

DNA-binding activity and subunit interaction of the *mariner* transposase

Lei Zhang, Angela Dawson and David J. Finnegan*

Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Edinburgh EH9 3JR, UK

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ABSTRACT

Mos1 is a member of the *mariner/Tc1* family of transposable elements originally identified in *Drosophila mauritiana*. It has 28 bp terminal inverted repeats and like other elements of this type it transposes by a cut and paste mechanism, inserts at TA dinucleotides and codes for a transposase. This is the only protein required for transposition *in vitro*. We have investigated the DNA binding properties of *Mos1* transposase and the role of transposase–transposase interactions in transposition. Purified transposase recognises the terminal inverted repeats of *Mos1* due to a DNA-binding domain in the N-terminal 120 amino acids. This requires a putative helix–turn–helix motif between residues 88 and 108. Binding is preferentially to the right hand end, which differs at four positions from the repeat at the left end. Cleavage of *Mos1* by transposase is also preferentially at the right hand end. Wild-type transposase monomers interact with each other in a yeast two-hybrid assay and we have used this to isolate mutations resulting in reduced interaction. These mutations lie along the length of the protein, indicating that transposase–transposase interactions are not due to a single interaction domain. One such mutation which retains both DNA-binding and catalytic activity has greatly reduced ability to excise *Mos1* from plasmid DNA through coordinate cleavage of the two ends and transposition *in vitro* is lowered to a level 20-fold below that of the wild-type. This suggests that transposase–transposase interaction is required to form a synaptic complex necessary for coordinate cleavage at the ends of *Mos1* during transposition. This mutant enzyme allows insertion at dinucleotides other than TA, including sequences with GC base pairs. This is the first example of a *mariner/Tc1* transposase with altered target specificity.

INTRODUCTION

Mariner is a transposable element with short terminal inverted repeats and was first identified during analysis of an unstable

mutation in the *white* gene of *Drosophila mauritiana*. This mutation results in a peach eye colour and is due to insertion of a 1.3 kb transposable element, *mariner*. This can excise in somatic cells to give mosaic eyes and in germ cells to give progeny with wild-type red eyes in the next generation. This particular element does not produce functional transposase and excises at high frequency in strains carrying autonomous *mariner* elements (1,2). The autonomous element *Mos1* was isolated from one such strain (3). *Mariner* and elements closely related to it, *mariner*-like elements (MLEs), have been found to occur naturally in other species of *Drosophila*, although not *Drosophila melanogaster*, and in a wide variety of insects, other arthropods, planaria, nematodes, fungi, fish and mammals, including man (4–8).

The *Mos1* element is 1286 bp long, has 28 bp imperfect terminal inverted repeats and contains a single open reading frame which codes for a 345 amino acid polypeptide (3). This is the transposase responsible for *mariner* transposition (9,10). Comparison of the putative amino acid sequences of the proteins encoded by MLEs from different families shows that they have an average of 34% sequence identity and that they are similar to the transposases encoded by another large family of elements, including the *Tc1* and *Tc3* elements found in *Caenorhabditis elegans* (11,12). The transposases encoded by *mariner/Tc1* elements contain conserved acidic residues equivalent to the D,D(35)E motif found in retroviral integrases and the transposases of several transposable elements in prokaryotes (13,14), where it is required for coordination of a divalent cation needed to catalyse phosphoryl transfer reactions during transposition (14). In the *Tc1* family this motif takes the form D,D(34)E whereas it is D,D(34)D in MLEs (15). This catalytic domain is located in the C-terminal two-thirds of the transposases encoded by MLEs. The N-terminal region contains the DNA-binding domain including a helix–turn–helix motif which is required for recognition of the terminal inverted repeats of the corresponding elements (16,17).

Our understanding of the mechanism by which *mariner/Tc1* elements transpose is based on the results of experiments using the transposases of *Tc1* (18), *Tc3* (19), *mariner* (10) and a reconstructed MLE, *Himar1* (20). These elements are believed to transpose by cut-and-paste mechanisms similar to that used by *Tn10* (21), *Tn5* (22) and *Tn7* (23) of *Escherichia coli* and *P* elements of *Drosophila* (24,25). Transposition of these elements is initiated by binding of transposase to each end of

*To whom correspondence should be addressed. Tel: +44 131 650 5377; Fax: +44 131 650 8650; Email: david.finnegan@ed.ac.uk

Present address:

Lei Zhang, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr Way, Oakland, CA 94609-1673, USA

the element, bringing the ends together to form a synaptic complex. Each DNA strand is then cleaved at, or near, the junction of the element and flanking DNA.

Cleavage at the 3'-end of each strand of *Tc1*, *Tc3* and *Himar1* occurs precisely at the junction of the element and flanking DNA. Cleavage at the 5'-end occurs within the element, 2 nt in the case of both *Tc1* and *Tc3* and 3 nt for *Himar1* (18–20,26). A transposable element released in this way is thought to integrate at a new site as the result of nucleophilic attack on the target DNA by the 3'-hydroxyl residues of the excised element followed by trans-esterification that covalently links each strand of the transposon to the target. This is always to the 5'-side of thymidine nucleotides at the sequence TA in the target. After integration the single-stranded gaps at the 5'-ends of the transposed element are repaired by host enzymes generating a TA target site duplication and replacing nucleotides lost at the ends of the element during excision.

We have purified the transposase encoded by *Mos1* after expression in *E.coli*. This can excise *Mos1* from plasmid DNA and mediate transposition of a marked element *in vitro*. We have shown that the protein recognises the inverted repeats at the ends of *Mos1* in a sequence-specific manner via a DNA-binding domain located within the N-terminal 120 amino acids and that it binds the right hand inverted repeat about five times more strongly than the left. Yeast two-hybrid assays have shown that transposase monomers bind to each other (27) and we have isolated mutations which reduce this interaction. These are distributed along the length of the protein, indicating that no single protein-protein interaction domain is involved. One of these mutations lies between the DNA-binding and catalytic domains of the protein and reduces coordinate cleavage at the ends of *mariner* during excision, reduces *in vitro* transposition 20-fold and alters target site selection, indicating the importance of interaction between transposase monomers during transposition.

MATERIALS AND METHODS

DNA constructs

DNA coding for amino acids 1–150 of the *Mos1* transposase was amplified from pBS*Mos1* using primers N6799 (GCCAT-ATGTCGAGTTTCGTGCCA) and A6499 (CGCATATGTTAATGCAAAAACGACTTCC). These primers contain a *NdeI* restriction site for insertion of the amplified fragment into pET-15b (Novagen, Madison, WI) after first cloning into pGEM-T (Promega). Deletion derivatives of this 150 amino acid sequence were made similarly using appropriate primers. In each case the downstream primer places a stop codon immediately following the transposase coding sequence. Site-directed mutagenesis was used to change codon 106 from CGC to GCC and codon 112 from AAG to GCG. The identity of each clone was confirmed by DNA sequencing.

The *Mos1* sequence encoding the *mariner* transposase was inserted into the expression vector pBCP368 (28). PCR was carried out using pBS*Mos1* as the template and primers GCCATATGTCGAGTTTCGTGCC and GCGAATTCTTAT-TCAAAGTATTTGCC, which contain sites for the restriction enzymes *NdeI* and *EcoRI*, respectively. The 1 kb fragment

generated in this way was initially inserted into the pGEM-T vector. The resulting plasmid, pGEM-*Mos1*, was digested with *EcoRI*, the cohesive end filled in using Klenow polymerase, digested with *NdeI* and the fragment containing the transposase gene was inserted into pBCP368 which had previously been digested with *NdeI* and *SmaI*. This gave the plasmid pBCP*Mos1*. The sequence of the transposase gene was confirmed at the end of the construction.

All recombinant plasmids were initially grown in strain DH5 α (29). Recombinant proteins were expressed in strain BL21 (DE3).

Protein purification

Cells expressing the N-terminal derivatives of *Mos1* transposase were grown in L broth at 37°C to an OD₅₉₅ of 0.6 and then induced with 1 mM IPTG for a further 4 h. The cells were harvested and lysed by sonication in 5 mM imidazole, 500 mM NaCl, 20 mM Tris-HCl pH 7.9. The lysate was centrifuged and the supernatant filtered through a 0.45 μ m filter before being loaded on a column of nickel resin (Novagen). Bound protein was eluted with TBS (150 mM NaCl, 20 mM Tris-HCl pH 7.9) with 50 mM EDTA and then dialysed against TBS.

The full-length *mariner* transposase used in these experiments was purified from *E.coli* strain BL21 DE3 carrying the plasmid pBCP*Mos1*. These cells were grown in L broth in an orbital shaker (200 r.p.m., 37°C) to an OD₅₉₅ of 0.4, when they were induced for 2 h by the addition of IPTG to 0.5 mM. Following induction the cells were harvested and stored at -20°C until required. The cells in the pellet from a 1 l culture were resuspended in 5 ml of 20 mM Tris-HCl pH 7.5, 10% glycerol, 2 mM MgCl₂, 1 mM DTT. Lysozyme was added to a concentration of 0.1 mg/ml and the cells incubated for 5 min at room temperature. They were then lysed by the addition of 5 ml of detergent buffer containing 25 mM Tris-HCl pH 7.5, 4 mM EDTA, 0.2 M NaCl, 1% deoxycholate, 1% NP-40, 1 mM DTT and incubated at room temperature for a further 15 min. MgCl₂ was added to a final concentration of 10 mM together with 100 μ l of a 2000 U/ml DNase I solution. The extract was pipetted up and down until the viscosity decreased and was left at room temperature for 10 min. The whole cell extract was then centrifuged at 20 000 g for 30 min. The pellet was washed three times in 0.5% NP-40, 1 mM EDTA, followed by one wash in 6 M urea before finally being resuspended in 1 ml of 25 mM Tris-HCl pH 7.5, 6 M guanidine hydrochloride, 5 mM DTT. After centrifugation at 13 000 g for 10 min the supernatant was diluted 100-fold into 25 mM Tris-HCl pH 7.5, 8 M urea, 5 mM DTT, 10% glycerol buffer and loaded onto a 2 ml fast flow CM Sepharose column (Sigma) pre-equilibrated with the same buffer supplemented with 50 mM NaCl. Under these conditions denatured *mariner* transposase bound to the column. Protein was renatured on the column by passing a 200 ml linear gradient of 8–0 M urea in buffer at a rate of 1 ml/min. Following renaturation bound protein was eluted with a 20 ml linear NaCl gradient of 50 mM–1.0 M in 25 mM Tris-HCl pH 7.5, 1 mM DTT, 10% glycerol. Fractions containing *mariner* transposase were identified by SDS-PAGE and further concentrated by spinning through a centricon column (30 kDa molecular weight cut-off). The protein was stored at -70°C at a concentration of 0.25–0.5 mg/ml.

Gel retardation assays

Purified protein was incubated at 25 nM with 50 ng of poly(dI-dC) on ice in binding buffer [25 mM HEPES pH 7.9, 100 mM NaCl, 5 mM dithiothreitol (DTT), 5 mM spermidine, 1 mg/ml BSA, 5 mM CaCl₂, 20% glycerol] for 10 min before 2 ng of probe DNA was added and incubated for a further 4 h at 4°C. The DNA-protein complexes were then fractionated on an 8% polyacrylamide gel.

In vitro excision of the *Mos1* element

Two hundred nanograms of supercoiled pMos plasmid (final concentration 1.89 nM), which contains the *Mos1* element within a fragment of DNA from *Drosophila simulans*, was incubated in 25 mM HEPES pH 7.5, 100 mM NaCl, 10% glycerol, 2 mM DTT, together with 1 µg of BSA and 1 µg of poly(dI-dC)·(dI-dC), 5 mM MgCl₂ or 5 mM Mn(CH₃COO)₂ and *Mos1* transposase to give a final concentration of 304 nM in a total volume of 20 µl at 30°C. Reactions were analysed by Southern blotting following separation on a 1% agarose gel (1×TAE). Fragments containing *Mos1* sequences were detected by hybridisation with ³²P-labelled pMos.

In vitro transposition assays and product analysis

In vitro transposition assays were carried out essentially as described for *P* element transposition by Kaufman and Rio (24). Three hundred nanograms (38 fmol) of donor plasmid, pRJM345MosTet, was incubated with 200 ng (25 fmol) of pBSKS+ tetramer in the presence of varying concentrations of renatured *Mos1* transposase and either 5 mM MgCl₂, 5 mM Mn(CH₃COO)₂ or 5 mM EDTA. Incubations were carried out at 30°C in 25 mM HEPES pH 7.9, 100 mM NaCl, 10% glycerol, 200 mg/ml acetylated BSA and 2 mM DTT for 60 min in a final volume of 20 µl. Reactions were stopped by the addition of 80 µl of STOP buffer (50 mM Tris-HCl pH 7.5, 0.5 mg/ml proteinase K, 10 mM EDTA, 250 µg/ml yeast tRNA) and incubated at 37°C for 1 h. Samples were then extracted once with an equal volume of phenol:chloroform (1:1) and ethanol precipitated before final resuspension in 10 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). One microlitre of each sample was used to transform DH5α cells by electroporation. The transformed cells were incubated at 37°C for 45–60 min with shaking before plating out 0.5 ml on L broth plates containing ampicillin (100 µg/ml) and tetracycline (12 µg/ml) and incubating overnight at 37°C. At the same time viable counts were performed on transformed cells and the number of ampicillin-resistant colonies calculated. Restriction enzyme analysis of DNA isolated from colonies growing on LB+amp+tet was used to check for the presence of the marked *mariner* element.

The DNA flanking *de novo* insertions of the *Mos1* Tet element was also sequenced using primers directed outward from the ends of the *Mos1* open reading frame. Primer V6892 anneals to nucleotides 123–101 of *Mos1* sequence (CTATGGTGGTTCGACAGTCAAGG) and primer V5054 (GGGAAAATGTGAGCTAGCGACGGC) anneals to nucleotides 1169–1194 of *Mos1*. Sequencing reactions were carried out in the presence of [³⁵S]dATP using the Sequenase v.2.0 DNA sequencing kit according to the manufacturer's instructions (Amersham, Little Chalfont, UK).

Yeast two-hybrid assay

Yeast two-hybrid assays (30) were carried out using the host strain L40 [MATa, *ade2*, *his3Δ200*, *trp1-901*, *leu2-3,112,ade2*, *lys2-801am*, *URA3::(lexAop)₈-lacZ/LYS2::(lexAop)₄-HIS3*; 31] transformed as described (32).

Western blotting

Yeast transformants were grown in 5 ml of selectable medium overnight at 30°C. Cells were then diluted into 50 ml of YPDA and incubated to an OD₆₀₀ of ~0.5. Aliquots of 3 ml of cells were pelleted and resuspended in 80 µl of 'crack buffer' (2% SDS, 80 mM Tris-HCl pH 6.8, 10% glycerol, 10 mM EDTA, 0.4 mg/ml bromophenol blue, 0.1 M DTT, 2 mM PMSF). About 40 µl of glass beads were added and the cells were vortexed for 1 min. After centrifugation for 3 min at 13 000 r.p.m., the supernatants were boiled for 3 min and fractionated on a 10% SDS-polyacrylamide gel.

Proteins separated by SDS-PAGE were transferred onto PVDF (Boehringer Mannheim) membranes, which were pre-soaked in methanol. Transfer was conducted at 40 V for 1.5 h in transfer buffer (9 g Tris, 43.2 g glycine to 3 l) using a Bio-Rad Trans-Blot cell. The membranes were incubated in 1% blocking solution (Boehringer Mannheim) overnight and then incubated with primary antibody (usually 1:1000–2000 dilution in 0.5% block) for 2 h. After washing with TBST for 1 h with four wash changes, the membranes were incubated with peroxidase-conjugated rabbit anti-mouse IgG (Santa Cruz) for 1 h. Following washing in several changes of TBST for 1 h, the bound antibodies were detected by chemiluminescence using a Boehringer Mannheim Chemiluminescence Western blotting kit. The membrane was incubated with detection solution for 1 min at room temperature and then exposed to film for 2–30 s.

RESULTS

DNA-binding domain of *mariner* transposase

The transposases of Tc1, Tc3 and *pogo* contain DNA-binding domains comprising helix-turn-helix motifs near their N-termini (16,17,33,34) and a helix-turn-helix motif is predicted to lie between residues 88 and 108 of the *Mos1* transposase (35). In order to determine whether this forms the DNA-binding domain responsible for recognising the *Mos1* terminal inverted repeats we have expressed various deletion derivatives of *Mos1* transposase in *E.coli* and have determined their DNA-binding properties in gel retardation experiments. The probe for these experiments was a 176 bp fragment containing 108 bp of DNA from the right end of *Mos1*, including the 28 bp inverted repeat, and 66 bp of flanking DNA. Since transposase also binds to DNA from the left end of *Mos1* (see Fig. 3) it must be recognising the terminal inverted repeats, since these are the only sequences in common between the two ends. We have confirmed this by DNase I protection (data not shown).

The N-terminal 150 amino acids of the transposase (N150) bound the right end of *Mos1* in a sequence-specific manner since binding was competed by an excess of unlabelled probe fragment but not by equivalent amounts of a 131 bp fragment containing the left hand inverted repeat of a *pogo* element (17; Fig. 1A, lanes 2 and 3). We have mapped in more detail the

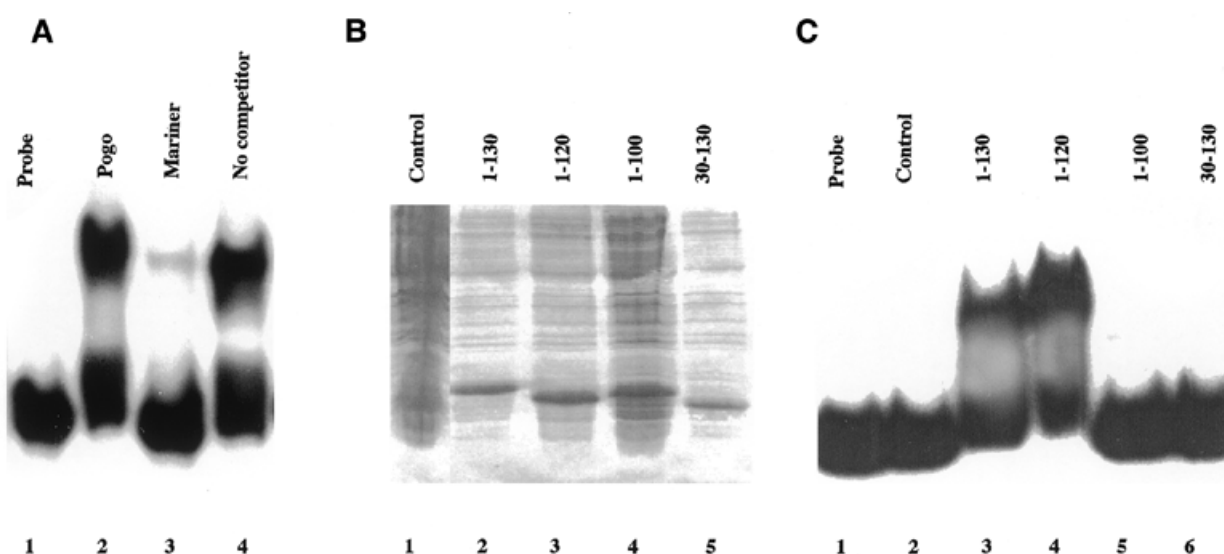


Figure 1. Binding of *Mos1* transposase to the ends of *Mos1* DNA. (A) A 32 P-labelled 176 bp fragment of DNA containing 108 bp from the right end of *Mos1*, including the inverted repeat, was mixed with 10 ng of purified N150 in the presence or absence of a 100-fold molar excess of competitor DNA as follows: lane 1, probe alone; lane 2, *pogo* DNA as competitor; lane 3, unlabelled probe DNA as competitor; lane 4, no competitor. (B) SDS-PAGE analysis of N-terminal deletion fragments of *Mos1* transposase expressed in *E.coli*. Soluble proteins from sonicated cells were loaded on the gel. Lane 1, extract of cells containing the plasmid vector; lanes 2–5, extracts of cells containing plasmids containing DNA coding for amino acids 1–130, 1–120, 1–100 and 30–130, respectively. (C) DNA-binding ability of proteins shown in (B). The 32 P-labelled fragment used in (A) was incubated with each of the extracts shown in (B) and fractionated on an 8% polyacrylamide gel. Lane 2, control extract; lane 3, amino acids 1–130; lane 4, amino acids 1–120; lane 5, amino acids 1–100; lane 6, amino acids 30–130.

region of transposase required for binding using soluble extracts of *E.coli* cells expressing derivatives of N150 (Fig. 1B). Binding was still detected if 30 amino acids were deleted from the C-terminus of N150 but not if a similar deletion was made from the N-terminus (Fig. 1C). This indicates that a sequence-specific binding domain lies within the N-terminal 120 residues of *Mos1* transposase. This appears to require the helix–turn–helix motif predicted to lie within this part of the protein, as changing the arginine at the seventh position of the second helix to alanine (R106A) abolishes DNA binding, whereas a similar mutation of the lysine six residues downstream and beyond the predicted helix (K112A) has no detectable effect (Fig. 2). This is consistent with the results of Augé-Gouillou *et al.* (36), who have determined that the DNA-binding domain of *Mos1* transposase is within the first 140 residues.

Transposases bind differentially to the two ends of *Mos1*

There are four mismatches between the inverted repeats of *Mos1*, including a difference at the first position, raising the possibility that the two ends of the element may be recognised by transposase with different efficiencies. We have investigated this by comparing the ability of cold left or right end competitor fragments to titrate transposase–DNA complexes formed with either left or right end probes. The right end competes ~5-fold more efficiently for binding of the left end probe than does the left end competitor (Fig. 3, compare lane 4, which contains 20 ng of right end competitor, with lane 10, containing 100 ng of left end competitor). We have obtained similar results using the right hand probe, for which 35 ng of the right hand end competitor was equivalent to 180 ng of left end competitor (data not shown). These results indicate that transposase binds preferentially to the right hand end of *Mos1*. Similar results have been reported by Augé-Gouillou *et al.*

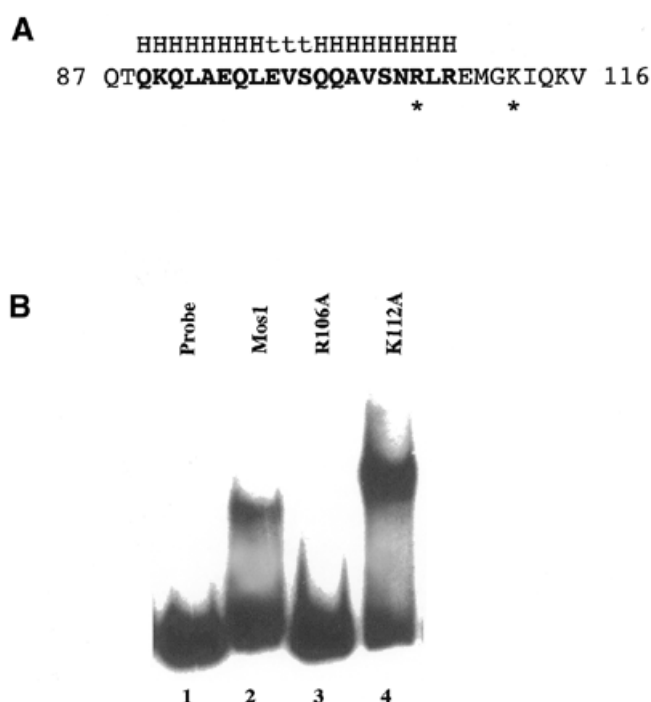


Figure 2. The DNA-binding domain of *Mos1* transposase contains a helix–turn–helix. (A) The region of *Mos1* transposase containing a putative helix–turn–helix. The amino acid residues concerned are indicated in bold, indicating those believed to be in the helices (H) and the turn (t). Asterisks indicated the residues mutated *in vitro*. (B) Gel retardation showing that mutation of Arg106 within the second helix abolishes DNA binding whereas mutation of Lys112 outside the helix does not. The same probe and conditions were used as in Figure 1A.

(37), who have shown that an element with two right hand ends transposes more efficiently than the wild-type.

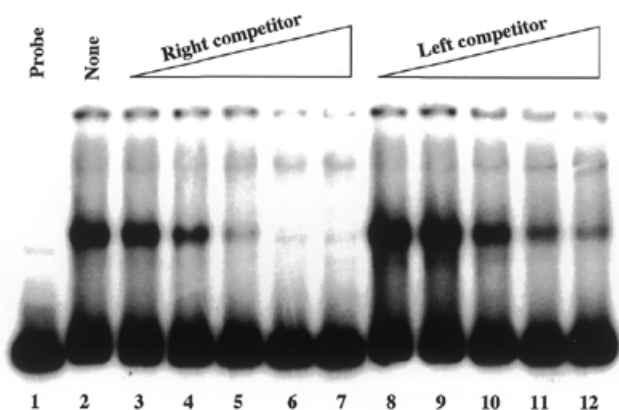


Figure 3. *Mos1* transposase binds preferentially to the right hand inverted repeat. A 190 bp fragment containing the first 120 bp of *Mos1* including the left hand inverted repeat was labelled with ^{32}P and used as probe (lane 1). This was mixed with 10 ng purified N150 (lane 2) together with increasing amounts of right hand end (lanes 3–7) or left hand end (lanes 8–12) competitor as follows: right end, 10 (lane 3), 20 (lane 4), 50 (lane 5), 100 (lane 6) and 200 ng (lane 7); left end, 20 (lane 8), 50 (lane 9), 100 (lane 10), 200 (lane 11) and 300 ng (lane 12).

Interaction of transposase monomers

Transposase has to recognise both ends of *mariner* to excise the element from one site and insert it at another and it is likely that the ends are brought together as a result of interactions between transposase bound to the left and right end inverted repeats. This has been demonstrated *in vitro* for Tn5 and Tn10, each of which forms a synaptic complex comprising two transposase molecules and the two ends of the corresponding element (38,39). A similar synaptic complex may be formed by *mariner* transposase; indeed, transposase monomers have been shown to interact in a yeast two-hybrid assay (27).

In order to identify the region of transposase required for this interaction and to determine whether or not this is required for transposition we have isolated mutations which show reduced interaction. Cells of the *Saccharomyces cerevisiae* strain L40 containing *lacZ* and *HIS3* reporter genes downstream of four and eight copies of the LexA binding site, respectively, were transfected with a derivative (pACTIIst-*Mos1*) of the plasmid pACTIIst (40), containing a gene coding for the GAL4 activation domain fused to the complete *Mos1* transposase sequence, and derivatives of pBTM116 (41), containing sequences coding for the DNA-binding domain of LexA fused to wild-type or mutant transposase sequences. Staining of colonies with X-gal gave a qualitative measure of the interaction of transposase monomers while the amount of 3-amino-1,2,3-triazole (3-AT) required to prevent growth by inhibition of His3 protein gave a quantitative measure of the interaction.

When both pACTIIst and pBTM116 contained the full-length transposase sequence there was a strong interaction, giving colonies which stained well with X-gal and grew on medium with 20 mM 3-AT. No staining was seen with L40 cells carrying either plasmid alone and these cells were unable to grow in the presence of 0.5 mM 3-AT. In order to detect any single region of the protein which might be required for protein–protein interaction we first tested various deletion derivatives of the protein for their ability to interact with full-length transposase. The deleted sequences coding for amino

acids 1–177, 1–300, 30–345, 90–345 and 170–345 were placed downstream of the LexA DNA-binding domain in pBTM116 and introduced into L40 cells together with pACTIIst-*Mos1*. In no case was the expression of *lacZ* or *HIS3* above background. This was not due to lack of expression of the deleted transposase fusions as this was checked using antibodies to the LexA DNA-binding domain (data not shown). These results suggest that residues required for the interaction of transposase monomers are distributed along the length of the protein.

We have analysed the interaction between transposase subunits further by screening for point mutations which reduce subunit interaction. The transposase coding sequence with 50 bp of flanking DNA was amplified by PCR from pBTM116-*Mos1* using *Taq* polymerase, either under standard conditions or conditions favouring the introduction of mutations (reduced concentration of one dNTP or with Mn^{2+} rather than Mg^{2+} ; 42). The amplified DNA was then introduced, together with pBTM116 DNA digested with *EcoRI* and *PstI*, into L40 cells carrying pACTIIst-*Mos1*. The transfected cells were plated on medium lacking Leu, Trp and His (–LWH) to select for transformants in which pBTM116 had incorporated a molecule of amplified DNA by gap repair recombination. Transformants were streaked on –LWH medium containing 0.5, 5 and 10 mM 3-AT to test their ability to form colonies, a reflection of the strength of the interaction between transposase monomers. About 10% of transformants grew poorly or not at all on 5 mM 3-AT, indicating a reduced interaction between monomers. The pBTM116-*Mos1* plasmids from these transformants were cloned in *E.coli* and then introduced into L40 cells containing pACTIIst-*Mos1* to check that they still showed reduced growth on 3-AT. Most gave a wild-type interaction.

The transposase coding sequence of each plasmid giving reproducibly reduced growth on 3-AT (Fig. 4A–C) was determined. Twenty-three mutant plasmids were sequenced and of these, 12 contained single base changes resulting in amino acid substitutions. The remainder had multiple base substitutions resulting in two or more amino acid changes. The single amino acid changes affecting the interaction of transposase monomers are distributed along the length of the protein but are concentrated in the N-terminal region containing the putative helix–turn–helix motif required for DNA binding (Fig. 4A). The reduced ability to stimulate reporter gene expression shown by the mutant proteins is not due to instability or reduced expression, except perhaps in the case of mutation D279G, since anti-LexA antibodies indicate that the mutant and wild-type fusion proteins are present at about the same level in extracts of soluble proteins (data not shown). Although some of these mutations may prevent interaction between transposase monomers by altering the conformation of the protein, the results suggest that *Mos1* transposase does not contain a single protein–protein interaction domain but that these interactions are due to sites along the length of the molecule.

Is interaction between transposase monomers required for transposition?

If interaction between transposase monomers is required to form a synaptic complex, a mutation which disrupts this interaction would reduce transposition. Since transposition requires both the DNA-binding and catalytic activities of transposase we chose for analysis a mutation lying between the DNA-binding and catalytic domains of the protein. Of the

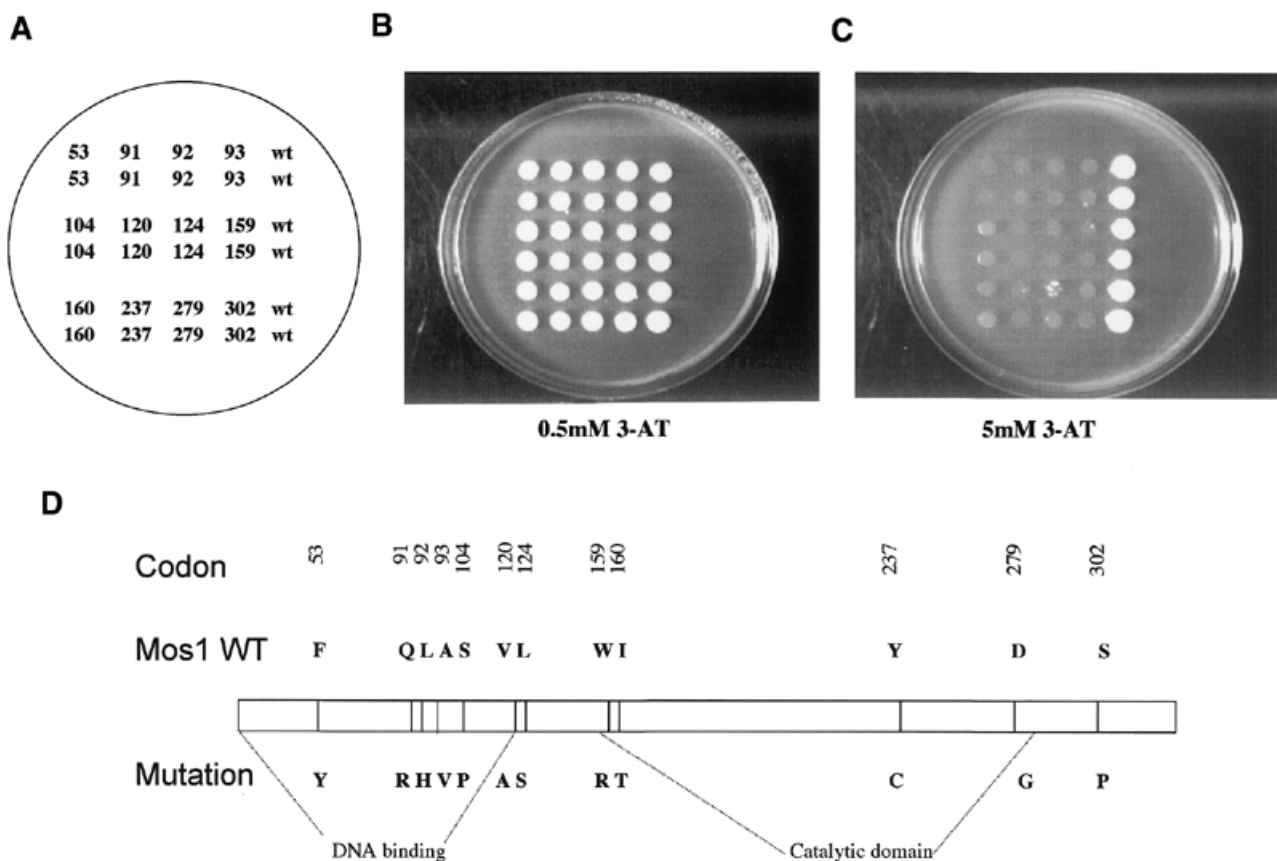


Figure 4. Mutations reducing interaction between *Mos1* transposase monomers. (A–C) Yeast cells expressing wild-type or one of 12 mutant transposases fused to the *lexA* protein were spotted on medium containing 0.5 (B) or 5 mM 3-AT (C). Only cells expressing the wild-type fusion can grow on the higher concentration. The position of each mutant is indicated in (A). (D) Map showing the position and identity of each of the 12 amino acid substitutions within *Mos1* transposase.

12 single amino acid substitutions affecting protein–protein interaction, six lie within the N-terminal 120 amino acids which we have shown are required for DNA binding, and of these five are within the putative helix–turn–helix motif. These are likely to have reduced DNA binding and we have confirmed this for one of them, S104P (data not shown). The catalytic domain presumably includes residues 156–284, the positions of the first and last residues of the DD(34)D triad. Four mutations lie within this region. Mutation L124S does not lie in either region (Fig. 4D).

We have purified L124S as described for wild-type transposase and confirmed that it recognises *mariner* ends in a gel retardation assay as described previously and tested its catalytic activity, together with that of wild-type transposase, in an *in vitro* excision assay using the plasmid pMos as substrate (Fig. 5A).

Wild-type transposase generated a 1.3 kb fragment, the size of the *Mos1* element, from supercoiled plasmid DNA, as well as a 6.7 kb fragment the size of the plasmid vector, and an 8 kb band the size of linear pMos. We have confirmed that, as expected, the 6.7 kb fragment hybridises to a pMos but not a *Mos1* probe and that the 8 kb band hybridises to both (Fig. 5B). Since the 1.3 and 6.7 kb bands were seen with even the lowest amounts of enzyme used, we conclude that cleavage at the two ends of *Mos1* is coordinated.

The mutant transposase, on the other hand, produced 6.7 and 1.3 kb bands only at the highest protein concentrations. This was not because of low catalytic activity, since the 8 kb full-length plasmid band, but not the 6.7 kb vector, accumulated in a similar manner with both the wild-type and mutant enzymes. This suggests that the mutant enzyme cuts the two ends of *Mos1* independently of each other (Fig. 5A).

We have confirmed that the 8 kb molecules are due to cleavage at a single end by purifying them and digesting with *SalI*, which cuts once within *mariner*. This would produce a 937 bp fragment if the 8 kb band had been cut at the right end of *mariner* or 349 bp if cut at the left end. Both fragments were generated from the 8 kb band produced by wild-type transposase but the 937 bp fragment was about 10 times more abundant than the 349 bp fragment (Fig. 5C), presumably reflecting the preferential binding to the right hand inverted repeat. A similar pattern was seen when the 8 kb fragment generated by the L124S transposase was digested with *SalI*, demonstrating that catalytic activity and preferential binding to the right hand inverted repeat are retained by the mutant enzyme. This indicates that its reduced ability to excise *Mos1* DNA is the result of reduced protein–protein interaction.

In order to determine the effect of reduced transposase–transposase interaction on transposition we have compared the efficiency of wild-type and mutant proteins to mediate transposition *in vitro* using a plasmid-based assay. The donor plasmid

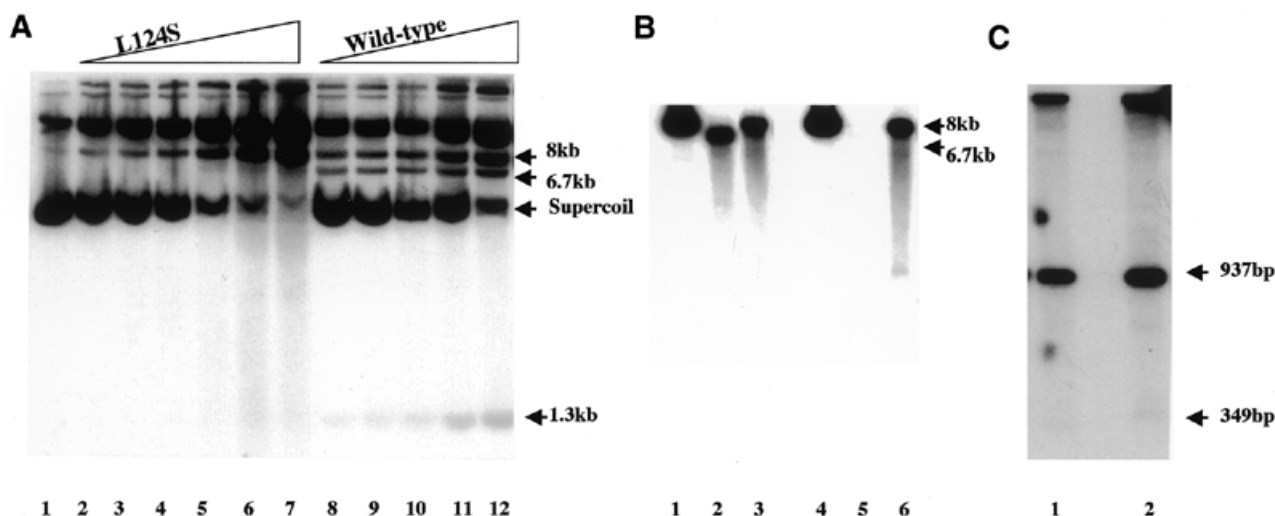


Figure 5. Protein-protein interaction is required for normal excision of *MosI* DNA by transposase. (A) Digestion of pMos DNA by wild-type (lanes 8–12) or L124S (lanes 2–7) transposase. Lane 1 contains undigested DNA. DNA was separated on a 1% agarose gel after digestion at 30°C and probed with ³²P-labelled pMos. The amounts of protein used are as follows: lanes 2 and 8, 37.5 nM; lanes 3 and 9, 75 nM; lanes 4 and 10, 150 nM; lanes 5 and 11, 300 nM; lanes 6 and 12, 600 nM; lane 7, 1200 nM. (B) The 8 and 6.7 kb fragments shown in (A) were extracted from gel slices, run on a fresh agarose gel, transferred to nylon filter and probed with either ³²P-labelled pMos (lanes 1–3) or *MosI* DNA (lanes 4–6). Lanes 1 and 4, linear pMos DNA; lanes 2 and 5, purified 6.7 kb fragment; lanes 3 and 6, purified 8 kb fragment. (C) The 8 kb fragments generated by wild-type (lane 1) and mutant (lane 2) transposase were extracted from a gel, digested with *SalI* and separated on a 1.3% agarose gel before hybridisation with ³²P-labelled *MosI*.

contained a derivative of *MosI* into which we had inserted a tetracycline resistance cassette while the target plasmid was a tetramer of pBSKS+ (24). After incubating these plasmids with transposase for 1 h at 30°C the mixture was electroporated into *E. coli* strain DH5 α (29) and the cells plated on nutrient agar containing either ampicillin alone or both ampicillin and tetracycline. Cells transfected with plasmids into which the marked *MosI* element had transposed would be Amp^R, Tet^R so the ratio of Amp^R to Amp^R, Tet^R colonies gives a measure of the transposition frequency. The donor plasmid is unable to replicate in DH5 α cells, reducing the chance that Amp^R, Tet^R colonies could arise by other means.

Transposition was 10-fold higher in the presence of Mn²⁺ than with Mg²⁺ and was dependent on the concentration of transposase. No Amp^R, Tet^R colonies were obtained in the absence of transposase or in the presence of 5 mM EDTA. The maximum frequency of transposition (0.8×10^{-3} per recipient plasmid) was obtained with transposase at a concentration of 12–24 nM, a ratio of protein to target DNA of 5:1. As we do not know what proportion of transposase molecules are active in our preparations we cannot say what ratio of active protein to DNA gave maximum activity. Transposition decreased rapidly at concentrations of transposase either above or below this, a 2-fold change in concentration reducing transposition ~2-fold. This differs from results reported previously in which transposition increased with increasing amounts of *MosI* transposase (10) but is similar to the behaviour of *HimarI* transposase (43).

Plasmid DNA isolated from 35 Amp^R, Tet^R colonies derived from transposition in the presence of Mg²⁺ and 11 from transposition in Mn²⁺ were analysed by restriction digestion and agarose gel electrophoresis. In each case the plasmid had increased in size by 2.7 kb, as expected if they were the result of transposition of the marked *mariner* element. We have sequenced the DNA flanking the transposed elements in these

Wild-type Transposase		Mariner		Wild-type Transposase	
		← Mariner →			
Mg ⁺⁺ 35/35	nnnnnnnnTA	CCAGGT.....ACCTGA		TA	TAnnnnnnnn
Mn ⁺⁺	gtgtgaaaTT	CCAGGT.....ACCTGA		TT	Tgttatccc
	aaaaataTT	CCAGGT.....ACCTGA		TT	aaagccta
	cctggcgtTA	CCAGGT.....ACCTGA		TA	cccaactt
	gcttcaatAT	CCAGGT.....ACCTGA		TA	atattgaa*
	cgataagcTT	CCAGGT.....ACCTGA		TT	gatatacga
	gatoctttTT	CCAGGT.....ACCTGA		TT	gataatct
	tcgccgcaTA	CCAGGT.....ACCTGA		TA	actatttc
	aaccgatTA	CCAGGT.....ACCTGA		TA	ccgccttt
	tctagaacTA	CCAGGT.....ACCTGA		TA	gtgatcc
	tcgccctaTA	CCAGGT.....ACCTGA		TA	gtgagtct
caggcaacTA	CCAGGT.....ACCTGA		TA	gtgatgaa	
L124S Transposase					
Mg ⁺⁺	taatccggTA	CCAGGT.....ACCTGA		TA	actatcgt
	attcgcccTA	CCAGGT.....ACCTGA		TA	atgtgagt
	tgctcaaaTA	CCAGGT.....ACCTGA		TA	atattgaa
	aaaggatcTC	CCAGGT.....ACCTGA		TC	aagaagat*
atgctcgtCA	CCAGGT.....ACCTGA		CA	ggggggcg	
Mn ⁺⁺	aaagtataTA	CCAGGT.....ACCTGA		TA	tgagtaaa
	tctgagaaTA	CCAGGT.....ACCTGA		TA	gtgatgc
	tcgccgcaTA	CCAGGT.....ACCTGA		TA	actatttc
	ccaggcttTA	CCAGGT.....ACCTGA		TA	acttttat
	atgctcgtCA	CCAGGT.....ACCTGA		CA	ggggggcg
	ttggagtcCA	CCAGGT.....ACCTGA		CA	gctttttt
	cttgagtcCA	CCAGGT.....ACCTGA		CA	accgggta
	taagacacGA	CCAGGT.....ACCTGA		GA	cttatcgc
	acggatcGA	CCAGGT.....ACCTGA		GA	taagcttg
	tcgattttTG	CCAGGT.....ACCTGA		TG	tgatgctc
	ggaatgtTG	CCAGGT.....ACCTGA		TG	aatactca
tagttcttTC	CCAGGT.....ACCTGA		TC	ctgcgcta	
aaaggatcTC	CCAGGT.....ACCTGA		TC	aagaagat	
atgagtgATA	CCAGGT.....ACCTGA		ATA	aacactgc	

Figure 6. Target site selection by wild-type and mutant transposase. Sequences flanking *MosI* insertions after *in vitro* transposition using either wild-type or mutant transposase in the presence of either Mg²⁺ or Mn²⁺. *, The three bases underlined were added to the sequence of the recipient plasmid during insertion. There is no target site duplication. †, Four insertions were recovered at this site.

plasmids to ensure that they are the result of bona fide transposition events (Fig. 6). All 35 insertions generated in the presence of Mg²⁺ were flanked by TA target site duplications,

as is the case for *mariner* elements transposing *in vivo* (44). The distribution of insertions amongst the 306 TA dinucleotides in the target plasmid was non-random ($P < 0.01$) and the site used most frequently is also a hot spot for insertion of *HimarI* (43). Six of the 11 insertions which took place in the presence of Mn^{2+} had also inserted at TA dinucleotides. The remainder had inserted at other A/T-containing dinucleotides, indicating that increased transposition in Mn^{2+} is at the expense of relaxed target site selection (Fig. 6).

In vitro transposition stimulated by the L124S mutant transposase was ~20-fold lower than with wild-type in the presence of Mn^{2+} and was reduced further in Mg^{2+} , indicating that the ability of transposase monomers to bind to each other is essential for normal transposition as well as for excision. We have analysed the target site duplications of insertions stimulated by L124S transposase under both conditions. While some of the insertions were into the dinucleotide TA the majority were at other sites, including some G/C-containing dinucleotides. This has not been reported previously for *mariner* or any other element of this type (Fig. 6). This change in target site selection may be due to a change in the conformation of the transpososome resulting from altered protein-protein interaction or possibly a direct effect of the L124S mutation.

DISCUSSION

Transposition of elements which move by excision and insertion requires recognition of the ends of the element by transposase, formation of a synaptic complex, cleavage at the ends of the transposon, capture of target DNA and integration to generate a characteristic target site duplication. All transposases studied so far recognise the ends of the corresponding element through an N-terminal DNA-binding domain, which is generally a helix-turn-helix motif. This has been most clearly demonstrated for Tc3, for which a polypeptide comprising the N-terminal 64 amino acids has been crystallised with its target DNA (16). We have shown that the same is true of *MosI* transposase, which has a DNA-binding domain within the N-terminal 120 amino acids, and that a helix-turn-helix predicted to run from residues 87 to 108 (35) is required for binding, as changing a basic residue (R106A) within the putative second helix abolishes binding whereas similar changes (K112A) just outside this helix had no detectable effect.

The transposases of Tc1 and Tc3 have both sequence-specific and sequence-non-specific DNA-binding domains, with the non-specific domain lying between a C-terminal catalytic domain and a sequence-specific binding region. In contrast, we have been able to detect only a single DNA-binding domain in the N-terminal region of *MosI* transposase. The N-terminal 30 amino acids of the transposase are required but are not sufficient for binding, possibly because they interact with the helix-turn-helix in binding to the terminal inverted repeats or because they are required for the correct conformation of the DNA-binding domain. We have been unable to test whether or not there is a DNA-binding domain within the catalytic region as attempts to express this part of the protein have yielded only insoluble proteins.

There are four mismatches between the 28 bp inverted repeats of *MosI* and transposase can distinguish between them, binding to the right hand repeat about five times more efficiently than to the left. Cleavage at the right hand end of *MosI*

is about 10 times more efficient than cleavage at the left end in *in vitro* excision assays. This is presumably the result of differential binding to the ends and suggests that the ends of *MosI* may not be equivalent during transposition. This is true of the ends of the *P* element of *D.melanogaster*, even though they have identical terminal inverted repeats (45). The *P* transposase does not bind to the inverted repeats but to a 10 bp sequence 9 bp from the left hand inverted repeats and 21 bases from the right (25). In this case it must be the sequence which lies between the transposase-binding sites and the terminal inverted repeats, or simply the distance of the transposase from the ends of the elements, which are critical for transposition (24).

Transposase molecules bound to the ends of a transposon associate to form a synaptic complex which may serve to ensure that cleavage of the ends is coordinated, reducing the possibility that cleavage of a single end, or of a fortuitous transposase-binding site, will produce a chromosome break. Tn10 (39) and Tn5 (38) form synaptic complexes which contain transposase dimers whereas bacteriophage Mu forms a synaptic complex with four monomers of MuA transposase, although only two of these take part in cleavage of the donor DNA (46).

There is no direct evidence for synaptic complex formation by transposases of the *mariner*/Tc1 family but the DNA-binding domain of Tc3 transposase forms a dimer when crystallised with DNA (16) and isolation of point mutations of *MosI* which reduce *in vivo* excision of *mariner* when heterozygous with wild-type *MosI* suggest that *MosI* transposase acts as an oligomer (27). Moreover, we have found that during *in vitro* excision the ends of *MosI* are cleaved coordinately, which presumably requires interaction between transposase molecules at either end.

We have confirmed earlier observations that *MosI* transposase interacts with itself in a yeast two-hybrid experiment (27) and have used this to isolate mutations which show reduced interaction. The results show that there is no single protein-protein interaction domain and these mutations were distributed along the length of the protein. Presumably interaction between transposase monomers involves regions of the protein brought together in the tertiary conformation. Several of the mutations lie within the helix-turn-helix required for recognition of the terminal inverted repeats. Sequence-specific DNA binding is not required for protein-protein interaction in the two-hybrid assay as the terminal inverted repeats are not present.

One mutation, L124S, which retains both DNA-binding and catalytic activities nevertheless has a 20-fold reduced ability to mediate transposition *in vitro*. It has also lost the ability to cleave the ends of *MosI* coordinately, suggesting that formation of a synaptic is required for *mariner* transposition. Although cleavage at the left and right ends is coordinated, it is not necessarily simultaneous, and preferential cleavage of the right end suggests that this is cleaved first. Since *MosI* transposase both binds and cleaves the right end preferentially it is likely that in the synaptic complex transposase monomers cleave the ends of the element *in cis* rather than *in trans*, as is the case for Tn5 (47) and Mu (48).

Surprisingly the L124S mutation also affects target site selection. *mariner*/Tc1 elements insert exclusively at TA dinucleotides *in vivo* and this specificity is retained *in vitro* if

transposition is carried out in the presence of Mg²⁺. In the presence of Mn²⁺ insertion is still preferentially at TA, although insertions at other T/A-rich sequences are found. Although the L124S mutant still generates 2 bp target site duplications during *in vitro* transposition these include many sequences with a G or C at either position.

Mutations affecting target site selection have been identified for Tn10 transposase. These alter two residues within the catalytic domain but do not significantly reduce transposition frequencies (49), suggesting that interaction between transposase monomers and formation of a synaptic complex are not affected. Changing the glutamate residue of the DDE motif also reduces target site selection (50). No mutation affecting target site selection has been reported previously for a member of the *mariner*/Tc1 family of transposases. Our results suggest that L124 is either directly involved in target site selection or that this has changed as a consequence of the reduced ability of mutant transposase monomers to form a normal synaptic complex, with this being a prerequisite for normal integration.

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REFERENCES

- Jacobson, J.W., Medhora, M.M. and Hartl, D.L. (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **83**, 8684–8688.
- Jacobson, J.W. and Hartl, D.L. (1985) Coupled instability of two X-linked genes in *Drosophila mauritiana*: germinal and somatic mutability. *Genetics*, **111**, 57–65.
- Medhora, M., Mruyama, K. and Hartl, D.L. (1991) Molecular and functional analysis of the *mariner* mutator element *Mos 1* in *Drosophila*. *Genetics*, **128**, 311–318.
- Oosumi, T., Belknap, W.R. and Garlick, B. (1995) *Mariner* transposons in humans. *Nature*, **378**, 672.
- Oosumi, T., Garlick, B. and Belknap, W.R. (1995) Identification and characterization of putative transposable DNA elements in solanaceous plants and *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA*, **92**, 8886–8890.
- Sedensky, M.M., Hudson, S.J., Everson, B. and Morgan, P.G. (1994) Identification of a *mariner*-like repetitive sequence in *C. elegans*. *Nucleic Acids Res.*, **22**, 1719–1723.
- Robertson, H.M. (1993) The *mariner* transposable element is widespread in insects. *Nature*, **362**, 241–245.
- Garcia-Fernandez, J., Bayascas-Ramirez, J.R., Marfany, G., Munoz-Marmol, A.M., Casali, A., Baguna, J. and Salo, E. (1995) High copy number of highly similar *mariner*-like transposons in planaria (Platyhelminthes): evidence for a trans-phyta horizontal transfer. *Mol. Biol. Evol.*, **12**, 421–431.
- Maruyama, K., Schoor, K.D. and Hartl, D.L. (1991) Identification of nucleotide substitutions necessary for *trans*-activation of *mariner* transposable elements in *Drosophila*: analysis of naturally occurring elements. *Genetics*, **128**, 777–784.
- Tosi, L.R.O. and Beverley, S.M. (2000) *cis* and *trans* factors affecting *Mos1 mariner* evolution and transposition *in vitro* and its potential for functional genomics. *Nucleic Acids Res.*, **28**, 784–790.
- Plasterk, R.H.A. (1996) The Tc1/*mariner* transposon family. *Curr. Top. Microbiol. Immunol.*, **204**, 125–143.
- Robertson, H.M. and Lampe, D.J. (1995) Distribution of transposable elements in arthropods. *Annu. Rev. Entomol.*, **40**, 333–357.
- Doak, T.G., Doerder, F.P., Jahn, C.L. and Herrick, G. (1994) A proposed superfamily of transposase genes: transposon-like elements in ciliated protozoa and a common “D35E” motif. *Proc. Natl Acad. Sci. USA*, **91**, 942–946.
- Grindley, N.D. and Leschziner, A.E. (1995) DNA transposition: from a black box to a color monitor. *Cell*, **83**, 1063–1066.
- Robertson, H.M. (1995) The Tc1-*mariner* superfamily of transposons in animals. *J. Insect Physiol.*, **41**, 99–105.
- van Pouderooyen, G., Ketting, R.F., Perrakis, A., Plasterk, R.H.A. and Sixma, T.K. (1997) Crystal structure of the specific DNA-binding domain of Tc3 transposase of *C. elegans* in complex with transposon DNA. *EMBO J.*, **16**, 6044–6054.
- Wang, H., Hartswood, E. and Finnegan, D.J. (1999) *Pogo* transposase contains a putative helix–turn–helix DNA binding domain that recognises a 12 bp sequence within the terminal inverted repeats. *Nucleic Acids Res.*, **27**, 455–461.
- Vos, J.C., De Baere, I. and Plasterk, R.H.A. (1996) Transposase is the only nematode protein required for *in vitro* transposition of Tc1. *Genes Dev.*, **10**, 755–761.
- van Luenen, H.G.A.M., Colloms, S.D. and Plasterk, R.H.A. (1994) The mechanism of transposition of Tc3 in *C. elegans*. *Cell*, **79**, 293–301.
- Lampe, D.J., Churchill, M.E.A. and Robertson, H.M. (1996) A purified *mariner* transposase is sufficient to mediate transposition *in vitro*. *EMBO J.*, **15**, 5470–5479.
- Kleckner, N., Chalmers, R.M., Kwon, D., Sakai, J. and Bolland, S. (1995) Tn10 and IS10 transposition and chromosome rearrangements: mechanism and regulation *in vivo* and *in vitro*. *Curr. Top. Microbiol. Immunol.*, **204**, 49–82.
- Bhasin, A., Goryshin, I.Y. and Reznikoff, W.S. (1999) Hairpin formation in Tn5 transposition. *J. Biol. Chem.*, **274**, 37021–37029.
- Bainton, R., Gamas, P. and Craig, N.L. (1991) Tn7 transposition *in vitro* proceeds through an excised transposon intermediate generated by staggered breaks in DNA. *Cell*, **65**, 805–816.
- Kaufman, P.D. and Rio, D.C. (1992) P element transposition *in vitro* proceeds by a cut-and-paste mechanism and uses GTP as a cofactor. *Cell*, **69**, 27–39.
- Beall, E.L. and Rio, D.C. (1997) *Drosophila* P-element transposase is a novel site-specific endonuclease. *Genes Dev.*, **11**, 2137–2151.
- Lampe, D.J., Churchill, M.E.A. and Robertson, H.M. (1997) A purified *mariner* transposase is sufficient to mediate transposition *in vitro*. *EMBO J.*, **16**, 4153.
- Lohe, A.R., Sullivan, D.T. and Hartl, D.L. (1996) Subunit interactions in the *mariner* transposase. *Genetics*, **144**, 1087–1095.
- Velterop, J.S., Dijkhuizen, M.A., van't Hof, R. and Postma, P.W. (1995) A versatile vector for controlled expression of genes in *Escherichia coli* and *Salmonella typhimurium*. *Gene*, **153**, 63–65.
- Hanahan, D. (1983) Studies on transformation of *E. coli* with plasmids. *J. Mol. Biol.*, **166**, 557–580.
- Vidal, M., Braun, P., Chen, E., Boeke, J.D. and Harlow, E. (1996) Genetic characterization of a mammalian protein-protein interaction domain by using a yeast reverse two-hybrid system. *Proc. Natl Acad. Sci. USA*, **93**, 10321–10326.
- Hollenberg, S.M., Sternglanz, R., Cheng, P.F. and Weintraub, H. (1995) Identification of a new family of tissue-specific basic helix-loop-helix proteins with a two-hybrid system. *Mol. Cell Biol.*, **15**, 3813–3822.
- Gietz, R.D. and Schiestl, R.H. (1995) Transforming yeast with DNA. *Methods Mol. Cell Biol.*, **5**, 255–269.
- Colloms, S.D., van Luenen, H.G.A.M. and Plasterk, R.H.A. (1994) DNA binding activities of the *Caenorhabditis elegans* Tc3 transposase. *Nucleic Acids Res.*, **22**, 5548–5554.
- Vos, J.C. and Plasterk, R.H. (1994) Tc1 transposase of *Caenorhabditis elegans* is an endonuclease with a bipartite DNA binding domain. *EMBO J.*, **13**, 6125–6132.
- Petrokovski, S. and Henikoff, S. (1997) A helix-turn-helix DNA-binding motif predicted for transposases of DNA transposons. *Mol. Gen. Genet.*, **254**, 689–695.
- Augé-Gouillou, C., Hamelin, M.H., Demattei, M.V., Periquet, G. and Bigot, Y. (2001) The ITR binding domain of the *Mariner* Mos-1 transposase. *Mol. Genet. Genomics*, **265**, 58–65.
- Augé-Gouillou, C., Hamelin, M.H., Demattei, M.V., Periquet, M. and Bigot, Y. (2001) The wild-type conformation of the Mos-1 inverted terminal repeats is suboptimal for transposition in bacteria. *Mol. Genet. Genomics*, **265**, 51–57.
- Davies, D.R., Goryshin, I.Y., Reznikoff, W.S. and Rayment, I. (2000) Three-dimensional structure of the Tn5 synaptic complex transposition intermediate. *Science*, **289**, 77–85.

39. Sakai, J., Chalmers, R.M. and Kleckner, N. (1995) Identification and characterization of a pre-cleavage synaptic complex that is an early intermediate in *Tn10* transposition. *EMBO J.*, **14**, 4374–4383.
40. Fromont-Racine, M., Rain, J.-C. and Legrain, P. (1997) Toward a functional analysis of the yeast genome through exhaustive two-hybrid screens. *Nature Genet.*, **16**, 277–280.
41. Vojtek, A.B., Hollenberg, S.M. and Cooper, J.A. (1993) Mammalian Ras interacts directly with the serine/threonine kinase Raf. *Cell*, **74**, 205–214.
42. Li, B. and Fields, S. (1993) Identification of mutations in p53 that affect its binding to SV40 large T antigen by using the yeast two-hybrid system. *FASEB J.*, **7**, 957–963.
43. Lampe, D.L., Grante, T.E. and Robertson, H.M. (1998) Factors affecting transposition by the *Himar1* mariner transposase *in vitro*. *Genetics*, **149**, 179–187.
44. Bryan, G., Garza, D. and Hartl, D. (1990) Insertion and excision of the transposable element *mariner* in *Drosophila*. *Genetics*, **125**, 103–114.
45. Mullins, M.C., Rio, D.C. and Rubin, G.M. (1989) *cis*-acting DNA sequence requirements for P-element transposition. *Genes Dev.*, **3**, 729–738.
46. Baker, T.A., Mizuuchi, M., Savilahti, H. and Mizuuchi, K. (1993) Division of labor among monomers within the Mu transposase tetramer. *Cell*, **74**, 723–733.
47. Naumann, T.A. and Reznikoff, W.S. (2000) Trans catalysis in Tn5 transposition. *Proc. Natl Acad. Sci. USA*, **97**, 8944–8949.
48. Savilahti, H. and Mizuuchi, K. (1996) Mu transpositional recombination: donor DNA cleavage and strand transfer in *trans* by the Mu transposase. *Cell*, **85**, 271–280.
49. Bender, J. and Kleckner, N. (1992) IS10 transposase mutations that specifically alter target site recognition. *EMBO J.*, **11**, 741–750.
50. Junop, M.S. and Haniford, D.B. (1997) Factors responsible for target site selection in Tn10 transposition: a role for the DDE motif in target DNA capture. *EMBO J.*, **16**, 2646–2655.