A Sex-Influenced Modifier in Drosophila That Affects a Broad Spectrum of Target Loci Including the Histone Repeats

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ABSTRACT

A second chromosomal trans-acting modifier, Lightener of white (Low), modulates the phenotypic expression of various alleles of the white eye color gene. This modifier has an unusually broad spectrum of effects associated with changes in chromosomal dosage, such as autosomal dosage compensation (BIRCHLER 1979, 1981; DEVLIN et al. 1982; BIRCHLER et al. 1989, 1990), aneuploid syndromes (BIRCHLER and NEWTON 1981; GUO and BIRCHLER 1994) and genetic suppression and enhancement (RABINOW and BIRCHLER 1989; CSINK et al. 1994a,b; BHADRA and BIRCHLER 1996; BHADRA et al. 1997).

Mechanistically, the binding of different trans-acting factors to regulatory sites is influenced by the local chromatin structure, that is, the DNA and histone packaging. In Drosophila, a tandem repeat of genes encoding five somatic histone proteins is located at 39D2-3 to 39E1-2 on the salivary gland chromosome map. Flies that are deficient for 39D-E die as embryos, yet flies that are haplo-deficient for the segment are viable and fertile, and show no phenotypic alteration (MOORE et al. 1983). Differential expression of histone genes is observed in other organisms (for review see ZLATANOVA and VAN HOLDE 1992; BOUVET et al. 1994; KANDOLF 1994; SHEN and GOROVSKY 1996). In yeast, an alteration of core histones in vivo affects the expression of many genes either positively or negatively. The histone H4 N-terminal end affects both gene activation and repression. Mutations in the H4 and H3 N-termini also exhibit promoter-specific effects, activating the expression of some genes but not others (FISHER-ADAMS and GRUNSTEIN 1995; SHEN and GOROVSKY 1996). However, the molecular mechanisms for the promoter-specific effects related to specific gene activation are unknown.

Chromatin structure is also thought to affect gene expression when a chromosomal rearrangement places a monitored gene next to an abnormal euchromatin-
heterochromatin junction. This phenomenon is known as position effect variegation (PEV) (Henikoff 1981, 1994, 1996; Hayashi et al. 1990; Karpen and Spradling 1990). PEV can be suppressed by a large number of euchromatic dominant mutations as well as by an extra Y chromosome (see reviews by Eisenberg 1989; Grigliatti 1991; Reuter and Spierer 1992). Many such suppressor loci, known as Su( var) s, show dosage effects; a single dose will suppress PEV relative to the normal two doses while an extra dose in hyperploids proportionately enhances variegation (Locke et al. 1988). The results of changing histone gene dosage suggested that deletion of the histone gene complex suppresses PEV (Moore et al. 1983).

A connection between the dosage effects described above and modification of PEV is suggested by the fact that a few haplo-suppressor/triple-enhancer genes controlling gene inactivation via PEV and acting as regulators of gene expression have been identified (Dorn et al. 1993; Birchler et al. 1994; Farkas et al. 1994; Bhadra and Birchler 1996). In the present study, we describe the recovery and characterization of a transacting modifier that inversely alters the expression of the histone genes in three developmental stages and exerts direct and inverse effects on several other genes depending on the tissue and developmental stage. We also provide evidence that changes in histone expression in Drosophila melanogaster alter specific gene expression.

MATERIALS AND METHODS

Flies were grown at 25° on Drosophila culture medium of cornmeal-yeast-agar containing fungicide. For description of the genetic mutations and chromosomal rearrangements see Lindsley and Zimm (1992) and Flybase (http://morgan.harvard.edu/fb.html).

Low was induced by gamma-irradiation (4000 rad) of white blood (w+) males as previously described (Csink et al. 1984a).

Localization: Genetic: The original mutation was recombined with chromosomes carrying different dominant marker mutations to provide an initial map position, namely, [ patriarchal region. For description of the genetic mutations and chromosomal rearrangements see Lindsley and Zimm (1992) and Flybase (http://morgan.harvard.edu/fb.html)].

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The progeny of the above crosses were scored after discarding the Curly flies that carry the Sm6a balancer chromosome. Low was localized relative to three second chromosomal dominant markers using the recessive lethality of Low. Flies that are recombinant between Low and a marker chromosome that move Low onto that chromosome will die as a Low homozygote. The number in each recombinant class will depend on the relative position and the proximity of the Low gene to the markers. In the first cross, Low is located between Tft and L based on the fact that the + class was the least frequent. Low was located proximal to Bl based on the failure to recover the Tft + recombinant class in the second cross.

Cytological: A series of deficiencies that span the 37D to 40B regions were tested for Low (Figure 1). Virgins of the balanced stock w+/w+; Low/In(2LR) Gla were crossed with males of each deficiency stock separately. All progeny males containing the balancer and having a modified eye color distinct from the normal white-bloom would indicate no complementation between the deficiency and Low. If the deficiency does not span the location of Low, both balancer and non-balancer males were recovered as a result of complementation. Two of the seven crosses produced only Curly and Glazed flies as a result of noncomplementation (Figure 1), indicating that Low is located within the 39E7-31 cytotical region. The recovery of Glazed males and females from each cross confirmed that the lethality of normal-winged progeny is attributed to the uncovering of the Low mutation rather than a semidominant lethality of the deficiencies.

Progeny analysis of Low mutants: Eye pigment assays were performed as described elsewhere (Rabinow et al. 1991).

Crosses for RNA extraction: Females of a stock w+/w+; Low/Sm6a were crossed to males carrying the T(2;3) CyO, Cy Tb ch translocated chromosome. The resulting F1 non-Curly winged females were again mated with Low/Sm6a males. Parental and recombinant normal-winged progeny were scored to determine the recombination frequency between each dominant marker and Low. Low is a recessive lethal, so the surviving scored offspring are all heterozygous for Low. The presence or absence of the various classes allows a determination of gene order. Preliminary mapping of the Low mutation indicated that it is located between the genes, Tufted (53.6 cytological location 37A3-6) and Lobe (72.0, 51A2-B1) (Table 1). We further mapped Low using a chromosome marked with Tufted and Bristle (54.8, 38A6) (Table 1). After scoring 1365 flies, we recovered four Bl and only two wild-type recombinants. The recovery of the wild-type recombinants showed that the gene is not located between Tft and Bl, while recovery of Bl recombinants demonstrated that Low is proximal to both markers (Table 1).

<table>
<thead>
<tr>
<th>Progeny phenotypes</th>
<th>No. scored</th>
<th>Percentage of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low/Tft Bl</td>
<td>1579</td>
<td>99.62</td>
</tr>
<tr>
<td>Tft</td>
<td>1439</td>
<td>90.38</td>
</tr>
<tr>
<td>+</td>
<td>79</td>
<td>4.96</td>
</tr>
<tr>
<td>+ +</td>
<td>57</td>
<td>3.58</td>
</tr>
<tr>
<td>+ + +</td>
<td>17</td>
<td>1.06</td>
</tr>
<tr>
<td>Low/In(2LR) Gla</td>
<td>2</td>
<td>0.13</td>
</tr>
</tbody>
</table>

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elsewhere (Birchler and Hiebert 1989; Birchler et al. 1990). Hybridization with different [32P]-labeled RNA probes (white, brown, scarlet, copper, Pgd, Ztw, Adh, histone repeat, rRNA and β-tubulin as a loading control) was carried out as previously described (Rabinow et al. 1991).

Because the modifier is effective on such a wide spectrum of genes, it is important to determine the abundance of specific transcripts per unit DNA template in the four segregating classes, since any one gene chosen as a standard might be affected. To address this question, we isolated total nucleic acid (DNA and RNA) using a technique described earlier (Rahia et al. 1990; Hiebert and Birchler 1994). Triplicate isolations of the four genotypes shown in Figure 2a were electrophoresed on 1% agarose. Separate DNase I and RNase A digestions confirmed that the upper and lower bands on ethidium-stained gels corresponded to genomic DNA and rRNA, respectively. In the same gel, a dilution series of identically prepared nucleic acid was electrophoresed (data not shown). A photographic negative of an ethidium-stained gel containing triplicate preparations (Figure 2a) was analyzed by laser scanning densitometry using the same parameters as described earlier (Hiebert and Birchler 1994). The measurements of the dilution series were used to establish the standard curve (data not shown). The DNA/18S rRNA and the DNA/28S rRNA ratios, calculated by the densitometric scans, are not significantly different between normal males and females (0–24 hr) or between Low/+ and +/+ males. The results of a similar experiment using Df(2L)DS6/CyO stocks show an equal expression between Df(2L)DS6/+ and +/+ classes (data not shown).

To determine whether the expression of the β-tubulin gene is equal between males and females and whether Low affects the β-tubulin transcripts, we hybridized an identical blot with the β-tubulin probe (Figure 2b). The level of β-tubulin did not vary between the sexes in either genotype indicating that the expression of β-tubulin is equal in 0–24 hr males and females per unit DNA and Low has no significant effect on β-tubulin transcripts (Figure 2b). Thus, we used β-tubulin as a gel loading control.

RESULTS

Isolation of Low From gamma-irradiation, a female fly homozygous for white-blood (w^{Bl}) was recovered that had a pale eye as opposed to the typical color. This mutation proved to be heritable in successive generations and was located to the second chromosome. Because this mutation lightened the white-blood eye color, it was termed ‘‘Lightener of white’’ (Low). In contrast, Low increases the level of pigment deposition in ocelli, testes and Malpighian tubules in which the product of white is also required for pigmentation.

![Diagram](https://example.com/diagram.png)
### TABLE 2

Modulation of white alleles and constructs by Low

<table>
<thead>
<tr>
<th>Allele</th>
<th>Interaction</th>
<th>white locus lesion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(w'^{a}) (apricot)</td>
<td>+</td>
<td>\textit{copia} retrotransposon insertion in second intron</td>
<td>GEHRING and PARO (1980); BINGHAM and JUDD (1981)</td>
</tr>
<tr>
<td>(w'^{d}) (apricot-4)</td>
<td>0</td>
<td>\textit{BEL} retrotransposon in intron 2</td>
<td>ZACHAR and BINGHAM (1982); GOLDBERG \textit{et al.} (1983)</td>
</tr>
<tr>
<td>(w'^{c}) (roo-in-copia)</td>
<td>0</td>
<td>Insertion of (B104) (roo) into \textit{copia} coral retrotransposon in intron 5</td>
<td>DAVIS \textit{et al.} (1987)</td>
</tr>
<tr>
<td>(w'^{b}) (buff)</td>
<td>+</td>
<td>(B104) in intron 4</td>
<td>A. CSINK, unpublished data</td>
</tr>
<tr>
<td>(w'^{b'}) (buff-2)</td>
<td>-</td>
<td>(B104) in 5' untranslated leader (antiparallel)</td>
<td>ZACHAR and BINGHAM (1982); O'HARE \textit{et al.} (1983, 1984)</td>
</tr>
<tr>
<td>(w'^{h}) (blood)</td>
<td>-</td>
<td>\textit{blood} retrotransposon in intron 2</td>
<td>ZACHAR and BINGHAM (1982); BINGHAM and CHAPMAN (1986)</td>
</tr>
<tr>
<td>(w'^{p5}) (spotted-55)</td>
<td>+</td>
<td>\textit{mdg3} retrotransposon in 5' untranslated leader</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{RM}) (apricot revertant)</td>
<td>0</td>
<td>Transposable element in \textit{copia} 5' LTR</td>
<td>MOUNT \textit{et al.} (1988)</td>
</tr>
<tr>
<td>(w'^{re}) (apricot revertant)</td>
<td>0</td>
<td>I-element insertion in \textit{copia} 3' LTR</td>
<td>MOUNT \textit{et al.} (1988)</td>
</tr>
<tr>
<td>(w'^{R1}) (apricot revertant)</td>
<td>0</td>
<td>Solo \textit{copia} LTR</td>
<td>CARBONARE and GEHRING (1985)</td>
</tr>
<tr>
<td>(w'^{i}) (ivory)</td>
<td>0</td>
<td>Duplication of sequences from intron 1 to start of exon 3</td>
<td>COLLINS and RUBIN (1982); KARESS and RUBIN (1982); O'HARE \textit{et al.} (1984)</td>
</tr>
<tr>
<td>(w'^{s}) (crimson)</td>
<td>0</td>
<td>\textit{FB} insertion revertant of (w'^{i})</td>
<td>COLLINS and RUBIN (1982); O'HARE \textit{et al.} (1983, 1984)</td>
</tr>
<tr>
<td>(w'^{c}) (spotted)</td>
<td>0</td>
<td>(B104) retrotransposon in 5' regulatory region</td>
<td>ZACHAR and BINGHAM (1982); O'HARE \textit{et al.} (1983, 1984)</td>
</tr>
<tr>
<td>(w'^{3}) (spotted-3)</td>
<td>0</td>
<td>Deficiency in 5' regulatory region</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{4}) (spotted-4)</td>
<td>0</td>
<td>Deficiency in 5' regulatory region</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{2}) (spotted-2)</td>
<td>0</td>
<td>Deficiency in 5' regulatory region</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{1}) (spotted-8ld5)</td>
<td>0</td>
<td>Deficiency in 5' regulatory region</td>
<td>O'HARE \textit{et al.} (1984)</td>
</tr>
<tr>
<td>(w'^{v}) (coffee)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{e}) (apricot-2)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{e'}) (apricot-3)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{e''}) (satsuma)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{e'''}) (colored)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{vR}) (Brownex)</td>
<td>+</td>
<td>Point</td>
<td>ZACHAR and BINGHAM (1982)</td>
</tr>
<tr>
<td>(w'^{d}) (honey)</td>
<td>+</td>
<td>(B104) element into \textit{Doc} element of (w'^{i}) (\textit{Doc} element)</td>
<td>ZACHAR and BINGHAM (1982); O'HARE \textit{et al.} (1984); HAZELRIGG (1987)</td>
</tr>
<tr>
<td>(w'^{s}) (cosin)</td>
<td>0</td>
<td>Transposable element reversion of (w'^{i})</td>
<td>ZACHAR and BINGHAM (1982); O'HARE \textit{et al.} (1984)</td>
</tr>
<tr>
<td>(w'^{R1}) (apricot-like)</td>
<td>0</td>
<td>P-M hybrid dysgenic revertant of (w'^{i}) (\textit{Doc} element)</td>
<td>O'HARE \textit{et al.} (1991)</td>
</tr>
<tr>
<td>(w'^{R2})</td>
<td>+</td>
<td>I-element insertion</td>
<td>SANG \textit{et al.} (1984); FAWCETT \textit{et al.} (1986)</td>
</tr>
<tr>
<td>(w'^{R3})</td>
<td>+</td>
<td>I-element insertion revertant of (w'^{i}) (\textit{Doc} element)</td>
<td>SANG \textit{et al.} (1984); FAWCETT \textit{et al.} (1986)</td>
</tr>
<tr>
<td>(w'^{R4})</td>
<td>+</td>
<td>I-element insertion</td>
<td>SANG \textit{et al.} (1984); FAWCETT \textit{et al.} (1986)</td>
</tr>
<tr>
<td>(w'^{R5})</td>
<td>+</td>
<td>I-element insertion</td>
<td>SANG \textit{et al.} (1984); FAWCETT \textit{et al.} (1986)</td>
</tr>
<tr>
<td>(w'^{R6})</td>
<td>+</td>
<td>I-element insertion</td>
<td>SANG \textit{et al.} (1984); FAWCETT \textit{et al.} (1986)</td>
</tr>
<tr>
<td>(z w'^{n}) (isoxanthopterinless)</td>
<td>0</td>
<td>Unknown</td>
<td>R. JONES, unpublished data</td>
</tr>
<tr>
<td>(z w'^{n})</td>
<td>0</td>
<td>\textit{BEL} retrotransposon in intron 1</td>
<td>ZACHAR and BINGHAM (1982); O'HARE \textit{et al.} (1984)</td>
</tr>
</tbody>
</table>
TABLE 2
Continued

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<tr>
<th>Allele</th>
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<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>zw0</td>
<td>+</td>
<td>copia insertion in intron 2</td>
<td>GEHRING and PARO (1980); BINGHAM and JUDD (1981)</td>
</tr>
<tr>
<td>Df(1;1)w6109</td>
<td>0</td>
<td>Duplication of white locus sequences</td>
<td>GREEN (1963); GUNARATNE et al. (1986)</td>
</tr>
<tr>
<td>w118; E6 (57B)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to BgII site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; E7 (94D)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to BgII site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; E8 (47D)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to BgII site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; F3 (64B)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to Scal site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; F4-1 (57B)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to Scal site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; F4-2 (21D)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to Scal site</td>
<td>LEVIS et al. (1985)</td>
</tr>
<tr>
<td>w118; F4-3 (97B)</td>
<td>0</td>
<td>Deletion of cis regulatory region 5' to Scal site</td>
<td>LEVIS et al. (1985)</td>
</tr>
</tbody>
</table>

Males of Low balanced stocks were crossed to virgins of each white allele or construct. The eye color of the Low/+ and SM6a/+ male offspring was compared. An elevation in the pigment level is designated by +, a reduction by −, and a lack of alteration by 0 in the interaction column.

**Cytological localization and genetic mapping:** The polytene chromosomes of Low heterozygotes showed no detectable chromosomal rearrangements. Genetic mapping was conducted using various dominant markers encompassing the entire second chromosome, as described in MATERIALS AND METHODS. These results placed Low proximal to both markers and closer to Bl. Further localization was performed by conducting a number of complementation tests between Low and the available deficiencies proximal to 37D and distal to the 40B regions as listed in Figure 1. The results of these crosses indicate that Low resides within the 39E7-F1 region.

To test the nature of the Low mutation, zw0 and zw1 were individually crossed to Df(2L)TW65/CyO and Df(2L)DS6/SM6a males. These two deficiencies most closely flank the Low gene. The former deletes Low and the nearby histone cluster while the latter removes only the region surrounding the histone genes. The eye color of the Curly and non-Curly brothers were compared. In both w mutant backgrounds, the eye color of the Df(2L)TW65/+ males is changed relative to the balancer control in the same manner as with the Low/+ flies. The corresponding trisomic for this region causes an increase in zw0 pigment (SABL and BIRCHLER 1993). In contrast, no alteration in the eye color of zw0 and zw1 was found in mutant males segregating for the Df(2L)DS6 chromosome (data not shown). Thus, the Df(2L)TW65 modulates the zw0 and zw1 eye phenotype as Low does, whereas Df(2L)DS6 does not alter the eye pigmentation. These results suggest that Low is a loss-of-function mutation.
Spectrum of Low interaction with white alleles: For the initial characterization of Low, the spectrum of its interaction with different molecularly defined white alleles was determined. The rationale was that this series would reveal whether Low effects only transposon-induced alleles or the white locus itself. For this purpose, 55 different white alleles, including insertions, deletions, presumptive point mutations and Adh promoter-white reporter fusions, were tested with Low. The results of these crosses are summarized in Table 2.

Low increases the eye color pigment of the six tested point mutations (w\textsuperscript{a3}, w\textsuperscript{a3}, w\textsuperscript{a3}, dal, w\textsuperscript{a4}, w\textsuperscript{a5}) (Figure 3a, Table 2). In contrast Low has no effect upon a series of white alleles that are mutant for cis-acting regulatory sequences, namely the white-spotted series (w\textsuperscript{dp}, w\textsuperscript{dp2}, w\textsuperscript{dp3}, w\textsuperscript{dp4}, w\textsuperscript{dp5}). Low strongly lightens the eye color of the transposon-induced allele, white blood (w\textsuperscript{blood}), whereas tests with white\textsuperscript{promot} (w\textsuperscript{p}), a copia retrotransposon-induced allele, showed a greater amount of pigment in the Low/+ flies. Additionally, all the revertants of w\textsuperscript{d} (w\textsuperscript{d\textsuperscript{mel}}, w\textsuperscript{d\textsuperscript{melt}}, w\textsuperscript{d\textsuperscript{melt}}) do not interact with the Low mutation. Other transposon-induced alleles are suppressed, such as w\textsuperscript{p}, w\textsuperscript{p2} (B10A) and w\textsuperscript{p} (coral). Other tested alleles (w\textsuperscript{w}, w\textsuperscript{w\textsuperscript{p}}) containing FB and BEL transposons do not respond to the Low mutation.

Another group of tested alleles are the revertants of the original w\textsuperscript{d} mutation (w\textsuperscript{uf}, w\textsuperscript{u\textsuperscript{p}}, w\textsuperscript{uf}, w\textsuperscript{uf\textsuperscript{2}}), which contains a Doc retrotransposon insertion into the 5' leader sequence of the white mRNA (Sang et al. 1984; O'Hare et al. 1991). The first two non-dosage-compensated revertants do not exhibit any discernible differences in eye color over their controls when tested with Low. The other two are properly dosage compensated and both are elevated in expression by the Low mutation. Another non-dosage-compensated allele, white-ivory (w\textsuperscript{iv}), also does not respond to Low (Table 2).

Additionally, a series of deletions of the 5' regulatory region of white were tested (Table 2). One set of constructs retains sequences from the Seo site (−360) to the transcriptional start point, and another set retains longer cis-acting regions from the BgII site (−1019) to the transcriptional start of white. We also examined the effect of Low on miniwhite and three different truncated miniwhite constructs that are progressively deleted at their regulatory sequences from the 5' end (Table 2). The original miniwhite is a small version of the wild-type
white gene that lacks 5′ upstream sequences, −305 bp preceding the transcriptional initiation site and a 3.0-kb region from the first intron. The 5′ termini of the miniwhite lesions are as follows: −113, −17 and +174 bp before the transcription start (QIAN and PIRROTTA 1995). The (−17) miniwhite bears an additional 40-bp synthetic oligonucleotide encoding four ZESTE protein binding sites. For expression in the eye, the (+174) miniwhite has the 5′ region replaced by the eye enhancer that is normally 1.2 kb from the start site at the 5′ end of the white. This construct is transcribed by readthrough from adjacent genes (QIAN and PIRROTTA 1995). No significant alteration in the eye color of these constructs was found in the presence of the Low mutation (Table 2). In contrast, the effect of Low on an Alcohol dehydrogenase (Adh) promoter-w reporter construct showed that Low causes a darker eye color of the adult flies (Figure 3c and Table 2). These results, in combination with those above suggest that the product of Low may modulate the Adh promoter.

In summary, almost all white alleles with lesions in the structural gene were suppressed in the presence of the Low mutation. Alleles that are 5′ cis-acting regulatory defects and 1′ experimentally synthesized deletions including miniwhite constructs, however, do not respond. These results suggest that the eye enhancer sequences are required for the Low effect on white. The alleles that lack dosage compensation between males and females are also unaffected suggesting that lesions that interfere with this regulatory mechanism block the action of Low.

Effect with other white modifiers: zeste: The zeste gene is involved in chromosomal pairing-dependent phenomena such as transvection and the suppression of white gene expression. The zeste-white interaction requires an allele of zeste, whose product is able to suppress the transcription of the wild-type white gene in the eye, resulting in a yellow eye color instead of wild-type red (GANS 1953; BINGHAM and ZACHAR 1985). This effect requires two copies of the white gene in close proximity produced either by homologous pairing or by tandem duplication of a 5‘ regulatory sequence (JACK and JUDD 1979; CHEN and PIRROTTA 1993). To test whether the two regulators zeste and Low interact with each other, a genetic cross was conducted, in which males of w<sup>118b</sup>; Low/SM6a were crossed to z Dp(1;1)w<sup>118b</sup>/z Dp(1;1)w<sup>118b</sup> females. This stock carries a tandemly duplicated white sequence, resulting in a yellow eye color in hemizygous zeste males instead of the normal red. The eye color of z Dp(1;1)w<sup>118b</sup>; Low/+ males is equivalent to their SM6a/+ brothers (data not shown), indicating that the action of Low on white is blocked by zeste.

To gain more information regarding this interaction, we used a dominant white allele, w<sup>p22</sup>, which causes a zeste-like yellow eye color in the presence of the wild-type zeste gene (BINGHAM and ZACHAR 1985). This allele results from an insertion of a Foldback transposon upstream of the regulatory sequences of white. A cross between +/Y; Low/SM6a males and w<sup>p22</sup> females generated males and females heterozygous for Low. The eye colors of the males and females are quite distinct. In males, it is almost wild-type red, while in females, the color is intermediate between the normal and mutant zeste. In both sexes, the color of Low/+ individuals is significantly reduced relative to the balancer control (data not shown). It appears that Low reduces the expression of white under these circumstances in contrast to the effect on point mutations in the protein encoding portion of white that were examined under the same circumstances.

su(w<sup>0</sup>) w<sup>0</sup>: The mutational effect of the suppressor of white-spotted gene increases the phenotypic expression of spotted alleles of white, which are lesions in the 5′ eye enhancer regulatory site. To determine the interaction of Low and su(w<sup>0</sup>), males of the Low/SM6a balanced stock were crossed to su(w<sup>0</sup>) w<sup>0</sup> females and the eye color of the two classes of F<sub>1</sub> males were compared. The absence of a difference between the two classes of flies suggests that su(w<sup>0</sup>) cannot restore an effect on w<sup>0</sup> by Low (data not shown), since Low has no effect on w<sup>0</sup> alone.

Phenotypic effect on other loci: In Drosophila, an altered expression of a homeobox gene causes the Bar mutation (KOJIMA et al. 1991). Bar H1 and Bar H2 comprise a complex at the Bar region that is essential for ommatidial differentiation. Analysis of the Bar mutation revealed that the proper functioning of Bar H1 along with Bar H2 is required for pigment cell formation, in which the white, brown and scarlet genes are expressed. During the analysis of the new modifier, it was found that Low suppresses the mutational effect of Bar (Figure 3b). To examine the generality of this response, three other dominant mutations, Lobe, Irregular facets and Drop, that affect the number of eye ommatidia, were also examined. The results show that Low suppresses the Lobe mutation, whereas there is no recognizable effect on the others (data not shown).

An additional test was performed using different X chromosomal mutations that affect eye color and wings of adults. Balanced stocks homozygous for two other X chromosomal genes, vermilion (v<sup>3</sup>/v<sup>3</sup>; Low/SM6a) and rudimentary (r<sup>0</sup>/r<sup>0</sup>; Low/SM6a), were constructed to detect any Low mutational effect phenotypically. Females homozygous for each mutation were individually crossed to normal males and the F<sub>1</sub> Low/+ males were compared to SM6a/+ males. Neither gene was affected (data not shown), suggesting that the dosage sensitive effect of Low is gene specific rather than for the X chromosome in general.

Position effect variegation: Effect on w<sup>116</sup>: When a rearrangement places heterochromatin next to the wild-type white (w<sup>+</sup>) gene, which is required for pigmentation of the eye, the inactivation can be visualized as
a variable pattern of pigmented and nonpigmented ommatidia (for reviews see Reuter and Spierer 1992; Henikoff 1996). \textit{w}^\textit{neb} is an inverted X chromosome with a breakpoint near the \textit{white} locus and has frequently been used for the isolation and genetic characterization of PEV modifying mutations. Various second site modifiers have been isolated that enhance or suppress \textit{w}^\textit{neb} variegation as well as variegating alleles of other genes (Locke et al. 1988; Wustmann et al. 1989; Talbert et al. 1994). To test for the effect of \textit{Low} on \textit{w}^\textit{neb} variegation, a stock was constructed that was homozygous for \textit{w}^\textit{neb} and carried the \textit{Low} mutation on the second chromosome. Females of this stock, when crossed with \textit{w}^\textit{neb} males, produce two types of progeny with respect to the dosage of functional copies of \textit{Low}. An increase in the number of red ommatidia in the eyes of \textit{Low}/+ heterozygotes indicates that in both sexes, \textit{Low} has a partial but consistent suppression of the \textit{w}^\textit{neb} variegation (Figure 3d).

Eye pigment assays of each class of flies demonstrate that the presence of \textit{Low} alters the effect of \textit{w}^\textit{neb} variegation by twofold over the control classes (Table 3). The degree of the effect is equal between males and females. To ensure that the suppression of PEV was solely due to \textit{Low} and not to a preexisting suppressor present on the progenitor chromosome, we analyzed the second chromosome from the parental \textit{w}^\textit{111} line for its effect on \textit{w}^\textit{neb}. The females from the \textit{w}^\textit{neb} strain were mated to males carrying the progenitor second chromosome, balanced with \textit{SM6a}, the same balancer used with the \textit{Low} mutation. The amount of pigment in \textit{w}^\textit{neb}; \textit{SM6a}/+ and \textit{w}^\textit{neb}; +/+ males and females was measured spectrophotometrically. There was a slight but nonsignificant effect of the progenitor chromosome on PEV particularly in males (Table 4). However, this effect does not approach the magnitude of the effect of the \textit{Low}/+ heterozygotes.

To determine whether the level of pigment increase by \textit{Low} is proportional to the degree of PEV suppression, the effect on the expression of wild-type \textit{white} \textit{white} was estimated in \textit{Low}/+ heterozygotes. Nearly equal amounts of pigment in \textit{Low}/+ and their normal brother (+/+) reveals that \textit{Low} heterozygotes have little effect on the pigment levels of \textit{white} (Table 4). This is the case because the amount of the \textit{white} product is not limiting on pigment deposition in wild-type eyes. It appears therefore that the level of pigment increase of the variegating allele correlates with the degree of \textit{Low} suppression. However, suppression by \textit{Low} is not as strong as some of the previously described suppressors (Reuter et al. 1990), but rather comparable in its strength with three other \textit{white} modifiers, \textit{Wow}, \textit{Lip} and \textit{Mow} (Birchler et al. 1994; Csink et al. 1994b; Bhadra and Birchler 1996).

\textit{Effect on \textit{y}^\textit{w}}: To test the generality of suppression, we also examined the effect of \textit{Low} on a variegating allele of \textit{yellow}. The \textit{yellow} gene displays position-effect variegation, when placed next to heterochromatin in the inversion, \textit{In(1)Yp}. In addition to \textit{w}^\textit{neb} suppression, \textit{Low} also reduces the frequency of \textit{yellow} variegation among the 80 or so triple row bristles along the anterior margin of the wing blade in \textit{In(1)Yp} flies. For segregating classes, \textit{Low}/\textit{SM6a} females carrying the \textit{In(1)Yp} chromosome were mated with normal males. The \texttt{FI} males segregating for \textit{Low} and the \textit{SM6a} balancer were scored. The number of wild-type and \textit{yellow} bristles of nine wing blades were counted in each class of flies and the results are summarized in Table 4. \textit{Low} suppresses \textit{yellow} bristle variegation nearly threefold (49%) above the compara-

### Table 3

<table>
<thead>
<tr>
<th>Cross</th>
<th>Genotype</th>
<th>Sex</th>
<th>n</th>
<th>OD$_{100}$</th>
<th>Ratio</th>
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<tr>
<td>\textit{w}^\textit{neb}, +/+ × \textit{w}^\textit{neb}/\textit{Y}; +/\textit{SM6a}</td>
<td>\textit{w}^\textit{neb}; +/+</td>
<td>M</td>
<td>4</td>
<td>0.137 ± 0.026</td>
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<td>\textit{w}^\textit{neb}; \textit{SM6a}/+</td>
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<td>0.164 ± 0.010</td>
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<tr>
<td></td>
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<td>0.185 ± 0.013</td>
<td></td>
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<tr>
<td></td>
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<td>4</td>
<td>0.201 ± 0.008</td>
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<td>\textit{w}^\textit{neb}, +/+ × \textit{w}^\textit{neb}/\textit{Y}; \textit{Low}/\textit{SM6a}</td>
<td>\textit{w}^\textit{neb}; \textit{Low}/+</td>
<td>M</td>
<td>4</td>
<td>0.265 ± 0.019</td>
<td>2.15*</td>
</tr>
<tr>
<td></td>
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<td>M</td>
<td>3</td>
<td>0.123 ± 0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>\textit{w}^\textit{neb}; \textit{Low}/+</td>
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<td>3</td>
<td>0.674 ± 0.027</td>
<td>2.15*</td>
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<td></td>
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<td>F</td>
<td>4</td>
<td>0.313 ± 0.019</td>
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<td>\textit{w}; +/+ × \textit{w}; \textit{Y}; \textit{Low}/\textit{SM6a}</td>
<td>\textit{w}; \textit{SM6a}/+</td>
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<td>2.824 ± 0.032</td>
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<tr>
<td></td>
<td>\textit{w}; \textit{Low}/+</td>
<td>F</td>
<td>4</td>
<td>2.979 ± 0.028</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Three crosses were used to generate the 12 different classes of progeny. The crosses are as follows: top, the progenitor second chromosome with \textit{w}^\textit{neb}; middle, the \textit{Low} mutation with \textit{w}^\textit{neb}; bottom, the \textit{Low} mutation with normal \textit{white}. The pigment concentration of segregating offspring was determined as described in MATERIALS AND METHODS. The OD$_{100}$ values denote means of values ± SE of \textit{n} number experiments performed. The OD$_{100}$ values between males and females in each \textit{w}^\textit{neb} genotype are statistically significant at the 95% level of confidence. Alteration of eye pigment by \textit{Low} is represented by the ratio of \textit{Low}/+ to \textit{SM6a}/+. The ratios marked with an asterisk are significantly different from 1.00 (P < 0.05).
In Table 5, the level of reduction or elevation is nearly twofold greater for males than females, indicating a sexual dimorphism similar to that observed in several mRNAs via Northern blots. This effect is greater in females than in males, with the level of reduction or elevation being nearly twofold greater in females than males. The sex-specific influence is also found with the other eye pigment loci, white and brown, as well as the retrotransposon copia (see below), but it shows a unique pattern in each case. The variation of the sexually dimorphic effect on each locus in different developmental stages suggests that reduction of the Low product concentration has a distinct sensitivity for each gene. The overall results regarding the effect of Low on pigment deposition genes suggest that despite different developmental fluctuations in the strength of the response, the transcript levels of all three genes appear to be modulated by Low in different ways.

Effect of Low on mRNA levels: To test whether the effect of Low on the phenotypic expression of several unrelated loci is correlated at the mRNA level, the developmental profile of several mRNAs via Northern blots was determined. Data listed in Table 5 illustrate the amount of white mRNA accumulated in different classes of progeny. The quantity of white mRNA in Low/+ larvae was reduced indicating a direct correlative effect. In contrast, the transcripts of white are strongly elevated in early Low/+ pupae suggesting that the response to Low shifts from a direct correlative effect to an inverse one at this stage. It appears that the transition from larvae to early pupae may act as a switch for the opposite type of dosage effect of Low. The abundance of white transcripts in this genotype also increased significantly in the mid-pupal and the adult stages (Table 5).

Several genetic and molecular studies suggest that the three genes, white, brown and scarlet, are required for pigment deposition (Dreessen et al. 1988; Tearle et al. 1989). To test whether Low regulates coordinately all three genes, we estimated bw and st mRNA quantity in the different genotypes and developmental stages as for white. The relative ratios between Low/+ and +/+ indicate that the transcript levels of brown in all tested stages are significantly reduced with the exception of male larvae (Table 5), where the level of brown transcript in Low/+ individuals is nearly exponentially increased.

The effect of Low on scarlet is quite distinct from white but similar to brown. The abundance of scarlet transcripts in Low/+ is significantly reduced in all tested developmental stages compared to the normal individuals producing a direct correlative effect throughout development (Table 5). This effect is greater in females than males, indicating a sexual dimorphism similar to Modifier of white (Mow) and Ultra female overexpression (Ufo) (Bhadra and Birchler 1996; Bhadra et al. 1997). The level of reduction or elevation is nearly twofold greater in females than males. The sex specific influence is also found with the other eye pigment loci, white and brown, as well as the retrotransposon copia (see below), but it shows a unique pattern in each case. The variation of the sexually dimorphic effect on each locus in different developmental stages suggests that reduction of the Low product concentration has a distinct sensitivity for each gene. The overall results regarding the effect of Low on pigment deposition genes suggest that despite different developmental fluctuations in the strength of the response, the transcript levels of all three genes appear to be modulated by Low in different ways.

To examine whether Low affects Adh transcripts, as suggested by the effect on the Adh-w constructs, an antisense probe of the Adh gene (Birchler et al. 1990) was hybridized to a set of blots as described above. Table 5 illustrates that Adh mRNA in the Low/+ genotype in larvae and adults is significantly reduced compared to the +/+ males at the 95% confidence level in statistical analysis. In contrast, in two separate pupal stages, the total transcript levels of the Adh gene is considerably higher in Low/+ compared to the +/+ individuals.

The steady-state level of copia transcripts of the different classes was measured to examine the basis of white apricot (w+) suppression by Low (Table 2). Table 5 demonstrates that an increase of the stable copia transcripts was found in Low/+ relative to the normal +/+ in adults and pupae. These data suggest that the phenotypic alteration of w+ by Low is a cumulative effect exerted by the modulation of white as well as copia.

Because of the differential effects between males and females, we next examined the amount of transcripts from two X chromosomal loci, Zw (G6PDH) and Pgd (6PGDH), to determine whether there was any relationship between Low and the process of dosage compensation (Baker et al. 1994; Kelly and Kuroda 1995). A nonsignificant variation of the Zw and Pgd transcripts was observed among all the developmental stages and genotypes (Table 5). Thus Low modulates gene expression on the individual transcript level but not on the chromosomal level. To test whether the differential response of Low in males and females interacts with the sex-specific lethal mutations, Sex-lethal (Lucchesi and Skrinsky 1981; Cline 1993) and male specific lethal-3 (msl-
<table>
<thead>
<tr>
<th>Genes</th>
<th>3rd instar larvae</th>
<th>Early pupae (24–48 hr)</th>
<th>Middle pupae (48–72 hr)</th>
<th>Adults (0–24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>white</td>
<td>0.53 ± 0.017*</td>
<td>0.21 ± 0.008*</td>
<td>2.78 ± 0.043*</td>
<td>2.94 ± 0.112*</td>
</tr>
<tr>
<td>copia</td>
<td>1.26 ± 0.051</td>
<td>1.25 ± 0.069</td>
<td>3.40 ± 0.089*</td>
<td>5.50 ± 0.128*</td>
</tr>
<tr>
<td>Adh</td>
<td>0.63 ± 0.014*</td>
<td>0.64 ± 0.021*</td>
<td>2.46 ± 0.107*</td>
<td>2.79 ± 0.111*</td>
</tr>
<tr>
<td>scarlet</td>
<td>0.70 ± 0.029*</td>
<td>0.37 ± 0.019*</td>
<td>0.56 ± 0.033*</td>
<td>0.50 ± 0.022*</td>
</tr>
<tr>
<td>histone</td>
<td>2.73 ± 0.085*</td>
<td>2.92 ± 0.107*</td>
<td>0.53 ± 0.031*</td>
<td>2.59 ± 0.107*</td>
</tr>
<tr>
<td>brown</td>
<td>8.66 ± 0.159*</td>
<td>0.32 ± 0.017*</td>
<td>0.49 ± 0.017*</td>
<td>0.53 ± 0.020*</td>
</tr>
<tr>
<td>Pgd (6PGDH)</td>
<td>1.17 ± 0.087</td>
<td>1.02 ± 0.071</td>
<td>1.06 ± 0.055</td>
<td>1.07 ± 0.034</td>
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<tr>
<td>Zw (G6PDH)</td>
<td>1.04 ± 0.051</td>
<td>1.10 ± 0.035</td>
<td>0.81 ± 0.027</td>
<td>0.97 ± 0.031</td>
</tr>
</tbody>
</table>

Values are mean ± SE (Low/+:+/+). The amount of radioactive label for each RNA on northern blots was measured using a Fuji BAS 2000 phosphorimagery. All the values were corrected for loading differences by rehybridizing each blot with the β-tubulin probe. Each value is the mean of three different assays ± SE. To compare Low/+ vs. the T(2;3) CyO, Tb ch/+ genotype, the individual transcript/β-tubulin ratios were calculated followed by the Low/+:+/+ ratios, which are presented in the table. * Significantly different from the value of 1.00 (P < 0.05).

**TABLE 5**
Quantitative estimation of the steady-state mRNA levels of eight loci from Low segregating progeny in four selected developmental stages

**Figure 4** — Northern hybridizations showing the effect of the Low mutation on histone transcripts in adults and larvae (a) and in early and mid pupae (b). The bottom panel of each row shows the re-probing with β-tubulin as a loading control. The genotypes are indicated at the top of each lane.
larvae and mid-pupae is elevated nearly two- to three-
fold when one copy of the Low gene is mutated (Low/+ ) compared to the progenitor chromosome (Figure
4 and Table 5). However, at the time of the major
developmental transition from larvae to early pupae,
the amount of the histone transcripts is reduced. This
result suggests that the Low mutation modulates the
expression of the histone repeats.

In eukaryotic nuclei, DNA is highly compacted with
histones. The Low mutation produces an increased
amount of histone transcripts in three developmental
stages. To determine whether the broad effect of the
Low mutation on gene expression is related to the accu-
mulation of the excess histone transcripts, we examined
the phenotypic expression of a number of the tested
genes, white (w<sup>e</sup>, w<sup>1</sup>, w<sup>3</sup>), Bar and the Adh-w<sup>1</sup> transgene
by altering the dosage of the histone repeats alone. We
compared the phenotypic effect of each mutation in
the F<sub>1</sub> Curly and non-Curly males from the crosses be-
tween the Df(2L)DS6/CyO males and each of the individ-
ual mutant females. No significant alteration of the pheno-
types suggested that these genes are not affected by
the histone deficiency (data not shown). A current
search of Flybase revealed that no small duplications
that only encompass the histone gene complex without
Low are available for testing.

**Histone dosage and gene expression:** A deficiency
of the histone repeats suppresses PEV, while a larger
duplication including the histone genes has no effect
(Moore et al. 1983). To determine whether a subtra-
ction of a chromosomal segment carrying the histone
tandem array has an impact on histone and unlinked
gene expression, the transcript level of selected genes
was examined in flies with a heterozygous deficiency
for the histones. Northern hybridization analyses of
the segregating classes was performed using the histone
repeat, white, Adh, copia and scarlet probes (Figure 5).
The data show that the deficiency reduces histone mRNA
levels in larvae and adults as well as the quantity of scarlet
and copia transcripts (Figure 5a, Table 6). However, this
deficiency does not affect the transcript levels of the
white and Adh loci in the two developmental stages ex-
amined (Figure 5b and Table 6). These results indicate
that the effects of the Low mutation and the histone
deficiency are distinct. Nevertheless, monosomy for
the histone repeats has an effect on the expression of spe-
cific unlinked genes as occurs in other species (Fisher-
Adams and Grunstein 1995; Shen and Gorovskv
1996); however, we cannot rule out the possibility that
another gene located within the deficient region of the
Df(2L)DS6 chromosome may contribute to these effects.

**DISCUSSION**

**A broad effect of Low on gene expression:** Low is a
unique dosage-sensitive modifier in a number of ways.
We chose nine randomly selected genes to examine the
effect of Low at the RNA level. Six of the nine selected
transcripts were modulated. Overall these data revealed
that the effect of Low on gene transcripts is broad and
dispersed throughout the genome. The Low mutational
effect is not limited to a particular tissue and stage. For
example, Adh is largely expressed in the fat bodies and
gut and is modulated by Low. In contrast, for white, the
opposite effect of Low was found in two separate tissues.
In addition, Low modifies two eye morphology muta-
tions, Bar and Lobe, indicating a diverse collection of
modified genes.

Low modulates the abundance of several unrelated
transcripts developmentally. However, the features at-
tributed to the Low gene seem to be rather unusual.
Low alters the level of brown and scarlet transcripts in a
direct correlative manner and the effect is greater in
adults. These results suggest that Low controls these
two separate but functionally related genes by a similar
mechanism. In contrast, Low modulation of white, his-
tone and many other transcripts switches from a positive
dosage effect to a negative one depending on the stage.
Thus, increases or decreases of gene expression could
be governed by a single gene. Such developmental dif-
cences have been reported previously for four sepa-
rate white modifiers, Inr-a (Rabinow et al. 1991), Wow
(Birchler et al. 1994), Mow (Bhadora and Birchler
1996) and Ufo (Bhadora et al. 1997).

**Aneuploid effects and Low** The classical view of an-
euploid syndromes is that they result from an imbalance
of structural gene products from the varied region rela-
tive to those from the remainder of the genome. How-
ever, the identification of modifiers such as Low sug-
gests that aneuploids will reduce or elevate gene expression
throughout the genome. Previous studies on aneu-
ploids have hypothesized that the reductions in gene
expression in both monosomes and trisomies is the
major molecular basis of the detrimental effects of an-
euploidy (Birchler and Newton 1981; Guo and
Birchler 1994), and Low would be a single locus exam-
ples of a gene responsible for such effects. The magni-
tude of the majority of dosage effects produced by Low
falls within the limits of a direct or inverse correlation
of gene expression and aneuploid dosage. The inverse
effect of genes such as Low in combination with a simul-
taneously varied structural gene could account at least
partially for the dosage compensation that occurs in
aneuploids (Birchler 1979; Devlin et al. 1982; Guo
and Birchler 1994).

**Regulatory sequences:** Low modulates white alleles
with lesions in the structural gene but does not interact
with the white-spotted series that deletes the presumptive
eye enhancer. The white-spotted mutations represent a
series of cis-inactivations of white alleles that delete white
regulatory sequences ranging from 590 to 1270 bp 5’
to the start site of the white transcription unit. These
mutations delete the eye enhancer element and the
region responsible for synapsis dependent transvection.
Figure 5.—Northern blots of total cellular RNA of the segregating $Df(2L)DS6/+\text{ and } ++\text{ individuals hybridized with antisense RNA probes (see MATERIALS AND METHODS for probe preparation)}$ prepared from histone, scarlet and copia genes in adults (a) and from the white and Adh genes in adults and larvae (b). The $\beta$-tubulin hybridization pattern below each blot acts as a loading control. The genotypes are indicated at the top of each lane. The names of the antisense probes are noted in each panel.

Effects (DAVISON et al. 1985). Similarly, two different series of truncated promoter constructs were tested (LEVIS et al. 1985), which both remove the eye enhancer. None of the spotted mutations as well as the experimentally synthesized constructs are phenotypically modulated by Low. These observations suggest that the common deleted region of all the constructs is required for the interaction with Low (directly or indirectly).

Relationship between gene modulation and PEV: We have shown that Low requires a complete regulatory region to interact with white. The suppression effect of Low on PEV suggests that Low might be involved in determining a particular chromatin configuration as found with other suppressors of PEV (HAYASHI et al. 1990; BELVAeva and ZHIMULEV 1991; DORN et al. 1993). Almost 200 dominant mutations that suppress or enhance PEV have been isolated by several research groups (REUTER and WOLFF 1981; SINCLAIR et al. 1983, 1989, 1992; LOCKE et al. 1988). To date, a large number of PEV suppressors have been characterized that en-

<table>
<thead>
<tr>
<th>Genes</th>
<th>3rd instar larvae</th>
<th>Adults (0–24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\delta$</td>
<td>$\varphi$</td>
</tr>
<tr>
<td>white</td>
<td>$1.09 \pm 0.045$</td>
<td>$1.08 \pm 0.031$</td>
</tr>
<tr>
<td>copia</td>
<td>$0.95 \pm 0.027$</td>
<td>$1.05 \pm 0.043$</td>
</tr>
<tr>
<td>Adh</td>
<td>$0.52 \pm 0.011^*$</td>
<td>$0.46 \pm 0.010^*$</td>
</tr>
</tbody>
</table>

Values are mean ratios $\pm SE [Df(2L)DS6/+;+/+]$. The band densities were measured using a Fuji 2000 Bass Phosphorimage. The values were obtained from three different Northern blots. The ratios were calculated by first dividing the specific gene transcript levels by the $\beta$-tubulin loading control. To generate the $Df(2L)DS6/+\text{ vs. } T(2;3)CyO, Tb ch/+\text{ ratios shown in the table, the specific gene transcript/}\beta$-tubulin ratios were used.

* Significantly different from a value of 1.00 ($P<0.05$).
code chromosomal binding proteins or their modifying enzymes (Sinclair et al. 1992; Reuter and Spierer 1992; Baksa et al. 1993; Dorn et al. 1993; Tschiersch et al. 1994; Seum et al. 1996). Some of them are also required for normal expression of homeotic genes, such as Trithoraxlike (Farkas et al. 1994) and E(var)3-93D (Dorn et al. 1993). It was suggested that these genes are involved in establishing or maintaining an open chromatin configuration, which is required for transcriptional initiation.

The elevated level of histone expression does not correlate with the suppression effect of Low on PEV, because histone deletion suppresses PEV and histone duplication has no effect (Moore et al. 1983). However, Low reduces the expression of the histone genes at the early pupal stage. It is potentially the case that the reduction of histone at this stage is responsible for the Low effect on PEV. However, the lack of a relationship between histone expression and the mRNA levels of other loci modified by Low does not support the general possibility that the effects of Low are a consequence solely of its effect on histone expression.

Possible mechanisms for Low action: The organization of the histone gene complex in Drosophila is distinct from other organisms (Chernyshev et al. 1980). It forms a tandem array containing ~100–150 copies. Depletion of H1 in Tetrahymena cells and H4 in yeast does not produce a global effect, but changes the expression of sensitive genes (Kim et al. 1988; for review see Zlatanova and Van Holde 1992). The results presented here and in other earlier work argue that the reduction of histone transcripts reduces specific gene expression and suppresses PEV.

Low was originally identified as a modifier of unrelated genes and a weak suppressor of PEV. Subsequently its role in modulation of histone transcripts was found. It is possible that the alteration in the amount of the histone transcripts associated with the Low mutation is responsible for the broad effects on gene expression. In this view, the chromatin structure of each gene is unique, established by a sequence specific placement of nucleoprotein complexes. An alteration in histone transcripts by Low (either reduction or elevation) causes a chromatin architectural change of the sensitive genes but not others. Counter to this explanation, however, is the fact that Low modulates the expression of other genes, not affected by the histidine deficiency. In other words, much of the specificity lies with the Low mutation. It is likely, therefore, that the full spectrum of modulations occurs due to a combination of the primary effect of Low together with the secondary effects of altering histone expression.

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