Mutational Analysis of the Mu Transposase

CONTRIBUTIONS OF TWO DISTINCT REGIONS OF DOMAIN II TO RECOMBINATION*

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Mu transposase is a member of a protein family that includes many transposases and the retroviral integrases. These recombinases catalyze the DNA cleavage and joining reactions essential for transpositional recombination. Here we demonstrate that, consistent with structural predictions, aspartate 336 of Mu transposase is required for catalysis of both DNA cleavage and DNA joining. This residue, although located 55 rather than 35 residues NH2-terminal of the essential glutamate, is undoubtedly the analog of the second aspartate of the Asp-Asp-Glu motif found in other family members. The core domain of Mu transposase consists of two subdomains: the NH2-terminal subdomain (IIA) contains the conserved Asp-Asp-Glu motif residues, whereas the smaller COOH-terminal subdomain (IIB) contains a large positively charged region exposed on its surface. To probe the function of domain IIB, we constructed mutant proteins carrying deletion or substitution mutations within this region. The activity of the deletion mutants revealed that domains IIA and IIB can be provided by different subunits in the transposase tetramer. Substitution mutations at two pairs of exposed lysine residues within the positively charged surface of domain IIB render transposase defective in transposition at a reaction step after DNA cleavage but prior to DNA joining. The severity of this defect depends on the structure of the DNA flanking the cleavage site. Thus, these data suggest that domain IIB is involved in manipulating the DNA near the cleavage site and that this function is important during the transition between the DNA cleavage and the DNA joining steps of recombination.

Transposons are genetic elements that move from one site on DNA to another by transpositional recombination. Most transposons encode a single transposase protein responsible for the DNA cleavage and DNA joining reactions required during the initial recombination steps (1, 2). Retroviral integration follows a very similar recombination pathway as transposition (3, 4), and recent studies clearly establish that the transposases and retroviral integrases are a protein family whose members contain regions of similar amino acid sequence, protein structure, and function (5, 6).

The transposase of bacteriophage Mu (MuA) is one of the best characterized members of the transposase/integrase family. MuA promotes two chemical steps during recombination: 1) donor DNA cleavage, which is the endonucleolytic cleavage of the phosphodiester bond between the element and the flanking host DNA, and 2) DNA strand transfer, the coupled DNA cleavage and joining reaction that covalently links the 3′ ends of the element DNA to the DNA at the target site (5) (reviewed in Refs. 7 and 8). The active form of MuA is a homotetramer that is bound simultaneously to the two ends of the Mu DNA (9). In this complex (called a transpososome), MuA holds the two DNA ends in a paired configuration and engages the DNA cleavage sites (9, 10). Assembly of tetrameric MuA activates the protein’s catalytic functions and ensures that the DNA cleavage and joining reactions occur in a coupled manner on the two ends of the Mu DNA (11, 12). Once assembled, the MuA tetramer stays associated with the donor DNA throughout the recombination reactions (10, 13).

MuA is a large (75-kDa monomer) multidomain protein (Fig. 1A). The minimal region required for DNA binding, cleavage, and strand transfer in vitro includes domain IB, the catalytic core (domain II), and the NH2-terminal portion of domain III (IIIA). These essential domains have the following functions. Domain IB is a bipartite site-specific DNA binding domain that recognizes six transposase binding sites located near the ends of the Mu genome (14, 15). Domain IIIA also binds DNA and has a cryptic nuclease activity implicated in catalysis of DNA cleavage (16). Domain II, also known as the catalytic core domain, participates directly in both cleavage and strand transfer (see below). The extreme NH2- and COOH-terminal subdomains of transposase regulate the protein’s activity. Domain IA is a winged-helix-turn-helix domain that binds the enhancer-like DNA element (the IAS); this interaction is normally required during transpososome assembly but can be dispensed with under certain in vitro conditions (17, 18). The carboxyl-terminal subdomain (IIIB) interacts with the activator protein, MuB (19, 20), and the chaperone, ClpX (21). MuB stimulates MuA and controls DNA target site choice (19, 22–24), whereas ClpX participates in disassembly of MuA-DNA complexes after recombination is complete (20, 25, 26).

The crystal structure of domain II of MuA revealed two subdomains, referred to as IIA and IIB. The overall topology of domain IIA (residues 258–490) can be superimposed on the structure of the core domain of HIV1-1 integrase (27). This region of MuA contains the phyleogenetically conserved catalytic triad of acidic amino acids known as the Asp-Asp-Glu motif (27, 28). These acidic residues are implicated in coordi-
nating divalent metal ions required for catalysis (29, 30). Domain IIB, which extends from amino acid 490 to amino acid 560, has a β-barrel structure with a large positively exposed patch on one side; this region has been suggested to be responsible for the nonspecific DNA binding activity of domain II (27). The function of domain IIB in transposition is unknown.

This paper extends our understanding of the functional organization of the MuA. We demonstrate that, consistent with the structural predictions, aspartate 336 in domain IIA is required for catalysis of cleavage and strand transfer. This residue corresponds to the second aspartate of the Asp-Asp-Glu motif of other transposases and the retroviral integrases. In contrast, mutations at several other conserved positions in domain IIA do not dramatically affect the catalytic activity of the protein, although two residues involved in contacts between domain IIA and IIB appear important for protein folding or function. Furthermore, we find that domains IIA and IIB can be provided by different subunits in the active transposase tetramer and suggest that domain IIB contacts and manipulates the DNA duplex near the cleavage site during recombination.

EXPERIMENTAL PROCEDURES

DNA—The target DNA was dSx174 RFI (Life Technologies, Inc.). The circular donor DNA was pSG1 or pMK586 (10, 28). The precleaved donor DNA was made by linearization of pKKn7 (identical to pMK426 between the two Mu DNA ends) with HindIII as described previously (7). Synthetic fragments for donor DNAs were synthesized by MIT Biopolymer Laboratory and labeled on the 5’ end with T4 polynucleotide kinase and [γ-32P]ATP. The oligonucleotides contained slightly modified versions of the natural R1 and R2 sequences of Mu DNA (see Fig. 7 and Ref. 12).

Proteins—MuB protein used in Fig. 2 was purified as described by Chambon et al., with the additional step described by Adzuma and Mizuochi (32) to remove aggregated protein. In the remaining experiments, MuB was the His-tagged version and was purified as described (21). HU protein was purified from the overproducing strain of R. McMacken (Johns Hopkins University, Baltimore, MD) by the method of Dixon and Kornberg (33) with the additional step of Mono S chromatography in 20 mM NaPO4 (pH 4.8), eluted with a gradient of 0–500 mM NaCl. MuA crude extract (300 to 600 mM NaCl. MuA crude extract assays (Fig. 3B) contained 1 μl of fraction prepared as described (35). The protein concentration of MuA preparations was determined either spectrophotometrically with the value of 280 μg/ml or by quantitating the intensity of Coomassie Blue-stained bands after SDS-polyacrylamide gel electrophoresis, in comparison with MuA standards of known concentration.

Site-directed Mutagenesis—Site-directed changes were introduced into the MuA gene using the method of Kunkel as described previously (28) or using the Chameleon double-stranded mutagenesis kit as recommended by the supplier (Stratagene). The mutated gene was cloned into pET-3a (pMK591), and the sequence was confirmed by sequencing.

Transposition Reactions—The standard reaction conditions were essentially as described previously (34). Reaction mixtures contained 25 mM Tris-Cl (pH 8 at room temperature), 150 mM NaCl, 10 mM MgCl2, 2 mM ATP, 1 mM dithiothreitol, 15% glycerol, 10 μg/ml of donor DNA, and 10 μg/ml of dSx174 RFI. The protein levels in 25-μl reactions were as follows: MuB, 6.5 pmol; HU, 3.0 pmol; and MuA between 0.13 and 3.0 pmol, with the level being 1.3 pmol, unless otherwise stated (see figure legends). Proteins were prepared by dilution of concentrated stock solutions in storage/dilution buffer described previously (19). Reactions using donor DNA fragments were done as described (35), contained 1 pmol of γ-32P-labeled fragment carrying the Mu end sequences, and lacked HU protein. MuB and MeSO (15%) were added as noted in the figure legends. The reactions were incubated at 30 °C for 20 to 60 min (or as described in the figure legends) stopped by the addition of 0.2 volume of a stop solution (0.1% bromphenol blue, 2.5% SDS, 50 mM EDTA, 25% glycerol), and portions of the samples were analyzed by electrophoresis on a 0.9% high gelling temperature-agarose gel with a constant circulation of the buffer (1 × TAB: 40 mM Tris acetate at pH 7.8, 5 mM sodium acetate, 1 mM EDTA). Reactions using oligonucleotide substrates were further analyzed as follows: gels were dried to filter paper, exposed on a Phosphor cassette (Molecular Dynamics), and scanned with a Molecular Dynamics BI455si. The cleavage products were analyzed on a denaturing 8% acrylamide gel; gels were dried and exposed in a Phosphor exposure cassette. Images were analyzed using ImagequantaNT, v4.2 software (Molecular Dynamics).

RESULTS

Aspartate 336 of Mu Transposase Is Essential for Catalysis of Cleavage and Strand Transfer—Site-directed mutagenesis and biochemical analysis of the mutated proteins established that Asp336 and Glu392 of MuA are required for catalysis of both donor DNA cleavage and DNA strand transfer (28, 36). These results, combined with amino acid sequence alignments, indicated that these residues were analogous of the first aspartate and the glutamate of the Asp-Asp-Glu motif first described for the retroviral integrases (30). However, sequence alignments failed to reveal an aspartate properly spaced to be “the middle Asp,” which is almost always positioned 34 or 35 residues NH2-terminal of the glutamate (Glu). Although located 55, rather than 35, residues NH2-terminal of Glu392, the sequence immediately neighboring Asp336 has some similarity to sequences neighboring the middle Asp in other transposases (Fig. 1B). Furthermore, the structure of domain II reveals that this residue is also located in a position similar to that of the second conserved aspartate in the retroviral integrase structures (27). The D336N substitution mutation was therefore constructed, and the protein purified and assayed for its ability to promote the individual steps of transposition.

The D336N mutant protein was defective in both cleavage and strand transfer. When assayed with a supercoiled mini-Mu plasmid as the donor DNA substrate, D336N had no detectable cleavage activity (Fig. 2, lane 3). Precleaved donor DNA was used to assay for the strand transfer step, independent of cleavage; D336N also had no detectable strand transfer activity (lane 6). More sensitive cleavage and strand transfer assays using radiolabeled DNAs confirmed that proteins carrying the D336N substitution had a profound defect in both chemical steps.

To assess whether D336N was specifically defective in catalysis, or whether its function was blocked at an earlier step, several tests were performed. Gel mobility shift assays and protein-protein cross-linking experiments confirmed that D336N retained specific DNA binding activity and assembled into tetramers (data not shown). Furthermore, when wild-type MuA and D336N were mixed, the presence of D336N reduced the amount of strand transfer products generated by wild-type MuA (Fig. 2, lane 7). Virtually all of these strand transfer products had only one of the two ends of the Mu DNA joined to the target DNA, a product that is only rarely formed by the wild-type protein. The presence of this single-ended strand transfer product indicated that D336N assembles with MuA to form mixed tetramers and that the presence of D336N in this complex blocks catalysis of strand transfer. Therefore, we conclude that the D336N derivative of MuA is specifically defective in catalysis of both the donor DNA cleavage and strand transfer steps in transposition, as has been shown previously for the D269N and E392Q mutant proteins (28).

Importance of Other Conserved Amino Acids in the Core Domain of Transposase—In contrast to the inhibitory effect of substitutions at Asp269, Asp336, and Glu392, alterations at several other acidic amino acids in domain IIA did not have a large impact on MuA activity. Four other acidic amino acids within this domain were altered by the following substitutions: D306N, E309Q, D312N, and D320A (Asp294 has been mutated 2 T. L. Williams and T. A. Baker, manuscript in preparation.)
previously (28)). The proteins were overexpressed, and their ability to catalyze the individual steps in transposition was assayed using crude extracts. Strand transfer of radiolabeled, precleaved fragments that are analogs of the right end of the Mu genome (Fig. 3A) was a very sensitive assay for transposase function; the results of this strand transfer assay with the mutant proteins is shown in Fig. 3B, lanes 7-9. In contrast, the D320A mutant protein was inactive (lane 13). Inspection of the position of this residue in the structure of domain II revealed that it is involved in a contact between domains IIA and IIB. The R534A mutant protein was also inactive (lane 6), and this residue is likewise involved in a domain IIA-IIB contact. Alteration of a neighboring hydrophobic residue by the V532A mutation did not have such a severe consequence (lane 5). The D320A and R534A proteins were not further characterized, although analysis of the extracts confirmed that they were expressed and present in the cell extract (data not shown).

Three additional mutant proteins carrying the changes N267A, K373T, and K373A were constructed and analyzed. Asn267 is two amino acids NH2-terminal of the first catalytic aspartate and has the potential to interact with the active-site residues. However, alanine substitution at this position had little effect on transposase activity (Fig. 3B, lane 10). Lys373 is previously (28)). The proteins were overexpressed, and their ability to catalyze the individual steps in transposition was assayed using crude extracts. Strand transfer of radiolabeled, precleaved fragments that are analogs of the right end of the Mu genome (Fig. 3A) was a very sensitive assay for transposase function; the results of this strand transfer assay with the mutant proteins is shown in Fig. 3B, lanes 7-9. In contrast, the D320A mutant protein was inactive (lane 13). Inspection of the position of this residue in the structure of domain II revealed that it is involved in a contact between domains IIA and IIB. The R534A mutant protein was also inactive (lane 6), and this residue is likewise involved in a domain IIA-IIB contact. Alteration of a neighboring hydrophobic residue by the V532A mutation did not have such a severe consequence (lane 5). The D320A and R534A proteins were not further characterized, although analysis of the extracts confirmed that they were expressed and present in the cell extract (data not shown).

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a surface exposed lysine and is the only residue other than the two active site asparagines that is present in an analogous position in MuA, HIV integrase, and RNaseH (see Fig. 1B and Ref. 27). Whereas the Lys to Thr substitution was inactive in the crude extracts (lane 12), the Lys to Ala mutant protein was clearly active (lane 11). The K373A protein was purified to near homogeneity and further characterized; its cleavage and strand transfer activities were similar to wild-type MuA (data not shown).

Domains IIA and IIB Can Be Provided by Different Subunits in the Transposase Tetramer—Domain II of MuA consists of two subdomains: residues 258–490 form a mixed a/b domain, which contains the Asp-Asp-Glu motif residues, and residues 491–560 form a small b-barrel (Fig. 4). To address whether domains IIA and IIB can function independently, we constructed a series of carboxyl-terminal truncations; the truncated proteins are referred to as MuA1-562, MuA1-527, and MuA1-490, with the last number being the final residue present in the deletion protein. MuA1-527 is missing about half of domain IIB (and inspection of the structure suggests it is unlikely that the remaining portion will be well folded), and the entire domain is missing from MuA1-490. The deletion proteins were overexpressed and purified. All three proteins bound specifically to Mu end DNA (data not shown), suggesting that at least domain I adopts a native tertiary structure in these deletion proteins. MuA1-562 and MuA1-527 had solubility properties similar to full-length MuA. MuA1-490 was considerably less soluble and lost activity during storage.

As expected from the previous characterization of MuA1-574, which is missing domain III (19), none of the deletion proteins had cleavage or strand transfer activity when assayed alone (Fig. 5, A and B, lanes 3–5). Furthermore, all three deletion proteins, like MuA1-574, were unable to assemble into homotramers (data not shown). However, when assayed in the presence of a full-length but defective MuA derivative carrying substitution mutations at two of the Asp-Asp-Glu-motif residues (D269N/E392Q, referred to as DE/NQ), the protein mixtures were active. The ability of the protein mixtures to support cleavage was assayed by observing the appearance of cleaved donor complexes (CDCs) after agarose gel electrophoresis. Cleaved donor complexes (also known as type I complexes) are formed when a MuA tetramer cleaves the donor plasmid and remains bound to the product DNA. The wild-type MuA sample (lane 1) was diluted 20-fold prior to loading. DNA species are labeled as in Figs. 2 and 3.

**Fig. 3.** Strand transfer activity of Domain II mutants. A, schematic of fragment assay for strand transfer. B, reactions were done under the standard conditions for the fragment assay, in the presence of MuB protein. Lanes 1–3, are a dilution series with an extract expressing wild-type MuA. Lanes 5–16 show the products generated by each of the mutant proteins as marked over each lane. S.E. is the single end strand transfer product, and DE is the double end strand transfer product. Although single-ended STPs are rarely formed by wild-type MuA with a circular donor DNA, they are not uncommon in this fragment assay.

**Fig. 4.** Structure of MuA domain II. Domain IIA (residues 248–489) is in the dark ribbon, whereas domain IIB (residues 490–560) is in lighter gray. The Asp-Asp-Glu motif residues and the solvent accessible lysines mutated in this study are displayed. The region marked "extra loop" corresponds to residues 354–356, which lie between Asp356 and Glu392.

**Fig. 5.** Deletion mutants missing domain IIB form mixed tetramers with DE/NQ and catalyze cleavage and strand transfer. Top, formation of CDCs in mixed tetramers with MuA DE/NQ. Reactions were under standard conditions, with pSG1 as the donor DNA, and lacking MuB and target DNA. Bottom, strand transfer by deletion mutants in mixed tetramers with DE/NQ. Reactions were under strand fragment assay conditions, in the absence of MuB. The wild-type MuA sample (lane 1) was diluted 20-fold prior to loading. DNA species are labeled as in Figs. 2 and 3.
Mutations in Mu Transposase

An electrostatic map of the structure of domain II shows a large, positively charged region on one face of domain IIB (27). Because the site-specific DNA binding determinants that interact with the Mu DNA are contributed by domain I, and domain II has nonspecific DNA binding activity (37), it has been proposed that domain IIB might interact with the DNA surrounding the cleavage site or the target DNA (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27). To study the role of the charged surface of domain IIB in transposition, four surface-exposed lysines were mutated to alanine. Two mutant proteins, each with two substitutions, KK/AA1 and KK/AA2, were constructed and characterized (27).
Asp336 of Mu Transposase Is Essential for Catalysis—Introduction of the conservative aspartate to asparagine substitution at position 336 inactivates the cleavage and strand transfer activities of Mu transposase (MuA). As DNA binding and assembly of MuA tetramers are essentially unaffected by this substitution, we conclude that aspartate 336 is important to the catalytic steps of recombination. Aspartate 336 is located in a similar position within the domain II structure of MuA to the second conserved aspartate of the Asp-Asp-Glu motif in the HIV-1 and ASV integrase core domain structures (5, 27). Mutations at these residues (Asp116 and Asp121, respectively), like Asp336 in MuA, inactivate the cleavage and strand transfer activities of the integrase proteins (30, 38). Thus, we conclude that Asp336 of MuA is the third residue of the conserved catalytic triad, known as the Asp-Asp-Glu motif, present in many representatives of the transposase/integrase protein family. These acidic residues have been suggested to coordinate an essential divalent metal ion in the transposase/integrase active site (reviewed in Refs. 5, 6, and 29). The two aspartates in a structure of the ASV integrase catalytic domain coordinate a Mn2+ or Mg2+ ion in the crystal structure solved in the presence of these cations, providing direct support for this hypothesis (39).

Aspartate 336 is located 55 residues NH2-terminal of the essential glutamate (Glu302), in contrast to the 35 residue spacing common to most members of the transposase/integrase family. As pointed out in Rice and Mizuuchi (27), the extra residues form an additional loop between α-helices B and C of domain IIA; this loop participates in an interdomain contact, contributing to the β-barrel structure of domain IIB (see Fig. 4). Although in transposases from Mu-like phage (e.g. from the Pseudomonas aeruginosa phage D3112, and the Mu-like phage from Hemophilus influenzae; see Fig. 1B) the longer spacing is conserved, this arrangement is unusual among members of the transposase/integrase family. Although the function of the interdomain contact provided by these residues is unknown, it may allow for tighter control between assembly and activation of catalytic function observed with Mu transposase compared with the integrases. For example, since our current results suggest that domain IIB may interact with the DNA flanking the cleavage site, it is attractive to consider that the domain IIA-domain IIB interdomain contact may participate in sensing the status of the donor DNA (i.e. uncleaved versus cleaved). The results of this mutagenesis study further highlight the importance of contacts between domain IIA and IIB. The only residues other than Asp336 that we mutated that had a severe inhibitory effect on MuA function (Asp320 and Arg534) both engage in interdomain contacts. Interestingly, Asp320 is involved in a bipartite interaction with Arg534 and Asp536, and substitution of Asp336 to Asn has been previously shown to reduce the activity of MuA in vivo (36).

Possible Functions of Domain IIB of MuA—Whereas domain IIA clearly carries an essential portion of the transposase active site, the role of domain IIB is much less clear. Domain IIB is a six-stranded β-barrel that, in contrast to domain IIA, is not highly related to the analogous region of HIV integrase (although integrase also has a small β-barrel domain just COOH-terminal of the catalytic core (40)). The most dramatic feature of domain IIB of MuA is the large region of positive electrostatic potential on one surface of the barrel, which led to the suggestion that this domain may be a DNA binding determinant that contacts the substrate DNA during recombination (27). Possible regions of DNA contact include the cleavage sites on the donor DNA, the target DNA, and the DNA flanking the cleavage site.

Characterization of two mutant proteins, each carrying two alanine substitutions at neighboring lysine residues within the positive patch on domain IIB, suggests that this region interacts with DNA flanking the cleavage site. The two mutant proteins had very similar defects, indicating that their behavior may in fact reflect the molecular consequences of altering
the positively charged patch. Under standard in vitro reaction conditions, both mutant proteins assembled and cleaved the donor DNA as well as wild-type MuA but were virtually unable to carry out the strand transfer. The most severe strand transfer defect was specifically observed with substrates (either circular or linear) carrying an extensive region of duplex DNA flanking the cleavage sites. Based on these data, we suggest that domain IIB may contact the DNA flanking the cleavage site and this contact may be especially important for manipulating the DNA during the transition between the cleavage and strand transfer steps of recombination.

The importance of distorting the DNA flanking the cleavage sites has been indicated by a number of studies of Mu transposition. Persuasive evidence for a MuA-dependent distortion of DNA near the cleavage site is provided by hydroxyl radical footprinting experiments of active transposase-DNA complexes that reveal a large enhancement on the noncleaved strand two and three nucleotides beyond the cleavage site (9). Furthermore, in a study using synthetic donor DNA fragments of different lengths, MuA efficiently melted DNA segments flanking the cleavage site that were 6 or 11 base pairs in length, thereby releasing the single-stranded segment on the cleaved strand (35). Based on our current results, it is attractive to consider that domain IIB plays a role in introducing this DNA distortion. However, direct physical data that domain IIB contacts this segment of DNA will be needed to establish this point.

If a role of domain IIB is to contact and manipulate the DNA flanking the cleavage sites, then the behavior of the KK/AA mutants may provide some insight into the molecular mechanism by which MuB stimulates catalysis of recombination. MuB clearly overcomes the defects exhibited by the mutant proteins, perhaps suggesting that MuB activates MuA by helping it to distort the flanking sequences. Consistent with this idea, MuB helps overcome the inhibitory effects exhibited by certain “toxic” flanking DNA sequences (41). Furthermore, although MuB stimulates many aspects of Mu transposition, under our standard reaction conditions, strand transfer is the step most responsive to MuB. Thus, the slow rate of strand transfer in the absence of MuB could reflect the relatively slow nature of the same cleavage-to-strand transfer transition defective in the domain IIB mutants when wild-type protein is not activated by MuB.

Organization of the Transposase Tetramer—Deletion version of MuA were constructed to determine whether the functions of domain IIA and domain IIB could be separated. Based on the ability of proteins missing all residues beyond amino acid 490 or 527 to provide the active site residues in domain IIA to an active tetramer (even at a low efficiency), we conclude that the two subdomains of domain II can function independently and that different subunits in the MuA tetramer can donate domain IIA than donate domains IIB and III to form a functional complex. This result agrees nicely with the suggestion that, if the putative DNA binding region in domain IIB has and important function in catalysis of recombination, then its large distance from the acidic residues of the active site would suggest a “trans” arrangement of these domains within the tetramer (27). The conclusion that domain IIA and domain IIB can be provided by different subunits in the tetramer was recently reported by Namgoong et al. (42) based on mixing experiments with proteins carrying a point mutation near the COOH terminus of domain IIB; our experiments allow this conclusion to be drawn for the function of the whole domain.

Domain IIA has both nonspecific DNA binding activity and a cryptic nuclease activity (16). This region of MuA has been implicated in contacting the substrate DNA at the cleavage site during transposition. There is some similarity between the mutants described here in domain IIB and those previously characterized in domain IIIA (16) However, the domain IIIA mutants affect tetramer assembly and cleavage more than they effect strand transfer (16), whereas the domain IIB mutants are most defective at the transition between the cleavage and strand transfer steps. Subunit mixing experiments also establish that both domain IIB and IIA can be provided by different subunits than provide the essential functions in domain IIA (20, 43). It is, however, premature to conclude that the same subunits donate domains IIB and IIA, although the similarity between the behavior of MuA1-574 (which is missing domain III) and MuA1-527 (which is missing both domain III and a substantial region of domain IIB) suggest that this is a likely possibility.

Structure and function analysis of different members of the transposase/integrate protein family demonstrates that the catalytic core domains, which appear to coordinate Mg2+ or Mn2+ in the protein’s active site, are very similar among different family members. The active sites of these proteins must also contain determinants involved in locating, binding, and activating the cleavage sites on the donor and target DNA. Although our understanding is far from complete, emerging evidence suggests that the molecular determinants responsible for these binding interactions are more diverse than those involved in metal binding. For example, two lysine residues in HIV integrase have recently been identified that interact with the cleavage site at the ends of the viral cDNA (44); analogous residues are not present in the MuA structure. Likewise, a number of mutations in Tn10 transposase that are likely to identify target DNA interaction residues lie in regions not conserved along many other transposases (45, 46). This diversity perhaps relates to the different recombination strategies adopted by different elements (i.e., replicative versus cut and paste transposition and transposition versus viral insertion). Establishing how MuA makes these DNA contacts and how these contacts change as the protein catalyzes the distinct steps of cleavage and strand transfer will be critical to understanding the recombination mechanism. Identifying all the active site components and assigning functions to the individual protein domains are important steps in this process.

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