Analysis of the Promoter of the Rh2 Opsin Gene in *Drosophila melanogaster*

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Manuscript received February 17, 1988
Accepted May 9, 1988

ABSTRACT

We have analyzed the *cis*-acting regulatory sequences of the *Drosophila melanogaster* Rh2 gene that encodes the protein component of a rhodopsin which is expressed in ocellar photoreceptor cells. DNA fragments containing the start point of transcription of the Rh2 gene were fused to either the *Escherichia coli* chloramphenicol acetyltransferase (CAT) or *lacZ* (β-galactosidase) genes and introduced into the Drosophila germline by *P*-element-mediated transformation. Expression of the *E. coli* genes was then used to assay the ability of various sequences from the Rh2 gene to confer upon the indicator genes the Rh2 pattern of expression. Fragments containing between 4.3 kb and 183 bp upstream of the start of transcription plus the first 32 bp of the 5′-untranslated leader were found to result in nearly identical levels of head-specific CAT expression. Deletion of Rh2 sequences distal to position −112 bp resulted in loss of detectable CAT expression from these Rh2/CAT fusion constructs. We have, therefore, defined a region essential for head-specific expression of the Rh2 gene to a region extending from −183 to −112. We have determined the DNA sequence of the Rh2 promoter from −448 to +32 and have found an 11-bp sequence which is also present in the upstream flanking sequences of two other photoreceptor-specific genes (*ninaE* and *ninaC*). By histochemical staining of β-galactosidase expressed under the control of the Rh2 promoter and by analyzing the effect of the *ocelliless* mutation on the expression of an Rh2/CAT fusion gene, we have been able to demonstrate that this promoter is active in ocelli.

RHODOPSIN is the major photoreceptor molecule of both vertebrate and invertebrate visual organs. It consists of a protein, opsin, covalently linked to a chromophore, usually 11-*cis* retinal (reviewed in HARGRAVE 1982). Four different opsin genes have been identified in the *Drosophila melanogaster* genome (ZUKER, COWMAN and RUBIN 1985; O'TOUSA *et al.* 1985; COWMAN, ZUKER and RUBIN 1986; ZUKER *et al.* 1987; MONTELL *et al.* 1987; FRYXELL and MEYEROWITZ 1987).

Compound eyes and ocelli are two types of light-sensing organs found in the head of most insects. The compound eye of Drosophila contains, in addition to other cell types, three physiologically distinct classes of photoreceptor cells: R1–R6, R7 and R8 (for review of the physiological differences between the photoreceptor classes see PAK 1979). Each of the isolated opsin genes is expressed in only a subpopulation of photoreceptor cells. Three ocelli are arranged triangularly on the vertex of the head (for review of the structure of insect ocelli see CHAPMAN 1982). The function of the ocelli is poorly understood, but it seems that they are capable of perceiving low-intensity light as well as influencing the orientation of the insect with respect to a light source, a function necessary for visual guidance (GOODMAN 1970; HU, REICHART and STARK 1978; HU and STARK 1980).

The Rh2 gene was cloned by virtue of its homology with the Rh1 gene encoded by the *ninaE* locus (COWMAN, ZUKER and RUBIN 1986). The Rh2 opsin is 381 amino acids long and the predicted amino acid structure has seven hydrophobic domains (COWMAN, ZUKER and RUBIN 1986), a characteristic shared by all known rhodopsins (OVCHINNIKOV 1982; NATHANS and HOGNESS 1983; 1984; NATHANS, THOMAS and HOGNESS 1986). COWMAN, ZUKER and RUBIN (1986) detected low level of expression of the endogenous Rh2 gene in the photoreceptor cell R8 by *in situ* hybridization using a probe homologous to the 3′-untranslated portion of the Rh2 gene. However, it now appears that this observation was an artifact due to cross-hybridization to another RNA sequence, presumably an as yet unidentified R8-specific opsin, and several independent lines of evidence suggest that the Rh2 gene is expressed exclusively in the ocelli. POLLOCK and BENZER (1988), by using a Rh2 gene-specific probe from the 5′-portion of the coding sequence, detected expression clearly above background only in the ocelli. [COWMAN, ZUKER and RUBIN (1986) did not examine sections containing the ocelli.] ZUKER *et al.* (1988) have overexpressed the Rh2 gene in photoreceptor cells R1–R6 by using a transcriptional fusion between the Rh1 promoter and the Rh2 coding sequences. By introducing this Rh1/Rh2 fusion gene into flies carrying the *ninaE* mutation, they were able to substitute the Rh2 opsin for the Rh1 opsin in

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photoreceptors R1–R6. Spectrophotometric examination of such flies showed that the R1–R6 cells now had spectral properties like those of ocelli, but clearly distinct from those of R8 cells (FEILER *et al.* 1988). Finally, FEILER *et al.* (1988) showed that the Rh2 gene is expressed at a level at least fivefold lower in *ocelliless* flies, which lack ocelli, than in wild-type flies.

Many genes have been reintroduced into *D. melanogaster* genome and, in general, these genes appear to be correctly regulated despite their presence at chromosomal locations that differ from that of the endogenous gene (SPRADLING and RUBIN 1983; SCHOLNICK, MORGAN and HIRSH 1983; GOLDBERG, POSAKONY and MANIATIS 1983; JOWETT 1985; MARSH, GIBBS and TIMMONS 1985; KALFAYAN, WAKIMOTO and SPRADLING 1984; HIROMI, KUROIWA and GEHRING 1985; KRUMM, ROTH and KORGE 1985; HAZELRIGG, LEVIS and RUBIN 1984; PIRROTTA, STELLER and BOZZETTI 1985; LEVIS, HAZELRIGG and RUBIN 1985; GARABEDIAN, HUNG and WENSINK 1985; DUDLER and TRAVERS 1984; MISMER and RUBIN 1987). For several developmentally regulated Drosophila promoters, analysis of intact and truncated promoters by *P*-element-mediated transformation has revealed the existence of multiple control elements, each being responsible for expression in a given tissue (LEVIS, HAZELRIGG and RUBIN 1985; PIRROTTA, STELLER and BOZZETTI 1985; GARABEDIAN, HUNG and WENSINK 1985; COHEN and MESELSON 1985; SCHOLNICK *et al.* 1986). Quantitative and qualitative regulatory elements have been separated during deletion analysis of the Sgs3 glue protein gene (BOUROUIS and RICHARDS 1985), the *ninaE* opsin gene (MISMER and RUBIN 1987) and the gene encoding the α-chain of larval serum protein 1 (DELANEY *et al.* 1987).

We have begun an analysis of the promoter sequences of the Rh2 gene using *in vitro* mutagenesis coupled with *P*-element-mediated germline transformation. We have made fusions between segments of the Rh2 gene and two bacterial indicator genes [chloramphenicol acetyltransferase (CAT) and *lacZ*, which encodes β-galactosidase]. We show that a promoter fragment extending from 183 bp upstream of the transcription initiation site plus 32 bp of the 5′-untranslated leader is capable of conferring upon bacterial indicator genes the pattern of expression characteristic of the endogenous Rh2 gene. Comparison of the DNA sequence of the Rh2 promoter with the sequence of promoters of two other genes expressed in photoreceptor cells (*ninaE* which encodes the opsin present in photoreceptor cells R1–R6 and *ninaC*, a protein needed for functional rhabdomere structure), revealed a conserved 11-bp sequence located in approximately the same position in all three promoters. Fusions between the Rh2 promoter and the *lacZ* gene were expressed in ocelli, consistent with the idea that this opsin comprises the photopigment of the ocellar photoreceptor cells.

MATERIALS AND METHODS

DNA manipulations and sequencing, *P*-element-mediated germline transformation and CAT assays: DNA manipulations, such as restriction endonuclease and Bal31 digests, nick-translation labeling of DNA and genomic DNA blotting, were carried out as previously described (MANIATIS, FRISCH and SAMBROOK 1982; MISMER and RUBIN 1987). The vectors used in *P*-element-mediated transformation have been previously described (RUBIN and SPRADLING 1983; STELLER and PIRROTTA 1985; MISMER and RUBIN 1987). Fly strains used in transformation were Canton-S or $rosy^{506}$. CAT activity in transformed flies was detected and quantified as previously described (GORMAN, MOFFAT and HOWARD 1982; MISMER and RUBIN 1987) or the autoradiographs were subjected to laser microdensitometry. Nucleotide sequence analysis was carried out as described (SANGER, NICKLEN and COULSON 1977; BANKIER and BARRELL 1983). The Rh2 DNA sequence in the region from −448 (*Sac*I site) to +32 was determined on both strands.

Construction of fusions between the Rh2 promoter and *E. coli* indicator modules: Various Rh2 promoter fragments were fused with the *E. coli* CAT or *lacZ* (β-galactosidase) genes in order to facilitate the analysis of transcriptional regulatory sequences. Diagrams of the gene fusions described are shown in Figure 2.

pP[*ry*; Rh2(−~4300/+32)-CAT]: A plasmid subclone of the *Bam*HI/*Sac*I genomic fragment encompassing the site of transcription initiation (see Figure 1) was made linear by digestion with *Bam*HI. Following limited Bal31 digestion and complete *Sac*I digestion, a population of partially deleted Rh2 promoter fragments was isolated from a preparative agarose gel and subcloned between the *Sac*I and *Sma*I sites of M13 mp18. Single-stranded DNA was then obtained from a number of subclones and the end-points of Bal31 deletion were determined by DNA sequencing. One of these clones had an end point at position +32 which is between the start of transcription and start of translation of the Rh2 gene. This promoter fragment was then subcloned from M13 RF into pUC12 as a *Hin*dIII/*Sac*I fragment. A pUC12 subclone of the 5-kb genomic *Bam*HI fragment containing the upstream sequences (see Figure 1) was subjected to *Sac*I partial digestion and complete *Hin*dIII digestion to result in cleavage at the *Sac*I site in the Drosophila DNA and the *Hin*dIII site in the polylinker. Into this *Hin*dIII/*Sac*I cleaved plasmid the *Hin*dIII/*Sac*I promoter fragment was introduced, thereby generating a 4.3-kb *Bam*HI genomic DNA fragment whose 3′-end is at nucleotide +32 (rather than several hundred base pairs into the coding sequence) in the polylinker of pUC12. In order to place a *Kpn*I site at the 3′-end (*i.e.*, at position +32), this *Bam*HI fragment was cloned into the *Bam*HI site of pUC19. In the appropriate orientation, a *Kpn*I site is placed at the 3′-end of the promoter fragment and an *Xba*I site at its 5′-end. The 4.3-kb promoter fragment was then excised from pUC19 by *Kpn*I/*Xba*I cleavage and cloned into the CAT fusion vector pDM47. This plasmid contains a CAT gene placed within the polylinker of plasmid pHSS7 (SEIFERT *et al.* 1986) and, therefore, between *Not*I sites which flank the polylinker. Several restriction endonuclease sites are available for the insertion of a promoter fragment: *Hin*dIII, *Sal*I, *Xba*I and *Kpn*I. The Rh2/CAT fusion was then cut out by *Not*I digestion and placed into the *Not*I site of the transformation vector pDM30 (MISMER and RUBIN 1987).

pP[hs-neo; Rh2(−~1300/+32)-CAT], pP[hs-neo; Rh2(−443/+32)-CAT], pP[hs-neo; Rh2(−309/+32)-CAT],

pP[hs-neo; Rh2(−266/+32)-CAT] and pP[hs-neo; Rh2(−112/+32)-CAT]: These constructs were obtained by cloning appropriate restriction enzyme fragments from the 4.3-kb promoter fragment into pDM26 (cut with *Kpn*I and *Sma*I) (MISMER and RUBIN 1987). Plasmid pP[hs-neo; Rh2(−~1300/+32)-CAT] was obtained by cloning a *Nco*I (end-repaired with Klenow to give a blunt end)/*Kpn*I fragment into pDM26. Plasmid pP[hs-neo; Rh2(−443/+32)-CAT] was a result of cloning a *Sac*I (end-repaired with T4 polymerase to give a blunt end)/*Kpn*I fragment into pDM26. Plasmid pP[hs-neo; Rh2(−309/+32)-CAT] was obtained by cleaving the plasmid pP[hs-neo; Rh2(−443/+32)-CAT] with *Sal*I and religating the plasmid. Plasmid pP[hs-neo; Rh2(−266/+32)-CAT] was obtained by cloning a *Pst*I (end-repaired by T4 polymerase)/*Kpn*I fragment into pDM26; pP[hs-neo; Rh2(−112/+32)-CAT] results from cloning a *Pvu*II/*Kpn*I fragment into pDM26.

pP[*ry*; Rh2(−208/+32)-CAT], pP[*ry*; Rh2(−183/+32)-CAT], pP[*ry*; Rh2(−107/32)-CAT] and pP[*ry*; Rh2(−70/+32)-CAT]: To obtain these plasmids, the plasmid pP[hs-neo; Rh2(−309/+32)-CAT] was linearized by digestion with *Xba*I and then subjected to limited Bal31 digestion. Subsequently, partially digested Bal31 products were cleaved by *Sac*I and the population of Rh2/CAT fragments was cloned into *Eco*RI/*Sac*I-cut Bluescript(+) (obtained from Stratagene Inc.). Upon obtaining the single-stranded DNA following superinfection of *E. coli* strain *nm522* by the helper M13 phage strain *R408*, the end point of the deletion in a number of clones was determined. Four of such deletions, with end points at nucleotides −208, −183, −107 and −70 have been cloned into a pHSS7 derivative as *Hin*dIII/*Sac*I fragments in order to place the Rh2/CAT fusion gene between the *Not*I sites. The corresponding *Not*I fragments were then cloned into *Not*I-cut pDM30.

pP[hs-neo; Rh2(−309/+32)-*lacZ*]: Plasmid pP[hs-neo; Rh2(−443/+32)-CAT] was digested with *Sal*I and *Kpn*I and the Rh2 promoter fragment was purified by agarose gel electrophoresis. The Rh2 promoter fragment was then introduced into *Sal*I/*Kpn*I-digested pDM79 (MISMER and RUBIN 1987) resulting in a transcriptional fusion between the Rh2 promoter and *lacZ* gene encoding β-galactosidase.

β-Galactosidase histochemical staining: The staining to detect *lacZ* expression in either whole mounts or cryostat sections of flies transformed by Rh2/*lacZ* fusion genes was carried out by the method of D. R. KANKEL and M. FITZGERALD (personal communication); see MISMER and RUBIN (1987) for a detailed description.

***In situ* hybridization to polytene chromosomes:** In order to determine the number and position of *P* element vector insertions into the genomes of the transformed flies, in situ hybridization to polytene chromosomes was carried out for lines where G418 selection was utilized for the isolation of transformant lines. Polytene chromosome squashes were prepared as previously described (ZUKER, COWMAN and RUBIN 1985). Squashes were hybridized with biotinylated DNA probes corresponding to plasmid pUC18. The procedure was carried out as described by LANGER-SOFER, LEVINE and WARD (1982) except that the DNA was labelled by nick translation using Bio-16-dUTP (ENZO Biochem) and hybridization sites were detected by using the Detek-I-HRP detection kit produced by ENZO Biochem.

RESULTS

The plasmid pP[*ry*; Rh2(−~4300/+32)-CAT] contains a transcriptional fusion between nucleotides −~4300/+32 of the *D. melanogaster* Rh2 gene (transcriptional initiation site is at nucleotide +1) and the *E. coli* CAT gene. The 5′-end of this fragment coincides with the *Bam*HI site located approximately 4.3 kb upstream of the transcription initiation site (Figure 1). Two transformant lines of pP[*ry*; Rh2(−~4300/+32)-CAT] in the *D. melanogaster* ry^{506} host strain were obtained. CAT activity in these lines was found to be restricted to the head (Figure 3). The Rh2 gene has been found to be expressed in the ocelli (POLLOCK and BENZER 1988; FEILER *et al.* 1988). Thus, the observed head-specific CAT activity is an indication that the promoter fragment used can specify, at least in part, the normal endogenous pattern of expression of the Rh2 gene. We are confident that if 10% or more of the CAT expression in any transformant line was in the body it would have been readily detected. The absence of body CAT activity is not due to body-specific degradation of CAT protein or its mRNA as can be seen by examining the CAT activity found in a transformant line containing a nearly identical CAT module fused to the *D. melanogaster* hsp70 promoter (gift of MARY C. MULLINS). In this line high levels of CAT activity are present in both the head and the body (data not shown). A transformant line P[*ry*; Rh2(−~4300/+32)-CAT]1 containing this Rh2 promoter fragment was assayed to determine the developmental timing of CAT expression (Figure 3). Expression of the Rh2 gene, as determined by RNA blotting (A. C. COWMAN, C. S. ZUKER and G. M. RUBIN, unpublished observation), begins during the late pupal period (48–60 hr after puparium formation) when the final stages of photoreceptor cell differentiation occur. The temporal pattern of CAT expression in this transformant line appears to follow that of the endogenous gene.

A set of promoter fragments extending from −~1300, −443, −309, −266, −208, −183, −112, −107 and −70 to +32 was constructed (see MATERIALS AND METHODS). Each of these was fused to CAT and assayed following its reintroduction into the genome by *P*-element-mediated transformation. Special care was taken to minimize the source of variation between the transformant lines: multiple, independent lines were obtained for the majority of the constructs analyzed and the flies assayed were of the same age. The

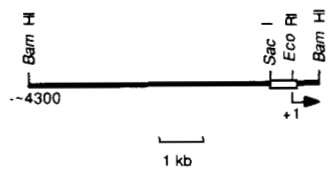

FIGURE 1.—Restriction enzyme map of the upstream flanking DNA and transcription initiation site of the Rh2 gene. The 5-kb *Bam*HI fragment containing the transcription and translation initiation sites was used to construct the initial Rh2 promoter fragment as described in MATERIALS AND METHODS. The arrow indicates the orientation of transcription as well as the approximate start of transcription (nucleotide +1) (COWMAN, ZUKER and RUBIN 1986). Not all *Eco*RI sites in this *Bam*HI fragment are shown. The sequence of the region shown as a stippled rectangle is given in Figure 5.

CONSTRUCT	LINE	RELATIVE LEVEL OF CAT ACTIVITY IN THE HEAD INDIVIDUAL LINES	MEAN
Rh2 CAT --~4300	1	100 ± 29 (n=2)	163 ± 89
	2	226 ± 26 (n=2)	
--1300	1	161 ± 54 (n=2)	106 ± 56
	2	108 ± 10 (n=2)	
	6	49 ± 6 (n=2)	
-443	1	25 ± 5 (n=2)	170 ± 211
	2	73 (n=1)	
	8	413 ± 57 (n=2)	
-309	1	61 ± 14 (n=2)	54 ± 11
	2	46 ± 7 (n=2)	
-266	1	25 ± 0.04 (n=2)	25 ± 0.04
-208	1	128 ± 7 (n=2)	118 ± 85
	2	84 ± 7 (n=2)	
	3	102 ± 1 (n=2)	
	6	178 ± 0.01 (n=2)	
	7	43 ± 1 (n=2)	
	8	58 ± 3 (n=2)	
	9	54 ± 6 (n=2)	
	10	251 ± 11 (n=2)	
	B	76 ± 11 (n=2)	
	N	34 ± 5 (n=2)	
	Z	287 ± 73 (n=2)	
-183	1	271 ± 41 (n=2)	110 ± 93
	3	56 ± 5 (n=2)	
	8	41 ± 0.5 (n=2)	
	9	83 ± 9 (n=2)	
	A	99 ± 14 (n=2)	
-112	1	<1 (n=2)	<1
	2	<1 (n=2)	
-107	1	<1 (n=2)	<1
	2	<1 (n=2)	
	6	<1 (n=2)	
	A	<1 (n=2)	
	B	<1 (n=2)	
	C	<1 (n=2)	
-70	1	<1 (n=2)	<1
	2	<1 (n=2)	
	3	<1 (n=2)	

FIGURE 2.—Table showing diagrams of the Rh2/CAT fusions and quantitative analysis of the CAT level in corresponding transformant lines. The CAT assay was carried out as previously described (MISMER and RUBIN 1987) except that longer incubation times were required (several hours when an extract from five fly heads or bodies was assayed). The level of CAT activity in each transformant line found after two independent assays is expressed as a percentage value of the reference line P[*ry*; Rh2(−~4300~/+32)-CAT]1. The mean level of activity for each construct is also presented. A level of activity below the limit of detection of the assay is represented as <1%. The diagrams outline the constructs used in this study. The Rh2 sequences are represented as a solid black rectangle and CAT sequences as a stippled rectangle. The 5′ extent of the Rh2 promoter fragment is shown as the number of base pairs upstream of the transcription initiation site that are still present in the construct; the 3′ end of the promoter fragment is always at position +32 of the 5′-untranslated leader. The constructs which were cloned into the transformation vector pDM30 (see MATERIALS AND METHODS) as *Not*I fragments are all in the same orientation; the Rh2 promoter fragment is always close to the 5′-end of the *P* element with the CAT gene separating Rh2 promoter and *rosy* gene sequences.

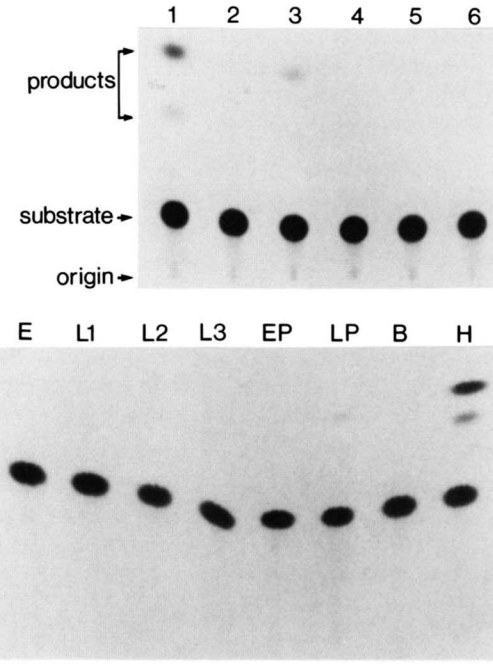

FIGURE 3.—Autoradiographs of the CAT assays of flies belonging to transformant lines carrying the Rh2-CAT fusions. CAT assays were carried out as described in MATERIALS AND METHODS and visualized by autoradiography. The top panel shows an autoradiograph of an assay in which extracts from either five heads or bodies of each transformant line were incubated with substrate for 2 hr. Lanes 1 and 2 represent head and body assays of line P[*ry*; Rh2(−~4300/+32)-CAT]1, respectively. Lanes 3 and 4 represent head and body assays of line P[*ry*; Rh2(−183/+32)-CAT]8, respectively. In these lines there is accumulation of the acetylated chloramphenicol (the product of the CAT enzyme) in the extract derived from heads, but not bodies indicating head-restricted expression of the Rh2 promoter. Extracts derived from the heads or bodies of flies of the transformant line P[*ry*; Rh2(−70/+32)-CAT]3 (lanes 5 and 6, respectively) shows no accumulation of the products of CAT assay indicating loss of expression from the Rh2 promoter. The lower panel shows an autoradiograph of a developmental CAT assay performed on transformant line P[*ry*; Rh2(−~4300/+32)-CAT]1 by analyzing five individuals belonging to different developmental stages. No activity can be detected in following stages: embryo (lane E), first, second and third larval instar (lanes L1, L2 and L3, respectively), early pupa (white, no adult structures discernible; lane EP) and adult body (lane B). CAT activity can be detected in stages that have begun or detected the morphogenesis of the rhabdomere, the light-sensing organelle of the retinula cells: late pupa (red eyes, darkening of the cuticle, but no adult bristles; lane LP) and adult head (lane H).

results of this analysis are shown in Figure 2. During the construction of each deletion, a novel junction was created between the *P* element sequences and Rh2 promoter sequences. Although it cannot be formally excluded, we believe that the creation of this novel junction did not influence tissue-specificity since the characteristic pattern of expression was never altered. Our results indicate that the promoter fragment extending from −183 to +32 directs expression of CAT indicator gene to the head at a level comparable to the fragment extending from −~4300 to +32. Therefore, it would appear that deletion of sequences located between approximately −4.3 kb and −183 bp has little effect upon the function of this promoter. Deletion to nucleotide −112, however, resulted in the loss of all detectable promoter function.

A fusion between the Rh2 promoter fragment extending from −309 to +32 and the *lacZ* gene was constructed as described in MATERIALS AND METHODS. Histochemical staining in this transformant line was restricted to the ocelli (Figure 4). Consistent staining

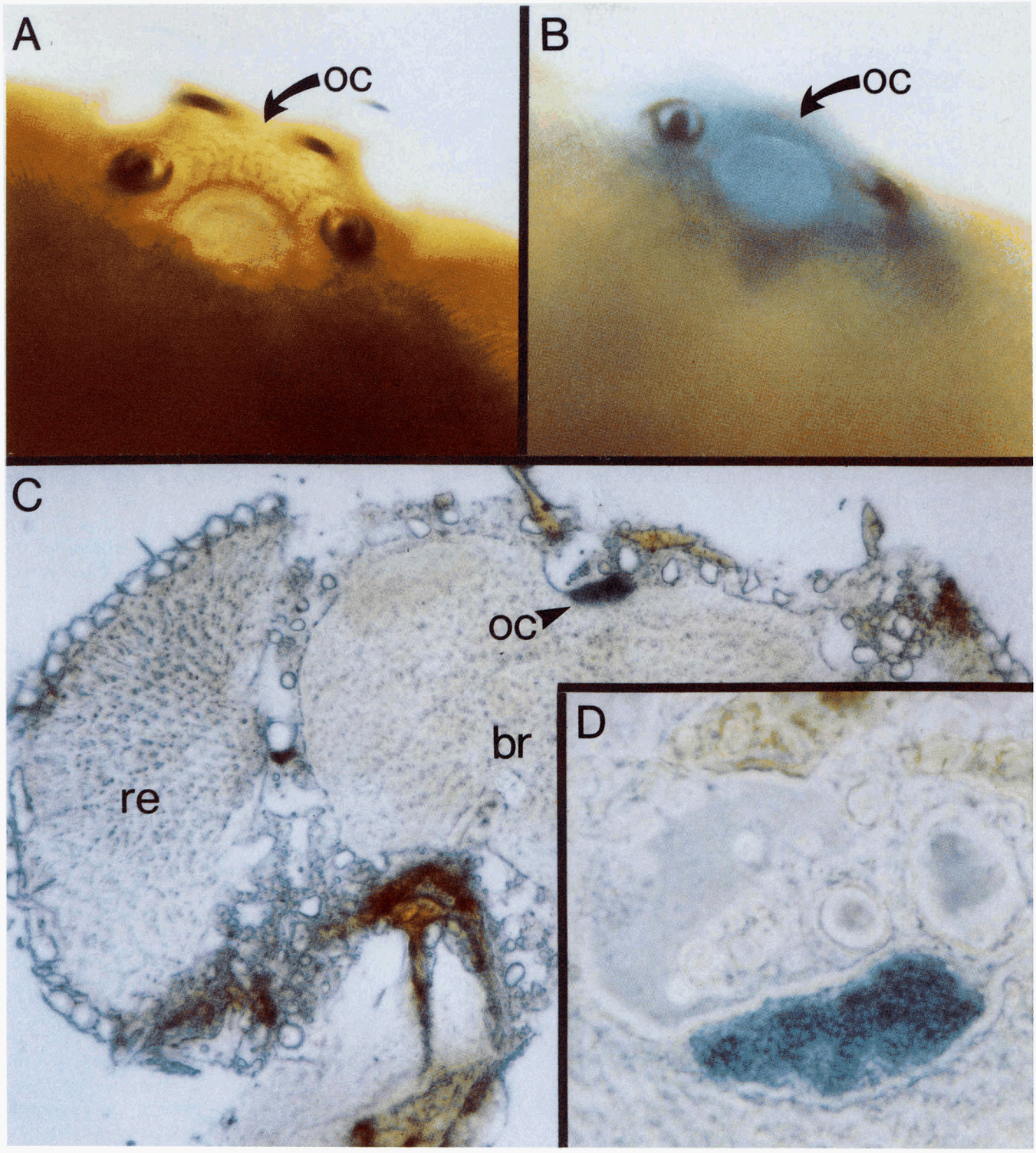

FIGURE 4.—β-Galactosidase histochemical staining of a transformant line carrying a Rh2/*lacZ* fusion. A transformant line in which the plasmid pP[hs-neo; Rh2(−309/+32)-*lacZ*] has integrated into the third chromosome (cytological position *75E*) has been crossed into a *white*^{1118} genetic background to facilitate the histochemical staining. Panels A and B show the photograph of the anterior-most median ocellus; the two more laterally arranged ocelli are out of focus. Two ocellar setae (large bristles), one on each side of the planoconvex lens of the median ocellus, are also visible. Wholemounts of w^{1118} and transformant heads were stained as indicated in MATERIALS AND METHODS. While no staining can be detected in the ocelli (oc) in the wholemount of a w^{1118} head (panel A), ocelli in the transformant head contain β-galactosidase activity (panel B). Panel C shows a sagittal cryostat section through the head of the transformed fly. While no staining is detectable in the retina (re) of the compound eye or the brain (br), staining can be seen in the ocelli (oc). A higher magnification of the ocellar region of the section shown in panel C is shown in panel D.

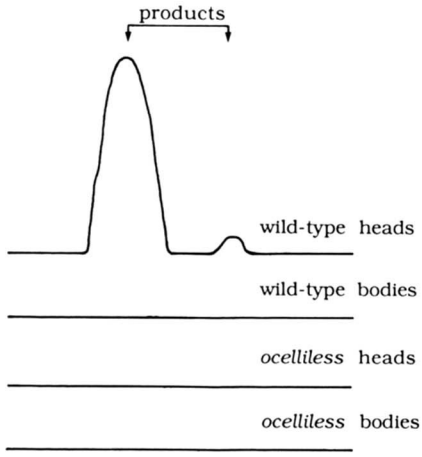

FIGURE 5.—Expression of the P[*ry*; Rh2(−~4300/+32)-CAT]1 insertion in heads and bodies of wild-type and *ocelliless* flies. Densitometer tracings of an autoradiograph of the CAT assays are shown. No CAT expression was detectable in wild-type bodies, *ocelliless* heads or *ocelliless* bodies indicating at least a 10-fold reduction in CAT activity when the P[*ry*; Rh2(−~4300/+32)-CAT]1 insertion was introduced into *ocelliless* flies.

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     SacI
-448 GAGCTCTACT ACTCTGGCAA GGATAAATGC GCCGCAGGAC TCGGCCAAGG   -399
       ↑
-398 CGAATCACTT GACGGAAACA AGCCATAACA ACAGTGGCAT ATATCTGCTA   -349
                                              SalI
-348 AACACGGAGA TAAGATCCTC CGGTGGACTG ATGTCCTCGT CGACCGACCC   -299
                                  PstI        ↑
-298 ATCCAACTCG AAGACCTCGA TGCCATCCTG CAGCCAAGGA GTGCAGCGAA   -249
                                        ↑
-248 AACTGTGCCA GAAATGTGGA CAGAAATTTC TGGCTGCCCT TTAGTGTCAA   -199
                                              ↑
-198 GAACTAATAT TCAAAGGCGG CAAATTAACC TTGCGCTTAG ATTATGTAGC   -149
                  ↑                     PvuII
-148 GTATGAAATT AAATAAAATT GACTTTTATC TGGCAGCTGT CCGCCGTCTA    - 99
                                        ↑     ↑
-  98 TAAGTCTTCT GCGCCTCTTT TGATGAGCGG CTAATTGGGT TAGCAAACAT    - 49
                                    ↑
-  48 CTATAAAGGG CTGGGAATTC GGCCTCCTGG CACAGATCCA CTTCGATGAG    +  2
+   3 CCATCGTCTA GTCAAGGGGT TGCGGGTAGC                        + 32
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FIGURE 6.—DNA sequence of the Rh2 promoter region. The Rh2 gene was sequenced from *Sac*I site at position −448 to position +32 in the 5′-untranslated leader as described in MATERIALS AND METHODS. Restriction enzyme sites used in construction of various promoter deletion constructs are underlined. The end points of deletion constructs used in shortening of the promoter are indicated by arrows. The sequence TATAAA which conforms to the consensus sequence for the TATA box (GOLDBERG 1979) is also underlined. The sequence CTAATTGAATT which can be found in the 5′-flanking sequences of several photoreceptor-specific genes in Drosophila (see Figure 7) is shaded.

above background was not observed in the retina of the compound eye. We have also introduced the P[*ry*; Rh2(−~4300/+32)-CAT]1 insertion into flies carrying the *ocelliless* mutation. These flies, which lack ocelli, did not show detectable levels of CAT activity (see Figure 5).

We have determined the nucleotide sequence of the Rh2 promoter in the region extending from −448 to +32 (Figure 6) and compared it with the sequence of the *ninaE* gene which encodes an opsin present in photoreceptor cells R1–R6 (MISMER and RUBIN 1987). We have found a region of similarity between these two promoters (Figure 7) located, in each case,

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Rh2:      -68   C T A A T T G G G T T  -58
ninaE:    -76   C T A A T T G A A T T  -66
ninaC:   -107   C T A A T T G A A T T  -97

CONSENSUS:      C T A A T T G R R T T
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FIGURE 7.—DNA sequence homology detected in three photoreceptor-specific promoters. By comparing the DNA sequences of promoter regions of genes Rh2, *ninaE* (MISMER and RUBIN 1987) and *ninaC* (MONTELL and RUBIN 1988) we have identified an 11 bp region of similarity. The position of this region relative to the start site of transcription is indicated in each case. In the sequence consensus, R denotes a purine base (adenosine or guanosine).

about 60 nucleotides upstream of the transcription start site. The same sequence can also be found about 100 nucleotides upstream of the transcription start site of the photoreceptor-specific gene *ninaC* (MONTELL and RUBIN 1988).

DISCUSSION

We have performed a preliminary analysis of the *cis*-acting sequences involved in the regulation of the Rh2 gene. We have succeeded in transferring the normal tissue-specific pattern of expression of this gene onto two distinct bacterial indicator genes by using solely sequences upstream of its transcriptional start point and a part of its 5′-untranslated leader. Our results indicate that no essential regulatory elements exist in the Rh2 coding sequence, introns or 3′-flanking sequences. The level of expression observed in transformant lines carrying the Rh2/CAT fusion genes is consistent with a high level of expression in the approximately 90 photoreceptor cells found in each of the three ocelli. In absolute terms this level is about 50 to 100 times lower per head than that observed from Rh1/CAT fusions (MISMER and RUBIN 1987). However, there are approximately 9000 R1–R6 photoreceptor cells (six in each of the 800 ommatidia in each eye) in each head and only 250–300 ocellar photoreceptors. Moreover, R1–R6 photoreceptor cells contain larger rhabdomeres than ocellar photoreceptor cells and thus are likely to have higher levels of rhodopsin per cell. For these reasons we believe that no essential quantitative regulatory elements are missing in the −183 to +32 promoter fragment. We have identified a region essential for the expression of the Rh2 gene in the transformed flies that is located between nucleotides −183 and −112. Furthermore, our deletion analysis indicates that a small promoter fragment, extending from −183 to +32, is sufficient to ensure head-restricted expression of the bacterial CAT gene. Another opsin gene, *ninaE*, requires a promoter fragment extending only from −215 to +67 to confer its pattern of expression onto bacterial indicator genes (MISMER and RUBIN 1987). Thus, the two opsin genes analyzed so far in *D. melanogaster* require relatively short promoter frag-

ments to obtain apparently normal expression in transformed flies.

The expression of Rh2/*lacZ* fusion in the ocelli agrees with the findings of POLLOCK (1988) and FEILER *et al.* (1988) that this opsin gene is expressed in ocelli. Moreover, the marked decrease in the absolute amount of CAT activity observed when the transformant line P[*ry*; Rh2(−~4300/+32)-CAT]-1 is introduced into the *ocelliless* genetic background is consistent with the notion that little or no Rh2 expression occurs elsewhere in the head.

An 11-bp region of nucleotide sequence similarity has been detected among the Rh2, *ninaE* and *ninaC* promoters. The functional significance of this sequence in the regulation of photoreceptor-specific genes is now being investigated by site-directed mutagenesis and *P*-element-mediated transformation. As a part of a systematic study of the *cis*-acting regulatory elements of the Rh1 gene, we have mutated this 11-bp conserved sequence and observed a 10–100-fold lowering of expression in transformant flies (D. MISMER and G. M. RUBIN, unpublished data). The promoter regions of several other sets of coordinately regulated Drosophila genes have been sequenced (PELHAM 1982; PELHAM and BIENZ 1982; COHEN and MESELSON 1985; MESTRIL *et al.* 1986; GARFINKEL, PRUITT and MEYEROWITZ 1983; DELANEY *et al.* 1987; LEVINE and SPRADLING 1985; WONG *et al.* 1985; RIDDIHOUGH and PELHAM 1986). A conserved heat-shock element is located just upstream of the TATA box in most heat-shock genes and is essential for heat-shock inducibility (PELHAM 1982; PELHAM and BIENZ 1982; MESTRIL *et al.* 1986). This sequence appears to represent a binding site for a transcription factor induced by heat-shock (PARKER and TOPOL 1984; WU 1984a, b; 1985). GARFINKEL, PRUITT and MEYEROWITZ (1983) examined the promoter sequences of three glue protein genes residing as a cluster in cytological region *68C*. They have detected two regions, 15 and 28 bp long, of nearly perfect identity in the promoter sequences of the glue protein genes Sgs-7 and Sgs-8 although the third member of the cluster, Sgs-3, does not contain these sequences within its promoter region. Similarly, DELANEY *et al.* (1987) found two regions of homology in the 5′-flanking sequences of three genes encoding larval serum protein 1 and a six-bp element has been found in the 5′-flanking sequences of several chorion protein genes (WONG *et al.* 1985; LEVINE and SPRADLING 1985). The importance of these sequences in gene regulation, with the exception of heat-shock control element, remains to be established.

The authors wish to thank ALAN COWMAN for the gift of the Rh2 gene, MARY MULLINS for donating a transformant line carrying the hsp70/CAT fusion and FRED HEFFRON for supplying the plasmid pHSS7. We particularly wish to thank C. S. ZUKER, J. POLLOCK and S. BENZER for communicating their results prior to publication. We thank Jim FRISTROM for his help with laser microdensitometry. DANIEL KALDERON and MATTHEW FREEMAN have improved this manuscript with their valuable comments. This research has been supported by National Institutes of Health grant GM33135 to G.M.R.

Note added in proof: These Rh2 promoter sequence data will apear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X07789.

LITERATURE CITED

BANKIER, A. T., and B. G. BARRELL, 1983 Shotgun DNA sequencing. pp. 1–33. In: *Techniques in Nucleic Acid Biochemistry*, Vol **B5**, R. A. FLAVELL, ed. Elsevier, Amsterdam.

BOUROUIS, M., and G. RICHARDS, 1985 Remote regulatory sequences of the Drosophila glue gene Sgs3 as revealed by *P*-element transformation. Cell **40:** 349–357.

CHAPMAN, R. F., 1982 *The Insects, Structure and Function.* Harvard University Press, Cambridge, MA.

COHEN, R. S., and M. MESELSON, 1985 Separate regulatory elements for the heat-inducible and ovarian expression of the Drosophila hsp26 gene. Cell **43:** 737–746.

COWMAN, A. C., C. S. ZUKER and G. M. RUBIN, 1986 An opsin gene expressed in only one photoreceptor cell type of the Drosophila eye. Cell **44:** 705–710.

DELANEY, S. J., C. E. SUNKEL, G. K. GENOVA-SEMINOVA, J. E. DAVIES and D. M. GLOVER, 1987 *cis*-Acting sequences sufficient for correct tissue and temporal specificity of larval serum protein 1 genes of Drosophila. EMBO J. **6:** 3849–3854.

DUDLER, R., and A. A. TRAVERS, 1984 Upstream elements necessary for optimal function of the hsp70 promoter in transformed flies. Cell **38:** 391–398.

FEILER, R., W. HARRIS, K. KIRSCHFELD, C. WEHRHAHN and C. ZUKER, 1988 Targeted missexpression of a *Drosophila* opsin gene leads to altered visual function. Nature **333:** 737–741.

FRYXELL, K. J., and E. M. MEYEROWITZ, 1987 An opsin gene that is expressed only in the R7 photoreceptor cell of Drosophila.EMBO J. **6:** 443–451.

GARABEDIAN, M. J., M.-C. HUNG and P. C. WENSINK, 1985 Independent control elements that determine yolk protein gene expression in alternative Drosophila tissues. Proc. Natl. Acad. Sci. USA **82:** 1396–1400.

GARFINKEL, M. D., R. E. PRUITT and E. M. MEYEROWITZ, 1983 DNA sequences, gene regulation and modular protein evolution in the Drosophila *68C* glue gene cluster. J. Mol. Biol. **168:** 765–789.

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.

GOLDBERG, M., 1979 PH.D. thesis, Stanford University, Stanford, Calif.

GOODMAN, L. J., 1970 The structure and function of the insect dorsal ocellus. Adv. Insect. Physiol. **7:** 97–195.

GORMAN, C. M., L. F. MOFFAT and B. H. HOWARD, 1982 Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:** 1044–1051.

HARGRAVE, P. A., 1982 Rhodopsin chemistry, structure and topography. pp. 1–51. In: *Progress in Retinal Research*, edited by N. OSBORNE and G. CHADER. Oxford University Press, Oxford, England.

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.

HIROMI, Y., A. KUROIWA and W. J. GEHRING, 1985 Control elements of the Drosophila segmentation gene *fushi tarazu.* Cell **43:** 603–613.

HU, K. G., and W. S. STARK, 1980 The roles of Drosophila ocelli

and compound eye in phototaxis. J. Comp. Physiol. **135:** 85–95.

HU, K. G., H. REICHART and W. S. STARK, 1978 Electrophysiological characterization of Drosophila ocelli. J. Comp. Physiol. **126:** 15–24.

JOWETT, T., 1985 The regulatory domain of a larval serum protein gene in *Drosophila melanogaster*. EMBO J. **4:** 3789–3795.

KALFAYAN, L., B. WAKIMOTO and A. C. SPRADLING, 1984 Analysis of transcriptional regulation of the s38 chorion gene of Drosophila by P element-mediated transformation. J. Embryol. Exp. Morph. **83**(Suppl.): 137–146.

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

LANGER-SOFER, P. R., M. LEVINE and D. C. WARD, 1982 Immunological method for mapping genes on Drosophila polytene chromosomes. Proc. Natl. Acad. Sci. USA **79:** 4381–4385.

LEVINE, J., and A. C. SPRADLING, 1985 DNA sequence of a 3.8 kilobase pair region controlling Drosophila chorion gene amplification. Chromosoma **92:** 136–142.

LEVIS, R., T. HAZELRIGG and G. M. RUBIN, 1985 Separable *cis*-acting control elements for expression of the *white* gene of Drosophila. EMBO J. **4:** 3489–3499.

MANIATIS, T., E. F. FRISCH and J. SAMBROOK, 1982 *Molecular Cloning, a Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MARSH, J. L., P. D. L. GIBBS and P. M. TIMMONS, 1985 Developmental control of transduced dopa decarboxylase genes in *D. melanogaster*. Mol. Gen. Genet. **198:** 393–403.

MESTRIL, R., P. SCHILLER, J. AMIN, H. KLAPPER, J. ANANTHAN and R. VOELLMY, 1986 Heatshock and ecdysone activation of the *Drosophila melanogaster* hsp23 gene; a sequence element implied in developmental regulation. EMBO J. **5:** 1667–1673.

MISMER, D., and G. M. RUBIN, 1987 Analysis of the promoter of the *ninaE* opsin gene in *Drosophila melanogaster*. Genetics **116:** 565–578.

MONTELL, C., and G. M. RUBIN, 1988 The *ninaC* locus of Drosophila encodes two photoreceptor cell specific proteins containing adjacent domains homologous to protein kinases and the myosin heavy chain head. Cell **52:** 757–772.

MONTELL, C., K. JONES, C. S. ZUKER and G. M. RUBIN, 1987 A second opsin gene expressed in the ultraviolet sensitive R7 photoreceptor cells of *Drosophila melanogaster*. J. Neurosci. **7:** 1558–1566.

NATHANS, J., and D. S. HOGNESS, 1983 Isolation, sequence analysis, and intron-exon arrangement of the gene encoding bovine rhodopsin. Cell **34:** 807–814.

NATHANS, J., and D. S. HOGNESS, 1984 Isolation and nucleotide sequence of the gene encoding human rhodopsin. Proc. Natl. Acad. Sci. USA **81:** 4851–4855.

NATHANS, J., D. THOMAS and D. S. HOGNESS, 1986 Molecular genetics of human color vision: the genes encoding blue, green and red pigments. Science **232:** 193–202.

O'TOUSA, J. E., W. BAEHR, R. L. MARTIN, J. HIRSH, W. L. PAK and M. L. APPLEBURY, 1985 The Drosophila *ninaE* gene encodes an opsin. Cell **40:** 839–850.

OVCHINNIKOV, Y. A., 1982 Rhodopsin and bacteriorhodopsin: structure-function relationships. FEBS Lett. **148:** 179–189.

PAK, W. L., 1979 Study of photoreceptor function using Drosophila mutants. pp. 67–99. In *Neurogenetics, Genetic Approaches to the Nervous System*, edited by X. D. BREAKFIELD. Elsevier North-Holland, New York.

PARKER, C. S., and J. TOPOL, 1984 A Drosophila RNA polymerase II transcription factor specific for the heat-shock gene binds to the regulatory site of an hsp70 gene. Cell **37:** 273–283.

PELHAM, H., 1982 A regulatory upstream promoter element in the Drosophila hsp70 gene. Cell **30:** 357–369.

PELHAM, H., and M. BIENZ, 1982 A synthetic heat-shock promoter element confers heat inducibility on the herpes simplex thymidine kinase gene. EMBO J. **1:** 1473–1477.

PIRROTTA, V., H. STELLER and M. P. BOZZETTI, 1985 Multiple upstream regulatory elements control the expression of the Drosophila *white* gene. EMBO J. **4:** 3501–3508.

POLLOCK, J. A., 1988 Transcript localization of four opsin genes in the three visual organs of *Drosophila;* Rh2 is ocellus specific. Nature **333:** 779–782.

RIDDIHOUGH, G., and H. R. B. PELHAM, 1986 Activation of the Drosophila hsp27 promoter by heat shock and by ecdysone involves independent and remote regulatory elements. EMBO J. **5:** 1653–1658.

RUBIN, G. M., and A. C. SPRADLING, 1983 Vectors for P element-mediated gene transfer in Drosophila. Nucleic Acids Res. **18:** 6341–6351.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

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

SCHOLNICK, S. B., S. J. BRAY, B. A. MORGAN, C. A. MCCORMICK and J. HIRSH, 1986 CNS and hypoderm regulatory elements of the *Drosophila melanogaster* dopa decarboxylase gene. Science **234:** 998–1002.

SEIFERT, H. S., E. Y. CHEN, M. SO and F. HEFFRON, 1986 Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83:** 735–739.

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.

STELLER, H., and V. PIRROTTA, 1985 A transposable P vector that confers selectable G418 resistance to Drosophila larvae. EMBO J. **4:** 167–171.

WONG, Y.-C., J. PUSTELL, N. SPOEREL and F. C. KAFATOS, 1985 Coding and potential regulatory sequences of a cluster of chorion genes in *Drosophila melanogaster*. Chromosoma **92:** 124–135.

WU, C., 1984a Two-protein binding sites in chromatin implicated in the activation of heat-shock genes. Nature **309:** 229–234.

WU, C., 1984b Activating protein factor binds *in vitro* to upstream control sequences in heat-shock chromatin. Nature **311:** 81–84.

WU, C., 1985 An exonuclease protection assay reveals heat-shock element and TATA box DNA-binding proteins in crude nuclear extracts. Nature **317:** 84–87.

ZUKER, C. S., A. F. COWMAN and G. M. RUBIN, 1985 Isolation and structure of a rhodopsin gene from *D. melanogaster*. Cell **40:** 851–858.

ZUKER, C. S., C. MONTELL, K. R. JONES, T. LAVERTY and G. M. RUBIN, 1987 A rhodopsin gene expressed in photoreceptor cell R7 of the Drosophila eye: homologies with other signal transducing molecules. J. Neurosci. **7:** 1550–1557.

ZUKER, C. S., D. MISMER, R. HARDY and G. M. RUBIN, 1988 Ectopic expression of a minor Drosophila opsin in the major photoreceptor cell class: a molecular genetic approach to distinguishing the role of primary receptor and cellular context. Cell **55:** 475–482.

Communicating editor: A. SPRADLING