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Expression of the *Drosophila white* gene under the control of the *hsp70* heat shock promoter

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We have used P-mediated transformation to introduce into the *Drosophila* genome transposons containing the *hsp-70* heat shock promoter (*hsp*) placed at different distances upstream of the *white* gene. Using these constructions we found a strong terminator 250 nucleotides upstream of the *white* transcription start. When all *white* upstream sequences are deleted and the *hsp* is fused to the *white* leader region, *white* transcription is under *hsp* control, however, flies carrying this construction have red eyes even at low temperature, due to the basal level of activity of the *hsp*. High levels of transcription are obtained after heat shock but about half of the transcripts are incompletely spliced. One transformed line carries the transposon integrated near the heterochromatin. These flies have very pale eyes but develop strongly pigmented, variegated eyes if heat shocked in the first 2 days of the pupal stage. Heat shocks after eclosion have no effect on eye pigmentation. The properties of these transposons also indicated that the control sequences required for *zeste* interaction lie >1 kb upstream of the *white* transcription start while those required for dosage compensation are close to the gene.

Key words: RNA processing/dosage compensation/*zeste* interaction/developmental specificity

Introduction

Genetic and molecular experiments show that the *white* gene is subject to several regulatory mechanisms that control its activity in different tissues at different developmental stages, its ability to be dosage compensated and its interaction with other loci such as *zeste* (Jack and Judd, 1979; O'Hare *et al.*, 1984; Pirrotta and Bröckl, 1984; Fjose *et al.*, 1984; Bingham and Zachar, 1985; Davison *et al.*, 1985). The telomere-proximal part of the locus, which is also the promoter-proximal end of the gene, is the site of mutations and rearrangements that affect these regulatory properties.

Germ line transformation studies have shown that transposons containing the *white* gene and 3 kb of its 5′-flanking sequence exhibit all the normal regulatory features (Hazelrigg *et al.*, 1984; Gehring *et al.*, 1984). To identify the sequence elements important for regulation, we have studied the effects of removing parts of the region preceding the promoter, most of the major intron and some of the 3′-flanking sequences (Pirrotta *et al.*, 1985). It remains possible that some regulatory effects, notably dosage compensation and *zeste* interaction, could be attributed to sequences in the transcribed part of the locus. In the work reported here, we removed completely the region preceding the start of transcription and placed the *white* gene under the control of an externally regulatable promoter. We chose for this purpose the *Drosophila hsp-70* heat shock promoter whose activity can be strongly induced by heat treatment in virtually all cells (Steller and Pirrotta, 1984; Bonner *et al.*, 1984). This promoter switch should reveal the importance of the 5′-flanking sequences for dosage compensation and *zeste* interaction. The inducibility of the *hsp-70* promoter should also allow us to determine the developmental period during which *white* expression is necessary for the pigmentation of the various structures. Finally, we expected that the strength of the *hsp-70* promoter would raise the abundance of the *white* mRNA and of *white* product from the very low normal levels [~0.0005% of poly(A)$^+$ RNA] to amounts that would facilitate a variety of biochemical studies.

Results

We have shown that a 456-bp DNA fragment of the *hsp-70* gene contains a fully functional and inducible heat shock promoter that includes 206 nucleotides of the untranslated leader sequence (Steller and Pirrotta, 1984). In our first attempt to place the *white* gene under heat shock control, we inserted this fragment, henceforth called the *hsp*, in the *Bgl*II site at position +4.8 in the map of the *white* gene (Figure 1), ~1 kb upstream from the normal transcription start site. The resulting construction, *Bg-hsp-w*, contains 9.4 kb of the *white* locus, from position +4.8 to −4.6.

It was assembled in the Carnegie-1 vector for P-mediated transformation (Rubin and Spradling, 1983) and injected into *Drosophila* embryos carrying a deletion of the *white* gene. Since the *Bg-hsp-w* transposon still contains ~1 kb of the regulatory region we expected that the *w* promoter would be at least partly active.

A number of independent transformed lines were derived from these experiments. They were detected by their various levels of eye pigmentation: some lines have very pale eyes, others range from orange to brown. None showed a temperature dependence. Flies raised at a constant temperature of 18° or 25°C and flies given periodic heat treatments every day of their life cycle had the same degree of pigmentation. In fact, the behavior of this transposon was identical to that of analogous transposons containing the same amount of *white* upstream sequences but lacking the *hsp* (Pirrotta *et al.*, 1985). From the lack of detectable temperature effect on pigmentation we concluded that the *white* expression in these transformed lines was due to residual activity of the *w* promoter and not to the *hsp*.

A strong terminator precedes the white promoter

The failure to obtain temperature-dependent pigmentation could be due to the lack of induction of the *hsp*, to inability to transcribe through to the *white* gene or to inefficient translation. We analysed the RNA produced by three independent transformed lines with or without heat treatment. Figure 2 shows a Northern blot of these RNAs hybridised with the HB7 probe (see Figure 3) which includes the first exon of the *white* gene as well as the normally

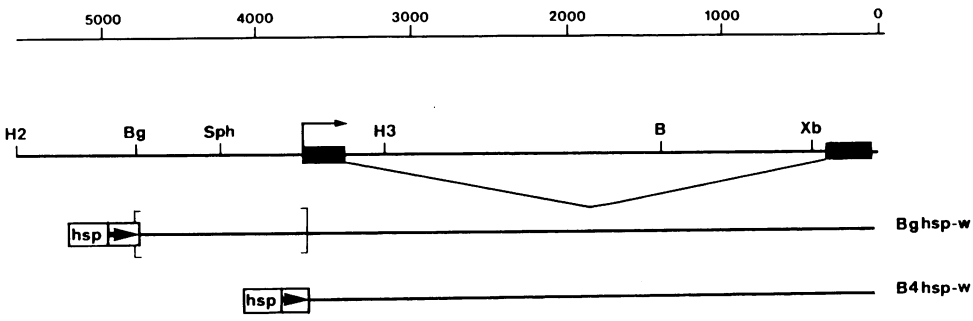

Fig. 1. The proximal part of the *white* gene and constructions used. Transcription, as determined in this paper, starts around position 3692 and its direction is shown by the arrow. Landmark restriction sites are indicated by: H2, *Hind*II; Bg, *Bgl*II; Sph, *Sph*I; H3, *Hind*III; B, *Bam*HI and Xb, *Xba*I. Only the first two exons, separated by the major intron, are shown. The *Bg-hsp-w* and *B4-hsp-w* constructions are shown below. For *Bg-hsp-w*, the heat shock promoter fragment was ligated at the *Bgl*II site. To produce *B4-hsp-w*, *Bg-hsp-w* was cut at the *Sph*I site and digested with *Bal*31 to the extent shown by the brackets.

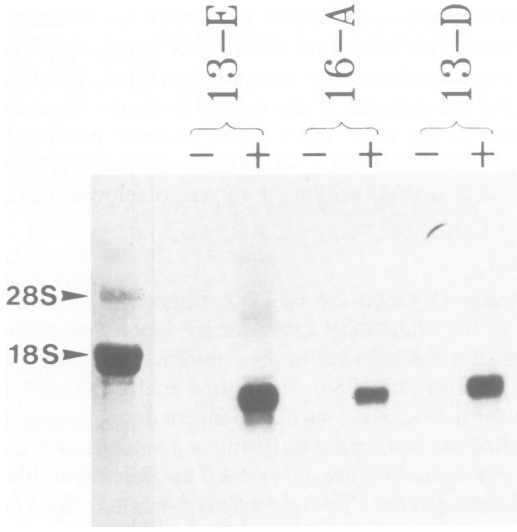

Fig. 2. Northern blot hybridisation of *Bg-hsp-w* RNA. Flies from three different lines transformed with the *Bg-hsp-w* transposon were heat shocked for 1 h at 37°C, their RNA extracted and one fly equivalent (~5 μg) was electrophoresed through a 1.2% agarose-formaldehyde gel. The RNA was blotted onto a nitrocellulose filter and hybridised with the HB7 probe (see Figure 3). The lane on the left shows total RNA stained with methylene blue, indicating the position of rRNA (3.6 kb and 1.9 kb). For each transformed line, the plus and minus lanes correspond to heat shocked and non-heat shocked flies.

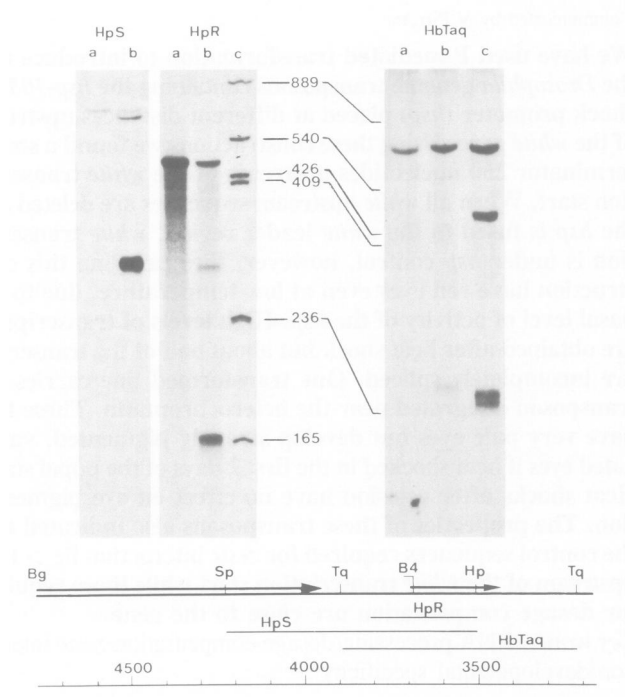

Fig. 3. S1 mapping. The diagram below shows the map fo the *Bgl*II-*Hind*III fragment from the proximal part of the *white* locus showing the extents of the different probes used. The entire fragment corresponds to the HB7 probe. The normal *white* transcription start and the first exon are indicated by the shorter arrow on the right. The longer arrow on the left shows the RNA produced from the *hsp-70* promoter in the *Bg-hsp-w* construction. The site of the *hsp-w* fusion in the *B4-hsp-w* transposon is indicated by B4 and the relevant restriction sites are marked Bg: *Bgl*II; Sp: *Sph*I; Tq: *Taq*I; Hp: *Hpa*II. The **left panel** shows the S1 mapping of *Bg-hsp-w* RNA extracted after heat shocking adult flies for 1 h at 37°C. **(a)** HpS probe + tRNA control, **(b)** HpS probe + *Bg-hsp-w* transposon DNA to localize the site of fusion. **(a)** HpR probe + tRNA control, **(b)** HpR probe + *B4-hsp-w* DNA, **(c)** molecular size marker. The **right panel** shows S1 mapping of the *white* transcription start **(a)** HbTaq probe + tRNA control, **(b)** HbTaq probe + 2 μg poly(A)$^+$ RNA from Canton S flies, **(c)** HbTaq probe + total RNA from *B4-hsp-w* flies after heat shock for 1 h at 37°C.

untranscribed region upstream of the *white* promoter which should be transcribed if *hsp* is activated. It is clear that heat treatment results in massive transcription of this region but that the RNA species detected is only ~1 kb long. This falls far short of the expected size of a read-through transcript: 2.5 kb wRNA + 1 kb upstream region + 0.2 kb *hsp* leader = 3.7 kb. Instead, it corresponds in length to an RNA which starts at *hsp* and stops just before the normal transcriptional start site of the *white* gene. In fact, the same filter shown in Figure 2, re-hybridised with probes from the distal part of the *w* gene, gave no detectable signal, indicating either that transcription is prematurely terminated or that the RNA is rapidly processed. The precise termination site is given by an S1 protection experiment with a *Hpa*II-*Sph* fragment (probe HpS) which yields a protected band of 278 nucleotides (Figure 3), locating the 3′ end of the RNA at position 3950 in the nucleotide sequence determined by O'Hare *et al.* (1984) and shown in Figure 5. An AATAAA sequence precedes this termination site by 24 nucleotides, the approximate distance usually found between the canonical cleavage-polyadenylation signal and the start of the poly(A) tract. Although we have not determined whether the RNA species produced is in fact polyadenylated, we suppose that the termination at this site is probably due to the normal polyadenylation machinery. A much fainter protected band of ~700 nucleotides visible in Figure 3 probably corresponds to transcripts that fail to terminate and continue through this site, through the first exon and into the first intron.

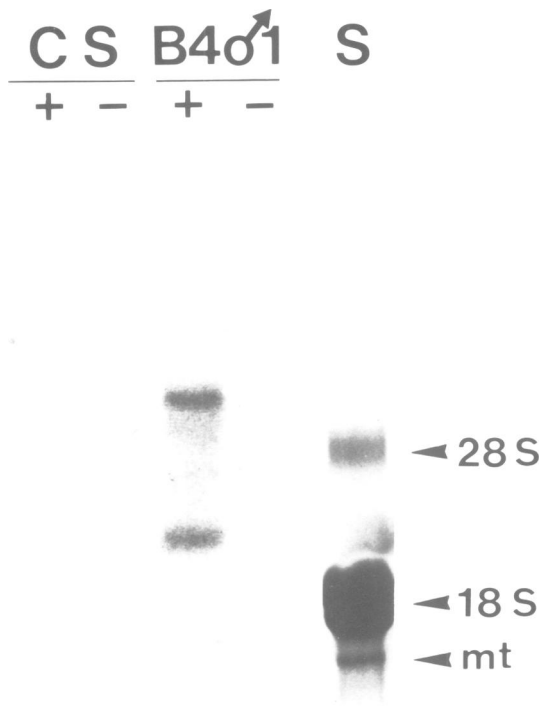

Fig. 4. Northern blot hybridisation of *B4-hsp-w* RNA. Wild-type Canton S flies (CS) and B4-1 flies (transformed with the *B4-hsp-w* transposon) were heat shocked for 1 h at 37°C and their RNA electrophoresed and blotted. The filter was hybridised with probe BS5 from the distal part of the *white* gene (Pirrotta and Bröckl, 1984). The plus and minus lanes correspond to heat shocked and non-heat shocked flies, respectively. **Lane S** represents total RNA stained with methylene blue and shows the 28S, 18S and mitochondrial RNA bands.

Removal of the terminator

Whatever its nature, it should be possible to remove the DNA sequence causing the block and at the same time bring the *white* coding sequence closer to the *hsp*. We achieved this by cleavage with *Sph*I and *Bal*31 digestion to obtain clones containing deletions of different extents in the region between *hsp* and the *white* transcription start. Restriction digests indicated that one of these clones, B4, should have deleted the entire *white* upstream sequence as well as a few nucleotides of the *white* untranslated leader.

When we reintroduced the B4 transposon into the fly genome, we were surprised to find that even when raised continuously at low temperature, all but one of the transformed lines showed very strongly pigmented eyes, virtually indistinguishable from wild-type flies. Heat treatment of these lines during development intensified the eye color but did not produce pigmentation in structures not normally pigmented. The exception is line B4-13 which at low temperature has nearly white eyes with a barely detectable yellow tinge. The presence of a few more darkly pigmented facets becomes noticeable with age. We will postpone for the moment a discussion of this line.

Heat shock-induced wRNA

To verify that we had removed the transcriptional block, we analysed the RNA produced by one of the red-eyed fly lines, B4-1, with or without heat shock treatment. Figure 4 shows a Northern blot of the total RNA extracted from a single fly per lane, hybridised with probe HB7 (see Figure 3) containing the first exon of the *white* gene. Because of the very low abundance of normal *white* mRNA, wild-type flies give no visible hybridisation with this probe. No signal was obtained with the transformed line at low temperature, indicating that, despite the red eyes, the *hsp* is not constitutively activated in these flies. After heat shock we detect two strong hybridising bands corresponding to RNA species of ~2.7 kb and ~6 kb. Neither Canton S control flies nor the pale B4-13 transformed line gave any detectable hybridisation after heat shock.

Of the two species produced by B4-1 after heat shock, the smaller RNA has the expected size of the *hsp-w* fusion transcript (2.5 kb from *white* + 0.2 kb from the *hsp* leader). The higher mol. wt. RNA species is of the size expected for an unspliced or partially spliced precursor which still contains the large first intron (3 kb) of the *white* gene.

In a 'chase' experiment in which the flies were returned to low temperature for various lengths of time after the heat shock, before their RNA was extracted, the higher mol. wt. band disappeared rapidly after returning to low temperature while the lower mol. wt. band persisted for several hours (not shown).

Although this experiment does not exclude the possibility that the higher band is simply degraded more rapidly, it suggests that during heat shock the splicing is incomplete either because the RNA is produced too rapidly, or because the splicing machinery is not fully active.

S1 mapping of the B4 RNA and of the normal white RNA

To understand the nature of these transcripts and to map their origin, we determined first the precise junction between *hsp* and *w* sequences in the B4 transposon. Figure 3 shows the fragment protected against S1 by the B4 DNA hybridised to the HpR probe. The band corresponds to a fragment of 166 nucleotides, placing the *hsp-w* fusion in B4 at position 3679 in the sequence. S1 mapping of the RNA isolated from the B4 line after heat shock shows two bands protected with the HbTaq probe. The lower band is just a few nucleotides shorter than the normal first exon (Figure 3, right hand panel).

This exon is normally 252 nucleotides long, not 310 as we originally reported (Pirrotta and Brockl, 1984). That length was based on an incorrect value of the mol. wt. marker used. Of the two bands protected by B4 RNA, we interpret the shorter one to represent the first exon, from the *hsp-w* junction to the end of the exon, while the longer one represents the unspliced RNA which continues into the first intron. These results allow us to position the normal transcription start of *w* RNA. Since the *hsp-w* junction is at position 3679 and the normal first exon is 10 nucleotides longer, we conclude that the normal start is at position 3689 ± 2. Figure 5 shows the nucleotide sequence of the *white* proximal region and summarises these results.

The S1 mapping confirms the splicing explanation for the two RNA species seen in the Northern hybridisation and shows that in the B4 transposon the entire promoter region is deleted up to and including the first few nucleotides of the normal *white* transcript. Since the entire *white* promoter is missing, we conclude that the *white* expression seen in these flies even without heat induction comes from the *hsp* and that even the uninduced level is sufficient to confer a red eye.

Dosage compensation and zeste interaction

Several mutations affecting dosage compensation and the interaction with *zeste* map in the regulatory region of the *white* gene. Hazelrigg *et al.* (1984) and Gehring *et al.* (1984) showed that *white* transposons including up to 3 kb upstream of the promoter are both dosage compensated and affected by *zeste*. We tested

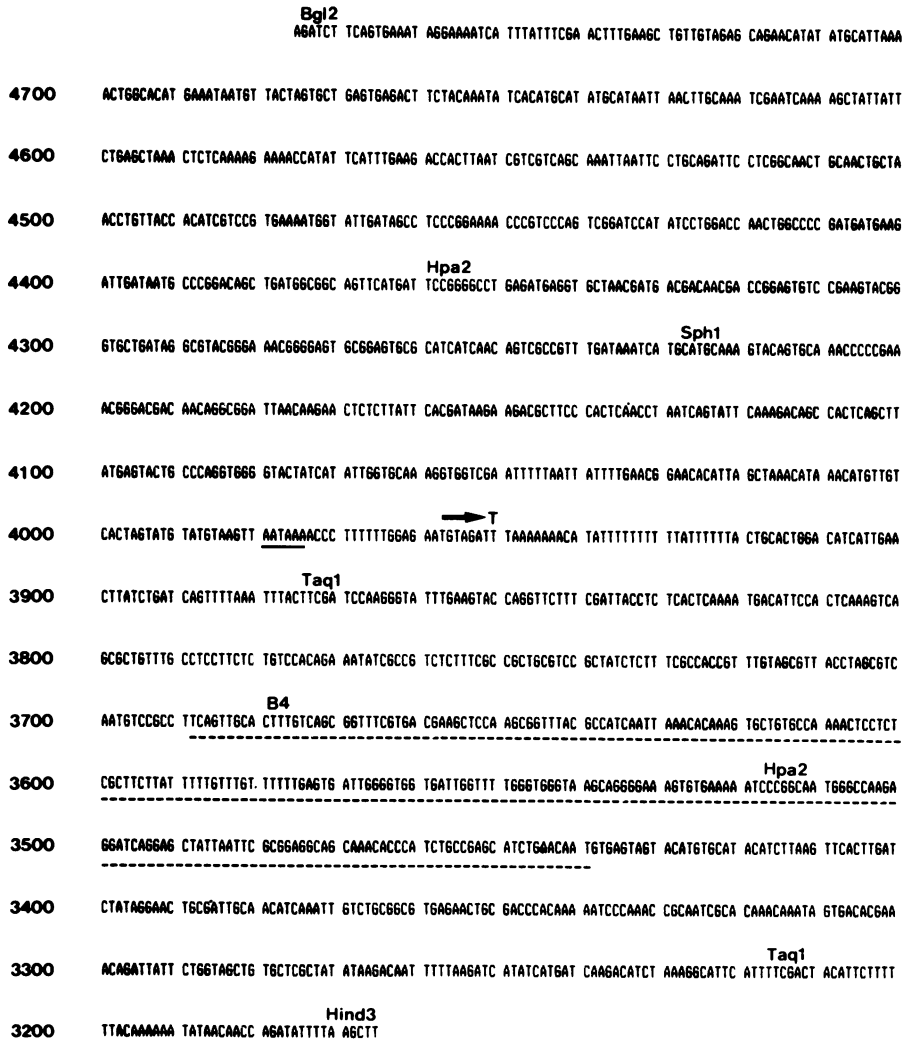

Fig. 5. Nucleotide sequence of the proximal part of the *white* locus. The sequence is taken from O'Hare *et al.* (1984) and the nucleotides are numbered according to their convention using the *copia* insertion site in the w^a mutant as the coordinate origin. The sequence shown is that of the *Bgl*II-*Hind*III fragment corresponding to the HB7 probe in Figure 1. Only the restriction sites relevant to this work are indicated. The arrow points to the termination site (T) of *Bg-hsp-w* RNA. The polyadenylation signal AATAAA preceding it is underlined. B4 marks the position of the *hsp-w* fusion in the B4 transposon and the dotted line marks the extent of the first exon with an uncertainty of ± 2 nucleotides.

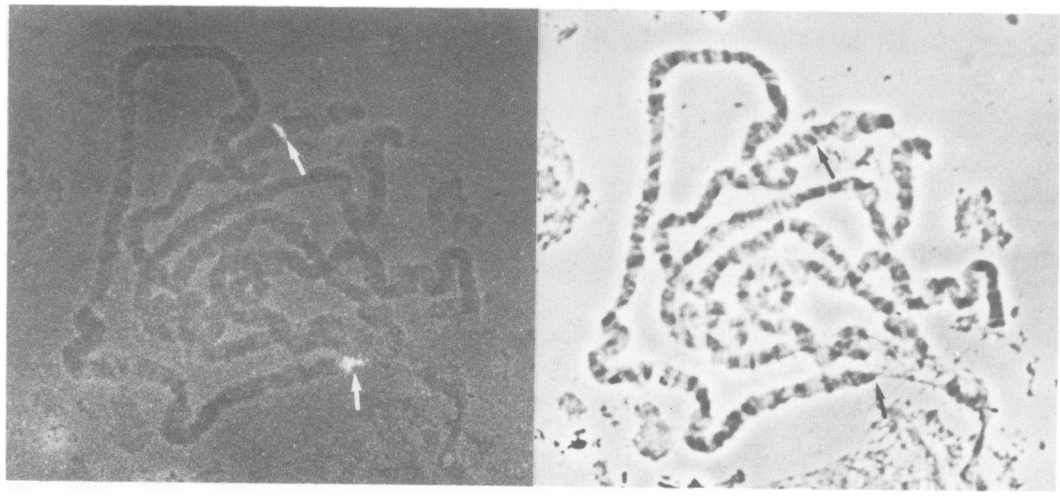

Fig. 6. *In situ* localization of the transposon in the B4-13 line. A salivary gland chromosome squash from B4-13 larvae was hybridised with *B4-hsp-w* transposon DNA labeled with biotinylated dUTP (Langer *et al.*, 1981). The hybridised DNA, detected by indirect immunofluorescence, is found near the base of chromosome 3R. A signal at 3C on the X chromosome is due to residual *white* sequences in the w^{67c23} host strain.

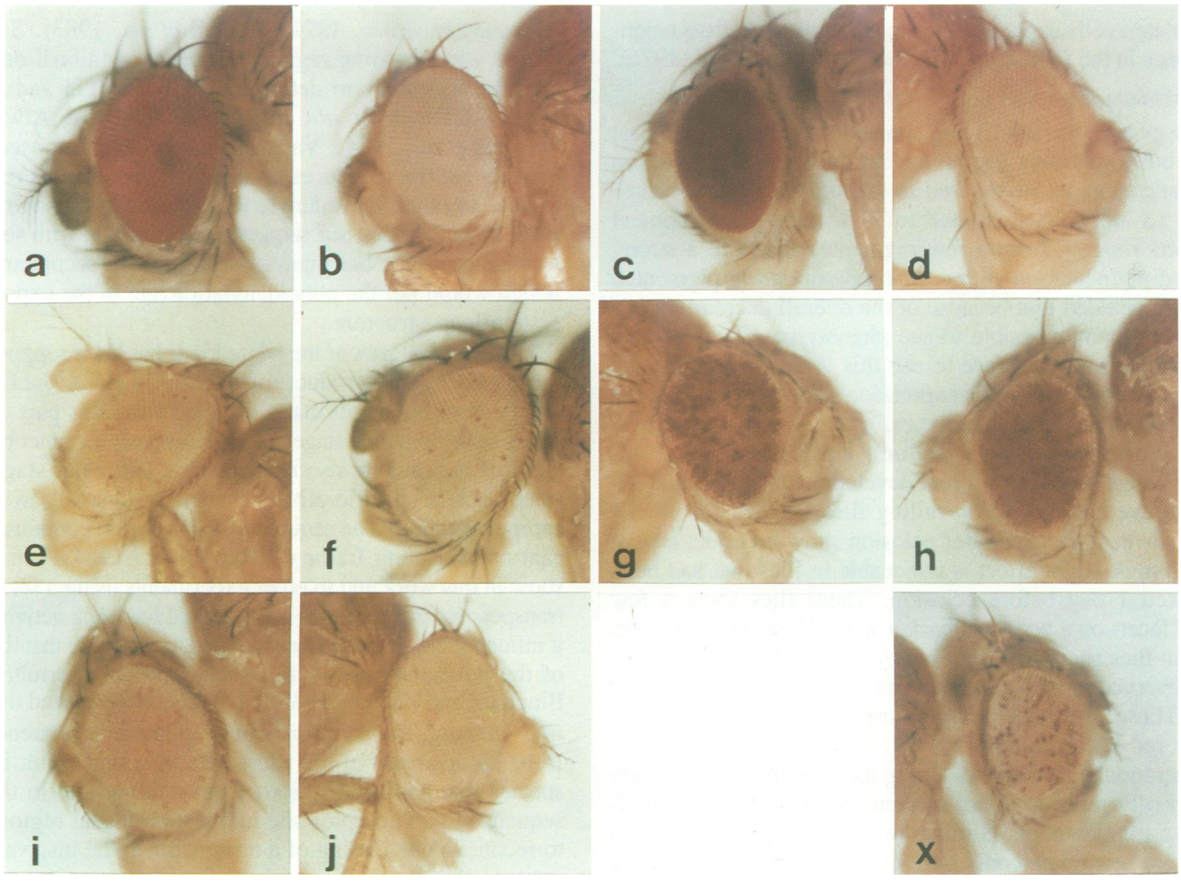

Fig. 7. Heat shock effect on eye pigmentation. Vials containing B4-13 flies at all developmental stages were administered three 1-h heat shock treatments at 37°C at 2-h intervals then returned to room temperature. Flies eclosing on successive days were collected and representative individuals were photographed. **(a)** Canton S fly, **(b)** w^{67c23} fly (lacking the *B4-hsp-w* transposon), **(c)** non-heat shocked B4-1 fly showing near normal pigmentation, **(d)** non-heat shocked B4-13 fly, **(e)** – **(j)** flies eclosing on successive days after the heat treatment, **(g)** and **(h)** represent flies that had been heat treated on the second and first day, respectively, after puparium formation. **(x)** shows that the spots such as those in **(f)** become more distinct with age.

the *Bg-hsp-w* and the *B4-hsp-w* transposon for dosage compensation by measuring the amount of pigment produced in male and female flies. As described in more detail elsewhere (Pirrotta *et al.*, 1985), the various lines carrying *Bg-hsp-w* differ by more than an order of magnitude in the amount of pigment they produce, but in all cases males are more strongly pigmented than females regardless of whether the transposon was integrated in the X or in the autosomes. In contrast, flies homozygous for the *B4-hsp-w* transposon contain 70 – 80% of the wild-type pigment levels and no sex difference in pigmentation was detectable in the homozygous or the heterozygous condition.

We tested the ability of autosomally integrated *Bg-hsp-w* and *B4-hsp-w* transposons to interact with *zeste* by two kinds of crosses. If T represents the transposon, one set of crosses produced males with genotype:

$$\frac{z\ w^{11E4}}{Y}\ ;\ \frac{T}{T}$$

The other set of crosses produced males with genotype:

$$\frac{z^{op6}\ w^{+}}{Y}\ ;\ \frac{T}{+}$$

The z^{op6} mutation is a strong z^1 mutation which gives the *zeste* effect even with a single copy of the *white* gene (Lifschytz and Green, 1984). No detectable *zeste* effect was obtained by either of these tests at 25°C. The failure to obtain a *zeste* effect for any of the lines transformed with either transposon suggests that specific sequences are involved in *zeste* interaction and that these sequences lie upstream of the *Bgl*II site at position 4775.

The B4-13 transformed line

The B4-13 line was exceptional for the extremely low degree of eye pigmentation in flies raised at low temperature. We considered two explanations for this result: the *hsp-w* transposon might have become rearranged in this line or, alternatively, the transposon might have inserted in a chromosomal site unfavorable for expression of even the *hsp*.

To distinguish between these possibilities, we induced secondary transposition events by a dysgenic cross. If the low activity is simply due to the site of insertion but not to alteration of the transposon, mobilisation to new chromosomal sites should allow the recovery of flies with a high level of eye pigmentation. We mobilised the P elements in the B4-13 line by a dysgenic cross and screened the following generation for strong eye pigmentation.

We found strongly pigmented flies at a frequency of ~1 in 200 in the F2 generation, indicating that the transposon itself was not rearranged and that the low expression was due to the chromosomal insertion site.

In situ hybridisation to polytene chromosomes of B4-13 flies shows that the transposon is inserted at the base of the right arm of the third chromosome (Figure 6). The hybridising region at the base of the chromosome extends partly into a filament connecting it to the chromocenter. Euchromatic genes transposed to the vicinity of heterochromatin often show a reduced activity and a characteristic variegated pattern of expression: groups of

clonally related cells express the gene more strongly than neighboring clones in the same tissue (Becker, 1960; Spofford, 1976).

Heat shock-dependent eye pigmentation

The *hsp-w* gene in the B4-13 line can still be induced upon heat treatment but to a lesser degree and in the variegating pattern characteristic of genes juxtaposed to the vicinity of heterochromatin. B4-13 individuals exposed to 37°C during development show effects ranging from a few pigmented facets to a strong background pigmentation with a multitude of darkly pigmented spots. We concluded that because of the overall decrease in *hsp* activity, it was now possible to see phenotypic effects of heat shock induction and therefore to use this line to determine the critical period for *white* gene expression necessary to give the various *white* dependent phenotypes.

To determine the critical period for eye pigmentation, we administered a heat shock at different developmental stages and examined the eye color of the resulting flies (Figure 7).

Individuals heat shocked after eclosion show no effect on eye pigmentation. A slight effect is noticeable in flies that had been heat shocked 1 day before eclosion. These flies show a few pigmented facets on a very pale background. The number of spots increases in flies shocked earlier but the major effect on pigmentation is observed in flies that had been treated 3 – 4 days before eclosion. These flies have a large number of dark red spots on an orange red background.

Administration of heat shocks during the third instar larval stage has a markedly reduced effect and earlier treatment has no effect on eye pigmentation. Similar results were obtained when the flies were heat treated only once or repeatedly, but a stronger and more uniform response was obtained by applying several short heat treatments instead of a single longer treatment.

Discussion

The aim of these experiments was to study the effects of substituting the promoter of the *white* gene with the heat shock promoter. To carry out the promoter switch we first installed the *hsp* fragment in a site 1 kb upstream of the normal *white* transcription start and then deleted the intervening region by *Bal*31 digestion. Flies transformed with the initial construction, *Bg-hsp-w*, contain only 1 kb of the regulatory region and are deficient in some aspects of the normal control mechanisms. As we report in more detail elsewhere (Pirrotta *et al.*, in preparation), expression appears to be hypercompensated in males and the gene fails to interact with *zeste*. The presence of the *hsp* in this position has no appreciable effect on the expression of the *white* gene at high or low temperature. The RNA mapping experiments show that this is due to premature termination of the RNA transcribed from *hsp*.

Terminator in the white upstream region

The transcription of the *Bg-hsp-w* transposon is efficiently terminated before reaching the *white* coding region. This termination could be explained in several ways: it could be due to a steric block such as a tightly bound protein or it might be caused by rapid processing of the transcript. The presence of a canonical cleavage polyadenylation signal 24 nucleotides upstream strongly suggests that processing and polyadenylation are responsible. This AATAAA is the only such sequence found in the 820 nucleotides between the *Bgl*II site at position +4774 and the first exon at position +3692 and it is surprisingly efficient, directing the termination of >90% of the transcripts under heat shock conditions.

The AATAAA signal is generally found in eukaryotic mRNAs, 11 – 30 nucleotides before the site of 3′ end formation (Proudfoot and Brownlee, 1976; Montell *et al.*, 1983). Since it also occurs within coding regions of genes, additional determinants must be necessary to determine cleavage (Gil and Proudfoot, 1984; McDevitt *et al.*, 1984; Sadofsky and Alwine, 1984; Woichik *et al.*, 1984). Various authors have proposed their candidates, but the sequences proposed are not always readily identifiable in other polyadenylation sites or in our own case. We note, however, that our sequence, in common with several other termination sites, has many possibilities for secondary structure which would put the polyadenylation site in the exposed part of a stem-loop structure.

The proximal part of the *white* locus has been supposed to code for a second gene product, or regulatory RNA (Jack and Judd, 1979). No RNA is normally detected from this part of the gene at any developmental stage tested. However, it cannot be excluded that this region is transcribed in some particular stage or tissue since such a specific RNA, especially if small, would easily escape detection. The sequence of this region contains no significant open reading frame (O'Hare *et al.*, 1984). Finally, as we show in this work and in other experiments using a series of *white* transposons, this region is not essential for *white* activity although it influences it. We are more inclined to conclude that the presence of the polyadenylation site is very probably fortuitous.

Bingham and Zachar (1985) have recently reported that in the w^{DZL} mutant, transcription can originate in a sequence inserted >10 kb upstream of the *white* promoter and result in an RNA species that has been spliced from a donor site in the inserted sequence to an acceptor site in the *white* distal region. In order to reconcile our finding of a terminator in the interval, we must suppose that the conformation of the primary transcript in the w^{DZL} mutant prevents polyadenylation at this site or that splicing can occur before the cleavage-polyadenylation machinery intervenes.

The B4-hsp-w fusion and the white transcription start

The S1 mapping experiments now give us the correct length of the first exon of the *white* gene, its position in the nucleotide sequence and the site of the *hsp* fusion relative to the normal start of *white* transcription. Our results are in good agreement with the position of the first exon proposed by O'Hare *et al.* (1984), except that we place the transcription start at position 3693 rather than 3737. The correct transcriptional start site is nearly coincident with the site of the insertion found in the w^h and w^e mutants. Because only the distal edge of this insertion was sequenced by O'Hare *et al.* (1984), we do not know the extent of the target site duplication it produced. The insertion could therefore be either just proximal or just distal to the normal start of transcription.

No recognisable TATA box precedes the start site at position 3693 but this is not surprising since a promoter as weak as that of the *white* gene might be expected to have a deviant sequence. Our first construction, *Bg-hsp-w*, contains ~1 kb of the 5′ control region of the *white* gene but lacks additional regulatory sequences, including the site of the w^{sp} mutations which lie further upstream of the *Bgl*II site at position 4774. The different lines transformed with this transposon show varying degrees of eye pigmentation, in all cases lower than the wild-type and independent of temperature. Because of the efficient termination of transcription starting from *hsp* and the lack of effect of heat shock on eye pigmentation, we conclude that the expression is due to residual activity of the *white* promoter. This is consistent with the results we obtain with another series of transposons containing progressive deletions of the 5′-flanking region of the *white* gene (Pirrotta *et al.*, 1985). The lines transformed with *Bg-hsp-w*

still show dosage compensation effects but none shows any sign of interaction with *zeste*, suggesting that the sequences recognised by *zeste* lie further upstream than the *Bgl*II site.

In the *B4-hsp-w* transposon, the entire promoter region of the *white* gene is deleted, including the first few nucleotides of the transcribed sequence. The *white* activity detected in lines transformed with this transposon must all come from the *hsp*. The fact that all but one of these lines have strong eye pigmentation even when raised at low temperature is consistent with the notion that the uninduced level of *hsp* promoter activity is comparable with the normal level of *white* transcription in the eye. In contrast to *Bg-hsp-w*, the *B4-hsp-w* transposon does not appear to be dosage compensated, suggesting that the sequences required for compensation lie between the transcription start and the *Bgl*II site. Not surprisingly this transposon does not interact with *zeste*.

The massive transcription initiated from the induced *hsp* in the *B4-hsp-w* transposon is easily detected even in the total RNA from a single fly. Two bands corresponding to the spliced and unspliced RNA are observed in roughly equal proportions. We have never detected unspliced transcripts coming from the normal *white* gene. The accumulation of unspliced precursor suggests that under heat shock conditions the splicing machinery is not capable of handling all the transcripts produced. This could be due to the high rate of transcription from *hsp*, to inefficiency of the splicing machinery under heat shock conditions or, possibly, to the *white* RNA being in general a poor substrate for splicing. Bonner *et al.* (1984), who tested a fusion of *hsp* with the *Adh* gene, found no evidence of inefficient splicing after heat shock. They, however, looked only at the kinetics of appearance of *Adh* activity and not at the RNA species produced. Inefficient processing of transcripts after heat shock has been reported for rRNA (Lengyel and Pardue, 1975; Rubin and Hogness, 1975) and attributed to abnormal assembly of nuclear ribonucleoproteins (Mayrand and Pederson, 1983).

Drosophila lines transformed with the *B4-hsp-w* transposon have a wild-type level of eye pigmentation both when reared at low temperature and when heat shocked periodically during development. We have shown previously that the *hsp* fragment used in this construction is inducible by heat shock in most tissues (Steller and Pirrotta, 1984). We conclude that high level expression of the *white* gene is not harmful to the fly nor does it lead to abnormal pigmentation of tissues other than those normally exhibiting *white*-dependent pigmentation. Since the precursors of the major eye pigments are normally synthesised in fat body cells and are transported to the pigment cells of the eye, we conclude that additional structures or gene products are necessary for pigment deposition and that these are tissue specific, as is the *white* product itself.

Position effects

It is well known from classical genetics that translocations of genes to the vicinity of heterochromatic regions can cause both lower activity and variegation: different levels of activity in different cells of a given tissue (see review by Spofford, 1976). The biochemical basis for this position-effect variegation is not known, but the differences between neighboring cells appear to be established at some stage in development and the corresponding levels of activity are then inherited by the clonal descendants of those cells (Becker, 1960). Our results with the B4-13 line indicate that even the *hsp* is subject to this effect.

Position-effect variegation is itself temperature dependent with higher temperatures (25°C) usually, but not always, partially suppressing variegation (Spofford, 1976). It is possible that this effect contributes to the temperature-dependent pigmentation of B4-13 flies although this contribution is unlikely to be a major one. In any case, the drastically lower level of activity of the *hsp* in the B4-13 line makes it possible to demonstrate temperature-dependent *white* activity and to study its effect at different stages of development. Our results show that eye pigmentation requires heat treatment of the early pupae. Transcription of the gene resulting from a heat shock in the late larva has a much weaker effect on the pigmentation of the adult eye. Heat shock in the late pupa or in young adult flies has even less effect. Unless pigment cells suddenly lose the ability to respond to heat shock, we can conclude that *white* function is required at a critical stage in eye development after which the structures or enzymatic activities necessary for pigment deposition are no longer available in the eye pigment cells. These results suggest that some of the eye color mutants may be defective in the temporal specificity of *white* expression.

Our results show that it is possible to use the *hsp* to regulate the expression of genes of interest during development, subject to certain limitations. It is evident from these experiments and others we report elsewhere (Steller and Pirrotta, 1985 and in preparation) that there is a basal level of expression of the *hsp* even at low temperature and that this level can be important, particularly if the rate of transcription required to produce the phenotype is very low, as in the case of the *white* gene. To bring the *hsp* under control in such cases it is possible to decrease its activity or its efficiency. We obtained this in this work by the fortuitous integration of the transposon near heterochromatin but it is also possible to manipulate the *hsp* promoter directly. In another case (Steller and Pirrotta, in preparation) we achieved this by interposing a foreign sequence between the *hsp* and the gene to decrease the translational potential of the RNA.

Materials and methods

Drosophila strains

Strain $w^{67c23(2)}$ obtained from M. Green contains a small male viable deletion of the *white* proximal region (Pirrotta *et al.*, 1983). Canton S Heidelberg and *C(1)DX, w cv* were from the local EMBL stock collection. The w^{67c23}; SM1 and w^{67c23}; *TM3 Ser/Sb* balancer stocks were made in our laboratory. All crosses were carried out at 25°C.

Construction of the white transposons

A 9-kb fragment containing the *white* gene was excised with *Eco*RI + *Bgl*II from clone λw11 (Pirrotta *et al.*, 1983), and inserted in a triple ligation together with a *Eco*RI-*Bam*HI fragment containing 456 bp of the *hsp-70* promoter region previously described (Steller and Pirrotta, 1984) into the Carnegie-1 vector (Rubin and Spradling, 1983) cut with *Eco*RI.

The resulting clone, *Bg-hsp-w*, contains the remaining linker sequences of Carnegie-1, in front of the *hsp* promoter. To delete the region between the *hsp* and the *white* transcription start, we cut *Bg-hsp-w* with *Sph*I which has a unique site roughly in the middle of this region and digested the ends generated with *Bal*31. After re-ligation we isolated clones containing deletions of different extents.

Germ line transformation and establishment of transformed lines

Embryos were collected in 30 min intervals from strain w^{67c23} and injected before pole cell formation as described earlier (Steller and Pirrotta, 1985). The DNA concentrations were 1 mg/ml for the *white* transposons and 100 μg/ml for either *pπ25.1* or *phs-π* (Steller and Pirrotta, in preparation) helper DNA.

The GO adults were individually mated to uninjected w^{67c23} partners and transformants identified in the F1 by eye pigmentation. Transformed flies were individually mated to *w* stocks with balancer chromosomes to map genetically the position of the transposon and establish stably transformed lines.

S1 mapping

Uniformly labelled, single-stranded probes were prepared from suitable subclones constructed in mp 8 (Messing *et al.*, 1981), hybridised to DNA or RNA and S1 digested as previously described (Pirrotta and Bröckl, 1984). To map the *B4-hsp-w* fusion we used 10 ng of the transposon DNA; for the transcript mapp-

ing we used one fly equivalent of total RNA extracted from heat-shocked flies or 2 μg of poly(A)$^+$ RNA from Canton S flies. Plasmid pUC8 cut with *Dde*I and pBR322 cut with *Hpa*II were used as size markers.

In situ hybridisation to polytene chromosomes

Polytene chromosome squashes were prepared according to Pardue and Gall (1975). Hybridisation with biotinylated DNA probes was as described by Langer *et al.* (1981). Signal detection was by direct immunofluorescence with goat anti-biotin and rhodamin-labeled rabbit anti-goat IgG (J.E.Edström, unpublished, protocol).

RNA extraction and analysis

Total *Drosophila* RNA was prepared by a slight modification of our earlier protocol (Steller and Pirrotta, 1984). Briefly, 1–10 flies were homogenized in 100–200 μl of GHCl buffer (7.5 M guanidine-HCl; 0.025 M sodium citrate pH 7.0; 5 mM DTT) containing 0.5% lauryl sarcosine and 0.2–0.5% DEP. The homogenate was extracted twice with an equal volume of phenol-chloroform-isoamylalcohol (25:24:1) and nucleic acids were precipitated by the addition of 0.025 volumes 1 M acetic acid and 0.5 volumes ethanol at −20°C for 5–24 h. The pellet was dissolved in half the original volume of GHCl buffer and reprecipitated twice more to remove DNA (Chirgwin *et al.*, 1979). Northern blot hybridisations were performed as described by Steller and Pirrotta (1984).

Heat shock-dependent eye pigmentation

Homozygously transformed flies of line B-13 were allowed to lay eggs for several days and the culture allowed to develop at 25°C. After removing all parental flies the developing population was heat shocked by incubating the vial for 1 h at 37°C, 2 h at 25°C, again 1 h at 37°C and 2 h at 25°C and repeated once more. The heat shocked culture was allowed to continue development at 25°C and the eye pigmentation of emerging flies analysed. For photographic reproduction, 2–3 day old flies were compared unless otherwise stated.

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