Local Transposition of a *hobo* Element Within the *decapentaplegic* Locus of Drosophila

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ABSTRACT

We have efficiently mobilized a phenotypically silent *hobo* transgene inserted within the *cis*-regulatory heldout region of the *decapentaplegic (dpp)* locus in *Drosophila melanogaster*. The goal of our experiment was to identify germline transmission of a local transposition event within the *dpp* locus that meets two specific criteria. First, excision of the *hobo* construct does not generate an adult mutant phenotype, suggesting minimal alteration to the original site of insertion. Second, we required a new insertion of the *hobo* transgene into the Haploinsufficient region of the locus ~25 kb away. Genetic and molecular criteria are used to evaluate candidate germlines. In a pilot study, this local transposition event occurred independently in two individuals. Both of the transposition events appear to be new insertions into the *dpp* transcription unit. One insertion is between the two protein-coding exons, and the other is in the 3′-untranslated region of exon three. Strains carrying these insertions are valuable new reagents for the analysis of *dpp* function and molecular evolution. These results further support the use of the *hobo* system as an important tool in Drosophila genetics.

ENDOGENOUS, transposable elements have been developed into powerful genetic tools in a wide variety of species. In *Drosophila melanogaster*, experimental systems based on the *P* and *hobo* elements are used widely. These transposons belong to the same superfamily of mobile elements, those displaying inverted terminal repeats and transposing via DNA intermediates (Hartl and Lozovskaya 1995). As a result, their respective genetic systems share many characteristics. For example, both *P* and *hobo* transposon systems are capable of efficient germline transformation (Blackman *et al.* 1987) and enhancer trapping mutagenesis (Smith *et al.* 1993). One feature of the *P*-element system not yet fully explored for *hobo* is local transposition (Tower *et al.* 1993; Zhang and Spradling 1993). Here, we describe experiments designed to recover new insertions in a specific genomic region that result from the local jump of a *hobo* transgene. Our strategy is based on the well-characterized molecular genetics of the *decapentaplegic* (*dpp*) locus.

The *dpp* gene encodes a secreted signaling protein belonging to the transforming growth factor-β (TGF-β) family (Padgett *et al.* 1987) that is required for a variety of developmental decisions. Members of the TGF-β family share the following features: precursor polypeptides are proteolytically cleaved to generate an N-terminal fragment (the proregion) thought to be involved in dimerization and a C-terminal fragment that forms the biologically active ligand (reviewed in Massagué 1990). Among the developmental events influenced by *dpp* are the determination of dorsal ectoderm in the early embryo (Irish and Gelbart 1987), gut morphogenesis (Immerglück *et al.* 1990; Panganiban *et al.* 1990), and proper differentiation of adult wings (Posakony *et al.* 1991). By several criteria, the developmental functions of *dpp* were separated into three genetic domains [shortvein (shv), Haploinsufficient (Hin), and imaginal disk specific (disk)] that span nearly 60 kb (St. Johnston *et al.* 1990).

The Hin region is a roughly 8-kb block of DNA that contains one of the five classes of *dpp* transcript (including the common protein-coding exons 2 and 3) and all regulatory sequences necessary for normal dorsal-ventral patterning of the embryo (Hoffmann and Goodman 1987). Embryos that contain only a single functional copy of this region are inviable because of severe defects along the dorsal-ventral axis (Wharton *et al.* 1993). On either side of the Hin region are large arrays of *cis*-regulatory sequences. The shv region lies distal to the Hin region. The shv region contains four alternatively spliced first exons and regulatory sequences that govern expression in the embryonic epidermis and midgut (St. Johnston *et al.* 1990; Hursh *et al.* 1993). The disk region lies proximal to the Hin region and is not transcribed. This region is composed of 25 kb of regulatory sequence beginning ~2 kb beyond the polyadenylation site in *dpp* transcripts. Sequences in the disk region control expression of *dpp* along the anterior/posterior compartment boundary of imaginal disks. Seven distinct enhancers have been

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identified using reporter genes, and each directs expression within a subset of the overall *dpp* pattern (Blackman *et al.* 1991). One of the most distant enhancers is phenotypically uncovered by a small deletion of 2 kb ($dpp^{d\text{-}ho}$; St. Johnston *et al.* 1990). When homozygous, this deletion fixes the wings in a heldout position because of the absence of specific sensory structures (Spencer *et al.* 1982). The specific sequences responsible for this phenotype are unknown.

A strain was created in which a *hobo* transgene was inserted within the heldout enhancer region (Smith *et al.* 1993). Interestingly, the strain H{Lw2}dpp^{151h} is homozygous viable and fertile, and there is no obvious mutant phenotype. However, the allele H{Lw2}dpp^{151a} created by imprecise excision of the *hobo* construct displayed the heldout phenotype when *in trans* to $dpp^{d\text{-}ho}$ (Smith *et al.* 1993). To critically examine the usefulness of *hobo* elements for local jumping mutagenesis, we designed an experiment to identify a specific local transposition event within the *dpp* locus. We desired a precise excision of H{Lw2}dpp^{151h} from the heldout region followed by a new insertion into the Hin region ~25 kb away. In a pilot screen, we identified two such events. Both transposition events appear to result in insertions within the *dpp* transcription unit. Strains containing these new insertions represent valuable reagents for the analysis of *dpp* and for further studies of the *hobo* element system.

MATERIALS AND METHODS

Fly strains and genetic characterization of *hobo* mobilization: All stocks were devoid of endogenous *hobo* elements (E strains) unless otherwise indicated. The original line $y^1\ w^{67c23}$; H{Lw2}dpp^{151h} containing a *hobo* transgene inserted in the heldout region of *dpp* is described in Smith *et al.* (1993). The *Sp Bl* *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ strain carrying a duplication of the *dpp* locus is described in Spencer *et al.* (1982). The composite parental strain $y^1\ w^{67c23}$; H{Lw2}dpp^{151h} *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ was created by recombination; individual w^+ (from the mini-*white* marked *hobo* construct) recombinant males were tested for the ability to suppress lethality resulting from *dpp* haplo-insufficiency by crossing to dpp^{H61}-bearing females. The dpp^{H61} line contains endogenous *hobo* elements (H strain). The *net* $dpp^{d\text{-}ho}$ *dp Sp cn sca bw* transvection tester strain was not examined for the presence of *hobo* elements. Heat-shock-induced mobilization of H{Lw2}dpp^{151h} by P{ry^+: HSH2}*CyO* was conducted as described in Calvi and Gelbart (1994) with the following modification: cultures were brooded every 2 days and heat shocked every 2 days for a total of three heat shocks for each of the three broods. All *dpp* mutant strains and visible phenotypic markers are described in FlyBase (1998).

Molecular characterization of *hobo* transposition within the *dpp* locus: Standard methods of DNA isolation, digestion, and Southern blot hybridization were used (Sambrook *et al.* 1989). To demonstrate *hobo* mobilization in each candidate line, genomic DNA was digested with *Ssp*I. A Southern blot containing this DNA was hybridized with pSV-β-gal (Promega, Madison, WI). pSV-β-gal is a probe for β-galactosidase sequences located in the H{Lw2} transgene. For the identification of new *hobo* insertions in the 8-kb Hin region, genomic DNA from each candidate line was digested with *Eco*RI. A Southern blot containing this DNA was hybridized with the *dpp* cDNA H1. H1 is a full-length cDNA reverse transcribed from a "class B" *dpp* transcript (Newfeld *et al.* 1997). The same Southern blot was then stripped of probe and rehybridized with pH{Lw2}. For the identification of new *hobo* insertions in a 5-kb region bounded by the two *dpp* protein-coding exons, genomic DNA from each candidate line was digested with *Nhe*I (bp 12732) and *Sca*I (bp 17630). A Southern blot containing this DNA was hybridized with H1 and then stripped and rehybridized with pH{Lw2}. For the identification of new *hobo* insertions in *dpp* protein-coding exons 2 and 3, genomic DNA from each candidate line was digested with *Xba*I. There are four *Xba*I sites in the Hin region (bp 12772, 13754, 14853, and 17073). The first pair of sites nearly brackets exon 2 (bp 12607–13474), and the second pair of sites brackets the exon 3 open reading frame (bp 15192–16090). Numbers in parentheses indicate the location of the feature in the *dpp* shv/Hin sequence (GenBank accession number U63857; Newfeld *et al.* 1997).

RESULTS

Mobilization of H{Lw2}dpp^{151h}: The starting point for our examination of *hobo* local transposition was the transgenic line H{Lw2}dpp^{151h} (Smith *et al.* 1993). We planned to identify a specific *hobo*-mediated event, precise excision of H{Lw2}dpp^{151h} from the heldout region followed by a new insertion into the Hin region. A composite physical and genetic map of the *dpp* locus (polytene subdivision 22F1-2 on chromosome arm 2L) in this transgenic line is shown in Figure 1A (adapted from Newfeld *et al.* 1997). Note the relative position of the Hin and heldout regions, at least 20 kb apart. The 2-kb heldout region contains the phenotypically silent *hobo* transgene, and the 8-kb Hin region is our local jump target.

Alterations in the Hin region are impossible to recover in a *dpp* diploid genome as a result of dominant lethality caused by *dpp* haplo-insufficiency. Therefore, we recombined a chromosomal duplication of the *dpp* locus [*Dp(2;2)DTD48* $dpp^{d\text{-}ho}$] onto chromosome arm 2R of this strain. A crucial part of our strategy required the duplicated copy of *dpp* be deleted for the heldout region.

H{Lw2}dpp^{151h} was mobilized using a stable source of *hobo* transposase under the control of the heat-shock promoter inserted on the *CyO* balancer chromosome (Figure 2; parental cross). Mass matings and multiple heat shocks were used to generate candidate individuals of the appropriate genotype. Under these conditions, somatic mobilization of the *hobo* construct was seen in nearly 100% of the adults containing the transgene and transposase (Figure 2; F_1 generation). This was determined by mosaicism for *white* expression (w^+) in the eye from the mini-*white*-marked *hobo* construct. This rate of somatic mobilization is similar to that seen by Calvi and Gelbart (1994) when examining individuals that contain two *hobo* transgenes undergoing a single heat-shock induction of the transposase.

Mobilization of H{Lw2}dpp^{151h} may result in a variety of genomic events at the *dpp* locus. Five examples of

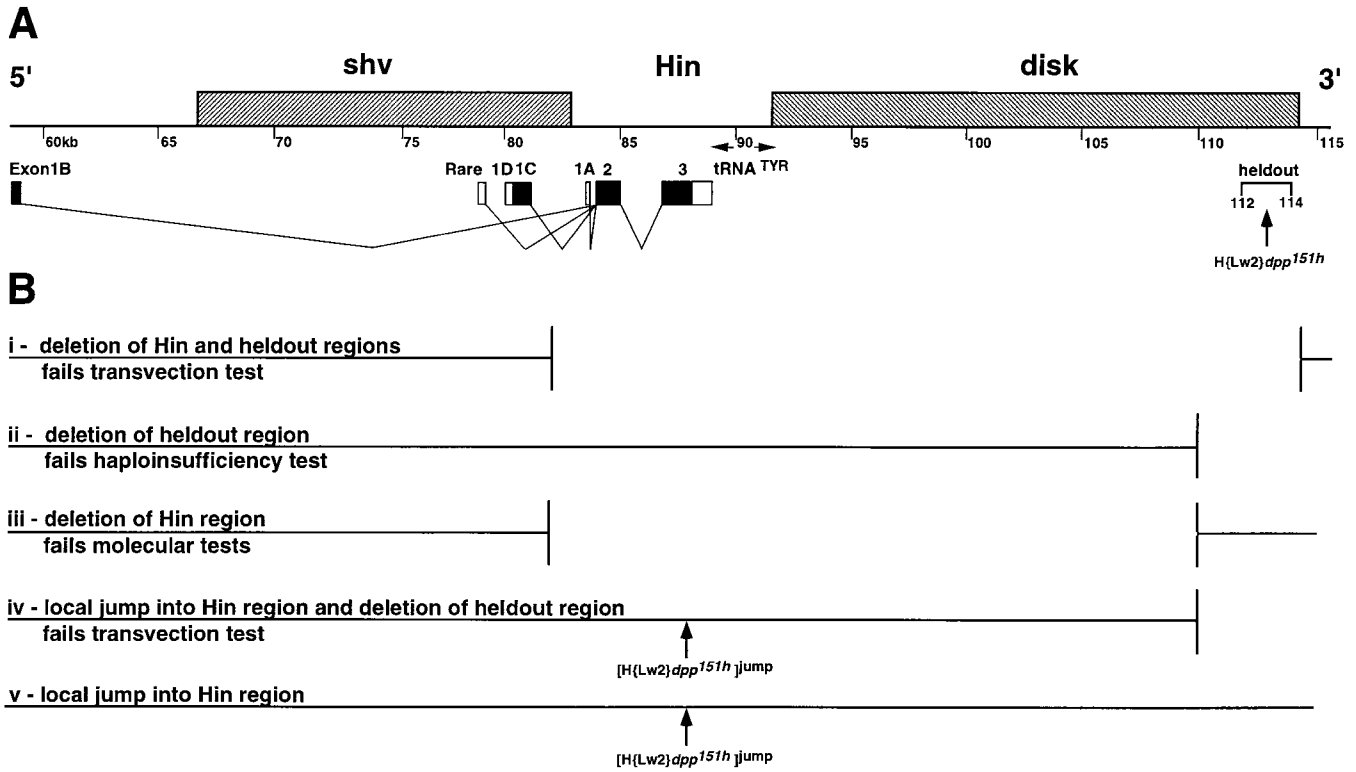

Figure 1.—Genetic and molecular map of the *dpp* locus. (A) Schematic of the *D. melanogaster dpp* locus with molecular coordinates from the 22F1-2 chromosome walk. The shaded rectangles above the coordinate line delineate the approximate locations of three genetically defined regions of the locus. The locations of exons and their splicing patterns are shown below the coordinate line; filled rectangles represent protein-coding sequences, and open rectangles indicate untranslated regions. The position and direction of transcription for two $tRNA^{Tyr}$ genes within the *dpp* locus are indicated by arrows. The coordinates of the portion of the disk region that, when deleted, lead to the heldout wing posture phenotype are indicated (St. Johnston *et al.* 1990). The *hobo* transgene H{Lw2}dpp^{151h} inserted within this 2-kb segment is also shown. (B) Schematic of simple molecular events that may result from the mobilization of H{Lw2}dpp^{151h} from the *dpp* disk region. Five possible scenarios are shown, and the experimental tests used to identify unwanted events (scenarios i–iv) are listed.

possible outcomes are shown in Figure 1B. Those listed are simple events, such as deletions or deletion / transposition combinations. The existence of a duplication of the *dpp* locus on chromosome arm 2R allows the recovery of unwanted Hin region deletions as well as Hin region *hobo* insertions. Four of the events (Figure 1B, i–iv) are unwanted outcomes, and the experimental tests used to identify these events are listed. Complex chromosomal events such as deletion / inversion combinations are also possible, but these will fail the transvection test.

Genetic characterization of [H{Lw2}dpp^{151h}]^{jump} candidate strains: In a pilot study, candidate males containing the transposition-capable genotype (Figure 2; F_1 generation) were individually mated to groups of dpp^{hr27}-bearing tester females. The dpp^{hr27} allele contains a nonconservative amino acid substitution in the proregion (Wharton *et al.* 1996) and acts as a strong hypomorph (Wharton *et al.* 1993). In the progeny of this cross, transpositions that affect the Hin region of H{Lw2}dpp^{151h} will generate phenotypically heldout adults. This is because the two remaining copies of *dpp* (sufficient to overcome haplo-insufficiency) are dpp^{hr27}, which encodes a defective protein, and the copy on 2R, which is a heldout allele ($dpp^{d\text{-}ho}$). The dpp^{hr27} and the $dpp^{d\text{-}ho}$ alleles are unable to complement each other and generate a wild-type wing phenotype through transvection because of their location on opposite chromosome arms. See below for a discussion of transvection at the *dpp* locus. However, a heldout phenotype is also generated in the progeny of this cross by two unwanted classes of transposition events. These are events that affect the heldout region only or both the heldout and Hin regions of H{Lw2}dpp^{151h}. Transposition resulting in Hin-affected alleles will be distinguished from both heldout-affected and heldout plus Hin-affected alleles in subsequent genetic tests.

The dpp^{hr27} tester females were also homozygous for z^1. We included z^1 because it acts as an enhancer of the heldout phenotype of $dpp^{d\text{-}ho}/dpp^{hr4}$ individuals (Gelbart and Wu 1982). We hoped it would also enhance the heldout phenotype of local jump heterozygotes in which the Hin region of H{Lw2}dpp^{151h} was affected. We focused our attention on male progeny that were hemizygous for z^1 and displayed heldout wings and w^+ eyes (Figure 2; F_2 generation). The presence of w^+ eyes indi-

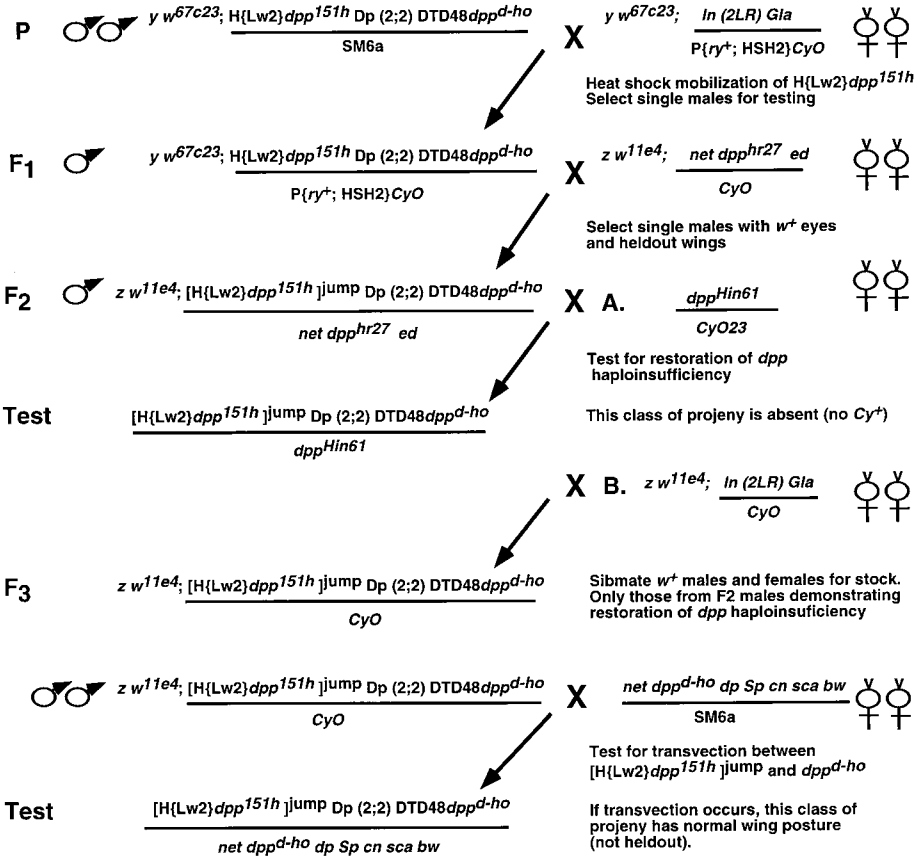

Figure 2.—Genetic scheme used in the identification of local transposition of H{Lw2}dpp^{151h}. The mobilization of H{Lw2}dpp^{151h} takes place in the F_1 male germline. For a description of genetic symbols, see FlyBase (1998). Individuals derived from test crosses were not maintained. Details are described in the text.

cates that the *hobo* transgene remains in the F_2 genotype after segregation of the homologs in the F_1 male germline, suggesting that a local jump is a possibility.

From ~500 fertile F_1 males, we recovered a total of 41 w^+ heldout males representing 30 different germline events. In several cases, multiple w^+ heldout males arose among the F_2 progeny of a single F_1 cross. For these clustered males, every individual was carried separately through the remainder of the scheme. In some clusters, distinctions between individuals were seen in the haploinsufficiency test. This indicates that under extremely efficient conditions of transposase induction, multiple independent events can occur in a single male germline. We also recovered w^+ heldout females (heterozygous for z^1) at a slightly lower rate. However, because of their location on distinct chromosome arms, 50% of all recombination events in these unbalanced females will separate [H{Lw2}dpp^{151h}]^{jump} and *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ in the next generation. This prevents the efficient recovery of Hin-affected alleles of [H{Lw2}dpp^{151h}]^{jump} in the F_3 generation. We chose not to characterize these females further.

Each of the F_2 w^+ heldout males was individually mated to groups of dpp^{H61}-bearing tester females (Figure 2; F_2 cross A). The dpp^{H61} allele is a 2-kb deletion that removes nearly all of the third exon, including the ligand domain (St. Johnston *et al.* 1990). Lines that carry this allele are haplo-insufficient for *dpp*. The line is maintained over a *dpp* embryonic rescue construct containing Hin region sequences inserted in the *CyO* balancer (*CyO23*; Wharton *et al.* 1993). In the test cross, the *dpp* rescue construct will segregate away from dpp^{H61}, allowing us to determine if *dpp* haplo-insufficiency has been restored through involvement of the Hin region in alleles of [H{Lw2}dpp^{151h}]^{jump}. Flies of the genotype [H{Lw2}dpp^{151h}]^{jump} *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ / dpp^{H61} will have only one functional copy of *dpp* (the one on 2R) if the Hin region of H{Lw2}dpp^{151h} has been affected by the *hobo* mobilization. These flies will not survive. As a result, the progeny class they would have contributed to (Cy^+) will be reduced. In addition, dpp^{H61} / dpp^{hr27} flies (also Cy^+) will die because of *dpp* haplo-insufficiency. Thus, the creation of a *dpp* haplo-insufficient allele at the [H{Lw2}dpp^{151h}]^{jump} locus in F_2 w^+ heldout males is easily detected by the absence of any Cy^+ progeny. Restoration of *dpp* haplo-insufficiency eliminates the possibility that the *hobo* mobilization affected only the heldout region of the *dpp* locus in [H{Lw2}dpp^{151h}]^{jump} strains (Figure 1B, ii).

Each of the F_2 w^+ heldout males was subsequently individually mated to groups of double-balancer females homozygous for z^1 (Figure 2; F_2 cross B) to generate F_3 individuals suitable for creating balanced stocks of each allele of [H{Lw2}dpp^{151h}]^{jump}. We were unable to use balanced progeny from the dpp^{H61} mating (Figure 2; F_2 cross A) because dpp^{H61} is an H strain. The presence of

endogenous *hobo* elements would result in the remobilization of [H{Lw2}*dpp*^{151h}]^{jump} at some future point in the stock. Sibmating of balanced F_3 individuals of the genotype [H{Lw2}*dpp*^{151h}]^{jump} *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$/*CyO* was performed only for lines derived from F_2 w^+ heldout males that demonstrated restoration of *dpp* haplo-insufficiency (Figure 2; F_2 test). As a second criterion for choosing F_3 progeny for stock construction, only F_2 cross B (Figure 2) progeny containing exclusively w^+ males and females of the appropriate genotype were chosen. The presence of w^+ eyes in every individual of the desired genotype indicates that the *hobo* transgene remains in the F_3 genotype after two generations of segregation from the original mobilization, again suggesting that a local jump remains a possibility. These F_3 individuals and stocks derived from them are again homozygous for z^1. We continued to maintain a z^1 background, hoping that the heldout phenotype would be enhanced in suitable genotypes during future experiments.

Nearly 50% of the F_2 w^+ heldout males were eliminated by these two criteria. Balanced F_3 stocks were created representing 24 F_2 w^+ heldout males from 11 different F_1 clusters. However, there was wide variation in the extent of *dpp* haplo-insufficiency in the F_3 lines. Six of the F_3 lines were essentially haplo-insufficient. These lines gave a small number of escapers (Cy^+) in the progeny of the cross to *dpp*^{H61}. In these lines, escapers appear at roughly the same rate as in the progeny of a cross between *dpp*^{H61} and wild type (Canton-S). Fourteen F_3 lines gave a moderate number of escapers compared to *dpp*^{H61} flies crossed to wild type, yet Cy^+ flies among the progeny were far fewer than expected by Mendelian ratios (33%). In these lines, we observed 10–30% of the expected number of Cy^+ progeny. Four F_3 lines gave a large number of escapers. In these lines, we observed 35–65% of the expected number of Cy^+ progeny.

We chose to pursue the F_3 lines with moderate and high escaper rates for the following reasons. First, the only reported *P*-element allele of *dpp* (*dpp*^{10638}; Twombly *et al.* 1996) is an insertion at the boundary between the shv and Hin regions (coordinate 83 on the *dpp* chromosome walk; St. Johnston *et al.* 1990). The insertion results in a recessive, embryonic-lethal *dpp* allele, not a haplo-insufficient allele. Second, the *dpp*^{e87} allele is a 0.5-kb deletion that spans the boundary of the shv and Hin regions. The *dpp*^{e87} allele is homozygous viable and fertile, but it fails to complement other recessive, embryonic-lethal *dpp* alleles (St. Johnston *et al.* 1990). We did not want to eliminate the possibility of recovering *hobo* insertions in this portion of the Hin region.

To further test the cosegregation of the w^+ eye phenotype with the [H{Lw2}*dpp*^{151h}]^{jump} chromosome, the F_3 stocks were examined for the presence of homozygous individuals (Cy^+). In each stock, these were uniformly heldout and w^+. The cosegregation of w^+ eyes and the otherwise unmarked [H{Lw2}*dpp*^{151h}]^{jump} chromosome through three generations implies an 87.5% probability (for each stock) that the *hobo* transgene resides on this chromosome. Proof that the *hobo* transgene is inserted in the *dpp* locus of the [H{Lw2}*dpp*^{151h}]^{jump} chromosome in the F_3 lines requires molecular data.

The final genetic test of the experiment exploits the allelic interaction known as transvection, which is defined as synapsis-dependent, intragenic complementation (Lewis 1954). Transvection at the *dpp* locus has been studied by Gelbart (1982). The best-characterized *dpp* genotypes that display transvection are heterozygous for *dpp*$^{d\text{-}ho}$ and any one of a number of recessive, lethal mutations in the Hin region (*e.g.*, *dpp*^{hr4}). Genotypes that contain this combination of mutant alleles are phenotypically wild type in the absence of any chromosomal rearrangement that interferes with the proper pairing of homologs. According to one model (Pirrota 1990), *dpp*$^{d\text{-}ho}$ complements *dpp*^{hr4} because the wild-type copy of the heldout enhancer on the *dpp*^{hr4} chromosome acts *in trans* to promote transcription of the wild-type DPP protein encoded by the *dpp*$^{d\text{-}ho}$ allele on the homolog. We used transvection to identify H{Lw2}*dpp*^{151h} mobilization events that affected both the original site of insertion in the heldout region and the Hin region (Figure 1B, i and iv), as well as mobilizations that involve complex chromosomal rearrangements.

Several males from each F_3 stock were crossed to groups of females carrying *dpp*$^{d\text{-}ho}$. *Trans*-heterozygous (Cy^+) progeny were examined for wing posture (Figure 2; F_3 test). These flies carry three copies of *dpp*—two copies of *dpp*$^{d\text{-}ho}$ and [H{Lw2}*dpp*^{151h}]^{jump}. The only possible location for a normal heldout enhancer is at [H{Lw2}*dpp*^{151h}]^{jump}. If the heldout enhancer is intact and if there have been no chromosomal rearrangements, the heldout enhancer should promote the transcription *in trans* of the wild-type DPP protein encoded by the *dpp*$^{d\text{-}ho}$ allele on the homolog. This results in wild-type wing posture. However, if the heldout enhancer is affected at the [H{Lw2}*dpp*^{151h}]^{jump} locus, or if there has been a chromosomal rearrangement, then the Cy^+ progeny will have a heldout phenotype. Two of the F_3 lines from a single F_1 cluster failed to show normal wing posture in this test.

Molecular characterization of [H{Lw2}*dpp*^{151h}]^{jump} candidate strains: Having identified and eliminated a number of lines that carry several distinct classes of unwanted chromosomal events (Figure 1B, i, ii, and iv), we conducted a molecular characterization of the remaining candidate lines. In the following experiments, candidate lines are organized into groups according to the number of escapers in the haplo-insufficiency test (see the legend to Figure 3 for details). Our first experiment was designed to provide molecular evidence of *hobo* mobilization in each candidate line. A Southern blot containing genomic DNA digested with *Ssp*I from balanced lines containing the original *hobo* transgene H{Lw2}*dpp*^{151h}, the recombinant parental line for our experiment H{Lw2}*dpp*^{151h} *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$, and all candi-

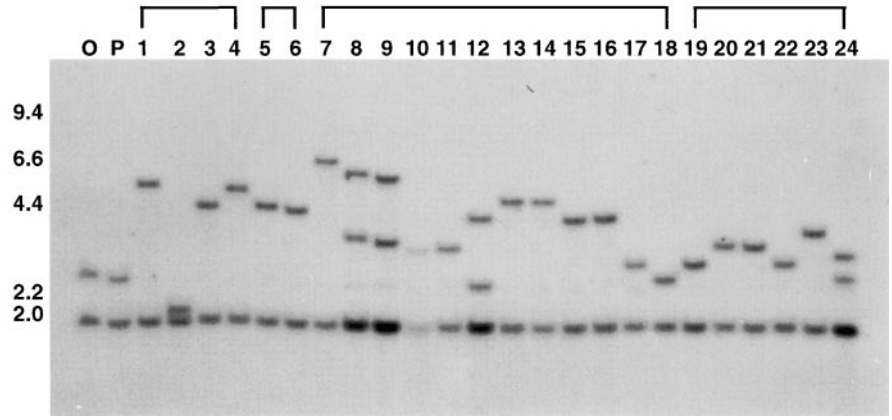

Figure 3.—Molecular evidence for the mobilization of H{Lw2}*dpp*^{151h}. Autoradiograph of a Southern blot containing *Ssp*I-digested genomic DNA from F_3 candidate [H{Lw2}*dpp*^{151h}]^{jump} balanced lines probed with pSV-β-gal. Restriction fragment size markers are indicated in kilobases. Lane O, original balanced line containing H{Lw2}*dpp*^{151h}; lane P, recombinant parental balanced line containing H{Lw2}*dpp*^{151h} *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$; lanes 1–24, F_3 balanced lines containing [H{Lw2}*dpp*^{151h}]^{jump} *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$. F_3 candidate lane groupings are as follows: 1–4, lines that showed large numbers of escapers in the haplo-insufficiency test; 5 and 6, lines that failed the transvection test; 7–18, lines that had a moderate number of haplo-insufficiency escapers; 19–24, haplo-insufficient lines with a small number of escapers. Individual lanes represent the following strains: 1, H{Lw2}*dpp*^{14}; 2, H{Lw2}*dpp*^{F18}; 3, H{Lw2}*dpp*^{F22}; 4, H{Lw2}*dpp*^{T2}; 5, H{Lw2}*dpp*^{15D}; 6, H{Lw2}*dpp*^{15B}; 7, H{Lw2}*dpp*^{F3}; 8, H{Lw2}*dpp*^{F4}; 9, H{Lw2}*dpp*^{F5}; 10, H{Lw2}*dpp*^{F6}; 11, H{Lw2}*dpp*^{F7}; 12, H{Lw2}*dpp*^{F11}; 13, H{Lw2}*dpp*^{F13}; 14, H{Lw2}*dpp*^{F14}; 15, H{Lw2}*dpp*^{F16}; 16, H{Lw2}*dpp*^{F17}; 17, H{Lw2}*dpp*^{F19}; 18, H{Lw2}*dpp*^{F21}; 19, H{Lw2}*dpp*^{F2}; 20, H{Lw2}*dpp*^{F8}; 21, H{Lw2}*dpp*^{F9}; 22, H{Lw2}*dpp*^{F10}; 23, H{Lw2}*dpp*^{F12}; 24, H{Lw2}*dpp*^{F20}.

date [H{Lw2}*dpp*^{151h}]^{jump} lines was hybridized with pSV-β-gal. This is a probe for the β-galactosidase sequences located in the H{Lw2} transgene. Since *Ssp*I has two restriction sites in the β-galactosidase gene, the probe will hybridize to two restriction fragments. One is an internal fragment of 2 kb that is unaffected by mobilization of the transgene. The other fragment contains the 3′ end of H{Lw2} and the genomic sequences flanking the transgene. If the *hobo* transgene in the [H{Lw2}*dpp*^{151h}]^{jump} chromosome no longer resides in the same location as in the original H{Lw2}*dpp*^{151h} or the parental H{Lw2}*dpp*^{151h} *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$ chromosome, the chimeric restriction fragment will change in size in the [H{Lw2}*dpp*^{151h}]^{jump} strains. Altered fragment size in a [H{Lw2}*dpp*^{151h}]^{jump} line reflects the incorporation of new flanking sequences.

As shown in Figure 3, nearly all candidate lines show chimeric fragments of a different size than in the original and parental lines. Only lane 18 appears similar to the parental lane. This could result from a new *hobo* insertion that is the same distance from a genomic *Ssp*I restriction site as the original insertion was from its flanking *Ssp*I site. Thus, the data suggest that we successfully mobilized the *hobo* transgene in all candidate lines. Intriguingly, several lanes in Figure 3 (lanes 8, 9, 12, and 24) display two chimeric fragments. These lanes also show an increase in the intensity of the signal from the internal fragment in comparison to the original and parental lines. This suggests that there are two *hobo* transgenes in each of these lines, and that they are inserted at different genomic locations. In two of the lanes in Figure 3 (lanes 12 and 24), one of the chimeric fragments appears similar in size to the parental fragment, suggesting that one copy of the transgene remains in the original location. We did not pursue this finding any further.

We focused our efforts on identifying new *hobo* insertions in the *dpp* Hin region. A Southern blot of genomic DNA digested with *Eco*RI from balanced lines containing the original *hobo* transgene, the recombinant parental line, and all candidate lines was probed with the *dpp* cDNA H1. In *Eco*RI-digested genomic DNA, the H1 probe will hybridize to an 8-kb fragment that contains the Hin region with its two protein-coding exons and to a 15-kb fragment that contains the distant 5′ noncoding exon (see Figure 1). *Eco*RI has three restriction sites within the *hobo* transgene and therefore a change in size of the 8-kb Hin region restriction fragment indicates a *hobo* insertion in the Hin region. The altered size of any Hin region fragment indicates that it is now defined by genomic and *hobo* transgene *Eco*RI sites.

As shown in Figure 4A, new restriction fragments are detected in lanes 2 and 12 (lines H{Lw2}*dpp*^{F18} and H{Lw2}*dpp*^{F11}). In lane 2, a single new fragment >8 kb is detected. In Figure 4 (lane 12), two fragments <8 kb are detected. This result suggests that new *hobo* insertions now flank the two protein-coding exons in H{Lw2}*dpp*^{F18} and separate the two protein-coding exons in H{Lw2}*dpp*^{F11}. In all lanes, the H1-hybridizing restriction fragments derived from *Dp(2;2)DTD48 dpp*$^{d\text{-}ho}$ and from the *CyO* balancer chromosome (8 and 15 kb) are unaffected. The H{Lw2}*dpp*^{F11} and H{Lw2}*dpp*^{F18} lines did not derive from the same F_2 w^+ heldout male germline, indicating that the new insertions are independent events.

We wanted to confirm that *hobo* transgene sequences are contained within the new restriction fragment detected with H1. This is important because the Hin region restriction fragment can also change in size if the mobilized *hobo* transgene deleted chromosomal material adjacent to its original site of insertion. If a *hobo*-induced deletion removes some but not all of the Hin region, the H1 probe will detect an altered Hin region fragment. However, if the altered fragment is the result

of a mobilization-induced deletion, *hobo* sequences may not be associated with the new Hin region fragment. We took the Southern blot shown in Figure 4A, removed the H1 probe, and reprobed it with pH{Lw2}, as shown in Figure 4B. We chose to use pH{Lw2} as a probe instead of pSV-β-gal so that we could detect chimeric restriction fragments containing the 5′ or the 3′ ends of the *hobo* transgene. This allows us to detect hybridization of *hobo* sequences to fragments that previously hybridized with H1 regardless of the orientation of the new transgene insertion.

Since *Eco*RI has three restriction sites within the *hobo* transgene, we predict four hybridizing fragments in each lane in this experiment. Two of the fragments are internal, and two are chimeric fragments that contain *hobo* sequences and either 5′ or 3′ flanking genomic DNA. The internal fragments are nearly equal in size. They appear in Figure 4B as a strongly hybridizing 4-kb doublet in all lanes. The chimeric fragments that contain flanking genomic DNA are of unpredictable size. The large (>20 kb) fragment or fragments hybridizing in all lanes, as well as other strongly hybridizing fragments, *e.g.*, Figure 4B, lanes 2, 8, 9, 12, and 24, likely represent these chimeric fragments. By overlaying the autoradiographs, it is clear that the new fragment in lane 2 and one of the new fragments in lane 12 from Figure 4A also hybridize to pH{Lw2}. These fragments are highlighted by arrows in Figure 4B. Taken together, the data in Figure 4 strongly support the existence of a new *hobo* transgene insertion in the Hin region in lines H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11}.

To further specify the location of the new *hobo* insertions in the H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11} strains, we conducted additional molecular analyses on these lines. We wanted to determine if the insertions were in a 5-kb region roughly bounded by the two *dpp* protein-coding exons. A Southern blot of genomic DNA digested with *Nhe*I and *Sca*I from balanced lines containing the original transgene, the recombinant parental line, and the H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11} lines was probed with H1. These enzymes each have a single restriction site within the Hin region. *Nhe*I does not cut in the *hobo* transgene. *Sca*I cuts very close to one end of the *hobo* transgene.

As shown in Figure 5A (left), new restriction fragments are detected in lanes 1 and 2 (H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11}, respectively). In lane 1, a single new fragment <5 kb is detected. In lane 2, two new fragments, one <5 kb and one >5 kb, are detected. This result is consistent with our *Eco*RI experiment, confirming that a new *hobo* insertion flanks the protein-coding exons in the H{Lw2}dpp^{F18} strain and separates the two protein-coding exons in the H{Lw2}dpp^{F11} line. In all lanes, the H1-hybridizing fragments derived from *Dp(2;2)DTD48*

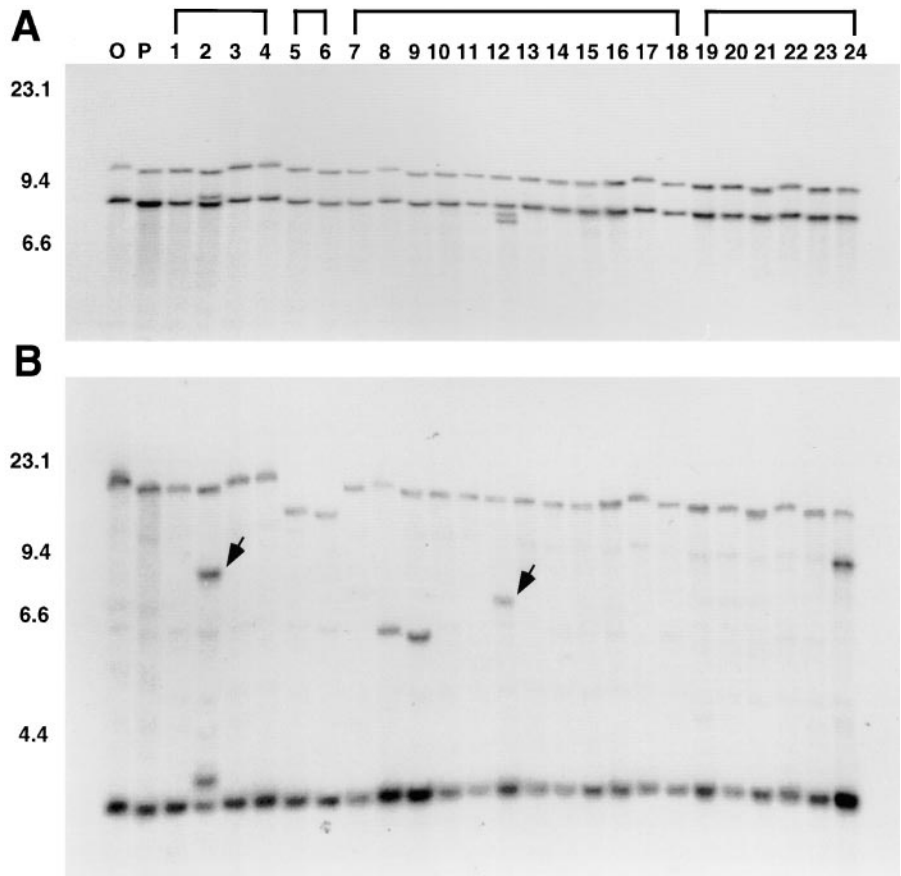

Figure 4.—Molecular identification of new *hobo* insertions in the *dpp* Hin region. Autoradiographs of a single Southern blot containing *Eco*RI-digested genomic DNA from F_3 [H{Lw2}dpp^{151h}]^{jump} balanced lines probed with (A) the *dpp* cDNA H1 or (B) pH{Lw2}. (A) New restriction fragments are detected in lanes 2 and 12 (H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11}). The wild-type restriction fragments derived from *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ and the *CyO* balancer chromosome remain unchanged. (B) Hybridizing restriction fragments highlighted by arrows in lanes 2 and 12 are the same fragments that show altered size in A. Note that A is an overnight exposure, and B is a 4-day exposure that leads to the difference in the sharpness of the autoradiographic signal from fragments that hybridize to both probes. The background of weakly hybridizing fragments seen in B is common on genomic Southern blots probed with *hobo* sequences (Blackman *et al.* 1987). Lane designations follow those in Figure 3.

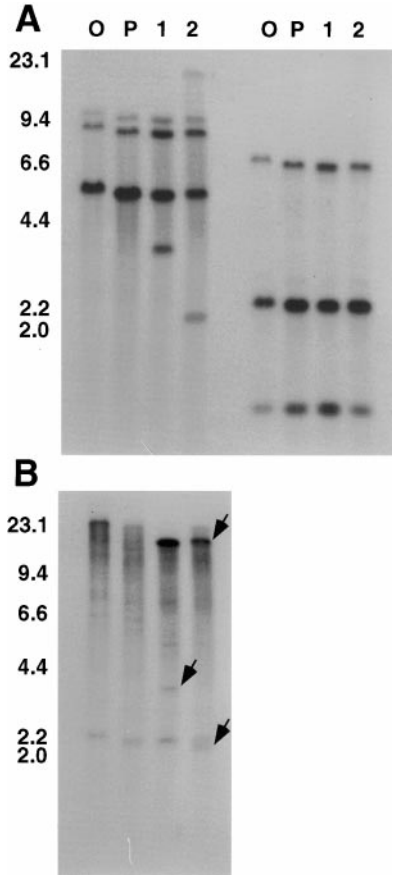

Figure 5.—Molecular specification of the location of new *hobo* insertions in the *dpp* Hin region. Autoradiographs of a single Southern blot containing restriction enzyme-digested genomic DNA from two F_3 [H{Lw2}dpp^{151h}]^{jump} balanced lines containing new *hobo* insertions in the Hin region (H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11}). The blot was probed with (A) the *dpp* cDNA H1 or (B) pH{Lw2}. In the four lanes on the left, the genomic DNA was cut with *Nhe*I and *Sca*I. In the four lanes on the right, the genomic DNA was cut with *Xba*I. (A) New restriction fragments are detected on the left in lanes 1 and 2 (*Nhe*I and *Sca*I). No new fragments are seen on the right (*Xba*I). The wild-type restriction fragments derived from *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$ and the *CyO* balancer chromosome remain unchanged. (B) Hybridizing restriction fragments highlighted by arrows in lanes 1 and 2 (*Nhe*I and *Sca*I) are the same fragments showing altered size in A. Lane O, original balanced line containing H{Lw2}dpp^{151h}; lane P, recombinant parental balanced line containing H{Lw2}dpp^{151h} *Dp(2;2)DTD48* $dpp^{d\text{-}ho}$; lane 1, H{Lw2}dpp^{F18}; lane 2, H{Lw2}dpp^{F11}.

$dpp^{d\text{-}ho}$ and from the *CyO* balancer chromosome (5 and 9 kb) are unaffected.

Our final diagnostic genomic digest was designed to determine if the insertions landed in the *dpp* open reading frame contained in exons 2 and 3. A Southern blot of genomic DNA digested with *Xba*I from balanced lines containing the original transgene, the recombinant parental line, and the H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11} lines was probed with H1. There are four *Xba*I sites in the Hin region. The first pair of sites nearly brackets exon 2 and delineates a 1-kb fragment. The second pair of sites brackets the coding region of exon 3 and defines a 2-kb fragment. *Xba*I does not cut in the *hobo* transgene. As shown in Figure 5A (right), no new fragments are detected.

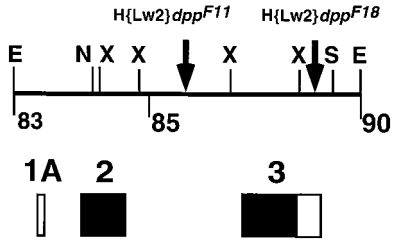

Figure 6.—Molecular map of new *hobo* insertions in the *dpp* Hin region. Schematic of the endogenous *dpp* Hin region from the *hobo*-bearing chromosome in lines H{Lw2}dpp^{F11} and H{Lw2}dpp^{F18}. The molecular coordinates and depiction of *dpp* exons follow that in Figure 1. The positions of restriction enzymes used for the molecular characterization of new *hobo* insertions in these strains are shown above the coordinate line. E, *Eco*RI; N, *Nhe*I; S, *Sca*I; X, *Xba*I. The *Nhe*I site is 145 bp from the splice acceptor site of exon 2. The distance between *Nhe*I and the closest *Xba*I site is 50 bp. The distance between the second and third *Xba*I sites (these sites define a restriction fragment not recognized by the *dpp* cDNA H1) is 1099 bp. The distance between *Sca*I and the closest *Xba*I site is 552 bp. The most likely location for the new *hobo* insertions in each of these lines is shown. For H{Lw2}dpp^{F18}, which flanks the protein-coding exons, this is the 552-bp region between the 3′ *Xba*I and the *Sca*I sites rather than the 50-bp region between the *Nhe*I and the 5′ *Xba*I sites.

We then took the Southern blot shown in Figure 5A, removed the H1 probe, and rehybridized it with pH{Lw2}. As described above, we were looking to confirm that *hobo* transgene sequences are coincident with the altered restriction fragments seen in Figure 5A. Since *Nhe*I does not cut in the transgene and *Sca*I cuts once in the transgene (1300 bp from the 5′ end), we expect two hybridizing chimeric fragments of unpredictable size. Both fragments will contain *hobo* sequences and flanking genomic DNA. Note the hybridizing restriction fragments highlighted by arrows in lanes 1 and 2 (Figure 5B). By overlaying the autoradiographs, it is clear that the new fragment in lane 1 and both new fragments in lane 2 from Figure 5A (left) also hybridize to pH{Lw2}. From the relative intensity of hybridization of the fragments in lane 1, it appears that the small fragment that also hybridizes to H1 contains just the 5′ end of the pH{Lw2} transgene. When hybridizing pH{Lw2} to the *Xba*I digest, we found no fragments hybridizing in common with those in Figure 5A (data not shown). Taken together, the data in Figure 5 strongly support the presence of new *hobo* insertions in both lines in the Hin region between *Nhe*I and *Sca*I, but not in the protein-coding regions of the two exons defined by *Xba*I.

A composite restriction map of the Hin region from lines H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11} that summarizes our molecular data is shown in Figure 6. We present

the most likely location for the new *hobo* insertions in these lines based upon the relative position of the restriction enzymes and the hybridization patterns of our Southern blots. The detection of only one new fragment in the H{Lw2}dpp^{F18} strain suggests that the most likely location for the new *hobo* insertion in this line is in the 3′-untranslated region of *dpp* exon 3. The detection of two new fragments in the H{Lw2}dpp^{F11} strain implies that the most likely location for the new *hobo* insertion in this line is the intron between the protein-coding exons.

DISCUSSION

The development of endogenous, transposable elements into powerful genetic tools has had an enormous impact on our understanding of organismal biology. This study further characterizes the *hobo* element system in *D. melanogaster* and compares its utility to the well-established *P*-element system. Relying upon the exquisite molecular genetics of the *dpp* locus, we have demonstrated that *hobo*, like *P*, is capable of local transposition. We recovered two chromosomes that experienced a precise excision and new insertion of a *hobo* transgene roughly 25 kb apart. The independent *hobo* local transposition events resulted in new insertions in the *dpp* transcription unit. One insertion is between the two protein-coding exons (line H{Lw2}dpp^{F11}), and the other is in the 3′ untranslated region of exon 3 (line H{Lw2}dpp^{F18}). A summary of the experiment describing the results of each genetic and molecular test is shown in Table 1.

Interestingly, line H{Lw2}dpp^{F11} had a moderate number of escapers in the *dpp* haplo-insufficiency test, and line H{Lw2}dpp^{F18} had a large number of escapers. In these lines, it seems unlikely that the new transgene insertions have a major affect on the DPP protein. In line H{Lw2}dpp^{F11}, the insertion could be removed from the *dpp* transcript by RNA splicing. In line H{Lw2}dpp^{F18}, the insertion could be removed by proper translation termination. The effect of the new insertions on *dpp* function may be caused by problems associated with the *dpp* transcript. The large transgene (13 kb) may interfere with splicing or translation termination to a limited extent in these lines.

TABLE 1

Summary of the *hobo* local transposition experiment

Experimental step	Number of individuals
Transposition-capable F_1 males	500
w^+ heldout F_2 males	41
Fail haplo-insufficiency test	17
Surviving F_3 candidates	24
Small number of escapers in haplo-insufficiency test	6
Moderate number of escapers in haplo-insufficiency test	14
Large number of escapers in haplo-insufficiency test	4
Fail transvection test	2
Fail molecular test	20
Local transposition within *dpp*, identified in one moderate and one large escaper candidate	2

None of the haplo-insufficient lines (those with a small number of escapers) contained a *hobo* insertion in the Hin region. One possible interpretation of our molecular data is that the haplo-insufficient lines resulted from large, *hobo* mobilization-induced deletions that removed the entire disk and Hin regions (Figure 1B, iii). The lines that showed escapers from *dpp* haplo-insufficiency that did not have *hobo* insertions in the Hin region may have smaller *hobo* mobilization-induced deletions. These deletions may remove varying amounts of chromosomal material between the heldout region and the Hin region. For example, a number of chromosomal inversions have breakpoints near the $tRNA^{Tyr}$ genes that remove all disk region sequences. These inversions are lethal as *trans*-heterozygotes (St. Johnston *et al.* 1990). Perhaps in the background of dpp^{H61} and $dpp^{d\text{-}ho}$, as in our haplo-insufficiency test, a percentage of dominant lethality is achieved.

A more intriguing possibility is that the various levels of *dpp* haplo-insufficiency in candidate lines without Hin region insertions are the result of new insertions in second-site enhancers of *dpp*. In the haplo-insufficiency test, the [H{Lw2}dpp^{151h}]^{jump} chromosome was paternal in origin. Thus, our experiment could be viewed as a mutational screen for zygotic enhancers of *dpp*. As described above, there is an 87.5% probability (for each stock) that the *hobo* transgene still resides on the second chromosome. Complementation tests of candidate lines without Hin region insertions using alleles from known *dpp* enhancers on chromosome 2, such as *Mothers against dpp* (Newfeld *et al.* 1996), *saxophone*, and *thickveins* (Brummel *et al.* 1994), would be the first step in evaluating this exciting possibility.

No *P*-element insertions have been reported in the portions of the Hin region where the new *hobo* insertions are suggesting that these elements have different insertional preferences. To critically test this proposal, we conducted an analogous excision/new insertion experiment using a *P*-element transgene (PZ; Mlodzik and Hiromi 1992). In the E32 strain, the PZ transgene is inserted in the 5′ untranslated region of the *out-at-first* gene, ~6 kb further from the *dpp* Hin region than H{Lw2}dpp^{151h} (Bergstrom *et al.* 1995; Merli *et al.* 1996). The *P*-element version of our mobilization/new insertion scheme contained several minor technical differences from the *hobo* experiment. In the F_1 cross, the tester allele was dpp^{hr4} carried on a chromosome marked with *Sp* and the DTD48 duplication of *dpp* on 2R. The genetic scheme was started by jumping the *P* element

in females, which was shown by Zhang and Spradling (1993) to be more efficient than jumping in males. In the *P*-element scheme, z^1 was not introduced.

We conducted a screen of 1200 fertile, transposition-capable females. Each female carried the E32 chromosome and a copy of the engineered *P*-element transposase Δ2-3 (reviewed in Rio 1990) inserted on the *CyO* balancer chromosome via a *hobo* transgene (*CyOHOP*1; B. Calvi and W. Gelbart, unpublished data). This screen generated 182 F_2 heldout progeny of both sexes (155 clusters). In this scheme, the continued presence of the transgene could not be followed by eye color, as in our *hobo* experiment. All F_2 heldout flies were tested for *dpp* haplo-insufficiency by crossing to flies carrying *In(2LR)Gla* over *CyO23*. If haplo-insufficiency for *dpp* is now present, no offspring of the genotype $E32^{jump}$ over *In(2LR)Gla* will survive, and the $E32^{jump}$ chromosome can be recovered over *CyO23*. Progeny carrying the $E32^{jump}$ chromosome are distinguishable from those inheriting the dpp^{hr4} chromosome using *Sp*. In this much larger screen, none of the 182 F_2 heldout flies demonstrated any level of *dpp* haplo-insufficiency.

Our interpretation of this result is that all the F_2 heldout flies in the *P*-element scheme had affected heldout enhancers only, reflecting mobilization-induced deletions. This suggests that the *dpp* Hin region is refractory to *P* insertion. A limited exception would be at the shv/Hin boundary, where dpp^{10638} is inserted. The results of our *P* and *hobo* experiments for insertion preference at the *dpp* locus support the findings of Smith *et al.* (1993), who detected distinct insertion preferences for *P* and *hobo* elements in a genome-wide survey. The expanded use of *hobo* transgenes will facilitate our understanding of aspects of the *D. melanogaster* genome that are not accessible with *P* elements.

The new *hobo* insertions adjacent to the *dpp* protein-coding exons may be suitable substrates for exploring another aspect of the *P*-element system that has not yet been demonstrated for *hobo*. These Hin region insertions are excellent candidates for experiments in gene replacement via transposable element-induced gap repair (reviewed in Gloor and Lankenau 1998). In this technique, the gap in the chromosome created by *P*-element excision is repaired from the DNA sequence of a homologous (but nonidentical) template at an ectopic chromosomal site. This process results in the replacement of sequences at the original site of *P*-element insertion and the loss of the *P* element. Using this method, tracts of ~1.4 kb of new sequence have been introduced onto otherwise wild-type chromosomes.

The first step in exploring the feasibility of this method for *hobo* is to determine the exact site of the H{Lw2}dpp^{F18} and H{Lw2}dpp^{F11} insertions in the *dpp* Hin region. This is done using restriction sites for plasmid rescue contained in the H{Lw2} construct (Smith *et al.* 1993). The exact location of the insertion is obtained by sequencing the genomic region of the rescued plasmid. Once the site of insertion is known, constructs that contain the desired gap repair template can be injected, and the gene replacement method can be tested in lines that contain both transgenes. One template that would provide valuable new information about *dpp* function, if successfully transferred, would be an epitope-tagged ligand for studying DPP protein activity. For improving our understanding of *dpp* molecular evolution, the possibility of transferring *dpp* homologs from other species (*e.g.*, grasshopper; Newfeld and Gelbart 1995) is very exciting. Larger questions about the developmental role and molecular evolution of the Drosophila TGF-β family could be addressed by replacing *dpp* with 60A (Wharton *et al.* 1991) or *screw* (Arora *et al.* 1994).

In summary, our studies have revealed similarities and differences between the *hobo* and *P* genetic systems. Both *hobo* and *P* are amenable to local jumping strategies, but the elements vary in their insertion site preference. *hobo* transgenes will insert into genomic regions refractory to *P* elements. These results suggest that the continued exploitation of both genetic systems is the best approach to understanding the genome of *D. melanogaster*. In addition, strains generated in our experiments are valuable new reagents for exploring the *hobo* genetic system and for understanding *dpp* function and molecular evolution.

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