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Genetics

Transcription of the *white* locus in *Drosophila melanogaster*

(*zeste*/intron/transposable elements/gene regulation/mutational target)

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ABSTRACT **Genetic studies of the *white* locus have shown that it has a distal region where structural mutations occur and a proximal region where regulatory mutations occur. To better understand the molecular basis of this genetic organization we have analyzed *white* locus transcription. A 2.7-kilobase transcript comprising 0.0005% of poly(A)-RNA was detected in RNA prepared from pupae or adults. The structure of this transcript helps clarify some unusual genetic properties of the locus. There is a small 5′ exon separated from the majority of the sequences found in the mature RNA by an intron of ≈2.8 kilobases. This 5′ exon is from the proximal region of the locus, whereas the main body of the RNA maps to the distal region. The mutationally silent region between the proximal and distal regions corresponds to the large intron. We have identified the family and determined the exact location of a number of transposable element insertions within the locus. These results show that transposable element insertions within introns can be without phenotypic effect. We have also investigated the effect on the *white* transcript of the *zeste* mutation, which represses *white* locus expression as judged by eye color phenotype. The RNA was unchanged in size or abundance in poly(A)-RNA from adult flies. This demonstrates that the *zeste–white* interaction does not occur by simply repressing transcription of the *white* locus in all tissues.**

The *white* locus of *Drosophila melanogaster* has long been the subject of intensive genetic studies (for a review see ref. 1). Mutations in *white* affect the degree of pigmentation of the adult eye, ocelli, and testis sheath and of the larval Malpighian tubules (2). The brick-red eye color of wild-type flies is due to the accumulation of both ommochrome (brown) and pteridine (red and yellow) pigments. Some *white* mutations, including deletions for the entire locus, result in the lack of all pigmentation, whereas other mutations reduce the amounts of one or both classes of pigment.

A number of different sites where mutations occur within the *white* locus have been separated by recombination and ordered on a genetic map of the locus (3, 4). These sites have been divided into distal and proximal regions with respect to the centromere, where the distal region is defined as including the site of the *white-apricot* (w^a) mutation and those mutations mapping distal to it (3). The DNA sequences for the wild-type locus (5, 6) and a number of mutants associated with insertions (6–11) have been isolated and characterized. The physical map constructed from these studies correlates well with the genetic map and shows in addition that the genetically defined distal and proximal regions are physically separated by some 3 kilobases (kb) in which no mutations map.

Genetic studies have led to the suggestion that the distal region contains structural sequences, whereas the proximal domain contains regulatory sequences (11–14). Most null mutations (3, 15, 16) and apparent point mutations (11) map to the distal region. A series of mutations associated with insertions into or deletions of the proximal region (11), the *white-spotted* mutations (w^{sp}, w^{sp2}, w^{sp3}, and w^{sp4}), have a mottled pattern of eye pigmentation. These mutations also partially complement many other *white* mutations (17). Other mutations in the proximal region affect the *zeste–white* interaction (see below) and dosage compensation (3). Proximal mutations have been proposed to affect *white* locus expression by altering a proximal transcript that regulates the production of a structural distal transcript (18) or by disrupting sequences that act at a distance to affect transcription of distal sequences (11).

The unusual interaction of the *zeste* (*z*) locus (19) with *white* illustrates how proximal and distal mutations have different effects on the regulation of *white* locus expression. Flies carrying the *zeste-1* (z^1) allele and two copies of the normal *white* locus (e.g., $z^1\ w^+/z^1\ w^+$) lack all pteridine pigments and have reduced amounts of ommochromes, producing zeste (lemon-yellow) colored eyes (19). Flies having only one copy of the *white* locus [e.g., $z^1\ w^+$/Y or $z^1\ w^+/z^1\ Df(w)$] have wild-type colored eyes. Some mutants mapping in the proximal region of *white* block the repression that *zeste* exerts on *white* (3). For example, $z^1\ w^+/z^1\ w^h$ flies have wild-type colored eyes. Thus, these proximal mutations are said to suppress the effect of z^1 on the expression of the *white* locus. In contrast, females heterozygous for a mutation in the distal portion of the locus (e.g., $z^1\ w^+/z^1\ w^a$) have zeste colored eyes and thus these mutations are not *zeste* suppressors.

In this paper, we identify a *white* locus transcript and map the DNA sequences coding for it with respect to the genetic and physical maps of the locus. We also investigate transcription of *white* in flies whose eye pigmentation has been reduced by the *zeste* mutation.

MATERIALS AND METHODS

D. melanogaster strains (Table 1) were raised at 25°C. Flies were collected 0–24 hr after eclosion and stored at −70°C. Total RNA was prepared from 0.5 to 1 g of flies by homogenization in a mixture of 10 ml of buffer (0.15 M Na acetate/5 mM EDTA/1% $NaDodSO_4$/50 mM Tris·HCl, pH 9/20 μg of polyvinyl sulfate per ml), 5 ml of phenol, and 5 ml of chloroform/isoamyl alcohol, 100:1 (vol/vol), by using a Tekmar homogenizer. The homogenate was extracted several times with phenol/chloroform and then precipitated with ethanol. The RNA was further deproteinized and poly(A)-containing RNA was isolated as described (21).

RNA was analyzed by electrophoresis on agarose gels containing formaldehyde (22) and was transferred to nitrocellulose

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Abbreviation: kb, kilobase(s).

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Table 1. *D. melanogaster* strains

Genotype	Phenotype	Source	Refs.
w^+/w^+ (P2 strain)	Brick-red	A. Spradling	
w^+/Y (P2 strain)	Brick-red	A. Spradling	
$z^1\ w^+/z^1\ w^+$	Yellow	W. Gelbart	19
$z^1\ w^+$/Y	Brick-red	W. Gelbart	19
w^{rdp+}/w^{rdp+}	Brick-red	B. Judd	18, 20
w^{rdp+}/Y	Brick-red	B. Judd	18, 20
$z^1\ w^{rdp+}/z^1\ w^{rdp+}$	Yellow	W. Gelbart	18, 20
$z^1\ w^{rdp+}$/Y	Yellow	W. Gelbart	18, 20
w^{DZL}/w^{DZL}	Yellow	P. Bingham	14
w^{DZL}/Y	Red-brown	P. Bingham	14
$z^1\ w^{DZL}/z^1\ w^{DZL}$	Yellow	P. Bingham	14
$z^1\ w^{DZL}$/Y	Yellow	P. Bingham	14

(23). The blots were hybridized with double-stranded probes prepared by nick-translation of plasmid subclones of the *white* locus or with strand-specific probes prepared from M13 subclones. To make strand-specific probes, a 15-base-pair primer (New England BioLabs) was hybridized with the single-stranded M13 subclone template and the primer was extended as described (24), except that no dideoxynucleotides were present. *Escherichia coli* DNA polymerase I Klenow fragment was used at 30 units/ml with [α^{32}P]dATP (400 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) at 1 μM and the other three dNTPs at 50 μM for 15 min at room temperature. After adding unlabeled dATP to 50 μM, incubation was continued for 15 min. The reaction products were then treated with an appropriate restriction endonuclease to release a single-stranded DNA whose 5′ and 3′ ends are defined by the primer and the restriction enzyme used. The strand-specific probes were then purified by electrophoresis on an alkaline agarose gel followed by elution by using an NA-45 DEAE membrane from Schleicher & Schuell (25). These single-stranded probes gave extremely low backgrounds and approximately a 5-fold stronger signal than corresponding double-stranded probes labeled by nick-translation, even when 1/10th as much radioactivity was used.

Filters were pretreated with 5× concentrated SET buffer (SET buffer: 0.15 M NaCl/0.03 M Tris·HCl, pH 8.0/2 mM EDTA), 50% formamide, 5× concentrated Denhardt's solution (Denhardt's solution: 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone), 100 μg of denatured salmon sperm DNA per ml, and 0.5% $NaDodSO_4$ overnight at 42°C and then hybridized with probe (typically 2–5 × 10^5 cpm per ml) under the same conditions for 40 hr. After washing at room temperature with several changes of 2× concentrated SET buffer/2× concentrated Denhardt's solution/0.5% $NaDodSO_4$ and then with several changes of 0.5× concentrated SET buffer/0.5% $NaDodSO_4$, the filters were exposed to x-ray film at −70°C by using Cronex intensifying screens. The filters were sometimes rehybridized to other *white* locus probes or, as a control for the amount of RNA loaded, to nick-translated pDmA2 (26), a probe for actin mRNAs.

RESULTS AND DISCUSSION

Structure of the Wild-Type *White* Locus Transcript and Its Relationship to the Genetic Map. A physical map of the *white* locus (5) is shown in Fig. 1. We have analyzed RNA transcripts of this locus in a series of RNA blotting experiments. By using double-stranded probes from the distal portion of the locus (coordinates −3.2 to −0.8), a low abundance, poly(A)-containing RNA was detected. The direction of transcription for this RNA was determined to be proximal to distal by using strand-specific probes from the same region. Probes for the other strand (coordinates −3.2 to +6.6) gave no detectable hybridization.

Fig. 2 shows the results of hybridization with a series of probes that span the locus. The positions of the probes are shown in Fig. 1, where filled bars indicate hybridization and open bars indicate the absence of hybridization. Control experiments in Fig. 2 show that the absence of hybridization is significant. For example, the filters in lanes 4 and 6 that showed no hybridization with the probe used were shown to hybridize with a second probe in lanes 8 and 9, respectively. Furthermore, in some instances a filter from a DNA blot was included with a filter from a RNA blot to show that the probe was capable of detecting homologous sequences; lanes 14, 16, and 18 show DNA filters that were included with the RNA filters shown in lanes 15, 17, and 19, respectively.

The results show that a single transcript band hybridizes to probes from both distal and proximal regions and suggest that there is a small 5′ exon separated from the majority of the sequences found in the mature RNA by an ≈2.8-kb intron. We believe it highly unlikely that sequences outside the region analyzed (coordinates −3.2 to +6.6) contribute to the structure of the *white* locus transcript. We have isolated a cloned cDNA for the *white* locus from a library made from pupal mRNAs (gift of M. Goldschmidt-Clermont and D. S. Hogness). The sequence of this clone was determined and found to terminate in a polyadenylylation signal followed by a poly(A) tract. By comparison with the genomic DNA sequence (unpublished data), this positions the 3′ end of the *white* locus transcript at approximately coordinate −2.2. Gene transfer experiments done in collaboration with T. Hazelrigg have shown that sequences between coordinates −5.1 and +6.6 are sufficient for wild-type eye pigmentation, whereas studies of chromosomal rearrangements (5) indicate that the sequences required for wild-type expression must lie between −3.2 and +10.6.

We estimate that we could have detected hybridization of as little as 50 base pairs in these experiments, but we cannot exclude the existence of additional smaller exons. Small introns may have escaped detection; introns would only be identified by the lack of hybridization of a probe and this requires that the sequences of the probe be totally contained within the intron. Thus, although the distal sequences appear from this analysis not to be further interrupted by introns, the genomic DNA sequence (unpublished data) suggests that that there are at least two more small introns in this region.

The size of this transcript was determined to be ≈2.6–2.7 kb by comparison with single-stranded DNA markers and with actin mRNAs. Its abundance was estimated by densitometry of appropriate autoradiograms. By comparison with actin [assumed to be 2–5% of poly(A)-containing RNA (26)], the 2.7-kb transcript was estimated to be 0.0005–0.001% of total adult poly(A)-RNA. Assuming that DNA and RNA hybridize equally well under the conditions used, an independent estimate of abundance can be made from the experiment shown in lanes 16 and 17 of Fig. 2. A comparison of the signal for 1 μg of genomic DNA (in which the hybridizing fragment represents 0.001%) with the signal for 2 μg of poly(A)-RNA suggests that this transcript represents ≈0.0005% of the adult poly(A)-RNA. It was also detectable in preparations of poly(A)-RNA from embryos or pupae. Preliminary studies on a number of *white* mutations associated with insertions of transposable elements have shown that this transcript is absent and transcripts of altered structures are present (unpublished data).

The organization of the *white* transcript is consistent with the behavior of the locus as a mutational target. We have precisely positioned and identified by family a number of transposable element insertions in the *white* locus (see Fig. 1) in the course of DNA sequence studies (unpublished data). When aligned

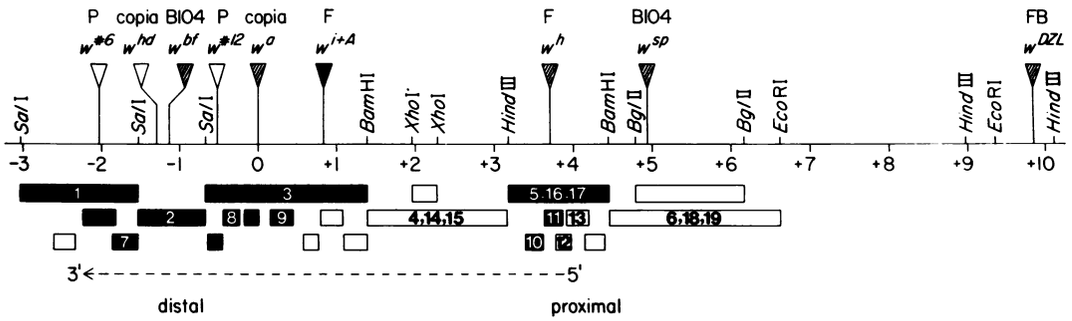

FIG. 1. Relationship of *white* locus transcript and transposable element insertions to the physical map. The positions of a number of insertions causing or associated with *white* mutations are shown on the restriction map of the locus. The coordinate system is that of Levis *et al.* (5), in which 1 unit corresponds to 1 kb. The DNA insertions have been cloned as part of previous studies in this laboratory [w^a (7); $w^{\#6}$, $w^{hd81b11}$ (abbreviated as w^{hd} in the figure), and $w^{\#12}$ (16); w^{i+A} (9); w^{DZL} (10)] and other laboratories [w^{bf}, w^h, and w^{sp} (11)]. We found that the map for w^{bf} given by Zachar and Bingham (11) was incorrect and that this insertion has the same map as a prototypical *B104* element (27). Insertions were classified by their restriction enzyme maps and terminal DNA sequences. The positions of insertion have all been determined to the nucleotide by DNA sequence analysis (unpublished data). The phenotypes (but not the size of the insertions) are indicated by the triangles: a solid triangle indicates that flies with this insertion have wild-type colored eyes; an open triangle indicates bleached white eyes; a shaded triangle indicates eyes with some degree of coloration. The allele and family of inserted element are shown above the positions of insertion. *B104* and *copia* are different families of *copia*-like elements. For more detailed descriptions of the various families see refs. 28 and 29. The bars show the extent of sequences in different probes used in RNA blotting experiments. Probes that hybridized to the 2.7-kb RNA are shown as solid bars; open bars show those probes that did not hybridize. The numbers on some of the bars show the lanes in Fig. 2 for which the probe was used. The probes were all M13 subclones of the genomic wild-type locus, except for one that was derived from a *white* locus cDNA (see text). Probes made from the M13 subclones shown would hybridize with transcripts whose direction of transcription was proximal to distal.

with the transcript, it can be seen that almost all of the insertions fall within the transcribed region (coordinates −2.2 to +3.7). Only the w^{sp} and w^{DZL} mutations definitely fall outside the region coding for the mature transcript. The extent of the proximal sequences that are found in the mature transcript (≈200–400 base pairs) in comparison with those from the distal region (≈2.5 kb) would make them a smaller target for mutational events and so explain the preponderance of distal mutations (3). It seems unlikely that many single base changes either in the large intron or in upstream regulatory sequences would produce a null allele, whereas most in the distal region would presumably alter the structure of the putative protein encoded by the *white* locus. This could explain why apparent point mutations appear to be limited to the distal region (11).

Despite their similar size, the major intron appears refractory to mutation by insertion of transposable elements when compared to the distal region of the locus. No mutations that alter eye color phenotype are found between w^a and w^h, whereas several insertion mutations are found in the distal region (see Fig. 1). This seems more likely to be due to the failure of such insertions to alter eye color phenotype rather than to the failure of transposable elements to insert into this region. Indeed, the one transposable element insertion known to lie within the major intron has no obvious phenotypic effect. This is the w^{i+A} allele, which was found as a phenotypically wild-type revertant of *white-ivory* (9). However, not all transposable element insertions within introns will be without phenotypic effect. The w^a mutation appears to be due to insertion of a *copia* element within one of the small introns of the distal region (unpublished data). Whether or not an insertion that falls within an intron has a phenotypic effect may depend on the nature of transcriptional control signals within the inserted element.

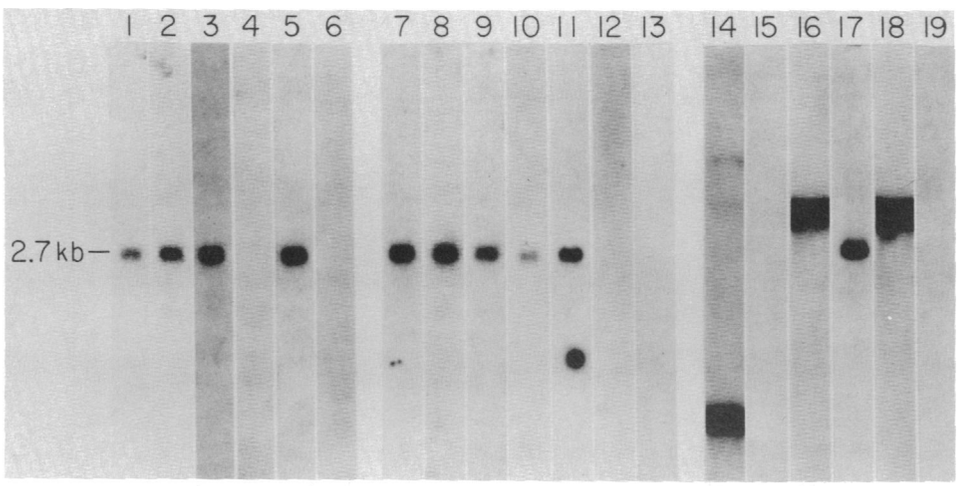

FIG. 2. RNA blot analysis of the wild-type *white* locus transcript. Lanes 1–13, 15, 17, and 19 show filters from a RNA blot in which aliquots of 2 μg of poly(A)-RNA from wild-type adult flies were transferred to nitrocellulose after electrophoresis on a formaldehyde/agarose gel. Lanes 14, 16, and 18 show filters in which 1-μg aliquots of a *Bam*HI/*Hin*dIII double-digest of Oregon R wild-type DNA were transferred to nitrocellulose after electrophoresis on an agarose gel. The *Bam*HI site at coordinate +4.5 is polymorphic and absent from Oregon R DNA (10, 11). The numbers of the lanes correspond to the numbers on the probes shown in Fig. 1. The filters in lanes 14 and 15, 16 and 17, and 18 and 19 were hybridized as pairs with an equal number of cpm of the indicated probes.

Zachar and Bingham (11) have shown that many *white* mutations with diverse effects on the level of pigmentation and on the degree of interaction with *zeste* are associated with different insertions into the *Hin*dIII–*Bam*HI interval (coordinates +3.2 to +4.5) where we have positioned the 5′ end of the RNA. These mutations include *white-spotted 55* (w^{sp55}), *white-zeste-mottled* (w^{zm}), and *white-1* (w^1) with its derivatives *white-honey* (w^h) and *white-eosin* (w^e) (2, 11). In contrast, mutations elsewhere in the locus can produce similar phenotypes even though they are associated with either different elements inserted at similar positions (e.g., w^a and w^{a4}) or both insertions into and deletions of the same region (e.g., w^{sp}, w^{sp2}, w^{sp3}, and w^{sp4}) (11).

The element inserted in w^h is related to the F family of transposable elements. Its terminal DNA sequence (unpublished data) has the poly(A) tract characteristic of F elements and shares extensive but interrupted homology with the terminal DNA sequence of a prototypical F element (29). The phenotype and properties of w^h contrast with the apparent absence of a phenotype for the F element inserted in w^{i+A}. Both w^h and w^e are spontaneous colored derivatives of the colorless *white-1* (w^1) mutant, apparently generated by internal rearrangement of the transposable element inserted in w^1 (11). The identification of these insertions as F elements shows that, as for *copia*-like transposable elements (30; reviewed in ref. 28), the expression of mutations associated with F-like elements may in some cases be modulated by unlinked genes. In this case, *enhancer of white-eosin* [$e(w^e)$] reduces the degree of pigmentation of the eyes of both w^h and w^e flies (3).

The Zeste Phenotype Does Not Correlate with Changes in *White* Locus Transcripts Isolated from Adult Flies. The effect of *zeste-1* on *white* locus expression was investigated by analysis of RNA prepared from wild-type males and females (z^+ w^+/Y and z^+ w^+/z^+ w^+) and *zeste-1* males and females (z^1 w^+/Y and z^1 w^+/z^1 w^+). Samples of poly(A)-RNA were analyzed by using a mixture of probes (coordinates −3.2 to +6.6) from that strand of the *white* locus shown to hybridize to the 2.7-kb RNA (Fig. 3). The same 2.7-kb RNA described above was detected in all of the samples. The filter was rehybridized with an actin probe to normalize for the amount of RNA loaded. It is clear that there is no large difference in the relative concentration of the 2.7-kb *white* transcript either between male and female flies or between wild-type and yellow-eyed flies. This comparison has been made by using two different preparations of RNA from z^1 w^+/Y and z^1 w^+/z^1 w^+ flies.

For the *zeste–white* interaction, two copies of the *white* locus have to be present (19) in close proximity in synapsed chromosomes (18, 31). Genetic studies indicate that only the proximal region is required to be present in two doses (20, 32). Thus, expression in males with tandem duplications of the proximal region (z^1 w^{rdp+}/Y) is repressed by *zeste* and the flies have yellow eyes (20, 32). The recent molecular characterization of such a tandem duplication (33) suggests that only sequences proximal to the *white-apricot* site (see Fig. 1) are required in two copies. A dominant mutation, *white-Dominant zeste-like* (w^{DZL}), has been described, which maps to the proximal end of the *white* locus and produces similar phenotypes to those seen with the *zeste-1* allele (14). This mutation acts in a synergistic fashion with *zeste-1*; though z^1 w^+/Y males have wild-type pigmentation and w^{DZL}/Y males have dark red-brown eyes, the double mutant z^1 w^{DZL}/Y males have yellow eyes.

Preparations of RNA were made from flies with various combinations of *zeste* and *white* alleles (Table 1) and their *white* locus transcripts were analyzed as above (Fig. 3). The 2.7-kb RNA was present in all of the samples. Its concentration in RNA from flies with zeste-colored eyes was not consistently lower than in RNA from wild-type flies. A smaller, much less abundant RNA was sometimes detected, but this RNA was not correlated with either genotype or phenotype and so it most likely represents a partial degradation product of the 2.7-kb RNA.

The phenotypic repression of *white* loci that are able to pair synaptically, but not of those that cannot pair, strongly suggests that *zeste* regulates the transcription of *white*, because other mechanisms would require *zeste* to distinguish between the products of paired and unpaired *white* loci (18). This dependence on the ability of chromosomes to pair in order to produce a phenotype is known as transvection and has been observed for other loci (31, 34–37). The results presented here show that there is no change in the size and no large change (if any) in the abundance of the *white* transcript we have identified when

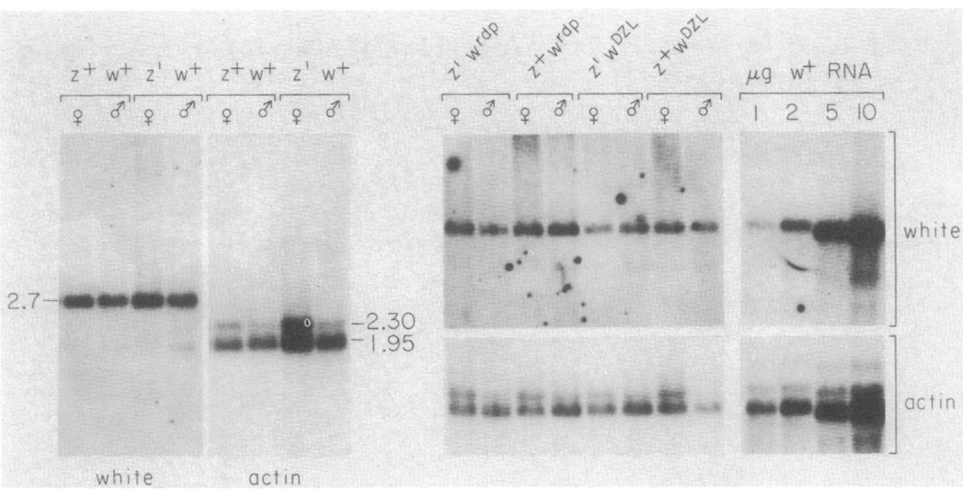

FIG. 3. The zeste phenotype does not correlate with changes in *white* locus transcripts from adult flies. Five-microgram aliquots of poly(A)-RNA from flies of the indicated genotype and gender were analyzed by RNA blotting. A range (1–10 μg) of poly(A)-RNA from mixed male and female wild-type flies was also analyzed (rightmost four lanes) as a control for the proportionality of autoradiographic signal to RNA concentration. The probe used to detect *white* locus transcripts was a mixture of probes 1–6 of Fig. 1. After exposure to x-ray film, the filters were then rehybridized with nick-translated plasmid pDmA2, a probe for actin mRNAs (26), to control for the amount of RNA loaded. The consistent difference in relative abundance of the 2.30- and 1.95-kb mRNAs between males and females probably occurs because these RNAs are present in ovaries of adult flies (26). Portions of the autoradiograms detecting both *white* and actin mRNAs are shown.

the zeste phenotype is produced by either z^1 or w^{DZL}. We consider our estimates for the abundance of the *white* transcript to be accurate to within a factor of 2. It is unlikely that a reduction in abundance of *white* transcripts within this margin of experimental error would be sufficient to account for the zeste phenotype. Female flies with only one copy of the *white* locus have lighter colored eyes than normal wild-type females and are presumed to have half as much *white* transcript. However, the effect on eye phenotype is much less pronounced than that of *zeste*. Moreover, in contrast to the effect of *zeste*, both eye pigments are present.

There could be several reasons for the apparent lack of an effect of *zeste* on *white* locus transcription. First, all of our experiments have analyzed poly(A)-RNA from adult flies and it is formally possible that unchanged expression of the *white* locus in other tissues is obscuring changes in transcription in the eye. Certainly there is genetic evidence that *white* expression is not limited to the eye (see *Introduction*). In particular, it should be noted that there is no apparent effect of *zeste* on the degree of pigmentation of Malpighian tubules or ocelli (19), suggesting that the interaction is indeed specific to the eye. However, z^1 affects primarily the accumulation in the eye of pteridine pigments (19), and these pigments do not appear to contribute to the coloration of Malpighian tubules and ocelli. Thus, z^1 might still act in these tissues without phenotypic effect. It is also possible that the effect on transcription is only apparent at earlier times in development and that the adult levels of transcription are unchanged. A detailed study of the developmental timing and tissue specificity of *white* locus transcription will be required to clarify these issues.

Another possible explanation is that there could be a subtle alteration in the structure of the *white* locus transcript when repressed by *zeste*. For instance, there might be a change in the splicing pattern such that the overall size of the spliced product remains unchanged. Further experiments on the structure of this transcript from flies with wild-type and zeste-colored eyes are necessary. In any case, it is clear that the phenotypic repression of *white* by *zeste* does not occur by a simple repression of *white* locus transcription in all tissues.

Note Added in Proof. Pirotta *et al.* (38) have recently reported a 2.4-kb poly(A)-RNA hybridizing to DNA probes from coordinates −3 to +1.6 of the *white* locus; this RNA is almost certainly the same as the transcript we report here.

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