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***pcdr*, a novel gene with sexually dimorphic expression in the pigment cells of the *Drosophila* eye**

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Abstract

We report the molecular cloning and characterization of *pcdr* (*pigment cell dehydrogenase reductase*), a *Drosophila* visual system-specific gene with novel properties of spatial, temporal and sexual regulation. Short chain dehydrogenase/reductases are a family of proteins that catalyze mechanistically conserved dehydrogenase/reductase reactions in a wide range of cells and tissues. These enzymes are required in a variety of reactions ranging from steroid metabolism and prostaglandin synthesis to alcohol detoxification. The *Drosophila pcdr* gene encodes a new member of this family, displaying 42% amino acid sequence identity to the mammalian prostaglandin dehydrogenase. *pcdr* expression is restricted to the visual system with very high levels found in the pigment cells. Interestingly, expression of *pcdr* mRNA is sexually dimorphic with males showing higher levels of expression than females. This sexual dimorphism is under the control of the sex differentiation cascade as *transformer* and *transformer 2* mutations shift females to a male-like level of expression. Finally, we demonstrate that a region of 335 nucleotides including sequences upstream and just downstream of the transcription start is sufficient to reproduce the normal expression pattern.

Introduction

The *Drosophila* visual system has been used as a powerful model system for the study of development, cell differentiation and signal transduction (for reviews see Dickson and Hafen 1993; Wolff and Ready 1993). The *Drosophila* compound eyes are composed of repeating arrays of unit eyes or ommatidia (for a review see Wolff and Ready 1993). Each ommatidium consists of eight photoreceptor neurons (R1-R8), a set of 1°, 2° and 3° pigment cells responsible

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for optically isolating each ommatidium, and four cone cells, which together with 1° pigment cells are involved in the synthesis of the corneal lens and the pseudocone chamber. R1-R6 cells express a blue-sensitive rhodopsin, R7 expresses opsins sensitive to UV light, and R8 cells are blue-green sensitive (reviewed in Dickson and Hafen 1993; Wolff and Ready 1993). Females have a mean of 776 ommatidia per eye while males have approximately 50 fewer. Visual-input mediated behavioral differences between males and females have been well documented in a variety of organisms. Many of these differences are clearly encoded at the processing level, but some may also reflect differences in the structure and organization of the visual system. For example, many dipterans have a highly specialized region of the compound eyes fully dedicated to tracking females during flight.

Sexual behavior in *Drosophila melanogaster* involves a defined set of behaviors performed in a specific order, as well as complicated signaling interactions between males and females (Hall 1994). Male behaviors include orienting toward the female, tapping her abdomen, following her, “singing” a species-specific courtship song by extending and vibrating one wing, licking and copulation. Virgin females can respond to male courtship with mild rejective behaviors or with behaviors that facilitate copulation. Mated females respond to male courtship with strong rejection responses such as ovipositor extrusion. A number of the male behaviors, such as finding a female, orientation, following, and appropriate positioning require visual input to be performed optimally. Indeed, loss of visual function substantially reduces mating efficiency, even in the laboratory (Hall 1994). The selective pressure on appropriate visual function in the wild is likely to be substantially greater.

In this paper we report the isolation of a new gene, *pcdr*, which is expressed at a very high level in the pigment cells of the eye and which may be one of the protein components of the corneal lens. Interestingly, the RNA product of this gene is expressed in a sexually dimorphic manner and this sexual dimorphism depends on the function of the sex differentiation regulatory genes *tra* and *tra2*.

Materials and methods

Fly stocks

All stocks were grown at 25°C on standard cornmeal-molasses-yeast-agar medium. A description of all the mutations used can be found in Lindsley and Zimm (1992). The genes *transformer* (*tra*, chromosome 3) and *transformer 2* (*tra2*, chromosome 2) are components of the sex differentiation cascade necessary for female development. In the absence of either of these genes, XX animals develop with male morphology and behavior, although the larger body size characteristic of females is retained. The X chromosome linked mutations *yellow* (yellow body color, *y*) or *white* (white eyes, *w*) are used to distinguish XX pseudo males from XY males in the following manner, using *w* and *tra* as examples: *w/w; tra/+* females are crossed to *+/Y; tra/+* males. XX progeny are heterozygous for *w* and *+* and have red (wild type) eyes. XY progeny are *w/Y* and have white eyes. *tra* mutant animals of either sex are identified because they are morphologically male, homozygous for recessive mutations linked to *tra* and lack dominant mutations on the wild-type chromosome.

Df(2R)trix (labeled *Trix* in the Fig. 5) is a small deletion which removes the *tra2* locus. Df(3L)st j7 (labeled *J7* in Fig. 5) is a small deletion removing both *tra* and the eye color mutation *scarlet*. It is also marked with the dominant bristle morphology mutant *Kinked* and the recessive eye color mutation *pink peach*. *tra etc* is a *tra* mutant chromosome marked with a number of recessive mutations including *scarlet*, *tra*, and *pink peach*.

The *tudor* locus must function in the maternal germline for proper development of germ cells in both male and female progeny. Thus, *tudor* mutant mothers produce sons and daughters that

lack germ cells. *SM1* and *CyO* are second chromosome balancer chromosomes marked with the dominant mutation *Curly*. Balancer chromosomes are multiply inverted (and therefore recombination suppressing) chromosomes marked with a dominant visible mutation and at least one recessive lethal mutation. Third chromosome balancers used (indicated by *Bal* in Fig. 5) included TM3 (marked with the dominant bristle morphology mutation *stubble* and the dominant wing morphology mutation *Beaded-Serrate*) and TM6B (marked with the dominant mutations *Tubby* and *Humoral*).

Cloning methods, DNA purification, DNA blots

The screening of recombinant phage libraries, plasmid subcloning and isolation, restriction digestion, and blotting were done as described in Sambrook et al. (1989). The screen was performed as described in Madigan et al. (1996) and Shieh et al. (1989). Genomic clones and cDNAs were sequenced after cloning into Blue-script (Stratagene). Sequencing was by the dideoxy chain termination method using the Sequenase Kit (United States Biochemical; manufacturer's protocol). Sequence analysis and assembly made use of the Sequencer, DNA Star and DNA Strider (Marck 1988) packages. Sequence comparisons were carried out by the BLAST network service (Altschul et al. 1990) and the Fasta program (Devereux et al. 1984) run remotely either at NCBI or EMBL. Salivary chromosome in situ hybridization was performed as described (McKeown et al. 1987). In vitro transcription and translation of cDNA was performed in reticulocyte lysates using the TNT translation kit from Promega.

Isolation of RNA and RNA blots

Drosophila total RNA was prepared as described in Ausubel et al. (1994). RNA was size fractionated on 1% formaldehyde-agarose gels, transferred to Hybond (Amersham), and probed according to Madigan et al. (1996). *Pcdr* probes were radioactively labeled by random priming as described (Sambrook et al. 1989). The RNA blots were also probed for the ribosomal protein *rp49* mRNA (O'Connell and Rosbash 1984) and for the *bendless* mRNAs (Muralidhar and Thomas 1993; the pSK-bendless plasmid was a gift from M. Muralidhar and J. Thomas). Blots were imaged on a molecular Dynamics PhosphorImager. Counts in the *pcdr* and *rp49* (or *bendless*) bands in each lane were measured to give an estimate of the quantity of products. The intensity of the *rp49* mRNA band or the sum of the intensity of the *bendless* mRNA bands was used as internal standard to normalize for loading differences. The final values in Fig. 5 were calculated as 100 times the ratio of the normalized level of *pcdr* RNA in females relative to the normalized level of *pcdr* RNA in males, where normalization was done by dividing the intensity of the *pcdr* band by the intensity of the band(s) for the RNAs from the gene used to normalize. For example, when *RP49* RNA was used to normalize, the values in Fig. 5 were calculated as follows:

$$100 \times (\text{intensity } pcd\text{r band in female lane} / \text{intensity } rp49 \text{ band in female lane}) / (\text{intensity } pcd\text{r band in male lane} / \text{intensity } rp49 \text{ band in male lane})$$

Construction of transgenes and germline transformation

The PstI-HinI fragment at the 5' end of *pcdr* was cloned into the PstI-BamHI region of the CasPeRlacZ vector (J. Posakony, personal communication). The HinI and BamHI sites were filled in with Klenow polymerase, allowing directional cloning into the PstI and BamHI sites. *Drosophila* transformation was done by standard techniques (McKeown et al. 1988).

Production of antibodies and immunostaining

Two peptides were synthesized containing the amino acids predicted to make up the C-terminus of the protein (248-LEIKPQWTGKEQAL-261) and the region determined by the protein (163-CLANEKYYQRSGIK-176). The peptides were cross-linked with glutaraldehyde to tuberculin

PPD (Statens Serum Institut, Copenhagen). The C-terminal peptide was injected into rats and the internal peptide was injected into rabbits. Both peptides were injected subcutaneously and boosted monthly. Sera were purified over a Bio-Rad Affi-Gel affinity column coupled with peptide.

Drosophila heads were frozen in O.C.T. Compound (Tissue-Tek) and cut in 10 micron sections with a cryostat. Samples were fixed with 3% formaldehyde, rinsed with phosphate-buffered saline (PBS) and incubated with the antibody in PBS overnight. After rinsing in PBS with 0.1% saponin, the samples were incubated with fluorescent secondary antibody for 1 h, rinsed and imaged with a Zeiss confocal microscope.

Histochemical assay of β -galactosidase activity

Flies were dissected in Ringer's solution and X-gal (5-bromo-4-chloro-3-indolyl-beta-galactopyranoside). Histochemical staining assays of beta-galactosidase activity were performed as described (Ashburner 1989). At least five independent transformed lines were examined to account for positional effects on expression. For tissue sections, 4-day-old adult fly heads were quick-frozen in O.C.T. compound and cut into 10- μ m-thick sections. Histochemical staining assays for β -galactosidase activity were performed as described (Ashburner 1989).

Results

Isolation of and molecular characterization of *pcdr*

In the course of independent screens for genes which show preferential expression in the visual system and genes which show sexually dimorphic expression, a new gene, *pcdr*, was isolated. To determine the cytogenetic location of *pcdr*, we carried out in situ hybridization to larval polytene chromosomes. The *pcdr* gene was localized to the third chromosome at position 72 E, between the left hand break point of Df(3L)st 6 and the left hand end of Df(3L)st 100.62 (data not shown; Belote et al. 1990; Lindsley and Zimm 1992). No mutants affecting visual function have been identified at this location.

We used an 2.0-kb genomic fragment of *pcdr* (see Fig. 1) to screen a *Drosophila* head cDNA library and isolated several cDNA clones. Using dideoxynucleoside triphosphate sequencing we determined the nucleotide sequence of one of those cDNA clones, and of the 2.0-kb genomic fragment. Figure 1B–C shows the nucleotide sequence and the deduced amino acid sequence of *pcdr*. The gene is divided into 3 exons by 2 introns of 56 and 66 nucleotides, respectively. The structure of the RNA and the position of the start of transcription were determined by comparison of genomic and cDNA sequences and by performing primer extension analysis. In vitro translation reactions primed with in vitro transcribed *pcdr* mRNA yielded a protein product of 29 kDa (data not shown); this is consistent with the expected sized of the deduced open reading frame.

pcdr encodes a short-chain dehydrogenase related protein

Comparison of the deduced amino acid sequence of Pcdr with previously sequenced genes and proteins revealed very high homology to the human 15-hydroxyprostaglandin dehydrogenase (42% amino acid identity; Jörnvall et al. 1995), the *Drosophila* fat body protein P6 (38% amino acid identity; Rat et al. 1991), the *Sarcophaga peregrina* development-specific 25 kDa protein (36% amino acid identity; Rat et al. 1991) and the *Drosophila* alcohol dehydrogenase protein (~30% amino acid identity; Rat et al. 1991; Fig. 2). All of these proteins are classified as members of the short-chain dehydrogenase/reductase family, and indeed, are part of the same subfamily of short chain dehydrogenases (SDR proteins; Jörnvall et al. 1995). The SDR group of enzymes includes a heterogeneous set of proteins found in bacteria, fungi, plants, insects

and mammals, which catalyze mechanistically conserved reactions. The dehydrogenase members of this family catalyze the conversion of ketones to alcohols as well as alcohols to ketones, coupled to the interconversion of NAD(P)H and NAD(P). Reductase members of the family can also catalyze reduction of C=C bonds as well as of C=O bonds. Presently, at least 57 different enzymes belong to this family (Jörnvall et al. 1995).

If Pcdr is a functional dehydrogenase/reductase, we expect it should contain residues that are highly conserved throughout the protein family, in particular those associated with aspects of enzyme function. In the alignment of the 57 short-chain dehydrogenases/reductases, only 8 residues are conserved in more than 90% of the proteins (Jörnvall et al. 1995). These are the glycines (Gly¹², Gly¹⁶, Gly¹⁸) involved in binding the adenine moiety of the cofactor NAD (H) or NADP(H), Tyr¹⁵⁰ and Lys¹⁵⁴, which are thought to be directly involved in catalysis, and Gly¹³⁰, Ser¹³⁷ and Pro¹⁸². All of these are conserved in the Pcdr protein. If we also consider residues conserved in 70–90% of the SDR proteins (Jörnvall et al. 1995), Pcdr shows identity in 11 out of 15 such residues. The conservation of *pcdr* gene structure, the conservation of general characteristics of the dehydrogenase/reductase protein family and the conservation of residues believed to be highly important in catalytic function are all consistent with Pcdr being a catalytically active member of the SDR protein family. The divergence between Pcdr and the other members of the family makes it difficult to predict what if any substrate there may be for Pcdr.

Temporal and spatial expression patterns

To examine the developmental profile of expression of the *pcdr* gene, we performed RNA blots on polyA+RNA isolated from wild-type flies at different stages of development. Figure 3 shows that *pcdr* RNA is first detectable by late pupation (8 and 9 days) and reaches maximum levels after eclosion (10 days). This profile matches the time when terminal differentiation of the eye occurs (Cagan and Ready 1989).

To determine the tissue specificity of *pcdr*, we isolated polyA+RNA from heads, thorax and abdomens of wild-type flies and from heads of flies carrying the *eyes absent* (*eya*) mutation; *eya* flies have a complete loss of the compound eyes. Figure 3 shows that the *pcdr* transcript is present in preparations of polyA+ RNA from wild-type heads, but not from bodies, or *eya* heads. Quantitation of *pcdr* mRNA in eyes compared to that of the *Drosophila* ribosomal protein probe (*rp49*) indicates that the abundance of *pcdr* transcript is about 5 to 10 times that of *rp49*, which makes *pcdr* one of the most abundantly expressed genes in *Drosophila*.

To study the distribution of Pcdr protein at the cellular level, we generated anti-Pcdr polyclonal antibodies using two synthetic peptides encompassing residues 248 to 261 and 163 to 176, and used these antibodies to examine Pcdr distribution in frozen tissue sections of wild-type adult animals. The results (Fig. 4 and data not shown) demonstrate that Pcdr expression occurs exclusively in the visual system, both in the compound eyes and ocelli (data not shown). Most staining is restricted to a region immediately above and to the side of the photoreceptor cells; this corresponds to the location of the primary pigment cells. In addition, there is some weak staining in the secondary pigment cells as well as in the lens. The results clearly show that the expression of *pcdr* is turned on during a period in which eye differentiation is occurring and that Pcdr is expressed in the primary pigment cells of the adult *Drosophila* eye.

Expression of *pcdr* is sexually dimorphic

Quantitation of *pcdr* mRNA levels in males and females showed that *pcdr* was expressed at higher levels in males than in females. On RNA blots, the level of *pcdr* mRNA in adult female heads is ~60% of that found in male heads (normalized to control RNAs; Fig. 5). Similar results were obtained when RNAs were isolated from individually dissected eyes as opposed

to whole heads (data not shown). This level of dimorphism is highly significant and is comparable to expression differences seen between females and males for X-linked genes that are not being dosage compensated. In addition, differences of about this level in gene expression can lead to substantial consequences when associated with genes encoding structural proteins (e.g. myosin heavy chains), nuclear regulatory proteins (e.g. Ubx) or cell signaling molecules (e.g. Notch; see Lindsley and Zimm 1992 for references and summaries of these phenotypes).

To determine if the sexual dimorphism in *pcdr* expression is actually the consequence of differences in sexual differentiation, we examined its expression in mutant backgrounds that affect sexual differentiation or germline formation. Targets of the somatic sex differentiation cascade are expected to show a dependence of their level of expression on the function of *tra* and *tra2*, which are necessary for female somatic differentiation, but not on the presence of a germline. RNAs were isolated from flies that were mutant for *tra* or *tra2* and from flies that lack a germline as the result of being offspring of homozygous *tudor* mothers. The observed RNA levels for males of different genotypes remains constant. As shown in Fig. 5, RNAs isolated from the heads of *tudor* flies exhibit the same 60% female/male ratio as seen for wild type. Thus, sex-specific differences in *pcdr* levels are not the indirect consequence of some factor linked to differences in sex-specific germline function. When RNAs from animals mutant in sex differentiation genes are examined a different result is seen. As shown in Fig. 5, females transformed into pseudo males by mutations in either *tra* or *tra2* show a substantial increase in the amount of *pcdr* RNA. The expression of *pcdr* RNA in XX pseudo males at or above the level seen in XY males indicates that the sexual dimorphism seen in *pcdr* expression is controlled by the sex differentiation cascade. The cause of the slightly higher than expected expression in XX pseudo males is not known.

Tissue-specific regulation is controlled by a small region just upstream of the translation start

pcdr shows a high degree of tissue specificity and remarkably high levels of expression. We wished to determine if this regulation is a property of a relatively small region near the gene or if sequences covering a wide area are necessary. To test these hypotheses, we placed a bacterial *lacZ* reporter gene under the control of a fragment of the 5' end of *pcdr* encompassing 335 nucleotides upstream and 28 nucleotides downstream of the transcription initiation site (Fig. 1). Multiple independent transgenic lines were isolated and analyzed for beta-galactosidase expression. Figure 4 shows that beta-galactosidase is found in the compound eyes, while other experiments show expression in ocelli but not elsewhere in the body (not shown). The staining is very intense after a short period of incubation, consistent with high levels of enzyme resulting from high levels of transcription. To determine more precisely where the beta-galactosidase reporter is expressed, we carried out histochemical staining on 10- μ m thick frozen tissue sections of adult heads (Fig. 4). Consistent with the antibody staining results, the beta-galactosidase staining is largely limited to the layer of cells below the corneal lens in the eye (Fig. 4D). Expectedly, when sections tangential to the surface of the eye are examined, the beta-galactosidase staining appears as a ring in the region just below the corneal lens (Fig. 4E). No staining is observed in the corneal lens, the pseudocone or in sections containing cells located further down in the ommatidia. The staining pattern is compatible with a specific expression of *pcdr-lacZ* in the primary pigment cells, ocelli and perhaps, to a lower level, in secondary pigment cells. This suggests that the 335 nucleotides upstream of the transcription start site are sufficient to drive appropriate tissue-specific expression.

Discussion

We report here the isolation and characterization of a new gene with high level expression in the pigment cells of the eye which displays sexual dimorphism under the control of the sex differentiation cascade. This gene encodes a potentially catalytically active novel member of the short chain dehydrogenase/reductase family of proteins. Spatially correct high level expression is conferred upon reporter genes by a short (335 nt) region at the 5' end of the gene.

The stunningly high level of *pcdr* expression, especially in primary pigment cells, raises important questions about the function of this protein. We suggest three possibilities which are not mutually exclusive: (1) *Pcdr* catalytic activity may be involved in some aspect of retinoid metabolism and this may be consistent with the tremendous demand for the chromophore required in rhodopsin biogenesis; (2) *Pcdr* protein function may be associated with the pigments present in pigment cells (involving possibly synthesis, recycling of the pigments and/or protection against light induced damage) and (3) our favored hypothesis, that *Pcdr* protein may be used as a structural component of the eye, possibly as part of the corneal lens.

The possibility that *Pcdr* may function enzymatically is suggested by the high conservation of functionally important residues within the deduced protein sequence. These include residues inferred to be involved in NAD or NADP binding as well as residues thought to be present at the active site of this class of dehydrogenases/reductases. One class of potential substrates for *Pcdr* activity is one of the retinoid derivatives. Recently, Simon and colleagues (Simon et al. 1995) isolated and partially characterized a 32-kDa member of the SDR protein family (P32), expressed specifically in the polarized retinal pigment epithelial cell of the bovine eye. They proposed that p32 is the 11-*cis* retinol dehydrogenase, which catalyzes the final step in the biosynthesis of the 11-*cis* retinaldehyde, the universal chromophore of the visual pigments (Simon et al. 1995). *Pcdr* and bovine p32 display 26% amino acid identity. However, it should be noted that flies use 3-hydroxy-11-*cis* retinal as opposed to 11-*cis*-retinal as the chromophore.

Another potential class of *Pcdr* substrates is the screening pigments of the eye. Both xanthommatin (the brown pigment) and the drospterins (the red pigments) are complex aromatic molecules with hydroxy or keto groups exhibiting flexible oxidation states (Phillips and Forrest 1980). The localization of the pigments in specific ommatidial cells suggests a function in preventing lateral light scattering between ommatidia. It is possible that *Pcdr* is involved in the synthesis of the pigments, or in maintaining an appropriate oxido-reduction state. A similar function has been observed for mammalian dehydrogenases in UV light protection. For instance, in the mouse cornea a major role has been postulated for aldehyde dehydrogenase and alcohol dehydrogenase in detoxifying peroxidic aldehydes generated through exposure to UV light. A possible direct role in absorbing UV light via a suicide inactivation mechanism has also been suggested (Downes et al. 1994). Although the mammalian dehydrogenases are structurally different from *Pcdr*, the functional parallels are interesting.

The possibility of *Pcdr* functioning as a lens structural component is suggested by the high levels of protein and RNA, by the spatially regulated expression, the observation of antibody staining in the lens, and by the precedent for enzymatically active proteins being used as lens proteins in mammals and some arthropods (Piatigorsky and Wistow 1991). The structural components of the lens, known as crystallins, are often enzymes recruited from various abundantly expressed housekeeping genes, including heat-shock proteins, stress-inducible enzymes, chaperones and dehydrogenases (deJong et al. 1989). Interestingly, most of the lens proteins showing enzymatic activities are oxidoreductases with the potential to sequester a reduced cofactor and function as UV filters (Piatigorsky and Wistow 1991). The connection to stress proteins may also hold true for invertebrates lenses. The major proteins of the squid

lens are related to glutathione S-transferase, which is important in the lens for detoxification and possibly for protection against peroxidation damage (deJong et al. 1989). To date, only one potential corneal lens protein has been identified in *Drosophila*. The gene for this protein, drosocrystallin, has not been cloned and the protein sequence is unknown. However, this protein is synthesized in the primary pigment cells (as is Pcdr) and transported to the cornea (Komori et al. 1992). If Pcdr is a crystallin, we are faced with the question of why we only detect low level of staining in the lens with our antibodies. It is possible that the highly ordered array of lens proteins is such that the Pcdr epitopes recognized by our peptide antibodies are not exposed or accessible to the antibodies.

Finally, what purpose might be served by the sexual dimorphism of *pcdr*? An interesting possibility is to consider Pcdr acting as a component of the lens complex. Males and females have a biological need to have optimal visual acuity at different distances. Males, for example, have a strong dependence on visual function for mating success (Quinn and Greenspan 1984), with substantial emphasis placed on finding and tracking females. Female receptivity, on the other hand, does not require such high visual acuity and involves judgments based on non-visual cues such as the quality of the male's "courtship song." (Quinn and Greenspan 1984). Therefore, it is possible that males need a different refractive index lens than females and that they may accomplish this, in part, by differential expression of lens proteins.

Determination of the exact role of *pcdr* in lens production, pigment maintenance or chromophore production as well as its possible role in visual aspects of courtship behavior await identification and characterization of mutants in the gene. Screens to isolate protein null alleles based on existing antibodies are now in progress.

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Fig 1A.

Map of the *pigment cell dehydrogenase reductase (pcdr)* region and structure of *pcdr* RNA. Restriction sites for the enzymes BamHI, HindIII and PstI are indicated by *B*, *H* and *P* respectively. *AUG* and *UAA* indicate the putative initiation and stop codons. The *dashed line* indicates the PstI-HinI fragment cloned into CasPeRlacZ vector. **B** Sequence of the 5' region of *pcdr* including the fragment PstI-HinI fragment cloned into CasPeRlacZ vector. The putative TATA box is *underlined*. The putative transcription start as determined by primer extension is indicated by a *plain arrow* (position 285) and the putative translation start site as determined by computer-assisted translation is indicated by a *dashed arrow* (position 360). The *italicized sequence* (starting at 311) indicates the sequences included in the longest cDNA.

C Sequence of one *pcdr* cDNA. The longest peptide sequence generated by computer-assisted translation is shown beneath the DNA sequence. The *bold* and *underlined* two dinucleotides **GA** form the boundaries of the two introns in the genomic DNA. The *underlined* **AATAAA** is a potential polyadenylation signal

Fig 2.

Sequence alignment of the conceptual translation of a *pcdr* clone (F3P7), human prostaglandin dehydrogenase (PGDH_HUMAN) and *Drosophila* fat body protein P6 (Fat Body P6). The alignments are based on the sequence alignment of SDR family members (short chain dehydrogenases) in Jörnvall et al. (1995). The aligned sequences in this figure are marked so as to designate amino acid residues that correspond to highly conserved residues in the Jörnvall et al. (1995) alignment. *Black squares* indicate residues present in >90% of the family members, *shaded squares* indicate residues present in 70–90% of the family members, and *circles* indicate residues present in 50–70% of the family members. A *small square above the sequence* indicates a position where the amino acids of the three shown sequences differ from

the amino acid present at that position in 70–90% of the family members, *small circles above the sequence* indicate a position where the amino acids of the three shown sequences differ from the amino acid found in 50–70% of family members. Putative cofactor binding domains are noted above the sequence

Fig 3A–C.

pcdr expression pattern. **A, B** Northern blots. Total RNAs prepared **A** from the abdomen, the thorax or the head of five pseudo male (lane 1), five male (lane 2) or five female flies (4 to 5 days old) or **B** from the body or the head of adult Canton-S (wild-type) flies or whole *eye-absent* (*eya*) flies were size fractionated and blotted to nitrocellulose prior to probing with the cDNA from *pcdr*. The blot shown in **A** has also been probed with DNA encoding ribosomal protein RP49. **C** Developmental time course. Total RNAs prepared from staged males were size fractionated and blotted to nitrocellulose prior to probing with a *pcdr* cDNA. The estimated RNA quantity compared to the quantity observed in male adult flies was plotted

Fig 4A.

A schematic diagram of a single ommatidium (redrawn from Wolff and Ready 1993). The orientation corresponds approximately to the orientation of the ommatidia in **B**, **C**, and **D**. Each facet within the eye is approximately 18 μm across (Wolff and Ready 1993). **B**, **C** Section through an adult eye immunostained with primary antibodies against Pcdr and fluorescent secondary antibodies viewed in bright light (**B**) or fluorescence (**C**). Note the strong fluorescence in the apical position of the eye occupied by the primary pigments cells as well as some fluorescence in the lens region above the pseudocone. Because ommatidia are staggered, and because the sections are thinner than the ommatidia, an individual section cuts through the lens and pseudocone region of individual ommatidia at various positions relative

to the pseudocone and the primary pigment cells. Some ommatidia are cut such that a portion of the pseudocone separates two primary pigment cells while other ommatidia are cut so that the section passes entirely through a primary pigment cell without entering the pseudocone.

D, E Histological staining of sections through the eye of transformed flies expressing β -galactosidase under the control of the 5' PstI-Hinfl fragment of *pcdr*. The section in **D**, as in **B** and **C**, is perpendicular to the surface of the eye and shows high levels of staining in the position of the primary pigment cells. The section in **E** is parallel to the surface of the eye. Because of the curvature of the eye, such a section intersects different ommatidia at different depths. Note the ommatidia at the left edge of the section showing clear staining around the ommatidia at an apical position consistent with primary pigment cell expression

Fig 5.

Quantitation of *pcdr* levels in adults heads. For each genotype the percent expression of *pcdr* in XX animals (“female”) relative to expression in XY animals (“male”) is calculated. When the levels are identical in XX and XY animals, this number is 100. A value lower than 100 indicates a higher expression level in XY animals compared to XX animals, a value higher than 100 indicates a lower expression level in XY animals compared to XX animals. In each case RNA was derived from isolated heads and normalization was done as described in Materials and methods. At least two independent sets of experiments have been done for each genotype and the RNA levels were compared only with those obtained during the same experiment. Note that in the *tra2/tra2*, *tra2/Trix*, *j7/traetc*, and *traetc/traetc* animals XX

animals develop with male morphology and behaviors. In such experiments XX pseudo males were distinguished from XY males on the basis of the X-linked markers *w* or *y* as described in the Materials and methods (*Trix Df(2R)trix*, *J7 Df(3L) st j7*, *traet* a third chromosome of the genotype *th st tra cp in ri p^p*). The *Cyo*, *SM1*, and *Bal* chromosomes are balancer chromosomes wild type for *tra2* or *tra* as described in the Materials and methods section. Normalization in **A** was relative to *rp49* RNA while in **B** normalization was to *bendless* RNAs