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# A Rhodopsin Gene Expressed in Photoreceptor Cell R7 of the *Drosophila* Eye: Homologies with Other Signal-Transducing Molecules

**Charles S. Zuker, Craig Montell, Kevin Jones, Todd Laverty, and Gerald M. Rubin**

Department of Biochemistry, University of California, Berkeley, California 94720

**We have isolated an opsin gene from *D. melanogaster* that is expressed in the ultraviolet-sensitive photoreceptor cell R7 of the *Drosophila* compound eye. This opsin gene contains no introns and encodes a 383 amino acid polypeptide that is approximately 35% homologous to the blue absorbing *ninaE* and Rh2 opsins, which are expressed in photoreceptor cells R1–6 and R8, respectively. Amino acid homologies between these different opsins and other signal-transducing molecules suggest an important role for the conserved domains of rhodopsin in the transduction of extracellular signals.**

Phototransduction, the neuronal excitation process triggered by light, provides an ideal model system for the study of sensory transduction in the nervous system. Rhodopsin, the major photoreceptor of both vertebrate and invertebrate eyes, consists of an apoprotein, opsin, covalently bound to a chromophore, generally 11-*cis*-retinal (reviewed in Fein and Szuts, 1982; Hargrave, 1982). Light activation of rhodopsin is the first step in the visual response, a biochemical cascade that converts the energy of an absorbed photon into a receptor potential (reviewed by Stryer, 1983, 1985; Kühn, 1984; Stieve, 1986). 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. These photoactivated rhodopsin molecules then trigger the cascade of events that results in a transient change of the cation conductances of the photoreceptor cell membrane (reviewed by Fain and Lisman, 1981). Several vertebrate opsins have been sequenced (Ovchinnikov et al., 1982; Hargrave et al., 1983; Pappin and Findlay, 1984) and the genes for bovine and human opsins have been analyzed (Nathans and Hogness, 1983, 1984; Nathans et al., 1986). These vertebrate opsins are highly homologous in amino acid sequence and structure.

*Drosophila* is an attractive experimental organism in which to study signal transduction in the visual system using a combined molecular, genetic, and physiological approach (see, for example, Pak, 1979; Hardie, 1983; Rubin, 1985). The compound eye of *Drosophila* contains 3 distinct classes of photoreceptor cells, R1–6, R7, and R8, distinguishable by their morphological arrangement and the spectral behavior of their corresponding visual pigments (reviewed by Hardie, 1983). In each of the approximately 800 ommatidia that make up the eye there are 6 outer (R1–R6) and 2 central (1 R7 and 1 R8) photoreceptor cells (Fig. 1). The photopigments found in the R1–R6 cells, the R7 cell, and the R8 cell differ in their absorption spectra (Harris et al., 1976) most likely because different opsin genes are expressed in these distinct classes of photoreceptor cells. The 6 peripheral cells (R1–6) contain the major visual pigment, a rhodopsin that absorbs maximally at 480 nm (Ostroy et al., 1974). The gene encoding this visual pigment has been isolated by virtue of its homology to the bovine opsin gene and has been shown to correspond to the genetically defined *ninaE* locus (O'Tousa et al., 1985; Zuker et al., 1985). Of the 2 central photoreceptor cells, R7 contains a UV-sensitive pigment and R8 a blue nonadapting pigment (Harris et al., 1976; Hillman et al., 1983). We have previously reported the isolation and analysis of a *Drosophila* opsin gene that is transcribed specifically in the R8 photoreceptor cell (Cowman et al., 1986). We report here the isolation and analysis of a novel opsin gene that is homologous to the opsins expressed in the R1–6 and R8 pho-

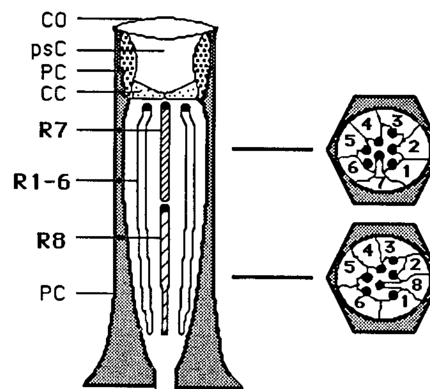


*Figure 1.* Adult ommatidial unit. The rhabdomeres (microvilli containing the visual pigments) of R1–R6 form an asymmetrical trapezoidal shape around the central rhabdomeres of the R7 and R8 cells. The R8 cell is located below the R7 cell and extends through the proximal half of the retina. *Inset* shows cross sections through the distal (*upper*) and proximal (*lower*) regions of the retina. *CO,* corneal lens; *psC,* pseudocone; *PC,* pigment cells; *CC,* cone cells; *R1–6, R7,* and *R8,* photoreceptor cells. Adapted from Tomlinson (1985).

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Correspondence should be addressed to Charles S. Zuker, Department of Biology, B-022, University of California at San Diego, La Jolla, CA 92093.

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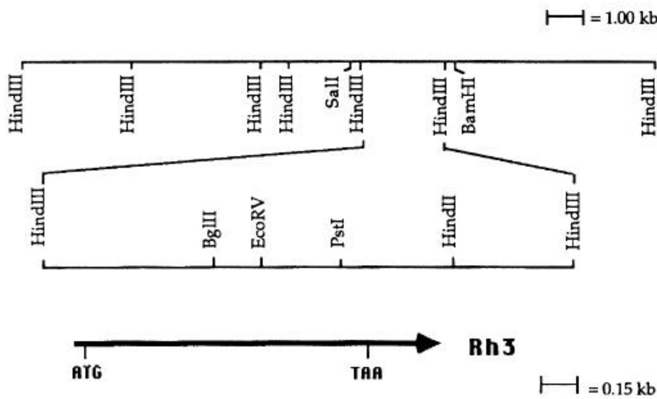


*Figure 2.* Restriction map of λDmRh3 and structure of the RNA it encodes. Shown is a map of λDmRh3 indicating the restriction sites for Hind III, *Bam* HI, and *Sal* I (*top map*). Also shown, in expanded view, is the region of the 2.4 kbase Hind III fragment encoding the RNA (*lower map*). The restriction sites for *Eco* RV, *Pst* I, *Bgl* II, and Hind II endonucleases are indicated. The *diagram below the map* shows the structure of the Rh3 RNA as deduced by comparison of the nucleotide sequences of a cDNA clone and the genomic clone, and S1 nuclease mapping and primer extension data (see Materials and Methods); note the lack of introns in Rh3.

toreceptor cells. We show that this opsin gene is transcribed in the UV-sensitive photoreceptor cell, R7, of the compound eye. In the accompanying paper (Montell et al., 1987), we describe the isolation and characterization of an additional opsin gene that is expressed in a nonoverlapping subset of R7 photoreceptor cells.

## Materials and Methods

*Isolation of poly(A)⁺ RNA.* RNA was extracted from the heads of the appropriate mutants, as described by O'Hare et al. (1983). Heads of adult flies (0–24 hr after eclosion) for Oregon R (P2), *sev* (sevenless; Harris et al., 1976), *ora* (outer rhabdomeres absent), and *sev ora* mutants (Harris et al., 1976) were separated from bodies as described by Oliver and Phillips (1970). 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 prehybridization were carried out exactly as described by Chung et al. (1981). Hybridizations with nick-translated DNA probes were carried out at 65°C in 750 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. Filters were washed in 0.2× SSC (1× SSC is 150 mM NaCl, 15 mM sodium citrate), 0.5% SDS at 65°C. The cDNA library (a gift from B. Yedvobnick and S. Artavanis-Tsakonas) was made from poly(A)⁺ RNA isolated from the heads of adult flies (0–24 hr after eclosion) of the Oregon R (P2) strain.

*DNA probes.* Nick-translation of DNA was carried out as described by Maniatis et al. (1982). All probes for *in situ* hybridization to head sections were made as described previously (Hafen et al., 1983).

*Oligonucleotide probes.* Two oligonucleotides were used as probes. The first [TATGTGCCIGAGGGTAA(C/T)CTGAC(C/T)] was 24 residues long, degenerate at positions 18 and 24, contained inosine at position 9, and encoded the peptide YVPEGNLT. The second [CAGGCCAAGAAGATGAATGTCAA(A/G)TCCCTI] was a 30-residue oligonucleotide, degenerate at position 24, contained inosine at position 30, and encoded the peptide QAKKMNVKSL. Synthetic oligonucleotides were purified by thin-layer chromatography on silica gel plates (Silica Gel 60 F-254; EM Reagents) in *n*-propanol : $NH_4OH$ : $H_2O$ (55:35:10), and were end-labeled with $\gamma$-$^{32}$P-ATP, as described by Maniatis et al. (1982). Hybridizations with end-labeled oligonucleotide probes were carried out at 42°C in 7× SSC, 0.1% bovine serum albumin, 0.1% PVP-40, 0.1% Ficoll. Filters were washed in 7× SSC, 0.5% SDS at 42°C. Four genome equivalents of a total genomic library (Maniatis et al., 1978) were screened.

In situ *hybridization to tissue sections.* Preparation of 8 μM frozen sections of adult heads and hybridization of $^3$H-labeled probes were as described by Hafen et al. (1983), except that the acid and pronase treatments were omitted in the pretreatment of the slides.

*DNA sequence analysis.* DNA sequencing was carried out on randomly sheared fragments according to the chain termination procedure of Sanger et al. (1977). M13 mp18 and mp19 were used as sequencing vectors and TG1 (the gift of Toby Gibson, MRC Laboratory of Molecular Biology, Cambridge, England) as the host strain. Reactions were carried out as described by Bankier and Barrell (1983) with $\alpha$-$^{35}$S-dATP as the radioactive nucleotide. The genomic sequence of the Rh3 opsin gene was determined over both strands. The complementary DNA (cDNA) sequence was determined on 1 strand.

*Primer extension and S1 nuclease analysis.* Primer extensions were carried out by hybridizing 5 ng of a synthetic 20 base oligonucleotide (complementary to positions +18 to +37) in separate 20 μl reactions to either an M13 clone containing the 0.75 kbase Hind III-*Bgl* II fragment of λDmRh3 (see Fig. 2), 20 μg of head poly(A)⁺ RNA, or 20 μg of body poly(A)⁺ RNA. Reverse transcription was then carried out as described for cDNA synthesis by Maniatis et al. (1982). S1 nuclease protection experiments were carried out as described by Maniatis et al. (1982). Single-stranded DNA probes used in the S1 endonuclease protection experiments were prepared by synthesizing a radiolabeled second strand on M13 templates. The newly synthesized material was separated from the template after restriction enzyme cleavage by boiling in 30% dimethyl sulfoxide (DMSO) and gel purified on a 1% agarose gel.

In situ *hybridization to polytene chromosomes.* Polytene chromosome squashes (Canton S strain) were prepared as previously described (Zuker et al., 1985). Hybridization with biotinylated DNA probes was carried out according to Langer-Sofer et al. (1982) with the following modifications: DNA was nick-translated using Bio-16-dUTP (Enzo Biochem)

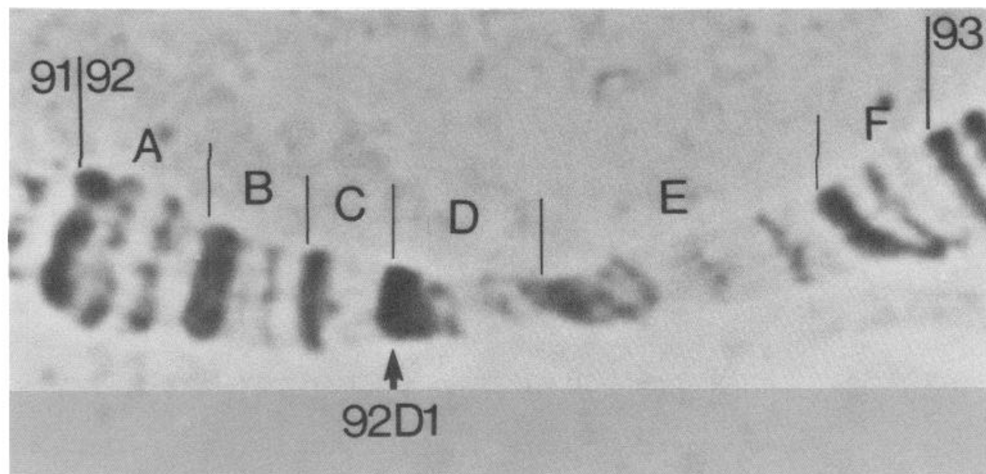


*Figure 3.* *In situ* hybridization to salivary gland chromosome squashes. λDmRh3 was biotinylated as described in Materials and Methods and used as a hybridization probe to determine its chromosomal location. Shown is the 92 region of chromosome III of *Drosophila melanogaster* (Canton S). The *arrow* indicates the site of hybridization at 92D1; no other sites of hybridization were observed.

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TTACTGCAACCCAAAATGGTCACTGCACTAACCTTCAGATGAGCTGCACTACACCCTCAATCGAGAATCA   -133
ATGCAAACGCAGTGCCAGCGAAAATGTCAGCAAGGGATTAGGCCAATCCCAAACGGGTAATCCCGCTGCG    -63
                                                                  *
ACAATGCTAATCCAATTCCGATGGGCCGTATAAAAGCCCCAAGCTGGGCTGGCTGTGATTTCGTCTTGG       7

CCCGCAGACCGGAGC ATG GAG TCC GGT AAC GTG TCG TCG AGT CTG TTT GGC AAC GTG    64
                MET Glu Ser Gly Asn Val Ser ser Ser Leu Phe Gly Asn Val    14

TCC ACC GCG CTG CGG CCG GAG GCG CGG CTC TCC GCC GAA ACG CGT CTG CTG GGC   118
Ser Thr Ala Leu Arg Pro Glu Ala Arg Leu Ser Ala Glu Thr Arg Leu Leu Gly    32

TGG AAT GTG CCG CCG GAG GAG CTG CGG CAC ATT CCC GAG CAC TGG CTA ACT TAC   172
Trp Asn Val Pro Pro Glu Glu Leu Arg His Ile Pro Glu His Trp Leu Thr Tyr    50

CCG GAG CCG CCC GAA TCG ATG AAC TAC CTG CTG GGC ACG CTC TAC ATC TTC TTC   226
Pro Glu Pro Pro Glu Ser Met Asn Tyr Leu Leu Gly Thr Leu Tyr Ile Phe Phe    68

ACC CTG ATG TCG ATG CTG GGC AAT GGA CTG GTG ATT TGG GTC TTC TCC GCA GCC   280
Thr Leu Met Ser Met Leu Gly Asn Gly Leu Val Ile Trp Val Phe Ser Ala Ala    86

AAA TCG CTG CGA ACT CCC TCC AAT ATA CTG GTC ATC AAT CTG GCC TTC TGC GAC   332
Lys Ser Leu Arg Thr Pro Ser Asn Ile Leu Val Ile Asn Leu Ala Phe Cys Asp   104

TTC ATG ATG ATG GTC AAG ACT CCG ATA TTC ATC TAC AAT AGC TTC CAC CAG GGA   388
Phe Met Met Met Val Lys Thr Pro Ile Phe Ile Tyr Asn Ser Phe His Gln Gly   122

TAT GCG CTG GGT CAT CTG GGA TGC CAG ATC TTT GGA ATC ATT GGC TCC TAT ACG   442
Tyr Ala Leu Gly His Leu Gly Cys Gln Ile Phe Gly Ile Ile Gly Ser Tyr Thr   140

GGA ATC GCT GCC GGT GCC ACC AAT GCG TTT ATA GCC TAC GAT CGA TTC AAT GTG   496
Gly Ile Ala Ala Gly Ala Thr Asn Ala Phe Ile Ala Tyr Asp Arg Phe Asn Val   158

ATC ACT CGA CCC ATG GAG GGC AAG ATG ACG CAT GGC AAG GCC ATT GCC ATG ATC   550
Ile Thr Arg Pro Met Glu Gly Lys Met Thr His Gly Lys Ala Ile Ala Met Ile   176

ATA TTC ATC TAC ATG TAC GCC ACT CCA TGG GTG GTT GCC TGC TAC ACG GAG ACT   604
Ile Phe Ile Tyr Met Tyr Ala Thr Pro Trp Val Val Ala Cys Tyr Thr Glu Thr   194

TGG GGC CGT TTT GTG CCG GAG GGA TAT CTG ACA TCC TGC ACC TTT GAC TAT CTC   658
Trp Gly Arg Phe Val Pro Glu Gly Thr Leu Thr Ser Cys Thr Phe Asp Tyr Leu   212

ACC GAT AAC TTC GAT ACG CGA CTC TTT GTG GCC TGC ATC TTC TTC TTC AGC TTC   712
Thr Asp Asn Phe Asp Thr Arg Leu Phe Val Ala Cys Ile Phe Phe Phe Ser Phe   230

GTG TGT CCC ACC ACG ATG ATC ACG TAC TAC TAC TCC CAG ATT GTG GGC CAT GTG   766
Val Cys Pro Thr Thr Met Ile Thr Tyr Tyr Tyr Ser Gln Ile Val Gly His Val   248

TTT AGC CAC GAG AAA GCA CTG CGG GAT CAG GCC AAG AAG ATG AAC GTG GAA TCG   820
Phe Ser His Glu Lys Ala Leu Arg Asp Gln Ala Lys Lys Met Asn Val Glu Ser   266

CTG CGT TCG AAT GTG GAC AAA AAC AAG GAG ACG GCG GAA ATC CGG ATA GCC AAA   874
Leu Arg Ser Asn Val Asp Lys Asn Lys Glu Thr Ala Glu Ile Arg Ile Ala Lys   284

GCG GCC ATC ACC ATA TGC TTC CTG TTC TTT TGC TCG TGG ACG CCG TAC GGA GTT   928
Ala Ala Ile Thr Ile Cys Phe Leu Phe Phe Cys Ser Trp Thr Pro Tyr Gly Val   302

ATG TCG CTG ATT GGC GCC TTT GGG GAT AAG ACC CTT TTG ACG CCC GGA GCC ACA   982
Met Ser Leu Ile Gly Ala Phe Gly Asp Lys Thr Leu Leu Thr Pro Gly Ala Thr   320

ATG ATT CCC GCC TGT GCC TGC AAA ATG GTG GCC TGC ATC GAT CCG TTC GTG TAC  1036
Met Ile Pro Ala Cys Ala Cys Lys Met Val Ala Cys Ile Asp Pro Phe Val Tyr   338

GCC ATA AGT CAT CCC AGA TAC CGC ATG GAG CTG CAG AAG CGA TGT CCC TGG CTG  1090
Ala Ile Ser His Pro Arg Tyr Arg Met Glu Leu Gln Lys Arg Cys Pro Trp Leu   356

GCG CTC AAC GAA AAG GCG CCG GAA TCG TCG GCT GTC GCC TCC ACC AGC ACC ACC  1144
Ala Leu Asn Glu Lys Ala Pro Glu Ser Ser Ala Val Ala Ser Thr Ser Thr Thr   374

CAG GAA CCA CAG CAG ACC ACC GCC GCC TAA TGCAAATTCCCACCCAACAGCCACCCATCC   1204
Gln Glu Pro Gln Gln Thr Thr Ala Ala                                       383

AAAAAATGCTGACAATGATGATGAACTGAAACGGCAACGAACTCGTAATACCAAACGCCAGGGAAAATTG   1274
TATAAATATTTCACGGAGTTTCTGCTAGAGTGCAAAGAGTTGAAAATATTTTCTTGTTATGATTTGTAGT   1344
CACTTTTGTGTTTATGAAAGAAATATAGTAAAATTACAGCAAGCTATGGAAAAAAGCGTTATATAAACTG   1414
TTTTTTTTTTTTAATTTCGTCGAACTAACCAAATAATGATGGAAGTTAAAGAGGATTTAATTTTACACAA   1434
CTTTTTTGTGTTAAAATATGGCCAAATGTAAGTAACTGAACATAAAAAGTTAAGTAAATACTCTTATCAA   1554
CTGAATAAGTCATTAACTCAGTTGACAACTTTAATAAAATAAATTCTTAAAAATCTTGAGTTGATATAAT   1624
CAGGTCATACTCAACGCATTGTGCAAAACACCTATTGTTTCACCAGGTTTACTTATGTTTATAGAAATCC   1694
TAAATGTTTGGTGTTTTGCTGATAACGAAAATGTGCATTCATTCGAAGGGGAAAAACTTCTTGATGTAAA   1764
AATGTAGGCTCAGTCCCGCTTTGAACTTTGCCCCCTTGTCAATTGTCAATCGTCCACTTGACGCGGCCAT   1834
TGCAGGGCACTCAGACGAGCCGAAGAAGGCGGCTTAATGCTCCCAGGAGCGGAGATGGCCAACAGCAAAA   1904
GAGTTGGCCAAGAACAATAGGGAATTGTGCCAGTTCCAATGGAATCCACAGACGGGAAAGCAGCTCTCTA   1974
ACTCTCTTGTGCTCGCTTACTTTCTCTGGAGAGCGAAAGCTT
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*Figure 4.* Nucleotide sequence and deduced amino acid sequence of *Drosophila* Rh3 opsin. The sequence shown was determined on both strands of the genomic clone (2.4 kbase Hind III fragment; see Fig. 1). The region from nucleotide 400 to 1270 was also sequenced in a cDNA clone. The *underlined region* at −28 to −34 shows the putative TATA box. The *star* at nucleotide +1 indicates the position of the start of transcription, as determined by primer extensions and S1 nuclease mapping data. The putative poly(A) addition signal is *underlined at position* ←

*1589–1599* (note the presence of 2 overlapping AATAAA consensus sites). The Rh3 opsin protein sequence is shown aligned under the nucleotide sequence.

and hybrids were detected using a Detek-I-HRP detection kit (Enzo Biochem).

## Results and Discussion

### *Isolation of λDmRh3, a DNA segment encoding a novel* Drosophila *opsin*

The different spectral sensitivities of the various photoreceptors of the *Drosophila* eye indicate the presence of multiple rhodopsins (reviewed in Hardie, 1983). The gene encoding the opsin expressed in the 6 peripheral photoreceptor cells (R1–6) has been isolated by virtue of its sequence homology to the bovine rhodopsin gene (O'Tousa et al., 1985; Zuker et al., 1985). This gene corresponds to the genetically identified *ninaE* locus (Scavarda et al., 1983). We have used the *ninaE* gene to search for cross-homologous sequences in the *Drosophila* genome and thereby isolated the gene encoding the opsin expressed in the central, blue-absorbing R8 photoreceptor cells (Rh2 opsin; Cowman et al., 1986); neither the *ninaE* nor the Rh2 opsin genes appear to be expressed in the central R7 photoreceptor cell (Zuker et al., 1985; Cowman et al., 1986). Therefore, at least one additional rhodopsin gene, the one encoding the opsin expressed in the R7 photoreceptor cell, must exist in the *Drosophila* genome. Low-stringency hybridizations of *Drosophila* genomic and cDNA libraries with the cloned *ninaE* and Rh2 probes did not reveal any additional homologous sequences. Assuming that functionally significant regions of rhodopsin might be conserved among the different opsin genes, we designed oligonucleotide probes (see Materials and Methods) corresponding to 2 of the most highly conserved regions between *ninaE* and Rh2 opsins in order to screen a genomic library with greater sensitivity. One of these regions encodes an 8 amino acid sequence that is conserved between the 2 *Drosophila* and bovine rhodopsins (Nathans and Hogness, 1984; O'Tousa et al., 1985; Zuker et al, 1985; Cowman et al., 1986). The other encodes a 10 amino acid sequence that is unique to *Drosophila* opsins (Zuker et al., 1985; Cowman et al., 1986); vertebrate opsins lack this 10 amino acid region. Positive clones were isolated and counterscreened with radiolabeled *ninaE* and Rh2 gene-specific probes to eliminate the *ninaE* and Rh2 cognate sequences. Three of the genomic clones that hybridized strongly with both oligonucleotide probes represented overlapping sequences, hereafter referred to as Rh3. Homology to the oligonucleotides was confined to a 2.4 kbase Hind III fragment (Fig. 2).

The *ninaE* and Rh2 genes have been cytogenetically mapped to chromosomal positions 92B8-11 and 91D1-2, respectively (Zuker et al., 1985; Cowman et al., 1986). The Rh3 clones were mapped by *in situ* hybridization to salivary gland chromosomes to chromosomal position 92D (Fig. 3). This cytogenetic location corresponds to that of λ512, a genomic clone isolated by Levy et al. (1982) on the basis of its encoding a head-specific transcript. No mutations affecting vision or visual input-mediated behavior have been isolated at or near this cytogenetic location.

### *Rh3 encodes an opsin*

We used the 2.4 kbase Hind III fragment of λDmRh3 (see Fig. 2) to screen a *Drosophila* cDNA library and isolated several

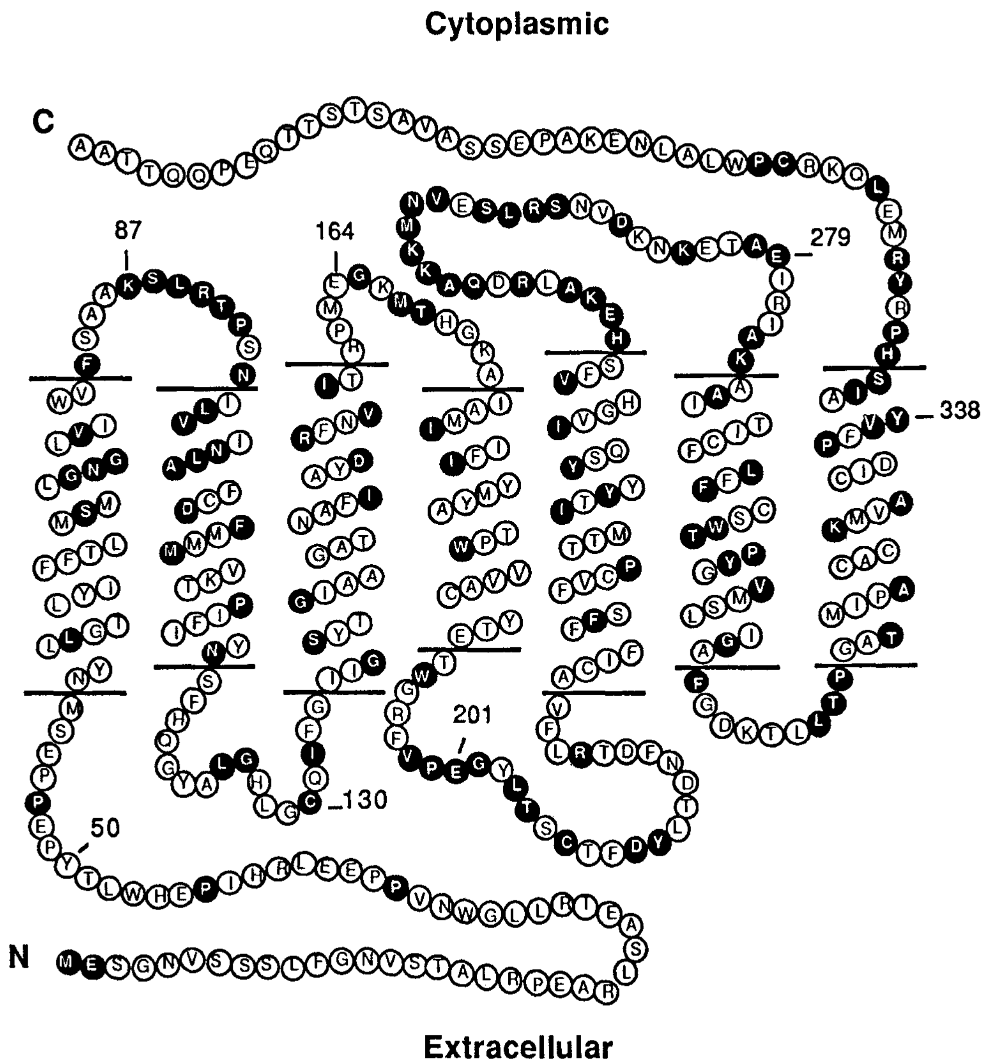


*Figure 5.* Proposed structure of *Drosophila* Rh3 opsin. Drawing is a modified version of the model of Hargrave (1982) and Ovchinnikov (1982). Putative transmembrane domains were determined by the algorithm of Kyte and Doolittle (1982). Amino acid residues are indicated by their *single letter codes. Black solid circles* indicate identities among the *Drosophila ninaE*, Rh2, and Rh3 opsins.

cDNA clones. Using M13 dideoxynucleoside triphosphate sequencing, we have determined the DNA sequence of one of those cDNA clones and of the 2.4 kbase Hind III genomic fragment of λDmRh3 (see Fig. 2). The genomic sequence we characterized included the same coding region as that present in λ512 (C. Montell and G. M. Rubin, unpublished observations; K. Fryxell and E. Meyerowitz, personal communication). Figure 4 shows the nucleotide sequence and the deduced amino acid sequence of the Rh3 gene. The structure of the RNA (Fig. 2) and the position of the start of transcription were determined by primer extension and S1 nuclease mapping analyses (data not shown).

Rh3 encodes a 383 amino acid polypeptide that shares 130 and 125 amino acid identities with the *ninaE* and Rh2 opsins, respectively; 116 of these residues are conserved between all 3 polypeptides. In addition, the protein encoded by Rh3 contains all of the structural features expected of a visual pigment protein: 7 hydrophobic domains separated by hydrophilic sequences, a presumed retinal-binding site in the seventh transmembrane domain (Lys 328), a series of potential phosphorylation sites (Ser and Thr residues) in the C-terminal region of the polypeptide chain, and a glycosylation site(s) in the extracytoplasmic face (Asn 5 and Asn 13). This is the only *Drosophila* opsin that contains more than 1 potential N-linked glycosylation site (consensus, Asn-X-Ser, Asn-X-Thr). Figure 5 shows the proposed structure of the Rh3 opsin, based on the algorithm of Kyte and Doolittle (1982) and the models of Ovchinnikov (1982) and Hargrave (1982). It is worth noting that amino acid residues 198–205 and 258–267, corresponding to oligonucleotide probes 1 and 2, are 71% (5/7) and 90% (9/10) conserved in Rh3 (see Materials and Methods). At the nucleotide level, oligonucleotide 1 shows 83% identity (20/24, including degenerate residues) with the corresponding region of the Rh3 gene, and the oligonucleotide 2 sequence is 86% conserved (26/30, including degenerate residues).

### *Rh3 is expressed in the UV-sensitive R7 photoreceptor cells*

Previous comparision of the amino acid sequences of the *ninaE* opsin with the Rh2 opsin revealed a high degree of homology (67%; Cowman et al., 1986). Both of these opsins have absorption maxima in the blue region of the spectra (reviewed by Hardie, 1983). In contrast, Rh3 is only approximately 35% homologous with the *ninaE* and Rh2 proteins (Fig. 5). In order to determine in which photoreceptor cell type the Rh3 opsin is expressed, and thus what spectral behavior it mediates, we examined the levels of mRNAs homologous to this gene in mutant

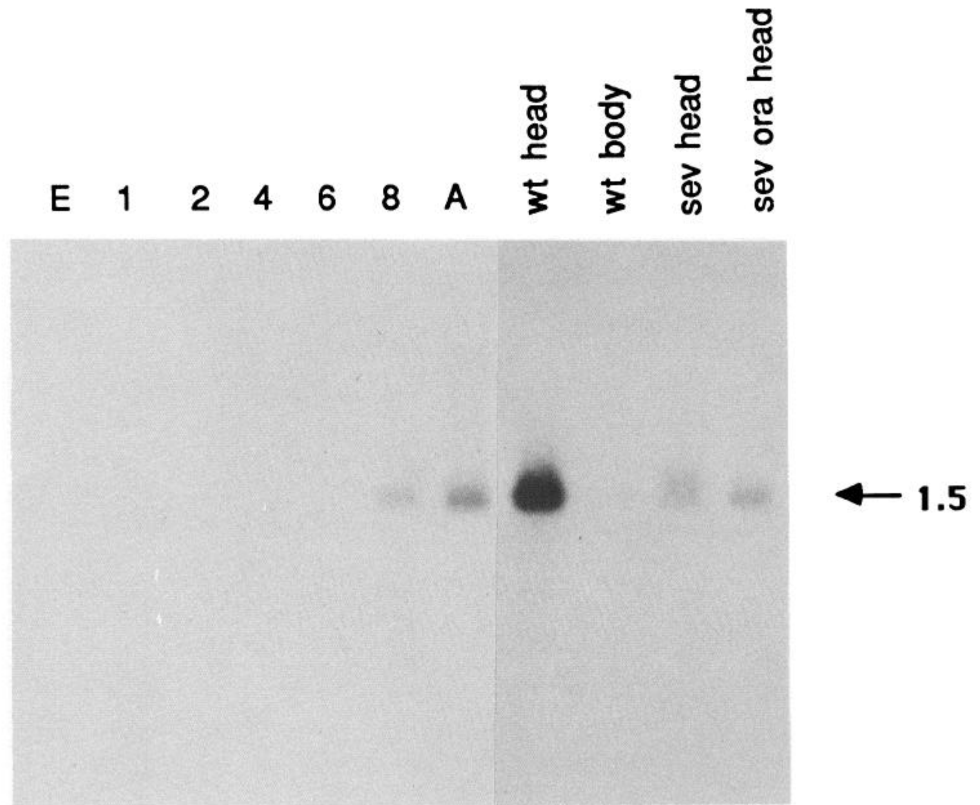


*Figure 6.* Expression of Rh3 transcripts. Poly(A)+ RNAs were extracted at different stages of development, from adult heads of wild-type, *sev*, and *sev ora* flies, and from wild-type adult bodies. The RNAs (2.0 μg/lane) were gel fractionated, blotted, and hybridized to a DNA probe derived from nucleotides 1070–2016 of Rh3, as described in Materials and Methods. *Lane E*, embryos; *lanes 1, 2, 4, 6, and 8*, days of development; *A*, newly eclosed adults. λ-Hind III and ΦX174 Hae III fragments were used as size markers.

strains that lack specific photoreceptor cells and have determined the sites of accumulation of these mRNAs by *in situ* hybridization to tissue sections of the wild-type eye.

We isolated total poly(A)+ RNA from wild-type Oregon R flies at different times during development, and from the heads and bodies of wild-type adult flies. We also isolated RNA from heads of flies homozygous for the sevenless (*sev*) mutation, which lack the central R7 photoreceptor cell, from the heads of *ora* (outer rhabdomeres absent) flies, which lack the 6 peripheral outer photoreceptor cells (R1–R6), and from the double mutant *sev ora*, which has only the central R8 photoreceptor cells (lacking R1–6 and R7). The RNAs were fractionated on agarose–formaldehyde gels and hybridized to a radiolabeled DNA fragment consisting of the 3′ region of Rh3 (nucleotides 1070–2016; Fig. 4). This sequence does not hybridize to any of the other *Drosophila* opsin genes. Rh3 hybridizes to a 1.5 kbase RNA

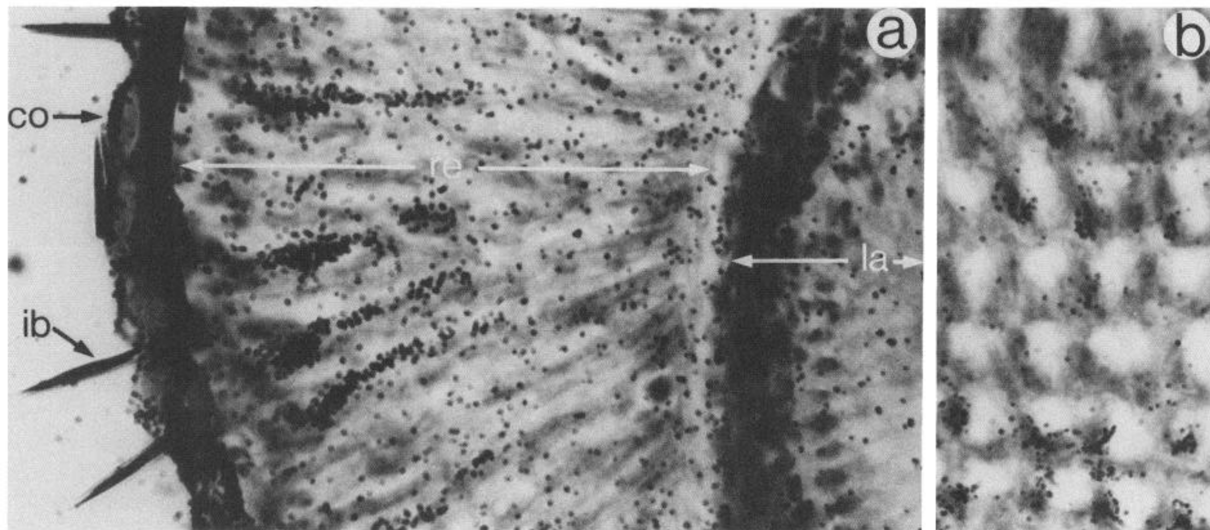


*Figure 7.* Spatial distribution of Rh3 transcripts in tissue sections of adult heads. Shown is the hybridization of an Rh3-specific probe to adult retinas (exposure, 30 d). Longitudinal (*a*) and tangential (*b*) sections of adult heads are shown. The R7 cell is located above the R8 cell and extends through the distal half of the retina (see Fig. 1). *Re,* retina; *la,* lamina ganglionaris; *co,* corneal lens; *ib,* interommatidial bristles.

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hGreen           MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWV-    52
hRed             MAQQWSLQRLAGRHPQDSYEDSTQSSIFTYTNSNSTRGPFEGPNYHIAPRWV-    52
hBlue                           MRKMSEEEFYLFKNISSVGPWDGPQYHIAPVWA-    33
hRhodopsin                    MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQ-   36
ha β-AR                         MGPPGNDSDFLLTTNGSHVPDHDVTEERDEAWVV    34
Dm Rh1            MESFAVAAAQLGPHFAPLS-NGSVVDKVTPDMAHLISPYWNQFPAMDPIW--    49
Dm Rh2       MERSHLPETPFDLAHSGPRFQAQSSGNGSVLDNVLPDMAHLNVPYWSRFAPMDPMM--    56
Dm Rh3   MESGNVSSSLFGNVSTALRPEARLSAETRLLG--WNVPPEELRHIP-EHWLTYPEPPESM    57
Dm Rh4          MEPLCNASEPPLRPEARSSGNGDLQFLGWNVPPDQIQYIP-EHWLTQLEPPASM    53

          *        * ***      *  *  *  *    ***  ** *     *            *   *
hG   |YHLTSVWMIFVVIASVFTNGLVLAA|TMKFKKLR--HPLN|WILVNLAVADLAETVIASTISVVN|QVYGYFVLGHPMCVLEG   130
hR   |YHLTSVWMIFVVTASVFTNGLVLAA|TMKFKKLR--HPLN|WILVNLAVADLAETVIASTISIVN|QVSGYFVLGHPMCVLEG   130
hB   |FYLQAAFMGTVFLIGFPLNAMVLVA|TLRYKKLR--QPLN|YILVNVSFGGFLLCIFSVFPVFVA|SCNGYFVFGRHVCALEG   111
hRh  |FSMLAAYMFLLIVLGFPINFLTLYV|TVQHKKLR--TPLN|YILLNLAVADLFMVLGGFTSTLYT|SLHGYFVFGPTGCNLEG   114
AR   |  GMAILMSVIVLAIVFGNVLVITA|--IAKFERLQTVTN|YFITSLACADLVMGLAVVPFGASH|ILMKMWNFGNFWCEFWT   110
Rh1  |AKILTAYMIMIGMISWCGNGVVIYI|FATTKSLR--TPAN|LLVINLAISDFGIMITNTPMMGIN|LYFETWVLGPMMCDIYA   127
Rh2  |SKILGLFTLAIMIISCCGNGVVVYI|FGGTKSLR--TPAN|LLVLNLAFSDFCMMASQSPVMIIN|FYYETWVLGPLWCDIYA   134
Rh3  |NYLLGTLYIFFTLMSMLGNGLVIWV|FSAAKSLR--TPSN|ILVINLAFCDFMMM-VKTPIFIYN|SFHQGYALGHLGCQIFG   134
Rh4  |HYMLGVFYIFLFCASTVGNGMVIWI|FSTSKSLR--TPSN|MFVLNLAVFDLIMC-LKAPIFIYN|SFHRGFALGNTWCQIFA   130

        *         **   **  *** **   *       *     *    *    *          *      * *         *
hG   |YTVSLCGITGLWSLAIISWERWMVVC|KPFGNVRF-DAKL|AIVGIAFSWIWAAVWTAPPIF|G-WSRYWPHGLKTSCGPDVF   208
hR   |YTVSLCGITGLWSLAIISWERWLVVC|KPFGNVRF-DAKL|AIVGIAFSWIWSAVWTAPPIF|G-WSRYWPHGLKTSCGPDVF    20
hB   |FLGTVAGLVTGWSLAFLAFERYIVIC|KPFGNFRF-SSKH|ALTVVLATWTIGIGVSIPPFF|G-WSRFIPEGLQCSCGPDWY   189
hRh  |FFATLGGEIALWSLVVLAIERYVVVC|KPMSNFRF-GENH|AIMGVAFTWVMALACAAPPLA|G-WSRYIPEGLQCSCGIDYY   192
AR   |SIDVLCVTASIETLCVIAVDRYIAIT|SPFKYQSLLTKNK|ARMVILMVWIVSGLTSFLPIQ|MHWYRATHQKAIDCYHKETC   190
Rh1  |GLGSAFGCSSIWSMCMISLDRYQVIV|KGMAGRP-MTIPL|ALGKIAYIWFMSSIWCLAPAF|G-WSRYVPEGNLTSCGIDYL   205
Rh2  |GCGSLFGCVSIWSMCMIAFDRYNVIV|KGINGTP-MTIKT|SIMKILFIWMMAVFWTVMPLI|G-WSAYVPEGNLTACSIDYM   212
Rh3  |IIGSYTGIAAGATNAFIAYDRFNVIT|RPMEGK--MTHGK|AIAMIIFIYMYATPWVVACYT|ETWGRFVPEGYLTSCTFDYL   212
Rh4  |SIGSYSGIGAGMTNAAIGYDRYNVIT|KPMNRN--MTFTK|AVIMNIIIWLYCTPWVVLPLT|QFWDRFVPEGYLTSCSFDYL   208

          *    *      *  **  * * *      *   *     *          *
hG   SGSSYPGVQS|YMIVLMVTCCITPLSIIVLCYLQVWLAI--|RAVAKQQKESESTQ-------------KAEKEVTR-----   268
hR   SGSSYPGVQS|YMIVLMVTCCIIPLAIIMLCYLQVWLAI--|RAVAKQQKESESTQ-------------KAEKEVTR-----   268
hB   TVGTKYRSES|YTWFLFIFCFIVPLSLICFSTYQLLRAL--|KAVAAQQQESATTQ-------------KAEREVSR-----   249
hRh  TLKPEVNNES|FVIYMFVVHFTIPMIIIFFCYGQLVFTV--|KEAAAQQQESATTQ-------------KAEKEVTR-----   252
AR   CDFFTNQAYA|--IASSIVSFYVPLVVMVFVYSR-VFQVAK|RQLQKIDKSEGRFHSPNLGQVEQDGR-SGHGLRRSSKFCL   266
Rh1  ERDWNPRSYL|--IFYSIFVYYIPLFLICYSYWFIIAAVSA|HEKAMREQAKKMNV-KSLRSSEDAEK-SAEGKLAK-----   276
Rh2  TRMWNPRSYL|--ITYSLFVYYTPLFLICYSYWFIIAAVAA|HEKAMREQAKKMNV-KSLRSSEDCDK-SAEGKLAK-----   283
Rh3  TDNFDTRLFV|--ACIFFFSFVCPTTMITYYYSQIVGHVFS|HEKALRDQAKKMNV-ESLRSNVDKNKETAEIRIAK-----   284
Rh4  SDNFDTRLFV|--GTIFFFSFVCPTLMILYYYSQIVGHVFS|HEKALREQAKKMNV-ESLRSNVDKSKETAEIRIAK-----   280

              *     *   ** **            * *        ▼  * *** ** *  * **     *
hG   ------|MVVVMV-LAFCFCWGPYAFFACFAA|ANPGYPFHP--|LMAALPAFFAKSATIYNPVIYVFM|NRQFRNCILQ---L   336
hR   ------|MVVVMI-FAYCVCWGPYTFFACFAA|ANPGYAFHP--|LMAALPAYFAKSATIYNPVIYVFM|NRQFRNCILQ---L   336
hB   ------|MVVVMV-GSFCVCYVPYAAFAMYMV|NNRNHGLDL--|RLVTIPSFFSKSACIYNPIIYCFM|NKQFQACAMK--MV   318
hRh  ------|MVIIMV-IAFLICWVPYASVAFYIF|THQGSNFGP--|IFMTIPAFFAKSAAIYNPVIYIMM|NKQFRNCMLT-TIC   322
AR   KEHKAL|KTLGIIMGTFTLCWLPFFIVNIVHV|--IQDNLIPKE|VYILLNWLGYVNSA-FNPLIYCRS|-PDFRIAFQELLCL   342
Rh1  ------|VALVTITLWFMA-WTPYLVINCMGL|F-KFEGLTP--|LNTIWGACFAKSAACYNPIVYGIS|HPKYRLALKE-KCP   345
Rh2  ------|VALTTISLWFMA-WTPYLVICYFGL|F-KIDGLTP--|LTTIWGATFAKTSAVYNPIVYGIS|HPKYRIVLKE-KCP   352
Rh3  ------|AAITICFLFFCS-WTPYGVMSLIGA|FGDKTLLTP--|GATMIPACACKMVACIDPFVYAIS|HPRYRMELQK-RCP   354
Rh4  ------|AAITICFLFFVS-WTPYGVMSLIGA|FGDKSLLTP--|GATMIPACTCKLVACIDPFVYAIS|HPRYRLELQK-RCP   350

                **        *
hG   FGKKVDDGSEL-SSASKTEVSSVSSVSPA                                              364
hR   FGKKVDDGSEL-SSASKTEVSSVSSVSPA                                              364
hB   CGKAMTDESDTCSS-QKTEVSTVSSTQVGPN                                            348
hRh  CGKNPLGDDEASATVSKTETSQVAPA                                                 348
AR   RRSSSKAYGNGYSSNGNGKTDYMGEASGCQLGQEKESERLCEDPPGTESFVNCQGTVPSLSLDSQGRNCSTNDSPL  418
Rh1  CCVFGKVDD-GKSSDAQSQ-ATASEAESKA                                             373
Rh2  MCVFGNTDE-PKPDAPASDTETTSEADSKA                                             381
Rh3  -WLALNEKA-PESSAVAS-TSTTQEPQQTTAA                                           383
Rh4  -WLGVNEKSGEISSAQS---TTTQEQQQTTAA                                           378
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*Figure 8.* Amino acid sequence homologies between the β-adrenergic receptor and the human and *Drosophila* opsins. Colinear alignment of the deduced amino acid sequences of the 4 human visual pigments (Nathans and Hogness, 1984; Nathans et al., 1986), the hamster β-adrenergic receptor (Dixon et al., 1986), and the 4 *Drosophila* opsins (O'Tousa et al., 1985; Zuker et al., 1985; Cowman et al., 1986; Montell et al., 1987). Amino acids are designated by their *single letter codes.* Alignment has been optimized for the largest number of identities with the least number of gaps. *Boxed areas* show the position of the 7 putative transmembrane domains of *Drosophila* opsins. *Stars above the sequence* indicate homologies between the β-adrenergic receptor and a minimum of 4 opsin genes. The *arrowhead* indicates the position of the lysine residue to which the retinal chromophore is thought to be bound.

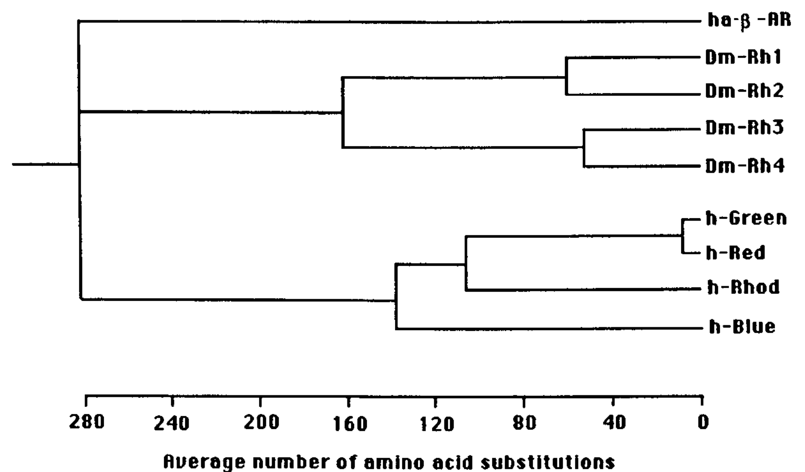


*Figure 9.* Phylogeny of visual pigments and the β-adrenergic receptor. This phylogenetic tree relating the different animal visual pigments and the β-adrenergic receptor was constructed on the basis of the principle of minimal mutation distances (parsimony principle). The data presented in Figure 8 were subjected to phylogenetic analysis by the Farris (1972) method. The most parsimonious tree is shown. Branch lengths (number of mutational events) are indicated in the *scale below the tree. Dm-Rh1* stands for the *Drosophila ninaE* gene, *Dm-Rh2, Dm-Rh3,* and *Dm-Rh4* refer to the *Drosophila* Rh2, Rh3, and Rh4 opsin genes. The hamster β-adrenergic receptor (Dixon et al., 1986) is referred to as *ha-β-AR. h-Green, h-Red,* and *h-Blue* refer to the human color opsins, and *h-Rhod* to human rhodopsin (Nathans and Hogness, 1984; Nathans et al., 1986).

species that accumulates in the late pupa and peaks after eclosion (Fig. 6). This RNA is present in the heads of wild-type flies or in flies carrying the *ora* mutation (data not shown). However, the RNA is greatly reduced in flies carrying the sevenless mutation (either *sev* or *sev ora*). The small amount of hybridization observed in the heads of *sev* and *sev ora* flies may represent expression of this gene in other cell types, as this RNA is also present in the heads of mutant flies lacking all photoreceptor cells of the eye (*glass*; data not shown). The spatial distribution of Rh3 transcripts was directly determined by hybridizing the radiolabeled Rh3 gene-specific probe to tissue sections of wild-type adult heads. Figure 7 shows that RNAs homologous to Rh3 are localized to the distal region of the retina; this is the location of the UV-sensitive R7 photoreceptor cells (see Fig. 1). This result, taken together with the severe reduction of Rh3 transcripts in mutants carrying the *sev* mutation (Fig. 6), indicates that the Rh3 opsin is transcribed in the central R7 photoreceptor cells of the compound eye.

The spectral and dichroic properties of the *Drosophila* R7 photoreceptor cells have not been studied in detail. These cells contain a bistable pigment system with sensitivity in the UV (330–350 nm) and metarhodopsin forms in the blue region of the spectrum (see Hardie, 1983; Hillman et al., 1983). We believe the differences between the primary sequences of the *ninaE* and Rh2 opsins, and the much more divergent Rh3 opsin, reflect the different spectral properties of these photopigments (see Montell et al., 1987).

### Drosophila *opsins contain amino acid sequence domains that are highly conserved with other signal-transducing proteins*

Vertebrate and invertebrate rhodopsins interact with at least 2 cytoplasmic proteins: a G-protein (transducin) and rhodopsin kinase (reviewed by Kühn, 1984; Stieve, 1986). The interaction between light-activated rhodopsin and transducin results in a cascade of effects that give rise to the photoreceptor potential. Evolutionary conservation of a transducin binding site has been postulated, as vertebrate transducin can be activated by vertebrate or invertebrate rhodopsin (Vandenberg and Montal, 1984). On the cytoplasmic face, the first and the third cytoplasmic loop of the *Drosophila* Rh3 opsin show a significant similarity to the corresponding regions of the *ninaE* and Rh2 opsins (see Fig. 5). However, only the first of these loops is conserved between *Drosophila* and vertebrate opsins (residues 87–94; Fig. 4; **KXLRXPXN**). The third cytoplasmic loop (Figs. 4 and 5, residues 251–284) contains a 10 amino acid insertion that is common to all *Drosophila* opsins analyzed to date (O'Tousa et al., 1985; Zuker et al., 1985; Cowman et al., 1986; Montell et al., 1987) but is absent from all vertebrate opsins (Ovchinnikov et al., 1982; Hargrave et al., 1983; Nathans and Hogness, 1983, 1984; Nathans et al., 1986).

We previously suggested that the first cytoplasmic loop of rhodopsin is involved in the interaction of rhodopsin with transducin (Zuker et al., 1985). Figure 8 presents sequence data indicating that this region also shows amino acid conservation between opsins (*Drosophila* and vertebrate) and the β-adrenergic receptor (β-AR), another signal-transducing molecule that interacts with a G-protein (Lefkowitz et al., 1983). Dixon et al. (1986) have recently shown that bovine rhodopsin and the hamster β-AR share significant amino acid sequence homology, particularly in the transmembrane domains. Functional conservation of catalytic components used for signal transduction in the amplification of the visual response and in the activation of adenylate cyclase-coupled β-ARs has recently been demonstrated, in that rhodpsin and β-AR can be properly phosphorylated by the other's kinase (Benovic et al., 1986). Sequence analyses of the additional *Drosophila* opsins (see Montell et al., 1987) and the recent isolation of the human color opsin genes (Nathans et al., 1986) have allowed us to place all of these proteins in a single phylogenetic tree. The phylogeny shown in Figure 9 was constructed on the basis of the principle of minimal mutation distances (Wilson, 1985; PAUP program, Illinois Natural History Survey, version 2.4.0). The most parsimonious tree favors the branching order shown. Remarkably, the vertebrate β-AR is equally related to *Drosophila* opsins as it is to those of vertebrates. The similarity in the number of mutations needed to account for the divergence of these sequences suggests that a single primordial "opsin-like" molecule was present in an ancestor common to vetebrates and invertebrates, and that it gave rise to vertebrate opsins, invertebrate rhodopsins, and the β-AR. Future studies involving the isolation and characterization of

the invertebrate homolog to the β-AR and other sensory receptors will help us dissect and understand similarities in signal-transduction mechanisms.

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