The *Lighten up* (*Lip*) Gene of *Drosophila melanogaster*, a Modifier of Retroelement Expression, Position Effect Variegation and *white* Locus Insertion Alleles

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ABSTRACT

We are interested in identifying single gene mutations that are involved in *trans*-acting dosage regulation in order to understand further the role of such genes in aneuploid syndromes, various types of dosage compensation as well as in regulatory mechanisms. The *Lighten up* (*Lip*) gene in *Drosophila melanogaster* was identified in a mutagenic screen to detect dominant second site modifiers of *white-blood* (w^{bl}), a retrotransposon induced allele of the *white* eye color locus. *Lip* specifically enhances the phenotype of w^{bl} as well as a subset of other retroelement insertion alleles of *white*, including the *copia*-induced allele, *white-apricot* (w^{a}), and six alleles caused by insertion of *I* elements. We isolated six alleles of *Lip* which are all recessive lethal, although phenotypically additive heteroallelic escapers were recovered in some combinations. *Lip* also suppresses position effect variegation, indicating that it may have a role in chromatin configuration. Additionally, *Lip* modifies the total transcript abundance of both the *blood* and *copia* retrotransposons, having an inverse effect on the steady state level of *blood* transcripts, while showing a non-additive effect on *copia* RNA.

A NUMBER of genes in Drosophila have been found that enhance or suppress mutations at a second locus in a *trans*-acting manner [see RUTLEDGE *et al.* (1988) for example]. Many of these modifiers show effects beyond their interactions with specific mutant loci. For instance, some modifier genes are recessive lethals, and are of interest not only for their ability to alter the target mutation, but also because they may function in the normal regulation of gene expression. Often a modifier interacts with only a subset of the mutant alleles at a given locus. An example of this, *Enhancer of white-apricot* ($E(w^{a})$), modifies only four alleles of *white* (w) all caused by retrotransposon insertions (BIRCHLER and HIEBERT 1989). The specificity of the interaction shows the complexity of the regulatory mechanisms working on both the *white* locus and the insertion elements causing the specific mutations. Transposons give rise to a substantial proportion of spontaneous mutations. Therefore, the characterization of the modifier genes that interact with an insertion mutation can reveal the many new regulatory mechanisms a gene can acquire by the insertion of a single transposable element.

To identify genes that regulate the *white* locus and/or its mutant alleles, our lab has performed a number of mutageneses. Some of the resulting modifiers of the *white-apricot* (w^{a}) allele have been described previously (BIRCHLER and HIEBERT 1989; BIRCHLER *et al.* 1989; RABINOW and BIRCHLER 1989; RABINOW *et al.* 1991; CSINK *et al.* 1994). In this paper we describe a gene, *Lighten up* (*Lip*), found as a result of a mutagenic screen on a stock carrying the w^{bl} mutation. Alleles of *Lip* enhance the phenotype of w^{bl}, which is caused by an antiparallel insertion of the *blood* retrotransposon into the second intron of the *white* locus (BINGHAM and CHAPMAN 1986). *Lip* also enhances two other retrotransposon induced *white* mutations w^{a} and *white-coral* (w^{co}) as well as six *white* alleles caused by insertion of *I* element retroposons (*i.e.*, retroelements that lack long terminal repeats). Interestingly, *Lip* suppresses *white-ivory* (w^{i}), a mutation caused by a duplication of part of the *white* locus.

Moreover, *Lip* was found to be a suppressor of position effect variegation (PEV). Position effect variegation refers to the inactivation of an otherwise wild-type gene when brought into close proximity to abnormal junctions of euchromatin and heterochromatin. A number of second site modifiers have been identified that make the inactivation more or less severe [seeWUSTMANN *et al.* (1989) for example]. Since PEV seems to involve the configuration of chromatin in the vicinity of the affected gene, suppressors of PEV (*Su(var)*s) are thought to influence the organization of heterochromatin. Many of the presently known *Su(var)*s are dosage sensitive (LOCKE *et al.* 1988). It is interesting, therefore that *Lip* identifies a locus that demonstrates the overlap between modifiers of gene expression and PEV. Additionally, *Lip*, like other genes we have previously described, modifies retrotransposon transcript abundance.

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MATERIALS AND METHODS

Fly stocks and cytology: Fly stocks were maintained on cornmeal-glucose-yeast media at 25°. Stocks used to map *Lip* were provided by the Indiana University Drosophila Stock Center. Various stocks containing *Su(var)* genes on the right arm of chromosome *3* were kindly provided by GUNTER REUTER (Martin Luther University, Halle, Germany). Larval salivary glands were dissected in 40% acetic acid and the polytene chromosomes stained with aceto-orcein.

Mutagenesis: Males of w^{bl} were exposed to 4000 rad of γ-irradiation from a ^{137}Cs source. Irradiated males were mated to w^{bl} females for three days and then removed. Females were regularly transferred to fresh medium. Approximately 17,500 progeny were screened for individuals showing lighter or darker eye color. From this mutagenesis we recovered eight flies containing enhancers of the w^{bl} mutation and two flies containing suppressors. Five of the enhanced flies proved to be alleles of the same locus, *Lip*.

RNA extraction: RNA was extracted using a guanidine-HCl method (COX 1968). Approximately 0.5 g of 2–24-hr adult flies were homogenized in 6 ml 8 M guanidine-HCl and precipitated with 50% ethanol. The RNA pellet was resuspended in 4 M guanidine-HCl and precipitated with 50% ethanol four times. The RNA pellet was resuspended in sterile water, centrifuged and the supernatant collected. This extraction was repeated three additional times and the supernatants pooled and precipitated with 0.3 M sodium acetate and 75% ethanol. The resulting pellet was dissolved in sterile water to the appropriate concentration.

Northern blots: Twenty micrograms of total cellular RNA were subjected to electrophoresis through 1.5% agarose formaldehyde gels (LEHRACH *et al.* 1977) and transferred to Biotrans nylon filters (ICN Inc.) according to manufacturer's instructions. Filters were prehybridized for 8 hr at 60° in 50% formamide, 5 × SSC, 10 × Denhardt's solution, 0.5% SDS, 0.2 mg/ml sonicated, single-stranded salmon sperm DNA and 9% dextran sulfate [for reagents see MANIATIS *et al.* (1982)]. Radioactive RNA probes (see below) were then added to a final concentration of 1.5 million cpm/ml and the hybridization continued for 20 hr at 60°. Filters were washed in 0.2 × SSC, 0.05% SDS, at 75° for 30 min three times and exposed to X-ray film for autoradiography. Quantitation of the bands on the Northern blot autoradiographs was carried out using a LKB Ultrascan XL densitometer. The exposures scanned are not necessarily those shown, since, in some cases, shorter exposure times were used to ensure that the linear range of the film was not exceeded.

The probe for the *blood* retrotransposon was isolated by screening a genomic lambda phage library of the w^{bl} line with a probe from the second intron and exon of wild-type *white*. A positive clone was obtained, found to contain an insertion and partially sequenced to confirm that it was homologous to that described by BINGHAM and CHAPMAN (1986). An internal *blood SalI-SalI* fragment was then subcloned into pBlue ScriptII (Promega) and used to produce antisense RNA probes. Other antisense RNA probes used in this study have been described previously. The partial *white* cDNA probe, pATe1, which spans exons 3–6 and the genomic *white* subclone pBS12.5Xh-Pv (BIRCHLER *et al.* 1989), which spans part of intron 1 and exon 2 were used to analyze the *white* transcripts. *Copia* RNA was probed using the ApaI-*Hin*dIII *copia* fragment cloned into pIBI (RABINOW and BIRCHLER 1989). Plasmid pBSBT1 was used to probe the blots with *β1-tubulin* (BIALOGAN *et al.* 1985). T7 or T3 RNA polymerase (Promega) was used to incorporate [^{32}P]UTP into antisense RNA according to manufacturer's instructions.

TABLE 1

Lethality of various heteroallelic combinations of *Lip*

	Lip^D	Lip^E	Lip^F	Lip^H	Lip^K	Lip^{Dem}
Lip^D	–					
Lip^E	+	–				
Lip^F	–	–	–			
Lip^H	–	+	–	–		
Lip^K	–	+	–	–	–	
Lip^{Dem}	–	+	+	–	–	–

–, no escapers; +, heteroallelic escapers recovered. Flies carrying each of the alleles of *Lip* over a marked balancer were crossed to all other *Lip* alleles over a marked balancer. Progeny were examined for the absence of balancers indicating heteroallelic escapers. Results are based on examination of 100–200 progeny from each cross.

Pigment determination: To quantitate the amount of eye pigment in flies of the variegating allele, w^{m4h}, flies were collected from the appropriate genotype and frozen on dry ice. These frozen flies were then vortexed to removed the heads from the bodies and 40 heads per genotype were collected for each assay. The forty heads were homogenized in 0.5 ml acidified methanol (0.1% HCl), centrifuged and the supernatant used to determine the absorbance at 480 nm (EPHRUSSI and HEROLD 1944).

RESULTS

Males from a stock containing w^{bl} were gamma irradiated and mated to w^{bl} females. The F_1 progeny revealed a number of flies with lightened eye color which were crossed again to w^{bl} flies. Stocks were established from those flies whose eye color change proved heritable. A number of these mutants were found to contain second site modifiers that mapped to chromosome *3* and were homozygous lethal; therefore the lines with these mutations were balanced over *TM3, Ser*. Five of these isolates were determined to be allelic to each other by their failure to complement fully the recessive lethality and, in cases where there were rare heteroallelic homozygous escapers, their additive phenotypic effect on w^{bl} (see Table 1). A second mutagenesis attempting to mobilize marked *P* elements in flies containing the w^{bl} mutation yielded yet another fly with lightened eyes. This mutation was also due to a second site modifier and shown to be an allele of the locus found in the previous mutagenesis. However, later analysis revealed that the flies containing this mutation carried no marked *P* elements.

Because of the enhancing mutational effect on w^{bl}, we named this locus *Lighten up* (*Lip*). Alleles from the γ-irradiation have been designated *D, E, F, H* and *K*, while the one from the attempted *P* element mobilization is Lip^{Dem} (*Deus ex machina*). All *Lip* alleles are completely homozygous lethal, but some of the heteroallelic combinations give escapers at various frequencies (Table 1). These heteroallelic escapers enhance the eye color of both w^{bl} and w^a to practically bleach white (Figure 1, A and B).

Modification of specific *white* alleles by *Lip*: To determine the extent and nature of the effect of *Lip* on the

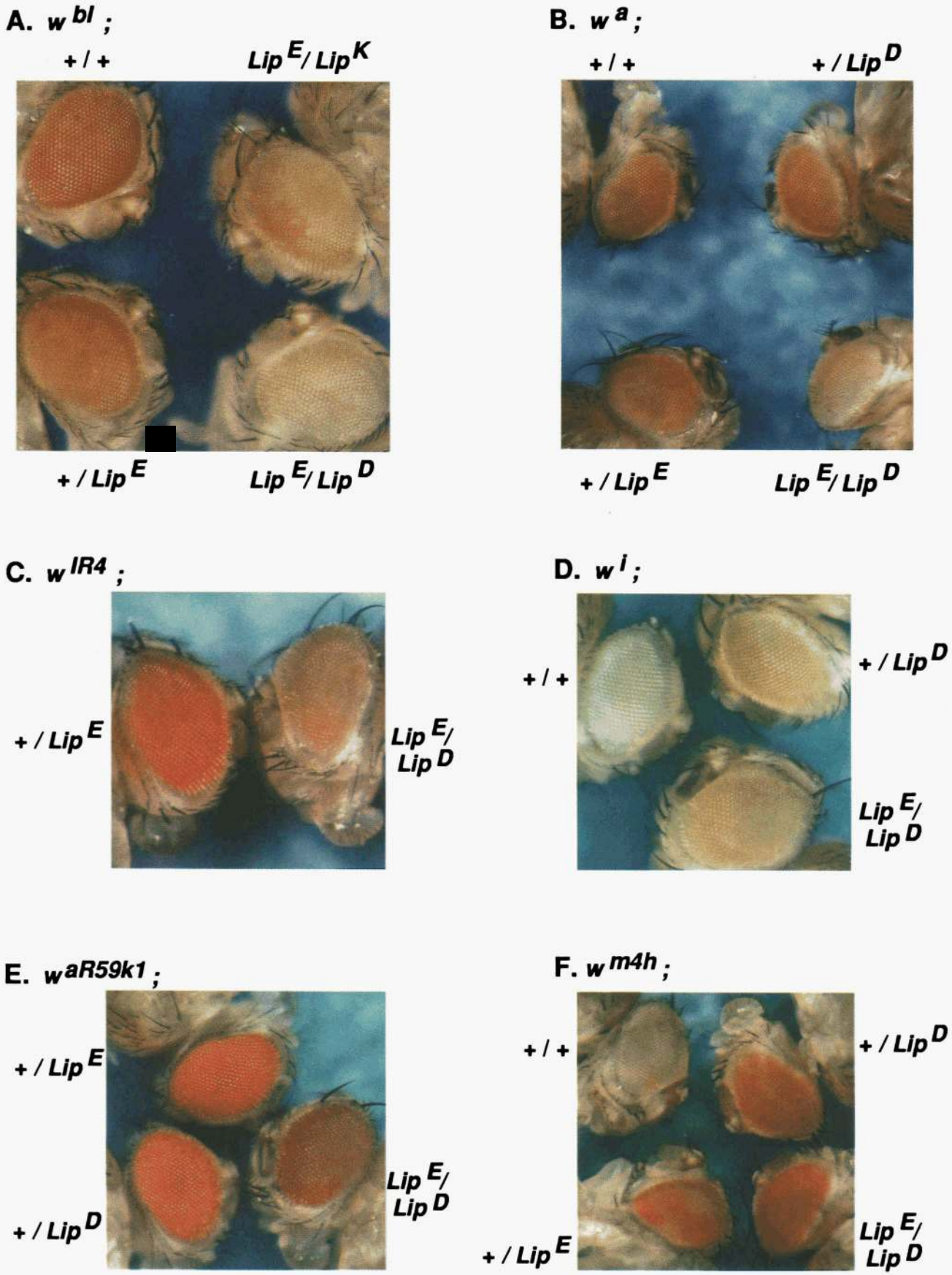

FIGURE 1.—Phenotypic effect of *Lip* on selected *white* alleles. The *Lip* genotypes are shown on each panel and the *white* allele analyzed is noted.

white locus, we examined the interaction of *Lip* with 40 alleles of *white*, including point mutations, transposable element insertions, rearrangements and an *Adh* promoter-*white* fusion construct (Table 2). These tests used both the Lip^D and Lip^E alleles and were performed by crossing females carrying the various *X*-linked *white* alleles to males that were *Lip* over a marked balancer. We then compared the eye color of the F_1 *Lip*/+ males to their Bal/+ brothers. The *white* alleles that were found to be modified by the two *Lip* alleles in the above screen were then tested in combination with the other four alleles of *Lip* to ensure that each of the alleles modified the same spectrum of *white* alleles. All of the alleles of *Lip* modified the same alleles of *white* in a similar manner.

Lip modifies none of the point mutations, indicating that this gene does not work on the *white* locus in general by simply reducing the amount of transcripts from the wild-type *white* promoter. However, *Lip* acts on a subset of the insertion mutations (Table 2). All of the alleles that *Lip* enhances result from the insertion of retroelements into the noncoding, transcribed region of the *white* gene. Three of these insertions involve long terminal repeat (LTR), retroviral-like retrotransposons, w^{bl}, w^a and *white-coral* (w^{co}). w^{bl} and w^a are caused by insertion of a *blood* (BINGHAM and CHAPMAN 1986) and *copia* (BINGHAM and JUDD 1981) retrotransposons, respectively, into the second intron of *white*. However, *copia* is inserted parallel to the direction of *white* transcription, while *blood* is antiparallel. w^{co} is caused by the antiparallel insertion of a previously undescribed 5.5-kb retrotransposon, which we have named *coral*, into the fifth intron of *white* (A. CSINK, unpublished). The other enhanced alleles all result from the insertion of the non-LTR retroposon *I* element in various locations (Figure 1c); however w^{IR2} also contains a *Doc* element (SANG *et al.* 1984; FAWCETT *et al.* 1986). In contrast to the above results, *Lip* suppresses the *white-ivory* allele (Figure 1D), which is not caused by the insertion of mobile DNA, but rather by the tandem duplication of wild-type *white* sequences from intron 1 to the start of exon 3 (KARESS and RUBIN 1982; O'HARE *et al.* 1984).

X chromosomes carrying the above *white* alleles which were found to be modified by *Lip*/+ were introduced into stocks which contained Lip^D or $Lip^E/TM3$. This allowed a test of the effect of *Lip/Lip* heteroallelic escapers on each of the modified *white* alleles. All of those alleles that were modified in the heterozygous form were more severely effected in the homozygotes (Figure 1).

We tested three revertants of w^a for interaction with *Lip* and showed all to be enhanced by *Lip* (Table 2). One of these, w^{aR59kl}, contains a solo *copia* LTR (CARBONARE and GEHRING 1985) (Figure 1E). This finding implies that, at least in the case of w^a, the internal sequences of the *copia* element are unnecessary for enhancement by *Lip* and that the sequences required for interaction with *copia* are contained within the LTR. The revertants w^{aRM} and w^{aR84h} disrupt the *copia* 5′ or 3′ LTR, respectively (MOUNT *et al.* 1988).

Effect of *Lip* on position effect variegation: The w^{m4h} chromosome contains an inversion that places the wild-type *white* gene in close proximity to *X* chromosome heterochromatin (REUTER and WOLFF 1981). This juxtaposition causes inactivation of the *white* gene in a mosaic manner, referred to as position effect variegation (PEV). Various second site modifiers have been isolated that enhance (*E(var)*) or suppress (*Su(var)*) w^{m4h}, as well as variegating alleles of other genes (LOCKE *et al.* 1988; WUSTMANN *et al.* 1989; HAYASHI *et al.* 1990). We tested the effect of *Lip* on w^{m4h} and found it to suppress PEV. The suppression of PEV by *Lip* is additive, in that the heteroallelic escapers are more strongly suppressed than the heterozygotes (Figure 1F). This suppression is not as strong as that which occurs with some of the previously described *Su(var)*s. For example, *Su(var)3-7*, *8*, *9* and *10* in the heterozygous condition give virtually wild-type eyes (REUTER *et al.* 1990), while *Lip* is more moderate in its suppression. This fact could explain why *Lip* was not recovered in previous mutagenic screens for suppressors of PEV. There are no previously identified *Su(var)*s in the region where *Lip* maps (see below) that show the characteristics of *Lip*, *i.e.*, moderate suppression and recessive lethality.

To ensure that this modification of PEV was not due to a preexisting suppressor present on the progenitor chromosome, we analyzed the third chromosome from the parental w^{bl} line for its effect on the w^{m4h}. Females from the w^{m4h} strain were crossed to males carrying the progenitor third chromosome heterozygous with the same balancer used with the *Lip* mutations. We quantitated the variegation by measuring spectrophotometrically the amount of pigment isolated from the eyes of the different genotypes. Additionally, the effect of three *Lip* alleles on w^{m4h} variegation was also measured (Table 3). While there was a small effect of the progenitor chromosome on position effect variegation, it was not nearly as large as the effect of the *Lip* alleles.To test the generality of the suppression of PEV, another variegating aberration was tested. Sb^V is an allele that is also subject to position effect variegation. However, *Sb* is a gain-of-function mutation, so variegation of heterochromatin into the *Sb* locus inactivates *Sb* and returns the phenotype to wild-type. The effect of *Lip* on Sb^V was examined by crossing females carrying three different *Lip* chromosomes over a marked balancer (*TM3, Ser*) to males carrying the Sb^V allele over a second marked balancer (*TM2, Ubx*). *Lip* was found to increase the number of mutant bristles, consistent with the interpretation that *Lip* decreases the spread of heterochromatin into euchromatin and acts as a suppressor of position effect variegation (Table 4).

TABLE 2

Effect of *Lip*/+ on alleles of *white*

Allele	Interaction	*White* locus lesion	Reference
w^a (apricot)	–	*copia* retrotransposon insertion in intron 2 (parallel)	GEHRING and PARO (1980); BINGHAM and JUDD (1981)
w^{bl} (blood)	–	*blood* retrotransposon in intron 2 (antiparallel)	ZACHAR and BINGHAM (1982); BINGHAM and CHAPMAN (1986)
w^{co} (coral)	–	*coral* retrotransposon in intron 5 (antiparallel)	A. CSINK (unpublished data)
w^{IR1}	–	*I* element insertion in intron 5 (antiparallel)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{IR2}	–	*I* element insertion revertant of w^1 (*Doc* element in 5′ untranslated leader)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{IR3}	–	*I* element insertion in intron 5 (antiparallel)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{IR4}	–	*I* element insertion in intron 5 (antiparallel)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{IR5}	–	*I* element insertion in 3′ untranslated region (parallel)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{IR6}	–	*I* element insertion in intron 1 (parallel)	SANG *et al.* (1984); FAWCETT *et al.* (1986)
w^{ric} (*roo*-in-*copia*)	–	insertion of *B104* (*roo*) into *copia*	DAVIS *et al.* (1987)
w^{aRM} (w^a revertant)	–	transposable element in *copia* 5′ LTR	MOUNT *et al.* (1988)
w^{aR84h} (w^a revertant)	–	*I* element insertion in *copia* 3′ LTR	MOUNT *et al.* (1988)
w^{aR59k1} (apricot revertant)	–	solo *copia* LTR	CARBONARE and GEHRING (1985)
w^i (ivory)	+	duplication of sequences from intron 1 to start of exon 3	COLLINS and RUBIN (1982); KARESS and RUBIN (1982); O'HARE *et al.* (1984)
w^{m4h}	+	chromosomal rearrangement which places w^+ near heterochromatin	REUTER and WOLFF (1981)
w^{a4} (apricot-4)	None	*BEL* retrotransposon in intron 2	ZACHAR and BINGHAM (1982); GOLDBERG *et al.* (1983)
w^{bf} (buff)	None	*B104* in intron 4	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1984)
w^{bf2} (buff-2)	None	*B104* in 5′ untranslated leader (antiparallel)	A. CSINK (unpublished)
w^h (honey)	None	*B104* element into *Doc* element of w^1	O'Hare *et al.* (1991)
w^{sp} (spotted)	None	*B104* retrotransposon in 5′ regulatory region	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1983, 1984)
w^{sp55} (spotted-55)	None	*mdg3* retrotransposon in 5′ untranslated leader (parallel)	ZACHAR and BINGHAM (1982); A. CSINK (unpublished data)
w^c (crimson)	None	*FB* insertion revertant of w^i	COLLINS and RUBIN (1982); KARESS and RUBIN (1982); O'HARE *et al.* (1984)
w^e (eosin)	None	Transposable element (*pogo*) reversion of w^i	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1984); HAZELRIGG (1987)
w^{apl} (apricot-like)	None	P-M hybrid dysgenic revertant of w^1	C. MCELWAIN (unpublished data)
w^{sp4} (spotted-4)	None	Deficiency in 5′ regulatory region	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1984)
w^{sp2} (spotted-2)	None	Deficiency in 5′ regulatory region	ZACHAR and BINGHAM (1982)
w^{sp81d5} (spotted-81d5)	None	Deficiency in 5′ regulatory region	DAVISON *et al.* (1985)
w^t (tinged)	None	Unknown	
w^{ec3} (ecru-3)	None	Unknown	
w^{mo} (mottled-orange)	None	Unknown	
w^{cf} (coffee)	None	Point	ZACHAR and BINGHAM (1982)
w^{a2} (apricot-2)	None	Point	ZACHAR and BINGHAM (1982)
w^{a3} (apricot-3)	None	Point	ZACHAR and BINGHAM (1982)
w^{sat} (satsuma)	None	Point	ZACHAR and BINGHAM (1982)
w^{col} (colored)	None	Point	ZACHAR and BINGHAM (1982)
w^{Bwx} (Brownex)	None	Point	ZACHAR and BINGHAM (1982)
z w^{is} (isoxanthopterinless)	None	Insertion in 5′ regulatory region	R. JONES (unpublished data)
w^{zm} (zeste-mottled)	None	*BEL* retrotransposon in intron 1	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1984)
z w^{zl} (zeste-light)	None	Derivative of w^{zm}	JUDD (1963)
z w^{zm}	None	*BEL* retrotransposon in intron 1	ZACHAR and BINGHAM (1982); O'HARE *et al.* (1984)
z w^a	None	*copia* insertion in intron 2	
z $Dp(1;1)w^{+61e19}$	None	Duplication of *white* locus sequences	GREEN 1963; GUNARATNE *et al.* (1986)
Adh-w #2	None	*Adh* promoter-*white* structural gene on chromosome *3*	BIRCHLER *et al.* (1990)

In this table, – denotes enhancement and + denotes suppression. Females carrying the various *X*-linked *white* alleles were crossed to males carrying *Lip* over a marked balancer. *Lip*/+ males were then compared to their *Bal*/+ brothers.

TABLE 3

Quantitation of suppression of position effect variegation by various *Lip* alleles

Genotype w^{m4h};	Mean absorbance 480 nm	Standard deviation	n	95% confidence interval	Ratio
+/+	0.406	0.074	3	0.084	1.58
+/*TM3, Ser*	0.257	0.052	3	0.059	
Lip^{Dem}/+	0.041	0.010	5	0.009	3.42
+/*TM3, Ser*	0.012	0.007	3	0.008	
Lip^{F}/+	0.110	0.013	4	0.012	4.40
+/*TM3, Ser*	0.025	0.004	3	0.005	
Lip^{D}/+	0.333	0.042	3	0.047	8.54
+/*TM3, Ser*	0.039	0.001	3	0.001	

w^{m4h} females were crossed to males that were Lip^{+} (from the progenitor w^{bl} stock), Lip^{Dem}, Lip^{F} or Lip^{D} all heterozygous with *TM3, Ser*. Males of the resulting two genotypes were compared. Each assay contained 40 heads. n is the number of assays performed on each genotype. The ratio is the absorbance of the *Lip*/+ genotype divided by the absorbance of the +/+ genotype. All comparisons within a cross are significant with at least 95% confidence. See MATERIALS AND METHODS for details.

TABLE 4

Effect of *Lip* on Sb^{V}

Genotype	Mean no. of Sb bristles	Standard deviation	n	95% confidence interval	Ratio
Sb^{V}/*TM3, Ser*	5.90	1.74	20	0.76	2.10
Lip^{Dem}/Sb^{V}	12.37	1.16	19	0.52	
Sb^{V}/*TM3 Ser*	6.90	2.02	20	0.89	1.71
Lip^{D}/Sb^{V}	11.82	1.78	11	1.05	
Sb^{V}/*TM3, Ser*	5.61	2.64	23	1.08	1.62
Lip^{F}/Sb^{V}	9.10	2.07	20	0.91	

Females that were Lip^{Dem}, Lip^{F} or Lip^{D} heterozygous with *TM3, Ser* were crossed to males that were Sb^{V}/*TM2, Ubx*. The sternopleural, notopleural, and humeral bristles (14 bristles total) were scored and the number of those that were stubble are shown. n is the number of flies scored. The ratio is the bristle number of the Sb^{V}/*Lip* genotype divided by the bristle number of the Sb^{V}/+ genotype. All comparisons within a cross are significant with at least 95% confidence. See MATERIALS AND METHODS for details.

It was possible that enhancement of retroelement induced mutations was a general attribute of suppressors of PEV that had not been noted previously. In order to test this, we set up four crosses with males carrying *Su(var)3-7*, *Su(var)3-8*, *Su(var)3-9* and *Su(var)3-10* (REUTER *et al.* 1990), to females containing the w^{bl} allele. We found that the w^{bl} allele was unchanged by any of the *Su(var)*s (data not shown).

Localization of the *Lip* gene: Preliminary mapping experiments indicated that *Lip* was located on the right arm of chromosome *3*. Cytological examination of the chromosomes of all six alleles revealed no shared breakpoints. More detailed mapping using Lip^{F}, which has no visible cytological rearrangements, placed the gene between *Lyra* (40.5, cytological location 70A3-5) and *Stubble* (58.2, 89B4) (Table 5). This mapping experiment (as well as the one below) used the recessive lethality of Lip^{F} as the marker. Briefly, the position of the lethal allele is determined by which recombinant class is missing, or in lowest abundance, when a recombinant chromosome is placed over *Lip*.

TABLE 5

Genetic localization of *Lip*

Progeny phenotypes	No. scored	Percen of total
$Lip^{F}/Ly\ Sb$ ♀♀ × Lip^{F}/*TM3, Ser*, ♂♂		
Ly Sb	1274	92.3
+ +	12	0.9
Ly +	58	4.2
+ *Sb*	36	2.6
$Lip^{F}/Ki\ Antp$ ♀♀ × Lip^{F}/*TM3, Ser* ♂♂		
Ki Antp	1722	99.83
+ +	2	0.12
Ki +	1	0.06
+ *Antp*	0	0.00

The progeny of the above crosses were scored after discarding the *Ser* flies that carry the third chromosome balancer *TM3*. These crosses were used to place *Lip* relative to four third chromosomal markers using the recessive lethality of *Lip*. Recombinants between the *Lip* and the marked chromosome that move *Lip* onto that chromosome will die as a *Lip* homozygote. The numbers of each of the recombinant classes will depend on the relative position and proximity of the *Lip* gene to the markers. In the top cross, *Lip* was placed between *Ly* and *Sb* based on the fact that the ++ class was the least frequent. *Lip* was placed proximal to *Ki* based on the failure to recover the + *Antp* recombinant class. Progeny testing confirmed the presumed genotype of the recombinants from the *Ki Antp* cross.

Lip^{E} is the phenotypically weakest and most viable in heteroallelic combinations with other alleles and shows sensitivity to other *Su(var)*s mentioned above. Therefore, we reasoned that Lip^{E} may contain an insertion of heterochromatin. Cytological examination of chromosome *3*R from Lip^{E} revealed that there was indeed an insertion near the cytological region 83D (Figure 2) that associated with the chromocenter. This result agreed with the preliminary mapping data and placed *Lip* quite close to *Kinked* (*Ki*). We further mapped Lip^{F} using a chromosome marked with *Ki* (47.2, 83DE) and *Antennapedia* (47.5, 84B1-2) (Table 5). After scoring 1725 flies, we recovered only one *Ki* and two wild-type recombinants. The recovery of the wild-type recombinants showed that the gene was not located between *Ki* and *Antp*, while the *Ki* recombinant demonstrated that the gene was proximal to both markers. These results concur with the cytological location of the Lip^{E} aberration. Unfortunately, this also placed it close to *Triplo-lethal* (which is also haplo lethal) and made deficiency mapping difficult. However, the fact that Lip^{E} is produced by a rearrangement involving heterochromatin implies that Lip^{E} (and by analogy the other *Lip* alleles) is a loss of function allele.

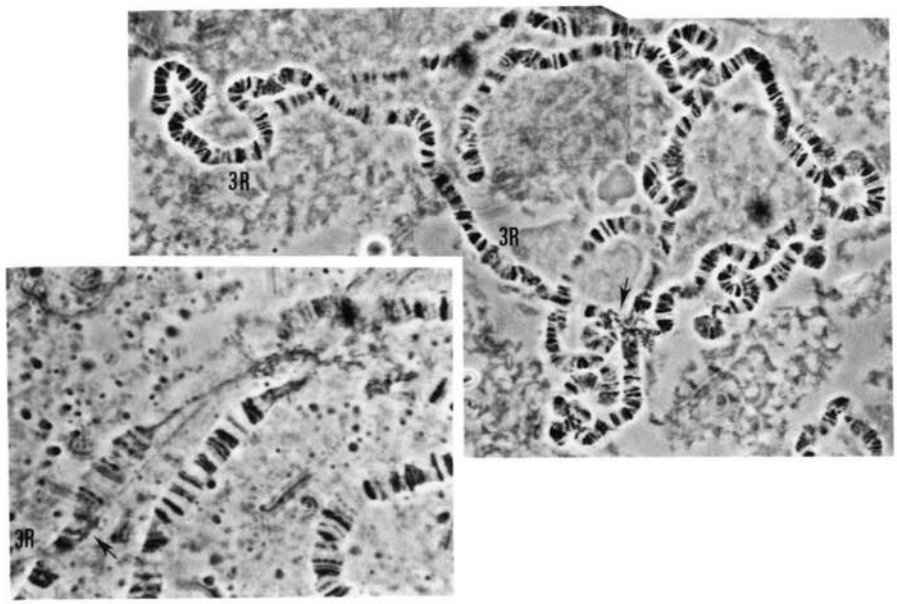

FIGURE 2.—Larval salivary gland polytene chromosome spread from $Lip^E/+$ larvae. The + chromosome is from the w^a stock. Arrows indicate the insertion at 83D. Inset shows a close-up of the affected region from a second spread.

While manipulating the Lip^E allele, it was found to segregate from second as well as third chromosome balancers, suggesting a rearrangement involving the two large autosomes. However, cytological examination (Figure 2 and other spreads not shown) revealed no rearrangements involving the euchromatin of both chromosomes *2* and *3*. Indeed, except for the insertion mentioned above, all chromosomes in Lip^E appear unchanged. This leads us to conclude that there may be a rearrangement in the centric heterochromatin of the two chromosomes which is not visible in the polytene chromosome spreads.

Effects of *Lip* on transcript levels of *white*, *copia* and *blood*: Since the mutant effect of w^a has been extensively studied, we investigated the molecular basis of *Lip* enhancement by examining the transcripts from this allele of *white*. The insertion of *copia* into the second intron of *white* results in the termination of *white* initiated transcripts in the 3′ LTR of *copia*. However, the incompleteness of this termination results in a low level of transcripts that continue to the 3′ terminus of *white*. The *copia* element is spliced out along with the remainder of intron 2, giving low levels of functional normal sized messenger RNA and hence the apricot phenotype. A diagram of *white-apricot* is shown in Figure 3, with the stable transcripts and probes used to detect them.

To analyze the effect of *Lip* on w^a, a segregating population was generated by crossing flies, homozygous on the *X* chromosome for w^a, but heterozygous for $Lip^D/+$ in one parent and $Lip^E/+$ in the other. The progeny were classified as Lip^D/Lip^E, Lip^E or $Lip^D/+$, and $+/+$. RNA was isolated from these phenotypic classes and used in northern blots to determine the effect of *Lip* on the transcript level of *white*, *copia* and *blood*.

The probe 3′ to the *copia* insertion in w^a (Figures 3 and 4A, Table 6) can detect three transcripts: (1) the wild-type sized *white* message (2.6 kb), (2) a transcript, present at low levels, initiated in the 5′ LTR of *copia*, reading through the 3′ LTR and terminating in *white* (7.9 kb) and (3) a transcript, rare in adults (not seen in these northern blots), initiated in the *copia* 3′ LTR and terminating in *white* (2.4 kb). The 2.6 kb wild-type *white* message is lowest in flies that are *Lip/Lip*, intermediate in *Lip*/+ and highest in +/+. This is consistent with the phenotypic effect of *Lip* on w^a, in that the pigment levels are correlated with the level of the full-length transcript. This result suggests that *Lip* produces a dosage effect on the RNA level of the wild-type *white* transcripts that is directly correlated with the number of functional *Lip* alleles present. The other transcript detected with the 3′ probe (7.9 kb), which is thought to initiate in the 5′ LTR does not vary in the same dosage dependent manner as the 2.6-kb message.

The probe used to detect transcripts 5′ to the *copia* insertion (Figures 3 and 4B, Table 6) in w^a is the second exon of *white*. The RNAs detected with this probe are those initiated at *white* and terminated in the 5′ (0.9 kb) and 3′ (5.8 kb) *copia* LTRs, three others that are spliced or terminated within *copia* (1.3, 2.7 and 2.9 kb) and a low level of normal sized *white* message (2.6 kb). The most abundant 5′ transcript is the 3′ LTR terminated one, which is increased only slightly in +/*Lip* and *Lip*/*Lip* flies. In contrast, the 2.9-kb message is inversely effected by the number of functional *Lip* alleles. Therefore, while *Lip* has a direct dosage effect on the 2.6-kb wild-type *white* message, it has an inverse dosage effect on the 2.9-kb message. The other transcripts seem to be unchanged by *Lip*.

We also measured the effect of *Lip* on the steady state RNA levels of the total genomic pool of *copia* and *blood* retrotransposons (Figure 4, C and D, Table 6). Total *copia* RNA is significantly elevated approximately 3-fold

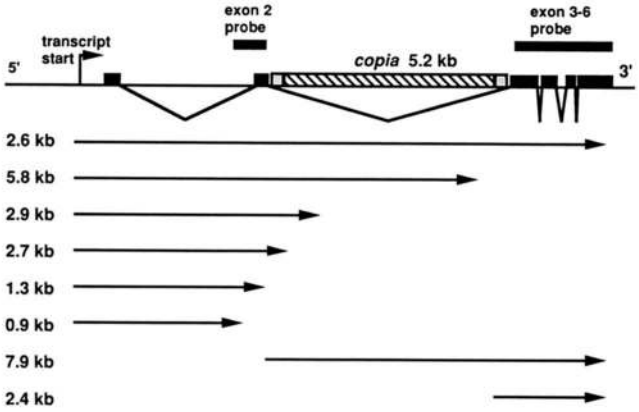

FIGURE 3.—Illustration of the w^a allele of the *white* locus and its transcripts. The arrowed lines show the origin and termination of the primary transcripts. The size is that of the processed, stable transcript. The two probes used to determine the transcripts with homology to *white* sequences 5′ and 3′ to *copia* are shown.

in the *Lip/Lip* class relative to +/+. However, the heterozygous class shows a significant reduction of approximately 2-fold in *copia* transcripts relative to the wild-type individuals (see Table 6 for 95% confidence intervals). The effect of *Lip* on *blood* transcript abundance is more linear, with the amount of *blood* RNA increasing with the decreasing number of functional *Lip* alleles.

It should be pointed out that these measurements were performed on a segregating population, which eliminates a number of potential problems that could arise when comparing the different *Lip* genotypes. Any differences in retrotransposon transcription due to variations in the original strains should be minimized by either independent assortment or, in the case of the third chromosome, recombination. As seen in Figure 2 and discussed above the Lip^E allele shows no major rearrangements and should be free to recombine along most of the third chromosome. Additionally, culturing all three phenotypic classes under the same conditions and separating them just prior to RNA collection controls for any environmental variation. Lastly, the fact that the homozygous class is heteroallelic excludes the possibility of secondarily induced modifiers being homozygous and therefore being the actual cause of the variation in retrotransposon expression. It is unlikely that a preexisting gene in the parental line is causing the variation we see in retrotransposon transcript levels, because other third chromosome modifiers isolated from the same mutagenic screen as the *Lip* alleles *D, E, F, H, K* show no effect on *copia* transcript abundance (U. BHADRA and J. A. BIRCHLER, unpublished). It is also unlikely that the altered levels of retrotransposon transcripts are due to segregation of elements of variable transcript activity closely linked to the *Lip* allele. If this were the case, the amount of transcript in the +/*Lip* flies would be intermediate between the +/+ and *Lip/Lip* flies. This is not the case for *copia* transcript abundance, which is nonadditive. The amount of blood transcript is intermediate but it seems improbable that the difference observed is due to active blood elements grouped around the *Lip* gene.

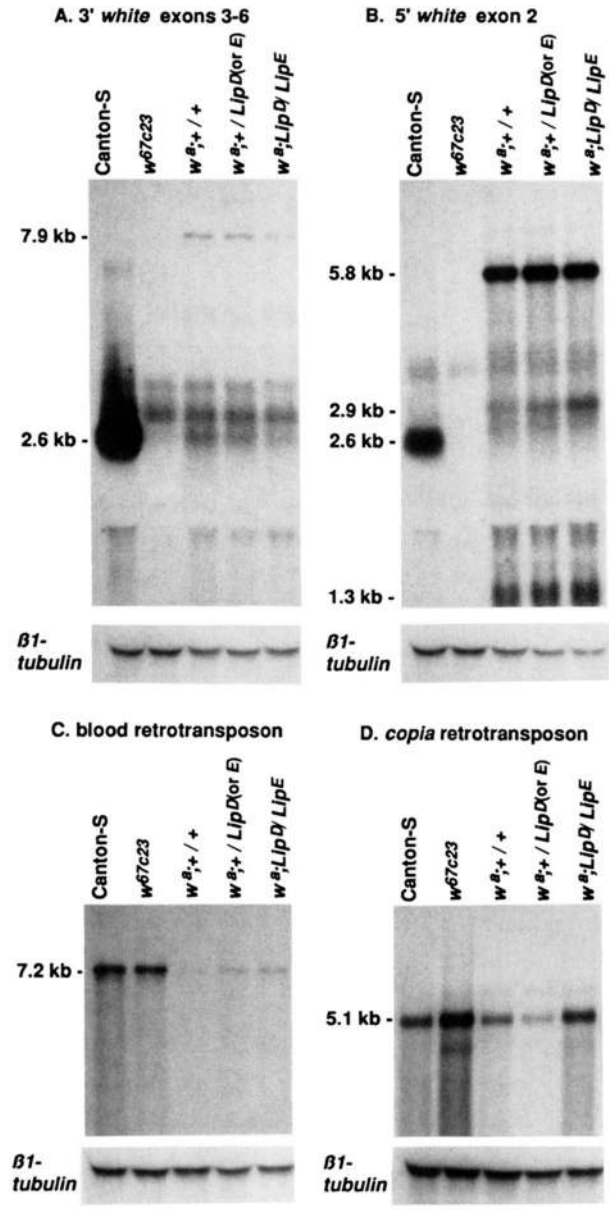

FIGURE 4.—Northern blots of total cellular RNA probed with (A) 3′ *white* (exons 3–6), (B) 5′ *white* (exon 2), (C) *blood*, (D) *copia* (see MATERIALS AND METHODS and Figure 3 for a description of probes). The bottom panel of each blot shows the reprobing with *β1-tubulin* as a loading control. Genotypes are indicated at the top of the lane. The lanes marked +/Lip^D (or ^{E}) contain a mixture of +/Lip^D and +/Lip^E. All of the flies are female. In blot B using the 5′ *white* probe, the 2.6-kb *white* transcript is not visible in the *Lip* segregating population because of the short exposure time and the fact that it is obscured by the 2.7-kb band. The transcripts common to the deficiency w^{67c23} and the other northern lanes are not due to transcription from the *white* locus.

DISCUSSION

A number of modifier genes of w^a that specifically effect insertion alleles of *white* have been described. The

TABLE 6

Quantitation of *copia*, *blood* and *white-apricot* transcripts from a segregating *Lip* population

Canton-S	*copia*	*blood*	*white* 2.6 kb (exons 3–6)
	0.331 (0.137)	2.700 (0.663)	11.037 (2.217)
w^{67c23}	1.193 (0.559)	1.958 (0.659)	
w^a; +/+	0.194 (0.054)	0.149 (0.047)	0.407 (0.003)
w^a; +/*Lip*	0.092 (0.022)	0.342 (0.025)	0.345 (0.010)
w^a; *Lip*/*Lip*	0.714 (0.098)	0.508 (0.064)	0.249 (0.011)
	white 7.9 kb (exons 3–6)	*white* 5.8 kb (exon 2)	*white* 2.9 kb (exon 2)
w^{67c23}			
w^a; +/+	0.333 (0.016)	0.250 (0.022)	0.166 (0.044)
w^a; +/*Lip*	0.414 (0.003)	0.371 (0.023)	0.312 (0.031)
w^a; *Lip*/*Lip*	0.252 (0.016)	0.379 (0.045)	0.522 (0.045)

The densities of the bands on the autoradiographs of the northern blots were determined using an LKB Ultrascan XL densitometer and the area under the peaks determined using the Pharmacia GelScanXL analysis program. Each mean is based on three replicate northern gels. The 95% confidence intervals are given in parentheses. All values were corrected for loading variation by reprobing each blot with a *β1-tubulin* probe and dividing each value by the intensity of the *β1-tubulin* hybridization in that lane. Units are arbitrary. The probe covering *white* exons 3–6 was used to detect the messages 3′ of the *white* insert as well as the wild type *white* message and the probe covering *white* exon 2 used to detect the messages 5′ of the *white* insert (see MATERIALS AND METHODS for probes).

genes which have been examined for the spectrum of *white* alleles listed in Table 2 include *mottler-of-white* (*mw*) (BIRCHLER *et al.* 1989), *E(w^a)* (BIRCHLER and HIEBERT 1989), *Darkener of apricot* (*Doa*) (RABINOW and BIRCHLER 1989), *Mosaic suppressor* (*Msu*) (CSINK *et al.* 1994) and *Lip*. Additionally, *su(w^a)* and *su(f)*, both modifiers of *w^a*, have been examined for effects on a subset of these *white* alleles (GREEN 1959). *Lip* is unique among these in a number of ways. First, *Lip* is the only one so far described that interacts with alleles that do not contain LTR-retrotransposons. All of the *I* element induced alleles in this study were enhanced by *Lip*. While the *I* element is a retroelement, it is considered a retroposon in that it contains a reverse transcriptase gene, transposes via an RNA intermediate, but is not retroviral-like in structure as are the LTR-retrotransposons. Secondly, *Lip* is the only one which effects the *copia* solo LTR revertant *w^{aR59k1}* [MOUNT *et al.* (1988) and above references]. The other four modifiers require internal *copia* sequences, or at least a larger insertion of DNA to have an effect.

Perhaps the most puzzling observation is *Lip* suppression of *w^i*. Since *w^i* is a tandem duplication from within intron 1 to within exon 3, there are no known new sequences introduced in this lesion. However, a decrease in the number of functional *Lip* alleles suppresses *w^i*, so *Lip* influences *w^i* expression differently than it does the *white* insertion alleles. There are other examples of modifier genes that show opposite effects on different alleles of *white* such as *Msu* and *Doa* which enhance *w^{sp55}* while suppressing *w^a* (RABINOW and BIRCHLER 1989; CSINK *et al.* 1994).

The last difference between the other modifiers and *Lip* is the suppression of position effect variegation. This observation implies that *Lip* has some role, however direct or indirect, in chromatin configuration. It is possible that *Lip* may have a effect on chromatin composition in the area of the *white* alleles it modifies, perhaps by interactions with the sequences contained within the element or by changing interactions between the element and the surrounding locus. Alternatively, *Lip* could be involved in the expression of multiple retroelements and produces an effect on PEV because heterochromatin is a repository of a great number of transposable elements (DEVLIN *et al.* 1990). With the reduction in the amount of the *Lip* gene product, the heterochromatic retroelements would no longer bind the same amount of *Lip* product (or a protein that *Lip* modifies), perhaps leading to a general alteration of heterochromatin. There are many dosage dependent modifiers of retroelement induced alleles (SABL and BIRCHLER 1993) and numerous dosage dependent modifiers of PEV (LOCKE *et al.* 1988; WUSTMANN *et al.* 1989; HAYASHI *et al.* 1990). The *Lip* mutation suggests an overlap between the two types of genes.

It appears unlikely that *Lip* produces its effect by modification of RNA splicing or termination. If *Lip* were influencing the transcriptional termination of the inserted elements in the modified *white* alleles, one would expect it to have different effects depending on the orientation of the inserted element. However, *Lip* has a similar effect on all of the *I* element insertions which include elements inserted both parallel and antiparallel (see Table 2). If *Lip* were influencing RNA splicing, one would not expect it to effect *w^{IR2}* or *w^{IR5}* which contain insertions into the 5′ leader and 3′ untranslated region, respectively. Therefore, the action of *Lip* probably involves other aspects of gene expression.

Lip is the third modifier gene we have found that has an effect on *copia* transcript abundance. This is by no means a general property of modifiers of *w^a* because, for example *E(w^a)* (BIRCHLER and HIEBERT 1989) and *mw* (BIRCHLER *et al.* 1989) have no effect on *copia* RNA levels. Interestingly, *copia* levels decrease 2-fold in the heterozygotes compared to the wild type while increasing 3-fold in the heteroallelic escapers. This profile is similar

to the action of *Msu* on *copia* transcript levels which show a significant decrease of 25% in the heterozygotes compared to wild-type homozygotes, but increases 3-fold in *Msu* homozygotes (CSINK *et al.* 1994). *Doa*, however, has no effect on the heterozygotes, but increases *copia* transcription 2-fold in the homozygotes (RABINOW *et al.* 1993). If one considers Lip^+ and Msu^+ alleles as modifiers of *copia* transcription then they can be considered overdominant in that the effect in the heterozygote falls outside the limits defined by the wild-type and mutant homozygotes. Interestingly, this is very similar to an effect that was observed by CSINK and MCDONALD (1990) on *copia* in some natural populations of *Drosophila melanogaster*. When two populations which had characteristically high and low transcript abundance were crossed, the F_1 had a *copia* transcript level that was lower than the low transcript line.

A situation analogous to the regulation of *copia* by *Lip* and *Msu* may be exhibited by the transcriptional regulator *Krüppel*. Low levels of the *Krüppel* protein (KR) activate expression of genes containing a KR binding site while higher KR concentrations repress the same genes (SAUER and JÄCKLE 1991). This directionally opposite regulatory effect is the result of the transcriptional activation by the KR monomer and repression by the dimer. The formation of the dimer is promoted by higher concentrations of KR (SAUER and JÄCKLE 1993). However, we note that *copia* expression is actually decreased by intermediate amounts of the *Lip* gene product. Therefore, the analogy holds for the overdominant nature of *Lip* regulation, but the direction of the effect is opposite.

The nonlinear effects of *Doa*, *Msu* and *Lip* on total *copia* transcript abundance seem to be uncorrelated with their effects on w^a, which are all additive. Indeed, *Msu* and *Lip* have opposite effects on the phenotype of w^a while having quite similar effects on *copia* transcripts. This lack of correlation between the *copia* abundance and the w^a phenotype is puzzling, but it should be pointed out that the *copia* at *white* is in a unique regulatory environment relative to the other copies of the middle repetitive element. Interactions between *copia* regulatory sequences and *white* regulatory sequences could give a regulatory profile different from that expected for *copia* elements in other parts of the genome. Additionally, there are almost certainly tissue specific regulatory differences between *white* and *copia*, which may be reflected in the total pool of *copia* transcripts.

The above discrepancy highlights the fact that there is not a simple relationship between the expression of the inserted DNA and the phenotype of the insertion alleles and supports the proposition that modifier genes are working through a variety of different mechanisms. The modifier genes from Drosophila that have been fully characterized have ranged from a transcription factor (*su(Hw)*) (PARKHURST *et al.* 1988) to a gene involved with RNA stability (*su(f)*) (MITCHELSON *et al.* 1993). Modifier genes provide a sensitive assay for identifying many loci involved in various aspects of gene expression.

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