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Identification of a fully-functional *hobo* transposable element and its use for germ-line transformation of *Drosophila*

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The transposable element *hobo* can be mobilized to induce a variety of genetic abnormalities within the germ-line of *Drosophila melanogaster*. Strains containing *hobos* have 3.0 kb elements and numerous smaller derivatives of the element. By analogy with other transposable element systems, it is likely that only the 3.0 kb elements are capable of inducing *hobo* mobilization. Here, we report that a cloned 3.0 kb *hobo*, called HFL1, is able to mediate germ-line transformation and therefore is an autonomous (fully-functional) transposable element. Germ-line transformation was observed when HFL1 and a marked *hobo* element were co-injected into recipient embryos devoid of endogenous *hobos*. Integration did not occur in the absence of the 3.0 kb element. A single copy of the marked *hobo* transposon inserted at each site, and the target sites were widely distributed throughout the genome. Integration occurred at (or very near) the termini of *hobo*, without internal rearrangement of the *hobo* or marker gene sequences. The *hobo* transformation system will allow us to determine the structural and regulatory features of *hobo* responsible for its mobilization and will provide novel approaches for the molecular and genetic manipulation of the *Drosophila* genome.

Key words: Drosophila melanogaster/germ-line transformation/*hobo* element/transposable elements

Introduction

In *Drosophila melanogaster*, the mobilization of the transposable element *hobo* within germ-line nuclei leads to a number of genetic aberrations associated with chromosomal instability (Blackman *et al.*, 1987; Hatzopoulos *et al.*, 1987; Yannopoulos *et al.*, 1987; Lim, 1988). In order to understand the molecular mechanisms governing *hobo* mobilization, we would like to determine the *cis*- and *trans*-acting functions necessary for the element's activity. However, the multiplicity of elements in *hobo*-containing genomes has precluded the straightforward analysis of the genetic factors influencing *hobo* mobilization.

Because of their large size and widespread presence in H (*hobo*-containing) strains, Streck *et al.* (1986) suggested that 3.0 kb *hobo* elements are autonomous (fully-functional) elements, i.e., elements capable of transposing themselves and mediating in *trans* the movement of other *hobo* elements. Their analysis of one 3.0 kb element, called $hobo_{108}$, revealed that it possesses 12 bp inverted terminal repeats and several open reading frames (Streck *et al.*, 1986). However, no functional evidence was available to determine if $hobo_{108}$ is an autonomous element. Indeed, we and others have observed sequence heterogeneity among 3.0 kb *hobo* elements (Lim, 1988; Blackman and Gelbart, unpublished). Thus, the size of an element cannot be sufficient criterion for its classification as autonomous.

Besides the 3.0 kb elements, H strains typically contain numerous, smaller derivatives of *hobo* (Streck *et al.*, 1986). These shorter elements, which are missing internal sequences but have intact termini (McGinnis *et al.*, 1983; Streck *et al.*, 1986) participate in the genome rearrangements associated with *hobo* mobilization (Blackman *et al.*, 1987; Hatzopoulos *et al.*, 1987; Yannopoulos *et al.*, 1987). Based on substantial evidence from other transposable element systems (Berg and Howe, 1989) it is unlikley that these deleted elements possess the *trans*-acting functions necessary for mobilization.

The *P* element of *D.melanogaster* (for review, see Engels, 1989) exhibits many genetic and structural parallels with *hobo*, but no obvious sequence similarity (for comparisons of *P* and *hobo*, see Louis and Yannopoulos, 1988; Blackman and Gelbart, 1989). The key to the present understanding of the *P* system was the identification of an autonomous *P* element. The method demonstrating its autonomy, germ-line transformation, is now one of the most important tools available to *Drosophila* molecular geneticists (Spradling, 1986). When injected in the presence of *P*-encoded transposase, *P* element-containing sequences integrate into the genome of the early embryo. This transposase activity can be supplied by the embryos themselves (if produced in a cross of a strain containing *P* elements to one lacking them) or by a co-injected plasmid carrying an autonomous *P* element (Spradling and Rubin, 1982; Rubin and Spradling, 1982).

In this report, we show that marked *hobo* elements, in the presence of 3.0 kb elements, can integrate with high efficiency into the *Drosophila* germ-line. By this transformation assay, we demonstrate the autonomous function of a cloned 3.0 kb *hobo*. We discuss the implications of the establishment of *hobo*-mediated germ-line transformation for the study of *hobo* itself, as well as for the development of new and more powerful tools for the manipulation of the *Drosophila* genome.

Results

Micro-injection of a marked hobo transposon

In order to follow by phenotype the germ-line transmission of integrated *hobo* sequences, we incorporated the rosy (ry^+) gene, required for normal eye pigmentation, within a *hobo* element. The ry^+ gene was inserted near the middle of a cloned *hobo* element, producing plasmid H[(ry^+)har1] (henceforth referred to as H[har1] (Figure 1). The insertion of the ry^+ gene disrupts the long open reading frame of

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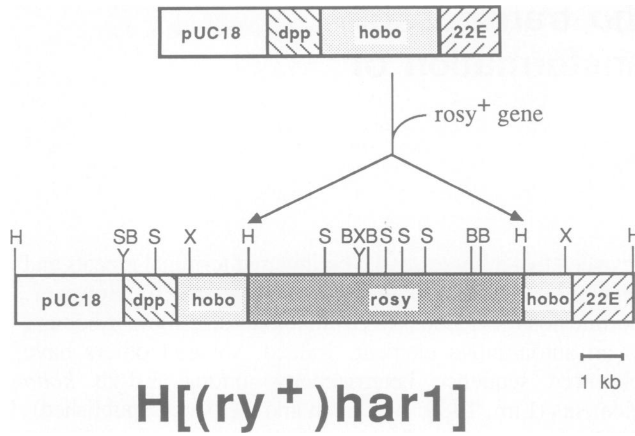

Fig. 1. Restriction map of the *hobo*-marker gene transposon. A phage DNA was isolated which contained one of the breakpoints resulting from an inversion between the decapentaplegic gene (*dpp*) in polytene region 22F1,2 and sequences from an unknown locus in region 22E1,2. A 3 kb *hobo* element was present at this inversion junction. A *Bam*HI–*Hin*dIII fragment from this phage, spanning the *hobo* element and containing 1.3 kb of *dpp* and 1.6 kb of 22E sequences, was subcloned into pUC18 (pictured at top of figure). This plasmid was partially digested with *Hin*dIII and a 7.3 kb *Hin*dIII fragment containing the intact rosy (ry^+) gene [taken from the Carnegie 20 vector (Rubin and Spradling, 1983) was inserted into the *hobo* element, producing plasmid H[ry^+)har1]. The rosy gene is transcribed from left to right as pictured. The presumptive *hobo* transcription unit also reads from left to right (Streck *et al.*, 1986). Restriction Sites: B, *Bam*HI; H, *Hin*dIII; S, *Sst*I; X, *Xho*I.

hobo (Streck *et al.*, 1986) presumably eliminating the production of *hobo*-encoded polypeptides contributed by this construct. Plasmid H[har1] also contains non-repetitive *Drosophila* genomic DNA flanking the *hobo* element (Figure 1). Three series of transformation experiments were performed to examine the ability of H[har1] to integrate into the genome and to define the factors required in *trans* to mediate its integration.

Series A: Injections into H × E embryos

First we determined if injected H[har1] DNA could be integrated into the genome of animals already capable of *hobo* mobilization. Our previous genetic analysis (Blackman *et al.* 1987) showed that *hobo* mobilization occurred within the germ-lines of progeny resulting from crosses of H strains to E strains (*hobo*-containing and *hobo*-lacking strains, respectively). Therefore we decided to use the ry^- offspring of an H × E mating as recipients for the injected H[har1] DNA (Figure 2). Both strains are ry^- and contain no *P* elements. Adults arising from these embryos were then backcrossed to the *cn*; ry^{42} strain and their progeny scored for ry^+ eye color.

Of the fertile G_0 adults surviving the injection procedure, 28% transmitted the ry^+ marker gene to their progeny (Table I). Lines were established from nearly all of the ry^+ G_1 individuals and the chromosomal linkage of the marker gene in each was determined. For any given G_1, the ry^+ activity always segregated with a single linkage group. However, independent integration events occurred in different germ-line cells of a single G_0 individual. These were detected because they showed linkage to different markers which were heterozygous in the injected G_0 individual. Only a single representative of each independently segregating insertion from a given G_0 was retained for further analysis. In this way 19 independent lines were established (Table II). From these results we conclude that H[har1] contains all sequences necessary in *cis* for its integration.

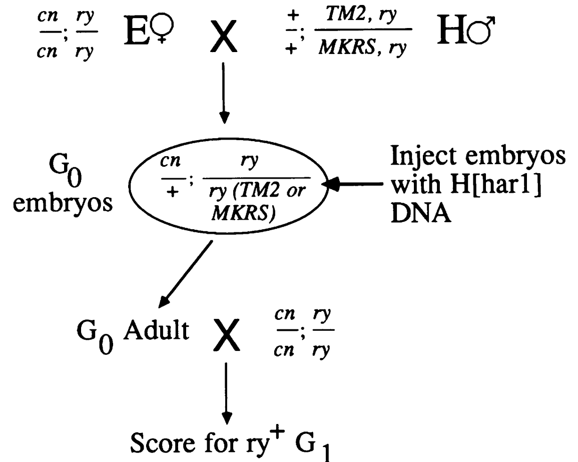

Fig. 2. Series A injections: mating scheme used to recover H[har1] transformants. Females of the E strain *cn; ry^{42}* were crossed to males of the H strain *In(3LR)TM2, ry Ubx^{130}/Tp(3;3) MKRS, ry^2 Sb.* Embryos produced by this cross were injected with H[har1] DNA. After eclosion, these G_0 adults were individually backcrossed to *cn; ry^{42}* flies and their progeny scored for eye color. The presence of G_1 adults with the ry^+ phenotype indicated that all or part of the transposon had been incorporated into the germ-line of the G_0 parent. Note that the major autosomes of the G_0 embryos were differentially marked.

Table I. Recovery data for the three transformation series

Injection series	No. fertile G_0s	No. G_0s with ry^+ progeny	% G_0s with ry^+ progeny
A	40	11	28
B	95	0	0
C	172	43	25

Table II. Series A: Recovery data and transposon sites for H[har1] transformed lines

	No. of G_1		Polytene location of transposon in line number			
G_0 fly	ry^+	Total	–1	–2	–3	–4
E5	27	189	*47A-C, 50A-B[b]	76A[e]	*79E-F[d]	60B-C[c]
E8	5	200	68E[f]	*34B[c]		
E14	12	85	*74A1,2[d]	*30D1,2[c]		
F19	20	143	*69B[d]			
G8	8	99	*39C-D[b]	*11C-D[a]	*90C-D[d]	
G9	1	60	*30D1,2[c]			
G12	6	132	92A[f]			
G13	2	26	*44C-D[b]			
G15	5	41	*38C-D[d], 64A-B			
G18	8	24	100E-F[e]	*56E[b]		
G21	14	59	*44C-D[c]			

Lines were established from each of the ry^+ G_1 individuals and the chromosomal association of each insertion was determined. For any G_0, only one line was retained for insertions on a given chromosome. Hence, additional independent insertions may have been missed if they had occurred on the same homolog.
*This line was analyzed molecularly by blot analysis.
[a]Insertion into X chromosome. [b]Insertion into cn^+ 2nd chromosome.
[c]Insertion into *cn* 2nd chromosome. [d]Insertion into ry^{42} 3rd chromosome.
[e]Insertion into *In(3LR)TM2* 3rd chromosome.
[f]Insertion into *Tp(3;3)MKRS* 3rd chromosome.

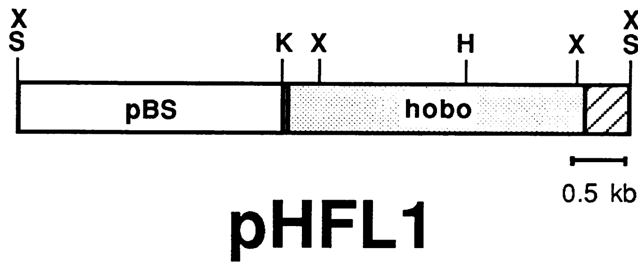

Fig. 3. Restriction map of a cloned autonomous *hobo* element. A 3.0 kb *hobo* element was cloned from the 94E region of the $dpp^{d\text{-}blk}$ strain, a strain known to induce *hobo* mobilization and to contain only two full-length elements (Blackman *et al.*, 1987). From this DNA, a 3.5 kb *HindIII-SalI* fragment was isolated which contained the entire *hobo* element plus 50–100 bp of flanking sequence on the *SalI* side and about 450 bp of flanking sequence at the *HindIII* end. The *HindIII* end was filled in with the large fragment of DNA polymerase I from *E.coli* and an *SstI* linker was added to the blunt end. The addition of the linker also created an *XhoI* site at that junction. This *SstI–SalI* fragment was inserted into the Bluescript-KS plasmid at its *SstI* and *XhoI* sites. The resulting DNA, pHFL1, contains only the *SstI* and *KpnI* sites of the plasmid's polylinker sequence. Restrictions sites: H, *HindIII*; K, *KpnI*; S, *SstI*; X, *XhoI*.

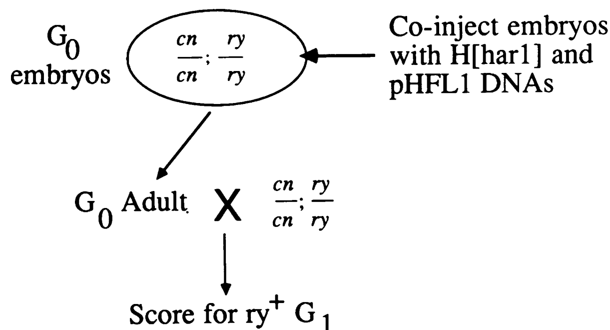

Fig. 4. Series B and C injections: mating scheme used to recover H[har1] transformants. Series C: embryos from the E strain *cn; ry^{42}* were co-injected with a mixture of H[har1] and pHFL1 DNAs. After eclosion, the G_0 adults were individually backcrossed to *cn; ry^{42}* flies and their progeny scored for eye color. All subsequent crosses employing these transformants were performed with E strains only. In Series B: only H[har1] DNA was injected into the *cn; ry^{42}* embryos. These injections were interspersed with the Series C injections.

Series B: Injections in the absence of 3.0 kb elements

To test whether H[har1] was also capable of contributing all *trans*-acting functions required for *hobo* integration, we injected the H[har1] plasmid alone into embryos of the *cn; ry^{42}* E strain. These injections were interspersed with those of Series C. Ninety-five fertile adults were recovered and backcrossed to *cn; ry^{42}* mates. None gave rise to ry^+ offspring (Table I). Because 72 of these fertile G_0s somatically expressed the rosy gene (i.e. the adults derived from the injected embryos were ry^+ in phenotype), we are confident that the DNA was effectively introduced into the embryos. We conclude that efficient H[har1] germ-line transformation requires additional genetic elements.

Series C: Injections with a co-injected autonomous hobo element

Having established that H[har1] can integrate only in the presence of additional *trans*-acting factors, we decided to use this assay as a means to identify a fully-functional *hobo* element. We have previously shown that the strain $dpp^{d\text{-}blk}$ is capable of promoting the mobilization of *hobo* elements (Blackman *et al.*, 1987). This strain contains numerous internally-deleted *hobos*, but only two 3.0 kb copies (Blackman *et al.*, 1987). The ability to promote *hobo* mobilization probably results from the action of at least one of the two full-length *hobos*. In support of this, we have observed that a strain derived from $dpp^{d\text{-}blk}$ which lacks these 3.0 kb elements was incapable of mobilizing the *hobo* element at *dpp* even though numerous defective elements were still present in the genome (unpublished observations). Thus, these 3.0 kb elements are likely sources for the *hobo* product(s) (presumably transposase) needed in *trans* for *hobo* mobilization. We cloned one of these elements, present in salivary gland polytene chromosome subdivision 94E, and used it to produce plasmid pHFL1 (Figure 3). pHFL1 contains less than 500 bp of flanking *Drosophila* sequences from 94E.

Table III. Series C co-injections with pHFL1: Recovery data and transposon sites for H[har1] transformed lines

G_0 fly	No. of G_1: ry^+	Total	Polytene location	G_0 fly	No. of G_1: ry^+	Total	Polytene location
*M1	32	133	25F	*M22	3	25	56E
M2	60	99	95A	M23	16	188	–
M3	3	68	24E	M24	49	150	–
M4	3	175	87D?[a]	M25	1	68	–
*M5-1	30	114	34D	M26	6	64	–
*M5-2	30	114	70C	M27	159	164	–
M6	61	154	98C	M28	18	150	–
M7	23	104	36A, 57E	M29	29	128	–
*M8	4	146	25F	M30	4	148	–
*M9	10	128	44D	M31	13	167	–
*M10	7	34	70C	M32	1	231	–
M11	6	103	9A	M33	8	209	–
M12	149	154	85A	M34	41	199	–
M13	18	178	18A	M35	117	233	–
*M14	170	226	22A	M36	3	173	–
*M15	154	262	26A	M37	1	120	–
*M16	1	124	70C	M38	116	257	–
*M17	11	118	97C	M39	28	213	–
*M18-1	1	147	34D	M40	8	67	–
M18-2	1	147	99F	M41	3	245	–
*M19	66	241	99A	M42	98	210	–
*M20	5	41	99A	M43	62	204	–
M21	2	38	65D				

For any G_0, only one line was retained for analysis, except for lines M5 and M18. Lines M5-2 and M18-2 were derived from lines M5-1 and M18-1, respectively, and each contains a ry^+ transposon which was segregating independently of the insertion mapped in the parental line (see text for details). Only lines derived from transformants M1 through M22 were analyzed by *in situ* hybridization.
*This line was analyzed molecularly by blot analysis.
[a]The signal from the rosy gene probe was observed only in 87D, the site of the endogenous rosy gene. It is possible that the transposon is integrated in the 87D region or in a site which is underreplicated in salivary gland polytene chromosomes. The ry^+ gene mapped genetically to the third chromosome in this line.

A mixture of H[har1] and pHFL1 DNAs were co-injected into *cy; ry^{42}* embryos and the resulting adults were testcrossed to ry^- E strains (Figure 4). In total, 25% of the fertile G_0 adults gave rise to ry^+ offspring (Table I). Thus, pHFL1 is competent to mediate the integration of other *hobo* elements.

A single line from each of 22 independent transformants (G_0 flies M1–M22) was retained for further analysis (Table III). During the course of our analysis, two lines (M5-1 and M18-1) displayed ry^+ insertions segregating on

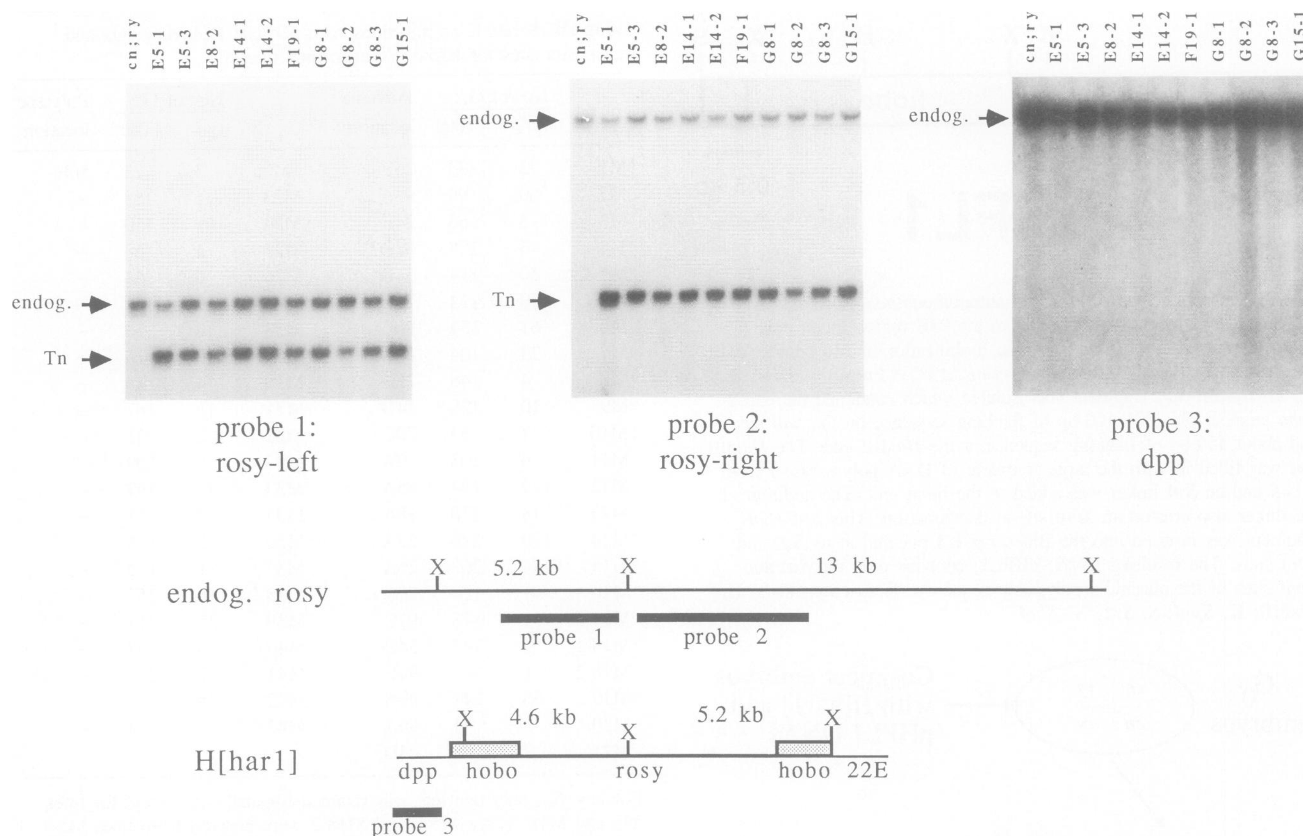

Fig. 5. Transposon integration occurs near or at the ends of the *hobo* element. **Top:** Genomic DNAs from 10 independent transformed lines and the *cn; ry*^{42} strain were digested with *Xho*I and electrophoresed in triplicate on separate agarose gels. Southern blots of these DNAs were probed with either the left or right half of the rosy gene or with the dpp sequences found in H[har1]. All lines were homozygous for the transposon insertion and the *ry*^{42} allele, except for line G8-1 which was hemizygous for the transposon. The autoradigram exposure of the blot probed with the dpp fragment (probe 3) is ~20 times as long as those presented in the other panels. Note that all three probes contained homology to the pUC18 sequences present in H[har1]. Since no bands were present in the same position in all three blots, none of the pUC18 sequences integrated into the *Drosophila* genome. Abbreviations used: endog., Expected position of the fragment resulting from the endogenous *ry*^{42} or *dpp* gene. Tn, Expected position of the fragment derived from the H[har1] transposon. **Bottom:** The *Xho*I restriction sites of the *ry*^{42} allele and the non-vector sequences of plasmid H[har1] are shown aligned at the internal *Xho*I site of rosy. The sizes of the *Xho*I fragments homologous to probes 1 and 2 are noted. Probe 3, specific for the dpp sequences of H[har1], is homologous to an endogenous 13 kb *Xho*I fragment.

two independent linkage groups. For each of these lines, insertions on each chromosome were retained for further analysis.

Molecular analysis of the transformants

We have analyzed homozygous *H[har1]; ry*^{42} flies from a total of 28 of the Series A and C transformed strains by whole genome Southern blot analysis. For each strain, genomic DNA was digested with *Xho*I, an enzyme which cuts near each end of the *hobo* element and once within the rosy gene in H[har1] (Figure 5). Southern blots of these DNAs were then probed with fragments corresponding to either left or right portions of the rosy gene (Figure 5, left and middle panels, respectively). The two predicted *Xho*I fragments from the transposon were present in each transformant, demonstrating that the entire rosy gene plus most or all of the *hobo* sequences of H[har1] were present in each genome. DNA from Series A and C lines gave identical results (data not shown).

Probes for DNA sequences flanking the *hobo* element of H[har1] were also employed. The flanking probes from the *dpp* gene (Figure 5, right panel) and from a segment of polytene band 22E1,2 (data not shown), only hybridized to endogenous genomic sequences. Sequences from the pUC18 plasmid vector of H[har1] were also undetectable in the genomic DNA (Figure 5). Thus, we conclude that no flanking DNA of H[har1] was incorporated into the transformants. The sensitivity of these blots was such that a maximum of 100 bp of flanking sequence could have escaped detection. Taken together with the results of the rosy probes, we infer that the exogenous DNA must have integrated at or near the termini of the *hobo* element, with all of the sequences internal to these termini inserted intact into the chromosome.

From the band intensities seen in the blots probed with rosy DNA, we infer that a single copy of the transposon was present in 26 of the 28 tested strains. This interpretation was confirmed by Southern blots employing restriction endonucleases which did not cut within the *hobo* element (data not shown) and by polytene chromosome *in situ* hybridization analysis (Tables II and III).

Two lines exhibited band intensities suggesting multiple copies of the transposon per genome. In one case *in situ* hybridization demonstrated that line E5-1 has two transposons integrated in different locations on chromosome 2 (Table II). In the second exception, line M18-1, analysis using a *hobo* element probe revealed both *Xho*I fragments characteristic of H[har1] and an additional 2.6 kb *Xho*I

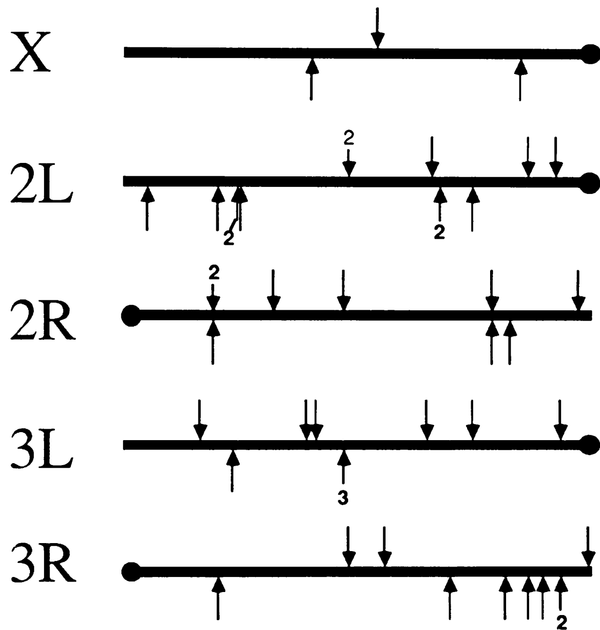

Fig. 6. Cytological positions of H[har1] insertion sites. Schematic representations of the major chromosome arms are shown with arrows noting the cytological positions of the transposon insertions. Integration sites were determined by *in situ* hybridization with a rosy gene probe. Arrows above the chromosome arm indicate insertions from Series A injections while those below are from Series C. The numbers associated with arrows indicate the number of occurrences of independent insertions at that site. The centromeric end of each arm is marked by a filled circle. For this diagram, each numbered polytene division is considered of equal length.

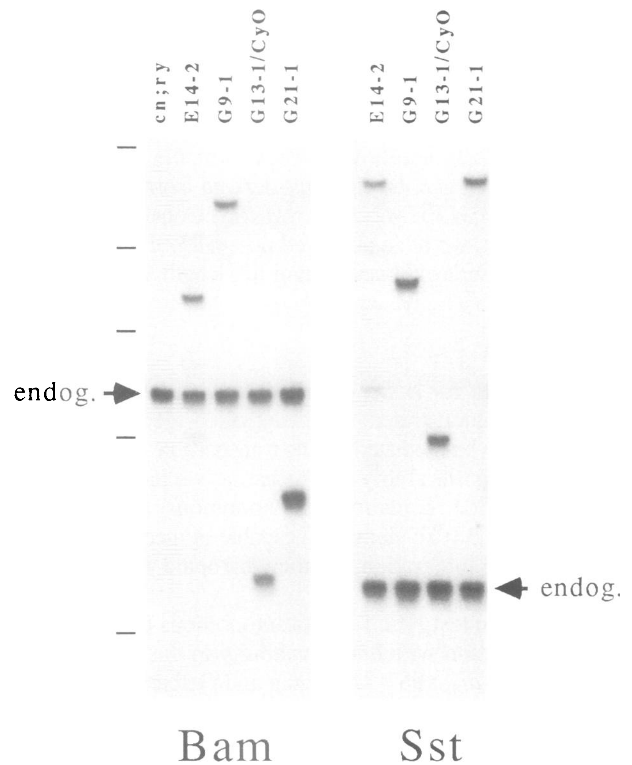

Fig. 7. Molecular analysis of strains with similar cytological insertion sites. Genomic DNAs from lines E14-2 and G9-1 (each integrating at 30D1,2) and lines G13-1 and G21-1 (each integrating at 44C-D) were digested with *Bam*HI or *Sst*I and examined by Southern blot analysis. A gel-purified *Bam*HI–*Hin*dIII fragment of the rosy gene (the rightmost one shown in Figure 1) was used to probe the filter. The transformant in line G13-1 is homozygous lethal; the G13-1 stain is heterozygous for the transposon and for the balancer chromosome *In(2LR)CyO*. Bands produced by the endogenous ry^{42} gene are noted with arrows. Additional bands in the E14-2 lanes are discussed in the text. Horizontal lines indicate the mobilities of mol. wt standards (in kb): 23, 9.4, 6.6, 4.4 and 2.3.

fragment (data not shown). This latter fragment presumably results from the integration of one or more pHFL1 *hobo* elements.

Distribution of transposon insertions in the genome

In situ hybridization with a rosy gene probe was used to ascertain the polytene chromosome position of the transposon in each of the 43 transformed lines (Table II and III, Figure 6). In general a single site of integration was present in the genome. In three cases however, two sites were observed. Lines E5-1 and M7 each contained two insertions on the same chromosome. In the third case, line G15-1, the second site on chromosome 3 contradicts both our linkage data and our Southern blot analysis (Figure 5). Most likely, the additional insertion represents a secondary mobilization event which occurred after the stock was established and which was segregating in the G15-1 strain. We presume mobilization was induced by autonomous *hobo* elements deriving from the *In(3LR)TM2/Tp(3;3)MKRS* ancestor.

Insertion sites mapped to the X chromosome and to each arm of the major autosomes (Figure 6). At the present time, we do not know if the apparent under-representation of X chromosome inserts reflects some preference in *hobo* integration or is an artifact of the mating schemes used to establish the lines for each transformant.

Independent insertions occurred at seven polytene positions (two insertions each at 25F, 30D1,2, 34D, 56E and 99A and three insertions each at 44C-D and 70C) (Figure 6). To determine if molecular hotspots existed at these cytological locations, Southern blot analysis of all 16 lines was undertaken using *Bam*HI and *Sst*I, two enzymes which cut within the rosy gene but not within the *hobo* element (Figure 1). These blots were then hybridized with a rosy probe. If integration had occurred at the identical position for any pair of insertions, then the same genomic restriction fragments would hybridize on the blots. Figure 7, displaying the data from two pairs of lines, shows that different fragments hybridized in each, demonstrating that the insertions occurred at different molecular positions. Using additional rosy fragment probes we also ruled out the possibility that the transposon insertions were present at the same site but in opposite orientations (data not shown). These results obtained for all seven sets of multiple insertions (data not shown). Thus, barring polymorphism of the *Bam*HI and *Sst*I sites adjacent to the insertions in these lines, we conclude that every transposon insertion was at a unique site in the genome.

There are indications that H[har1], once integrated, can be re-mobilized by endogenous *hobo* elements. In Figure 7, the gel lanes from line E14-2 contain several faint bands in addition to the prominent bands produced by the endogenous rosy gene and the original transposon insertion. These minor bands do not result from the partial digestion of the genomic DNA because at least one of the bands represents a fragment which is smaller than either of the two expected fragments in that gel lane. Rather, we believe that the faint bands are indicative of the transposition of the *hobo*

transposon into new chromosomal locations. A comparison of two preparations of genomic DNAs from line E14-2, isolated three months apart, indicates that the number of additional sites increased with time; we have observed this same phenomenon in other lines resulting from the Series A injections (data not shown). These unstable lines probably contain full-length *hobo* elements derived from their H strain progenitor, *In(3LR)TM2/Tp(3;3)MKRS*. Consistent with this interpretation, we have observed no evidence of transposon instability in Series C lines lacking full-length *hobo* elements.

Discussion

Requirements for hobo mobilization

We have demonstrated that controlled germ-line transformation can be mediated by the transposable element *hobo*. Further, using this ability as a bioassay, we have shown that plasmid pHFL1 contains an autonomous *hobo* element (HFL1). This 3.0 kb element is capable of mediating in *trans* the germ-line integration of other disrupted *hobo* elements as well as itself.

We opted to test HFL1 for its autonomous function based on its association with *hobo* instability in the $dpp^{d\text{-}blk}$ strain (Blackman *et al.*, 1987; Blackman and Gelbart, unpublished data). It is unclear at this time how HFL1 compares to the sequenced 3.0 kb $hobo_{108}$ element (Streck *et al.*, 1986). We have observed no restriction site differences between HFL1 and $hobo_{108}$, although there is at least one RFLP difference between the two (J.Lim, personal communication). The sequence of a functionally autonomous element, such as HFL1, will be needed for a complete analysis of the *hobo* system. Given the availability of *hobo*-mediated germ-line transformation and the sequence of an autonomous element, we will be in a position to molecularly dissect the *cis* and *trans* requirements for *hobo* mobilization.

The mobilization of both endogenous defective elements and micro-injected transposons appears to be dependent on the contribution of a *trans*-acting product of a full-length element. The true function of the *hobo*-encoded product remains unknown. We presume, but have not demonstrated, that this activity is a transposase. Because the results of the Series A and C injections were essentially identical, it is likely that the *trans*-acting products of HFL1 and the endogenous autonomous *hobo* elements are interchangeable.

Conditions regulating the presence or absence of the active *trans*-acting factor have not been determined. We have preliminary evidence, using transformants of a *hobo* transposon containing a cell-autonomous marker gene expressed in eye tissues (the *white* gene), that *hobo* mobilization does not readily occur in these somatic cells (Blackman and Gelbart, unpublished results). Thus, as with the *P* element system (Engels, 1979; McElwain, 1986), *hobo* activity may be restricted to germ-line cells. By transformation and *in vitro* mutagenesis techniques analogous to those used for the *P* system (Karess and Rubin, 1984; Laski *et al.*, 1986; Rio *et al.*, 1986), we can now examine the features of *hobo* responsible for this tissue specificity.

By Southern blot analysis, we have shown that integration occurs at or very near the termini of the *hobo* element. The conclusion that the entire *hobo* element is inserted is supported by the observation that the transposons in several lines continue to be mobile, a process which, by analogy with the *P* element (O'Hare and Rubin, 1983; Rubin and Spradling, 1983; Karess and Rubin, 1984; O'Hare, 1985) and other transposons (Berg and Howe, 1989), is likely to require the terminal *hobo* sequences.

Comparison of hobo- and P-mediated transformation

The properties of *hobo*-mediated transformation described above, along with the observation that only a single copy of the transposon integrates at any of a large number of genomic sites, are identical to those found for *P* element-mediated transformation (Spradling and Rubin, 1982; Rubin and Spradling, 1982). No other mobile element in *Drosophila* has been reported to mediate germ-line transformation. Elements bearing inverted terminal repeats, like *P* and *hobo*, might be singularly capable of efficient introduction into the germ-line of *Drosophila*.

Transformation efficiencies of *hobo*- and *P*-mediated transposon insertion are also quite comparable. Spradling (1986) reports transformation frequencies in the range of 10–35% (per fertile G_0) for *P* element/rosy constructs ~10 kb in size. Our *hobo*/rosy construct, H[har1], also ~10 kb, displayed a 25% transformation frequency. The median cluster size of *P*-mediated rosy transformants is 4.5% (Spradling, 1986), whereas H[har1] yielded a median cluster size of 12% for comparable transformants (Series C, Table III).

Applications of hobo/P transformation vectors

While much less information is available on *hobo* than on *P*, it is clear that each element can be mobilized in appropriate genetic backgrounds (Louis and Yannopoulos, 1988; Engels, 1989; Blackman and Gelbart, 1989). It is likely that the same products which promote this genetic instability are used to mediate germ-line transformation. Current evidence suggests that cross-mobilization, e.g., mobilization of *hobo* elements by *P* transposase, does not occur between *P* and *hobo* (or occurs infrequently) (Eggleston *et al.*, 1988). However, this issue of cross-mobilization needs to be addressed more thoroughly. If cross-mobilization indeed does not occur, then the availability of two transformation systems offers the potential for development of a new generation of transformation vectors and for substantial enhancement in the versatility of transformation-based functional assays and directed mutagenesis.

Two independent systems would permit the introduction of modified *P* and *hobo* elements into the genome using the other transposable element. For example, a *hobo* element can be inserted within the *cis*-regulatory region of a gene of interest. This entire gene/*hobo* construct can then be inserted into a *P* element vector and introduced into an E strain genome by *P*-mediated transformation. Subsequent mobilization of the *hobo* element can be used to produce a nested array of deletions initiated at the site of the *hobo* element and extending into the *cis*-regulatory region for varying lengths. The *hobo* element may prove particularly useful for such purposes, as adjacent deletions beginning at the element appear to be a frequent consequence of *hobo* mobilization (Blackman *et al.*, 1987).

Other types of constructs can also be envisioned. One possibility is the introduction of immobile *P* or *hobo* elements into any chromosome of interest, to serve as a source of transposase in mobilization and mutagenesis experiments. While the current immobile *P* elements (Cooley *et al.*, 1988;

Robertson *et al.*, 19088) have proven to be exceedingly useful, the alterations of the termini which immobilized them were rare chance events, limiting the likelihood that a large array of such immobile elements will be produced around the genome. The development of a second independent transformation system permits much more efficient engineering and integration of these immobile transposase sources. Furthermore, a *hobo* system analogous to the *P* element-mediated *Jumpstarter* system (Cooley *et al.*, 1988) can be developed and the two used together for such transposon mutageneses. Due to the insertion site preferences of *P*, a number of loci have been refractory to insertion or mutagenesis by *P* elements. The combined use of *hobo* and *P* in these screens should provide a wider spectrum of mutations associated with transposon tags.

In conclusion, the *hobo*-mediated transformation system will allow us to define the structural and regulatory components controlling *hobo* mobilization. Only one other *Drosophila* transposable element, *P*, is currently amenable to such analysis. A study of the similarities and differences between the two systems may reveal much about the general mechanisms of transposable element mobilization. An important byproduct of our studies will be the establishment of an effective *hobo* system for transposon mutagenesis and germ-line transformation. When employed in concert with *P*-mediated transformation, the *hobo* system will provide important additions to the repertoire of molecular and genetic techniques available for *Drosophila*.

Materials and methods

Drosophila strains

Tp(3;3)MKRS, a rearranged third chromosome containing the markers *kar*, ry^2 and *Sb*, is described by Gelbart and Chovnick (1979). Other mutations and chromosomes used in these studies, unless otherwise indicated in the text, are described in Lindsley and Grell (1968).

Injections

Procedures used routinely for P element-mediated germ-line transformation (Spradling, 1986) were employed in the present experiments. Embryos were collected at 25°C and dechorionated with 50% bleach before desiccation. DNA in 5 mM KCl, 0.1 mM $NaPO_4$, pH 6.8, was injected into the embryos at room temperature (about 20−22°C). Concentrations of the plasmids were as follows: Series A and B−H[har1] 300 μg/ml; Series C−H[har1] 300 μg/ml and pHFL1 100 μg/ml. The embryos were left at room temperature until hatching, after which they were transferred to standard *Drosophila* food and allowed to develop at 25°C. Subsequent crosses were performed at 25°C. Stocks were maintained at room temperature.

Southern blot analysis

For each line, genomic DNA from 25−30 flies was digested with restriction endonuclease and aliquots were loaded on 0.7% agarose gels. After electrophoresis, the nucleic acid was transferred to Zetaprobe membrane using alkali (Reed and Mann, 1985). Hybridizations and washes were performed by the method of Church and Gilbert (1984).

In situ hybridizations

In situ hybridization of biotinylated probes to salivary gland polytene chromosomes was performed, with minor modifications, as described by Engels *et al.* (1986) using the BluGene detection system (BRL). In most cases, a single individual from each line was analyzed.

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