Dosage Compensation of the copia Retrotransposon in Drosophila melanogaster

John C. Hiebert and James A. Birchler

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138
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ABSTRACT

Dosage compensation in Drosophila has been studied at the steady state RNA level for several single-copy genes; however, an important point is addressed by analyzing a repetitive, transposable element for dosage compensation. The two issues of gene-specific cis control and genomic position can be studied by determining the extent of dosage compensation of a transposable element at different chromosomal locations. To determine whether the multicopy copia transposable element can dosage compensate, we used the X-linked white-africot (w') mutation in which a copia element is present. The extent of dosage compensation was determined for the white and copia promoters in larvae and adults in two different genomic locations of the w' allele. We conclude that copia is able to dosage compensate, and that the white promoter and the copia promoter are not coordinate in their dosage compensation abilities when assayed under these various conditions. Thus, two transcriptional units, one within the other, both of which are able to dosage compensate, do so differently in response to developmental stage and genomic position.

Dosage compensation in Drosophila is the equivalence of expression of genes linked to the X chromosome, despite unequal dosages in the two sexes (Muller, League and Offermann 1931). Unlike placental mammals in which dosage compensation is achieved by random single X inactivation during female embryonic development, Drosophila uses a different system, as evidenced by the fact that both female X chromosomes are transcriptionally active. Almost all wild-type X-linked loci that have been examined are dosage compensated in males. It has been shown that dosage compensation exists at the level of steady state RNA abundance, and occurs via control of transcriptional initiation (Mukherjee and Beer-Mann 1965).

Two broad parameters are demonstrably important to a gene's ability to dosage compensate. One is cis-regulatory control and the other is genomic position. The best-studied examples of mutations which fail to dosage compensate have lesions in their 5' -regulatory regions. An example of the X-linked white locus is white-eosin (w') which is a partial revertant of the null allele, white-one. This allele exhibits no dosage compensation (Smith and Lucchesi 1969). The lesion in w' is a secondary insertion which may introduce a novel promoter (O'Hare et al. 1991). Also, the white-spotted alleles show abnormal dosage compensation and contain lesions in their 5'-cis-regulatory regions (Zachar and Bingham 1982). Certain strains show only partial dosage compensation for the wild-type allele of the X linked Sgs-4 gene, and the effect has been localized to the 5'-cis-regulatory region (Kaiser, Furia and Glover 1986; Korge 1981).

The influence of cis-regulatory control is further established by relocations of certain genes, which exhibit dosage compensation independent of genomic position. For example, the X-linked white (Hazelrigg, Levis and Rubin 1984) and Sgs-4 (Krumm, Roth and Korge 1985) genes, when transformed to autosomal sites, showed greater expression in males, which indicated that these genes continued to compensate at ectopic autosomal positions.

A second determinant in dosage compensation is genomic position, as evidenced by P element-mediated gene transfer experiments. Genes that have been derived from autosomes and relocated to the X chromosome include rosy (Spradling and Rubin 1983), Adh (Goldberg, Posakony and Maniatis 1983; Laurie-Ahlberg and Stam 1987; Sass and Meselson 1991), and Ddc (Scholnick, Morgan and Hirsh 1983). All three were found to dosage compensate, suggesting either that a quality specific to the X can induce dosage compensation along its length, or that a property of the the autosomes prevents response to the dosage compensation mechanism. Similarly, the X-linked LSP1-a gene, normally not dosage compensated, was found to compensate when relocated to ectopic sites on the X (Ghosh et al. 1989).

All available data on dosage compensation are from single-copy loci. In contrast, we were interested in analyzing whether middle repetitive retrotransposons
would exhibit dosage compensation. For several reasons they may be considered outside the normal system of dosage compensation in Drosophila. The analysis of retrotransposons addresses the nature of the compensation mechanism in terms of whether it is a gene-specific mechanism or a more general regulatory system that can be usurped from the host. If dosage compensation evolves by selection of altered X-linked promoters, and so is a specifically evolved process, one would expect multicopy transposons not to respond. Based on this hypothesis, retrotransposons would have no obvious requirement to dosage compensate since their products do not contribute to host viability. Moreover, it would be difficult to evolve compensation mechanisms since a transposable element resides at many genomic locations, and can shift between the X and autosomes. Furthermore, some compensation mechanisms since a transposable element resides at many genomic locations, and can shift between the X and autosomes. Furthermore, some retrotransposons would have had little evolutionary time to develop appropriate cis control elements for compensation.

Experimentally it is difficult to distinguish transcripts from an individual transposable element due to the presence of multiple identical transcribing copies. We were able to approach this problem by identifying a single copia retrotransposon transcript, distinguishable electrophoretically from all others in the genome. This copia element resides in the second intron of the white gene, producing the hypomorphic white-apricot (w*) allele. Some transcripts which initiate in the 5’-long terminal repeat (LTR) of copia fail to terminate in the 3’-LTR. This readthrough transcription produces an RNA species of greater molecular weight than that of copia, and is detectable as a discrete band on northern blots using downstream white probes.

This system allowed detection of transcripts, using a single probe, from three different, but closely linked promoters. These are the white promoter and the two LTRs of copia. A strain bearing a transposition of the w* allele to chromosome 3 (TE89) permitted an identical analysis when w* is autosomally linked.

In this report we demonstrate that copia does exhibit dosage compensation and that this response shows developmental and position dependence. In w* larvae, the copia-initiated transcript was not dosage compensated, whereas it was in adults. The TE89 copia-initiated transcript was not dosage compensated at either stage. The sex-specific responses of the three closely linked promoters differed, despite the fact that the copia transcriptional unit is contained entirely within that of white. Also, the response to genomic position differed for the internal transcription units and the external white gene.

MATERIALS AND METHODS

Fly stocks: Flies were maintained at 25° on Instant Drosophila Medium (Carolina Biological Supply). The TE89 strain is of the genotype, y Df(1) w* rs* /y; Y; TE89, in which the insertion maps to 98F (ISING and BLOCK 1981).

RNA isolation: RNA was extracted by the guanidine-HCl method (COX 1968). Adults from 0 to 24 hr of age and third instar larvae were harvested and frozen at -80°. All northern gel lanes represent total RNA. Flies and larvae were homogenized in 8 M guanidine-HCl (ULTRAPURE SCHWARZ/MANN) at a concentration of 1 mg/ml tissue, then RNA was precipitated in 0.5 volume ethanol. Four more extractions with 4 M guanidine-HCl and ethanol precipitations followed. Finally the RNA was extracted from the pellet three times with sterile water, the second time at 56°. After ethanol precipitation from the water extractions, the RNA was dissolved in sterile water and stored at -80°.

Northern analysis: Total RNA was separated on formaldehyde-agarose gels (1.5%) (LEHRACH et al. 1977) at 21 μg/lane. Gels were run at approximately 50 V for 18 hr. Formaldehyde was present in the tank buffer at the same concentration as in the gel (6.7%). The RNA was capillary transferred to Biotrans nylon membrane overnight using 20 × SSC, then UV cross-linked to the filter (CHURCH and GILBERT 1984), and baked under vacuum at 75° for 2 hr.

Molecular weight measurements were made using RNA standards (0.24–9.5 kb) and the protocol from Bethesda Research Laboratories, Life Technologies Inc. Hybridizations were performed as described in BIRCHLER and HIEBERT (1989). Band intensities were determined with the LKB Ultrascan laser scanning densitometer, and analyzed with LKB GelScan interface and software package.

RNA probe preparation: Radioactive RNA probes were made using constructs of the white fragment depicted in Figure 1. An 854-bp SalI fragment containing exons 4 and 5 was inserted into pIBI 76 to make pIBI 12.3 SS (probe E4-5). Similarly, a 1267-bp HindIII/BamHI fragment containing exon 1 was inserted into pIBI 76 to make pIBI 11.5 HB (probe E1). Both constructs were transcribed in vitro from the T7 promoter to make 32P-labeled antisense RNA probes. A 32P-labeled antisense β-tubulin probe served as a loading control (BIALOGAN, FAULKENBURG and RENKAWITZ-POHL 1985).

RESULTS

Identification of a marked copia element in w*:
The w* mutation is caused by a parallel insertion of the copia retrotransposon into the second intron of the X-linked white gene (BINGHAM and JUDD 1981; BINGHAM, LEVIS and RUBIN 1981) (Figure 1). The abundance of normal white mRNA in w* is greatly reduced as compared with wild-type flies, resulting in a yellow-orange eye color phenotype, intermediate between the wild-type and null alleles of white. The w* allele is moderately overcompensated in males, conferring slightly more pigment (Figure 2).

Previous studies have delimited the homologies for most of the white-initiated RNA species (LEVIS, O’HARE and RUBIN 1984; ZACHAR et al. 1985). The mutant effect of copia in w* is premature termination of white-initiated transcripts in the 3’-LTR of copia. A low level of transcription, however, reads through the LTR termination signal to terminate at the normal...
3'-end of white. Splicing of this transcript removes introns, including the one containing copia, to yield the wild type mRNA of 2.6 kb. Other RNAs include a 2.4-kb transcript which initiates in the 3'-LTR of copia (ZACHAR et al. 1985), and several terminated within copia (MOUNT, GREEN and RUBIN 1988) and Figure 3 above. Figure 1 illustrates these RNA species diagrammatically.

The transcripts from \( w^a \) can be distinguished on northern blots using probes that lie 5' (E1) and 3' (E4-5) to the copia insertion (Figure 1). Therefore, we could detect transcripts initiated in the white promoter and terminated in copia (E1 probe) as well as those initiated in copia and terminated in white (E4-5 probe). Northern blot analysis using these probes onto total \( w^a \) RNA is shown in Figure 3 (panels A and B, lane 4). All RNA species characteristic of \( w^a \) are clearly present.

In addition to confirming the presence of transcripts described previously we have identified an additional \( w^a \) RNA species of 7.9 kb. It is detectable by the E4-5 probe but not by the E1 probe (Figure 3, panels A and B, lane 4), nor is it detected by an RNA probe specific for exon 2 (data not shown). Its size and hybridization pattern is consistent with its being copia-initiated, reading through the 3'-LTR, and terminating at the 3'-end of white. The structure of the 7.9-kb transcript was confirmed by northern analysis of two partial revertants of \( w^a \) that have secondary insertions in the 5'-LTR and the 3'-LTR of copia. See Table 1 for stock descriptions.

The \( w^\text{Rm} \) allele is caused by an insertion of 2.3 kb in the 5'-LTR of copia. The 7.9-kb transcript is absent in E4-5-probed blots of \( w^\text{Rm} \), while a band of greater molecular weight is present, consistent with the 2.3-kb insertion (Figure 3, panel B, lane 3). Similarly, the E1 probe detects the insertion in \( w^\text{Rm} \) as a shift upward of the copia-terminated transcript (Figure 3, panel A, lane 3).

The \( w^\text{Rsh} \) allele is caused by an 83-bp insertion in the 3'-LTR. Probe E4-5 reveals a transcript of slightly over 7.9 kb for \( w^\text{Rsh} \), consistent with the size of the
insertion (Figure 3, panel B, lane 5). Probe E1 also detects a slight increase in the copia-terminated RNA species (Figure 3, panel A, lane 5). These RNA profiles show that the 7.9 kb transcript contains the entire copia element, because its size is affected by insertions into either the 5'-LTR or the 3'-LTR of the copia element.

**Analysis of copia readthrough and wild-type white transcripts:** The copia-initiated readthrough transcript was used as a gauge of the level of expression of copia in $w^e$, separate from other copia transcription in the genome. Thus, analysis of this transcript provided an assay for dosage compensation of a single copia retrotransposon. Further, expression from the white promoter, upstream of copia, is detectable at 2.6 kb using the same probe. This allowed a comparison of expression levels from both LTRs of copia as well as the white promoter. By this approach it was tested whether the three tightly linked promoters are concordant in their responses to sex and genomic position.

To test for genomic position dependence we employed the TE89 strain (Ising and Ramel 1976), which bears a transposition of the entire $w^e$ and rough-$est^+$ loci to chromosome 3. The TE89 transposition was spontaneous, caused by two foldback elements which flank the insertion (Paro, Goldberg and Gehring 1983). The TE89 strain used in these experiments carries a deletion of the entire white locus (see MATERIALS AND METHODS). The phenotypes of $w^e$ and TE89 are shown in Figure 2. The white promoter continues to exhibit dosage compensation in TE89 males, resulting in their having darker eye color than females. Using TE89 we analyzed the same transcripts detected for $w^e$, but deriving from a distinct, autosomal position. The rationale is that any difference between the two strains in ability to dosage compensate could be attributed to sequences outside the limits of the TE89 transposon—i.e., a position dependent effect.

Northern blots of duplicate total RNA isolations, extracted in parallel from $w^e$ and TE89 larvae and adults were hybridized to the E4-5 probe (Figure 4). The 2.6-kb white-initiated and 7.9-kb copia-initiated transcripts were analyzed by scanning densitometry of autoradiograms. Male/female ratios for white- and copia-initiated transcript bands show that the separate experiments gave equivalent results (Table 2). Also, the band densities for white-initiated 2.6-kb RNA (functional white message) in $w^e$ and TE89 adults correlate with phenotypic eye pigment differences between the sexes (Figure 2). This indicates that the RNA blot technique used here will detect differences in RNA abundance within the twofold range predicted in dosage compensation experiments. The correlation of 2.6-kb message abundance to phenotype is possible because the $w^e$ mutation is hypomorphic, and small changes in mRNA concentration are reflected at the phenotypic level. The following are descriptions of the behavior of the two transcripts in various genetic conditions: male or female, larval or adult, and X-linked or autosomal.

The copia-initiated readthrough transcript is clearly present in both strains. A representative transcript profile is shown in Figure 4, after hybridization with probe E4-5. The transcript is visible in all $w^e$ channels

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**Table 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lesion</th>
<th>Reference</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w^{111X}$</td>
<td>Deletion of promoter, exon 1, into intron 1</td>
<td>Hazlerigg, Levis and Rubin (1984)</td>
<td>R. Levis</td>
</tr>
<tr>
<td>$w^e$</td>
<td>5.2-kb parallel copia insertion into intron 2</td>
<td>Bingham and Judor (1981), Gehring and Paro (1980)</td>
<td>Bowling Green*</td>
</tr>
<tr>
<td>$w^{658}$</td>
<td>2.3-kb insertion into 5'-LTR of copia in $w^e$</td>
<td>Mount, Green and Rubin (1988)</td>
<td>M. Green</td>
</tr>
<tr>
<td>$w^{234}$</td>
<td>83-bp insertion into 3'-LTR of copia in $w^e$</td>
<td>Mount, Green and Rubin (1988)</td>
<td>L. Rabnow</td>
</tr>
<tr>
<td>TE89</td>
<td>Transposition of $w^e$ and $est^+$ to chromosome 3</td>
<td>Ising and Ramel (1976)</td>
<td>G. Ising</td>
</tr>
</tbody>
</table>

* Bowling Green Drosophila Stock Center, Bowling Green, Ohio.
The abundance of functional white message is consistent with the phenotypes of $w^a$ and TE89 adults abundantly in larvae than adults (PARKHURST and CORCES 1987). This indicates that the dos- age effect of dosage compensation of $w^a$ is not a generalized sexual dimorphism of copia expression, but is specific to this insertion. Also, the developmental profiles of $w^a$ and TE89 differ for the copia readthrough transcript (Figure 4), but this difference is not reflected in overall copia RNA, which is more abundant in larvae than adults (PARKHURST and CORCES 1987).

The abundance of functional white message is consistent with the phenotypes of $w^a$ and TE89 adults. The male/female ratio of this transcript in W" is not a generalized sexual dimorphism of copia expression, but is specific to this insertion. Also, the developmental profiles of $w^a$ and TE89 differ for the copia readthrough transcript (Figure 4), but this difference is not reflected in overall copia RNA, which is more abundant in larvae than adults (PARKHURST and CORCES 1987).

**TABLE 2**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Stage</th>
<th>Transcript</th>
<th>$n$</th>
<th>Male/Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>$w^a$</td>
<td>Larval</td>
<td>copia-initiated</td>
<td>3</td>
<td>0.58 ± 0.09*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>white-initiated</td>
<td>3</td>
<td>1.41 ± 0.08</td>
</tr>
<tr>
<td>Adult</td>
<td>copia-initiated</td>
<td>3</td>
<td>1.10 ± 0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>white-initiated</td>
<td>3</td>
<td>1.28 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>TE89</td>
<td>Larval</td>
<td>copia-initiated</td>
<td>3</td>
<td>0.87 ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>white-initiated</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>copia-initiated</td>
<td>3</td>
<td>1.11 ± 0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>white-initiated</td>
<td>3</td>
<td>1.87 ± 0.22*</td>
<td></td>
</tr>
</tbody>
</table>

Band densities were measured by scanning laser densitometry (see MATERIALS AND METHODS). Male/Female ratios are means (± SD) of $n$ number of ratios, obtained by scanning multiple northern blot autoradiograms of the type shown in Figure 4. Band density values were adjusted for loading differences prior to determining ratios. A ratio for TE89 larval white-initiated expression is not shown due to the rarity of that RNA species (Figure 4, lanes 1 and 2). ND = no data. * The indicated ratios were determined to differ from 1.0 with greater than 95% confidence.

(lanes 5–8). It is not dosage compensated in $w^a$ larvae, in which females show a greater amount; however, it is compensated in $w^a$ adults in which levels are equivalent. The male/female ratio of this transcript in $w^a$ larvae differs from 1.0 with greater than 95% confidence (Table 2). In contrast, in TE89 the same transcript is equally expressed in males and females at both stages, showing that copia fails to dosage compensate in the autosomal position (Table 2). In TE89 larvae the 2.6-kb message is very low compared to $w^a$ larvae. This strong position effect prevented an accurate analysis of this transcript in TE89 larvae.

The 3'-LTR-initiated species at 2.4-kb also exhibits a position dependence. This transcript is found at high levels only in larval males of the $w^a$ strain (Figure 4, lane 5), with other classes exhibiting levels insufficient to accurately measure. Thus, the two identical LTRs of copia differ greatly in their expression patterns. Also, in TE89 a position dependence for the 3' LTR is detected in that the 2.4-kb transcript is present in very low abundance in larvae or adults of either sex.

A separate test of the ability of copia to dosage compensate was done by analyzing RNAs from males and females which each carried only one copy of $w^a$. In this case, dosage compensation would be revealed by a difference between males and females. The cross, $w^{118}$ males (a deficiency mutant of white) to $w^a$ females yielded progeny with only one copy of $w^a$ in both sexes. For these progeny, the prediction is that the male/female ratio would be one if the copia in $w^a$ does not compensate, and two if it does compensate. A greater-than-expected amount of compensation for both sexes was observed; however, in accordance with the above results, a significant level of dosage compensation was observed only in adults (Table 3).

**DISCUSSION**

These results show that three promoters in very close proximity respond to a new chromosomal position in three different ways. copia is uncoupled from...
white in its ability to dosage compensate, both from the perspective of developmental stage as well as genomic position. Given the very close association of the transcription units, this uncoupling emphasizes promoter dependence in dosage compensation; however, the importance of genomic position is also demonstrated by the failure of copia to compensate at an autosomal position.

copia is sensitive to developmental and genomic changes which do not affect the white promoter. Other workers have shown that white, positioned ectopically in P element constructs, exhibits dosage compensation whether linked to the X or an autosome (HAZELRIGG, LEVIS and RUBIN 1984). Similarly, we found that the 2.6-kb white-initiated transcript exhibited no marked position dependence in adults for sex-specific expression i.e., adult males consistently expressed white at twice the rate per gene dose as adult females. In contrast, copia exhibits a strong position dependence for sex-specific expression. Sexually dimorphic expression of copia in w" adults differs from TE89 where copia does not dosage compensate. Thus, one transcription unit within another can respond differently to genomic position in a sex-specific fashion. From this, it is clear that promoter specific factors are important in a gene’s ability to dosage compensate. A mechanistic whereby a molecule or chromatin conformation spreads along the chromosome, and is solely responsible for the positional determination of dosage compensation, is doubtful in light of these results.

The developmental specificity, whereby the copia-initiated transcription shifts to compensation in w" adults suggests that control of dosage compensation is sufficiently complex to involve a temporal shift in some part of the mechanism. Two other cases of developmental dependence in dosage compensation were shown by relocation experiments of the Adh (GOLDBERG, POSAKONY and MANIATIS 1983) and Ddc (SCHOLNICK, MORGAN and HIRSH 1983) genes. Dosage compensation of copia is affected by genetic background (compare Tables 2 and 3), but the developmental shift remains intact, suggesting that the temporal effect is separable from the genetic background effect.

copia has a differential ability to dosage compensate depending on genomic location, whereby in w" adults it does compensate, and in TE89 adults it does not. This result was unexpected because the TE89 transposon is large, spanning two polytene bands (ISING and BLOCK 1981). One possibility is that genetic background differences between the two strains accounts for the difference. Another is that the X chromosome contains cis determinants for dosage compensation a large distance from the white regulatory region which influence copia to dosage compensate in w" adults. Alternatively, the autosomes may contain sequences which inhibit copia from responding to dosage compensation signals.

For reasons proposed earlier in this report, transposable elements might not be expected to contain cis-acting dosage compensation determinants. If copia carries no such sequences, the fact that it can dosage compensate would suggest that all genes have the intrinsic ability to respond to the compensation mechanism, but are secondarily augmented by promoter-specific cis determinants.

Other workers have proposed cis-acting sequences to explain the maintenance of dosage compensation for chromosomal transpositions involving large portions of the X to autosomes, and also to explain the sensitivity of smaller portions (i.e., single gene transformants) to position effects [LUCCHESI and MANNING (1987) and references therein]. Dosage compensation may result from interactions of short-range and long-range cis determinants, as evidenced here by the limits of the TE89 transposon defining short-range determinants, and outside sequences on the X or chromosome 3 defining long-range determinants. Our results suggest that such sequences, as well as developmental stage and genetic background, may be differentially interpreted by individual promoters within a very limited distance.

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