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Isolation and Structure of a Rhodopsin Gene from D. melanogaster

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Summary

Using a novel method for detecting cross-homologous nucleic acid sequences we have isolated the gene coding for the major rhodopsin of Drosophila melanogaster and mapped it to chromosomal region 92B8–11. Comparison of cDNA and genomic DNA sequences indicates that the gene is divided into five exons. The amino acid sequence deduced from the nucleotide sequence is 373 residues long, and the polypeptide chain contains seven hydrophobic segments that appear to correspond to the seven transmembrane segments characteristic of other rhodopsins. Three regions of Drosophila rhodopsin are highly conserved with the corresponding domains of bovine rhodopsin, suggesting an important role for these polypeptide regions.

Introduction

Rhodopsin is the major photoreceptor of both vertebrate and invertebrate eyes (reviewed in Fein and Szuts, 1982). It consists of an apoprotein, opsin, covalently attached to a vitamin A derived chromophore, generally 11-*cis*-retinal. Photoactivation of rhodopsin is the first step in a complex process that converts the energy of an absorbed photon into a change in membrane potential. The chromophore is isomerized by light from the 11-*cis* to the all-*trans* configuration, which in turn leads to a conformational change in the opsin moiety. Such photoactivated rhodopsin molecules then trigger the cascade of events that results in a receptor potential (reviewed by Stryer, 1984). The genes for bovine and human rhodopsin have been isolated and their nucleotide sequences determined (Nathans and Hogness, 1983, 1984). These mammalian opsins are both 348 residues long and are highly homologous in structure.

Drosophila provides an attractive experimental system in which to study the molecular basis of phototransduction. The compound eye of Drosophila contains three distinct classes of photoreceptors (reviewed by Pak and Grabowski, 1978). In each of the approximately 800 ommatidia that make up the eye there are six outer (R1–R6) and two central (one R7 and one R8) photoreceptor cells. The photopigments found in the R1–R6 cells, the R7 cell, and the R8 cell differ in their absorption spectra, most likely because different opsins are expressed in these three classes of photoreceptors. Several loci at which mutations affect phototransduction have been identified (reviewed by Hall, 1982), including one, *ninaE*, that appears to be the structural gene for the R1–R6 opsin (Scavarda et al., 1983).

Using a novel approach based on the high thermal stability of RNA–RNA hybrids (Britten and Kohne, 1966; Cox et al., 1984) we were able to isolate, from a genomic library, the gene coding for a major species of Drosophila rhodopsin by virtue of its weak sequence homology with a cloned bovine rhodopsin gene. This approach should be useful for the isolation of heterologous sequences where homology is insufficient to allow use of standard DNA–DNA hybridizations. We report the details of the isolation and the nucleotide sequence analysis of this gene and of a cDNA copy of its mRNA. In this issue, O'Tousa et al. (1985) also report isolating the gene encoding the major rhodopsin of D. melanogaster.

Results and Discussion

Isolation of a Drosophila Genomic Clone Homologous to Bovine Rhodopsin

RNA–RNA hybrids are more stable than either DNA–DNA or DNA–RNA duplexes (Britten and Kohne, 1966; Casey and Davidson, 1977; Cox et al., 1984). Therefore, by using a radioactively labeled RNA probe, it should be possible in many cases to detect RNA–RNA homology even if no hybridization is detected with a DNA probe.

We first attempted to isolate a clone for Drosophila rhodopsin by screening a genomic library with a probe prepared by nick translating a bovine rhodopsin cDNA clone (see Experimental Procedures); however, even under conditions of reduced stringency, no reproducible hybridization signal was detected either in this screen or in DNA blots to Drosophila genomic DNA (data not shown). We next tested whether Drosophila melanogaster contains an RNA species that could be detected by hybridization to a ^{32}P-labeled, single-stranded RNA probe synthesized from the same bovine rhodopsin cDNA clone. The RNA encoding rhodopsin is expected to be specifically localized and abundant in the photoreceptor cells of the eye and to be large enough to direct the synthesis of a protein of approximately 40 kd (Larrivee et al., 1981). We have used the heads of adult flies as a source of tissue enriched in photoreceptor cells and adult bodies as a source of tissue lacking rhodopsin. At low hybridization stringencies (Figure 1, 20% formamide) a large number of Drosophila poly(A)$^+$ RNA species complementary to the bovine rhodopsin RNA probe are detected, in both head and body. As the stringency of hybridization is increased, however, we detect only a single major RNA species. This RNA is approximately 1.5 kb and is limited to the head fraction (Figure 1, 40% formamide). No hybridization was detected to similar RNA blots using a nick-translated DNA probe (data not shown).

In order to isolate the genomic clone encoding this 1.5 kb head-specific Drosophila RNA we used the bovine antisense RNA probe to hybrid-select the complementary

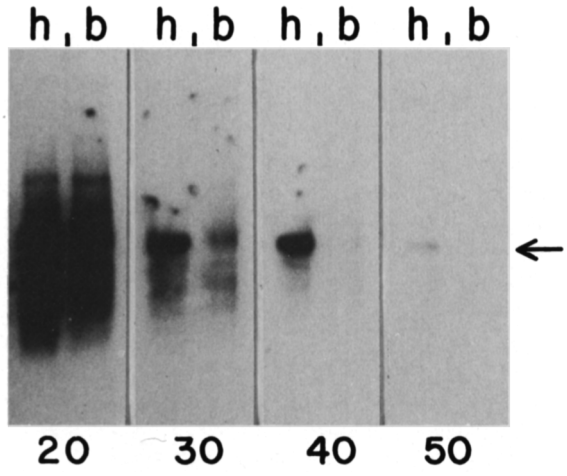

Figure 1. Homology between a Bovine Rhodopsin cRNA and Drosophila RNA Species

Polyadenylated RNA was isolated from heads (h) and bodies (b) of Oregon R flies, gel fractionated (2 μg per lane), and blotted onto nitrocellulose paper as described in Experimental Procedures. The RNA blots were then hybridized with a ^{32}P-labeled, single-stranded RNA probe derived from the 1.5 kb Bam HI–Hind III fragment of a cloned bovine rhodopsin cDNA (Nathans and Hogness, 1983) in 5× SSC at 65°C in 20%, 30%, 40%, or 50% formamide, as indicated. After 18 hr the blots were washed and exposed to X-ray film for 18 hr at −70°C with an intensifying screen. The arrow indicates the migration of the 1.5 kb RNA. Lambda Hind III and ϕX174 Hae III fragments were used as size markers.

Drosophila RNA species (see Experimental Procedures). The hybrid-selected Drosophila RNA was then used as a template for cDNA synthesis. This ^{32}P-labeled cDNA was used to screen both a complete genomic library and a genomic library previously enriched for sequences encoding RNAs expressed to a higher level in the head than in the body (see Experimental Procedures). Two positive clones were isolated from the total genomic library and seven from the enriched library. At least eight of these clones are derived from the same genomic region (see below), and one, λDmrh1, was chosen for further analysis.

We tested the hypothesis that our failure to detect Drosophila sequences homologous to bovine rhodopsin DNA probes resulted from the low stability of DNA–DNA hybrids relative to RNA–RNA duplexes. We measured the extent of hybridization, at various stringencies, between nick-translated bovine rhodopsin sequences and DNA blots of the putative Drosophila rhodopsin clone λDmrh1. We could detect weak hybridization in reactions containing 10% formamide at 42°C, but not in those containing 30% formamide (data not shown). In contrast, RNA–RNA hybrids between these same nucleotide sequences are stable in up to 40% formamide at 65°C (see Figure 1). Thus the low T_m of the Drosophila–bovine rhodopsin DNA hybrid can explain our inability to isolate the Drosophila gene by direct screening of genomic libraries.

DmRh1 Maps in Chromosomal Position 92B8–11

The six peripheral photoreceptors (R1–R6) of the Drosophila compound eye all appear to contain the same rhodopsin (Ostroy et al., 1974; Harris et al., 1976). Several mutations that reduce the amount of R1–R6 rhodopsin

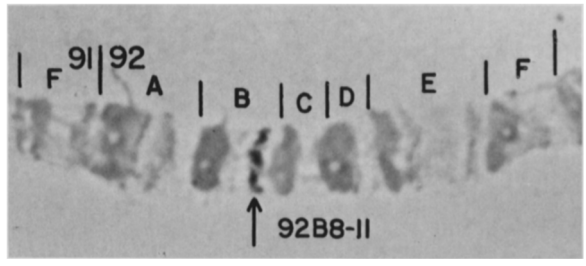

Figure 2. In Situ Hybridization to Salivary Gland Chromosome Squashes

A 1.5 kb cDNA clone complementary to the 5.4 kb Hind III fragment of Dmrh1 was biotinylated as described in Experimental Procedures and used as a hybridization probe to determine the chromosomal location of Dmrh1. Identical results were obtained with the 5.4 kb Hind III fragment or the entire lambda clone. However, the smaller cDNA probe provided better resolution.

Shown is the region 91–92 of chromosome III of D. melanogaster (Canton S). The arrow indicates the site of hybridization; no other sites of hybridization were observed.

have been isolated (reviewed by Pak, 1979). One of the loci, *ninaE*, has been shown to map in the salivary band region 92A–92B, and to have genetic properties expected of the R1–R6 opsin structural gene (Scavarda et al., 1983). Eight of the nine genomic clones we isolated cross-hybridize, and each was mapped by in situ hybridization to chromosomal position 92B8–11 (Figure 2), within the limits of the cytogenetic map position of *ninaE*. The ninth clone carried a moderately repetitive sequence and thus did not give a unique map position.

The rhodopsin in the R1–R6 rhabdomeres is expected to be expressed late in the fly's developmental cycle, during terminal differentiation of the photoreceptor cells. Hybridization of cDNA probes prepared from head and body poly(A)$^+$ RNA to restriction digests of λDmRh1 showed that the 1.5 kb head-specific RNA is contained on the 5.4 kb Hind III fragment (Figure 3; data not shown). To determine the developmental profile of expression of this putative rhodopsin mRNA, poly(A)$^+$ RNA was isolated from wild-type Oregon R flies at different times during development, fractionated on a formaldehyde gel, and hybridized to a nick-translated clone containing the 5.4 kb Hind III fragment of λDmrh1. Figure 4 shows that the 1.5 kb RNA species accumulates only by late pupation (8 and 9 days) and reaches maximum levels after eclosion (10 days). Comparing the intensity of the signal obtained with that observed after hybridization to a Drosophila actin probe (Fyrberg et al., 1983) we estimate that the abundance of this putative rhodopsin mRNA to be about twice that of actin mRNA in the heads of newly eclosed adults (Figure 4). In order to delineate the tissue specificity of the putative rhodopsin mRNA we isolated poly(A)$^+$ RNA from heads and bodies of wild-type flies and from heads of flies carrying the *sine oculis* mutation. Flies carrying the *sine oculis* mutation have nearly a complete loss of eyes (Fischbach and Technau, 1984). RNA from late third instar imaginal discs was also prepared to test for early expression of this gene. The 1.5 kb RNA complementary to λDmRh1 is present in preparations of poly(A)$^+$ RNA from wild-type heads, but not from bodies, imaginal discs, or *sine oculis* heads

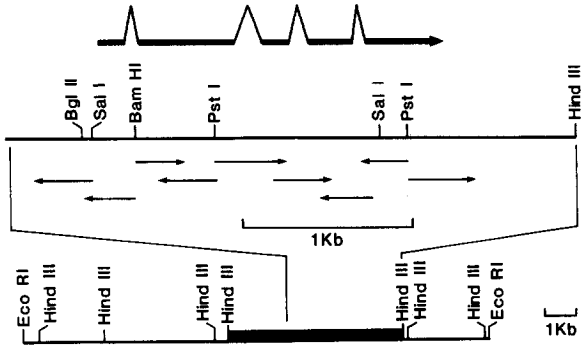

Figure 3. Restriction Map of Dmrh1 and Structure of the RNA It Encodes

The diagram shows a map of Dmrh1 indicating the restriction sites for Eco RI and Hind III (bottom map). Also shown, in expanded view, is the region of the 5.4 kb Hind III fragment encoding the RNA (upper map). The arrows underneath this map indicate those regions of the genomic clone that were sequenced and the diagram above the map shows the structure of the RNA as deduced by comparison of the nucleotide sequence of a cDNA clone (determined in both strands) and the genomic clone.

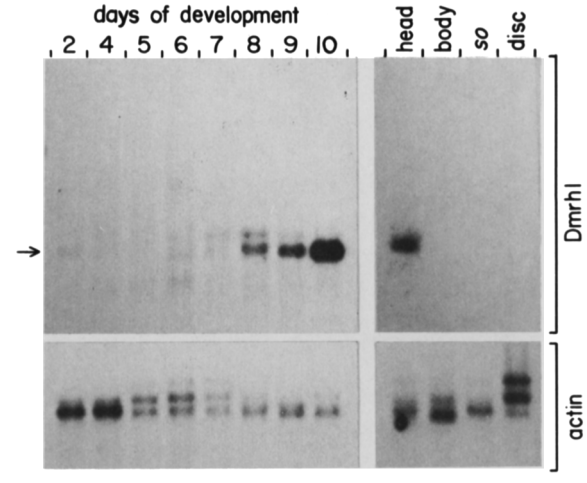

Figure 4. Accumulation and Localization of Dmrh1 Transcript during Development

Poly(A)$^+$ RNAs were extracted at different stages of development and from adult heads, adult bodies, and late third instar imaginal discs of a wild-type strain and from adult heads of the *sine oculis* mutant strain. The RNAs (1 μg per lane, 0.8 μg from *sine oculis*) were gel fractionated, blotted, and hybridized as described in Experimental Procedures. The upper panels show hybridization to the 5.4 kb Hind III fragment of λDmrh1 (see Figure 3). After exposure to X-ray film (48 hr, left panel, and 12 hr, right panel), the blots were boiled for 2 min in 10 mM Tris (pH 8.0), 1 mM EDTA, to remove the hybridized probe and were rehybridized with nick-translated pDMA2 plasmid (Fyrberg et al., 1983), a probe for actin RNAs, to control for the amount of RNA loaded. These blots were exposed for 5 hr (left panel) and 12 hr (right panel), respectively. The arrow indicates the migration of the 1.5 kb RNA. Lambda Hind III and ϕX174 Hae III fragments were used as size markers.

(Figure 4). Thus, the cytogenetic location, size, abundance, tissue specifity, and developmental time course of expression of this RNA are all consistent with its being an mRNA for a major species of Drosophila rhodopsin.

The 1.5 kb Putative Rhodopsin mRNA Is Encoded in Five Exons

We used the 5.4 kb Hind III fragment of λDmrh1 (see Figure 3) to screen a Drosophila cDNA library (gift from B. Yedvobnick and S. Artavanis-Tsakonas) and isolated several nearly full length cDNA clones. Using M13 dideoxynucleoside triphosphate sequencing we have determined the DNA sequence of one of those cDNAs and of a 2.5 kb segment of λDmrh1 (see Figure 3) that encodes the 1.5 kb putative rhodopsin mRNA. Comparison of these two DNA sequences reveals that the gene for the 1.5 kb RNA is divided into five exons of 179, 561, 148, 278, and 384 bp, separated by introns of 63, 187, 58, and 68 bp. The position of the second of these introns is precisely conserved between bovine and Drosophila sequences, and the third and fourth are located only 18 and 10 nucleotides, respectively, from the corresponding positions of the introns in the bovine sequence (Nathans and Hogness, 1983; 1984; see Figure 6).

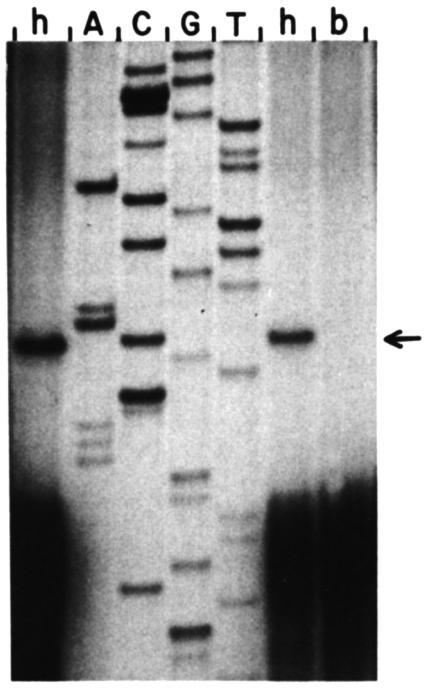

Figure 5. Primer Extension Analysis of Dmrh1 RNA

A 5′-labeled 23-mer synthetic oligonucleotide complementary to nucleotides 39 to 61 of the RNA was hybridized to poly(A)$^+$ RNA (1 μg) isolated from either adult heads (h) or adult bodies (b). The primer was then extended with reverse transcriptase as described in Experimental Procedures. The products of reverse transcription were electrophoresed in a 9% acrylamide gel along with the products of dideoxy sequencing reactions of the 3.5 kb Bam HI–Hind III fragment of Dmrh1 (see Figure 3) after priming with the same 23-mer synthetic oligonucleotide. A, C, G, and T refer to the specific sequencing reactions. The arrow indicates the position of the extended primer. Transcription initiation at the same site was obtained in vitro in transcription reactions templated with the 5.4 kb Hind III fragment (U. Heberlein and R. Tjian, personal communication).

To determine the site of transcription initiation in Dmrh1 we carried out primer extensions with RNA isolated from adult heads and adult bodies. The results (Figure 5) position the 5′ end of the RNA at the G residue (position 1) in Figure 6. The mRNA encoding Dmrh1 rhodopsin is 1550 nucleotides long, having a 5′ untranslated leader of 171 nucleotides and a 3′ untranslated segment of 260 nucleotides, excluding the poly(A) tail.

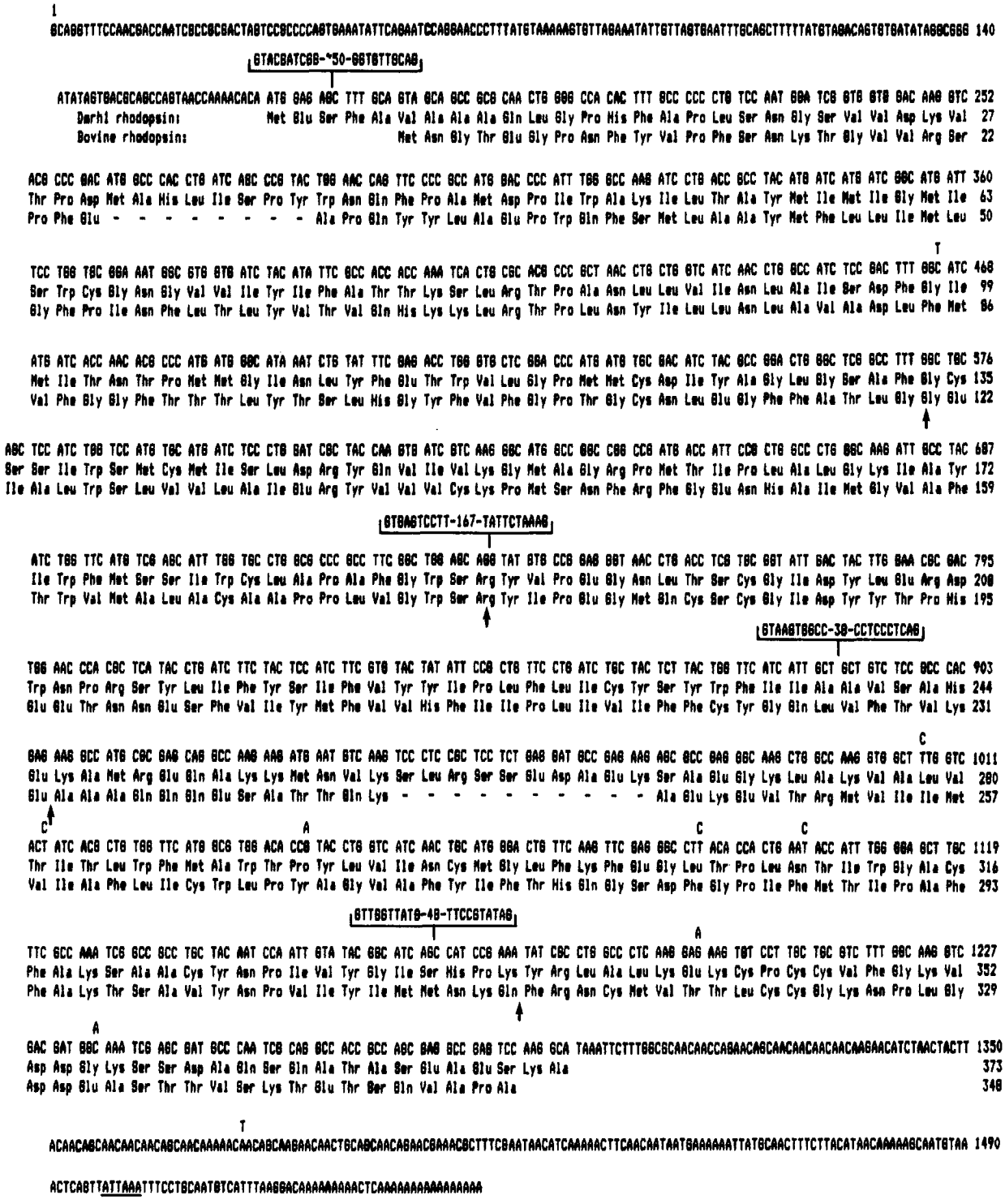

Figure 6. Nucleotide Sequence and Deduced Amino Acid Sequence of Drosophila Rhodopsin

The sequence from nucleotides 10 to 1556 was determined on both strands of the cDNA clone. This region was also sequenced in the genomic clone as indicated in Figure 3. The single nucleotides above positions 465, 1006, 1014, 1044, 1089, 1101, 1197, and 1236 show the differences in the coding region between the cDNA (Oregon R, P2 strain) and the genomic (Canton S) sequence; none of these polymorphisms would result in a change in the encoded amino acid. The sequence from 1 to 10 was determined by primer extension (see Figure 5). The brackets indicate the positions and the nucleotide sequences at the junctions of the four introns. The arrows show the positions of the four introns in the bovine gene. The ATTAAA sequence that probably signals poly(A)$^+$ addition (nucleotides 1499–1504) is underlined. When aligning the Drosophila and bovine rhodopsin (Nathans and Hogness, 1983) amino acid sequences it was necessary to introduce two gaps (at residues 30 and 258 of the Drosophila sequence). Additional regions could be aligned by the insertions of more gaps to reveal greater apparent homology—for example, in the C-terminal domain the two adjacent Cys residues of the Drosophila (positions 346 and 347) and bovine (positions 322 and 323) sequences could be aligned by adding a gap of one amino acid.

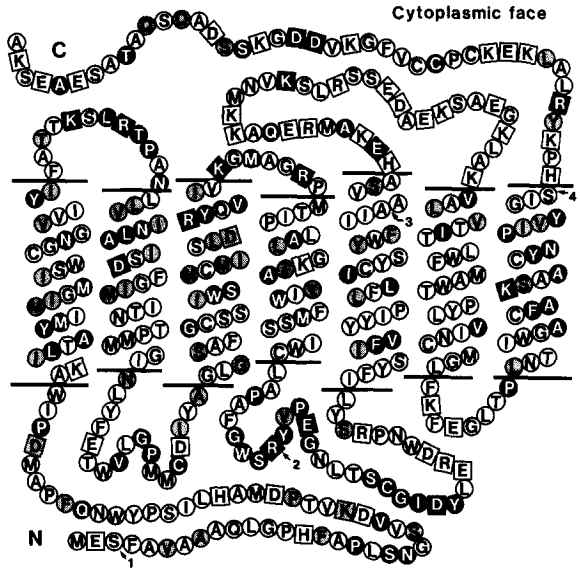

Figure 7. Proposed Structure of Drosophila Rhodopsin

The drawing is a modified version of the model of Ovchinnikov (1982). Amino acid residues are indicated by their single-letter code. Black solid circles indicate identities between the Drosophila and bovine sequences (see Figure 6). Those positions at which the Drosophila and bovine rhodopsin differ are indicated by open circles, and shaded circles denote conservative changes (M:V:I:L, A:G, F:Y, S:T:Q:N, K:R, and E:D). Squared residues indicate the positions of the charged (E,D and K,R,H) amino acids. The arrows indicate the positions of the four introns in the Drosophila gene.

The Protein Encoded by λDmrh1 Contains Amino Acid Sequence Domains That Are Highly Conserved in Those of Bovine Rhodopsin

Figure 6 shows the nucleotide sequence of the cDNA and the corresponding predicted amino acid sequence. Also shown is the amino acid sequence of bovine rhodopsin (Nathans and Hogness, 1983). The similarity between the structures of these two proteins (as discussed in detail below) clearly indicates that the protein encoded by λDmrh1 is a Drosophila rhodopsin.

The amino acid sequences of both bovine rhodopsin and bacteriorhodopsin are characterized by seven hydrophobic domains separated by hydrophilic sequences (Ovchinnikov et al., 1979, 1982; Nathans and Hogness, 1983; Khorana et al., 1979). Extensive studies on the topography of rhodopsins have revealed that the seven hydrophobic domains correspond to seven transmembrane segments (reviewed in Ovchinnikov, 1982). Analysis of the deduced amino acid sequence of the rhodopsin encoded by DmRh1 shows that over 50% of its residues are hydrophobic. More importantly, comparison of the amino acid sequence with that of bovine rhodopsin shows identity at 83 positions (Figure 6). It should be noted, however, that two gaps had to be introduced to align the two amino acid sequences. Based on the algorithm of Kyte and Doolittle (1982) and the model of Ovchinnikov (1982) we propose a structure for Drosophila rhodopsin that includes seven transmembrane segments, as shown in Figure 7.

Diptera and mammals are separated by over 500 million years of evolution (Holmquist et al., 1976). Thus the amino acid conservation between Drosophila and bovine rhodopsin is likely to reflect the presence of functionally significant domains in the polypeptide. Although the two proteins are only 22% homologous in amino acid sequence, 30 of the 83 positions conserved between bovine and Drosophila rhodopsin are located within three short polypeptide segments (see Figure 7).

The C-terminal intramembrane segment of bovine rhodopsin and bacteriorhodopsin contains the lysine residue responsible for chromophore attachment. In both proteins, the retinal is bound stoichiometrically by a Schiff's base linkage to the ε-amino group of lysine (reviewed in Ovchinnikov, 1982). Drosophila rhodopsin contains a single lysine in the seventh transmembrane domain (position 319), and it is in the same position in this domain as in bovine rhodopsin. Moreover, seven of the 11 amino acids flanking this lysine are identical in the bovine and Drosophila polypeptides.

Vertebrate rhodopsin is known to interact with at least three cytoplasmic proteins—transducin, a 48 kd protein, and rhodopsin kinase (Kuhn, 1978; Stryer, 1983, 1984). The interaction between light-activated rhodopsin and transducin results in a cascade of effects that eventually leads to a change in the ion permeabilities of the photoreceptor membrane. This change gives rise to the receptor potential. Assuming that analogous interactions are involved in the phototransduction process in Drosophila, similarities in the cytoplasmic aspect of bovine and Drosophila rhodopsins might be expected. In particular, conservation of a transducin binding site is indicated by the observation that vertebrate transducin can be activated by vertebrate or invertebrate rhodopsin (reviewed in Stryer, 1984). Previous studies, based on protease sensitivity experiments, have implicated the third cytoplasmic loop of bovine rhodopsin in its interaction with transducin (Kuhn and Hargrave, 1981). However, Drosophila and bovine rhodopsin show no significant sequence conservation in this domain. In contrast, six of the 12 amino acids that form the first cytoplasmic loop (residues 75–86, see Figure 7) are identical in the Drosophila and bovine sequences. Moreover, the conservation is restricted to the C-terminal part of the loop.

In mammals, light-activated rhodopsin is known to be deactivated by the action of rhodopsin kinase, which phosphorylates a series of serine and threonine residues located near rhodopsin's C terminus (reviewed by Hargrave, 1982; Miller and Dratz, 1983). Bovine rhodopsin contains three serines and six threonines in its hydrophilic C-terminal domain. Drosophila rhodopsin contains five serines and one threonine within the last 20 amino acids of the polypeptide chain (Figure 7). By analogy, we suggest that some, or all, of these residues serve as substrates for phosphorylation.

Rhodopsin is not known to interact with extracellular factors. However, the second extracellular loop of Drosophila and bovine rhodopsin contain 15 (out of 21) identical amino acid residues (Figures 6 and 7). The high degree of conservation of this domain strongly suggests it plays an important role in the structural integrity of rhodopsin, in its proper subcellular localization, or in its interaction with yet unknown factors involved in pho-

totransduction. It is noteworthy that in the rhodopsin gene encoded by Dmrh1, as well as in the human and bovine rhodopsin genes, the DNA encoding this conserved protein domain is split into two exons. Moreover, this region is highly conserved between human rhodopsin and the opsins involved in human color vision (J. Nathans, personal communication).

The spectral location of the λ_{max} of rhodopsin varies greatly among different species (reviewed in Fein and Szuts, 1982). It has been suggested that the number and distribution of charged residues inside the membrane are responsible for shifting the absorption maximum of rhodopsin (Mathies and Stryer, 1976). The Drosophila R1–R6 rhodopsin absorbs maximally at 470 nm (Harris et al., 1976) while bovine rhodopsin has a λ_{max} of 500 nm (Fein and Szuts, 1982). Interestingly, compared with bovine, Drosophila rhodopsin contains one additional positively charged residue within the membrane domain.

Concluding Remarks

We have isolated a Drosophila rhodopsin gene by homology to a cloned bovine rhodopsin cDNA with which it only shares 22% homology at the amino acid level. At the DNA level, the most significant homology between the Drosophila and the bovine gene is a 45 nucleotide segment that contains 34 identities (positions 740–785 and 530–575, respectively). This DNA segment encodes the amino acid residues seen in the conserved third extracytoplasmic loop (Figure 7). The method we used relies on the fact that RNA–RNA hybrids are more stable than either DNA–DNA or DNA–RNA duplexes and should be generally applicable as a means for isolating genes showing weak sequence homology.

Three regions of striking homology were found between the Drosophila and the bovine rhodopsin polypeptide chains, suggesting these regions have important functional or structural significance. Site-directed mutagenesis in combination with P-element-mediated DNA transformation should enable us to assign functional roles to these, and to other, polypeptide domains in the photoactivation and phototransduction process. The availability of mutations in the *ninaE* locus that abolish function of the endogenous rhodopsin gene (Scavarda et al., 1983) should greatly facilitate this type of analysis.

The different absorption spectra of the photopigments found in the three classes of photoreceptors of the compound eye of Drosophila suggest they are encoded by three opsin genes. In fact, we have isolated a number of sequences homologous to the major species of rhodopsin and are in the process of analyzing them.

Experimental Procedures

Isolation of Cytoplasmic RNA

RNA was extracted from various developmental stages of D. melanogaster (Oregon R, P2 strain) as described by O'Hare et al. (1983). Heads of adults flies (0–24 hr after eclosion) were separated from bodies as described by Oliver and Phillips (1970). Flies homozygous for the *sine oculis* mutation were grown at 29°C, a temperature at which the phenotype is most penetrant (Fischbach and Technau, 1984), and RNA was extracted only from those individuals that lacked over 95% of the eye structure. RNA was isolated from imaginal discs (a gift from O. Eugene and J. Fristrom) that had been prepared as described by Eugene et al. (1979). These disc preparations consisted of approximately 10% eye imaginal discs. Poly(A)$^+$ RNA was isolated by affinity chromatography on oligo(dT) cellulose columns (Blumberg and Lodish, 1980).

Blotting and Hybridization of DNA and RNA

Fractionation of the RNAs on formaldehyde gels, transfer onto nitrocellulose paper, and prehybridizations were carried out exactly as described by Chung et al. (1981). Hybridizations with nick-translated DNA probes were carried out at 65°C in 650 mM NaCl, 100 mM NaH_2PO_4 (pH 6.8), 75 mM sodium citrate, 0.04% bovine serum albumin, 0.04% PVP-40, 0.04% Ficoll, 0.5% SDS. Hybridizations with RNA probes were carried out at 65°C, in the same buffer, with the indicated amount of formamide. Filters were washed in 2× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate), 0.5% SDS, at 65°C when DNA probes were used, or in 0.3× SSC, 0.5% SDS, at 65°C for RNA probes. Genomic libraries were screened by the method of Benton and Davis (1977), except for the screens with nick-translated bovine rhodopsin, which were carried out in 5× SSC at 42°C. We screened four genome equivalents in the case of the genomic library screens. The "head-enriched" genomic library was selected from four genome equivalents of a total genomic library (Maniatis et al., 1978) as described by Davis et al. (1984) and will be described elsewhere (Cowman and Rubin, unpublished). The cDNA library (gift from B. Yedvobnick and S. Artavanis-Tsakonas) was made from poly(A)$^+$ RNA isolated from the heads of adult (0–24 hr after eclosion) flies of the Oregon R P2 strain.

RNA, DNA, and cDNA Probes

RNA probes were produced by in vitro transcription of linearized DNA templates containing the bacteriophage SP6 promoter (pSP64 and pSP65, Promega Biotec) exactly as recommended by the manufacturer (Promega Biotech). cDNA synthesis and nick translation of DNA templates were carried out as described by Maniatis et al. (1982).

RNA Selection

Drosophila poly(A)$^+$ RNA species complementary to the 1.5 kb Bam HI–Hind III fragment of a bovine rhodopsin cDNA clone (Nathans and Hogness, 1983) were isolated by either of two methods. In the first method, 25–50 μg of bovine rhodopsin RNA was synthesized with SP6 RNA polymerase from the bovine rhodopsin DNA fragment after it had been subcloned into the pSP65 vector (Promega Biotech). The newly synthesized RNA (cRNA) was phenol extracted and bound to DBM paper as described by Goldberg et al. (1979). Then 50 μg of poly(A)$^+$ RNA from D. melanogaster heads was hybridized to the paper-bound RNA in 250 μl of 40% formamide (v/v), 0.75 M NaCl, 20 mM NaH_2PO_4 (pH 6.8), at 65°C for 4 hr. The paper was washed, and bound RNA was eluted as described by Goldberg et al. (1979). The eluted RNA was ethanol precipitated and used as a template for cDNA synthesis primed with either oligo(dT) or random oligonucleotide (PL Biochemicals) primers as described by Maniatis et al. (1982).

In the second method, hybridization reactions were carried out in solution and the RNA–RNA hybrids were isolated by hydroxyapatite chromatography. ^{32}P-labeled bovine rhodopsin cRNA (1–5 μg; 1 × 10^7 cpm/μg), synthesized as described above with SP6 RNA polymerase, was hybridized with 15 μg of poly(A)$^+$ Drosophila RNA in 50 μl of 40% formamide (v/v), 0.75 M NaCl, 20 mM NaH_2PO_4 (pH 6.8), 0.1% SDS, at 65°C. After 4 hr, the reaction mixture was diluted into 10 volumes of 0.12 M phosphate buffer (pH 6.8), 0.1% SDS, and subjected to hydroxyapatite chromatography as described by Davis et al. (1984). The eluted RNA–RNA hybrid was denatured by boiling and used as a template for cDNA synthesis primed with oligo(dT). This approach is as effective as the previous one, but it is significantly simpler. However, one should be aware of endogenous RNAs with extensive secondary structure that may bind to hydroxyapatite at high salt. The two methods were used interchangeably.

DNA Sequence Analysis and Primer Extension

DNA sequencing was carried out according to the chain termination procedure of Sanger et al. (1977). M13 mp18 and mp19 were used as sequencing vectors, and JM101 as the host strain. Reactions were carried out as described by Bankier and Barrell (1983) with ^{35}S-dATP as

the radioactive nucleotide. The sequence of the entire cDNA clone was determined on both strands. The regions of the genomic clone that were sequenced are shown in Figure 3.

Primer extensions were carried out by hybridizing 5 ng of a synthetic 23 base oligonucleotide (complementary to positions 39–61) in separate 20 μl reactions to an M13 clone containing the 3.5 kb Bam HI–Hind III fragment of λDmrh 1 (see Figure 3), 2 μg of head poly(A)$^+$ RNA, and 2 μg of body poly(A)$^+$ RNA. Reverse transcription was then carried out as described for cDNA synthesis by Maniatis et al. (1982).

In Situ Hybridization to Polytene Chromosomes

Polytene chromosome squashes (Canton S strain) were prepared as described by J. K. Lim (unpublished procedure). Hybridization with biotinylated DNA probes were carried out according to Langer-Sofer et al. (1982) with the following modifications: DNA was nick-translated using Bio-16-dUTP (ENZO Biochem), and hybrids were detected using the Detek-I-HRP detection kit (ENZO Biochem).

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Note Added in Proof

Upon exchanging manuscripts with O'Tousa and collaborators (Cell, this issue) three nucleotide differences in the coding region were found (positions 674, 723, and 726; GCCCTGGGCAAG and GGCGCCCG-CCTTCGGC). We resequenced this area by the chemical method (Maxam and Gilbert, Meth. Enzymol. *65*, 499–560, 1980) and found that the sequence agrees with that reported by O'Tousa et al. We believe our original error was due to sequencing gel artifacts often associated with G-C-rich areas in M13 dideoxynucleoside triphosphate sequencing runs. Figures 6 and 7 were modified after submission of the manuscript to incorporate these changes. We thank O'Tousa and collaborators for communicating their results prior to publication.