Copyright © 1992 by the Genetics Society of America

Dosage Compensation of the *copia* Retrotransposon in *Drosophila melanogaster*

John C. Hiebert and James A. Birchler[1]

The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Manuscript received September 11, 1991
Accepted for publication November 15, 1991

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-apricot* (w^a) 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^a 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 BEERMANN 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^e) 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^e 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

[1] Current address: Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211.

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 transposable elements may be present via recent interspecific horizontal transfer (MOUNT and RUBIN 1985), and 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^a) 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^a allele to chromosome *3* (*TE89*) permitted an identical analysis when w^a 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^a 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⁻ rst⁻/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 ml/g 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 2202 Ultroscan 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 *Sal*I fragment containing exons 4 and 5 was inserted into IBI 76 to make pIBI 12.3 SS (probe E4-5). Similarly, a 1267-bp *Hin*dIII/*Bam*HI fragment containing exon 1 was inserted into IBI 76 to make pIBI 11.5 HB (probe E1). Both constructs were transcribed *in vitro* from the T7 promoter to make ^{32}P-labeled antisense RNA probes. A ^{32}P-labeled antisense β_1-*tubulin* probe served as a loading control (BIALOGAN, FAULKENBURG and RENKAWITZ-POHL 1985).

RESULTS

Identification of a marked *copia* element in w^a: The w^a 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^a 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^a 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^a 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

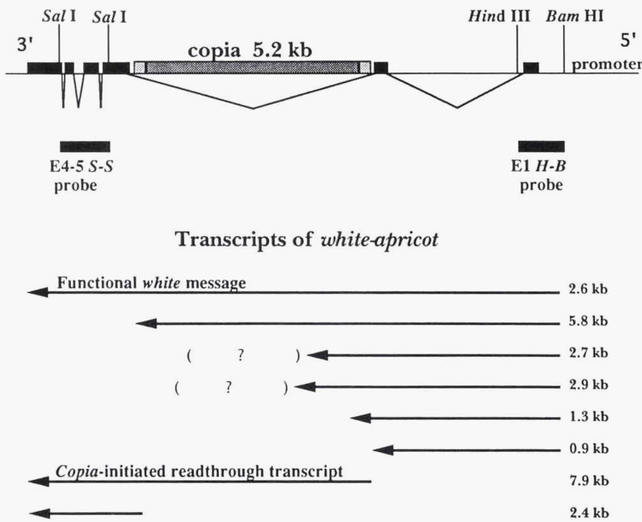

FIGURE 1.—Genomic map and transcripts of w^a. Selected restriction sites and positions of the probes used in the Northern analysis are shown.

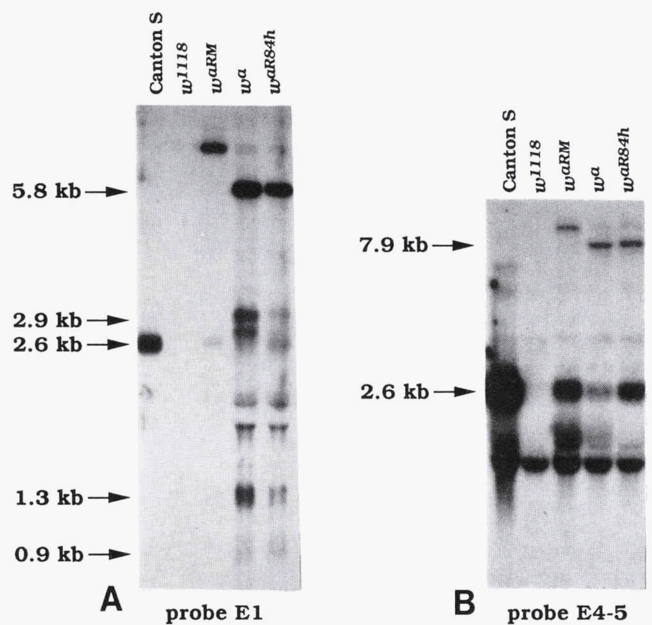

FIGURE 3.—Identification of the *copia*-initiated readthrough transcript by northern analysis of w^a and revertants. The genotype is indicated above each lane. The RNA probe used is indicated below each panel. All hybridizing transcripts are diagrammed in Figure 1. The *copia*-initiated/*white*-terminated readthrough RNA is 7.9 kb in w^a and is detected with only the E4-5 probe. Insertions in the LTRs of *copia* (w^{aRM} and w^{aR84h}) affect the mobility of the transcript. The hybridization near 2.1 kb (B) is a male-specific transcript, not deriving from the *white* gene, as evidenced by its presence in the deficiency strain w^{1118}. The absence of hybridization just below 2.2 kb is due to the prevalence of ribosomal RNA at this molecular weight.

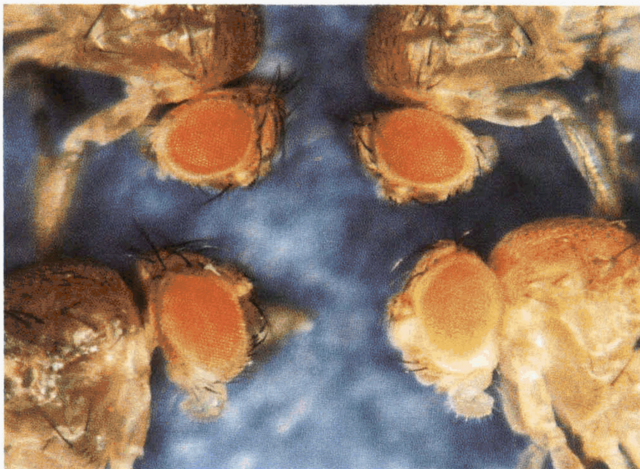

FIGURE 2.—Phenotypes of w^a and *TE89*. On the left are w^a/Y males (top) and w^a/w^a females (bottom). On the right are *TE89/TE89* males (top) and *TE89/TE89* females (bottom). Flies were reared at 25° and aged 4 days before photographing.

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^{aRM} 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^{aRM}, 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^{aRM} as a shift upward of the *copia*-terminated transcript (Figure 3, panel A, lane 3).

The w^{aR84h} 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^{aR84h}, consistent with the size of the

TABLE 1

***white* alleles used in this study**

Genotype	Lesion	Reference	Source
w^{1118}	Deletion of promoter, exon 1, into intron 1	HAZELRIGG, LEVIS AND RUBIN (1984)	R. Levis
w^{a}	5.2-kb parallel *copia* insertion into intron 2	BINGHAM AND JUDD (1981), GEHRING AND PARO (1980)	Bowling Green[a]
w^{aRM}	2.3-kb insertion into 5′-LTR of *copia* in w^a	MOUNT, GREEN AND RUBIN (1988)	M. GREEN
w^{aR84h}	83-bp insertion into 3′-LTR of *copia* in w^a	MOUNT, GREEN AND RUBIN (1988)	L. RABINOW
TE89	Transposition of w^a and rst^+ to chromosome *3*	ISING AND RAMEL (1976)	G. ISING

[a] Bowling Green Drosophila Stock Center, Bowling Green, Ohio.

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^a, 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^a and *roughest*$^+$ 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^a 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^a, 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^a 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

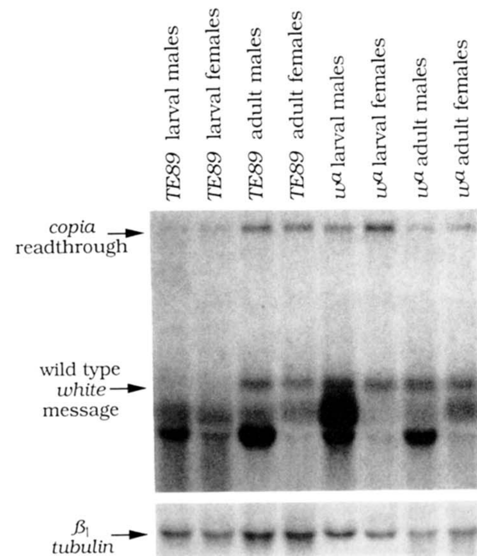

FIGURE 4.—Dosage compensation of the *copia*-initiated readthrough transcript. The RNA probe used was E4-5. All hybridizing transcripts are diagrammed in Figure 1 and shown in Figure 3. The wild type message (*white*-initiated) is indicated at 2.6 kb. The 5′-LTR *copia* readthrough transcript (*copia*-initiated) is indicated at 7.9 kb. The 3′-LTR *copia*-initiated transcript (2.4 kb) is present just below wild type in w^a larval males.

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^a 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^a 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^a channels

TABLE 2

Relative abundance of *copia*-initiated and *white*-initiated transcripts in *white-apricot* and *TE89*

Strain	Stage	Transcript	n	Male/Female
w^a	Larval	*copia*-initiated	3	0.58 ± 0.09*
		white-initiated	3	1.41 ± 0.08
	Adult	*copia*-initiated	3	1.10 ± 0.29
		white-initiated	3	1.28 ± 0.13
TE89	Larval	*copia*-initiated	3	0.87 ± 0.07
		white-initiated	ND	
	Adult	*copia*-initiated	3	1.11 ± 0.19
		white-initiated	3	1.87 ± 0.22*

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 (Figure 4, lanes 1–4). If the dosage compensation response of copia were intact in *TE89* then males would exhibit twice the expression level as females, because both male and female *TE89* flies have two copies of w^a.

The difference in abundance of the *copia* readthrough transcript in males and females is not attributable to a *copia*-specific effect. Previous studies of *copia* expression in w^a adults (BIRCHLER and HIEBERT 1989; BIRCHLER, HIEBERT and RABINOW 1989), and *copia* RNA levels in w^a and *TE89* larvae and adults (our unpublished data) show that total *copia* RNA levels are equal between males and females at both stages. *copia* transcripts are found in the midgut and fat body of larvae and adults, and not in the testis (MCDONALD *et al.* 1988), consistent with equivalent expression between sexes. This indicates that the dosage effect and dosage compensation of the w^a *copia* 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 (Figure 2). The band intensity of the 2.6-kb transcript is greater in w^a males than in females (Figure 4, lanes 7 and 8, and Table 2); thus, the *white* gene is slightly overcompensated in adult males at the RNA level. A dimorphism is found in *TE89* for the 2.6-kb transcript. Adult males express it at about twice the level of females (Figure 4, lanes 3 and 4, and Table 2), consistent with the phenotype of *TE89* flies (Figure 2, right). Thus, dosage compensation for *white*-initiated transcription is maintained in the autosomal position in adults, consistent with previous accounts of relocations of the *white* locus. The male/female ratio of this transcript differs from 1.0 with greater than 95% confidence (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.

TABLE 3

Relative abundance of the *copia*-initiated readthrough transcript in w^a/Y males and their w^a/w^{1118} female siblings

Stage	n	Male/Female
Larval	3	1.59 ± 0.46
Adult	3	3.42 ± 0.41*

Band densities were measured as in Table 2.

* The indicated ratio was determined to differ from 1.0 with greater than 95% confidence.

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^{1118} 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^a 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 dusage compensate. A mechanism 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^a 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^a 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^a 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.

Research was sponsored by the National Science Foundation Grant DMB-8811175. J.C.H. thanks AMY CSINK for helpful discussions.

LITERATURE CITED

BIALOGAN, S., D. FAULKENBURG and D. RENKAWITZ-POHL, 1985 Characterization and developmental expression of β_1-*tubulin* genes in *Drosophila melanogaster*. EMBO J. **3:** 2543–2548.

BINGHAM, P. M., and B. H. JUDD, 1981 A copy of the copia transposable element is very tightly linked to the w^a allele at the *white* locus of *D. melanogaster*. Cell **25:** 705–711.

BINGHAM, P. M., R. LEVIS and G. M. RUBIN, 1981 Cloning of DNA sequences from the *white* locus of *D. melanogaster* by a novel and general method. Cell **25:** 693–704.

BIRCHLER, J. A., and J. C. HIEBERT, 1989 Interaction of the *Enhancer of white-apricot* with transposable element alleles at the *white* locus in *Drosophila melanogaster*. Genetics **122:** 129–138.

BIRCHLER, J. A., J. C. HIEBERT and L. RABINOW, 1989 Interaction of the *mottler of white* with transposable element alleles at the white locus in *Drosophila melanogaster*. Genes Dev. **3:** 73–84.

CHURCH, G. M., and W. GILBERT, 1984 Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:** 1991–1995.

COX, R. A., 1968 The use of guanidium chloride in the isolation of nucleic acids. Methods. Enzymol. **12:** 120–129.

GEHRING, W. J., and R. PARO, 1980 Isolation of a hybrid plasmid with homologous sequences to a transposing element of *Drosophila*. Cell **19:** 857–904.

GHOSH, S., R. N. CHATTERJEE, D. BUNICK, J. E. MANNING and J. C. LUCCHESI, 1989 The *LSP1-a* gene of *Drosophila melanogaster* exhibits dosage compepsation when it is relocated to a different site on the X chromosome. EMBO J. **8:** 1191–1196.

GOLDBERG, D. A., J. W. POSAKONY and T. MANIATIS, 1983 Correct developmental expression of a cloned alcohol

dehydrogenase gene transduced into the *Drosophila* germ line. Cell **34:** 59–73.

HAZELRIGG, T., R. LEVIS and G. M. RUBIN, 1984 Transformation of *white* locus DNA in *Drosophila*: Dosage compensation, *zeste* interaction, and position effects. Cell **36:** 469–481.

ISING, G., and K. BLOCK, 1981 Derivation-dependent distribution of insertion sites for a *Drosophila* transposon. Cold Spring Harbor Symp. Quant. Biol. **45:** 527–549.

ISING, G., and C. RAMEL, 1976 The behavior of a transposable element in *Drosophila melanogaster*, pp. 947–954 in *The Genetics and Biology of Drosophila*, Vol. 1B, edited by M. ASHBURNER and E. NOVITSKI. Academic Press, London.

KAISER, K., M. FURIA and D. M. GLOVER, 1986 Dosage compensation at the *Sgs-4* locus of *Drosophila melanogaster*. J. Mol. Biol. **187:** 529–536.

KORGE, G., 1981 Genetic analysis of the larval secretion gene *Sgs-4* and its regulatory chromosome sites in *Drosophila melanogaster*. Chromosoma **84:** 373–390.

KRUMM, A., G. E. ROTH and G. KORGE, 1985 Transformation of salivary secretion protein gene *Sgs-4* and its regulatory chromosome sites in *Drosophila*: stage- and tissue-specific regulation, dosage compensation, and position effect. Proc. Natl. Acad. Sci. USA **82:** 5055–5059.

LAURIE-AHLBERG, C. C., and L. F. STAM, 1987 Use of *P*-mediated transformation to identify the molecular basis of naturally occurring variants affecting *Adh* expression in *Drosophila melanogaster*. Genetics **115:** 129–140.

LEHRACH, H., D. DIAMOND, J. M. WOZNEY and H. BOEDTKER, 1977 RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry 16: 4743–4751.

LEVIS, R., K. O'HARE and G. M. RUBIN, 1984 Effects of transposable element insertions on RNA encoded by the *white* gene of *Drosophila*. Cell **38:** 471–481.

LUCCHESI, J. C., and J. E. MANNING, 1987 Gene dosage compensation in *Drosophila melanogaster*. Adv. Genet. **24:** 371–429.

MCDONALD, J. F., D. J. STRAND, M. R. BROWN, S. M. PASKEWITZ, A. K. CSINK and S. H. VOSS, 1988 Evidence of host-mediated regulation of retroviral element expression at the posttranscriptional level, pp. 219–234 in *Eukaryotic Transposable Elements as Mutagenic Agents*, edited by M. E. LAMBERT, J. F. MCDONALD and I. B. WEINSTEIN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MOUNT, S. M., M. M. GREEN and G. M. RUBIN, 1988 Partial revertants of the transposable element-associated suppressible allele *white^{apricot}* in *Drosophila melanogaster*. Genetics **118:** 221–234.

MOUNT, S. M., and G. M. RUBIN, 1985 Complete nucleotide sequence of the *Drosophila* transposable element copia: homology between copia and retroviral proteins. Mol. Cell. Biol. **7:** 1630–1638.

MUKHERJEE, A. S., and W. BEERMANN, 1965 Synthesis of ribonucleic acid by the X-chromosomes of *Drosophila melanogaster* and the problem of dosage compensation. Nature **207:** 785–786.

MULLER, H. J., B. B. LEAGUE and C. A. OFFERMANN, 1931-Effects of dosage changes of sex-linked genes, and the compensatory effects of other gene differences between male and female. Anat. Rec. Suppl. **51:** 110.

O'HARE, K., M. R. K. ALLEY, T. E. CULLINGFORD, A. DRIVER and M. J. SANDERSON, 1991 DNA sequence of the Doc retroposon in the *white-one* mutant of *Drosophila melanogaster* and of secondary insertions in the phenotypically altered derivatives *white-honey* and *white-eosin*. Mol. Gen. Genet. **225:** 17–24.

PARKHURST, S. M., and V. G. CORCES, 1987 Developmental expression of *Drosophila melanogaster* retrovirus-like transposable elements. EMBO J. **6:** 419–424.

PARO, R., M. L. GOLDBERG and W. J. GEHRING, 1983 Molecular analysis of large transposable elements carrying the *white* locus of *Drosophila melanogaster*. EMBO J. **2:** 853–860.

SASS, H., and M. MESELSON, 1991 Dosage compensation of the *pseudoobscura HSP82* gene and the *melanogaster Adh* gene at ectopic sites in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **88:** 6795–6799.

SCHOLNICK, S. B., B. A. MORGAN and J. HIRSH, 1983 The cloned dopa decarboxylase gene is developmentally regualted when reintegrated into the *Drosphila* genome. Cell **34:** 37–45.

SMITH, P. D., and J. C. LUCCHESI, 1969 The role of sexuality in dosage compensation in *Drosophila*. Genetics **61:** 607–618.

SPRADLING, A. C., and G. M. RUBIN, 1983 The effect of chromosomal position on the expression of the *Drosophila* xanthine dehydrogenase gene. Cell **34:** 47–57.

ZACHAR, Z., and P. M. BINGHAM, 1982 Regulation of *white* locus expression: the structure of mutant alleles at the *white* locus in *Drosophila melanogaster*. Cell **30:** 529–541.

ZACHAR, Z., D. DAVISON, D. GARZA and P. M. BINGHAM, 1985 A detailed developmental and structural study of the transcriptional effects of insertion of the copia transposon into the *white* locus of *Drosophila melanogaster*. Genetics **111:** 495–515.

Communicating editor: A. CHOVNICK