

# Does the proposed DSE motif form the active center in the *Hermes* transposase?

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## Abstract

Donor cleavage and strand transfer are two functions performed by transposases during transposition of class II transposable elements. Within transposable elements, the only active center described, to date, facilitating both functions, is the so-called DDE motif. A second motif, R-K-H/K-R-H/W-Y, is found in the site-specific recombinases of the tyrosine recombinase family. While present in many bacterial insertion sequences as well as in the eukaryotic family of *mariner/Tc1* elements, the DDE motif was considered absent in other classes of eukaryotic class II elements such as *P*, and *hAT* and *piggyBac*. Based on sequence alignments of a *hobo*-like element from the nematode *Caenorhabditis elegans*, to a variety of other *hAT* transposases and several members of the *mariner/Tc1* group, Bigot et al. [Gene 174 (1996) 265] proposed the presence of a DSE motif in *hAT* transposases. In the present study we tested if each of these three residues is required for transposition of the *Hermes* element, a member of the *hAT* family commonly used for insect transformation. While D402N and E572Q mutations lead to knock-out of *Hermes* function, mutations S535A and S535D did not affect transposition frequency or the choice of integration sites. These data give the first experimental support that D402 and E572 are indeed required for transposition of *Hermes*. Furthermore, this study indicates that the active center of the *Hermes* transposase differs from the proposed DSE motif. It remains to be shown if other residues also form the active site of this transposase. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** *Hermes*; Transposase; Active center; DSE motif; DDE motif; Transposition

## 1. Introduction

The *Hermes* transposable element, originally isolated from the genome of the common house fly, *Musca domestica*, is currently used to transform a wide variety of insect species (Atkinson et al., 2001). Unfortunately, little is known about the basic biology of this element. As part of our effort to understand the biology of the *Hermes* transposable element, we tested the hypothesis that the active center of the *Hermes* transposase consists of a variation of a DDE motif, a catalytic triad found in many pro- and eukaryotic transposases (Bigot et al., 1996).

Transposable elements are grouped into two classes based on their mechanism of movement. Class II elements transpose via a DNA intermediate through a cut-and-paste mechanism, in which excision at the donor site is followed by reintegration of the same element without replication at

the target site somewhere else. Their overall structure is conserved and usually comes in two parts: terminal inverted repeats (ITRs) flank an open reading frame coding for a transposase, the enzyme that facilitates the movement of the element (Capy et al., 1998).

Our current knowledge on the mechanism of transposition of eukaryotic class II transposable elements is based on data obtained from a few well-studied examples, such as the *P* element from *Drosophila melanogaster*, the *Mos1* element from *Drosophila mauritiana*, the *Tc1* element from *Caenorhabditis elegans*, and the *Activator* (*Ac*) element from *Zea mays* (Engels, 1979; Jacobson et al., 1986; Emmons et al., 1983; McClintock, 1950). The molecular mechanism of transposition seems to be similar in these elements. Transposition begins by binding of one or several transposase molecules to a region close to the ITRs (Kaufman et al., 1989; Becker and Kunze, 1997; Hartl et al., 1997). The target site is opened by a staggered cut and, upon cleavage of the ITRs, ligation occurs between the 3'-end of the transposable element and the 5'-end of the target site (Kaufman and Rio, 1992). The excision site is usually

Abbreviations: ITR, inverted terminal repeat; *Ac*, *Activator*; PCR, polymerase chain reaction

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closed by gap repair using the intact sister chromatid (Engels, 1979; Rommens et al., 1993).

The chemical steps involved in transposable element transposition are carried out by the transposase. Cleavage and strand transfer of the element from donor to target site appear to be single step transesterification reactions, with a water molecule acting as the nucleophile during cleavage and with the 3'-OH group of the element acting as the nucleophile during transfer (Mizuuchi, 1992). Over the last 10 years it has been shown that both reactions are performed by a single active site in the transposase with the active center being a triad of three nucleophilic amino acid residues, the DDE motif (for the three residues, aspartate, aspartate, and glutamate). This catalytic triad captures bivalent cations, such as magnesium, which in turn direct the water molecules to the phosphodiester bond. This motif was found initially in the bacteriophage Mu and the integrase of HIV (Baker and Luo, 1994; Engelman and Craigie, 1992), and was subsequently found in the majority of prokaryotic and eukaryotic transposases (for review see Mahillon and Chandler, 1998; Capy et al., 1997).

While the DDE motif can be found in most class II transposable elements, there are exceptions. For example, the IS91 family of transposable elements has adopted a rolling circle mechanism of integration, and shows significant similarity to enzymes associated with replicons. Members of the IS110 family encode a new type of site-specific recombinase, the IS1 transposase, which shows similarity to the integrase protein of bacteriophage  $\lambda$ , and for several other insertion sequences an active center remains to be described (Mahillon and Chandler, 1998, and references therein). Among eukaryotic transposable element families, the DDE motif is not present universally. While the motif and variations thereof seem to be present in transposases belonging to the large *mariner/Tc1* family of eukaryotic class II transposable elements, other families of eukaryotic elements do not contain the motif. This was also considered to be the case for the *P* element and *hAT* element families (Capy et al., 1998).

Based on sequence alignments of six *hAT* transposases with three *mariner*, and one *Tc1* transposase, Bigot et al. (1996) proposed the existence of a DDE motif among members of the *hAT* family. According to their alignment, the second aspartate of the triad was replaced by a serine in *Ac*, *HFL1* and *Hermes*. This would be predicted to lead to a different mechanism of cleavage. Serine, a primary alcohol, rather than positioning a water molecule as the nucleophile, could act as the nucleophile and facilitate a covalent binding of the transposase to its target DNA during catalysis. The hypothesis of a DSE motif was subsequently disputed by Lerat et al. (1999) based on the lack of secondary structure similarity between *hobo* transposase and members of the *mariner/Tc1* superfamily. Unfortunately, no experimental data were available to support either hypothesis.

This study tested the hypothesis that a DSE motif formed the active center of the *Hermes* transposase. All three resi-

dues, D402, S535, and E572 were mutated and the resulting mutated transposases were tested for activity using an in vivo inter-plasmid transposition assay performed in *D. melanogaster* embryos (Sarkar et al., 1997). While mutations D402N and E572Q abolished transposase activity, the mutations of S535 to alanine and aspartate did not effect activity when compared to wild-type transposase. Furthermore, the target site preferences that were previously described for the wild-type transposase (Sarkar et al., 1997) were not changed by transposase mutants S535A or S535D. This study provides the first experimental data to support the hypothesis that the positive charge of residues D402 and E572 indeed are required for transposition. Nonetheless, based on results presented in this paper, we conclude that D402, S535, E572 do not constitute the catalytic center of *Hermes* transposase because one of these three amino acids is not essential for activity.

## 2. Material and methods

### 2.1. Helper plasmid mutagenesis

The *Hermes* transposase mutations were introduced directly into the helper plasmid pKSHHH1.4 (Sarkar et al., 1997), using the ExSite PCR-based site-directed mutagenesis kit from Stratagene. 1.67  $\mu\text{g}$  of pKSHHH was amplified using primer pairs listed in Table 1 and the following PCR program: Cycle 1: 94 °C for 4 min, 60 °C for 2 min, 72 °C for 2 min; cycles 2–9: 94 °C for 1 min, 56 °C for 2 min, 72 °C for 1 min, followed by an additional step at 72 °C for 5 min. All reactions were performed according to the manufacturers specifications.

To simplify screening for correctly mutated helper plasmids, mutagenesis primers were designed such that they additionally to the desired amino acid mutation also introduced or abolished a restriction site without changing the coding sequence of the helper plasmid at the point of introduction (see Table 1). Mutation D402N was screened by addition of *BspEI* site. Helper plasmids S535A and S535D contained an additional *EcoRI* site compared to wild-type helper, and mutation E572Q was screened through absence of an *Eco47III* restriction site. All mutated helper plasmids were sequenced using M13 forward and reverse primers to verify correct mutations and to ensure integrity of the *Hermes* open reading frame before microinjections were commenced.

### 2.2. In vivo transposition assays

Inter-plasmid transposition assays were performed essentially as described by Sarkar et al. (1997). Preblastoderm embryos of the Canton S *w* strain of *D. melanogaster* were injected with 2  $\mu\text{g}/\mu\text{l}$  of plasmid mix containing a 1:2:1 ratio of donor plasmid pHermesKSacO $\alpha$ , target plasmid pGDV1, and helper plasmid pKSHHH or mutations thereof. Embryos were allowed to develop over night and heat-

Table 1  
PCR primers used for introduction of *Hermes* transposase DSE mutations<sup>a</sup>

Oligo name	Sequence 5'–3'
D402N	GCAACGTTGCAGATATAGCAAATTTGAAAGTG
Hermes 1594-1621R	CAACGTCGGAGAACATATTTCTTTTAC
S535A	GTAAGAAATAGTTATTTTAGCCGAAGATTTTAAAG
Hermes 1976-2002R	GATAGAATTCAAATTCATCGCTTGGAC
S535D	GTAAGAAATAGTTATTTTAGACGAAGATTTTAAAG
Hermes 1976-2002R	GATAGAATTCAAATTCATCGCTTGGAC
E572Q	GCAGCATCGCAAAGGACATTTTCCCTAGC
Hermes 2097-2124R	GCTACTTGCAGGTATTGATAATAACGAC

<sup>a</sup> Primer pairs used to introduce four mutations in the *Hermes* open reading frame are listed. The numbers in the forward primers refer to amino acid position, and the letters follow the one-letter code for amino acids. Numbers in the reverse primers (indicated by R), show location of the primer in helper plasmid pKHSHH indicated by base pair position. To simplify screening for correct mutations, the primers introduced or removed restriction sites, which did not alter the amino acid composition of *Hermes* (Addition of *BspEI* site, primer *Hermes* 1594-1621R; addition of *EcoRI* site, primer *Hermes* 1976-2002R; removal of *Eco47III* site, primer *Hermes* 2097-2124R).

shocked 14–18 h post-injection at 37 °C for 1 h. After an additional incubation period of 1 h at room temperature, plasmid DNA was recovered from the embryos (Sarkar et al., 1997), and resuspended at 1 µl/10 embryos in 10 mM Tris, pH 8.

To test for transposition events, cells of *Escherichia coli* strain DH10B were transformed by electroporation with recovered plasmid DNA. An aliquot of the transformed bacteria from each experiment was tested for ampicillin resistance, and positives were scored to estimate the total number of donor plasmids recovered. The remainder of the electroporated bacteria was tested for chloramphenicol and kanamycin resistance, ampicillin sensitivity, and LacZ expression, identifying a transposition event of the *Hermes* element from the donor to the target plasmid. Per experiment up to 50 of the putative transposition events were further analysed by diagnostic *PstI* restriction enzyme digest, and a small proportion of positive plasmids was sequenced to determine the site of integration of the *Hermes* element into pGDV1. Rate of transposition for each experiment was scored as the ratio of number of transposition events per 10,000 donor plasmids screened. For each of the helper plasmids (wild-type, D402N, S535A, S535D and E572Q) as well as the negative control (no helper), transposition assays were performed in triplicate.

### 3. Results and discussion

Bigot et al. (1996) proposed, based on sequence alignments, the presence of a DDE like motif in the transposases of functional *hAT* elements. This was a controversial proposal since there had been no structural or functional basis for grouping *hAT* elements with transposable elements that contain a clear DDE motif. According to Bigot et al. (1996), the second aspartate in the triad was replaced by a serine, leading to a DSE motif in *hAT* transposases. A theoretical consequence of this alteration would be a two-step mechanism of donor cleavage, where the hydroxyl-group

Table 2  
Transposition frequencies for wild-type *Hermes* transposase and DSE mutants in *Drosophila melanogaster*<sup>a</sup>

<i>Hermes</i> transposase	No. of plasmids screened <sup>b</sup>	No. of transpositions	Rate per 10 <sup>4</sup> plasmids screened <sup>c</sup>
Positive control	50,900	25	4.91
	13,300	18	13.53
	82,350	174	21.13
	33,850	237	70.01
Negative control	50,400	0	0.00
	237,700	0	0.00
	13,150	0	0.00
D402N	168,940	0	0.00
	209,150	0	0.00
	36,100	0	0.00
S535A	204,850	124	6.05
	468,200	320	6.84
	170,850	339	19.84
S535D	125,150	81	6.47
	330,550	484	14.64
	155,415	120	7.72
E572Q	79,300	0	0.00
	282,950	0	0.00
	155,415	0	0.00

<sup>a</sup> Transposition assays were performed according to Sarkar et al. (1997). The four *Hermes* transposase mutations D402N, S535A, S535D and E572Q were tested in *D. melanogaster* embryos. While D402N and E572Q abolished function of the transposase, mutation of serine at position 535 to either alanine or aspartate did not change the transpositional activity of *Hermes* transposase. Positive control experiments were performed using wild-type KHSHH helper plasmid. No helper plasmid was injected in the negative control experiment. Letters follow the one-letter code for amino acids, and numbers represent position of the amino acid in the *Hermes* transposase.

<sup>b</sup> Estimated total number of donor plasmids recovered from injected embryos.

<sup>c</sup> Rate of transposition events per total number of donor plasmids. See Section 2.2 for a more detailed description of the assay.

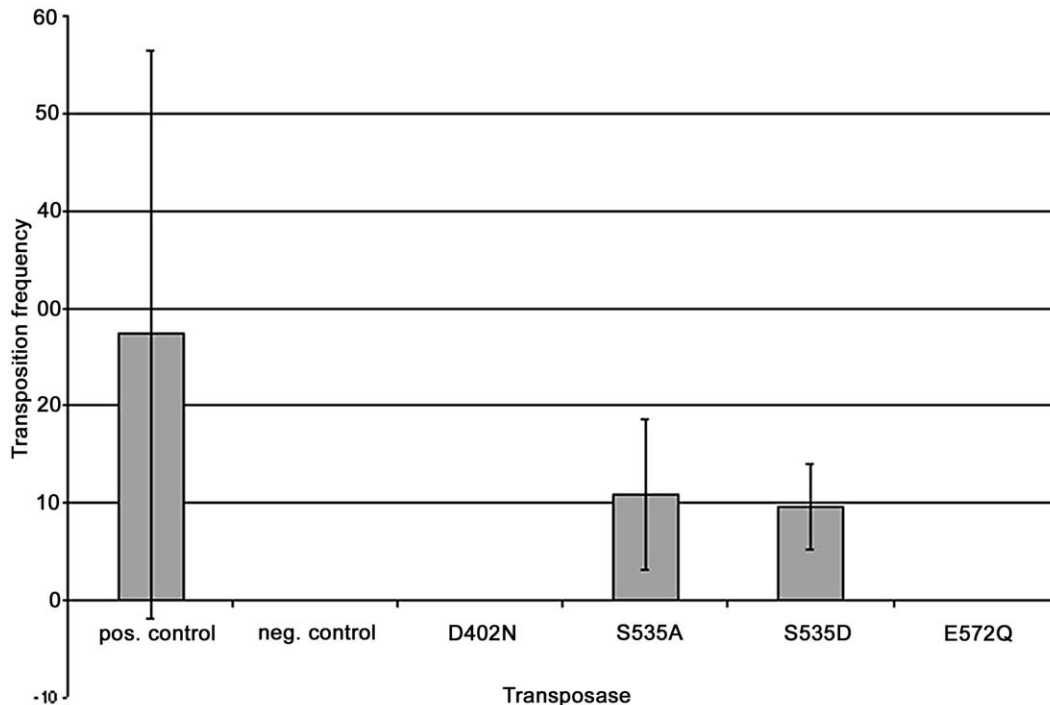


Fig. 1. Frequency of *Hermes* element transposition. Transposition frequencies obtained from *Drosophila melanogaster* using four mutated versions of the *Hermes* helper are shown (see Table 2). Error bars represent one standard deviation. While average transposition frequencies of S535A and S535D were reduced by 50% compared to positive control, differences were not statistically significant.

of the serine acted as the nucleophile leading to a covalent intermediate between DNA and transposase. This mechanism of donor cleavage is found in nature and is used by tyrosine recombinases such as phage P1 Cre protein, where the hydroxy-group of a tyrosine or serine in the active center is necessary for recombination (Grainge and Jayaram, 1999).

If the active center of the *Hermes* transposase is formed by residues D402, S535 and E572, as Bigot et al. (1996) propose, then all three amino acids would be predicted to be essential for function of the transposase. To test this hypothesis, four point mutations, D402N, S535A, S535D, and E572Q, were separately introduced into the transposase and tested individually in inter-plasmid transposition assays performed in *D. melanogaster* embryos (Sarkar et al., 1997). These assays were chosen since, to date, it has not been possible to chemically isolate functional *Hermes* transposase, the prerequisite for establishment of in vitro assays, which are commonly used to analyse mechanisms of transposition. We therefore used the only available quantitative test for *Hermes* transposase activity.

The average rate of transposition observed for wild-type transposase in the experiments described here was 27.4 transpositions per  $10^4$  donor plasmids screened (see Table 2). Variation between experiments was high with one standard deviation as large as 29.2 (see Fig. 1). As seen previously, frequencies for wild-type helper injections were subject to relatively high variability between experiments in inter-plasmid transposition assays (Lobo et al.,

1999), which may be caused, for example, by differences in fitness and survival of the injected embryos.

The exchange of aspartate for asparagine at position 402 resulted in a knockout mutant of the *Hermes* transposase. Transposition assays using the D402N helper did not result in the recovery of any transposition events, despite screening twice the number of donor plasmids as for the positive control and roughly the same number of donor plasmids as for the negative control (see Table 2 and Fig. 1).

The change of charge at position 572 by replacement of glutamate with glutamine resulted in a similar null mutant of the *Hermes* transposase. Injection of the E572Q helper did not lead to any transposition event with roughly the same number of donor plasmids screened as for the D402N mutation (see Table 2 and Fig. 1).

These results clearly indicate that the aspartate residue at position 402 and the glutamate residue at position 572 of the *Hermes* transposase are absolutely required for the transposase function. The role of the serine residue at position 535 was explored through two separate mutations: S535A (non-conservative change from polar to non polar) and S535D (non-conservative change from polar to acidic).

The transposition frequencies that were obtained for the *Hermes* helper plasmids containing mutations coding for the changes S535A and S535D, were  $10.91 \pm 7.75$  and  $9.61 \pm 4.40/10^4$  plasmids screened, respectively. These values are within the transposition frequencies obtained with the *Hermes* wild-type helper (see Table 2 and Fig.

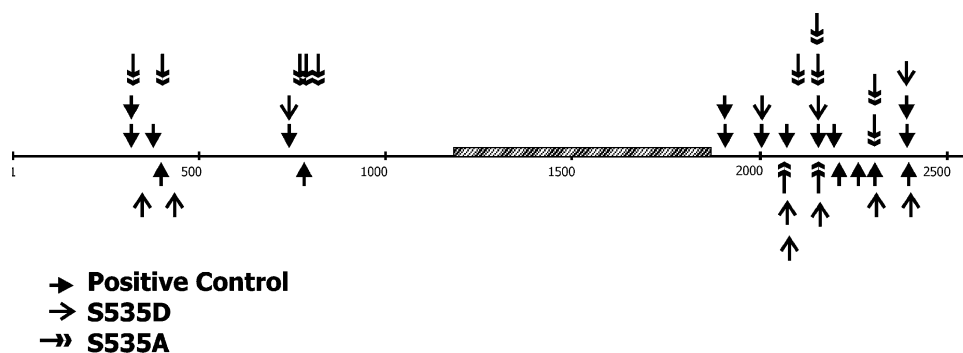


Fig. 2. Distribution of *Hermes* integrations into target plasmid pGDV1. Arrows indicate individual integrations into the target plasmid pGDV1, represented by the horizontal line. Arrows shown above the line represent integrations of the *Hermes* element in forward and arrows below represent integrations in reverse orientation. Integrations clustered in three regions, around 400 bp, around 800 bp, and between bp 1900 and 2400. Hatched area indicates position of chloramphenicol acetyltransferase gene (bp 1193–1873). Numbers refer to nucleotide positions within the pGDV1, and different arrowheads refer to the different helper plasmids used in the experiments.

1). Similar to the positive control, transposition frequencies were subject to relatively high variability between experiments. The wild-type levels of transposition activity obtained with both amino acid substitutions at position 535 refute the proposal of Bigot et al. (1996) that this serine residue constitutes part a DDE-like triad since the non-conservative substitutions tested would have been expected to have eliminated, or at least severely reduced, transposition activity if this residue formed part of a DDE-like triad.

A second possibility is that the S535 residue contributes to sequence-specific target capture (and subsequently target

site selection) of the *Hermes* transposase. The DDE motif of Tn10 has been shown, using gel mobility assays, to affect sequence-specific target capture of the Tn10 transposase (Allingham et al., 1999). We therefore investigated whether changes to the S535 residue of the *Hermes* transposase might also affect target capture, as measured by changes in the target site selection of the mutated transposase. A maximum of 12 transposition clones from each set of assays were randomly selected and sequenced to determine points of integration of the *Hermes* element into the target plasmid pGDV1. The results are shown in Table 3 and are graphically represented in Fig. 2. Integrations were found in three areas of the target plasmid, centered around base pair 350 and 750, and between base pairs 1900 and 2400. Integrations obtained with each of the three helper plasmids were found in these regions. The hot spots for *Hermes* element integration using wild-type helper were previously described to be positions 736, 2303, and 2154 (Sarkar et al., 1997). The S535D mutation led to integrations at positions 2154 and 2303 among others, and S535A as well as wild-type helper produced transposition events at position 736. Taken together, the distribution of integration sites did not differ between the experiments using all active forms of the *Hermes* transposase.

In summary, while the D402N and E572Q mutants abolished function of the transposase, the S535A and S535D mutants did not have a significant influence on either transpositional activity or the choice of integration site of the *Hermes* element indicating that these particular residues do not constitute the DSE triad proposed by Bigot et al. (1996). In the case of both the D402N and E572Q substitutions, the charge on the residues was reversed, and the substitution of serine to alanine in position 535 abolished a hydroxyl-group thought to be involved directly in lysis of the phosphodiester bond. The substitution of S535D changed the motif from a DSE to DDE, testing if the latter is more efficient in transposition. We therefore propose that, while D402 and E572 are residues that are required for the transposition of the element, S535 is not. The structure of

Table 3

Integration sites of *Hermes* elements into pGDV1 obtained from experiments with *Hermes* transposase S535A, S535D, and wild-type transposase<sup>a</sup>

S535A		S535D		Wild-type	
346	R	317	F	319	F
430	R	390	F	319	F
736	F	767	F	339	F
2005	F	789	F	396	R
2077	R	810	F	736	F
2077	R	2063	R	778	R
2153	F	2100	F	1904	F
2157	R	2154	F	1904	F
2310	R	2154	F	2002	F
2390	F	2155	R	2070	F
2401	R	2303	F	2161	R
		2304	F	2198	F
				2218	R
				2267	R
				2312	R
				2390	F
				2396	F
				2401	R

<sup>a</sup> The integration sites of a small number of randomly selected transposition events from inter-plasmid transposition experiments using *Hermes* helper plasmids S535A, S535D, and wild-type transposase are shown. The numbers indicate integration sites of the *Hermes* element into pGDV1, referring to the base pair of pGDV1 adjacent to the right hand end of the *Hermes* element. F and R refer to orientation of the element with respect to the target plasmid.

the active center is therefore different from the DSE motif proposed by Bigot et al. (1996). Two possibilities for the location of the active center can be envisaged. One is that D402 and E572 still form part of a DDE-like triad but that the central residue in this triad remains unidentified in the *Hermes* transposase. Several aspartate and glutamate residues are located close to the S535 residue and so may potentially function as the central residue of such a triad. The alternative possibility is that the *Hermes* transposase does not contain a DDE active center. We are currently investigating the nature of this center in *Hermes*.

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