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Interplasmid transposition of *Drosophila hobo* elements in non-drosophilid insects

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Abstract A modified *hobo* element from *Drosophila melanogaster* was introduced into embryos of the housefly, *Musca domestica* (family Muscidae) and the Queensland fruitfly, *Bactrocera tryoni* (family Tephritidae) to assess its ability to transpose. *Hobo* was capable of transposition in these species and transposition products had all of the hallmarks of *hobo* transposition products recovered from *D. melanogaster*, including the movement only of sequences precisely delimited by the inverted terminal repeats of *hobo*, the creation of an 8 bp duplication of the insertion site and an absolute requirement for *hobo*-encoded transposase. Transposition of *hobo* into the target gene resulted in a non-random distribution of insertion sites, with 10 of 38 independent insertions into the same nucleotide position. The results indicate that *hobo* can transpose in heterologous species, further demonstrating the similarty of *hobo* to *Ac* (*Activator*) of *Zea mays* and *Tam3* of *Antirrhinum majus*. *Hobo* has excellent potential to act as a gene vector or gene tagging agent in non-drosophilid insects.

Key words Transposable element · *hobo* · Gene vectors · Transposon tagging · Transgenic insects

Introduction

There is an enormous need to identify and isolate transposable elements that will permit the development of gene vectors and gene-tagging agents for non-drosophilid insects. In the absence of this technology the complete repertoire of molecular genetic tools and techniques cannot be applied fully to problems in insect biology that continue to impact on world health and agriculture. Sophisticated means of manipulating genotypes such as gene transfer, mutant rescue, transposon tagging and enhancer/promoter trapping will be crucial for understanding a myriad of important questions in insect biology, including those related to anopheline mosquito/*Plasmodium* interactions and mechanisms of insecticide resistance. In addition, insect control measures that rely on genetic methods, such as those involving the mass release of sterilized males or the release of insecticide-resistant beneficial insects, could profit from the availability of additional tools for manipulating insect genotypes and phenotypes (O'Brochta and Handler 1993).

Since the development of gene vector technology for *Drosophila melanogaster* (Rubin and Spradling 1982; Spradling and Rubin 1982) two approaches have been undertaken to identify or construct such a system for non-drosophilid insects. First, there have been attempts to identify and isolate new transposable elements with mobility properties conducive to their development into gene vectors and gene-tagging agents for non-drosophilid insects. Recently, some progress has been made, with the discovery of *Drosophila mariner*-like elements in a wide range of insect species and the discovery of *hobo*- and *P*-related elements in a more limited range of non-drosophilid insects (Anxolabehere and Periquet 1987; Atkinson et al. 1993; Perkins and Howells 1992; Robertson 1993). However, direct evidence for the mobility of these elements in either the species from which they were isolated or in heterologous species has not been reported.

A second approach to developing gene vectors for use in non-drosophilid insects has been to adapt transposable elements already isolated from either insect or non-insect systems. Most notably, efforts have been

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made to use the *P* element from *D. melanogaster* to transform a number of non-drosophilid species including mosquitos, tephritid fruitflies and grasshoppers (Handler et al. 1993; McGrane et al. 1988; Miller et al. 1987; Morris et al. 1989; Walker 1989). Using this approach, transgenic insects have occasionally been generated, however, these have been the product of low frequency, illegitimate recombination between the host's genomic DNA and injected plasmid DNA (McGrane et al. 1988; Miller et al. 1987; Morris et al. 1989). Despite these discouraging results, few elements have been tested to the extent required to confidently exclude them, or this approach, from consideration for gene vector development. For example, the *hobo* element from *D. melanogaster* has received relatively little attention as a potential gene vector for non-drosophilid insects, despite its demonstrated ability to act as a vector in *D. melanogaster* (Blackman et al. 1989). Here we report that the *hobo* element has remarkable potential to act as a gene vector in non-drosophilid insects.

Based on its structure, the *hobo* element is included in a category of transposable elements that includes the *P* and *mariner* elements of *Drosophila*, *Tc1* of *Caenorhabditis elegans*, the *Ac* element of *Zea mays*, and *Tam3* of *Antirrhinum majus* (Blackman and Gelbart 1989; Finnegan 1989). Elements within this group are characterized by having short, inverted, terminally repeated DNA sequences and an essential "transposase"-coding region. In general, the short inverted repeat-type elements are thought to transpose via a "cut and paste" mechanism whereby the donor element is excised from one location and is inserted into a new site. Upon excision, the donor site is repaired in ways that can yield insights into the mechanism of transposition and/or the post-excision repair process. *Hobo* excision results in the precise or nearly precise removal of all sequences delimited by the terminal repeat sequences of the element. Excision or post-excision repair results in the addition of approximately 8–20 additional nucleotides at the empty donor site which appear related to sequences originally flanking the *hobo* element (Atkinson et al. 1993). Excision of *Ac* and *Tam3* results in products very similar to those generated by *hobo* excision (Coen et al. 1989). In contrast, excision of the *P* element, under similar conditions, results in short (1–8 bp) fragments of the terminal sequences of *P* being left at the empty donor site without the addition of extra nucleotides (O'Brochta et al. 1991; O'Brochta and Handler 1988). Thus, based on the excision footprints, which reflect certain aspects of transposition, *hobo* appears more similar to *Ac* and *Tam3* than to *P*. In addition, the transposase genes of *hobo*, *Ac* and *Tam3* share limited sequence similarity at the amino acid level (Calvi et al. 1991; Feldmar and Kunze 1991). Taken together, the similarities in element structure, nucleotide sequence similarity, amino acid sequence similarity, excision mechanisms and mobility properties identify *hobo*, *Ac* and *Tam3* as members of a family of related transposable elements. We refer to this family as the *hAT* element family (Atkinson et al. 1993) and have recently identified additional members from a number of non-drosophilid insects, including *Musca vetustissima*, *Lucilia cuprina* (family Calliphoridae) and *Bactrocera* (*Dacus*) *tryoni* (family Tephritidae) (our unpublished results).

Here, we demonstrate that a modified *hobo* element from *D. melanogaster* can transpose when introduced into developing embryos of the house fly, *M. domestica*, and the Queensland fruitfly, *B. tryoni*. These data represent the first example of a cloned mobile genetic element transposing in a non-drosophilid insect and suggest that the *hobo* element will be useful as a gene vector in *M. domestica*, *B. tryoni* and perhaps other non-drosophilid species of insects.

Materials and methods

Insects

A *D. melanogaster* E-strain (*cn; ry*) was used in all experiments; see Lindsley and Zimm (1992) for a description of individual loci. This strain does not contain active *hobo* elements and readily supports the movement of *hobo* in the presence of *hobo* transposase (Streck et al. 1986).

The *M. domestica* strain used in all experiments was an insecticide-susceptible strain derived from flies caught in the wild and maintained at the USDA Livestock Insect Laboratory, Beltsville, Md. since 1980.

B. tryoni was obtained from Dr. B.S. Fletcher (CSIRO, Entomology) and originated from a wild population caught near Wollongong, New South Wales, Australia and has been maintained in the laboratory since 1989.

Plasmids

The donor plasmid, phoboKan, was constructed by replacing the 700 bp *Xba*I fragment of the HFL1 *hobo* element (Blackman et al. 1989) with the kanamycin resistance gene from pACYC184 (Chang and Cohen 1978). The kanamycin-marked *hobo* element and flanking genomic DNA from chromosomal region 94E was inserted into the multicloning site of pBCKS$^+$ (Stratagene). The target plasmid, pUCsacRB, was constructed by inserting the *Bacillus subtilis sacRB* gene from pUCD800 (Gay et al. 1985) into the multicloning site of pUC19 (Yanisch-Perron et al. 1985). The *sacRB* gene encodes for the enzyme levansucrase (sucrose: 2,6-ß-D-fructan 6-ß-D-fructosyltransferase; EC 2.4.10) which, when expressed in *Escherichia coli* in the presence of 5% sucrose, results in cell lysis (Gay et al. 1985). The helper plasmid, pKhsphobo, was constructed by inserting the 2.6 kb *Xho*I fragment from the HFL1 *hobo* element 3′ of a *D. melanogaster hsp70* promoter contained on pK19 (Pridmore 1987). All plasmids were constructed using standard molecular techniques as described in Sambrook et al. (1989).

Transposition assay

We developed a transient *in vivo* transposition assay that has permitted us to identify and isolate the products of inter-plasmid transpositions of the *hobo* element (Fig. 1). Transposition of *hobo* from the donor plasmid into the *sacRB* gene of the target plasmid results in

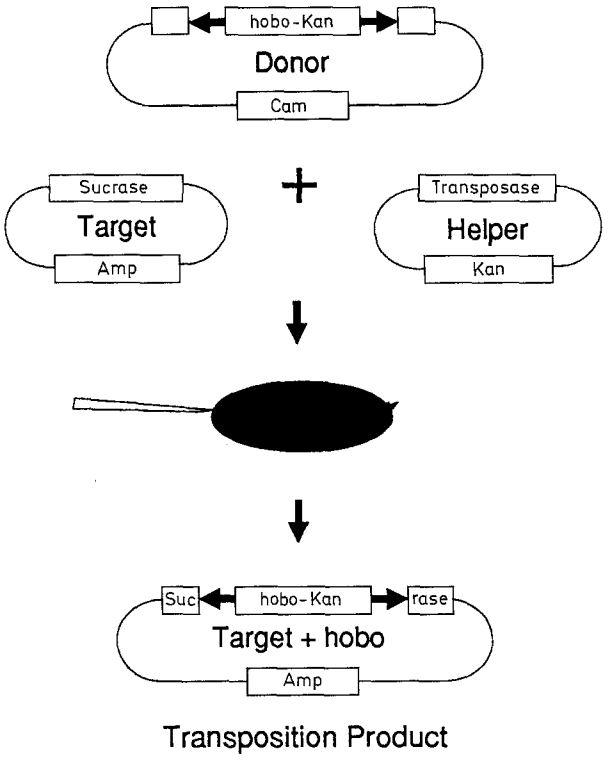

Fig. 1 *Hobo* transposition assay permitting the detection and recovery of inter-plasmid transpositions of *hobo*. Donor, target and helper plasmids were co-injected into developing insect embryos and subsequently recovered. Recovered plasmids were introduced into *Escherichia coli* and ampicillin, kanamycin and sucrose resistant plasmids resulting from the transposition of *hobo* from the donor to the target plasmid were selected. *Arrowheads* represent the inverted terminal repeats of *hobo*

the transfer of the kanamycin resistance gene to the target plasmid and elimination of levansucrase expression. Thus, transposition products can be selected for in *E. coli* as ampicillin-, kanamycin- and sucrose-resistant clones. *D. melanogaster* eggs were collected on agar plates containing yeast paste, manually dechorionated and injected as previously described (Spradling and Rubin 1982). *M. domestica* eggs were collected on a sponge soaked in warm 5% ammonium hydroxide, dechorionated using 50% household bleach and injected as described for *D. melanogaster*. *B. tryoni* eggs were collected from punctured apple skins then dechorionated and injected as for *M. domestica*. An appropriate mixture of CsCl-purified plasmid DNA was injected into preblastoderm embryos at concentrations of 1 mg/ml target plasmid, 0.5 mg/ml donor plasmid and 0.5 mg/ml helper plasmid (for *D. melanogaster* and *M. domestica*) or 0.33 mg/ml of each (for *B. tryoni*). After injection, embryos were incubated in an oxygen-rich atmosphere for approximately 18 h at 24 °C after which time the embryos were heat shocked at 37 °C for 1 h (*D. melanogaster*, *B. tryoni*) or 43 °C for 2.5 h (*M. domestica*). Embryos were allowed to recover at room temperature for 1 h and viable embryos, indicated by advanced embryogenesis, were recovered. Plasmid DNA was isolated from embryos using the method of Hirt (1967) and recovered plasmid DNA was resuspended in water at a concentration of 5 embryo-equivalents of plasmid DNA per μl. Two microliters of plasmid DNA were used to transform *E. coli* (strain JM83) by electroporation. Transformants were incubated in LB broth at 37 °C for 1 h then diluted to 10 ml with LB broth containing ampicillin (0.1 mg/ml), kanamycin (0.05 mg/ml) and sucrose (10%). The diluted cells were divided into 1 ml aliquots and incubated, with agitation, at 37 °C for 16 h. Plasmid DNA, prepared from those cultures that had become turbid, was used for restriction mapping and sequencing of the putative transposition products. The nucleotide sequence at the junction between the *hobo* element and target DNA was determined using primers specific for *hobo* sequences at the left and right ends of the element.

Transposition events were considered independent if inserted *hobo* elements were in different sites or orientations in the target plasmid, if the events were isolated from pools of DNA recovered from independent sets of injected embryos, or if the events were recovered from separate instances of *E. coli* transformation.

Results

Transposition

Using this assay we isolated 15 independent *hobo* transposition products from *D. melanogaster* after screening 7.9×10^5 target plasmids from a total of six experiments (Table 1, Fig. 2a). No transposition events were recovered in the absence of *hobo* transposase-encoding helper plasmids (Table 1). Transposition products recovered from *D. melanogaster* embryos in this plasmid-based, *in vivo* assay had all of the hallmarks of inter- and intrachromosomal *hobo* transpositions in *D. melanogaster*. Only those sequences delimited by the termini of *hobo* transposed and 8 bp duplications were created at the integration sites.

Using the same assay for transposition, *hobo* transposition products were also recovered from embryos of the house fly, *M. domestica*, and the Queensland fruitfly, *B. tryoni*. Following injection of *M. domestica* embryos with the three assay plasmids, 3.6×10^6 target plasmids were recovered and of these, 18 were the products of *hobo* transposition. From *B. tryoni* 2.7×10^6 target plasmids were recovered, including five *hobo* transposition products. In both of these species, transposition occurred only if transposase-encoding helper plasmids were injected (Table 1). Except for differences in the specific sites of insertion within the target gene (*sacRB*), the transposition products recovered from *M. domestica*

Table 1 *hobo* Transposase-dependent Transposition

	Drosophila melanogaster		*Musca domestica*		*Bactrocera tryoni*	
hobo transposase	−	+	−	+	−	+
experiments	2	6	16	10	4	12
target plasmids screened ($\times 10^5$)	1.5	7.9	15	36	30	27
transpositions	0	15	0	18	0	5

a

Drosophila melanogaster

242	A C C A G T T G C A	A T C C A A A C	G A G A G T C T A A
436	A G A A G C A G A C	C G C T A A C A	C A G T A C A T A A
504	A C A A G C A A C A	G T A T T A A C	C T T T A C T A C C
688	C A G A A G A G A T	A T T T T T A A	T T G T G G A C G A
819	T A A A A A T G C G	G A T G A C A C	A T C G A T T T A C
1042a	A A A C A C T G A C	A A C T G C A C	A A G T T A A C G T
1042b	A C G T T A A C T T	G T G C A G T T	G T C A G T G T T T
1084a	T C A G A C A G C T	C T T T G A A C	A T C A A C G G T G
1084b	C A C C G T T G A T	G T T C A A A G	A G C T G T C C T G A
1145	A A C T G C T G T A	C A T T T T G A	T A C G T T T T T C
1175	T C G C C T G A G C	T G T A G T T G	C C T T C A T C G A
1210	A C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1583	A T A A C C A A G C	A T G T A A A T	A T C G T T A G A C
1608	A G T T A A A G A A	T T A G A A A C	A T A A C C A A G C
1672	C A T C G T T A G G	A T C A A G A T	C C A T T T T T A A

Musca domestica

469	A C T T T T T G A T	G T T C A T C G	T T C A T G T C T T
760	A G C C G T G A T A	G T T T G C G A	C A G T G C C G T C
777	T G C A A A G A C G	A T G T G G T A	G C C G T G A T A G
819	G T A A A T C G A T	G T G T C A T C	C G C A T T T T T A
1065a	A C A A G T T A A C	G T A T C A G C	A T C A G A C A G C
1065b	A C A A G T T A A C	G T A T C A G C	A T C A G A C A G C
1084	C A C C G T T G A T	G T T C A A A G	A G C T G T C T G A
1210a	C T T C T A C G T A	G T G A G G A T	C T C T C A G C G T
1210b	C T T C T A C G T A	G T G A G G A T	C T C T C A G C G T
1210c	C T T C T A C G T A	G T G A G G A T	C T C T C A G C G T
1210d	C C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1210e	C C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1210f	C T T C T A C G T A	G T G A G G A T	C T C T C A G C G T
1221	G T G G C C T T T A	T C T T C T A C	G T A G T G A G G A
1276	C T T C G C C T T G	G T A G C C A T	C T T C A G T T C C
1431	T T T T T T C A G T	G T G T A A T C	A T C G T T T A G C
1712	T T C G C T T G A G	G T A C A G C G	A A G T G T G A G T
1731	T A A T C A C G A C	A T T G T T T C	C T T T C G C T T G

Bactrocera (Dacus) tryoni

979	G G A T T T T T C C	G T C A G A T G	T A A A T G T G G C
1210g	A C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1210h	A C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1210i	A C G C T G A G A G	A T C C T C A C	T A C G T A G A A G
1391	A G A G C G C C G T	T T G C T A A C	T C A G C C G T G C

b

Consensus insertion sites:

in *sacRB* $G^{47}T^{84}T^{29}T^{37}A^{32}A^{37}A^{63}C^{50}$

in chromosomes $C^{36}T^{64}T^{45}T^{45}C^{45}A^{36}A^{91}C^{91}$

Fig. 2a Sequence comparison of *hobo* insertion sites from the three species tested. Shown is the DNA sequence of the *sacRB* gene (5′–3′) prior to *hobo* insertion, with the 8 bp sequence that is duplicated upon *hobo* insertion and the 10 nucleotides flanking the insertion site. The alignment is made relative to the inserted *hobo* element such that the transposase coding region is oriented from 5′ to 3′. Nucleotides between the *vertical lines* are the sequences duplicated upon insertion. **b** The consensus insertion site determined in this experiment after comparing 38 independent insertion events is shown, as is the consensus insertion site determined by Streck et al. (1986) . *Superscript numbers* indicate the percentage of insertion sites that contain the designated nucleotide at that site

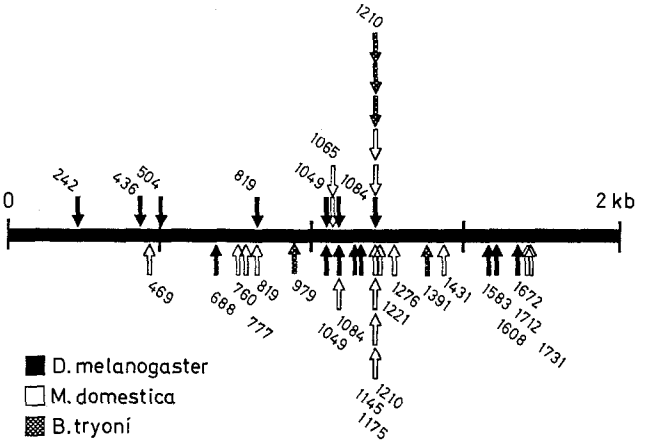

Fig. 3 Map of the 2 kb *sacRB* target gene showing the position of independent insertions of *hobo*. The *black bar* represents the *sacRB* gene. *Arrows* above the *bar*, pointing down refer to *hobo* element insertions in which the 5′ end of the transposase coding region is left-most and *arrows* below the *bar*, pointing up refer to elements inserted in the opposite orientation. Numbers refer to the nucleotide position of the insertion within *sacRB*

and *B. tryoni* were indistinguishable from those obtained from *D. melanogaster*, in that only sequences precisely delimited by the termini of *hobo* transposed and in all cases a duplication of 8 bp was created at the site of insertion.

Insertion sites

A comparison of the 38 transposition products recovered from *D. melanogaster*, *M. domestica* and *B. tryoni* identified a weak consensus insertion sequence, 5′GTTTAAAC 3′ (Fig. 2b). This palindromic sequence is similar to the consensus insertion sequence observed by Streck et al. (1986) after comparing the chromosomal sites of insertion of 11 *hobo* elements in *D. melanogaster* (Fig. 2b). Furthermore, the *hobo* insertion events within the 2 kb target sequence were distributed in a distinctly non-random manner in all species tested (Fig. 3). Of the 38 independent insertion events, 20 occurred within the interval between nucleotides 1049 and 1276, and remarkably, 10 independent transpositions resulted in insertions at nucleotide position 1210 of the *sacRB* gene (1 from *D. melanogaster*, 6 from *M. domestica*, 3 from *B. tryoni*). The insertion site at position 1210 (5′ATCCTCAC 3′) differs by only a single nucleotide from the 8 bp insertion site present on the *hobo* donor plasmid (5′ATCCTGAC 3′). Similarly, there are four other sites into which *hobo* independently inserted on at least two separate occasions, although these sites do not strongly resemble that on the donor plasmid.

Discussion

The *hobo* element from *D. melanogaster* is a member of a family of elements (*hAT* elements) that includes the *Ac* element from *Z. mays* and the *Tam3* element from *A. majus*, based on criteria such as structural organization, primary nucleotide sequence, protein coding capacity and the sequence of empty sites left following element excision (Atkinson et al. 1993; Calvi et al. 1991; Feldmar and Kunze 1991). Here we report data that demonstrate that *hobo*, like *Ac* and *Tam3*, is capable of transposition in a heterologous cellular environment. When introduced into the embryonic soma of developing *M. domestica* and *B. tryoni* embryos in which *hobo*-encoded transposase was being expressed, *hobo* elements from donor plasmids were capable of

transposing to target sequences located on separate plasmids. Significantly, these transpositions were indistingable from those recovered from *D. melanogaster* embryos in that there was an absolute requirement for *hobo*-encoded transposase, transposition involved only sequences precisely delimited by the inverted terminal repeats of the *hobo* element and 8 bp duplications were generated at the site of insertion in the target sequence. These common features of *hobo* element transposition in *D. melanogaster*, *M. domestica* and *B. tryoni* suggest that the mechanism of transposition is similar, if not identical, among these species.

Hobo transposition products were not recovered with equal frequency from the three species of Diptera tested. Transposition products were recovered approximately 4 fourfold less frequently from *M. domestica* embryos (1 event per 200 000 target plasmids) and approximately ten-fold less frequently from *B. tryoni* embryos (1 per 540 000 target plasmids) than from *D. melanogaster* (1 per 52 000) target plasmids). The basis of these frequency differences is presently unknown but may have resulted from differences in input plasmid concentrations, variation in the expression of the *D. melanogaster hsp70* promoter-containing helper plasmid and/or the presence of species-specific cellular factors capable of interacting with the *hobo* transposable element system.

Hobo-related transposable elements exist in both the *M. domestica* and *B. tryoni* strains used in these experiments (Atkinson et al. 1993 and our unpublished results). Atkinson et al. (1993) demonstrated that there is an endogenous *hobo* transposase-like activity in the embryos of *M. domestica* capable of mobilizing *hobo*, albeit imprecisely, and that this species contains approximately 50 copies of a sequence strongly resembling *hobo* transposase. Subsequently, we have shown these transposase-like coding regions to be part of a transposable element system (*Hermes*) that contains short inverted terminal repeats identical at 10 of the first 12 positions to those found in *hobo*. Conceptual translation of the long open reading frame (1839 bp) of *Hermes* indicates that it encodes a polypeptide that is 55% identical and 71% similar to the *hobo* transposase (W.D. Warren, unpublished). The presence of such a similar transposable element system in *M. domestica* probably accounts for the cross-mobilization reported by Atkinson et al. (1993) and perhaps for the ability of embryos of this species to support *hobo* transposition, as reported here. Similarly, *B. tryoni* contains approximately 30–50 copies of a *hobo*-like sequence (*Homer*). Partial sequencing of a cloned *Homer* element revealed an open reading frame encoding a polypeptide that is 53% identical to the corresponding region of *hobo* transposase (P.W. Atkinson, unpublished). At this time it is unclear whether the existence of an endogenous *hobo*-like transposable element system is a prerequisite for the transposition of *hobo* in a heterologous host. Additional studies on the mobility of *hobo* in heterologous systems with species lacking representatives of this transposable element family will permit the "host-range" of *hobo* and its determinants to be defined more precisely.

Excision of *hobo* in *M. domestica* has been reported to be unlike that observed in *D. melanogaster*, resulting in the imprecise removal of *hobo* sequences and the deletion of flanking DNA (Atkinson et al. 1993). Although excision was observed in both the presence and absence of *hobo*-encoded transposase, transposition of *hobo* in *M. domestica* was not observed in the absence of *hobo*-encoded transposase. These results could indicate that *Hermes*-encoded transposase is capable of interacting with *hobo*, as inferred by the generation of excision products, yet this interaction is insufficient to promote forward transposition.

A strong clustering of *hobo* insertions was observed in the target gene (*sacRB*) in the three species tested. Ten independent insertions occurred at precisely the same site while four other sites received at least two independent insertions of the *hobo* element. Little is known about the factors that influence target site selection of transposable elements, although the primary nucleotide sequence of the target probably plays a role for some elements (Capel et al. 1993). Parameters of more significance are likely to include chromatin structure at the target site and the proximity of the target site to the donor element (Pryciak and Varmus 1992). For example, *hobo*, *Ac* and *P* have been shown to insert preferentially at sites flanking the donor site (Jones et al. 1990; Sheen et al. 1993; Zhang and Spradling 1993). In this respect it is interesting that the preferred site of insertion of *hobo* into the *sacRB* target gene matched the site from which the *hobo* element originated at seven out of eight positions. This suggests that either there is a site preference that is determined primarily by the nucleotide sequence of the insertion site or that the sequences flanking the donor element influence where subsequent insertions occur. The transposition assay used in this investigation should provide us with the opportunity to explore the parameters determining target site selection.

Because of their relatively small size, simple structure and their presumed cut and paste (DNA to DNA) mechanism of transposition, transposable elements that have short inverted terminal repeats have been of some importance in the development of gene vectors and gene tagging strategies in a variety of systems. *P* and *hobo* elements from *D. melanogaster* and *mariner* elements from *D. mauritiana* have been used as gene vectors in *D. melanogaster* and closely related drosophilids (Blackman et al. 1989; Lidholm et al. 1993; Rubin and Spradling 1982). The elements *Ac* and *Tam3* from plants have been used as gene tagging agents, facilitating the identification and isolation of genes from agriculturally important plant species in which these elements can transpose (Wienand and Saedler 1988). The utility of *Ac* and *Tam3* has been greatly extended as a result of their ability to transpose when introduced into the cells of a variety of "non-host"

species. Consequently, the gene-tagging agent developed for corn can be used in tomatoes, petunia, carrots, and other plant species. The ability of *Ac* and *Tam3* to be mobilized in heterologous species allows plant scientists to identify and isolate genes and to probe the molecular genetics of species for which we know relatively little. Significantly, *hobo* shows some of the same properties, notably the ability to transpose in non-host species, that make *Ac* such a valuable tool for plant molecular biologists. Accordingly, we are currently examining the ability of *hobo* to transpose from a donor plasmid to the chromosomes of *M. domestica*, *B. tryoni* and other insect species.

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