Analysis of P Element Transposase Protein-DNA Interactions during the Early Stages of Transposition*

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P elements are a family of transposable elements found in Drosophila that move by using a cut-and-paste mechanism and that encode a transposase protein that uses GTP as a cofactor for transposition. Here we used atomic force microscopy to visualize the initial interaction of transposase protein with P element DNA. The transposase first binds to one of the two P element ends, in the presence or absence of GTP, prior to synopsis. In the absence of GTP, these complexes remain stable but do not proceed to synopsis. In the presence of GTP or nonhydrolyzable GTP analogs, synopsis happens rapidly, whereas DNA cleavage is slow. Both atomic force microscopy and standard biochemical methods have been used to show that the P element transposase exists as a pre-formed tetramer that initially binds to either one of the two P element ends in the absence of GTP prior to synopsis. This initial single end binding may explain some of the aberrant P element-induced rearrangements observed in vivo, such as hybrid end insertion. The allosteric effect of GTP in promoting synopsis by P element transposase may be to orient a second site-specific DNA binding domain in the tetramer allowing recognition of a second high affinity transposase-binding site at the other transposon end.

Mobile genetic elements are ubiquitous among both prokaryotic and eukaryotic organisms (1). Genome sequencing projects have shown that transposable elements make up a substantial fraction of eukaryotic genomes, including 49% of the human genome (2, 3). These mobile elements can lead to mutations and genome rearrangements and appear to play a role in genome evolution (4, 5). The mechanisms by which transposons are mobilized can be grouped based upon whether there is a DNA or an RNA intermediate (1, 4, 5). P elements use a cut-and-paste mechanism with a DNA intermediate, related to those used by the Tn5, Tn10, and Tn7 prokaryotic transposons (16); for the mariner family transposase, Mos1 is a dimer or tetramer (22–25); and for the mariner family transposase, DNA binding actually promotes tetramer formation (17–19). In V(D)J recombination, a dimeric complex of RAG2 and a dimer or trimer of RAG1 protein recognizes the recombination signal sequence (20, 21); for the Hermes transposase a hexamer is the active species (16); for the mariner family transposase, Mos1 is a dimer or tetramer (22–25); and for the mariner family member, Himar 1, is a tetramer (26). For Tn5 and Tn10, the protein acts as a dimer, with a single active site acting in trans at each transposon end (27–30). Thus, it has been of interest to understand the oligomeric state of transposase during P element transposition.

Here we use atomic force microscopy (AFM)2 to visualize and quantitate the initial transposase protein-DNA complexes formed on P element DNA ends in the absence of GTP, prior to synopsis of the P element termini. Biochemical methods show that P element transposase exists as a tetramer in solution, in the absence of DNA. We have also used AFM to determine the volume of the transposase protein, in the absence and presence of DNA, and we used this information to determine the native

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This paper is dedicated to our friend and colleague, Nick Cozzarelli.

‡ The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1 and 2.

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2 The abbreviations used are: AFM, atomic force microscopy; DTT, dithiothreitol; GTPγS, guanosine 5′-3′-(thio)triphosphate; GMP-PCP, guanylyl β,γ-methyleneimido[triphosphate; GTP-PNP, guanosine-5′-[(β,γ-imido)triphosphate; LM-PCR, ligand-mediated PCR.

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molecular weight of the protein by comparison with the relative volumes of a set of standard proteins; these measurements also indicate that the P element transposase exists as a tetramer in both conditions. This method of AFM protein volume determination has been used previously to analyze the size of protein-DNA complexes involved in transcription and DNA repair (31, 32). Our AFM data indicate that a pre-formed transposase tetramer initially interacts with either P element terminus, in the absence of GTP, prior to GTP-dependent synaptic complex formation. These studies also show that synapsis is rapid with GTP or non-hydrolyzable GTP analogs but that donor DNA cleavage occurs more slowly. These studies show initial binding of a pre-formed tetramer of P element transposase to one P element end and suggest that the GTP cofactor somehow orients a second DNA binding domain in the oligomer to interact with the high affinity transposase-binding site near the other P element end leading to synaptic complex formation. This two-stage binding/synapsis might explain how inappropriate pairing in vivo of two P element ends from different P elements might give rise to ectopic chromosomal rearrangements sometimes observed, such as hybrid ends insertions and deletions (33–35).

EXPERIMENTAL PROCEDURES

Protein Standards for Protein Volume Measurements—RNA polymerase holoenzyme was kindly provided by Dr. Caroline Kane (University of California, Berkeley). Alcohol dehydrogenase, bovine serum albumin, and thyroglobulin were purchased from Sigma.

Protein Purification and Excision Activity Assay—P element transposase tagged at the C terminus was purified as described previously (10, 14), and activity assays by LM-PCR to detect donor DNA cleavage were performed as described (11).

DNA Preparation—The DNA substrate, containing a 0.6-kbp P element transposase protein binds as a pre-formed tetramer to one of the two P element ends prior to synapsis. A, diagram of P element excision and integration; B, upon brief incubation of the protein with DNA (1–30 min), transposase protein binds to either one of the two transposon ends of a 629-bp P element flanked by 300 and 600-bp DNA tails (300–600). Diagrams at the top of each panel indicate the position at which the transposase binds along the DNA molecule. The transposase proteins bound to DNA are indicated by white arrows. These images were from reactions performed in the absence of GTP.
element flanked by 0.3- and 0.6-kb adjacent non-P element DNA, was prepared as described (14). The DNA substrate, containing 2.9-kb full-length P element flanked by 0.2- and 0.3-kb adjacent non-P element DNA, was prepared the same way using Prr25 as a template (36), and high fidelity Taq polymerase was used in the PCR step.

Transposase Assembly and Excision Reactions—Assembly and excision assays were carried out as described (9, 11, 14, 37). P element donor cleavage reactions were carried out by mixing 1 µl of purified 3′ polyoma epitope-tagged transposase (~50 µg/ml) in HEGK buffer (25 mM Hepes-KOH, pH 7.6, 20% glycerol, 0.5 mM DTT, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.4 mM KCl) with 1 ml of 50 nM P element donor DNA and 4 ml of HEGK buffer (25 mM Hepes-KOH, pH 7.6, 20% glycerol, 0.5 mM DTT, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride). After incubation on ice for 10 min, the mixture was added to 14 µl of 10 mM Mg(OAc)2 in HEGK buffer, with or without 2 mM GTP, and incubated at 30 °C for the indicated times. The final protein concentration of protein and DNA in the 20-µl assay was 28.5 and 2.5 nM respectively. These reaction conditions are similar to those used previously for donor cleavage and strand transfer in bulk solution reactions (11, 37). Thus, in the DNA binding reaction the DNA concentration was 2.5 nM, and the protein concentration (as a monomer) was typically ~28.5 nM giving a ratio of 11 monomeric proteins per DNA molecule. In the GTP analog experiments, GTP, GMP-PCP, and GMP-PNP were used at a final concentration of 2 mM.

Gel Filtration Chromatography—Approximately 3.5 µg of immunoaffinity-purified transposase protein was resolved on a Superdex 200 PC3.2/30 column (GE Healthcare) on a SMART system at 4 °C in HEGK buffer (25 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 300 mM KCl, 0.5 mM DTT, and 10% glycerol) at a flow rate of 40 µl/min. 40-µl fractions were collected and assayed by SDS-PAGE and immunoblotting (10, 37). Standard proteins used to calibrate the column were bovine serum albumin (66 kDa), catalase (232 kDa), and thyroglobulin (669 kDa) and were run in the same buffer as transposase.

Glycerol Gradient Velocity Sedimentation—Glycerol gradient centrifugation (38) was carried out in a 4-ml gradient with an SW60 rotor at 42,000 rpm for 16 h at 4 °C, in an L90M ultracentrifuge (Beckman). Approximately 3 µg of transposase was layered on top of a freshly poured gradient in HGKD buffer (25 mM Hepes-KOH, pH 7.6, 1 mM EDTA, 300 mM KCl, 0.5 mM DTT, and various % glycerol as indicated in Fig. 3, B and C). 150-µl fractions were collected from the top and assayed for transposase by SDS-PAGE and immunoblotting (10, 37). Standard proteins used to calibrate the column were bovine serum albumin (66 kDa), catalase (232 kDa), and thyroglobulin (669 kDa) and were run in a parallel gradient in the same buffer as transposase. For the experiment with GTP or GTP and strand transfer oligonucleotide (P1/P2–17, 37), 0.5 mM GTP and 1 mM Mg(OAc)2 were preincubated with ~3 µg of transposase at room temperature for 15 min in gradient buffer or 0.5 mM GTP and 1 mM Mg(OAc)2 along with a 10-fold molar excess of P element 3′ end strand transfer oligo. Following the preincubation, the samples were loaded on top of 4-ml glycerol gradients and centrifuged as above.

RESULTS

Initial P Element Transposase-DNA Complexes Form at One Transposon End Prior to Synapsis—The P element transposase-DNA complex formation reaction occurs in stages, with initial transposase binding, synapsis, nonconcerted DNA cleavage, and the target capture and integration (Fig. 1A). Our previous studies have shown that P element transposase binding to its internal site-specific recognition sites on P element DNA is not affected by the presence or absence of GTP, but GTP can promote synopsis of P element ends (14). Therefore, we were interested in determining how the P element transposase interacted with the P element termini prior to synaptonal complex formation. To investigate the early protein-DNA complexes that formed from 1 to 30 min, transposase protein was incubated with a linear DNA carrying a 0.6-kb P element flanked by flanking sequences of 300 bp at the 5′ end and 600 bp at the 3′ end (Fig. 1B, top). Upon brief incubation of the protein and DNA (1–30 min) at a molar ratio of ~11 transposase monomers to each P element DNA, the linear DNA was bound by transposase at either one end or the other (Fig. 1B) as evidenced by a protein-DNA complex formed with...
either a short (0.3 kb) or long (0.6 kb) tail. There appeared to be equal numbers of transposase bound to either the 5' or 3' transposon end, suggesting that the two ends have similar affinities for the protein, as had been suggested by earlier DNase I footprinting assays (15). In the experiments presented here, and those published previously (14), nonspecific DNA binding is seen to vary from experiment to experiment. However, in some cases transposase molecules were observed to be bound to DNA ends or at random to incorrect sites on the P element-containing DNA fragment (data not shown and see Ref. 14). These nonspecific binding events were 10–100-fold less abundant than site-specific DNA binding and this number is consistent with previous measurements of site-specific versus nonspecific DNA binding by the P element transposase made by DNase I footprinting (15). More importantly, even though the ratio of transposase monomers to P element DNA is ∼11:1, we did not observe two transposase complexes bound at the two transposon ends on the same DNA molecule, in over 100,000 DNA molecules examined that had transposase bound. Even when the transposase concentration was raised another 10-fold, no increase in either nonspecific binding or two transposase complexes on a single P element were observed (data not shown). Given the low occurrence of two ends being bound, it seems reasonable to propose that it is the single end-bound transposase complexes that proceed to synapsis. However, it may be the case that these stable one-end-bound complexes are stable, but more loosely bound protein-DNA complexes are lost during the sample preparation for AFM. It seems to be the case that once a single end of the transposon is bound by transposase, the second end cannot be stably bound by a second transposase complex. It may simply involve differential stability of protein-DNA interactions, since footprinting studies showed that the protein has high nonspecific DNA (15), yet nonspecific DNA binding is not frequently observed in the AFM assays (see Ref. 14 and this study).

Quantitation of these time course experiments, in the presence or absence of GTP, indicated that DNA binding by the transposase protein was rapid and, as had been observed using DNase I footprinting (9, 15), occurred in both the absence or presence of GTP (Fig. 2A). These experiments show that initial protein-DNA complexes form first on either P element end (Fig. 2A), and that they form prior to the GTP-dependent synapsis observed previously (14). These findings rule out the possibility of the transposase recognizing both P element ends and oligomerizing on the DNA. In fact, measurements indicate that a pre-formed tetramer binds to one P element end prior to synapsis (see below).

Time Course of the Formation and Reactivity of Early P Element Transposase-DNA Complexes—Our previous studies indicated that GTP allows the transposase to form synaptic complexes containing both P element ends at 30 min (14). However, we had not examined earlier time points. Given the observed single end binding (see Fig. 1 and Fig. 2A), we therefore quantitated formation of both synaptic and singly or dou-
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**A.**

- Fract. 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
- Glycerol gradient: 15% – 30%
- 669 kDa 322 kDa 66 kDa

**B.**

- Glycerol gradient: 15% – 30%
- Fract. 1 3 5 7 9 11 13 15 17 19
- 66 kDa 159 kDa 232 kDa 669 kDa

**C.**

- Glycerol gradient: 15% – 35%
- Fract. 1 3 5 7 9 11 13 15 17 19
- 66 kDa 159 kDa 232 kDa 669 kDa

**FIGURE 3. Biochemical fractionation of P element transposase by gel filtration chromatography and glycerol gradient velocity sedimentation.**

A, P element transposase chromatograms as a tetramer in a gel filtration experiment. About 3.5 mg of transposase was loaded onto the Superdex 200 column in 300 mM KCl buffer (see “Experimental Procedures”). Column fractions were monitored for the presence of transposase by SDS-PAGE followed by immunoblotting. Standard proteins used to calibrate the column were bovine serum albumin (66 kDa), catalase (232 kDa), and thyroglobulin (669 kDa), and their elution positions are indicated by arrows. Fraction (Fract.) numbers are indicated above the figure. Although the transposase protein seemed to be spreading across the column profile, perhaps because of interaction between the protein and the column matrix, the peak elution position (fractions 12–16) corresponded to an apparent molecular mass of ~350 kDa. B, P element transposase showed a single peak from glycerol gradient velocity sedimentation corresponding to an apparent molecular mass of ~170 kDa in several repetitions. Standard proteins (bovine serum albumin (66 kDa), alcohol dehydrogenase (159 kDa), catalase (232 kDa), and thyroglobulin (669 kDa)) were centrifuged in a parallel gradient. C, apparent size of P transposase determined by velocity sedimentation is not altered in the absence of GTP (top panel), in the presence of GTP (middle panel), or in the presence of GTP and an excess of a pre-cleaved strand transfer oligonucleotide (bottom panel). The centrifugation was performed as the same as in B. Fractions were taken and assayed by SDS-PAGE and immunoblotting. The locations and sizes of the standard proteins are marked below the bottom panel.

Purified Native Transposase Exists as a Tetramer and Is the Form That Interacts Initially with One P Element End—One universal observation about the transposase/integrase superfamily of polynucleotide transferases is that they invariably act as oligomeric enzymes, with at least two active sites, one positioned at each transposon end (1, 12). Because of the unusual nature of the P element cleavage site, a staggered break with a 17-nucleotide single strand extension (11), it was of interest to determine the oligomeric state of the transposase protein in the absence of DNA and when it initially interacts with one P element terminus. Therefore, we performed gel filtration chromatography on a Superdex 200 column in 300 mM NaCl to determine the native molecular weight of the protein, monitoring the column fractions by immunoblotting (Fig. 3A). The peak of the transposase protein in fractions 12–16 appears to be consistent with a tetramer of ~360 kDa (four 87-kDa monomer subunits). The protein also appears to interact nonspecifically with the resin, because there is a weak protein signal across the column profile. Velocity sedimentation in 15–30% glycerol gradients was also used to ascertain the native size of the transposase protein oligomer. In this experiment, the protein sediments with an apparent molecular mass of ~150–200 kDa (Fig. 3B). However, this mass is not consistent with either the gel filtration (see above) or the volume measurements by AFM (see below and Fig. 4). Other oligomeric protein complexes, such as *Drosophila* origin recognition complex, behave anomalously in velocity sedimentation gradients because of aberrant hydrodynamic properties (38). Nonetheless, both the gel filtration and gradient peak fractions are active for donor DNA cleavage using an LM-PCR-based assay (data not shown; see Ref. 14). More importantly, we also directly examined both the gel filtration and gradient peak fractions to visualize DNA binding and to take volume measurements. These direct measurements of the size-fractionated transposase showed that the protein in both the gel filtration and glycerol gradient fractions was the same size and that it was a tetramer when prepared for...
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AFM at pH 7 (see below and Fig. 4B). Additionally, the behavior of the protein in the gradients was not altered in the presence or absence of GTP or by a duplex oligonucleotide that corresponds to a pre-cleaved P element end that can be bound to the protein and is used as a substrate for strand transfer (37) (Fig. 3C). Taken together these data are consistent with the active form of the P element transposase being a tetramer and the oligomer being pre-formed in the absence of P element DNA.

As an independent means to determine the mass and oligomeric state of the transposase, we turned to AFM. AFM can be used to determine the volume of a protein, which by comparison to the volumes of a set of standard proteins can be used to determine the molecular weight of the protein. These sizes were determined by measuring the volumes of a set of standard proteins (bovine serum albumin, alcohol dehydrogenase, E. coli RNA polymerase, and thyroglobulin), plotting a standard curve and determining the relative molecular weight of the P element transposase sizes from the measured volumes (Fig. 4A). This type of molecular weight determination has previously been done for the DNA enhancer-binding protein NtrC and for other proteins (31, 39). Transposase protein, in the absence of DNA, was spotted on mica, rinsed with water, and imaged by AFM. The volumes of the protein species were determined and plotted as a function of numbers in the population (supplemental Fig. 1A). Three prevalent volume distributions were observed, consistent with monomer, dimer, and tetramer sizes, with dimeric and monomeric sizes predominating. However, we subsequently found out that low pH water washes of the mica grid prior to AFM imaging caused dissociation of the predominantly tetrameric protein into dimers and monomers (supplemental Fig. 2, A and B). This effect of low pH on the oligomeric state of transposase could also be seen with transposase-DNA complexes (supplemental Fig. 1B). However, and most importantly, when neutral pH (pH 7) wash water was used to image single end protein DNA complexes formed on P element DNA in the absence of GTP, either from the gel filtration column or glycerol gradient fractions, the predominant form of the protein bound to DNA was a tetramer (Fig. 4B), consistent with the observations of the protein in the absence of DNA (Fig. 4A and supplemental Fig. 1A and Fig. 2B) and the chromatographic behavior by gel filtration (Fig. 3A). Because in the presence of low pH water more monomers and dimers were observed compared with samples prepared with pH 7 water, we conclude that dissociation of tetrameric transposase complexes can occur during sample preparation with low pH water. These observations clearly show that sample preparation, and more specifically the pH of the water wash prior to imaging, could influence the level of the transposase oligomers but that the protein species that is active for transposition is a tetramer. In fact, LM-PCR assays for donor DNA cleavage (14), using both the peak gel filtration and glycerol gradient fractions that were imaged by AFM, indicate that these fractions, although dilute, exhibit donor DNA cleavage activity. Effects of pH of the water used in AFM sample preparation have been noted by others (40). A tetramer is formed even in the absence of P element DNA. Given the presence in the primary transposase amino acid sequence of a leucine zipper and a second protein interaction region (41, 42), as well as the staggered nature of the P element cleavage sites (11), it seems reasonable that the P element transposase might be active as a tetramer. Our results with the AFM volume measurements of the gel filtration and glycerol gradient fractions are indicative of higher order oligomerization of the transposase, in the absence of DNA, to a

FIGURE 4. Distribution of P element transposase oligomeric states. A, molecular weight determination of transposase oligomeric states. A standard curve was obtained by measuring the volumes of four different proteins as follows: bovine serum albumin (66 kDa), alcohol dehydrogenase (140 kDa), E. coli RNA polymerase holoenzyme (479 kDa), and thyroglobulin (669 kDa). Both transposase and standard proteins were imaged separately and measured the same way. The red squares along the standard curve represent the predicted volumes for the monomeric, dimeric, and tetrameric oligomeric states of transposase; the yellow triangles represent the volumes experimentally measured. The molecular masses corresponding to the measured volumes are as follows: 87 kDa (monomer), 174 kDa (dimer), and 348 kDa (tetramer). Samples of P element transposase were deposited on freshly cleaved mica for 1 min, rinsed with water, and imaged by AFM. The volume measurements were carried out according to the protocol developed by V. Holmes (56). B, peak protein fractions from both the gel filtration chromatography and glycerol gradient velocity sedimentation experiments showed predominantly tetramers by AFM. Under our experimental conditions (a 30-min pre-incubation of the protein fraction with P element DNA in the absence of GTP), we observed the majority of the transposase protein binding to a single P element DNA end without synopsis. Protein size (volume) was measured as described under “Experimental Procedures.” The bar graphs show that comparable multimeric states are observed in both fractions, with the majority of oligomers as the tetrameric form both the gel filtration and glycerol gradient methods of the protein size estimation.
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pre-formed tetramer and that the tetrameric form is what binds initially to one of the two P element ends prior to synapsis.

Correct Synapsis of Full-length 2.9-kb P Elements—Our previous studies by AFM of P element transposase used small, but mobile, internally deleted P elements (14) (see Fig. 1, above). We therefore wanted to see if we could visualize the synapsis of full-length 2.9-kb P elements, and at the same time, if we could visualize smaller looped DNA molecules that might represent molecules in the process of “sliding,” a possible mechanism by which DNA-binding proteins find binding sites on DNA (43, 44). This mode of DNA site recognition contrasts to intersegmental transfer in which repeated collisions between proteins bound at one site and other parts of the DNA allow a bound protein to locate a second binding site (43, 44). Therefore, a 3.4-kb DNA was amplified carrying a full-length 2.9-kb P element along with 0.2- and 0.3-kb flanking DNA “tails” (200–2900-300), and this DNA molecule was used to form synaptic complexes with P element transposase. Although these images are more difficult to analyze because of the “crossing” of the long internal portion of the P element DNA, we did observe normal synaptic complexes with the appropriate tail length and the correctly sized P element segment (Fig. 5A). However, quantitation of these synaptic complexes at 30 min showed only 1.3% correctly synapsed molecules (Fig. 5B), in contrast to 16.7% for the 0.6-kb P element (300–600-600) (see Fig. 2B, above). Additionally for this long DNA, although essentially no incorrectly sized loops (i.e. one site bound with an incorrect loop size) were observed, initial time points indicated nonspecific complexes (loops with neither tail of the correct length) were formed (4.47%), which dissociated over time (0.42%) (Fig. 5B). Because in these complexes there is no tail length of the correct size for engagement at one of the two P element ends, we believe these represent nonspecific protein binding, which has also been observed for this protein in DNase I footprinting assays (15). This type of incorrect complex was not observed (<0.01%) for the small 0.6-kb P element (data not shown). Thus, even with full-length P elements we can observe correctly synapsed molecules but at a lower frequency than when a smaller 0.6-kb P element was used (300–600-600) (14). See Fig. 2B and Fig. 3B. The two tails of the synaptic complexes are indicated by white arrows. B, single-site binding (i.e. one site at one P element end bound with an incorrect loop size), nonspecifically bound synapsed or looped complexes (i.e. transposase not bound at either P element end), and correctly synapsed molecules for the full-length 2.9-kb P element DNA quantitated as a percentage of the total DNAs observed at 30-min and 6-h incubation times.

Nonhydrolyzable GTP Analogs Support Normal Synapsis and Donor DNA Cleavage—Our initial characterization of the P element transposition in vitro showed that both GTP and nonhydrolyzable GTP analogs supported both catalytic steps of P ele-
Nonhydrolyzable GTP analogs support normal levels of transposase-mediated P element synapsis and excision activity. The results presented here show that the initial protein-DNA complexes formed between P element transposase and the transposon ends occur by interaction of a pre-formed tetramer with either one of the two termini. Many DNA-binding proteins that act on two sites on DNA are composed of multiple subunits. These proteins can often come either preassembled as oligomers, assemble on the DNA, or assemble on protein (43, 44). In the case of P elements, it appears that the initial contact of the protein with one end of the transposon DNA occurs via interactions of one site-specific DNA binding domain within the tetramer with one high affinity transposase-binding site located internally near the transposon terminus (15, 42). It is this pre-assembled oligomer that then synapses the P element ends, presumably by a second site-specific protein-DNA interaction at the opposite transposon end that carries a transposase-binding site (15, 42). Because of the unusual nature of the P element DNA cleavage, leaving a staggered double strand break, with a 17-nucleotide 3′ extension (11), it would seem to make sense that two catalytic domains would be required to cleave each transposon end, so that a tetramer would donate two subunits to each terminal donor DNA cleavage. Additionally, at high protein concentrations the truncated KP repressor protein, which carried the N-terminal THAP DNA binding domain, can also bind to the sub-terminal 11-bp inverted repeats (42). However, in footprinting assays with single transposon ends, the full-length transposase protein does not appear to stably interact with these internal repeats (15). Nonetheless, it seems possible that two DNA binding domains in the tetrameric transposase synaptic complex might interact with two DNA-binding sites at each transposon end to stabilize the complex.

In other transposon systems, different strategies are used to assemble synaptic complexes (12, 18, 45, 46). In the case of Tn5 and Tn10, a dimeric complex is active at the transposon ends (27–30), yet the state of the protein in solution and whether monomers bind each transposon end with subsequent synapsis or whether dimers bind to one end first is not known (29, 47). In the case of bacteriophage Mu, whereas each transposon end has three binding sites for the monomeric transposase, binding of monomeric subunits to these sites results in the DNA-induced assembly of an active and stable tetramer, in which two catalytically active subunits perform DNA cleavage and joining in trans (17, 19, 45, 48–51). It has been shown recently that the eukaryotic Hermes transposase exists in solution and in crystals as a hexamer (16). Another eukaryotic transposase, Mosl, seems to function as a dimer or tetramer (22–25), whereas a related mariner transposase, Himar 1, functions as a tetramer (26). Unlike the case of bacteriophage Mu, where a monomeric protein undergoes DNA-induced tetramer formation (18, 19, 49), we have no evidence for oligomerization of the P element transposase protein on P element DNA, because we consistently see what appears to be a tetramer on one of the P element ends prior to synapsis and in fact can visualize tetramers of P element transposase in the absence of DNA. Although the stoi-
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