

Transposable element interactions in insects: crossmobilization of *hobo* and *Hermes*

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Abstract

There are four non-drosophilid insect gene vector systems available that have been constructed from the short inverted repeat-type transposable elements *Minos*, *piggyBac*, *mariner* and *Hermes*. These elements (with the possible exception of *piggyBac*) are members of transposable element families that appear to be widespread in nature. Because these transposable element families are large it is possible that an insect species targeted for transformation will contain related transposable elements. The data presented here begin to address directly the question of interaction between diverged but related members of transposable element families. We tested the ability of the *hAT* elements *hobo* and *Hermes* to interact and cause crossmobilization. Using plasmid-based and chromosome-based element mobility assays we found that the terminal sequences of *hobo* and *Hermes* were almost equally good substrates for *hobo* transposase. However, this ability to crossmobilize was not reciprocal. *Hermes* transposase was only rarely able to cause the excision of *hobo* elements from plasmids and was never observed from germline chromosomes. These results have important implications for transgenic insect studies in the future.

Keywords: gene vector, *Hermes*, *hobo*, transgenic, transposable element.

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Introduction

Non-drosophilid insect transformation is an emerging technology that is likely to have a wide range of applications within insect biology (O'Brochta & Atkinson, 1996). Efforts within this field over the last decade have focused on the identification, isolation and development of mobile genetic elements that will serve as efficient gene vectors. There are now at least four gene vector systems available for the germline transformation of non-drosophilid insects, all of which are based on short inverted repeat transposable elements (Medhora *et al.*, 1988; Loukeris *et al.*, 1995; O'Brochta *et al.*, 1996; Gueiros-Fiho & Beverley, 1997; Sarkar *et al.*, 1997a,b; Coates *et al.*, 1998; Fadool *et al.*, 1998; Handler *et al.*, 1998; Jasinskiene *et al.*, 1998). The successful development of these gene transfer systems will accelerate efforts to develop transgenic insects and to explore their utility as insect pest management tools.

The vectors currently under development for non-drosophilid insects that appear to have the most promise are derived from transposable elements that belong to large and widely distributed transposable element families. Elements from such families appear to have the ability to function in foreign hosts, which has made them attractive candidates for vector development. Unfortunately the size and distribution of these transposable element families make it quite possible that the target insect being used as a host in a transformation experiment will contain a transposable element system from the same family. The presence of a related transposable element system could compromise the effectiveness of the vector. In this paper we begin to test the ability of related members of a transposable element family, *hobo* and *Hermes*, to interact.

Hermes and *hobo* elements are members of the widely distributed *hAT* family of transposable elements. *Hermes* was originally isolated from *Musca domestica* (family, Muscidae), is 2.75 kb in length with 17-bp terminal inverted repeats (Atkinson *et al.*, 1993; Warren *et al.*, 1994). *hobo* was isolated from *Drosophila melanogaster* (family, Drosophilidae) and is

≈3 kb in length with 12-bp terminal inverted repeats (Streck *et al.*, 1986). These elements are structurally related and have transposase-coding regions that are 55% identical at the amino acid level (Warren *et al.*, 1994). Using plasmid-based *in vivo* excision assays and chromosome-based excision assays we tested directly the ability of the *hobo* element from *D. melanogaster* and the *Hermes* element of *M. domestica* to interact in such a way as to cause vector destabilization and excision. We found that *Hermes* and *hobo* transposases were not only capable of interacting with their own terminal sequences to result in element excision but also of interacting with the terminal sequences of the other element to result in element crossmobilization. We suggest that these results have implications for future vector development and deployment efforts.

Most field applications of transgenic insect technology currently under serious consideration involve mass releases of transgenic insects or the establishment of a wild transgenic insect population. Experience gained through mass rearing strains of insects constructed with conventional genetic tools demonstrate that even levels of instability resulting from recombination at frequencies as low as 0.07% are intolerable and illustrate the severe stability demands that will be placed on genetically engineered strains (Franz *et al.*, 1994). More ambitious plans for releasing and spreading transgenes through wild insect populations will require, among other things, the transposable element vector to have a very well-defined set of mobility properties that will include high rates of movement initially to facilitate spread and stability of the desired genotype once equilibrium has been reached.

Field releases of transgenic insects will also result in regulatory and biosafety concerns. To address these concerns some understanding of the behaviour of gene vectors in the target and non-target insects will be essential. Again, stability appears to be one characteristic that will be relevant in evaluating a vector's ability to be used outside the laboratory. A potential threat to the stability of any transgene or gene vector is the presence of factors within the host that might interact with the vector to cause it to either continue to move

when such movement is undesirable or to inhibit its movement and prevent, for example, the spread of the transgene through a population. Serious concerns about containment of the transgene within the target species can also be expected, given the well-documented cases of horizontal gene transfer involving transposable elements that have occurred in the recent past (Kidwell, 1992; Simmons, 1992; Robertson & Lampe, 1995). Given the significant progress in the development of transgenic insect technology over the last few years it is essential that we begin to explore the behaviour of transgenes and transgenic vectors in 'foreign' insect hosts.

Results

Hermes/hobo interactions: plasmid-based mobility tests

hAT element excision is transposase dependent. Transpositional recombination of short inverted repeat-type elements appears to proceed by a cut-and-paste mechanism whereby transposable element excision is a requisite step. We used transposable element excision as an indicator of element movement because it is simple to measure and is a sensitive measure of transposase activity. When introduced into a strain of *D. melanogaster* that lacks any detectable *hobo* elements (Canton-S), *hobo* and *Hermes* element movement has been found to be completely dependent upon the expression of their respective transposases (Table 1) (Atkinson *et al.*, 1993; O'Brochta *et al.*, 1994; Sarkar *et al.*, 1997a,b). Furthermore, transposable element excision events have never been recovered following the direct transformation of the excision assay plasmids in *Escherichia coli* strain DH10B. Consequently, all *hobo* and *Hermes* excision events recovered in these experiments occurred in the embryonic cells of the developing insect used to perform the excision assay and were transposase dependent.

Homologous element/transposase interactions. *Hermes* transposable elements readily excised from excision indicator plasmids in the presence of *Hermes* transposase in *D. melanogaster* embryos (Table 1). Of the 15 300 *Hermes* excision indicator plasmids

Reporter	Transposase	Plasmids	Excisions	Frequency ($\times 10^{-3}$)
<i>Hermes</i>	None	42 000	0	0
<i>Hermes</i>	<i>hspHermes</i>	15 300	33	2.16
<i>Hermes</i>	<i>hspHobo</i>	357 266	105	0.29
<i>hobo</i>	None	110 000	0	0
<i>hobo</i>	<i>hspHobo</i>	130 100	43	0.33
<i>hobo</i>	<i>hspHermes</i>	190 300	3	0.015

Table 1. *Hermes* and *hobo* excision from plasmids.

At least three independent injection experiments were done for each combination of reporter and helper.

Table 2. Homologous element/transposase interactions in insect embryos.

<i>n</i> ^a	Left flank ^b	Left ITR ^c	Additional nucleotides at breakpoint	Right ITR ^c	Right flank ^b
<i>Hermes reporter with Hermes transposase in D. melanogaster</i>					
2	0		AC		-2
1	-1		ATACAC		-2
1	-2		AATACAGACTGTAATACCCTCCAC		-2
1	-8		TACCAACCCTCCAC		-2
1	-37		A		-93
1	-41				-94
1	-113				-88
1	-166				-48
2	-166				-51
1	-166				-75
1	-173				-133
1	-220				-90
2	-215		C		-110
1	-152				-260
1	-362				-227
1	-210				-384
1	-699				-175
1	-209				-664
1	-805				-87
1	-574				-383
1	-508				-487
1	-862				-481
<i>hobo reporter with hobo transposase in D. melanogaster</i>					
2	-4		AATCAGGATTCCTGAT		-19
5	-6		AATCAGGATTCCTGAT		-19
1	-174				-64
1	-91				-218
1	-93				-220
1	-326				-52

^a Number of independent events recovered. ^b Value refers to the number of bases deleted from the DNA flanking the inverted terminal repeat (ITR). Zero indicates that none of the flanking DNA was deleted. ^c Value refers to the number of bases of the inverted terminal repeat that were present in the excision product. No value indicates the complete absence of the ITR.

screened, thirty-three excision events (0.216%) were recovered. Of the thirty-three excision events, twenty-five were analysed by restriction mapping using *NotI* and DNA sequencing (Table 2). Excision of *Hermes* in which the entire transposable element was removed leaving all or some of the flanking 8-bp direct duplication was observed in five cases. In these cases a small number of nucleotides were also added at the point of recombination (Atkinson *et al.*, 1993). Eighty per cent of the excision events involved not only the complete excision of the *Hermes* element but the removal of large regions of the DNA flanking the element. In those cases involving the deletion of sequences flanking the transposable element, our analysis of the terminal sequences of the excised DNA did not reveal any similarity to the terminal inverted repeats of *Hermes*. In *D. melanogaster* deletion breakpoints were most likely to be located near the original element integration site suggesting that following excision there was exonuclease activity that was responsible for removing the flanking sequences.

A similar pattern of excision products was found

when *hobo* excision was mediated by *hobo* transposase in *D. melanogaster* (Table 2). Although the classes of excision products were similar to those found with *Hermes*, the frequency of *hobo* excision mediated by *hobo* transposase was less than observed with *Hermes* (Table 1). Of the 130 000 excision indicator plasmids recovered and screened only forty-three (0.033%) were excision events. The DNA sequence was determined for fourteen events and seven resulted from the complete removal of *hobo*, the deletion of a few flanking nucleotides and the addition of sixteen nucleotides at the breakpoint. The structure of these events was generally similar to those reported by others under similar conditions although not identical (Atkinson *et al.*, 1993; Handler & Gomez, 1995). We also observed excision events in which extensive regions of the flanking DNA were deleted. These types of highly imprecise excision events resemble those seen with *Hermes* transposase mediated excision of *Hermes* in *D. melanogaster*. Such events were not reported by Atkinson *et al.* (1993) although they have been subsequently observed

in our laboratories (unpublished data). The reason for the differences between the results reported here and those reported by Atkinson *et al.* (1993) are not clear but may be related to the fact that the elements used in the present experiment were flanked by different sequences from those used in earlier studies. The influence of sequences flanking a transposon on element excision has not been extensively investigated. In some cases flanking DNA did influence element remobilization (Elick *et al.*, 1997). The distribution of breakpoints resembled that resulting from *Hermes* excision in *D. melanogaster* and was consistent with the idea that an exonuclease activity was responsible for the deletion of flanking sequences. Deletions did not appear to arise because of the presence of cryptic terminal sequences within the flanking DNA.

Heterologous element/transposase interactions. The *Hermes* element could be mobilized by the expression of *hobo* transposase. Of the 357 266 *Hermes* excision indicator plasmids recovered, 105 (0.03%) were excision products (Table 1). Unlike *Hermes* excision events recovered using *Hermes* transposase all events recovered from this experiment were imprecise (Table 3). The structure of the imprecise excision events recovered in this experiment was distinctly different, however, from that of the imprecise excision events recovered when the homologous transposase was used. All of the excision events analysed (16/16) contained at least one breakpoint within the *Hermes* excision indicator element resulting in an internal deletion of the element and leaving one of the terminal inverted repeats. Two of the sixteen events also had their second deletion breakpoint within the *Hermes* excision indicator plasmid resulting in an excision

product containing both terminal inverted repeats but missing internal sequences. The remaining excision events (14/16) contained deletions in the flanking DNA. In thirteen of the fourteen events with this general structure the right terminal inverted repeat was deleted. Extra nucleotides not present in the original indicator plasmid were occasionally found at the deletion breakpoints.

The *hobo* element also appeared to be mobilized by *Hermes* transposase (Table 1). These events, however, were much less frequent than those seen with any other combination of terminal sequences and transposase. Only three excision events were found after recovering and screening 190 300 indicator plasmids. These events arose from the imprecise excision of *hobo* and were similar in structure to the *Hermes* events recovered when using *hobo* transposase (Table 3). Although we have never observed the excision of *hobo* in the absence of transposase in *D. melanogaster* E strains (Atkinson *et al.*, 1993) an analysis of the *Hermes* transposase/*hobo* element data using a *G*-test of independence (Sokal & Rohlf, 1981) did not reveal any significant effect of *Hermes* transposase on *hobo* excision. The rare nature of the apparent crossmobilization events do not permit us to conclude unequivocally that *Hermes* transposase can interact with *hobo* elements.

Hermes/hobo interactions: chromosome-based mobility tests

Results similar to those obtained with the plasmid assay were obtained when reporter elements and transposase genes were located on chromosomes. *Hermes* transposase-mediated excision of *Hermes* elements in the strain *Hermes-w⁺-515* occurred in

<i>n</i> ^a	Left flank ^b	Left ITR ^c	Additional nucleotides at breakpoint	Right ITR ^c	Right flank ^b
<i>Hermes</i> reporter with <i>hobo</i> transposase in <i>D. melanogaster</i>					
1	0	+ 459	CATTA		- 124
1	0	+ 277	TGTGACGGTGAT	+ 549	0
1	0	+ 970		+ 53	0
1	0	+ 67			- 1000
1	0	+ 371			- 300
2	0	+ 383			- 617
1	0	+ 1094			- 906
1	0	+ 738			- 2000
1	0	+ 738			nd
1	0	+ 746			- 254
1	0	nd			nd
1	nd			nd	0
<i>hobo</i> reporter with <i>Hermes</i> transposase in <i>D. melanogaster</i>					
1	- 186				- 140
1	0	+ 308	CGGTATACGGTATA	+ 247	0
1	0	+ 697			3- 140

^a, ^b and ^c As for Table 2. nd, precise breakpoint not determined but localized to a region.

Table 3. Heterologous element/transposase interactions in insect embryos.

Table 4. *Hermes* and *hobo* excision in *D. melanogaster* germlines.

Genotype		Germlines			Progeny		
Reporter	Helper	Tested	Excisions	%	Screened	Excisions	%
<i>Hermes-w⁺-520</i>	<i>hspHermes-2</i>	134	36	26.4	9041	50	0.55
	<i>hspHermes-48</i>	85	15	17.6	6840	33	0.48
<i>Hermes-w⁺-515</i>	<i>hspHermes-2</i>	132	108	81.8	9190	1144	12.4
	<i>hspHermes-48</i>	134	124	92.5	8491	1034	12.2
	<i>hspHobo</i> (HSH2)	419	10	2.4	26685	167	0.63
	<i>Gla</i> (no transposase)	NA	NA	NA	12178	0	0
<i>hobo-w⁺ 120</i>	<i>hspHobo</i> (HSH2)	271	45	16.7	17410	168	0.96
	<i>hspHermes-2</i>	115	0	0	12298	0	0
	<i>Gla</i> (no transposase)	NA	NA	NA	13836	0	0

NA, not applicable, matings for this experiment were done *en mass*.

80–90% of the genomes tested regardless of the chromosomal source of *Hermes* transposase (Table 4). Approximately 12% of all the progeny arising from these crosses contained chromosomes from which *Hermes* had excised. Using the *Hermes* reporter strain *Hermes-w⁺-520* which contains the *Hermes-w⁺* element located in a different position on the second chromosome than in *Hermes-w⁺-515* excision occurred in only 17–26% of the germlines tested and only 0.5% of the progeny contained chromosomes from which *Hermes* excised (Table 4). As with *Hermes-w⁺-515* two chromosomal sources of *Hermes* transposase did not result in very different excision frequencies.

As was observed using plasmid-based mobility assays, chromosomally located *hobo* elements in the presence of *hobo* transposase excised at lower frequencies than *Hermes* elements (Table 4). Seventeen per cent of the germlines containing *hobo-w⁺* and *hsp-hobo::transposase* produced excision events and, from those germlines yielding excision events, only 1% of the progeny contained chromosomes resulting from excision.

Hermes excision also occurred in the presence of *hobo* transposase (Table 4). Under the conditions used in these experiments, 2% of the germlines tested that contained *Hermes-w⁺* and *hsp-hobo::transposase* produced excision events. In those germlines in which *Hermes* was mobile only 0.6% of the progeny contained chromosomes resulting from excision. In one of the three experiments performed to test the mobility of *Hermes* in the presence of *hobo* transposase we also recovered what appeared to be *Hermes* transposition events. These events occurred in ten of the 139 germlines tested and consisted of 173 progeny out of 6961 progeny screened. Transposition events were recognized as exceptional progeny arising from *CyO::hspHobo/Hermes-w⁺-515* that had been crossed to *yw^{67c23}*. The exceptional progeny contained the *CyO* balancer chromosome and had red eyes. These indivi-

duals are likely to have arisen from the transposition of *Hermes-w⁺* from the second chromosome to either the *CyO* balancer, chromosome 3 or the X chromosome, although we cannot rule out the involvement of a chromosomal translocation or a transposase-independent illegitimate recombination event. Transposition events arising in experiments involving *Hermes* reporter elements and *Hermes* helper-containing chromosomes were more difficult to detect because of the presence of *w⁺* on the *CyO::hspHermes* balancer and were not recorded.

In contrast to the plasmid-based excision assays, *Hermes* transposase-mediated excision of chromosomally integrated *hobo* elements was not observed in this experiment. No *hobo* element excision was observed after screening 12 298 chromosomes from 115 germlines (Table 4).

Discussion

Transposable elements are proving to be particularly useful as tools for manipulating insect and plant genomes and are currently being developed as tools for vertebrate genome manipulation (Ivics *et al.*, 1997). The transposable elements that have proven to be the most valuable or with the most potential value as gene vectors are members of transposable element families that are widespread in nature. Although we do not understand the details of how transposable elements become distributed within taxa it is likely that one of the reasons why some elements are widespread is because they have the ability to function when introduced into a foreign host, permitting their establishment and eventual spread in the new host. Elements with the ability to function more or less autonomously from the host genome not only have the potential to be successfully transferred horizontally but also have the potential to serve as gene vectors. Members of the *hobo*, *Ac*, *Tam3* (*hAT*) family of transposable elements have the ability to function in foreign hosts, show

evidence of being transferred horizontally between taxa and have been developed as gene vectors or gene-tagging agents with broad host ranges.

hAT elements have been discovered and isolated from a number of widely divergent taxa. *hAT* elements have been found in the plants *Zea mays* (*Ac/Ds*) (Federoff *et al.*, 1983), *Antirrhinum majus* (*Tam3*) (Sommer *et al.*, 1985), *Arabidopsis thaliana* (*Tag1*) (Tsay *et al.*, 1993) and *Nicotiana tobacum* (*Slide*) (Grappin *et al.*, 1996); the fungi *Tolypocladium inflatum* (*restless*) (Kempken & Kueck, 1996) and *Ascobolus immersus* (*Ascot-1*) (Colot *et al.*, 1998); the fish *Oryzias latipes* (*Tol2*) (Koga *et al.*, 1996); and the insects, *Lucilia cuprina* (*hermit*) (Coates *et al.*, 1996), *Bactrocera tryoni* (*homer*) (O'Brochta & Atkinson, 1996), *Musca vetustissima* (*Hector*) (Warren *et al.*, 1995), *Bactrocera dorsalis* (*hopper*) (Handler & Gomez, 1997), *Drosophila melanogaster* (*hobo*) (Streck *et al.*, 1986) and *Musca domestica* (*Hermes*) (Warren *et al.*, 1994). *Hermes* has been developed into an efficient germline transformation vector for *D. melanogaster* and can serve a similar function, although less efficiently, in the mosquito *Aedes aegypti* (O'Brochta *et al.*, 1996; Jasinskiene *et al.*, 1998) and the Mediterranean fruit fly, *Ceratitidis capitata* (unpublished data). It is likely that *hAT* elements and *Hermes* in particular will also be useful in other insect species (Sarkar *et al.*, 1997a).

Given the widespread distribution of elements from the *hAT* family of transposons it is possible that when vectors or gene tagging agents constructed from these elements are used in hosts other than the ones from which the elements were originally isolated, there will be related transposable element systems present in the genome of the new host. Use of gene vectors and tagging agents based on transposable elements in species that contain functional members of these families of elements may compromise the effectiveness of these transposable element tools in a number of ways. First, transposase produced by endogenous elements may destabilize integrated heterologous elements and transgenes. Second, the transposase associated with the gene vector system used in the creation of the transgenic insect may cause the movement of endogenous transposable elements, resulting in mutation or reduced viability. Finally, the transposase from an endogenous transposable element system may interfere with the functioning of the transposase associated with the vector system by blocking binding sites or through the formation of non-functional transposase multimers, thereby reducing the frequency at which transgenic organisms are produced. Consequently, understanding the potential of related transposable elements to interact will help us choose the appropriate vector system and to predict the behaviour of these

elements following their introduction into a foreign host.

In this paper we demonstrated directly the ability of the *hobo* element from *D. melanogaster* and the *Hermes* element of *M. domestica* to interact in such a way as to cause vector destabilization. We found that *Hermes* and *hobo* transposases were not only capable of interacting with their own terminal sequences but also of interacting with the terminal sequences of the other element. In this experiment we concentrated on measuring element excision because it is a sensitive method for detecting element movement; however, we were also able to occasionally see element transposition. Using both a plasmid-based and a chromosome-based transposable element mobility assay we were able to detect the ability of *hobo* transposase to mediate the excision of *hobo* and *Hermes* elements. Surprisingly, there was little difference between the rates of excision observed from plasmid substrates containing *hobo* and *Hermes* termini in the presence of *hobo* transposase. Using a plasmid-based excision assay we observed *hobo* and *Hermes* excision events in one out of every donor plasmids screened when *hobo* transposase was supplied. Under these conditions *Hermes* terminal sequences are as good a substrate for *hobo* transposase as are *hobo* terminal sequences. When using our chromosome-based mobility assay we saw somewhat more *hobo* excisions than *Hermes* excisions in the presence of *hobo* transposase; however, *Hermes* excisions were not rare, supporting the conclusion that *Hermes* terminal sequences are good substrates for *hobo* transposase. Interestingly, the ability of these related transposable elements to crossmobilize was not reciprocal. *Hermes* transposase was much less effective at moving *hobo* elements compared to *hobo* transposase's ability to mobilize *Hermes* elements. Only one *hobo* excision event out of approximately every 100 000 donor plasmids screened was recovered when *Hermes* transposase was supplied. Excision of *hobo* from chromosomes was undetectable in our experiment when *Hermes* transposase was supplied. Clearly, *hobo* terminal sequences are not a good substrate for *Hermes* transposase.

The terminal inverted repeats of short inverted repeat-type transposable elements may serve as binding sites for the transposase, as appears to be the case for the *mariner*-like elements (Lampe & Robertson, 1996), or they may act only as recombination signal sequences indicating where strand cleavage should occur but with transposase binding to DNA at subterminal sequences, as appears to be the case for *Drosophila P* elements (Kaufman *et al.*, 1989). The transposase may also bind at both the terminal and

subterminal regions of the element as is the case for *Ac* (Becker & Kunze, 1997). *Hermes* and *hobo* are uniquely related and this similarity may account for the non-reciprocal nature of the interactions observed. The terminal inverted repeats of *Hermes* are 17 bp in length while those of *hobo* are only 12. However, 10 of the first 12 bp of the *Hermes* terminal inverted repeat are identical to those of *hobo* (Warren *et al.*, 1994). Therefore, the *Hermes* terminal inverted repeat essentially has a *hobo* terminal repeat embedded within it. This may account, in part, for the observed behaviour of *Hermes* terminal sequences in the presence of *hobo* transposase and why they are good substrates for *hobo* transposase.

The structures of the excision products resulting from *hobo* transposase-mediated excision of *hobo* and *Hermes* elements were similar, although there were some distinctive differences. *hobo* transposase-mediated excision of *hobo* elements resulted in the complete removal of the *hobo* element as well as the deletion of varying amounts of flanking DNA. In addition, extra nucleotides were added at the excision breakpoint. *hobo* excision products with this structure have been reported by others and are common for *hAT* elements (Coen *et al.*, 1989; Fedoroff, 1989; Atkinson *et al.*, 1993; Handler & Gomez, 1995; Colot *et al.*, 1998). The removal of sequences flanking the *hobo* element appeared to be due to the action of an exonuclease activity and not due to the presence of sequences that served as cryptic terminal repeats. The *hobo* transposase-mediated *Hermes* excision events had a somewhat different structure. The most notable difference was the presence in some cases of *Hermes* terminal sequences and deletion of sequences internal to the *Hermes* element. *Hermes* transposase-mediated excision of *Hermes* elements in *D. melanogaster* never resulted in excision products with such a structure, suggesting that there are very distinct differences between how these transposases interact with the *Hermes* substrate in *D. melanogaster*. These interactions may lead to either altered cleavage patterns or to altered post-cleavage repair processes. While there are clear similarities between these *hobo* transposase substrates, there are also significant differences that seem to be important in mediating transposase activity. Because the terminal inverted repeat sequences are nearly identical, these results suggest that subterminal sequences play an important role in mediating the excision reaction of *hobo* and *Hermes*. This would be consistent with what is known about the *cis* sequence requirements of other transposable elements such as *P* (Mullins *et al.*, 1989) and *Ac* (Coup-land *et al.*, 1989).

The structure of *Hermes* transposase-mediated

Hermes excision events in both *D. melanogaster* and *M. domestica* greatly resembled that of *hobo*-mediated *hobo* excisions. That is, excision products usually had deletions of various sizes encompassing the DNA flanking the element with the occasional addition of extra nucleotides at the breakpoint. The similarity in the structures of the *Hermes* excision products recovered from *D. melanogaster* and *M. domestica* suggests that there is little host influence on *Hermes* movement. This conclusion is consistent with the findings of Sarkar *et al.* (1997a) who found that transpositional recombination of *Hermes* in these two species also appeared host independent as illustrated by the similarities in the integration site preferences displayed by the element in these and other species. Host independence appears not to be the case for the *hobo* system. Atkinson *et al.* (1993) reported the structure of *hobo* excision events recovered in *D. melanogaster* and *M. domestica* and found them to be quite different in these two hosts suggesting an influence of the host on element movement and/or subsequent repair processes. The data presented here begin to illustrate differences between these two related transposable element systems which might be significant in assessing their suitability as gene vectors.

The phenomenon of transposable element cross-mobilization has rarely been tested directly but has been suggested by ourselves and others. For example, Atkinson *et al.* (1993) reported that *hobo* elements present on plasmids could excise in the absence of *hobo*-encoded transposase when introduced into the developing embryos of the housefly, *M. domestica*, whereas under similar conditions in *D. melanogaster*, *hobo* excision would absolutely require *hobo*-encoded transposase. The authors concluded that *M. domestica* embryos contain a *hobo* transposase-like activity capable of interacting with and mobilizing *hobo*. They subsequently demonstrated the presence of the functional *hobo*-like transposable element system, *Hermes*, in *M. domestica*. Similarly, Handler & Gomez (1995) reported *hobo* excision from plasmid substrates that were introduced into the embryos of a number of *Drosophila* species in the absence of any experimentally provided *hobo* transposase and suggested that this also represented crossmobilization by endogenous *hobo*-like elements as was reported in *Musca*. Fraser *et al.* (1995) reported what also appeared to be transposase-independent movement of the *piggyBac* element in various cell lines and concluded that the cell lines likely contained a *piggyBac*-related transposase activity. The unique retrotransposon *Penelope* from *D. virilis*, when injected into developing *D. virilis* embryos, resulted in the induction of visible mutations (Evgen'ev *et al.*, 1997).

These mutations were due to the mobilization of two unrelated elements, *Ulysses* and *Paris*. The authors proposed that *Penelope* was directly responsible for this transposable element crossmobilization although the mechanism was not clear. A member of the *P* element family of elements was isolated from the drosophilid *Scaptomyza pallida*. This element was 76% identical to the canonical *P* element of *D. melanogaster* yet was still capable of interacting with and mobilizing nonautonomous *P* elements in *D. melanogaster* (Simonelig & Anxolabehere, 1991). Finally, the *Ubiquitous* and *Ac* transposable element systems of *Zea mays* are related and appear to be capable of interacting (Pisabarro *et al.*, 1991).

The results reported here have important implications for future work on transgenic insects and the development of insect gene vectors. The data presented here call attention to an issue that is likely to be a concern for those using transgenic insect technology; namely, there is the potential for transposable elements belonging to the same family to interact even when they have transposases that share only 55% amino acid sequence identity and that this interaction can result in element crossmobilization, leading to the destabilization and excision of an integrated vector. Other forms of interaction are possible such as transposase inactivation through the formation of heteromultimers or through occlusion of binding sites. There are a number of implications of our results. First, the choice of a gene vector may be influenced by the potential of that vector to interact with endogenous elements. Second, the potential for related elements to interact argues strongly for the development of multiple independent insect gene vector systems so that the most appropriate vector can be chosen for that particular species. Third, element interaction and vector destabilization suggest that host insects be examined for the presence of transposable element family members related to the elements to be used as gene vectors in this species. Finally, future efforts to modify and improve transposable element-based gene vectors might focus on increasing specificities and limiting element crossmobilization.

Experimental procedures

Plasmid-based excision assay

The plasmid-based transposable element excision assay was performed essentially as described (O'Brochta & Handler, 1988). Combinations of *hobo* and *Hermes* excision indicator plasmids and transposase-encoding helper plasmids were mixed, coprecipitated and resuspended in 5 mM KCl; 0.1 mM PO₄ (pH 7.8) so the concentration of each plasmid was $\approx 0.5 \mu\text{g}/\mu\text{l}$. Control injections were performed using DNA solutions containing only the excision indicator plasmids.

The *Hermes* excision indicator plasmid used was pHermesKSacO α (Sarkar *et al.*, 1997a) and consists of a non-autonomous *Hermes* element containing a marker gene cassette with the kanamycin resistance gene, the sucrose gene (*SacRB*) from *Bacillus subtilis*, the ColE1 origin of replication from pBR322, and the α peptide coding region of the β -galactosidase gene. The *hobo* excision indicator plasmid, phoboSacO α , was similar to the *Hermes* excision indicator plasmid and was constructed by inserting the terminal sequences (≈ 1 kb of each end) of *hobo* into a plasmid containing the *M. domestica* genomic sequences found flanking the *Hermes* excision indicator plasmid. The *hobo* and *Hermes* excision indicator plasmids therefore contained elements embedded in an identical sequence environment consisting of 0.936 kb of *M. domestica* genomic DNA flanking the left end and 1.99 kb of DNA flanking the right end. The *hobo* excision indicator contained only the sucrose gene, the origin of replication and the lacZ α peptide coding region. Both excision indicator plasmids contained *NotI* sites at positions 0.936 kb from the left terminal repeat and 1.99 kb from the right terminal repeat.

The plasmids pKSHSH and pKhsphobo were used as helper plasmids to express *Hermes* and *hobo* transposase, respectively (O'Brochta *et al.*, 1994; Sarkar *et al.*, 1997a,b). Both plasmids contain a transposase gene under the regulatory control of the *hsp70* promoter from *D. melanogaster*.

The plasmid mixture was injected into preblastoderm embryos as described (Spradling, 1986). All injections into *D. melanogaster* were done into the wild-type strain Canton-S, which does not contain endogenous *hobo* elements (Streck *et al.*, 1986). After injection, the embryos were allowed to develop at 25°C for ≈ 18 h in a humid, oxygen-rich atmosphere. The developed embryos were heat-shocked at 37°C for 1 h, allowed to recover at 25°C and viable embryos, as indicated by advanced stages of embryogenesis, were collected. Low-molecular-weight DNA was extracted from the embryos as described (Hirt, 1967) and used to transform the *E. coli* strain DH10B by electroporation. *Hermes* and *hobo* excision events were both recognized as plasmids conferring sucrose resistance to the host and had no β -galactosidase activity. Plasmids arising from precise excision events and digested with *NotI* will yield a 3 kb band corresponding to the pBS vector (Stratagene, La Jolla California) and is expected to be present in all excision events and a 2.9 kb band corresponding to the *M. domestica* genomic DNA flanking the transposable element. Excisions resulting in deletion of DNA sequences flanking the transposable element will result in a *NotI* fragment less than 2.9 kb, while excisions resulting from internal deletions of the transposable element will result in a *NotI* fragment greater than 2.9 kb. *NotI* restriction endonuclease digestion of the plasmids isolated from these colonies therefore was used to confirm the nature and extent of the excision event. The DNA sequence of the excision reaction products was determined with the aid of an ABI 373 automated sequencer (PE Applied Biosystems, Foster City, California).

Chromosome-based excision assay

Transgenic *D. melanogaster* containing either *Hermes* or *hobo* elements carrying the dominant eye colour marker, mini-*white*, were used as excision indicators. Two *Hermes* indicator strains (*Hermes-w*⁺-515 and *Hermes-w*⁺-520) and a

single *hobo* strain (*hobo-w⁺*-120) were used in these experiments. Strains *Hermes-w⁺*-515 and -520 were created by transforming *yw^{67c23}* with the vector p*Hermes-w⁺* using the helper plasmid pSHH1.9 as a transposase source (O'Brochta *et al.*, 1996). Both *Hermes* excision indicator strains used in this experiment contain the *Hermes-w⁺* element inserted in the second chromosome. The *hobo-w⁺*-120 strain was created by transforming *yw^{67c23}* using a *P* element vector carrying the *hobo-w⁺* element. Strain *hobo-w⁺*-120 contains a single *P* element insertion in the second chromosome. *Hermes* and *hobo* transposases were supplied by second chromosome CyO-containing balancers with either *hsp70Hermes::transposase* (*hspHermes-2* and *hspHermes-48*) or *hsp70hobo::transposase* (HSH2) (Calvi & Gelbart, 1994). The transposase helper chromosomes were created using *P* element vectors containing the *hsp-transposase* transgenes and in the case of the *Hermes* helpers they also contained a *w⁺* gene.

Excision assays were performed by crossing excision indicator lines to an appropriate *CyO::hsptransposase/Gla* helper line. Heterozygous progeny received one heat shock of 37°C for 1 h during the first third of larval development. Red-eyed, curly winged progeny were selected and crossed individually to a *yw^{67c23}* tester strain. Straight-winged progeny were scored for the presence of red or white eyes. Straight-winged, white-eyed progeny were classified as excision events. All crosses were performed on standard cornmeal-based fly medium at 25°C.

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