Mosaic suppressor, a Gene in Drosophila That Modifies Retrotransposon Expression and Interacts With zeste

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ABSTRACT

A newly identified locus in Drosophila melanogaster, Mosaic suppressor (Msu), is described. This gene modifies the expression of white-apricot (w*), which is a copia retrotransposon-induced allele of the white gene. In addition to suppressing w* in a mosaic fashion, this mutation suppresses or enhances the expression of several other retrotransposon-induced white alleles. Mutations in Msu alter copia transcript abundance and may regulate the expression of several other retrotransposons. While each of the two Msu isolates is homozygous lethal, heteroallelic escapers occur at a low frequency. These escapers act not only as strong suppressors of w*, but also as a recessive enhancer of synaptic-dependent gene expression at white. The mutation described here suggests a connection between the regulation of specific transcriptional units such as retrotransposons and more global synapsis-dependent regulatory effects.

OUR laboratory is interested in dosage-sensitive modifiers of gene expression and their involvement in various phenomena such as suppression and enhancement, dosage compensation and aneuploid syndromes. In particular, we concentrate on a collection of regulatory loci that are implicated in regulating the expression of the white (w) eye color locus and the retrotransposon, copia in Drosophila melanogaster. The impetus for these studies derives from the observation that aneuploid segments produce a trans-acting dosage effect on unlinked genes (BIRCHLER 1979; BIRCHLER et al. 1990; RABINOW et al. 1991). To test whether such effects could be reduced to the action of single genes, mutageneses were conducted to test for dominant modifiers of the white-apricot (w*) allele of the white eye color locus. White-apricot is caused by the parallel insertion of the retrotransposon copia into the second intron of white (GEHRING and PARO 1980; BINGHAM and JUDD 1981). Modifiers that alter the expression of white or copia, or affect the splicing or termination of the copia element within white, will potentially alter the phenotype of w*.

A fraction of these modifiers has been described elsewhere (BIRCHLER and HIEBERT 1989; BIRCHLER et al. 1989; RABINOW and BIRCHLER 1989; RABINOW et al. 1991). In this paper we describe an unusual modifier, Mosaic suppressor (Msu), which suppresses white-apricot in a mosaic fashion with the suppression being more intense in the posterior portion of the eye. Msu alters copia transcript abundance and, since it also modifies several other retrotransposon-induced white mutants, may regulate the expression of the retrotransposons that cause those mutations as well.

In addition, Msu modifies the phenotype of the regulatory gene zeste, which encodes a DNA binding protein that alters the expression of several genes including bithorax (KAUFMAN et al. 1973), white (GANS 1953) and decapentaplegic (GELBART 1982; GELBART and Wu 1982) in a synapsis-dependent fashion. The mutation described here demonstrates a connection between the regulation of retroviral-like elements and synthesis-dependent regulatory effects.

MATERIALS AND METHODS

Fly stocks and cytology: Fly stocks were maintained on cornmeal-glucose-yeast media at 25°. The mutagenes which produced the two Msu alleles have been previously described (BIRCHLER et al. 1989; RABINOW et al. 1991). Larval salivary glands were dissected in 40% acetic acid and the polytene chromosomes stained with aceto-orcein.

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 extracted three times with sterile water, the extractions 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 μg of total cellular RNA were electrophoresed through 1.5% agarose formaldehyde gels (LEHRACH et al., 1977) and transferred to Biotrans nylon filters (ICN Inc.) according to manufacturers' instructions. Filters were prehybridized for 8 hr at 60° in 50% formamide, 5× SSC, 10× Denhardt's, 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 contin-

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ued for 20 hr at 60°C. Filters were washed in 0.2X SSC, 0.05% SDS, at 75°C 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 in Figure 6, since, in some cases, shorter exposure times were used to ensure that the linear range of the film was not exceeded.

Antisense RNA probes used in this study have been described previously. The partial white cDNA probe, pATel1, which spans exons 3–6, and the genomic white subclone pBS12.5Xh-Pv, which spans part of intron 1 and exon 2, were used to analyze the white transcripts (BIRCHLER et al. 1989). Copia RNA was probed using the Apal-HindIII copia fragment cloned into pIBI (RABINOW and BIRCHLER 1989). M. GOLDBERG provided a plasmid containing the cDNA clone of zeste (MANSURHANI et al. 1988). Plasmid pBSBT1 was used to probe the blots with βtubulin (BIAL-OGAN et al. 1985). T7 or T3 RNA polymerase (Promega) was used to incorporate 32P-UTP into antisense RNA according to manufacturers’ instructions.

RESULTS

In a chemical mutagenesis involving ethyl methane sulfonate (EMS) (BIRCHLER et al. 1989), a female fly carrying w+ on the X chromosome was found that had a mottled stripe of brilliant red at the posterior of the eye on an otherwise apricot background. The mutation producing this phenotype proved to be heritable and was located to the third chromosome. Because of the nature of the mutation it was termed, Mosaic suppressor (Msu). Years later in the course of a gamma irradiation mutagenesis (RABINOW et al. 1991), a similar mutation (Msu2) was recovered. Each of these chromosomes by themselves contains a recessive lethal but heteroallelic Msu/Msu2 flies are recovered at a frequency of approximately 4% of the expected value. This heteroallelic genotype very strongly suppresses the apricot phenotype (Figure 1a). Males and females of the genotype Msu/Msu2 are completely sterile when allowed to mate with the opposite sex from a wild-type Canton-S stock. Interestingly, male heteroallelic escapers are recovered at about 50% lower frequency than female escapers.

In addition to the suppression in the eye, testes were also examined for any effect since white is expressed greatly in that tissue as well. With both Msu and Msu2, there is an increase in pigment of the testes of males over siblings, both of which are carrying w+ on their X chromosomes (data not shown).

Modification by Msu of specific white alleles: To gain some initial information about the nature of this suppression, the two different chromosomes were tested for an effect on forty different alleles of the white locus. This collection of alleles includes various types of transposable element insertions, point mutations, promoter lesions, rearrangement alleles and an Adh promoter-white reporter construct. The rationale for this screen is that modifiers that are effective on all the structural gene lesions are implicated in affecting the white locus while those specific for transposable element insertions are believed to affect the phenotype because of an interaction with the respective elements. These tests were performed by simply crossing females carrying the various X-linked white alleles to males that were Msu or Msu2 over a marked balancer and comparing the eye color of the F, Msu/+ or Msu2/+ males to their Bal/+ brothers. The results are shown in Table 1 and the major points are summarized below.

There is a mosaic suppression of three alleles that were tested: wR, wRo and white-roo-in-copia. wRo and wR are both retrotransposon insertions in the second intron of white; the former being a copia (BINGHAM and JUDD 1981) element and the latter, BEL (ZACHAR and BINGHAM 1982; GOLDBERG et al. 1983). wR is a secondary insertion of a B104 retrotransposon into the copia in wR. In addition, there was a more subtle and nonmosaic suppression of white-buff, white-buff2 and white-honey (Figure 1b). White-buff is an insertion of a B104 retrotransposon in the fourth intron (O’HARE et al. 1984). White-honey is a reversion allele of white-one (O’HARE et al. 1991) caused by a secondary insertion of a B104 element into the Doc element of w1 in the untranslated 5′ leader of white (DRIVER et al. 1989). White-buff2 is caused by a B104 element inserted eight nucleotides 5′ to the start of white translation (A. CSINK, unpublished data). Lastly, Msu enhances the phenotype of white-spotted 55, which is an insertion of the mdg3 retrotransposon within the untranslated leader of white (ZACHAR and BINGHAM 1982; A. CSINK, unpublished data).

Also shown in Table 1 is the response of three different types of revertants of white-apricot. There is no effect on wR/3R4w, which is a solo LTR derivative of wR (CARBONARE and GEHRING 1985). The level of pigment is quite high in this revertant so the test was extended to wR/3R4w/118 females. Since w118 is a white deletion, the eye pigment is further reduced and it is easier to test for suppression. Even in this configuration, however, there is no elevation of the pigment level to that characteristic of homozygous females, indicating the lack of interaction between Msu and the wR/3R4w copia LTR. This result suggests that the sequences required for an effect of Msu are present in the body of the copia element rather than in the LTR. A second revertant is wR/3R6, which is an insertion allele in the 3′ LTR of copia. This allele is suppressed in the wR/3R6/118 configuration. The last revertant tested was wR/3R6, an insertion allele in the 5′ LTR of copia (MOUNT et al. 1988). Interestingly, this wR revertant is enhanced by Msu alleles.

To examine the allele specificity further, each of the responsive alleles was tested with the mutant combination Msu/Msu2 by first producing a stock of the white allele with the respective modifier allele balanced
FIGURE 1.—Flies of the following genotypes (from top to bottom, left to right). (A) (top left) $w^+$; +/+ , (top right) $w^+$; +/?Msu, (bottom left) $w^+$; +/?Msu², (bottom right) $w^+$; Msu/Msu². (B) (top left) $w^{N}$; +/+ , (top right) $w^{N}$; +/?Msu, (bottom left) $w^{N}$; +/?Msu², (bottom right) $w^{N}$; Msu/Msu². (C) male (left) $z^{l}$ Dp(1;1)w^{neb'/1}; +/+ , (right) $z^{l}$ Dp(1;1)w^{neb'/1}; Msu/Msu². (D) male (left) $w^+$; +/?Msu², (right) $w^+$; +/+/?Msu². (E) male (left) $z^{l}$ Dp(1;1)w^{neb'/1}; +/?Msu², (right) $z^{l}$ Dp(1;1)w^{neb'/1}; +/?Msu²/Msu. (F) females (left) $z^{l}$ Dp(1;1)w^{neb'/1}; +/+Msu², (right) $z^{l}$ Dp(1;1)w^{neb'/1}; +/?Msu²/Msu. (G) female (top left) $w^{neb'/1}$; +/+ , (top right) $w^{neb'/1}$; +/+Msu, (bottom left) $w^{neb'/1}$; +/+Msu², (bottom right) $w^{neb'/1}$; +/?Msu²/Msu.
### Table 1

**Effect of Msu on selected alleles of the white gene**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Interaction</th>
<th>White locus lesion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>w&lt;sup&gt;a&lt;/sup&gt; (apricot)</td>
<td>+</td>
<td>copia retrotransposon insertion in second intron</td>
<td>GEHRING and Paro (1980); Bingham and Judd (1981)</td>
</tr>
<tr>
<td>w&lt;sup&gt;a&lt;/sup&gt; (apricot-4)</td>
<td>+</td>
<td>BEL retrotransposon in intron 2</td>
<td>ZACHAR and Bingham (1982); Goldberg, Paro and GeHRING (1983)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (woo-in-copia)</td>
<td>+</td>
<td>Insertion of B104 (woo) into copia</td>
<td>DAVIS, SHEN and JUDD (1987)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (buff)</td>
<td>* (non-mosaic)</td>
<td>B104 in intron 4</td>
<td>ZACHAR and Bingham (1982); O'HARE et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (buff-2)</td>
<td>* (non-mosaic)</td>
<td>B104 in 5&lt;sup&gt;’&lt;/sup&gt; leader</td>
<td>A. CSINK (unpublished data)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (honey)</td>
<td>* (non-mosaic)</td>
<td>mdg3 retrotransposon insertion in 5&lt;sup&gt;’&lt;/sup&gt;</td>
<td>ZACHAR and Bingham (1982); A. CSINK (unpublished data)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (spotted-55)</td>
<td>-</td>
<td>untranscribed leader</td>
<td></td>
</tr>
<tr>
<td>w&lt;sup&gt;ab&lt;/sup&gt; (apricot revertant)</td>
<td>-</td>
<td>Transposable element in copia 5&lt;sup&gt;’&lt;/sup&gt; LTR</td>
<td>MOUNT, GREEN and Rubin (1988)</td>
</tr>
<tr>
<td>w&lt;sup&gt;ab&lt;/sup&gt; (apricot revertant)</td>
<td>+ (non-mosaic)</td>
<td>I element insertion in copia 5&lt;sup&gt;’&lt;/sup&gt; LTR</td>
<td>MOUNT, GREEN and Rubin (1988)</td>
</tr>
<tr>
<td>w&lt;sup&gt;ab&lt;/sup&gt; (spotted-55)</td>
<td>None</td>
<td>Solo copia LTR</td>
<td>CARBONARE and GeHRING (1985)</td>
</tr>
<tr>
<td>w&lt;sup&gt;b&lt;/sup&gt; (crimson)</td>
<td>None</td>
<td>FB insertion revertant of w&lt;sup&gt;’&lt;/sup&gt;</td>
<td>ZACHAR and Bingham (1982); O'HARE et al. (1983, 1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (spotted-4)</td>
<td>None</td>
<td>Deficiency in 5&lt;sup&gt;’&lt;/sup&gt; regulatory region</td>
<td>ZACHAR and Bingham (1982); O'HARE et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (spotted-2)</td>
<td>None</td>
<td>Deficiency in 5&lt;sup&gt;’&lt;/sup&gt; regulatory region</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;de&lt;/sup&gt; (spotted-81d5)</td>
<td>None</td>
<td>Deficiency in 5&lt;sup&gt;’&lt;/sup&gt; regulatory region</td>
<td>DAVISON et al. (1985)</td>
</tr>
<tr>
<td>w&lt;sup&gt;c&lt;/sup&gt; (coral)</td>
<td>None</td>
<td>coral retrotransposon insertion in intron 3</td>
<td>A. CSINK (unpublished data)</td>
</tr>
<tr>
<td>w&lt;sup&gt;c&lt;/sup&gt; (tinged)</td>
<td>None</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>w&lt;sup&gt;c&lt;/sup&gt; (ecru-3)</td>
<td>None</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>w&lt;sup&gt;c&lt;/sup&gt; (mottled-orange)</td>
<td>None</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>w&lt;sup&gt;c&lt;/sup&gt; (coffee)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (apricot-2)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (apricot-3)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (satuma)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (colored)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (Brownex)</td>
<td>None</td>
<td>Point</td>
<td>ZACHAR and Bingham (1982); BINGHAM and CHAPMAN (1986)</td>
</tr>
<tr>
<td>w&lt;sup&gt;d&lt;/sup&gt; (blood)</td>
<td>None</td>
<td>blood retrotransposon insertion in intron 2</td>
<td>ZACHAR and Bingham (1982); BINGHAM and CHAPMAN (1986)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e&lt;/sup&gt; (eixin)</td>
<td>None</td>
<td>Transposable element reversion of w’ (Doc element)</td>
<td>ZACHAR and Bingham (1982); O’HARE et al. (1984); HAZELRIGG (1987)</td>
</tr>
<tr>
<td>w&lt;sup&gt;ef&lt;/sup&gt; (apricot-like)</td>
<td>None</td>
<td>P-M hybrid dysgenic revertant of w’ (Doc element)</td>
<td>C. McELWAIN (unpublished data)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e1&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e2&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion revertant of w’ (Doc element)</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e3&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e4&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e5&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;e6&lt;/sup&gt;</td>
<td>None</td>
<td>I element insertion</td>
<td>SANG et al. (1984)</td>
</tr>
<tr>
<td>z w&lt;sup&gt;b&lt;/sup&gt;</td>
<td>None</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>(isoxanthopterinless)</td>
<td>None</td>
<td>BEL retrotransposon insertion in intron 1</td>
<td>ZACHAR and Bingham (1982); O’HARE et al. (1984)</td>
</tr>
<tr>
<td>w&lt;sup&gt;mm&lt;/sup&gt; (zeste-mottled)</td>
<td>None</td>
<td>BEL retrotransposon insertion in intron 1</td>
<td>ZACHAR and Bingham (1982); O’HARE et al. (1984)</td>
</tr>
<tr>
<td>z w&lt;sup&gt;mm&lt;/sup&gt; (zeste-light)</td>
<td>None</td>
<td>Derivative of w&lt;sup&gt;mm&lt;/sup&gt;</td>
<td>JUDD (1965)</td>
</tr>
<tr>
<td>z w&lt;sup&gt;mm&lt;/sup&gt; (Haploid)</td>
<td>None</td>
<td>BEL retrotransposon insertion in intron 1</td>
<td>ZACHAR and Bingham (1982); O’HARE et al. (1984)</td>
</tr>
<tr>
<td>z Dp(1;1) w&lt;sup&gt;mm&lt;/sup&gt;</td>
<td>None</td>
<td>copia insertion in intron 2</td>
<td>GREEN (1965); GUNARATNE et al. (1986)</td>
</tr>
<tr>
<td>Adh-w #2</td>
<td>None</td>
<td>Duplication of white locus sequences</td>
<td>BIRCHLER et al. (1990)</td>
</tr>
</tbody>
</table>

Except where noted, + denotes mosaic suppression in+/Msu, * denotes Msu/+ suppression and Msu/Msu<sup>2</sup> enhancement, – denotes enhancement.
on the third chromosome. In addition to the responsive alleles, two others were included (white-zeste-mottled and white-zeste-light). They are caused by BEL insertion, as is \( w^{+} \), in the first large intron of white (O’HARE et al. 1984) but are phenotypically dark and might not have been recognized as being affected by the heterozygous Msu or Msu\(^2\). The Msu/Msu\(^2\) combination strongly suppresses white-apricot, apricot\(^4\) and ric, while further enhancing \( w^{+} \). In contrast, this combination strongly enhanced the phenotype of white-buff, buff2 and honey, producing a bleach white color even though they were suppressed by the heterozygotes (Figure 1b).

**Msu enhancement of the zeste effect:** The effect of the zeste gene on white is an example of pairing dependent regulation. A recessive, X-linked, gain-of-function mutation in the zeste locus, zeste-one, gives \( w^{+} \) females with bright yellow-orange eyes. However, the males carrying the \( z' \) allele have wild-type eyes. The mutant effect can be seen in males if they carry a tandem duplication of \( w^{+} \). Therefore, two copies of \( w \) in close association are required for the action of \( z' \) (JACK and JUDD 1979).

As noted above, we tested two other alleles, white-zeste-light and white-zeste-mottled. Alone their phenotype is basically wild type, but in the presence of \( z' \) these \( w \) alleles produce a mottled phenotype. \( w^{+} \) was tested without \( z' \) in the genotype while the chromosome with \( w^{dl} \) had \( z' \). Interestingly, while there is no observable effect in the heterozygotes of Msu, the heteroallelic combination produced white eyes with the \( z' \) \( w^{dl} \) chromosome but had no effect on \( w^{+} \). This result could be interpreted in at least two ways. Either the Msu mutation is a recessive enhancer of \( z' \), or a specific enhancer of the \( w^{dl} \) allele, perhaps with the requirement for \( z' \).

To test these possibilities, several X chromosomes were substituted into the balanced Msu and Msu\(^2\) stocks. These included zeste\(^1\) Dp(1;1) \( w^{61c*} \) (GREEN 1963), zeste\(^2\), and Canton-S wild type. The respective X chromosome stocks with opposite alleles of Msu were crossed together and the heteroallelic class scored for an effect on eye color. The zeste-white duplication stock is enhanced in both sexes. The zeste stock alone is enhanced in females and has no effect in males. The Canton S X chromosome is normal in eye color. The results of these experiments indicate that Msu interacts with the zeste effect in the above test as opposed to the retroelement insertion (Figure 1c).

The interaction with the zeste phenomenon was examined further. BINGHAM and ZACHAR (1985) characterized an allele of white, referred to as Dominant zeste like (w\(^{dzl}\)), which produces a mutant phenotype as a heterozygote in females and a much less mutant phenotype in hemizygous males. This allele was incorporated into a balanced stock of Msu\(^2\). The new stock was then crossed by the Canton S, Msu stock. In heterozygous \( w^{dzl}/+ \) (Canton S) females, the Msu/Msu\(^2\) genotype enhances \( w^{dzl} \), while the other three genotypes (Msu/+, Msu\(^2\)/+ and +/+ ) appear identical (Figure 1g). The males that were Msu/Msu\(^2\) were not enhanced.

To test whether the \( z \) mutation could be made dominant by the enhancing effect of Msu, females were produced that were heterozygous for Canton S wild type and the \( z \) mutation in the presence of Msu/Msu\(^2\). The \( z/+; \) Msu/Msu\(^2\) did not appear to be any different than the other classes, indicating that the enhancement effect does not produce a dominant effect.

**Localization of the Msu gene:** Attempts to map the Msu\(^2\) allele against two different multiply marked third chromosomes suggested that the chromosome carried a rearrangement in the right arm and that the mutation was associated with it. Use of the Msu chromosome in recombination experiments also indicated the presence of a rearranged chromosome. Cytological examination of polytene chromosomes from both Msu alleles revealed a number of breakpoints on the third chromosome (Figure 2). We found inversions with breakpoints near the centric heterochromatin and 98F in both alleles, although there were other breaks in the third chromosome unique to each allele.

To examine the nature of the Msu mutations at the 98F breakpoint, we performed two crosses using a Y-autosome translocation line (T(Y;3)R128) to produce a partial trisomic from 97F to the end of 3R (LINDSLEY et al. 1972). The first cross (Figure 3) produced a partial trisomic that contained Msu and two wild-type copies of the 98F region. This genotype no longer suppressed \( w^{+} \), although, of course, the heterozygous euploid did (Figure 1d). The second cross (Figure 4) was designed to determine whether this region had...
any effect on the recessive enhancement of *zeste*. *Msu*/*Msu*²/+ males from this cross do not enhance *zeste* (Figure 1e). The females from this same cross provide a useful control in the following way. Jack and Judd (1979) showed that in females containing *w*°/z *Dp(1;1)w°*, *zeste* became slightly dominant and the females had mottled orange eyes. *Msu*/+ females show slight motting and the *Msu/Msu*² females, a greater amount, demonstrating that *Msu/Msu*² enhances the *zeste* effect under these circumstances also (Figure 1f). However, the females in our cross did not display the *zeste* phenotype as strongly as those in the earlier study probably because one of the *X* chromosomes in our cross was inverted, therefore inhibiting pairing among the three *white* genes. These results suggest that the suppression of *w*° and enhancement of *z* are due to loss-of-function alleles at *Msu* and are consistent with the common 98F breakpoint in the two alleles being the responsible lesion for both mutational effects.

We also localized the *Msu* gene by deficiency mapping using *Df(3R)3450* (98E3;99A6-8). Females that were *w*°; *Msu*²/TM3, *Sb*, *Ser* × *w*°/Y; *Msu*²/Df(3R)3450 progeny were found to suppress *w*° to almost wild type. Additionally, we crossed *z* *Dp(1;1)w°*61c19; *Msu*²/TM3, Sb Ser females to the above deficiency males and obtained white eyed flies of the genotype *z* *Dp(1;1)w°*61c19/Y; *Msu*²/Df(3R)3450. This result shows that *Msu*²/Df(3R)3450 enhances *z* in the same manner as the *Msu*²/Msu heteroallelic escapers. Because the breakpoint involved with *Msu* is extremely close if not coincident with the *Darkener of apricot* locus (Rabinow and Birchler 1989), experiments were performed to test whether *Msu* is an allele of *Doa*. The first distinction between the two mutations is in the allele specificity at *white*. *Doa* suppresses *w*° and enhances *w°*₅₃ and has no effect on any other *white* allele. As noted above, *Msu* effects a broader range of alleles. Second, when *Msu* and *Msu*² are crossed to the *Doa* alleles, *hd1* and *dem*, the degree of
suppression of is not to the level of wild type as is characteristic of _Msu_ and _Doa_ homozygous escapers (Rabinow and Birchler 1989). The two combinations of mutations were also fully complementary with regard to viability in contrast to _Msu/ Msu<sup>2</sup> or _Doa<sup>at1</sup>/ _Doa<sup>atm</sup>_. To test for complementation of the enhancement of _zeste_, the _Msu_ and _Msu<sup>2</sup>_ stocks carrying the X chromosome with _zeste_ and the _white_ duplication, were crossed by the two _Doa_ alleles and the male progeny scored for the enhancement of _zeste_ in the _Msu/Doa_ or _Msu<sup>2</sup>/Doa_ classes. None of the heterozygous combinations exhibited a _zeste_ enhancement. Therefore, by these genetic criteria, _Msu_ and _Doa_ are discrete complementation groups, although these tests do not rule out a molecular relationship.

**Attempted isolation of _Msu_ revertants:** Given the mosaic nature of the phenotype of _Msu_, attempts to revert the mutations were performed to test whether the original alleles were gain-of-function. The first attempt was by gamma irradiation, which generated several isolates that were reduced in pigment but only one which returned _Msu_ to the normal apricot phenotype and none that reverted _Msu<sup>2</sup>_. Subsequently, chemical mutagenesis was conducted with ethyl methane sulfonate (EMS) and a potential revertant of _Msu<sup>2</sup>_ was recovered. Further analysis of these presumptive revertants revealed that a second site suppressor (not an _Msu_ revertant) had been induced in both cases, that was present in the left arm of chromosome 3 and that could be recombined away. Since the _Msu_ breakpoints for both the alleles were near the centric heterochromatin, we tested these second site mutations for suppression of position effect variegation (PEV) by combining them with _white-mottled-4h_. This _white_ allele is caused by a rearrangement of the X chromosome that moves a wild-type _white_ gene near heterochromatin and leads to a patchy _white_ inactivation. We found that these second site suppressors of _Msu_ were indeed suppressors of PEV. Thus, the basis of this suppression appears to be due to the reversal of position effect at the _Msu_ locus and _Msu_ may be subject to modification by other modifiers of PEV.

**Effects of _Msu_ on transcript levels of _white_, _zeste_ and _copia_:** We investigated the molecular basis of the action of _Msu_ by examining the transcripts emanating from _w<sup>*</sup>_, which is caused by a parallel insertion of the retrotransposon, _copia_, into the second intron of _white_ (Levis et al. 1982). The mutant effect is believed to occur when _white_ initiated transcripts terminate in the second LTR of _copia_. However, this termination is not absolute, resulting 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, resulting in a low level of functional normal sized messenger RNA. A diagram of _white-apriot_ is shown in Figure 5, with the stable transcripts (described in

![Figure 5](https://example.com/f5.png)

**Figure 5.** Illustration of the _w<sup>*</sup>_ allele of the _white_ locus and the transcripts it produces. The two probes used to determine the _5'_ and _3'_ originating transcripts are shown.

**Zachar et al. 1985; Mount et al. 1988; Hiebert and Birchler 1992** and probes used in this study noted.

To analyze the effect of _Msu_ on _w<sup>*</sup>_, a segregating population was generated by crossing flies, homozygous on the X chromosome for _w<sup>*</sup>_ but heterozygous for _Msu/+_ in one parent and _Msu<sup>2</sup>/+ in the other. The progeny were classified as _Msu/Msu<sup>2</sup>_, _Msu_ or _Msu<sup>2</sup>/+ and +/+_. RNA was isolated from each progeny class and used in Northern blots that were probed with _white_ gene probes _5'_ and _3'_ to the _copia_ element in _white_, followed by a loading control using β1-tubulin. The Northern blots are shown in Figure 6, a and b. The probe _3'_ (exon 4–6, Figure 5) to _copia_ can detect three transcripts: (1) the normal 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 _5' LTR_ of the _copia_ element and terminating in _white_ (2.4 kb). The two transcripts detected here are in greatest abundance in _Msu/Msu<sup>2</sup>_ flies, intermediate in heterozygotes and lowest in +/+ (Figure 6a, Table 2). This is consistent with the phenotypic effect of _Msu_ on _w<sup>*</sup>_, in that the pigment levels are correlated with the level of the 2.6-kb transcript. This result suggests that _Msu_ produces a dosage effect on the RNA level of these _white_ transcripts that is inversely correlated with the number of functional _Msu_ alleles present.

The _5'_ probe used involves the second exon of _white_ (Figure 5). The RNAs detected with this probe are those initiated at _white_ and terminated in the _5' LTR_ (0.9 kb) and _3' LTR_ (5.8 kb). Three other transcripts are spliced or terminated within _copia_ (1.3 kb, 2.7 kb and 2.9 kb) and a low level of normal sized _white_ message (2.6 kb) is also detected. The most abundant of these RNA's is the _3' LTR_ terminated species, which is reduced slightly in the _Msu/Msu<sup>2</sup>_ males, but which is otherwise unaffected (Figure 6b, Table 2). These observations suggest that _Msu_ has little or no
modulating effect on the *white* promoter, but is acting via the *copia* element in the second intron.

To examine further whether there is evidence for the modulation of *copia*, total *copia* RNA was estimated in the above genotypes. Indeed, total *copia* RNA is elevated 2–3 fold in the *Msu/Msu* class relative to +/+ . However, the heterozygous class shows a slight reduction (20–35%) relative to the wild-type individuals (Figure 6c, Tables 2 and 3). This is slightly different than the predicted effect based on the response of the *copia* at *white*, but has been observed in four replicate gels (Table 3). The basis of this discrepancy between the *copia* at *white* and the total population is not known. We do note, however, that there is also a nonadditive heterozygous effect on the phenotypic level with the *white* alleles *buff*, *buff2* and *honey* albeit in the opposite direction.

It should be pointed out that these measurements were performed on segregating populations. Therefore, any differences in retrotransposon transcription

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**Figure 6.**—Northern blots of total cellular RNA probed with (A) 3’ *white* (exon 4-6), (B) 5’ *white* (exon 2), (C) *copia*, (D) *zeste* (see MATERIALS AND METHODS for 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 *Msu*+/+ contain a mixture of *Msu*/+ and *Msu*2+/+. The Canton-S flies were female, the *w67cz3* (a *white* deletion mutant) were males, the first three lanes from the *Msu* segregating population were females and the last three male. In blot B using the 5’ *white* probe the 2.6-kb *white* transcript is not visible in the *Msu* 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 *w67cz3* and the other Northern lanes are not due to transcription from the *white* locus.
due to variation in the original strains would be minimized by either independent assortment or, in the case of the third chromosome, recombination. Indeed, the original line used in the mutagenesis that produced Msu was the w̄ stock, which provided the wild-type chromosome in the segregating population. On the other hand, the Msu chromosome was derived from a different line and contains 3R inversions, so recombination cannot homogenize this chromosome arm. However, we do not think this fact compromises the results for the following reasons. If the differences between copia transcription in the +/- and Msu/Msu² flies were due to variation in the activity of copia elements on 3R (i.e., the Msu chromosome contained especially active elements) then the expected copia

transcript abundance in the Msu/+ should be intermediate between the two homozygous classes. It is also possible that the parental chromosome on which Msu was induced contained a trans-acting modifier of copia. However, such a hypothetical gene would not be homozygous in the heteroallelic combination (Msu/Msu²). Half of the flies in the Msu or Msu²/+ class would be of the same chromosome composition as the heteroallelic combination. Therefore, once again one would expect the heterozygous flies to be intermediate between the homozygotes. This is not the case. Secondarily induced or naturally occurring modifiers would not be homozygous in the Msu/Msu² combination. Therefore, we believe that the observed differences are due to the Msu mutation, acting directly or indirectly.

Since Msu is a recessive enhancer of zeste we tested for an effect of Msu on zeste RNA abundance. We found a slight (25–35%) decrease of zeste RNA in Msu/Msu² flies relative to Msu/0+, but no major or significant change (Figure 6d, Table 2). Additionally, we tested for an effect of Msu on copia transcript abundance in 1–2 day pupae, but again found no difference between Msu/+ and Msu/Msu² genotypes (data not shown).

**DISCUSSION**

Modifier genes can act upon their target loci in a great variety of ways. We have shown here that Msu interacts with the copia retrotransposon present in w̄. In addition, it interacts only with white alleles containing the copia, BEL, B104 and med3 retrotransposons, although some of these alleles contain additional insertions (the I and Doc retroposons). Other retrotransposon insertion alleles such as w⁸ and w⁸ were tested, but were unaffected by Msu. Therefore, Msu is not a general modifier of retrotransposon insertions. Because the insertion sites of these elements are in different locations within the gene (except that none are found in the protein coding region, but all interrupt the primary transcript), it is unlikely that Msu is specific for the affected alleles due to their insertion site. Instead, Msu may interact with these five retrotransposons through a common regulatory mechanism.

Msu acts upon w̄ in a dosage dependent manner, as has been shown for other modifiers of w̄, with the amount of suppression decreasing with the number of functional Msu alleles. However, it does not act on the total abundance of copia RNA in the same manner. The Msu/+/ flies actually have slightly less copia transcripts than +/- flies whereas those transcripts from the Msu/Msu² flies are greatly increased. Indeed, transcripts originating in the copia within the w̄ allele seem to be behaving differently than the total pool of copia RNA in adults. We observe this in the 5' LTR initiated copia-white fusion message from w̄ (Figures
5 and 6a) which is affected in a dosage dependent manner. This discrepancy could indicate that there is an interaction among the \( w^c \) copia, Msu and the surrounding white gene. Interestingly, the effect of Msu on the white alleles buff, buff-2 and honey follows the pattern of copia levels, in that Msu/+ has the directionally opposite effect of Msu/Msu\(^+\), i.e., Msu/+ suppresses these alleles, but Msu/Msu\(^-\) enhances them both relative to +/+.

It is intriguing that Msu, a specific regulator of retrotransposon expression, is also involved in chromosomal pairing "dependent gene expression", as shown by its recessive enhancement of zeste. It has been shown that the zeste protein can serve as a transcriptional activator of the Ubx gene in vitro (Biggin et al. 1988), but its role in vivo is uncertain since flies deleted for the entire zeste gene are Ubx\(^-\) (Goldberg et al. 1989). It is difficult to determine whether the small decrease in zeste RNA abundance in adult Msu/Msu\(^-\) flies is relevant to the enhancement of zeste. While the decrease is consistent in males and females (as is the enhancement of zeste), it is not of the magnitude one would expect given the extreme effect of Msu on the zeste phenotype. Nor do we see a change in zeste RNA in pupae. However, it is quite possible that zeste RNA is more strongly affected by Msu in a subset of the tissues of the fly relevant to eye color, but does not dramatically alter the zeste RNA abundance in the majority of other tissues. In any case, it is uncertain how a change in the amount of product from the \( z^1 \) mutation, a recessive gain-of-function gene, would effect the phenotype. The observation that \( z^2//z^2 \) flies are zeste (unenhanced) (Gans 1953; Jack and Judd 1979) suggests that a decrease of zeste RNA by half would have no effect on the zeste phenotype. Indeed, deletion of the entire zeste locus has little effect on eye color (Goldberg et al. 1989). The mechanism of enhancement of the zeste phenotype will require further study.

The fact that Msu modifies \( w^{DZL} \) without the presence of the \( z^1 \) mutation indicates that it is not merely interacting with \( z^1 \), but rather may play its own role in transvection. It is possible that the role of Msu in transvection involves interaction with the \( z^1 \) product. However, it is not simply an equivalent of zeste\(^1\), since it has no effect on wild type white as does \( z^1 \). A number of other modifiers of zeste have been previously described including Enhancer of zeste, Suppressor of zeste 2 and Sex comb on midleg (Kalisch and Rasmuson 1974; Persson 1976; Wu et al. 1989; Jones and Gelbart 1990). Interestingly, these loci produce other phenotypic effects that include them in the Polycomb-group of homeotic genes. However, all of these modifiers of zeste exhibit a dominant effect and Msu is therefore the first locus found that is a recessive enhancer of zeste.

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