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Twenty *Drosophila* visual system cDNA clones: One is a homolog of human arrestin

(eye/retina/photoreceptor/phototransduction/*Arr* gene)

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ABSTRACT **From a group of 436 *Drosophila melanogaster* cDNA clones, we selected 39 that are expressed exclusively or predominantly in the adult visual system. By sequence analysis, 20 of the clones appear to represent previously unreported distinct cDNAs. The corresponding transcripts are detected in the retina and optic lobes. The genes are scattered throughout the genome, some near mutations known to affect eye function. One of these clones has been identified, by sequence analysis, as the structural gene (*Arr*) for a *Drosophila* homolog of human arrestin. Vertebrate arrestin interacts with rhodopsin in phototransduction and has been associated with an autoimmune form of uveitis in primates. The presence of an arrestin homolog in *Drosophila* suggests that both the vertebrate and invertebrate phototransduction cascades are regulated in a similar manner.**

Previous molecular screens have identified important *Drosophila* eye-specific molecules that are required for proper eye development and phototransduction. Levy *et al.* (1) isolated 20 genomic clones that hybridized to transcripts more highly expressed in head than in body. Seven of these were shown to be eye-specific, including the genes for opsin Rh3, transient receptor potential (*trp*), and neither inactivation nor afterpotential C (*ninaC*); (2–4). Shieh *et al.* (5) identified eye-specific genes using, as a probe, total wild-type head cDNA that was subtracted with poly(A)$^+$ RNA from bodies and heads of the mutant eyes absent (*eya*). The *eya* mutation removes the eyes and reduces the optic lobes but leaves the light-detecting ocelli intact (6). Their screen identified many eye-specific genes, including *ninaA*, a cyclophilin homolog (5), and an eye-specific protein kinase C (7).

Recently, 436 *Drosophila* cDNA clones corresponding to genes expressed predominantly in the head but not in the early embryo were isolated, and their expression patterns described (8). Comparison of Northern (RNA) blots of RNA from wild-type and from *eya* heads led to the identification of 39 clones, described here, that correspond to mRNAs expressed in wild-type but reduced in *eya* heads. By DNA sequencing, we have identified 20 of the clones as being cDNAs that are distinct from each other and do not correspond to previously reported cDNAs. In addition, four previously sequenced genes expressed in the retina were also found in our collection, some more than once, confirming the validity of the approach. One of the new clones‖ corresponds to a *Drosophila* gene with strong sequence similarity to the vertebrate phototransduction molecule arrestin. The *Drosophila* arrestin gene has been independently cloned by Smith *et al.* (9).

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MATERIALS AND METHODS

DNA Purification, Isolation of RNA, Northern Blots, and Southern Blots. These were performed as described (8).

DNA Sequence Analysis. cDNA inserts were isolated by digesting phage DNA with *Eco*RI and *Xba* I and subcloning into either SK$^+$ or KS$^+$ Bluescript vectors (Stratagene). Either single-stranded phage DNA or double-stranded plasmid templates were sequenced with the United States Biochemical sequencing kit.

Isolation of Longer cDNA and Genomic Clones. Longer cDNAs were isolated by screening the unsubtracted *Drosophila* λ SWAJ2 head cDNA library (8) with radiolabeled probes (10). Over 50,000 plaques were screened for each clone tested. Positives were picked, replated, reprobed, plaque purified, and confirmed by Southern blots. Genomic clones were isolated from the Maniatis *Drosophila* genomic library (11).

Tissue *in Situ* Hybridization. Third instar larvae, prepupae, or adult flies were frozen in Tissuetek OCT embedding compound on dry ice and equilibrated at −20°C. Frozen sections were cut at 6 μm and processed for hybridization by the method of Hafen and Levine (12), omitting the Pronase step. Nick-translated, ^{3}H-labeled DNA probes were prepared with all four nucleotides labeled to a specific activity of about 5×10^7 cpm/μg.

Chromosomal *in Situ* Mapping. Biotinylated DNA probes were hybridized to larval salivary chromosomes as in ref. 13, except that the acetic anhydride treatment of the slides was omitted. The probes were λ SWAJ2 or SWAJ3 (8) cDNAs subcloned into Bluescript. Plasmids were randomly primed to incorporate Bio-16-dUTP (Enzo Biochemicals), or ^{35}S-labeled dATP and ethanol precipitated with 1/10th vol of 3M ammonium acetate. The hybridization buffer contained dextran sulfate at a final concentration of 10% (wt/vol).

Materials. T4 DNA polymerase, *Eco*RI, and *Xba* I were purchased from New England Biolabs. RNasin, T7 RNA polymerase, SP6 RNA polymerase, and cRNA transcription systems were from Promega Biotec. *Spe* I restriction enzyme and helper phage were from Stratagene. Radioactive nucleotide triphosphates, Hybond-N paper, and cDNA cloning kits were from Amersham. All other enzymes and oligo(dT)-cellulose were from Boehringer Mannheim.

RESULTS

Identification of Visual System-Specific Clones. We identified genes that are expressed predominantly in the eye by

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. M30140).

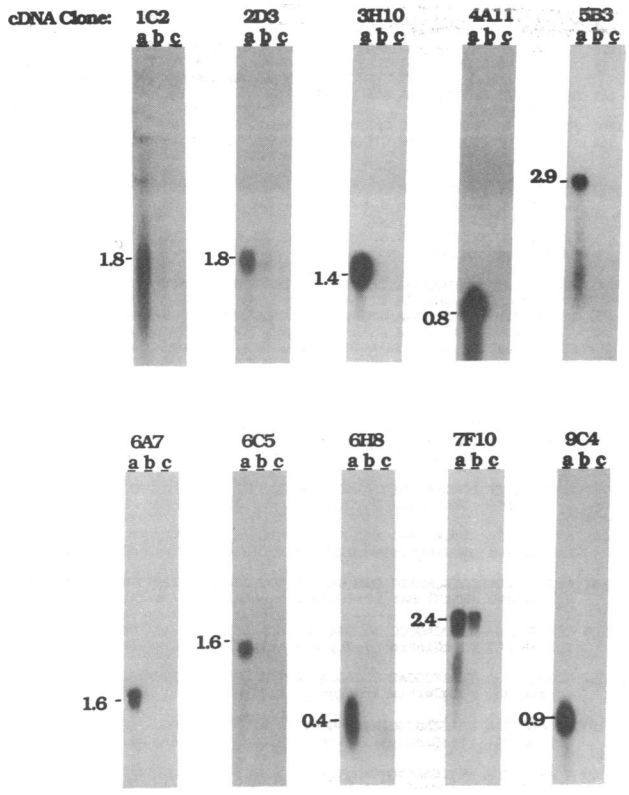

FIG. 1. RNA gel blots of poly(A)$^+$ RNA probed with different radioactive cRNAs to identify eye-specific transcripts. In each case, RNA was from wild-type heads (a), heads of *eya* (b), or wild-type bodies (c). Each lane contained 0.5 μg of poly(A)$^+$ RNA. The cRNA probes in these examples recognized transcripts of various sizes, reduced in the *eya* mutant, and not detected in wild-type bodies.

determining which wild-type head cDNAs hybridized to transcripts that were reduced or undetectable in an eyeless mutant. Antisense cRNA was synthesized *in vitro* from each of 436 head-not-early embryo *Drosophila* cDNA clones and used to probe Northern blots of poly(A)$^+$ RNA extracted separately from wild-type heads, *eya* mutant heads, and wild-type bodies (8). Body RNA was included as a negative control because the adult visual system is restricted to the head. Thirty-nine cDNAs detected transcripts expressed in wild-type heads that were reduced in *eya* heads and not detected in bodies. Examples of the various expression patterns are illustrated in Fig. 1; transcript sizes in the examples shown ranged from 0.4 to 2.9 kilobases (kb). Clone 7F10 is an example of a cDNA with a signal that is still detectable in *eya* but at a reduced level, suggesting that the expression of the corresponding gene is not restricted to the retina.

The Corresponding Genes Are Located at Many Sites. The cDNA clones in the original collection were rather short; therefore, homologous, longer cDNAs and genomic clones were isolated for hybridization *in situ* to third instar larval polytene chromosomes. The genes are scattered throughout the genome (Table 1), with some near to, or coincident with, previously identified mutant eye loci. Sixteen of the original 39 clones mapped to single bands; two mapped to multiple bands, suggesting that they correspond to repeated elements in the genome.

Identification of Previously Reported Genes. The 39 putative visual system cDNAs were tested for homology with previously cloned eye-specific genes. Eleven hybridized with the *Drosophila* gene *ninaE* (structural gene for opsin *Rh1*) (13, 14), two hybridized to opsin *Rh4* (15), and one each with clones 549 and 557 of Levy *et al.* (1). The fact that our screen for eye clones detected these previously identified eye-specific molecules demonstrated the effectiveness of the screening procedure. Our failure to detect opsin *Rh2* was consistent with the fact that it is expressed only in the ocelli (16–18), which are intact in the *eya* mutant.

Sequence Analysis Identifies Other Distinct cDNAs. We sequenced 25 clones that did not cross-hybridize with *ninaE*. Each contained a poly(A)$^+$ stretch corresponding to the 3′ end of a directionally cloned transcript and a consensus

Table 1. Twenty distinct eye cDNA clones

Clone	Expression onset	Transcript size, kb	Approximate gene location	Tissue *in situ*
7F10	24-hr embryo	2.4	66BC	Retina, cortex
6H8	Late 2nd instar	0.4	46F–47A	Negative
9C5	25-hr pupa	9.2	93AB	ND
10A2	25-hr pupa	1.3	95AB	ND
2D3	50-hr pupa	1.8	2D–F	Retina
2G1	50-hr pupa	3.6	46E	ND
9C4	50-hr pupa	0.9	83AB	Retina
1C2	After 75-hr pupa	Smear	Multiple sites	ND
2G8	After 75-hr pupa	0.8	ND	ND
9H9	After 75-hr pupa	2.9	10E	Retina
3H10	**After 75-hr pupa (arrestin)**	**1.4**	**36D**	**Retina, ocellus, larval photoreceptor**
5B3	After 75-hr pupa	2.9	72D	Retina, cortex
4A11	After 75-hr pupa	0.8	72E	Retina, cortex
3C12	After 75-hr pupa	0.8	62DE	ND
8H7	After 75 hr pupa	0.7	67EF	Negative
1D10	After 75 hr pupa	0.9	17A, 86F	ND
6A7	After 75 hr pupa	1.6	ND	Retina, cortex
6C5	After 75 hr pupa	1.6	ND	Negative
6F7	After 75 hr pupa	0.8	ND	Negative
7H1	After 75 hr pupa	0.8	ND	Retina

ND, not determined. Transcript size and expression onset were determined by Northern blots. Onset is listed as the earliest time a transcript could be detected by this method. Gene localization was determined by hybridization to salivary gland polytene chromosomes. Some clones hybridized to more than one site on the chromosomes, possibly due to repeated sequences in the genome. Tissue *in situ* expression was determined in cryostat sections of heads by using ^{3}H-labeled cDNA probes. A negative result in the tissue *in situ* experiment indicates that the transcript was difficult to detect with that particular probe.

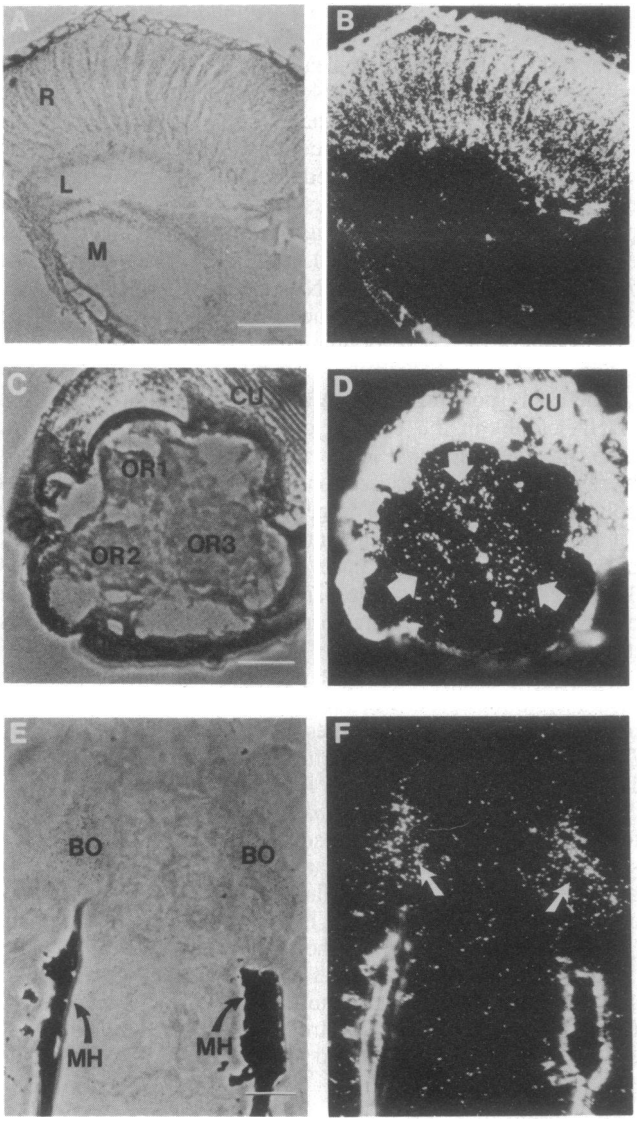

FIG. 2. Expression of the arrestin-homologous RNA in the three distinct visual organs of the fly. The endogenous transcript pattern is revealed autoradiographically in 6-μm cryostat sections after *in situ* hybridization with ^{3}H-labeled probe made from 3H10 cDNA. Phase illumination shows the tissue morphology; darkfield illumination of the same field of view shows the silver grain signal. (*A* and *B*) Adult compound eye in radial section of 1-day-old adult. (*A*) Phase: R, retina; L, lamina; M, medulla. (*B*) Darkfield, indicating abundant RNA expression in the retina. (Bar = 50 μm.) (*C* and *D*) Three adult ocelli, in a grazing tangential section of the dorsal head of a 1-day-old adult. (*C*) Phase shows the retinal layers (OR1, OR2, and OR3) of the three ocelli, surrounded by head cuticle (CU). (*D*) In darkfield, white arrows indicate the positions of the ocelli. The arrestin RNA is expressed, but at a reduced level compared with the compound eye. (Bar = 25 μm.) (*E* and *F*) Bolwig's organs in horizontal section of a third instar larva. *E* reveals the two clusters of larval photoreceptor neurons (BO). They lie within the cephalopharyngeal skeleton, just anterior to the indicated parts of the mouth hooks (MH) at the base of the protocerebral bridge. In the darkfield view, white arrows indicate the photoreceptor organs, which show robust expression of the arrestin RNA. (Note: The apparent signals in CU and MH are artifactual; the probe tends to stick to cuticular material.) (Bar = 25 μm.)

poly(A)$^+$ addition sequence. Each sequence was compared with all the others and with the GenBank nucleic acid data base. Two cDNAs corresponded to *norpA* (no receptor potential) (19) and one to opsin *Rh3* (2, 20). Three clones were identical but not previously reported. This yielded 20 distinct, not previously reported *Drosophila* cDNAs (Table 1).

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   1 AATCGATTTC TCGAGCTATT TCTGTACATT CACCTGTCCT GCATTGCTAA TACGCCGTGT
  61 TGCTCGCGCG TTATTTAATG TTTGAGCCAT CGATGTCGAT GTCGTGCTGC AATGTCAATA
 121 TCAAAGACAC TGCGCACAGC AAGGTTGCCG AAGCCGTAGT AGTTTACCGC CGTGCAATTG
 181 CTGAATTTCT GCTGTGCGGC TAATTGAATT TAGAGGGGCG ACAGGTGCCA CAATGCCAGG
                                    ← 11 bp conserved sequence
 241 TATAAATGCC GGATTGCCAA AGAGCGCTAA TTAATAGCCT AGTGGACCAC GCAACGCGGC
     ← TATA box                          ← Transcription start
 301 GTATACCATC GAGAACGAGC GCGAAACGTT AAAGGCACAT CCAAAGTTTA AACTATTTCC
 361 GCAGAGATTT TGATAAACAG CTCCAAAATG GTGGTCAATT TCAAGGTGTT CAAGAAGTGT
                           Met ValValAsnP heLysValPh eLysLysCys

 421 TCCCCGAACA ACATGATCAC GCTCTACATG AACAGGCGTG ATTTTGTAGA TTCCGTGACT
     SerProAsnA snMetIleTh rLeuTyrMet AsnArgArgA spPheValAs pSerValThr

 481 CAGGTGGAAC CCATTGGTAG GTGTCACAGA CCGAAACCCT TGAGCAATGG GATTTACGAA
     GlnValGluP roIleA

 541 TGAGGAAATC CATCAAAAAA TAAATTCGTG TAGGAATTGG TACCCATATT CGATTGAAGT
 601 ATCTTATAGT TTGAAAATAA CTTCAGTGTA ACTTTTGTTT CAACTTAACA CATTGGAATT
 661 TTTAATACCT TCCTTGAAAA GTGATATCAA ATCAAAATTA TATTATAAAA ACTCCATTTC
 721 GAATCTGCAT ATGCCGACCA GCAAATATAA ACTCAGGGAG TTATTCAAAA TTGCATCTGA
 781 ATTCAATAGC CTAGGGAGTT AAGTTAAATT GGCTGCGCTA GCAGCTAACC AAGTTCATCG
 841 ATTACGCGAG CAAGCAAACC AAGAGGCTGG GCGCTTGTAA ATAATATTCT CCAATTAATA

 901 AGCGTCCTCT TGCAGATGGA ATCATTGTGC TGGACGATGA GTACGTGCGC CAGAACCGCA
                   spGly IleIleValL euAspAspGl uTyrValArg GlnAsnArgL

 961 AGATCTTCGT GCAGTTGGTC TGCAATTTCC GATATGGGCG CGAGGACGAC GAGATGATCG
     ysIlePheVa lGlnLeuVal CysAsnPheA rgTyrGlyAr gGluAspAsp GluMetIleG

1021 GTCTGCGGTT CCAGAAGGAA CTGACCCTGG TCTCGCAGCA GGTGTGCCCA CCCCAGAAGC
     lyLeuArgPh eGlnLysGlu LeuThrLeuV alSerGlnGl nValCysPro ProGlnLysG

1081 AGGACATCCA GTTGACCAAG ATGCAGGAGC GTCTGCTGAA GAAGCTTGGC TCCAATGCCT
     lnAspIleGl nLeuThrLys MetGlnGluA rgLeuLeuLy sLysLeuGly SerAsnAlaT

1141 ATCCCTTCGT GATGCAGATG CCACCAAGCT CGCCGGCCTC GGTGGTTCTC CAGCAGAAGG
     yrProPheVa lMetGlnMet ProProSerS erProAlaSe rValValLeu GlnGlnLysA

1201 CCAGTGACGA GAGCCAGCCC TGCGGAGTCC AGTACTTCGT AAAGATCTTT ACCGGAGACA
     laSerAspGl uSerGlnPro CysGlyValG lnTyrPheVa lLysIlePhe ThrGlyAspS

1261 GCGACTGCGA TCGATCGCAT CGCAGGAGCA CCATTAACCT GGGCATCCGC AAGGTGCAGT
     erAspCysAs pArgSerHis ArgArgSerT hrIleAsnLe uGlyIleArg LysValGlnT

1321 ACGCACCGAC CAAGCAGGGC ATCCAGCCCT GCACCGTCGT TCGCAAGGAC TTCCTTCTGT
     yrAlaProTh rLysGlnGly IleGlnProC ysThrValVa lArgLysAsp PheLeuLeuS

1381 CGCCCGGAGA GCTCGAACTG GAGGTCACCC TCGACAAGCA GCTGTACCAT CACGGCGAGA
     erProGlyGl uLeuGluLeu GluValThrL euAspLysGl nLeuTyrHis HisGlyGluL

1441 AGATCTCGGT GAACATCTGC GTACGCAACA ACTCCAACAA GGTGGTGAAG AAGATCAAGG
     ysIleSerVa lAsnIleCys ValArgAsnA snSerAsnLy sValValLys LysIleLysA

1501 CCATGGTGCA GCAGGGCGTC GATGTGGTCC TTTTCCAGAA CGGTCAGTTC CGCAACACGA
     laMetValGl nGlnGlyVal AspValValL euPheGlnAs nGlyGlnPhe ArgAsnThrI

1561 TCGCCTTCAT GGAGACGAGC GAGGGATGTC CCCTGAACCC GGGATCCAGC CTGCAGAAGG
     leAlaPheMe tGluThrSer GluGlyCysP roLeuAsnPr oGlySerSer LeuGlnLysV

1621 TCATGTATCT GGTGCCCACC CTGGTGGCCA ATTGCGACCG CGCAGGCATC GCCGTTGAGG
     alMetTyrLe uValProThr LeuValAlaA snCysAspAr gAlaGlyIle AlaValGluG

1681 GTGATATCAA GCGCAAAGAC ACAGCTCTGG CCTCGACCAC ACTGTGAGTA AAATTTATTC
     lyAspIleLy sArgLysAsp ThrAlaLeuA laSerThrTh rLe

1741 ACATCATAGC TTAGCAGATG AAACATTAAT ATTATACTCT ATTAAGTATC AACTTAAAAT
1801 CATACCATAA AATCAATCAA ATTTTAAAGT TAGGAACCTT TTTAAAAATC GTATTTTCCC
1861 GGTGACTAAC AGTTCTTTAG CTAAATGTGT TTACAAAATG GCATAAAACG CATACTAATA
1921 CTAAGTGAAA AATGCATATT TAAAATTCTA TTTCAGTATT GCCAGTCAGG ATGCGAGGGA
                                        uIle AlaSerGlnA spAlaArgAs

1981 TGCCTTTGGC ATAATTGTTT CATATGCTGT GAAGGTCAAG CTTTTCTTGG GAGCCCTGGG
     pAlaPheGly IleIleValS erTyrAlaVa lLysValLys LeuPheLeuG lyAlaLeuGl

2041 CGGCGAGCTC TGCGCTGAGC TACCATTTAT TCTGATGCAC CCGAAGGTAA TAAAAGGTGT
     yGlyGluLeu CysAlaGluL euProPheIl eLeuMetHis ProLys

2101 GCCCAAATAT TTGAATAGTT ATTGAAAGGC AATCAATTAT TTACAGCCAA GTCGCAAGGC
                                                 ProS erArgLysAl

2161 CCAACTGGAA GCCGAGGGCT CCATTGAGGC CTAAACTGAA AGGGCTACCT CAACCAACGA
     aGlnLeuGlu AlaGluGlyS erIleGluAl a

2221 AAAAAATGGC GTATTTCTAC AAGTCAAACC GATTTTTGTA GATCCTAAAA AATGCTGATG
2281 TTGCTGAAAT GTTCTGAACT GCAGTCGTCG TACTTTTCCT ATATAGCAAA TCCAATATCC
2341 ATATATTGTA TGTGTGTATG TGTATTATAT TTATAACTCA ACTAACAATT AAATATGAAC
                                  poly(A) add site
2401 AGAGTTTATG T
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FIG. 3. Nucleotide sequence of the *Drosophila* arrestin gene. The 11-bp conserved sequence found in the promoters of genes expressed specifically in *Drosophila* photoreceptor cells (22), TATA box, transcription initiation site, and putative polyadenylylation sites are shown. The transcription start site indicates the major primer-extension product that we detect. Introns are indicated as interruptions in the deduced amino acid sequence. A poly(A)$^+$ tract after nucleotide 2411 was found in the cDNA.

Temporal Expression. From each of the 20 distinct clones, antisense cRNA was synthesized and used to probe Northern blots of poly(A)$^+$ RNA isolated from various developmental stages. Several different patterns were observed. For example, the transcript detected by clone 6H8 was seen as early as the late second larval instar. Clone 2D3 first detected a transcript in the 50-hr pupa. Clone 7F10 identified an RNA expressed at low levels in all the stages tested. Two cDNAs

identified RNAs that were not expressed until the 75-hr pupa, and eleven were not expressed until even later.

Tissue *in Situ* Hybridization. The distributions of the endogenous mRNAs were analyzed on cryostat sections of normal adult heads, and the results for 13 of the eye clones are given in Table 1. One common pattern was expression of RNA in the retina only, consistent with Northern blots of RNA from the *eya* mutant. Another pattern is demonstrated by clone 7F10, which shows expression in the retina and the entire cortex of the adult brain. This pattern is consistent with the Northern blot; the *eya* mutant gives a reduced, but positive signal, presumably due to brain expression.

Molecular Analysis of Clone 3H10. Clone 3H10 demonstrated an expression pattern consistent with a role in phototransduction. The RNA bloomed in the late pupa, when the light-gathering rhabdomeres are assembled and begin to function, and when known phototransduction genes begin to be expressed (3, 13, 21). The *in situ* hybridization on tissue showed that it was expressed in the compound eyes and ocelli of the adult and also in the larval photoreceptor organ (Fig. 2). Although the larval photoreceptor organ represents too little tissue to give a positive Northern blot under the conditions used, *in situ* hybridization did reveal a strong local signal.

By using the original 0.4-kb 3H10 cDNA as a probe, we isolated a 1.4-kb cDNA (3H10-1) that corresponded to the size of the transcript detected on Northern blots. We also isolated genomic clones. Genomic clones were digested and hybridized to cDNA 3H10-1 to identify a 9.0-kb *Xba* I fragment that contained the entire cDNA 3H10-1 sequence. The cDNA clones 3H10 and 3H10-1 and the genomic *Xba* I fragment were sequenced (Fig. 3). Comparison of the cDNA to genomic DNA revealed that the gene contains three introns of 419, 233, and 60 base pairs (bp), all demonstrating the consensus GT at the 5′ end of the intron and AG at the 3′ end. The methionine initiation codon at nucleotide position 390 is the first methionine in the open reading frame and shows a 7 of 9-bp identity with the consensus translational initiation site (23). Based upon primer-extension experiments (data not shown), a major putative transcription start site has been mapped to the thymidine at position 272 and a minor start site at position 268. The putative TATA box is located at nucleotide positions 241–246. Beginning at position 200 is a conserved 11-bp sequence that corresponds to a consensus sequence found upstream of the coding regions of several genes that are expressed specifically in photoreceptor cells (22). At the 3′ end of the transcription unit, a putative polyadenylylation sequence (positions 2387–2393) is positioned 19 bp upstream of the poly(A) region.

The 3H10 Gene Has Structural Similarity to Vertebrate Arrestin. The *Drosophila* 3H10 cDNA sequence was used to search the GenBank data base. The sequence is similar to the cDNA reported for the bovine phototransduction molecule arrestin (24) and a similar molecule isolated from a human retina cDNA library (25). Fig. 4 illustrates a comparison of the predicted *Drosophila* and human arrestin primary amino acid sequences. The amino acid sequences are 45% identical. In addition, 12% are conservative changes. Fig. 5 illustrates that the molecules are also closely colinear. We will designate the *Drosophila* arrestin gene by the symbol *Arr*.

DISCUSSION

Molecular screening offers several advantages over screening for mutations affecting specific traits. Traits that are lethal or too subtle to be recognized as anatomical, physiological, or behavioral changes, might escape detection genetically. Because all features of the visual system might be expected to be related to one or more RNA species expressed at distinct phases in development, a molecular approach should provide a more comprehensive picture. It also avoids the often time-consuming steps needed to clone a gene identified by mutation. A potential drawback, however, is that some of the transcripts we have identified may not be necessary for visual system function.

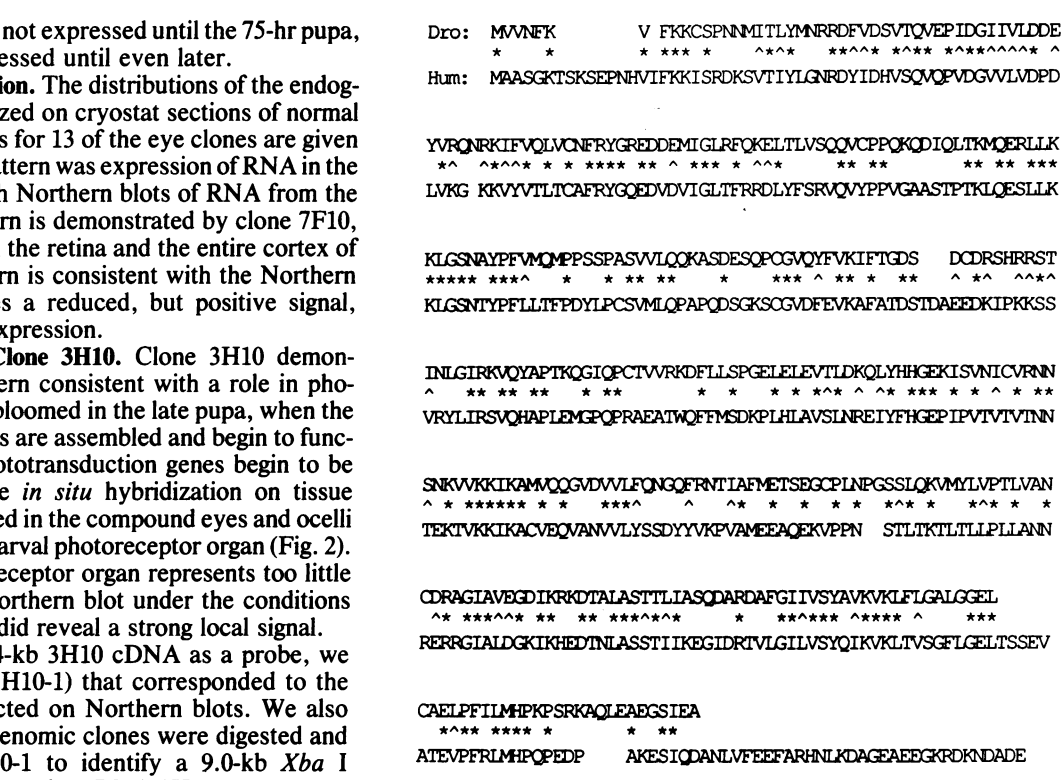

FIG. 4. Amino acid sequence comparison of *Drosophila* arrestin with the human homolog (25). Amino acids are designated by single-letter code. Identities are represented by *; changes with conserved types of amino acids (T=S, F=Y, D=E, L=I=V, and K=R) are designated by ^.

As judged by DNA sequencing, cross-hybridization, and similar map positions, our screen for cDNA clones has identified some of the same genes as the genomic clones found by Shieh *et al.* (5). The overlap appears to be 20% or

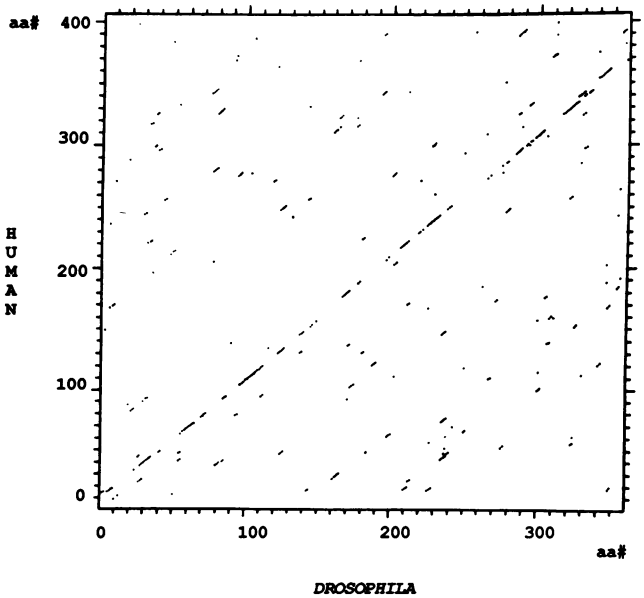

FIG. 5. Dot matrix comparison of *Drosophila* arrestin and human homolog. Windows of 5 amino acids are sequentially compared, and for every 3 identical residues a dot is scored by the sequence analysis program.

less (C. Zuker, personal communication). Moreover, many of the genes are represented by a single clone. Thus, it is clear that many more eye-specific genes remain to be found.

In certain cases, tissue *in situ* hybridization analysis proved more sensitive than Northern blots. This can be true when the gene is expressed in a relatively small number of cells, as is the case for the cDNAs expressed in the larval photoreceptor organ (Fig. 2). The organ consists of only two dozen photoreceptor cells in the anterior of the animal, and RNA extracted from whole larvae will contain only a small contribution from these cells.

Based upon their sequence similarity and tissue-specific expression, the *Drosophila Arr* gene product and vertebrate arrestin may be functional homologs, both molecules negatively regulating the transduction process. Data suggest that bovine arrestin binds to phosphorylated metarhodopsin II, thus inhibiting the transduction cascade (26, 27). Rhodopsin in flies is also phosphorylated upon light activation (28, 29), which further suggests that *Drosophila* arrestin and rhodopsin binding may be similar to vertebrates. It is of interest to note that the *Drosophila* homolog lacks the 31 C-terminal amino acids of human arrestin, suggesting that this segment does not have a necessary function, or that the truncated *Drosophila* molecule functions by a slightly different mechanism than the vertebrate molecule.

Several *Drosophila* visual system-specific genes show sequence similarity with genes from vertebrate species, reflecting possible similarities in function. The phototransduction cascade is initiated by the absorption of photons by chromophores bound to opsin proteins. The opsin molecules have both *Drosophila* and vertebrate counterparts (13, 14, 30). The identification of a *Drosophila* arrestin molecule makes it possible to examine the regulation of the invertebrate phototransduction cascade, which may improve our understanding of the process in vertebrates. The availability of many distinct eye-specific *Drosophila* cDNAs will allow us to investigate the extent to which these molecules are conserved between species.

Recently, a technique for accessing the function of the gene corresponding to a cDNA clone has been developed for the *Drosophila* system by Ballinger and Benzer (31) and Kaiser and Goodwin (Kaiser, K. and Goodwin, S. F., personal communication). The technique consists of creating large numbers of mutant flies with *P* element insertions. Individual flies with transposon inserts in or near the cloned gene of interest are identified by polymerase chain reaction amplification by using oligonucleotide primers derived from the sequences of the *P* element and the cDNA in question. This method can be applied to the clones described here; this may allow use of the fly system to identify the functions of conserved genes through specific mutations.

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