Effect of Mutations in the C-terminal Domain of Mu B on DNA Binding and Interactions with Mu A Transposase*

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Bacteriophage Mu transposition requires two phage-encoded proteins, the transposase, Mu A, and an accessory protein, Mu B. Mu B is an ATP-dependent DNA-binding protein that is required for target capture and target immunity and is an allosteric activator of transpososome function. The recent NMR structure of the C-terminal domain of Mu B (Mu B223–312) revealed that there is a patch of positively charged residues on the solvent-exposed surface. This patch may be responsible for the nonspecific DNA binding activity displayed by the purified Mu B223–312 peptide. We show that mutations of three lysine residues within this patch completely abolish nonspecific DNA binding of the C-terminal peptide (Mu B223–312). To determine how this DNA binding activity affects transposition we mutated these lysine residues in the full-length protein. The full-length protein carrying all three mutations was deficient in both strand transfer and allosteric activation of transpososome function but retained ATPase activity. Peptide binding studies also revealed that this patch of basic residues within the C-terminal domain of Mu B is within a region of the protein that interacts directly with Mu A. Thus, we conclude that this protein segment contributes to both DNA binding and protein-protein contacts with the Mu transposase.

Bacteriophage Mu is a temperate phage that undergoes two forms of transposition during its life cycle. The first form of transposition occurs when Mu DNA integrates into its host genome through a nonrepetitive transposition event called conservative integration (1–3). The second form occurs after integration, when the Mu DNA amplifies itself through a process known as replicative transposition (4). The conservative integration reaction has never been reproduced in vitro, but the replicative transposition pathway has been characterized extensively using purified substrate and both host-encoded and phage-encoded proteins (5).

Mu replicative transposition can be performed in vitro using a supercoiled mini-Mu substrate, which contains the left end binding region (attL), the right end binding region (attR), and the enhancer element within a donor DNA molecule. The reaction occurs through a series of higher order nucleoprotein complexes called transpososomes. Transpososome formation up to and including strand transfer occurs in the presence of two phage-encoded proteins, Mu A and Mu B, and two host encoded proteins, HU and IHF (Fig. 1). In the presence of the mini-Mu substrate, Mu A, HU, IHF, and an appropriate divalent metal ion, the initial LER complex is formed. The LER complex is a transitory three-site complex that through numerous protein-protein and protein-DNA interactions brings together the left end, the right end, and the enhancer elements (6). The first stable complex formed is the Type 0 (stable synaptic complex) (7, 8). The Type 0 complex is characterized by the engagement of the Mu ends by the active site and accumulates in the presence of magnesium, which does not support strand cleavage. In the presence of magnesium, the 3′-ends of the Mu DNA are nicked, and the Type 0 complex is quickly converted to Type 1 complex (cleaved donor complex) (9, 10). The addition of Mu B, ATP, and target DNA results in the formation of the Type 2 complex (strand transfer complex) in which the 3′-ends of the Mu DNA are transferred to a target DNA molecule (9, 10). The Type 2 complex is the most stable transpososome and must be destabilized by the host-encoded ClpX ATPase before replication can occur (11–14). Target capture can also occur at the LER or Type 0 stage of the reaction (15). In the absence of target DNA, Mu B and ATP stimulate intramolecular strand transfer, whereby the 3′-ends of the Mu DNA are transferred into a new DNA site within the donor molecule (16, 17).

The Mu A protein (transposase) is 663 amino acids in length (18) and is able to perform all of the chemical steps in the transposition reaction. Mu A can be divided into three distinct globular domains (19). Domain I is responsible for DNA binding of the enhancer element and the Mu ends (20, 21). Domain II can be divided into two functionally distinct and complementary subdomains: domain ΙΙα, which contains the conserved DDE motif believed to coordinate the divalent metal ion (22, 23), and domain ΙΙβ, which is involved with transpososome assembly and may bind DNA (24, 25). Domain III also contains two subdomains, domain ΙΙΙα and domain ΙΙΙβ. Domain ΙΙΙα has functional similarities to domain ΙΙβ, and they may both constitute a single functional domain (24–26). Domain IIIβ is a protein-protein interaction region that contains sequences bound by Mu B and ClpX (12, 17, 27, 28).

The 312-residue phage-encoded protein Mu B is an ATP-dependent DNA-binding protein required for efficient Mu DNA transposition (16, 29). Mu B is able to capture target DNA and interact with all of the transpososome complexes (15). Mu B is
also an allosteric activator of the transposase and can stimulate transpososome formation on both mutant donor DNA substrates and with partially functional mutant forms of Mu A (17, 26, 30–32). Along with target capture and transpososome formation, Mu B is also responsible for preventing Mu from transposing into itself, a process known as target immunity. During target immunity, Mu A stimulates the release of Mu B bound to DNA through the hydrolysis of ATP (33–35).

The Mu B protein has two globular domains (36). The 25-kDa N-terminal domain contains nonspecific DNA binding activity and has an ATPase motif (29, 36). The 11-kDa C-terminal domain is also able to bind nonspecifically to DNA (40). Site-directed mutagenesis of the N-terminal bipartite nucleotide binding motif (Walker A and Walker B boxes) results in the loss of ATPase activity (37). However, the isolated N-terminal domain by itself is unable to hydrolyze ATP (36). Mutations in the N-terminal domain of Mu B have been shown to affect target capture, but these mutants retain their ability to interact with the transpososome complex (37, 38). A small truncation of the C-terminal domain of Mu B blocks replicative transposition but not integration, whereas longer truncations affect both replicative and integrative transposition in vivo (39).

The C-terminal domain of Mu B (Mu B223–312) has a four-helix bundle (Fig. 2) and has a similar fold to that of the N-terminal region of DnaB (see Ref. 40). Examination of the charged residues within the peptide revealed that there is a positively charged patch of amino acids on the surface of the structure. It was proposed that this patch may be involved in the DNA binding activity that has been associated with the C-terminal domain. In this study, we first confirmed that these residues are responsible for binding DNA, and then we investigated how these positively charged residues contribute to the in vitro DNA binding activity.
transposition reaction. We found that by changing three lysine residues in the C-terminal domain of Mu B to alanine residues, we generated a mutant protein that could no longer capture target or interact with the Mu transpososome. Peptide binding experiments further reveal that this region of Mu B can participate directly in Mu A-Mu B protein-protein contacts.

EXPERIMENTAL PROCEDURES

DNA, Reagents, and Enzymes—The 6.5-kb mini-Mu plasmid pH80 has been previously described (6). The 7.2-kb mutant mini-Mu plasmid pH9251A has an A to G transition at the Mu left end terminal base pair (32). The target DNA was used as the 5.2-kb plasmid pH80. All plasmids were purified using a cesium chloride gradient and dialyzed extensively.

Mutagenesis and Protein Purification—The HU (41) and IHF (42) proteins were purified as previously described. Mu A proteins were purified as described previously (43) through the P-11 step. No dithiothreitol was added to the wash step or the elution step of the P-11 column, and the protein was induced in the presence of 1% (w/v) glucose, which improved the induction and purity after the P-11 column.

To introduce mutations into Mu B223-313 and the full-length Mu B genes, site-directed mutagenesis was performed using the QuickChange site-directed mutagenesis kit purchased from Stratagene, with a modified protocol (48). Two complementary oligonucleotides containing the desired mutation sequence were purchased (Sigma). Two separate 25-μl PCRs were set up (as per standard QuickChange protocol) containing 250 pmol of each mutant primer in separate reactions with 50 ng of wild-type Mu B expression plasmid DNA. Each separate PCR was amplified for three cycles of 95 °C for 30 s, 55 °C for 30 s, and 68 °C for 12 min with 0.5 units of Pfu Turbo DNA polymerase. The two amplification reactions were then combined to create a 50-μl PCR containing both primers and their amplification products from the first three cycles. This new reaction was supplemented with an additional 0.5 units of Pfu Turbo DNA polymerase, and the reaction was run for 18 cycles of 95 °C for 30 s, 55 °C for 30 s, and 68 °C for 12 min, followed by an additional extension for 7 min at 68 °C. The amplification product was 312 nucleotides in length. The PCR products were then digested with DpnI for 90 min at 37 °C and then transformed into electrocompetent DH5α cells. The mutant plasmids were then purified and sequenced.

Mutagenesis and purification of the Mu B223–313 gene was performed using the Mu B223–313 gene expression construct, pH805 from the strain GC1876, described previously (40). One liter of cells was grown in LB in a 4-liter Fernbach flask in the presence of 50 μg/ml ampicillin and 1% (w/v) glucose at 37 °C, with a rotation of 225 rpm, to an A590 of 0.65. The flask was then transferred to 18 °C, with a rotation of 225 rpm and incubated for 45 min, and the cells were induced with 1 μM isopropyl-1-thio-β-D-galactopyranoside for 18 h. The cells were harvested by spin- ning the cells at 3,000 × g for 10 min, washed, and suspended in 20 μl of Tris-HCl, pH 7.5, plus 5 μM EDTA. The solution was 312 nucleotides in length. The eluate was digested with 1 unit of thrombin/mg of protein for 45 min at room temperature. The digested protein was then filtered (0.22-μm filter) and loaded (1 μl/min) onto a 1-ml Resource H column (Amersham Biosciences), equilibrated with 25 mM Tris-HCl, pH 7.5, using a high pressure liquid chromatograph (Waters). The native 312 peptide was eluted from the Mono 15S column using a 0–700 mM NaCl (Tris-HCl, pH 7.5) gradient for 40 min at a flow rate of 1 ml/min. Peak fractions were collected and dialyzed against 20 mM phosphate, pH 6.8, plus 1.5 μM NaCl.

Mutagenesis and purification was performed on the full-length construct using the Mu B gene expression plasmid pH802 (38). Mu B was purified as previously described (32) with the following changes: the ethanol pellet was resuspended in 25 mM Tris-HCl, pH 8.8, plus 1 μM EDTA, 10 mM MSH, and 7.0 μl of water, and the DEAE-Sepharose column was equilibrated with 25 mM Tris-HCl, pH 8.8, plus 1 μM EDTA, 10 mM MSH, and 6.2 μl of water and eluted with 25 mM Tris-HCl, pH 6.8, plus 1 mM EDTA, 10 mM MSH, and 6.2 μl of water. The CM-Sepharose column was equilibrated with 25 mM Tris-HCl, pH 6.8, plus 1 μM EDTA, 10 mM MSH, 6.8 μl of water, and 25 mM NaCl, and the protein was loaded and washed with 4 ml of equilibration buffer before being eluted with 7 ml of 25 mM Tris-HCl, pH 7.4, plus 1 μM EDTA, 10 mM MSH, 6.8 μl of water, and 100 mM NaCl. The 3K protein was purified as described above using SP-Sepharose instead of CM-Sepharose. All of the proteins were greater than 95% pure with the exception of the 3R protein, which was greater than 90% pure as judged by SDS-PAGE.

DNA Binding Assays—The Mu B223–313 DNA binding assays were performed using various concentrations of peptide (described in the legends of Figs. 3 and 5) in the presence of 25 mM Hepes-NaOH, pH 7.6, 150 mM NaCl, and 15 μg/ml DNA (pSD7). The reactions were incubated at 30 °C for 10 min.

Affinity co-electrophoresis was performed essentially as described previously (44). Protein was embedded into 1% LMP agarose and 32P-end-labeled 70-bp double-stranded DNA was run through the gel. The final protein concentration in the gel varied between 22.5 nM and 1.44 μM as described throughout. The gels and electrophoresis buffer contained 25 mM Tris-HCl, pH 8.0, 0.1 mM mg bovine serum albumin, 10 mM magnesium acetate, 50 mM potassium acetate, and 0.5 mM ATP. Gels were run at 4 °C with circulating buffer at 4.2 V cm−1 for 5 h. The gels were dried and scanned using a PhosphorImager S and quantified using ImageQuant software from Amersham Biosciences. The fraction of DNA bound was taken as the percentage of radioactivity in the top half of each lane.

In Vitro Reactions—The Type 2 reactions were performed under standard reaction conditions (1×): 15 μg/ml supercoiled mini-Mu plasmid, 3 mg/ml (44 nm) Mu A protein, 3.5 mg/ml (315 nm) HU, 0.2 μg/ml (18 nm) IHF, 5 mg/ml (140 nm) Mu B, 2 μM ATP, 20 μg/ml target DNA, 25 mM Hepes-NaOH, pH 7.6, plus 140 μM NaCl and 10 mM MgCl2. Reactions were incubated at 30 °C for various amounts of time as described in the legends of Figs. 4 and 6. The intramolecular strand transfer reactions were performed at 2.5× reaction conditions in the absence of target DNA at Mu B concentrations of 140 and 280 nm as indicated.

ATPase Assay—The ATPase assays were performed essentially as previously described (16). Reactions contained 35 μg/ml (980 nm) Mu B, 1 μM (4.3 μM) IHF, 50 μM MgCl2, 3 mg/ml bovine serum albumin, 0.1 μM (5 μM) ATP (20 μc), and 25 mM Tris, pH 7.8, and, where indicated, 40 μg/ml (586 nm) Mu A and 50 μg/ml pSD7. The reactions were incubated at 25 °C for 60 min and stopped by the addition of 1 ml of activated charcoal (16%/w/v Norit A in H2O). The reactions were incubated on ice for 10 min in a 1-ml Eppendorf tube and centrifuged for 10 min at 30,000 × g in a table top centrifuge. Following centrifugation, the supernatant was filtered through a 0.22-μm filter and was analyzed.

Electrophoresis and DNA Quantification—Electrophoresis for the in vitro reactions was in TAE buffer in 1% agarose horizontal slab gels (200 mm in length) run as described in the Fig. 4 legend. Loading dye (2 μl 0.1% 100× TBE, plus 1 μl 1× TBE, 5% [v/v] sucrose, and 0.08% [w/v] bromphenol blue) was visualized by staining gels with ethidium bromide (0.5 μg/ml) for 30 min followed by a 30-min destaining in water. Gel documentation and quantification of fluorescent intensity made use of a camera and Alphaimager Software (version 4.03) from Alpha Innotech Corp. (San Leandro, CA).

Peptide-Mu A Binding—A cellulose filter containing synthetic peptide corresponding to the entire Mu B protein sequence was purchased from the Massachusetts Institute of Technology Biopolymers facility using an Abimed instrument. Each peptide consisted of 12 residues of Mu B sequence plus two additional β-alanines at the C-terminal end and was covalently attached to the filter via a C-terminal polyethylene glycol linker. The filter was blocked essentially as described (45), except that the buffer was as follows: 10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol, 10 mM MgCl2, 5% glycerol, 0.1% Tween. For blocking, this solution also contained 0.1% nonfat powdered milk, whereas for Mu A binding, it contained ~1 μM Mu A or Mu A-α-fib.

RESULTS

DNA Binding Activity of the C-terminal Domain Mutant of Mu B223–312 Peptide—The NMR solution structure of the Mu B223–312 peptide revealed the presence of a large number of positively charged residues on the surface of the structure (40). Three lysine residues (positions 233, 235, and 236) located near the N terminus of helix one were predicted to be part of a potential DNA binding patch. To analyze whether these residues affect nonspecific DNA binding, lysines 233, 235, and 236, along with
lysine 284 (not predicted to be part of the patch) was changed to alanine residues (K233A-Bc, K235A-Bc, K236A-Bc, and K284A-Bc). A triple alanine substitution was also made, incorporating the changes at positions Lys 233, Lys235, and Lys236 (3K-Bc). The K233A-Bc, K235A-Bc, and the K236A-Bc showed reduced DNA binding relative to wild-type peptide (Wt-Bc). The K284A-Bc peptide, which was not predicted to be part of the DNA binding pocket, showed DNA binding equivalent to wild type. The 3K-Bc peptide, however, did not show any detectable DNA binding at even the highest peptide concentration. To determine whether the 3K-Bc peptide was properly folded, far UV circular dichroism was performed on the Wt-Bc and the 3K-Bc peptides; these spectra contained similar absorbance peaks at 208 and 222 indicative of an α-helical protein (data not shown). The failure of the 3K-Bc peptide to bind DNA therefore did not appear to be the result of an overall change in the secondary structure of the peptide.

**Type 2 Formation with the Full-length C-terminal Mu B Mutants**—To further analyze the role of the DNA binding activity of the C-terminal domain of Mu B, identical mutations from the Mu B 223–312 peptides were made in the full-length protein. The C-terminal mutant proteins were analyzed for their ability to promote Type 2 complex formation (Fig. 4). The mutant proteins K233A, K235A, and K236A all displayed robust levels of Type 2 complex formation but at somewhat different efficiencies; K235A and K236A showed somewhat decreased efficiency of Type 1 to Type 2 conversion. The 3K mutant, however, did not form any Type 2 complex under standard reaction conditions. Interestingly, the single mutant proteins also formed varying amounts of intramolecular strand transfer products. In contrast, the 3K mutant did not stimulate any intramolecular strand transfer, suggesting that this protein may be profoundly defective in interacting with the transposase tetramer.

**DNA Binding Activity of the Full-length C-terminal Mu B Mutants**—To analyze the effect that the C-terminal mutations had on DNA binding of the full-length Mu B protein, affinity co-electrophoresis assays were performed (see “Experimental Procedures”). Affinity co-electrophoresis assays involve embedding various amounts of protein in an agarose gel and electrophoretically passing radiolabeled DNA through the embedded gel. This assay is particularly useful for studying interactions of Mu B with DNA. Full-length Mu B protein has a tendency to aggregate, and its DNA binding activity is not easily measured with other assays (37). DNA binding was determined by the ability of the embedded proteins to complex with the DNA and inhibit migration through the gel. The protein concentration required for half-maximal DNA binding was calculated in the presence of ATP and was 85 nM for wild-type protein, 90 nM for K233A, 136 nM for K235A, 100 nM for K236A, and 623 nM for the 3K full-length proteins. Single residue mutations on the full-length protein, therefore, displayed only a slight decrease in DNA binding affinity compared with the wild-type protein. In contrast, the 3K mutation had over a 7-fold decrease in affinity for DNA. These results were qualitatively similar with those from the gel shift assays with the mutant peptides (Fig. 3B) and suggest that each of these mutations have a modest affect on the ability of the full-length Mu B to interact with DNA, whereas the triple mutant has a much more profound affect.

**Stimulation of Strand Cleavage Using the Full-length Mu B Mutants**—Mu B suppresses the defects in assembly and cleavage observed with certain partially functional mutant substrates (31, 46, 47) and Mu A derivatives (25, 26, 30). To further
investigate the ability of full-length mutant Mu B proteins to stimulate the Mu A tetramer, we performed intramolecular strand transfer reactions with both substrate and transposase mutants (Fig. 6). The intramolecular assays allow for investigation of Mu B stimulation of strand transfer by Mu A, regardless of whether or not the Mu B protein can directly bind to the relaxed donor DNA, is an unstable form of the Type 2 complex where the target DNA is still supercoiled. The nomenclature for the mutant proteins is described in Fig. 3, except that all of the mutations are present in the full-length protein.
target DNA. The mutant substrate pMS9A1 (pA1GL) contains a single nucleotide change from A to G at the left end terminal base pair that supports a 20% level of DNA cleavage under normal reaction conditions (31, 46). Wild-type Mu B stimulated the cleavage reaction on this substrate over 3-fold. K233A stimulated DNA cleavage as well or better than wild-type Mu B. K235A and K236A stimulated the reaction of pMS9A1 over 2-fold. The triple mutant 3K, however, did not stimulate the reaction to any significant extent.

Mu B can also stimulate catalytically defective transposase mutants (25, 26, 30). The Mu A\textsubscript{ban} protein is a quadruple oligomerization domain (32) that performs DNA cleavage at less than 20% the efficiency observed for the wild-type Mu A under standard reaction conditions. Wild-type Mu B, K233A, K235A, and K236A all stimulated the reaction with this mutant transposase over 2-fold. The 3K mutant, however, did not stimulate the reaction to any significant extent.

The 3K mutant protein is compromised in its ability to interact with the Mu A tetramer.

**ATPase Activity of the Mu B Mutants**—The N-terminal domain of Mu B has been shown to be responsible for the ATP hydrolysis activity of the full-length protein (37). To determine whether the C-terminal domain mutants caused any major conformational changes to the structure of Mu B, the mutant proteins were tested for their ability to hydrolyze ATP (Fig. 7). All of the full-length mutant Mu B proteins hydrolyzed ATP at a rate that was equal to or greater than that observed with the wild-type protein, suggesting no major conformational defects in the overall structure of the proteins. The reason for the apparent increase in ATPase activity displayed by the mutant proteins is not entirely clear, but it may result from variability in the amount of active Mu B present in each protein preparation. The final step of the Mu B purification procedure requires that the protein be refolded from its denatured state (see "Experimental Procedures"). It has been noted that up to 50% of the Mu B protein is inactive after purification (35). Since the ATPase activity of Mu B is highly cooperative, small changes in the amount of active protein could lead to large differences in ATPase activity.

The ATPase activity of the mutant Mu B proteins was stimulated by the addition of either Mu A or DNA (data not shown) but most significantly by the addition of both of these factors (Fig. 7). These results suggested that Mu A and DNA could still interact with Mu B to stimulate ATP hydrolysis. These data therefore suggest that: 1) the interactions between the mutant Mu B protein and Mu A and DNA are stabilized under ATPase assay conditions or 2) that the Mu A and DNA interactions required for stimulation of the Mu B ATPase activity are different or less demanding than the interactions needed to stimulate the transposase tetramer, since no allosteric activation of Mu A was observed with the 3K mutant (Figs. 4 and 5). The ATPase activity of Mu B is also stimulated by the oligomerization of the protein and Mu B has been shown to oligomerize in the presence of ATP or ATP\textsubscript{γS} (35). To confirm the ability of the Mu B mutants to oligomerize, their ATPase activity was shown to be concentration-dependent (data not shown). To further demonstrate protein oligomerization, we incubated the Mu B variants with a chemical cross-linking reagent in the absence and presence of either ATP or ATP\textsubscript{γS}. We found that the mutants formed oligomers in the presence of ATP or ATP\textsubscript{γS} (data not shown). These results confirm that the mutant Mu B proteins retained the ability to interact with themselves and further suggest that the mutational changes did not cause any major defects in the overall structure of the proteins.

**The Important Lysines Lie within a Region of Mu B That Interacts with Mu A**—The results presented above indicated

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1 The abbreviation used is: ATP\textsubscript{γS}, adenosine 5'-(3-thiotriphosphate).
that the positively charged region in the C-terminal domain of Mu B is involved in Mu B-DNA interactions but also suggest that this region may be involved in protein-protein interactions between Mu A and Mu B. To directly determine regions of the Mu B protein that have the capacity to bind to Mu A, we constructed a library of 12 amino acid peptides representing the entire Mu B protein sequence immobilized on a solid support. This peptide filter was then incubated with Mu A, and peptide-Mu A complexes where detected using anti-Mu A antiserum (Fig. 8). Although several regions of the Mu B sequence clearly bound Mu A in this experiment, the strongest interaction was observed with peptides initiating at positions Glu217–Lys235. This region corresponds well to the N-terminal portion of the C-terminal domain, the region containing the positively charged patch. Interestingly, the peptide initiating with Lys235 was much poorer at Mu A binding than its neighbor, which carried both Lys235 and Lys236, indicating either that the presence of both lysines, or Lys236 in particular, is critical for Mu A binding. Control experiments in which Mu A residues 217–615 was used in place of wild-type Mu A revealed much less interaction with this family of peptides (data not shown), indicating that the observed binding was mediated by the C-terminal domain of Mu A.

**DISCUSSION**

The bacteriophage Mu B protein is an ATP-regulated DNA binding protein that makes protein-protein contacts with the Mu A transposase to stimulate transposition and control target site choice. Partial proteolysis studies have previously shown that Mu B has two domains, an N-terminal domain of 25 kDa and a C-terminal domain of 11 kDa (36). Analysis of the solution structure of the Mu B223–312 peptide revealed a positively charged patch on the surface of the C-terminal domain. It was proposed that these residues may be responsible for DNA binding (40). We found that single residue alanine substitutions at Lys233, Lys235, and Lys236 slightly decreased the DNA binding activity of the Mu B223–312 peptide and that a triple mutation of these three residues completely inhibited DNA binding. Likewise, the DNA binding activity of the full-length 3K mutant protein was over 7 times weaker than that of the wild-type protein. Furthermore, functional assays of Mu B-stimulated transpososome activity and physical Mu A binding experiments revealed that this region of Mu B makes important contacts with the transposase. These results strongly suggest that this region in the C-terminal domain of Mu B plays an important role in both the protein-DNA and protein-protein contacts needed to regulate transposition.

Most of the previously characterized Mu B mutants appear to weaken the protein’s affinity for DNA without strongly compromising its ability to interact with Mu A and allosterically activate the transpososome. Mutations in this class include those within or near the ATPase motif in the N-terminal domain (e.g. K106A, C99Y, and an insertion at residue 101) (37, 38, 46). It is not known whether these mutations are directly inhibiting DNA binding or if the reduced DNA binding activity of these mutant proteins is a result of other conformational changes related to the ATP-binding pocket. In addition, a Mu B mutant lacking residues 295–312 from the C-terminal region of the protein is also in this functional class (50). Interestingly, this protein is able to promote integrative but not replicative transposition in vivo (39, 50).

In contrast, the 3K mutant described here is defective in both DNA binding and Mu A interaction. Initial analysis suggests that these defects may be a direct result of the amino acid changes, rather than an indirect effect on the folded state of the protein. In support of this conclusion, peptide binding experiments revealed that the major region of Mu B that binds to Mu A resides between residues 217 and 235, a segment that corresponds to the N-terminal region of domain II. Thus, this analysis has added a useful component to our understanding of the structure/function map of Mu B. Interestingly, the Mu B223–312 peptide has structural similarities with the N-terminal domain of the helicase DnaB. Our findings suggest that these two domains may also have functional similarities, since the N-terminal domain of DnaB was also shown to be responsible for protein-protein interactions with the primase DnaG (48, 49). It is also of interest that lysines 233, 235, and 236 are in a region of Mu B that is disordered but near the start of helix 1 in the solution structure (40). Interaction of this region with Mu A (and/or DNA) may help to stabilize an ordered structure in this region of the protein.
An interesting feature of the ATPase activity of the mutant Mu B proteins is that they are stimulated by the combined presence of Mu A and DNA. These findings seem contradictory to our results showing that the mutant proteins had decreased DNA binding activity and that the 5K mutant could not interact with the transpososome. This discrepancy is best explained in one of two ways. The first explanation is that Mu B interactions with DNA and Mu A are stabilized under ATPase assay conditions. Since the ATPase activity of Mu B is quite weak, ATPase assays (relative to transposition assays) are typically performed under higher Mu B concentrations for a longer incubation period and in the presence of glycerol and bovine serum albumin. These conditions are known to rescue a variety of Mu transposition defects. Another explanation may be that the Mu A-Mu B interactions that occur during the stimulation of the ATPase activity are different from the Mu A-Mu B interactions required for recruitment of target to the transpososome or for stimulation of strand transfer. Consistent with this hypothesis, we find several distinct regions of Mu B that interact specifically with Mu A, as assayed using the peptide library. It has been proposed that monomers of Mu A can stimulate ATP hydrolysis and DNA dissociation of Mu B from DNA (34, 51); however, only a multimer of Mu A has been shown to form a stable target capture complex with Mu B. The stimulation of the ATPase activity by Mu A and DNA may therefore reflect differences between the Mu A-Mu B interactions required to cycle on and off of DNA during target immunity and the Mu A-Mu B interactions required during target capture by the transpososome. It is not clear from our studies whether the positive residue patch directly binds Mu A and DNA or whether mutation of these residues is causing other local conformational changes which inhibit these interactions. However, the peptide studies for both DNA binding and Mu A binding suggest that both activities may be direct.

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