Analysis of the Promoter of the ninaE Opsin Gene in
Drosophila melanogaster

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ABSTRACT
We have analyzed the cis-acting regulatory sequences of the ninaE gene. This gene encodes the major Drosophila melanogaster opsin, the protein component of the primary chromophore of photoreceptor cells R1–R6 of the adult eye. DNA fragments containing the start point of transcription of the ninaE gene were fused to either the Escherichia coli chloramphenicol acetyltransferase or lacZ (β-galactosidase) gene 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 ninaE gene to confer the ninaE pattern of expression. Fragments containing between 2.8 kb and 215 bp of the sequences upstream of the start of transcription plus the first 67 bp of the untranslated leader were able to direct nearly wild-type expression. We have identified three separable control regions in the ninaE promoter. The first, which has the properties of an enhancer element, is located between nucleotides −501 and −219. The removal of this sequence had little effect on promoter function; this sequence appears to be redundant. However, it appears to be able to substitute for the second control region which is located between nucleotides −215 and −162, and which also affects the level of output from this promoter. Removal of these two control regions resulted in a 30-fold decrease in expression; however tissue specificity was not affected. The third control region, located downstream from nucleotide −120, appears to be absolutely necessary for promoter function in the absence of the first two regulatory sequences. Examination of larvae containing fusion genes expressing β-galactosidase suggests that the ninaE gene is also expressed in a subset of cells in the larval photoreceptor organ.

ADULT Drosophila, like many other insects, have compound eyes consisting of a number of identical units called ommatidia (for review of insect ommatidia, see CHAPMAN 1982). In the Drosophila eye, each of the approximately 800 ommatidia contains 20 precisely arranged cells, eight of which are photoreceptor cells. The six outer photoreceptor cells, R1–R6, encircle the two inner cells, R7 and R8. The Drosophila compound eye develops from cells in the eye-antennal imaginal disc. At the start of the third larval instar, cells in the eye disc are undifferentiated. Differentiation of the disc epithelium starts at the posterior edge of the disc; associated with the differentiation process a morphogenetic furrow, moves across the disc in a posterior-anterior direction over a 3-day period (READY, HANSON and BENZER 1976). Posterior to the furrow cells assemble into clusters that will give rise to adult ommatidia. During the assembly process, undifferentiated cells apparently become determined by interpreting positional cues in their local environment (READY, HANSON and BENZER 1976; TOLLMAN 1985; TOLLMAN and READY 1987). No determinative lineage relationships have been detected between cells in the developing eye (READY, HANSON and BENZER 1976; LAWRENCE and GREEN 1979). The end result is a highly ordered retina made up of several different cell types that differ not only in the positions that they occupy within the ommatidium, but also in their patterns of gene expression.

Each of the four different opsin genes that have been identified in the Drosophila melanogaster genome is expressed in only a subpopulation of photoreceptor cells (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). Photoreceptor cells R1–R6 appear to contain the same rhodopsin (OSTROY, WILSON and PAK 1974; HARRIS, STARK and WALKER 1976) which is encoded by the ninaE locus (ZUKER, COWMAN and RUBIN 1985; O’TOUSA et al. 1985). This gene is also referred to as Rhl, being the first rhodopsin gene isolated. The predicted product of the ninaE locus is 373 amino acids long and has seven hydrophobic domains (ZUKER, COWMAN and RUBIN 1985; O’TOUSA et al. 1985), a characteristic shared by all known rhodopsins (OVCHINNIKOV 1982; NATHANS and HOG-
We have begun an analysis of the promoter sequences of *ninaE* locus using in vitro mutagenesis coupled with P-element-mediated germline transformation. We have made fusions between segments of the *ninaE* gene and two bacterial indicator genes (chloramphenicol acetyltransferase and *lacZ*, which encodes β-galactosidase). We show that as little as 120 bp upstream of the transcription initiation site plus 67 bp of 5′-untranslated leader are sufficient to direct expression to retinula cells R1–R6, albeit at reduced levels. Sequences located further upstream influence the level of expression from this promoter.

**MATERIALS AND METHODS**

*P*-Element-mediated transformation and modification of preexisting *Drosophila* transformation vectors: *P*-element-mediated transformation was carried out essentially as previously described (Spradling and Rubin 1982; Rubin and Spradling 1982). Drosophila embryos were dechorionated either mechanically or by submersion in 1:1 (v/v) commercial bleach:dH2O for 2–3 min. For injection, DNA was dissolved in 5 mM KCl, 0.1 mM NaH2PO4 (pH 6.8) and mixed with the helper plasmid p25.7 (Kares and Rubin 1984) to give final concentrations of approximately 2 mg/ml and 0.5 mg/ml, respectively.

Transformation vectors, containing either rosy (Rubin and Spradling 1983) or G418 resistance markers (Steller and Pirrotta 1985) were used. Selection for G418 resistant flies was carried out using commercially available dehydrated fly food (Carolina Biologicals) made according to the supplier's instructions but containing C4 18 sulfate at 350–500 µg/ml. G418 sulfate was purchased from Gibco. Drosophila strains injected were either ry506 when derivatives of the *rosy*-containing Carnegie vectors were used or Canton-S when pUCsheo derivatives were used. These stocks were reared on cornmeal-agar food.

To facilitate manipulations of the *ninaE* promoter, a number of modifications were introduced into preexisting transformation vectors. These vectors are diagrammed in Figure 1. In order to increase their unique cloning sites, Carnegie 30 and pUCsheo were modified by replacing part of their polylinkers by a portion of the M13 mp19 polylinker. pDM23 is a Carnegie 30 derivative in which XhoI, KpnI and Sall are unique cloning sites. pDM24 is a pUCsheo derivative containing unique Sall, XhoI, BanHI, Smal, KpnI, SacI and EcoRI sites. By inserting an 8-bp oligonucleotide encoding the cleavage site of NotI (GCCGCCGCC) into the *HpaI* site of Carnegie 20 and into the *SmaI* site of pUCsheo, the vectors pDM30 and pDM25 were obtained.

The plasmid pC4CAT (gift of C. Thummel and D. Hogness) contains a chloramphenicol acetyltransferase transcription fusion module within the polylinker of Carnegie 4. In order to place a KpnI site at the 5′-end of this module, pC4CAT was digested with Smal and an 8-bp KpnI linker sequence (CGGTTACCC) was inserted. The *KpnI* site was then excised from the resulting plasmid by digestion with KpnI and SacI and cloned into pDM24 to generate the plasmid pDM26. The plasmid pDM79 was constructed by transferring the *lacZ* (β-galactosidase) transcriptional fusion module from p2-galAUG (gift of C. Thummel and D. Hogness) as an EcoRI fragment into pDM24. The transcription start of this modified *lacZ* gene was taken from Drosophila alcohol dehydrogenase gene. The Adh segment used was the Sau3AI fragment extending from +34 to +160, which contains the translation initiator codon AUG (+1) represents the start of transcription from the proximal Adh.

**FIGURE 1.—Modification of vectors for *P*-element-mediated germline transformation. Both Carnegie and pUCsheo vectors were modified by introducing novel unique restriction enzyme sites into their polylinkers as described in MATERIALS AND METHODS. Only the polynucleotide sequences were modified; the remainder of the vector is the same as the previously published parent plasmids (Rubin and Spradling 1985; Steller and Pirrotta 1985). In the Carnegie vector system, transformants are recognized by the rescue of *ry* eye color. The utility of these vectors is limited by the scarcity of unique restriction enzyme sites suitable for the cloning of DNA fragments to be transformed. Two vectors, pDM23 and pDM30, have additional unique restriction enzyme sites within their polylinkers which were introduced as described in MATERIALS AND METHODS. Plasmid pDM25, a Carnegie 30 derivative, contains unique XhoI, SacI and *KpnI* sites in its polylinker sequence. Plasmid pDM30, a Carnegie 20 derivative, contains an unique NotI site within its polylinker. This enzyme possesses an octanucleotide recognition sequence that rarely occurs in the *D. melanogaster* DNA. pDM30 is used in conjunction with plasmid pHSS7 (Seifert et al. 1986) which has a polylinker bordered by NotI sites. The desired fragment is first cloned into the polylinker of the pHSS7 vector, removed from pHSS7 by NotI digestion and then recloned into either pDM30 or pDM25, a pUCsheo derivative with a NotI site. Other unique polylinker sites in pDM25 are EcoRI, BamHI and SacI. The plasmid pDM24 is a pUCsheo derivative where the unique polylinker sites are: EcoRI, KpnI, SacI, Smal, BamHI and XhoI. The *E. coli* indicator genes *CAT* and β-galactosidase were cloned into pDM24 to create plasmids suitable for gene fusion experiments. Plasmid pDM26 contains a *CAT* module as a 3′-*KpnI* fragment within its polylinker. A promoter can be inserted into the SacI, XhoI, BamHI, Smal or KpnI sites in order to effect the formation of a transcriptional fusion between the *CAT* gene and the promoter. Although SacI is also a unique site, it cannot be used since it is located 3′ to the *CAT* gene. The plasmid pDM79 permits the insertion of a promoter into a SacI, BamHI or *KpnI* site in order to form a transcriptional fusion between the promoter and *lacZ* gene, which has been cloned into pDM24 as an EcoRI fragment.
promoter). No Adh control regions have been reported in these sequences (GOLDBERG, POSAKONY and MANIATIS 1983; POSAKONY, FISCHER and MANIATIS 1985; HEBERLEIN, ENGLAND and TJIAN 1985). In pDM79, unique SalI, BamHI and KpnI sites are located in the 5′-untranslated leader of this Adh/lacZ fusion gene.

**DNA sequence analysis of ninaE promoter:** Nucleotide sequence analysis was carried out by the dideoxy chain termination method (SANGER, NICKLEN and COULSON 1977). Randomly sheared fragments, 300–600 bp long, of the ninaE promoter were cloned into Smal-cut M13 mp18 and sequenced. The host strain used for the growth of M13 was Escherichia coli TG1. Sequencing reactions utilized α-[32P]dATP as the radioactively labeled nucleotide and were carried out essentially as described by BANKEK and BARRELL (1985). The sequence of the ninaE promoter was determined on both strands in the region from -833 bp to +67 bp. The DNA sequence from -260 to -1 was also confirmed by the chemical degradation method (MAXAM and GILBERT 1977). The single-stranded DNAs used as templates for dideoxy chain termination sequencing were obtained from either M13 of pEMBL vectors according to published procedures (DENTE, CESARENI and CORTESE 1983).

**DNA manipulations:** DNA manipulations, such as restriction endonuclease digests and nick-translating label of DNA, were generally performed as previously described (MANIATIS, FRISCH and SAMBROOK 1982). Restriction enzymes were purchased from New England Biolabs and Boehringer Mannheim Biochemicals. DNA polymerase large fragment (Klenow) was purchased from Bethesda Research Laboratories. Bal31 degradation of DNA was carried out in 0.6 M NaCl, 12 mM MgCl₂, 12 mM CaCl₂, 1 mM EDTA and 20 mM Tris (pH 8.0). For each 3.5 μg of linear DNA, about 1 unit of Bal31 was used at room temperature which gave a digestion rate of approximately 50 bp/min. In order to insert the XhoI linkers (CCTCGAGG) at random into the ninaE promoter, plasmids containing the promoter were linearized by the DNaseI/Mn²⁺ procedure (MELCAR and GOLDTHWAITE 1968).

**Construction of ninaE promoter-CAT fusions:** Various ninaE promoter fragments were fused with the E. coli CAT gene in order to facilitate the analysis of the regulatory sequences of this rhodopsin gene. Diagrams of all of the fusions described are shown in Figure 2.

\[ \text{pP}[\text{hs-neo}; \text{Rhl}(-2800/+67)-\text{CAT}] \]: A plasmid subclone of the genomic HindIII/BamHI fragment of the ninaE gene (see Figure 3) that contains the 5′-untranslated leader and flanking sequences was made linear by digestion with BamHI. Following limited Bal31 degradation and HindIII digestion, a population of partially deleted ninaE promoter fragments was isolated from a preparative agarose gel and subcloned into the vector pEMBL19+ which has been cut with HindIII and Smal. pEMBL19+ is the same as pEMBL19 (DENTE, CESARENI and CORTESE 1983) except that it contains the polylinker of M13 mp19 instead of M13 mp19 (gift of H. ROHKA). 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 the clones contained an end-point at position +67 which is between the start of transcription and the start of translation of the ninaE gene. This ninaE promoter fragment was then cloned as a HindIII fragment (site filled in using DNA polymerase I large fragment)/KpnI (this KpnI site at the 3′ end of the promoter fragment, originates from the polylinker of pEMBL19+) fragment into Smal- and KpnI-digested pDM26.

\[ \text{pP}[\text{hs-neo}; \text{Rhl}(-835/+67)-\text{CAT}] \]: This plasmid was constructed by cleaving pP[hs-neo; Rhl(-2800/+67)-CAT] with BamHI (the BamHI site is in the polylinker of pDM26) and BglII (position -833 of the Rhl promoter) and religating.

\[ \text{pP}[\text{rhl}; \text{Rhl}(-448/+67)-\text{CAT}] \]: The Rhl promoter fragment was subcloned from pEMBL19+ as a HindIII/EcoRI fragment (the EcoRI site is in the pEMBL19+ polylinker) into pHSS7. The plasmid pHSS7 (SEIFERT et al. 1986) has a polylinker bordered by NolI sites. The resulting plasmid was then subjected to DNAseI cleavage in the presence of Mn²⁺. This results in a population of randomly linearized molecules into which XhoI linkers were then inserted to create plasmid molecules with unique XhoI sites. Since the vector pHSS7 contains virtually no nonessential sequences (SEIFERT et al. 1986), only one out of 51 independent linker insertions was outside the ninaE promoter fragment. The positions of XhoI linker insertions were first mapped by restriction enzyme digestion and the position of those downstream from BglII site at -833 were determined by sequencing. One of the insertions mapped at position -448, by cleaving the plasmid with XhoI and HindIII, repairing the ends with DNA polymerase I large fragment in the presence of all four dNTPs and recircularization, a deletion to position -448 was obtained. The CAT gene was then subcloned into this plasmid as a KpnI/SacI fragment. The Rhl-CAT fusion was then excised from pHSS7 by NolI cleavage and cloned into the NolI site of pDM30.

\[ \text{pP}[\text{rhl}; \text{Rhl}(-162/+67)-\text{CAT}] \]: The same set of manipulations as above were carried out using a linker insertion at position -162 to obtain this plasmid. In the plasmid containing the original XhoI linker insertion, an internal deletion occurred, resulting in the XhoI linker being placed between nucleotides -19 and -162.

In order to construct plasmids pP[ry; Rhl(-252/+67)-CAT], pP[ry; Rhl(-215/+67)-CAT], pP[ry; Rhl(-181/+67)-CAT], pP[ry; Rhl(-166/+67)-CAT], pP[ry; Rhl(-105/+67)-CAT], pP[ry; Rhl(-86/+67)-CAT], pP[ry; Rhl(-46/+67)-CAT], and pP[ry; Rhl(-55/+67)-CAT], the plasmid with the XhoI linker insertion at position -448 was cleaved with XhoI and then subjected to limited Bal31 digestion. After the Bal31 digestion, plasmid molecules were cleaved with HindIII, repaired with DNA polymerase I large fragment in the presence of all four dNTPs, and recircularized by DNA ligation. The endpoints of the resultant deletions were determined by subcloning the appropriate portion of the Rh1 promoter into M13 vectors and sequencing. The KpnI/SacI CAT module was then cloned into each deletion plasmid and the Rh1-CAT fusion was transferred into pDM30 as a NolI fragment.

\[ \text{pP}[\text{rhl}; \text{Rhl}(-2800/+701, A, -448/+67)-\text{CAT}] \]: This plasmid was constructed by combining two different XhoI linker insertions, one at -701 and the other at -448. A HindIII/XhoI fragment containing the 5′-part of the ninaE promoter fragment was isolated from the XhoI linker insertion at position -701. It was then cloned into the plasmid containing the XhoI linker insertion at -448, after the corresponding HindIII/XhoI fragment was removed. This results in an internal deletion between the sites of the two XhoI linker insertions (i.e., from -701 to -448). After the KpnI/SacI CAT fragment was cloned into this plasmid, the Rh1-CAT fragment was transferred to pDM30 as a NolI fragment.

\[ \text{pP}[\text{rhl}; \text{Rhl}(-162/+67)-\text{CAT} \text{Rhl}(-501 \text{ and } -219) \text{ and } \text{pP}[\text{rhl}; \text{Rhl}(-162/+67)-\text{CAT} \text{Rhl}(-219/-501)] \]: The plasmid containing the XhoI linker insertion between nucleotides -162 and -219 was digested with EcoRI (+67) and XhoI (-501) and cloned into EcoRI/SmaI digested pDM30. This places the part of the M13 polylinker adjacent to the AvaI site of the ninaE promoter. The -219 to -501 region was then isolated from the replicative form of the M13 plasmid, a XhoI (-219)/SalI (M13 polylinker; -501) frag-
<table>
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<tr>
<th>CONSTRUCT</th>
<th>LINE</th>
<th>CHROMOSOME</th>
<th>RELATIVE LEVEL OF CAT ACTIVITY IN THE HEAD INDIVIDUAL LINES</th>
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<td>88 ± 17</td>
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D. Mismer and G. M. Rubin
**ninaE Opius Promoter**

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<tr>
<th>Construct</th>
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<th>Chromosome</th>
<th>Relative Level of CAT Activity in the Head Individual Lines</th>
<th>Mean</th>
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<td>49 ± 2 (n = 3)</td>
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<td>57 ± 6 (n = 3)</td>
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<tr>
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<td>3</td>
<td>33 ± 0.1 (n = 2)</td>
<td>39 ± 17</td>
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pP [ry; Rh1 (-501/-219, Δ (-162/+67) - CAT]  

<table>
<thead>
<tr>
<th>Construct</th>
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<th>Mean</th>
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<td>25 ± 2 (n = 3)</td>
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<td>5</td>
<td>2</td>
<td>13 ± 0.1 (n = 2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>X</td>
<td>4 ± 1 (n = 2)</td>
<td></td>
</tr>
</tbody>
</table>

pP [ry; Rh1 (-162/+67) - CAT - Rh1 (-501/-219)]  

<table>
<thead>
<tr>
<th>Construct</th>
<th>Line</th>
<th>Chromosome</th>
<th>Relative Level of CAT Activity in the Head Individual Lines</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>+67 -219</td>
<td>1</td>
<td>X</td>
<td>58 ± 3 (n = 3)</td>
<td></td>
</tr>
<tr>
<td>-162 -501</td>
<td>2</td>
<td>2</td>
<td>32 ± 7 (n = 3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>30 ± 8 (n = 3)</td>
<td>40 ± 15</td>
</tr>
</tbody>
</table>

pP [ry; Rh1 (-162/+67) - CAT - Rh1 (-219/-501)]

**Figure 2.—Diagrams of the ninaE-CAT fusions and quantitative analysis of the CAT level in corresponding transformant lines. The CAT assay is described in MATERIALS AND METHODS. The chromosome of P-element insertion is shown; the cytological position of transposon insertion, when determined, is shown in parentheses. The level of CAT activity found in each line after a number of separate assays (usually two to three) is expressed as a percentage value of the reference line pP[hs-neo; Rh1 (~2800/+67) - CAT]. The mean level of activity for a construct is also presented. A level of activity below the limit of detection of the assay is represented as <0.005. The diagrams outline the constructs used in this study. ninaE promoter sequences are shown as a solid black rectangle and CAT sequences as a stippled rectangle. The 5' extent of the Rh1 promoter fragment is shown as the number of bp upstream of the transcription initiation site that are still retained in the construct; the 3' end of the promoter fragment was always at +67 bp in the 5' untranslated leader. The constructs which were cloned into the pDM30 transformation vector (see MATERIALS AND METHODS) as NotI fragments are all in the same orientation; the Rh1 promoter sequence is always close to 5' end of the P element with the CAT gene being between the Rh1 promoter and ry sequences.**

**MATERIALS AND METHODS**

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. Polytenic chromosome squashes were

**Construction of ninaE promoter – lacZ gene fusions:**

To more precisely determine the pattern of expression from the ninaE promoter, two promoter fragments were fused to β-galactosidase indicator gene. Plasmid pP[hs-neo; Rh1 (~833/+67) - β-gal] was constructed by cloning the BglII (~833)/KpnI (~67) ninaE promoter fragment into the vector pDM79 which had been digested with BamHI and KpnI. The plasmid pP[hs-neo; Rh1 (~120/+67) - β-gal] was obtained by cleaving pP[hs-neo; Rh1 (~833/+67) - β-gal] with SalI and religating the plasmid. Diagrams of these two constructs are shown in panel E of Figure 6.

**Chromaphenicol acetyltransferase assays:** Flies to be assayed were manually decapitated using a razor blade and their heads and bodies were placed into separate 1.5 ml microcentrifuge tubes. After freezing in a −80°C freezer for 15 min fly heads or bodies were homogenized in 100 μl of 0.25 M Tris (pH 7.7). The homogenate was frozen by placing in a −80°C freezer for several minutes, thawed and sonicated. Microtubes were then placed in a 65°C water bath for 5 min followed by centrifugation in a microcentrifuge for 10 min at 4°C. An aliquot of the supernatant, 20 μl of 4 mM acetyl-coenzyme A, 1 μl of 14C-labeled chloramphenicol (0.2 μCi) and 0.25 M Tris (pH 7.7) were combined to give a final volume of 140 μl. After incubation at 37°C reactions were stopped by extraction with 500 μl of ethyl acetate. Further treatment of the samples is identical to a previously published procedure (Gorman, Moffat and Howard 1981).

After preliminary assays of the transformant lines belonging to each construct, assays were quantitated by allowing the acetylation reaction to proceed for a limited period of time during which the reaction was in the linear range (10 min to 2 hr depending on the level of activity observed in the preliminary assays for that particular construct). To more accurately quantitate the low activity found in certain lines, an extract of the control line P[hs-neo; Rh1 (~2800/+67) - CAT]1 was diluted 1:1000, 1:100 and 1:20 with an extract of ry506 host flies and the observed level of acetylation in extracts of the low-level lines was directly compared to that observed in the diluted control extracts. The amount of acetylation was determined by liquid scintillation counting of that portion of the thin layer chromatography plates, used to separate the reaction products, that contained the 14C-labeled reaction products (1-O-acetyl- and 3-O-acetyl-chloramphenicol). The scintillation cocktail used was Betamax (Westchem Corp.).

**In situ hybridization to polytene chromosomes:** 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. Polytenic chromosome squashes were
FIGURE 3.—A restriction enzyme map of the 5.4 kb HindIII fragment containing the entire coding region of the ninE gene and approximately 2.8 kb of 5'-flanking sequence is shown. Below the map, the intron/exon arrangement of the ninE transcript is outlined.

 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 labeled 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.

Isolation of D. melanogaster genomic DNA and DNA blotting: In lines transformed with constructs in the pDM30 vector the chromosome of insertion was determined by segregation against appropriate balancers in a ry background. Subsequently, the number of insertions in the particular chromosome was determined by carrying out DNA

FIGURE 4.—Autoradiographs of chloramphenicol acetyltransferase assays of flies belonging to transformant lines carrying RhI-CAT fusions. CAT assays were carried out as described in MATERIALS AND METHODS and visualized by autoradiography. Panel A shows an autoradiograph of an assay in which extracts from either five heads or five bodies of each transformant line were incubated with substrate for two hours. Lanes 1 and 2 represent head and body assays of line P[y]; RhI(-162/+67)-CAT17, respectively. There is little accumulation of major monoacetylated product in this assay indicating the presence of only a low level of CAT activity in the heads of these flies. No activity can be detected in the body extract. In contrast, head extracts from transformant lines P[hs-neo]; RhI(-833/+67)-CAT14, P[y]; RhI(-448/+67)-CAT6 and P[hs-neo]; RhI(-2800/+67)-CAT11 (lanes 3, 5 and 7, respectively) show an abundance of both monoacetylated products as well as the appearance of the diacetylated chloramphenicol indicating a high level of CAT activity. No CAT activity is detectable under these conditions in the extract from the bodies of flies from these same transformant lines (lanes 4, 6 and 8, respectively). In order to test whether the low level of CAT activity detected in transformant line P[y]; RhI(-162/+67)-CAT17 is completely head-restricted, we have carried out same type of assay using ten-times more material (50 vs. 5 flies). The results are shown in panel B. Although an appreciable level of chloramphenicol acetylation is now observed in the head extract of line P[y]; RhI(-162/+67)-CAT17 (lane 1), no activity can be detected in the body extract (lane 2). On the other hand, in transformant line P[y]; RhI(-162/+67)-CAT11, in addition to a high level of chloramphenicol acetylation in the head extract (lane 3), there is a low level of acetylation in the body extract (lane 4). This transformant
blots to genomic DNA isolated from each transformant line. The restriction enzyme digest utilized to determine the number of ends was either SacI or XhoI depending on the construct and the hybridization probes was 32P-labeled CAT DNA. The number of bands observed corresponds to the number of 5'-P-elements ends. DNA blotting was carried out as previously described (MANIATIS, FRISCH and SAMBROOK 1982). Rapid small scale isolation of DNA from flies of the different transformant lines was performed by the procedure of STELLER and PIRROTTA (1986).

**In situ hybridization to tissue sections:** Adult D. melanogaster heads were sectioned (8 μm frozen cryostat sections) and treated as described by HAFEN et al. (1983), except that the acid and pronase pretreatments were omitted.

β-Galactosidase histochemical staining: Histochemical staining for β-galactosidase was carried out as described by D. R. KANKEL and M. FITZGERALD (personal communication). Cryostat sections (8 μm) were fixed in 2% glutaraldehyde in disodium phosphate buffer (pH 7.5). After the sections were washed free from the fixative, a staining gel consisting of 100 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2% gelatin in citric acid/phosphate buffer (pH 7.8) was applied. Although D. melanogaster adults possess endogenous β-galactosidase activity, the pH optimum for this enzyme is approximately 5 and it is largely inactive at the pH used during the histochemical staining procedure. For the wholemount staining of the anterior part of larvae and adult heads, the same procedure was used except that the staining solution did not contain gelatin.

**RESULTS**

Fragments containing either 2.8 kb or 0.9 kb of the *ninaE* promoter appear to be able to direct CAT and β-galactosidase expression to retinula cells R1-R6: The plasmid p[*hs-neo*; *Rh1*-(-2800/+67)-CAT] contains a transcriptional fusion between nucleotides −2800/+67 of the *D. melanogaster* *ninaE* promoter (transcription initiation site is at nucleotide +1) and the *E. coli* chloramphenicol acetyltransferase (CAT) gene. The 5' end of this fragment coincides with that of the 5.4 kb genomic HindIII fragment shown in Figure 3. This 5.4-kb DNA segment is sufficient to rescue the *ninaE* mutant phenotype when introduced into the Drosophila germline by *P*-element transformation (C. S. ZUKER, D. MISMER and G. M. RUBIN, unpublished data). Following microinjection of p[*hs-neo*; Rh1(−2800/+67)-CAT] into *D. melanogaster* Canton-S embryos, a transformant line was obtained. CAT activity in this transformant line was found to be restricted to the head (Figure 4A). The *ninaE* gene is expressed solely in the visual system (O'TOUNA et al. 1985; ZUKER, COWMAN and RUBIN 1985), which in the adult is found only in the head. Thus, the observed head-specific CAT activity is an indication that the promoter fragment reestablishes, at least in part, the pattern of expression of the *ninaE* gene. 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 M. C. MULLINS). In this line high levels of CAT activity are present in both the head and body (data not shown).

The 2.8-kb promoter fragment was shortened to contain 833 bp of upstream sequences. When transformant lines were obtained for this construct, p[*hs-neo*; Rh1(−833/+67)-CAT], their CAT activity was also restricted to the head and was similar in level to that found in the transformant line p[*hs-neo*; Rh1(−2800/+67)-CAT] (Figure 2A). The nucleotide sequence of this promoter fragment, extending from −833 bp to +67 bp, is shown in Figure 5.

In order to more precisely determine the tissue specificity of expression directed by the −833/+67 promoter fragment we constructed a fusion between this fragment and the *E. coli lacZ* gene, p[*hs-neo*; Rh1(−833/+67)-β-gal]. The pattern of β-galactosidase staining for the *E. coli lacZ* gene in the body and head of Canton-S embryos is similar to that described above for the CAT assay (Figure 6A). The body-to-head ratio of staining was determined by quantifying the blue color for both the head and the body. The promoter fragment −833/+67 contains most of the body-specific enhancer sequences that are present in the full-length *ninaE* promoter, as shown by the effect of the tandemly repeated fragment −162/−219 targeting the body region of the *ninaE* promoter (Figure 6B). The third transformant line belonging to this construct *pP*[hs-neo; *Rh1*(−162/+67)-CAT-Rh1(−501/-219)]3 and *pP*[hs-neo; *Rh1*(−215/+67)-CAT-Rh1(−501/-219)]3 show an assay of a head extract of line *pP*[hs-neo; *Rh1*(−162/+67)-CAT]1; a low level of chloramphenicol acetylation is observed. Although *pP*[hs-neo; *Rh1*(−162/+67)-CAT]1 contains some activity, this was not reproducibly seen and probably represents minor contamination of the CAT assay performed on the transformant line *pP*[hs-neo; *Rh1*(−215/+67)-CAT] by analysing five individuals belonging to different developmental stages. No activity can be detected in following stages: embryo (lane 1), first, second and third larval instar (lanes 2, 3 and 4, respectively), early pupa (white, no adult structures discernible: lane 5) and body (lane 8). CAT activity can be detected in the stages that have begun or completed the morphogenesis of the rhabdomere, the light-sensing organelle of the retinula cells: late pupa (red eyes, darkening of the cuticle but no adult structures discernible: lane 5) and body (lane 8). CAT activity in this transformant line was found to be restricted to the head (Figure 4A). The *ninaE* gene is expressed solely in the visual system (O'TOUNA et al. 1985; ZUKER, COWMAN and RUBIN 1985), which in the adult is found only in the head. Thus, the observed head-specific CAT activity is an indication that the promoter fragment reestablishes, at least in part, the pattern of expression of the *ninaE* gene. 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 M. C. MULLINS). In this line high levels of CAT activity are present in both the head and body (data not shown).

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D. Mismer and G. M. Rubin

\[
\begin{align*}
\text{FIGURE 5.} & \quad -833 \ AATGTTGCCC \ ACGATCGATC \ AATGCTACAC \ CAGCTGACAG \ TTATTTCGGT \ \text{Sal I} \\
& \quad -763 \ GGGCCAAAAGC \ GTGCGATACC \ ATGGGCAAT \ TTGAACTGCA \ CCCATTTCA \ GCTGATGTG \\
& \quad -693 \ TGTGCCCTCC \ CAAAAGGC \ AACAAGGATG \ TTTTAACAC \ AAGCTGGACT \ \text{Bgl II} \\
& \quad -623 \ GGGCCACCAAA \ AAAGAAAGA \ AAAACAAAA \ GCCTGGGAAA \ GTGACATCG \\
& \quad -553 \ CACAAAATAAC \ CATACAACCG \ CTGAGGTCGCT \ GGGGTTTAC \ \text{Aha III} \\
& \quad -483 \ CTTCCTAAAAG \ CTGGCATTCT \ TTTTAAGATA \ ATCCAAGATT \ AGCAGAGCCC \\
& \quad -413 \ CGGCACACTG \ GTGTATCTTG \ AATGGGCGAT \ CGCGACTCTG \ TTGGGATTAG \\
& \quad -343 \ CGCCACACCAC \ TTGACAGCTA \ AGAGAAATAG \ AGGAGGAAAT \ AGACGGTTA \\
& \quad -273 \ CCATTGTGGG \ TTTTCTTACA \ TATTGCTTGCA \ AATGGGACCA \ CATTAGTAT \\
& \quad -203 \ AAATTTCGTTT \ CAGGAGGTTG \ CTTGAGTTCC \ GTTATGGATA \ TTATGATTAT \\
& \quad -133 \ AATGCCTTTA \ ACTATTGACA \ TTGGCCCATT \ GCGATGTGCG \ CTTGCTTTCC \\
& \quad -63 \ AAGAGCAGGG \ GTTACAGAGG \ CATTGGGAAA \ ACATGGACGTA \ ATTGCAGGTT \\
& \quad +1 \ +8 \ TGCAGGGG \ CATTGGC \ CATGACGGATA \ CCACAGCGGC \ CAAACGAGCATAGGAC \ ATTGCAGGTT \\
& \quad +67 \\
\end{align*}
\]

\[\text{DNA sequence of the \textit{ninaE} promoter region. The \textit{ninaE} gene was sequenced from the BglII site located 833 bp upstream from the site of transcription initiation to the position +67 in the 5'-untranslated leader. Selected restriction enzyme sites are indicated. The sequence TATAAAA that conforms to the consensus sequence for the TATA box (COLOBERG 1979) and is located between nucleotides -31 and -25, is also underlined. No large (i.e., greater than 10 bp) direct or inverted repeats exist within the sequence.}\]
FIGURE 6.—β-Galactosidase histochemical staining of Rh1-β-galactosidase fusions. Panel E shows the diagram of the plasmids containing Rh1-β-galactosidase fusions used to obtain the transformant lines analyzed. Plasmids pP[hs-neo; Rh1(-833/+67)-β-gal] and pP[hs-neo; Rh1(-120/+67)-β-gal] contain 833 and 120 bp upstream of the transcription initiation site of the ninaE gene, respectively. Transformant lines containing these fusion genes were crossed into a white genetic background to facilitate the histochemical staining. Two transformant lines were obtained for plasmid pP[hs-neo; Rh1(-833/+67)-β-gal]; the sites of transposon insertion are at 72DE and 96F. Panel A shows a phase photomicrograph of a head section of a fly from the transformant line P[hs-neo; Rh1(-833/+67)-β-gal] after β-gal histochemical staining. Staining can be seen in the retina and lamina (labeled RE and LA, respectively; ME specifies medulla, another synaptic region of the optic lobe). The same pattern of staining has been observed in another transformant line P[hs-neo; Rh1(-833/+67)-β-gal] which carries the same construct (data not shown). Panels C and D show wholemount β-gal histochemical staining of the larval photoreceptor organ in the larvae belonging to line P[hs-neo; Rh1(-833/+67)-β-gal]. Arrows in C indicate the position of larval photoreceptor organ. Only four to five cells out of approximately 12 cells present in larval photoreceptor show detectable levels of β-gal activity. Panel B shows the pattern of staining observed in an adult head section of a fly from transformant line P[hs-neo; Rh1(-120/+67)-β-gal] in which the transposon is inserted at position 43E on 2R. The pattern of staining observed corresponds closely to the observed in the line P[hs-neo; Rh1(-833/+67)-β-gal] indicating that the tissue-specificity of expression has not been altered.
of the constructs analyzed, flies assayed were of the same age (7 days) and every line contained only a single P-element insertion into the genome. The 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 ninaE 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. At least two different control regions have been detected. Removal of the first control region, located between nucleotides −215 and −162, resulted in at least a 30-fold reduction in CAT activity. However, the low activity observed was still head-restricted (Figure 4, A and B). Moreover, in a transformant line, P[ks-neo; Rh1(−120/+67)-β-gal]1, in which nucleotides −120 bp to +67 bp are fused to laeZ, β-galactosidase expression is limited to the retinula cells R1–R6 (Figure 6B). These results indicate that sequences upstream of nucleotide −120 are not essential for the proper tissue-specificity of the ninaE promoter. The second control region found 3′ to the nucleotide −120 is essential for promoter function in the absence of the upstream element(s) controlling the level of expression; removal of sequences between −120 and −105 reduces the CAT activity to an undetectable level.

The smallest promoter fragment that still behaves essentially as the largest promoter fragment used in this study extends from −215 to +67 (Figure 2). A transformant line containing this promoter fragment, P[yr; Rh1(−215/+67)-CAT]7, was assayed to determine the developmental timing of CAT expression (Figure 4C). Expression of the ninaE gene, as detected by RNA blotting (ZUKER, COWMAN and RUBIN 1985; O’TOUSA et al. 1985), 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. The absence of detectable CAT activity in larval stages is probably due not to the lack of expression of the fusion gene in the larval photoreceptor organ but rather to the small number of cells expressing the gene. That the level of activity in adults is higher than in pupae is expected since ninaE mRNA levels are known to increase during adult life.

The removal of promoter sequences to position −215 did have a small quantitative effect. We wished to test whether this small effect resulted from the removal of an enhancer-like element. Enhancers are DNA sequences that can elevate the level of expression of a given promoter in position- and orientation-independent manner (for review, see SERFLING, JASIN and SCHAFFNER 1985). Often, promoters contain multiple, partially redundant sequences capable of exerting an enhancer effect. Deletion of any one of these sequences reduces the quantitative level of expression from the promoter only slightly in comparison with the reduction observed when all enhancer sequences are absent. By analogy, the 5′-flanking sequence of the ninaE gene may contain a number of enhancer sequences which may, at least in part, substitute for one another. We constructed the plasmids pP[yr; Rh1(−162/+67)-CAT-Rh1(−501/−219)] and pP[yr; Rh1(−162/+67)-CAT-Rh1(−219/−501)] in which the sequences from −501 to −219 are placed downstream from a ninaE/CAT gene fusion containing nucleotides −162 to +67 of the ninaE promoter. CAT expression in the lines obtained after transformation of these plasmids was restricted to the head and was comparable in level to that found in the transformant lines containing more than 215 bp of upstream sequences (Figure 4D) or about 20- to 30-fold greater than in transformants with only 162 bp of upstream sequences (Figure 2). This strongly suggests that the sequences located between nucleotides −219 and −501 behave as an enhancer; they can function 3′ to the promoter fragment, in both orientations, and over a distance of approximately 1.6 kb. Moreover, it appears that these sequences are partially redundant since in their absence there is only a two-fold reduction in expression.

In one of our constructs, an internal deletion within the 2.8-kb promoter fragment that removes the segment of DNA between the two SalI sites (−816 to −120 bp), a low level of expression was seen in bodies of the five independent transformant lines while no activity was detected in heads (data not shown). The repression of head activity is particularly striking since the lines corresponding to the plasmids pP[yr; Rh1(−120/+67)-CAT] have a significant level of head-specific CAT activity and no detectable activity in the body. We believe that this pattern of expression is due to the overriding effect of another enhancer-like element located between −2800 and −833 bp. The relevance, if any, of this control element to the regulation of the endogenous ninaE gene is unclear.

**DISCUSSION**

We have performed a preliminary analysis of the cis-acting sequences involved in regulation of the ninaE locus. We have succeeded in transferring the tissue-specific pattern of expression of this gene onto two distinct bacterial indicator genes by using solely ninaE sequences upstream of its transcription start point and a part of its 5′-untranslated leader. Our results indicate that no essential regulatory elements exist in the ninaE coding sequence, introns or 3′-flanking sequences. Deletion analysis revealed the presence of at least three separable cis-acting promoter elements (see Figure 7). Removal of both control elements I and II (Figure 7) resulted in at least a 30-fold reduction in the expression from this pro-
CONTROL ELEMENTS OF THE \textit{ninaE} PROMOTER

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{ninaE_promoter.png}
\caption{Schematic representation of the cis-acting regulatory elements of the \textit{ninaE} promoter. The position and properties of the control elements II and III have been determined from the deletion analysis of the promoter sequences carried out by \textit{P}-element-mediated transformation. Control element I, which has the properties of an enhancer, has been detected by placing the DNA fragment containing sequences between nucleotides -219 and -501 downstream from a truncated \textit{ninaE} promoter. This enhancer element is redundant; its removal has only a twofold effect on promoter efficiency. Control element II appears also to be a quantitative regulatory element. Control element III, located between nucleotides -166 and +67, contains all the sequences necessary for proper tissue-specificity of this promoter and can function independently from elements I and II, albeit at reduced levels.}
\end{figure}

The remaining low level of expression was still head-restricted, indicating that elements I and II are not the sole determinants of tissue specificity; a fragment that retains only 120 bp upstream of the transcription initiation site still appears to direct expression to the retinula cells R1–R6. Therefore, all the regulatory elements necessary for the proper tissue-specific expression from this promoter are located between nucleotides -120 and +67 (element III in Figure 7) and can function independently from elements I and II which appear to control transcriptional efficiency. We have not rigorously determined whether the transcription initiation point characteristic of the endogenous gene (Zuker, Cowman and Rubin 1985; O'Tousa et al. 1985) is used in our transformant lines. However, our constructs initiate transcription in an \textit{in vitro} transcription system derived from a Kc cell nuclear extract (prepared according to Heberlein, England and Tjian 1985) at a site identical to that found for the endogenous \textit{ninaE} gene (data not shown). It is, therefore, highly likely that the same transcription initiation site is used in the transformant lines.

For several developmentally regulated \textit{Drosophila} promoters, analysis by \textit{P}-element-mediated transformation has revealed the existence of multiple control elements. Separate control elements required for the expression in testes and Malpighian tubules have been described for the \textit{white} gene (Levis, Hazelrigg and Rubin 1985; Pirotta, Steller and Bozzetti 1985). Different control elements determine the expression of two yolk protein genes \textit{yp1} and \textit{yp2} in the ovaries and fat body (Garabedian, Hung and Wensink 1985). Separate control elements have also been implicated in the activation of hsp27 and hsp23 by the heat shock and ecdysteroids (Riddihough and Pelham 1986; Mestral et al. 1986). Cohen and Meselson (1985) demonstrated separate elements needed for heat-shock inducible and ovarian expression of hsp26 gene. Bourouis and Richards (1985) have separated the quantitative and qualitative control elements in the Sg33 glue protein gene. All of the regulatory elements identified in these studies are located 5' to the gene. However, Fischer and Maniatis (1986) described a regulatory element of \textit{Drosophila mulleri} Adh2 gene that is located at least 600 bp downstream of the Adh2 polyadenylation site; this element is essential for the expression of Adh2 gene in the larval Malpighian tubules after introduction of the \textit{D. mulleri} Adh2 gene into the germline of \textit{D. melanogaster}. In \textit{D. melanogaster} dopa decarboxylase gene separate elements appear to regulate expression in CNS and hypoderm (Scholnick et al. 1986). Thus, multiple control regions appear to be a common characteristic of developmentally regulated genes in \textit{D. melanogaster}.

Many genes have been reintroduced into the \textit{D. melanogaster} germline and, in general, these genes appear to be correctly regulated despite their presence at chromosomal positions that differ from that of the endogenous gene (Spradling and Rubin 1983; Scholnick, Morgan and Hersh 1983; Goldberg, Posakony and Maniatis 1983; Jowett 1985; Marsh, Gibbs and Timmons 1985; Kalfayan, Wako moto and Spradling 1984; Krumm, Roth and Korge 1985; Hazelrigg, Levis and Rubin 1984; Pirotta, Steller and Bozzetti 1985; Levis, Hazelrigg and Rubin 1985). However, the minimal length of the promoter needed to mimic the qualitative and quantitative regulation of the endogenous gene is highly variable and has been carefully determined in only a few cases. The \textit{ninaE} promoter analyzed in this study requires one of the shortest promoters, less than 300 bp, to obtain apparently normal expression. On the other extreme, 6.1 kb of 5'-flanking sequence is necessary to obtain the rescue of \textit{fushi tarazu} phenotype (Hiromi, Kuroiwa and Gehring 1985).

Some quantitative variation among different transformant lines carrying the same construct has been observed in all studies using \textit{P}-element-mediated transformation. The variation observed in the present study appears to be the greatest for constructs missing control elements I and II; presumably the truncated \textit{ninaE} promoter stripped of these elements becomes more responsive to other control sequences present in the region of transposon integration. Also, a higher level of expression was obtained when G418 was used to select transformants. This is probably due to the fact that high level expression of the G418 resistance gene is required to confer G418 resistance (Steller et al. 1985).
and Pirrotta 1985). Therefore, one may be biased toward recovering G418-resistant lines that carry insertions into chromosomal regions favoring a high level of gene expression. Random chromosomal locations are more probable when using ry as a marker, since less than 2% of wild type ry expression is needed to rescue the rosy eye color phenotype.

Control element I, located between nucleotides −501 and −219, appears to have the properties of an enhancer element. This sequence is capable of elevating the level of expression from the ninaE promoter, in the absence of control element II, in a position- and orientation-independent manner. Experiments are now in progress to determine whether the control element II, located between nucleotides −215 and −166, also behaves as an enhancer. Several other position- and orientation-independent control elements have been reported in the Drosophila genome: the yolk protein 1 enhancer (Garabedian, Shepherd and Wensink 1986), the alcohol dehydrogenase enhancer that acts on the proximal Adh promoter (Posakony, Fischer and Maniatis 1985), the ftz enhancer (Hiromi, Kuroia and Gehring 1985) and the Sgs4 enhancer (Shermoen et al. 1987). The Adh, yp1 and Sgs4 enhancers appear to be tissue-specific elements that are capable of conferring a pattern of expression characteristic of their parent genes onto a heterologous promoter element. Similar tissue-specific enhancers have been identified in the genes of the mammalian immune system (Neuberger 1983; Banerji, Olson and Schaffner 1985; Gillies et al. 1983; Picard and Schaffner 1984; Queen and Baltimore 1983). Although the removal of the enhancer sequence identified in this study does not alter the tissue-specific expression of the ninaE promoter, it cannot be ruled out that the enhancer itself carries determinants of tissue-specific expression. This enhancer is apparently redundant; in its absence expression is reduced at most twofold. Enhancer redundancy has not been previously demonstrated in D. melanogaster, although it is common in the enhancer regions of several animal viruses (for review, see Serfling, Jasin and Schaffner 1985). Also, three partially redundant enhancer elements have been identified in the mouse α-fetoprotein gene (Hammer et al. 1987). Although all three enhancers are functionally equivalent in transient transfection assays, in vivo these elements varied in their ability to modulate expression in different tissues.

Fusions between the ninaE promoter fragments (extending from either −833 bp or −120 bp to +67 bp) and the lacZ gene have been useful in demonstrating the tissue-specific expression of this promoter fragment. It is noteworthy that β-galactosidase molecules diffuse into the axons of the photoreceptor cells R1–R6. If such intracellular diffusion generally occurs in nerve cells, then expression of β-galactosidase becomes a powerful technique for staining a subset of neurons.

Expression of the ninaE–β-galactosidase fusion gene in the larval photoreceptor was an unexpected observation since larvae lacking a functional ninaE gene behave as their wild-type counterparts in assays of phototactic behavior (Hotta and Keng 1984). Our analysis suggested that the ninaE gene is expressed in only a subset of the cells belonging to the larval photoreceptor and thus it is likely that other adult opsins are expressed in different subsets of larval photoreceptor cells. This hypothesis is now being tested by constructing fusions between other adult opsins and appropriate indicator genes.

The functional differences between cells within the ommatidium must result in large part from transcriptional regulatory decisions that are made during ommatidial assembly and subsequent morphogenesis. Regulatory identities are established in the absence of cell division in a relatively short timespan in a previously homogenous cell population. Our long-term goal is to understand how the positional cues used in the assembly process are interpreted and lead to differences in transcription. By identifying the cis- and trans-acting regulators of genes which are restricted to specific photoreceptor subpopulations we hope to ultimately reconstruct the hierarchy of decisions that result in a particular cell type. Understanding the regulatory differences between the different cell types and how they are brought about should increase our knowledge of the regulatory mechanisms occurring during the final stages of organogenesis.

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