HMK-tagged proteins*

Type I reactions with HMK-tagged proteins were performed in 20 μl solutions containing 25 mM Hepes (pH 7·8), 10 mM MgCl$_2$, 100 mM NaCl, 1 mM DTT, at 30°C for 20 min, followed by addition of 1 μg of heparin to remove loosely bound protein from the DNA. Labelling reactions were done by addition of 1 μl (γ-^{32}P) ATP (10 μCi) and 1u HMK (Sigma P2645), at 30°C for 5 min. Free (γ-^{32}P) ATP was removed by passing the reaction mixture over a Centri-sep gel filtration spin column (Princeton Separation). After agarose gel electrophoresis, the gel was dried and exposed to film on a BioRad Molecular Imager.

2.3 *Mu DNA cleavage and strand transfer*

Type I cleavage reactions and type II strand transfer reactions were carried out as described by Namgoong *et al* (1998a).

2.4 *Determination of Mu end cleavage*

Two methods were employed to detect cleavage at the left and right Mu ends. Primer extension analysis was carried out as described by Namgoong and Harshey (1998), while end-labelling of appropriately digested restriction fragments with (α-^{32}P) cordycepin phosphate and terminal nucleotidyl transferase was carried out as described by Jiang *et al* (1999).

3. Results

3.1 *Domain III**a** function of MuA subunits at L1 or R2 sites is not required for DNA cleavage or strand transfer*

3.1a *Domain III**a** function of the L1 subunit in cleavage of R1*: Under normal reaction conditions, subunits contributing DDE domains occupy specific positions within the MuA tetramer (see figure 2A). If III*a* domains participate in catalysis (see figure 2B), the expectation is that the positions of subunits contributing these domains will also be specific. This section describes the III*a* function of subunits at L1 and R2 only (see figure 1B). This is because the altered att site functions poorly at R1 due to positional effects (Namgoong and Harshey 1998).

Three separate arrangements of MuA tetramers were configured as shown in figures 3 and 4. The double asterisk denotes presence of the altered att site to which the R146V DNA-binding variant specifically binds. Note that although the plasmids used in this study contain all six att sites (see figure 1B), for clarity only those on which the tetramer footprints are indicated. Note also that MuA with a wild-type att DNA-binding domain can also recognize altered att sites; therefore, in all reactions containing a MuA (R146V) variant plus a second protein, the substrate was pre-incubated with the R146V-containing protein prior to the addition of its partner protein, ensuring that the altered sites were selectively blocked from binding to the protein without the R146V substitution.

In the first set of experiments (figure 3), a domain III*a*$^-$ DDE$^+$ mutant [MuA (R146V) (Δ560-663)] was placed at L1 and a DDE$^-$ III*a*$^+$ mutant [MuA (E392A)] at all other sites on plasmid pL1** (figure 3A). Generation of a type I complex (figure 3B, lane 4) indicated that cleavage had occurred in this equimolar mixture of proteins, but not in controls with either protein alone (figure 3B, lanes 2 and 3; the DDE$^-$ protein can assemble an uncleaved type 0

complex, while the IIIa⁻ protein is assembly-defective by itself). Cleavage also occurred with wild-type MuA on this substrate (figure 3B, lane 5).

To determine if the C-terminal deletion protein was indeed incorporated into the type I complex, we adopted a strategy for direct protein detection in the agarose gel. To do so, we engineered a five amino acid N-terminal tag on MuA (and its C-terminal deletion derivative) which carries the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase from bovine heart muscle and can be phosphorylated at the serine residue (see §2; proteins carrying the tag are identified with a superscript 'K'). This modification (with or without phosphorylation) did not profoundly affect the reactivity of the proteins in the transposition assays. An autoradiogram of reactions identical to those in figure 3B, lanes 4 and 5, but with phosphorylated MuA (R146V) (Δ560-663)^K and MuA^K is shown in figure 3B, lanes 6 and 7. Labelled protein was detected in both type I complexes, showing that the C-terminal deletion was indeed present in the mixed tetramer.

In order to determine which Mu end underwent cleavage in the protein mixtures, the type I band was gel isolated and subjected to primer extension analysis using primers designed to monitor left (L) or right (R) end cleavage (see §2) (figure 3C). While products indicative of both end cleavages (lanes L and R) were identified in the complex formed with wild-type MuA from figure 3B, lane 5, only right end cleavage was observed in the mixed complex formed in figure 3B, lane 4 (absence of product band in lane L). This was consistent with earlier results where a DDE subunit placed at L1 catalyzed cleavage of R1 (Namgoong and Harshey 1998). These results show in addition that the domain IIIa function of the L1 subunit is apparently not required for R1 cleavage.

3.1b *Domain IIIa function of the L1 subunit in cleavage of L1*: While the results presented above show that the domain IIIa function of the subunit placed on L1 is not required for R1 cleavage, they do not exclude the possibility that domain IIIa has a catalytic function that is provided either in *cis* (i.e. from the L1 subunit for the cleavage of L1, and from the R1 subunit for the cleavage of R1), or in *trans* (from the R2 subunit for the cleavage of either L1 or R1). To test these possibilities, we first examined domain IIIa contribution from the L1 subunit for the cleavage of L1. As shown in figure 4A, all subunits carried an intact DDE domain in this arrangement, with the L1 subunit deleted for domain IIIa. The rationale for this experiment was that if domain IIIa of the L1 subunit contributed catalytic residues for left end cleavage, then inspite of the presence of functional DDE domains at both L1 and R1, the left Mu end should not get cleaved. The results are shown in figure 4B. Lanes 1 and 5 are

Figure 3. Domain IIIa function of the L1 subunit for cleavage of the right end of Mu. (**A**) Arrangement of mutant (hatched + double asterisk) and wild-type att sites on supercoiled plasmid pL1**. MuA (ovals) X = DDE⁻ subunit; DDE = DDE⁺ subunit; grey subunit = R146V variant; apple-sized 'bite' = domain IIIa⁻. Arrowhead indicates cleaved Mu end. (**B**) Complementation between MuA (R146V) (Δ560-663) and MuA (E392A) for DNA cleavage (type I complex formation). Lane 1 is a no-protein control, while lanes 2, 3 and 5 are controls with indicated proteins (0·4 μg each). In lane 4, 0·2 μg of MuA (E392A) were mixed with equal amounts of MuA (R146V) (Δ560-663). Samples were electrophoresed on a 1% agarose gel and visualized by staining with ethidium bromide. Lanes 6 and 7 are autoradiograms of reactions similar to those in lanes 4 and 5 except with phosphorylated, kinase-tagged MuA (R146V) (Δ560-663)^K and MuA^K. The smear of radioactivity in lane 6 is lkely due to the C-terminal deletion variant found free in solution as a result of inefficient incorporation and/or instability of the mixed type I complex. The positions of supercoiled (sc) and open circular (oc) forms of the donor (D) plasmid pL1** are indicated, as are those of type 0 and type I complexes. (**C**) Determination of Mu end cleavage. Type I complexes formed in lanes 5 (MuA) and 4 (E392A + R146V (Δ560-663)) in **B** were hybridized with radiolabelled primers designed to detect left or right end cleavage (see §2). Chain-extension products were analysed on a 6% denaturing polyacrylamide gel. L and R indicate left and right end-specific primer extension products, respectively.

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controls with no MuA and MuA (R146V) (Δ560-663), respectively. Since wild-type MuA is reactive on the pL1** substrate by itself (lane 2), suboptimal amounts (0·1 μg) of wild-type MuA (just sufficient to detect complex formation (lane 3) were mixed with MuA (R146V) (Δ560-663) (0·3 μg) to stimulate type I formation. Primer extension analysis of cleaved complexes formed in figure 4B, lane 4, showed that both ends underwent cleavage in this arrangement (figure 4C). In parallel reactions with kinase-tagged proteins, MuA (R146V) (Δ560-663)[K] was seen to be incorporated in the type I complex formed under these conditions (data not shown). We conclude that the III*a* domain of the L1 subunit does not participate in cleavage of L1 in *cis*.

3.1c   *Domain III*a *function of the R2 subunit in cleavage*:   To test if domain III*a* from the R2 subunit is required for cleavage of L1 or R1, experiments similar to those in figure 4B,C were performed except with the pR2** substrate (figure 4D). At suboptimal concentrations of wild-type MuA (figure 4E, lane 3), addition of MuA (R146V) (Δ560-663) stimulated type I formation

(figure 4E, lane 4). Both Mu ends underwent equivalent cleavages in this complex (figure 4F). Thus, the III*a* domain of the R2 subunit does not participate in cleavage of either L1 or R1 in *trans*.

3.1d   *Domain III*a *function of the L1 and R2 subunits in strand transfer*:   To test whether cleavage-competent configurations assembled with a domain III*a* deletion variant were also capable of strand transfer, we examined the ability of each of the tetramer arrangements shown in figures 3 and 4 to support strand transfer by including pUC19 target DNA, MuB protein and ATP in the reactions (see figure 1A). The data are summarised in table 1 (all reactions used supercoiled donor substrates). While wild-type MuA (table 1A) generated double-ended strand transfer products (DEP) on pL1**, no strand transfer was detected (table 1B) in the arrangement where the domain III*a*⁻ subunit was at L1 and the right end alone underwent cleavage (figure 3A). We had shown earlier that a singly cleaved right end generates single-end strand transfer products (SEP), albeit poorly, when similarly oriented tetramers carry an intact domain III*a* (table 1C; Nam-

Figure 4.   Domain III*a* function of the subunits at L1 or R2 in Mu end cleavage. (**A, D**) Arrangement of MuA subunits on pL1** and pR2** respectively. Symbols as in figure 3A. (**B, E**) Complementation between MuA (R146V) (Δ560-663) and wild-type MuA for DNA cleavage (type I complex formation), when suboptimal amounts of wild-type MuA (0·1 μg; lane 3) were added to 0·3 μg of MuA (R146V) (Δ560-663). Lanes 1, 2 and 5 are indicated controls (0·4 μg proteins in lanes 2 and 5). Symbols as in figure 3B. (**C, F**) Determination of Mu end cleavage by primer extension. Type I complexes formed in lanes 2 and 4 in **B** and **E**, respectively, were analysed as described in figure 3C.

goong and Harshey 1998). Comparison of the results in B and C might suggest that domain III*a* plays a role in strand transfer. However, when the tetramer arrangement in figure 4A was tested for strand transfer, in spite of the fact that L1 position was still occupied by a domain III*a*⁻ subunit, double-end strand transfer was detected (table 1D). Thus, domain III*a* is not required for strand transfer of either end. Strand transfer reactions performed using the MuA subunit arrangement shown in figure 4D, also showed the presence of double-ended strand transfer

products (table 1E). Thus, domain III*a* function at R2 is also not required for the strand transfer of either end.

In summary, the data in figures 3, 4 and table 1 suggest that domain III*a* does not provide a catalytic function for cleavage or strand transfer of Mu ends. The finding that III*a* function of the L1 subunit is required only when the right end alone has undergone cleavage but not when both ends are cleaved (compare table 1B and D), is indicative of a structural rather than a catalytic impediment to strand transfer in these mixed tetramers.

3.2 A domain III*a*⁻ subunit is preferentially excluded from R1 when randomly mixed with a domain III*a*⁺ partner

Yang *et al* (1995) had observed that mixtures of MuA (E392A) and MuA (Δ560-663) on wild-type substrates could perform cleavage but not strand transfer. In light of the results in table 1B where singly cleaved R end complexes do not support strand transfer when a III*a*⁻ subunit is at L1, we wondered whether only a single end had undergone cleavage in the protein mixtures tested by Yang *et al* (1995). We therefore repeated their experiment (figure 5), and analysed the type I complexes by end-labelling, a procedure that allows a better quantitation of the proportion of left and right end cleavages (see Jiang *et al* 1999).

The donor plasmid pJMM carries all wild-type att sites (Jiang *et al* 1999). Wild-type MuA showed efficient type I complex formation on this substrate (figure 5A, lane a). While, MuA (Δ560-663) and MuA (E392A) showed no type I activity on their own (figure 5A, lanes b and f, respectively), mixtures of these two proteins in various indicated molar ratios gave rise to the type I product (figure 5A, lanes c–e). DNA from type I complexes formed in lanes a and d was extracted as described by Jiang *et al* (1999) and digested with *Bam*HI and *Xba*I in one case, and with *Bam*HI and *Aat*II in the other (figure 5B). The 3′-hydroxyl ends generated from the digestion as well as those produced by cleavage of Mu ends were radio-actively labelled with ³²P and fractionated by electrophoresis in denaturing polyacrylamide gels. The diagnostic bands for left end cleavage are LC1 and LC2; those for right end cleavage are RC1 and RC2. For more details, the reader may refer to Jiang *et al* (1999).

No cleavage was detected at the Mu ends in control DNA not reacted with transposase (figure 5C, lanes 1 and 4). The type I complex formed by wild-type MuA (figure 5A, lane a) had undergone efficient cleavage at both L and R ends (figure 5C, lanes 2 and 5; LC1, LC2 and RC1 in lane 2 and RC1, RC2 and LC1 in lane 5). By comparison, the type I product from the d reaction shown in figure 5A showed nearly exclusive cleavage at the right end

Table 1. Strand transfer proficiency of oriented MuA tetramers incorporating a domain III*a*⁻ subunit at L1 or R2 on supercoiled donor substrates.

(RC1 with undetectable LC1 and LC2 in lane 3; RC1 and RC2 in lane 6). Note that the disappearance of the RU1 band in lanes 5 and 6 is indicative of complete right end cleavage.

The results in figure 5 show that the domain IIIa⁻ subunit preferentially occupies L1 in this protein mixture. These results explain why Yang *et al* (1995) failed to observe strand transfer by this pair of proteins, since the combination of a singly cleaved right end and a domain IIIa⁻ subunit at L1 (figure 3) is structurally impaired in transposition (table 1B). The data in figure 5C also show that for the L1 end to have undergone cleavage (albeit poorly), a domain IIIa⁻ DDE⁺ subunit was likely positioned at R1 in a small proportion of complexes.

To summarize the results thus far (figures 3–5 and table 1), cleavage at L1 does not depend on an intact domain IIIa at L1, R1 or R2. Cleavage at R1 does not depend on an intact domain IIIa at L1 or R2. While we could not assess if the domain IIIa of the R1 subunit is required for cleavage of R1, such a function is unlikely given the results with the L1 subunit. Strand transfer of cleaved left and right ends does not depend on domain IIIa function of the subunits at L1 or R2. While domain IIIa does not participate in the catalysis of transposition, it appears to provide a structural role; this role must be critical at the R1 site since a IIIa⁻ subunit is preferentially excluded from R1 when randomly mixed with a domain IIIa⁺ partner.

Figure 5. Domain IIIa⁻ subunit is excluded from R1 on wild-type substrates. (**A**) Complementation between MuA (Δ560-663) and MuA (E392A) for type I complex formation on pJMM donor substrate (lanes c–e). Lanes a, b and f are indicated controls (0·4 μg protein each). Symbols as in figure 3B. (**B**) End-labelling strategy to assay left and right end cleavages in the type I complex. The top and bottom strands of the Mu genome are represented by unfilled and filled bars, respectively. The diamonds indicate the strand cleavage positions. Double digestion with *Bam*HI-*Xba*I or *Bam*HI-*Aat*II, followed by 3′ end labelling (indicated by the asterisk) would give rise to the indicated radioactive products. They can be revealed by electrophoresis in denaturing polyacrylamide gels (see **C**). Uncleaved attL generates an LU doublet consisting of a 95 nt fragment from the bottom strand, which has the same length as the fragment from the top strand; uncleaved attR generates RU1 from the top strand, which differs in length by 8 nt from the corresponding bottom strand fragment RU2. The products specific to left and right end cleavages are denoted by LC and RC, respectively. (**C**) The type I product from reactions in lanes a and d of **A**, were analysed by the strategy outlined in **B** and are shown in lanes 2/5 and 3/6 respectively. Lanes 1 and 4 represent the substrate DNA that was not treated with MuA. B/X and B/A stand for *Bam*HI-*Xba*I and *Bam*HI-*Aat*II restriction digestions.

3.3 *At least two subunits must encode IIIb function for MuB-mediated strand transfer*

The MuB protein interacts with MuA through domain III*b* and modulates its efficiency at several stages in the transposition pathway prior to strand transfer (see Chaconas *et al* 1996). In addition, MuB is responsible for binding target DNA and transporting it to the MuA tetramer for strand transfer. In all of the experiments described above, the III*a*⁻ subunit is also deleted in domain III*b* (see figure 1C). Thus, one can see that domain III*b* function at either L1 or R2 is not required for MuB-assisted strand transfer (see table 1). A similar conclusion was reached in earlier experiments using complementing mixtures of proteins carrying a domain III*b* deletion and/or a DDE⁻ mutation (Mizuuchi *et al* 1995). These experiments did not involve the use of oriented tetramers as reported here. Mizuuchi *et al* (1995) concluded that not all four MuA subunits need to interact with MuB and that the position of MuB-interacting subunits is not unique.

To determine the minimal number of subunits required for MuB interaction, we set up several different arrangements of MuA tetramers (table 2). These included placing a single wild-type (DDE⁺) subunit at either at L1 or R2 with domain III*b*⁻ subunits at the other sites, diagonal placement of two wild-type or two III*b*⁻ subunits at L1-R2, as well as diagonal combinations of domain III*b*⁻ and DDE⁻ subunits. The data for two of these arrangements are shown in figure 6, while those for all of them are summarised in table 2.

Experiments shown in figure 6 test strand transfer from plasmid pL1** when a single functional domain III*b*⁺ subunit, MuA (R146V), was placed at L1 and MuA (Δ609-663) (domain III*b*⁻) subunits were at the other sites (figure 6A). Strand transfer reactions were similar to cleavage reactions described under figures 3–5, except they included pUC19 target DNA, MuB protein and ATP. SDS was added to the reactions prior to electrophoresis, in order to dissociate DNA-protein complexes. Therefore, the type I complex would be detected as the open circular plasmid in this assay [Donor(oc)]. While wild-type MuA generated double-ended strand transfer products that migrated as a series of distinct bands (reflecting the distribution of topoisomers retained in the Mu sequence of the donor plasmid) just above the open circular form of the target DNA (DEP, lane 5), no strand transfer products were detected with a mixture of MuA (R146V) and MuA (Δ609-663) (figure 6B, lane 4). Controls with either no protein, or with MuA (R146V) and MuA (Δ609-663) alone are shown in figure 6B, lanes 1–3. The increase in the open circular form of the donor plasmid in lanes 3 and 4 is indicative of Mu end cleavage. These results are tabulated in table 2B. Similar results were seen with this protein mixture on the pR2** substrate (table 2C). Thus, it appears that a single domain III*b*⁺ subunit cannot support MuB-mediated strand transfer from the L1 or R2 positions.

When two MuA (R146V) (domain III*b*⁺) subunits were placed at L1 and R2 on the pL1**-R2** substrate, with MuA (Δ609-663) (domain III*b*⁻) subunits at the other sites (figure 6C), strand transfer was observed with the

Table 2. Strand transfer proficiency of oriented tetramers incorporating a domain III*b*⁻ subunit at L1, R1 and R2 on supercoiled donor substrates.

	Strand transfer
A	DEP
B	–
C	–
D	DEP
E	DEP
F	SEP

protein mixture (figure 6D, lane 4) but not with either protein alone (figure 6D, lanes 2 and 3). Thus, two domain IIIb^+ subunits placed diagonally can support MuB-mediated strand transfer (table 1D). Similar results were obtained in the reciprocal arrangement, when domain IIIb^- subunits at L1 and R2 were paired with IIIb^+ subunits at the other sites (table 2E). Strand-transfer (single-ended) was also supported when IIIb^- subunits at L1 and R2 were paired with DDE$^-$ subunits at the other sites (table 2F), suggesting that MuB can transport target DNA to the catalytically active subunit at L1 while interacting with catalytically inactive subunits. As expected from these results, a single domain IIIb^- subunit at L1 or R2 was also functional in double end strand transfer when paired with wild-type subunits at the other sites (data not shown).

We conclude that MuB-mediated strand transfer requires at least two subunits in the MuA tetramer to harbour domain IIIb; these subunits function when placed diagonally and need not be catalytically proficient. We were unable to test if vertical or horizontal arrangements of pairs of IIIb subunits would be functional because of the inherantly poor activity of the R1** site.

4. Discussion

Yang *et al* (1995) had suggested that an active site for strand cleavage or strand transfer is assembled by sharing structural/catalytic residues between DDE and IIIa domains from separate MuA monomers, and that all four MuA subunits contribute both these shared domains for complete Mu transposition. The basis of the proposal was the observation that when the tetramer is assembled by two MuA variants, one lacking a functional DDE domain and the other lacking a functional domain IIIa, either

Figure 6. Position of domain IIIb subunits for MuB-mediated strand transfer. (**A**) Arrangement of wild-type and domain IIIb^- mutant MuA subunits on pL1**. Truncated subunit = domain IIIb^-. Other symbols as in figure 3A. (**B**) Complementation between mixtures (lane 4) of MuA (R146V) and MuA (Δ609-663) for strand transfer in the presence of target DNA, MuB protein and ATP. Lanes 1–3 are indicated controls (0·4 μg protein, when included). SDS was added prior to electrophoresis. Position of open circular (oc) and supercoiled forms of the target (T) plasmid, as well as double-end strand transfer products (DEP) is indicated. Other symbols as in figure 3B. (**C**) Arrangement of wild-type and domain IIIb^- mutant MuA subunits on pL1**-pR2**. Strand transferred target DNA is indicated by wavy lines. (**D**) Reactions as in B.

strand cleavage within a supercoiled substrate or strand transfer of a precleaved substrate, but not strand cleavage plus strand transfer, can be performed. The inspiration for the shared active model was derived from experiments in the Flp recombination system, where a monomer of Flp bound to its recognition site orients an adjacent phosphodiester for nucleophilic attack by the active site tyrosine derived in *trans* from a second Flp monomer (Chen *et al* 1992). In the Mu active site, DDE residues were proposed to provide the nucleophile while III*a* residues could orient the scissile phosphodiester (figure 2B; Yang *et al* 1995). The demonstration of a non-specific nuclease activity associated with domain III*a* heightened this speculation (Wu and Chaconas 1995). Analysis of transposition from linear substrates under altered reaction conditions (e.g. inclusion of DMSO in the reaction; Yang *et al* 1996), was not inconsistent with the Yang *et al* (1995) proposal.

Using oriented tetramers, recent experiments on supercoiled Mu substrates have shown that unlike the results derived from linear substrates, only two DDE$^+$ subunits (located at L1 and R1 and acting in *trans*) sequentially carry out strand cleavage followed by strand transfer (figure 2A; Namgoong and Harshey 1998). (The *trans* action of DDE was also observed on linear substrates employing two right ends; Aldaz *et al* 1996; Savilahti and Mizuuchi 1996.) To reconcile the results of Namgoong and Harshey (1998) with those of Yang *et al* (1995), both of which were performed under normal reaction conditions using supercoiled substrates, two possibilities suggested themselves. The first was that although the same DDE domains are used during cleavage and strand transfer, perhaps different III*a* domains are required during these two steps. The second was that the III*a* deletion variant used in the experiments of Yang *et al* (1995) might be responsible for the different results since this domain was intact in the experiments of Namgoong and Harshey (1998). Support for the former scenario would be consistent with a catalytic function, while that for the latter would indicate a structural role for domain III*a*. The present study was undertaken to dissect the importance of domain to III*a* to transposition by designing experiments that would distinguish between the two possibilities.

4.1 *No unique MuA subunit contributes domain III*a* function to strand cleavage or strand transfer*

The first reported study using the altered specificity variant MuA(R146V) (Namgoong and Harshey 1998) provided convincing evidence that a single DDE$^+$ MuA subunit placed at a particular att site functioned in a specific manner, and thus did not undergo rearrangement during assembly on supercoiled substrates. Interpretation of the data in this study is based on this assumption.

The results presented in figures 3–5 and table 1 show that a MuA subunit missing domain III*a* can function from L1 and R2 (two of the three 'core' att sites L1, R1, R2), without affecting double-end cleavage or strand transfer; domain III*a* function of the R1 subunit is also not required for L1 cleavage. Thus, there appears to be no critical position for the functioning of domain III*a* in transposition. The simplest interpretation of these results is that domain III*a* does not contribute residues to the catalytic pocket of the transposition active site, since if that had been the case, we would have expected such a contribution to be exquisitely position-specific. Several observations suggest that a more plausible function for this domain is to impart structural integrity to the transpososome during assembly, cleavage and transition from the cleavage to strand transfer mode. For example, all mutants in this domain are affected in assembly of the MuA tetramer (see figures 3–4; Baker *et al* 1991; Wu and Chaconas 1995; Krementsova *et al* 1998; Namgoong *et al* 1998a). Secondly, singly-cleaved right end complexes containing a domain III*a* deleted subunit fail to mature into strand transfer products (table 1B) and are unstable (our unpublished data). The latter results provide an explanation for why Yang *et al* (1995) observed cleavage but not strand transfer with a complementing pair of DDE$^+$ III*a*$^-$ and DDE$^-$ III*a*$^+$ variants on wild-type supercoiled substrates. We have shown in this study that the right end of Mu undergoes preferential cleavage under these conditions (figure 5). That this same complementing pair of MuA variants could strand transfer pre-cleaved linear substrates (Yang *et al* 1995), is consistent with our finding that doubly cleaved ends can undergo strand transfer even when one subunit carries a domain III*a* deletion (table 1D). Domain III*a*–deleted subunits positioned at R1 on pre-cleaved R1-R2 oligonucleotide substrates have been shown to support strand transfer as well (Aldaz *et al* 1996). Also, pre-cleaved Mu ends are able to rescue MuA assembly defects manifested on uncleaved substrates (Surette *et al* 1991; Namgoong *et al* 1994; Kim *et al* 1995; Wu and Chaconas 1995). The MuB protein used in strand transfer assays from linear substrates also assists in suppression of assembly defects (Namgoong *et al* 1998a).

Although all of these results favour a structural role for domain III*a*, we cannot rigorously exclude the possibility that there is an inherent flexibility in the catalytic contribution of the domain III*a* domain, and that alternate modes of protein-DNA associations may become manifest when one subunit in the tetramer is deleted for domain III*a*. Such alternate associations were indeed observed in the contribution of DDE domains on supercoiled vs linear substrates (Namgoong and Harshey 1998). However, in contrast to linear substrates, we imagine that a supercoiled

substrate must pose constraints on the number of protein-DNA arrangements that can be catalytically fruitful. Another line of evidence against a catalytic role for domain III*a* comes from the experiments of Wu and Chaconas (1995). These authors observed that when the domain III*a* peptide harbouring the non-specific nuclease activity carried multiple mutations in a stretch of basic amino acid residues, it simultaneously lost both DNA binding and nuclease activities; however, when these mutations were transferred to the full length MuA protein, the resultant MuA$_{BAN}$ protein was primarily defective in assembly. The mutant could be rescued for assembly and cleavage (at approximately half the efficiency of the wild-type protein), by the addition of either 10% dimethyl sulfoxide (DMSO) or MuB protein (plus ATP). On a pre-cleaved substrate, the strand transfer activities of MuA$_{BAN}$ and wild-type MuA were indistinguishable (Wu and Chaconas 1995). These results argue strongly against a catalytic role for domain III*a* in either DNA cleavage or strand transfer under the reaction conditions employed *in vitro*.

4.2 *Active sites and the role of domain III*a* in transposition*

The MuA transposase tetramer mediates two strand breakage-joining reactions via hydrolysis followed by transesterification. In principle, four active sites could each carry out one cleavage or one joining reaction. Alternatively, two active sites could sequentially carry out two cleavages followed by two joining reactions. The results obtained in this study, combined with those of Namgoong and Harshey (1998), are most easily accommodated by the latter scenario. Since only mutants in the DDE residues of MuA have thus far found to be unconditionally defective in transposition (Baker and Luo 1994; Kim *et al* 1995), the 'active sites' for Mu transposition are likely constituted solely of DDE residues. Domain III*a* residues appear not to contribute directly to the catalytic pockets, but rather to the structural integrity/flexibility of the transpososome. The relevance of the nuclease activity associated with domain III*a* is not clear at present, but it appears not to be involved in the chemistry of transposition *in vitro*, since mutants without this activity can be rescued for transposition under altered reaction conditions (Wu and Chaconas 1995). The recently solved structure of the Tn*5* synaptic complex show that DDE residues are likely the sole catalytic determinants of the active site of this transposase (Davies *et al* 2000).

If only two subunits of MuA perform the entire chemistry of transposition, what is the role of the other two subunits? It is likely that they contribute structurally or allosterically to the catalytic competence of the tetramer, since the catalytic prowess of MuA is manifested only within the context of the tetramer. Alternatively, or in addition, these subunits may be involved in initial target binding. While there is no direct evidence for involvement of any particular MuA domain in target recognition, the large positive charge potential of domain II*b* inferred from the crystal struture of domain II (Rice and Mizuuchi 1995), combined with a non-specific DNA-binding activity associated with this domain (Nakayama *et al* 1987), have led to speculations for a target-binding function for domain II*b*. However, although several mutations in this domain impair intramolecular DNA strand transfer, MuB-assisted intermolecular strand transfer is unaffected (Krementsova *et al* 1998; Namgoong *et al* 1998a). The role of MuB is discussed in more detail in the next section.

Tn*5* transposition is catalyzed by a transposase dimer which, like the Mu reaction, acts in *trans* (Davies *et al* 2000). A two-active site model has also been proposed for Tn*10* transposition, even though this reaction involves four single-strand breaks (two at each end) and two strand joinings (Bolland and Kleckner 1996). The oligomeric state of the Tn*10* transposition apparatus is not yet known. In contrast, there is evidence that in Tn*7* transposition, which like Tn*10* proceeds by a double-strand cleavage and single-strand joining reactions, four separate active sites perform four DNA cleavage reactions (Sarnovsky *et al* 1996). It is surmised that the Tn*7* transpososome consists of at least two subunits each of two distinct proteins, TnsA and TnsB. While TnsA mediates cleavage at the 5′ ends, TnsB mediates cleavage and joining at the 3′ ends. Each of these proteins encodes a DDE motif (Sarnovsky *et al* 1996). Whether the same subunits of TnsB sequentially mediate the cleavage and joining of 3′ ends, as appears to be the case for MuA, is not as yet known.

In summary, domain III*a* of MuA appears to play a role in transpososome assembly, but is unlikely to be involved in catalysis of transposition. While a catalytic role for DDE residues has been demonstrated for two subunits (those at L1 and R1; Namgoong and Harshey 1998) within the MuA tetramer, no specific catalytic function can yet be assigned to the other two subunits. These subunits may play an entirely structural role, might be involved in initial target recognition/binding, or may play a role in post-transposition events such as Mu DNA replication (Levchenko *et al* 1995; Kruklitis *et al* 1996).

4.3 *Specificity of domain III*b* in MuB-assisted strand transfer*

The MuB protein plays several roles in Mu transposition. It is not only an allosteric effector, stimulating the assembly, cleavage and strand transfer activities of MuA, but also captures target DNA and influences target-site selec-

tion (see Chaconas *et al* 1996). Residues in domain III*b* of MuA are essential for interaction with MuB protein, and thus for MuB function. The quaternary structure of the active form(s) of MuB is not known.

We have examined the specificity of domain III*b* function in MuB-assisted intermolecular strand transfer, and found that (i) a single III*b* domain$^+$ subunit at L1 or R2 is not sufficient, and (ii) two III*b* domains are sufficient, but their positions are not unique (figure 6 and table 2; we were only able to test diagonal placements). The non-specific nature of the function of domain III*b* subunits has also been demonstrated in an earlier study (Mizuuchi *et al* 1995), which did not involve the use of oriented tetramers or determine the minimal number of III*b*$^+$ subunits necessary for function. While the non-unique positioning of III*b* subunits can be rationalized for the allosteric-effector function of MuB, it is perhaps harder to rationalize for the target-transporter function. In the former case, a conformational change in a pair of MuA subunits could conceivably be propagated to the other two subunits by protein-protein interactions. In the latter case, the target DNA is likely delivered to specific sites on MuA, since, although MuB binds target DNA and influences target selection, the sequence of the selected sites is the same in the presence of absence of MuB (Mizuuchi and Mizuuchi 1993). How can specific delivery of target DNA be achieved from a non-specific position? Our results would argue that once MuB docks (non-specifically) on the MuA tetramer, it simply serves as an anchor to increase the local concentration of target DNA for specific capture by the catalytical MuA subunits.

Acknowledgements

References

Aldaz H, Schuster E and Baker T A 1996 The interwoven architecture of the Mu transposase couples DNA synapsis to catalysis; *Cell* **85** 257–269

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, Mizuuchi M and Mizuuchi K 1991 MuB protein allosterically activates strand transfer by the transposase of phage Mu; *Cell* **65** 1003–1013

Bolland S and Kleckner N 1996 The three chemical steps of Tn*10*/IS*10* transposition involve repeated utilization of a single active site; *Cell* **84** 223–233

Chaconas G, Lavoie B D and Watson M A 1996 DNA transposition – jumping gene machine, some assembly required; *Curr. Biol.* **7** 817–820

Chen J-W, Lee J and Jayaram M 1992 DNA cleavage in trans by the active site tyrosine during Flp recombination: switching protein partners before exchanging strands; *Cell* **69** 647–658

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

Davies D R, Goryshin I Y, Reznikoff W S and Rayment I 2000 Three-dimensional structure of the Tn*5* synaptic complex transposition intermediate; *Science* **289** 77–85

Grindley N D and Leschziner A E 1995 DNA transposition: from a black box to a colour monitor; *Cell* **83** 1063–1066

Harshey R M and Cuneo S 1986 Carboxyl-terminal mutants of phage Mu transposase; *J. Genet.* **65** 159–174

Jiang H, Yang J-Y and Harshey R M 1999 Criss-crossed interactions between the enhancer and the att sites of phage Mu during DNA transposition; *EMBO J.* **18** 3845–3855

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

Krementsova E, Giffin M J, Pincus D and Baker T A 1998 Mutational analysis of the Mu transposase. Contributions of two distinct regions of domain II to recombination; *J. Biol. Chem.* **273** 31358–31365

Kruklitis R, Welty D J and Nakai H 1996 ClpX protein of *Escherichia coli* activates bacteriophage Mu transposase in the strand transfer complex for initiation of Mu DNA synthesis; *EMBO J.* **15** 935–944

Kuo C F, Zou A, Jayaram M, Getzoff E and Harshey R M 1991 DNA-protein complexes during attachment-site synapsis in Mu DNA transposition; *EMBO J.* **10** 1585–1591

Lavoie B D and Chaconas G 1995 Transposition of phage Mu DNA; *Curr. Top. Microbiol. Immunol.* **204** 83–102

Leung P and Harshey R M 1991 Two mutations of phage Mu transposase that affect strand transfer or interactions with B protein lie in distinct polypeptide domains; *J. Mol. Biol.* **219** 189–199

Levchenko I, Luo L and Baker T A 1995 Disassembly of the Mu transposase tetramer by the ClpX chaperone; *Genes Dev.* **9** 2399–2408

Levchenko I, Yamauchi M and Baker T A 1997 ClpX and MuB interact with overlapping regions of Mu transposase: implications for control of the transposition pathway; *Genes Dev.* **11** 1561–1572

Li B L, Langer J A, Schwartz B and Pestka S 1989 Creation of phosphorylation sites in proteins: construction of a phosphorylatable human interferon alpha; *Proc. Natl. Acad. Sci. USA* **86** 558–562

Maxwell A, Craigie R and Mizuuchi K 1987 B protein of bacteriophage Mu is an ATPase that preferentially stimulates intermolecular DNA strand transfer; *Proc. Natl. Acad. Sci. USA.* **84** 699–703

Mizuuchi K 1992 Polynucleotidyl transfer reactions in transpositional DNA recombination; *Annu. Rev. Biochem.* **61** 1011–1051

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

Mizuuchi M and Mizuuchi K 1993 Target site selection in transposition of phage Mu; *Cold Spring Harbor Symp. Quant. Biol.* **58** 515–523

Naigamwalla D Z and Chaconas G 1997 A new set of Mu DNA transposition intermediates: alternate pathways of target capture preceding strand transfer; *EMBO J.* **16** 5227–5234

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

Namgoong S-Y and Harshey R M 1998 The same two monomers within a MuA tetramer provide the DDE domains for the strand cleavage and strand transfer steps of transposition; *EMBO J.* **17** 3775–3785

Namgoong S-Y, Jayaram M, Kim K and Harshey R M 1994 DNA-protein cooperativity in the assembly and stabilization of Mu strand transfer complex. Relevance of DNA phasing and att site cleavage; *J. Mol. Biol.* **238** 514–527

Namgoong S-Y, Kim K, Saxena P, Jayaram M, Giedroc D P and Harshey R M 1998a Mutational analysis of domain II*b* of bacteriophage Mu transposase: domains II*a* and II*b* belong to different catalytic complementation groups; *J. Mol. Biol.* **275** 221–232

Namgoong S-Y, Sankaralingam S and Harshey R M 1998b Altering the DNA-binding specificity of Mu transposase *in vitro*; *Nucleic Acids Res.* **26** 3521–3527

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

Sarnovsky R J, May E W and Craig N L 1996 The Tn7 transposase is a heteromeric complex in which DNA breakage and joining activities are distributed between different gene products; *EMBO J.* **15** 6348–6361

Savilahti H and Mizuuchi K 1996 Mu Transpositional Recombination: donor DNA cleavage and strand transfer in trans by the Mu transposase; *Cell* **85** 271–280

Surette M G, Harkness T and Chaconas G 1991 Stimulation of the Mu A protein-mediated strand cleavage reaction by the Mu B protein, and the requirement of DNA nicking for stable type 1 transpososome formation. *In vitro* transposition characteristics of mini-Mu plasmids carrying terminal base pair mutations; *J. Biol. Chem.* **266** 3118–3124

Watson M A and Chaconas G 1996 Three-site synapsis during Mu DNA transposition: A critical intermediate preceding engagement of the active site; *Cell* **85** 435–445

Wu Z and Chaconas G 1994 Characterization of a region in phage Mu transposase that is involved in interaction with the Mu B protein; *J. Biol. Chem.* **269** 28829–28833

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

Yamauchi M and Baker T A 1998 An ATP-ADP switch in MuB controls progression of the Mu transposition pathway; *EMBO J.* **17** 5509–5518

Yang J-Y, Jayaram M and Harshey R M 1996 Positional information within the Mu transposase tetramer: catalytic contributions of individual monomers; *Cell* **85** 447–455

Yang J-Y, Kim K, Jayaram M and Harshey R M 1995 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