Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium

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ABSTRACT A visual pigment-like protein, referred to as peropsin, has been identified by large-scale sequencing of cDNAs derived from human ocular tissues. The corresponding mRNA was found only in the eye, where it is localized to the retinal pigment epithelium (RPE). Peropsin immunoreactivity, visualized by light and electron microscopy, localizes the protein to the apical face of the RPE, and most prominently to the microvilli that surround the photoreceptor outer segments. These observations suggest that peropsin may play a role in RPE physiology either by detecting light directly or by monitoring the concentration of retinoids or other photoreceptor-derived compounds.

Visual pigments are retinal-binding chromoproteins that constitute a subfamily of G protein-coupled receptors. The most extensively studied visual pigments are those located in retinal photoreceptors. These proteins detect light by monitoring the 11-cis to all-trans photoisomerization of a covalently bound retinal chromophore. Within this group, pairwise comparisons among vertebrate family members typically show greater than 40% amino acid identity, whereas pairwise comparisons between vertebrate and invertebrate visual pigments show approximately 20% amino acid identity. All visual pigments described to date have the following features: (i) a lysine in the middle of the seventh putative transmembrane segment, corresponding to Lys-296 in bovine rhodopsin, which is the site of covalent binding of the chromophore via a retinylidene Schiff base; (ii) a pair of cysteines, corresponding to Cys-110 and Cys-187 in bovine rhodopsin, which are presumed to form a disulfide bond connecting the first and second extracellular loops; (iii) the sequence (glu-asp)-arg-tyr, or a close match to this sequence, at the beginning of the second cytosolic loop; and (iv) one or more serine or threonine residues in the cytosolic carboxyl terminus, which in bovine rhodopsin are the sites of light-dependent phosphorylation by rhodopsin kinase (1).

Molecular cloning studies have revealed the sequences of three members of the visual pigment family that are either present in nonretinal tissues or that have been hypothesized to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction. Pinopsin is a pineal-specific visual pigment identified in chickens that is likely to play a role other than phototransduction.

In this paper we report the identification of a visual pigment-like protein that is present in the RPE, where it is localized to the microvilli that surround the photoreceptor outer segments. This protein may play a role in RPE physiology either by detecting light directly or by monitoring the concentration of retinoids or other photoreceptor-derived compounds.
described (22). A 0.7 kb probe containing part of the second exon of the mouse peropsin gene detected a fragment of 5.0 kb in \textit{PvuII}-digested C57BL/6 DNA and a fragment of 2.9 kb was detected in \textit{PvuII}-digested \textit{M. spretus} DNA. The presence or absence of the 2.9-kb \textit{PvuII} \textit{M. spretus}-specific fragment was followed in backcross mice. A description of the probes and restriction fragment length polymorphisms for the loci linked to \textit{Rrh}, including \textit{Egf} and \textit{Nfkb1}, has been reported previously (23). Recombination distances were calculated using MAP MANAGER, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

\textbf{RNase Protection and Northern Blot Hybridization.} Ten micrograms of total RNA from each mouse tissue or 10 \mu g of yeast tRNA was used for the RNase protection assay. For RNA blotting, 10 \mu g of bovine RNA was resolved by agarose gel electrophoresis under denaturing conditions, blotted, and hybridized with a human peropsin coding region probe (20).

\textbf{In Situ Hybridization.} \textit{In situ} hybridization was performed with digoxigenin-labeled riboprobes essentially as described (24) using 20 \mu g of TotalRNA from each mouse tissue or 10 \mu g of yeast tRNA was used for the RNase protection assay. For RNA blotting, 10 \mu g of bovine RNA was resolved by agarose gel electrophoresis under denaturing conditions, blotted, and hybridized with a human peropsin coding region probe (20).

\textbf{Production of Antibodies and Immunoblotting.} Rabbits were immunized with a synthetic peptide corresponding to the carboxyl-terminal 20 residues of mouse peropsin conjugated via its amino terminus to BSA (25). For affinity purification, carboxyl-terminal 20 residues of mouse peropsin conjugated were immunized with a synthetic peptide corresponding to the amino terminus of the maltose binding protein. The maltose binding protein–peropsin fusion was purified by amylose affinity chromatography, coupled to an Affigel matrix (Bio-Rad), and used to affinity purify anti-peropsin antibodies. Protein extracts from mouse eye (following removal of the lens) or brain were prepared by homogenizing the tissue in SDS sample buffer, and insoluble material was removed by microcentrifugation.

\textbf{Immunohistochemistry.} For plastic embedding, CD1 mice were perfused with PBS/4\% paraformaldehyde for light microscopy, or PBS/1\% glutaraldehyde/4\% paraformaldehyde for electron microscopy. The anterior chamber and lens were removed, and eye cups were further incubated in the same fixative for 3 hr at 4°C and embedded in Unicryl resin (BBI, Cardiff, U.K.). For light microscopy, 1 \mu m sections were stained, as described below for the staining of frozen sections, with affinity-purified rabbit anti-peropsin and then incubated with goat anti-rabbit antibodies conjugated to 1 nm gold particles followed by silver enhancement. For post-embedding immuno-electron microscopy, 0.1 \mu m sections were blocked in 10 mM Tris (pH 7.5), 150 mM NaCl, 0.5\% Tween 20 containing 5\% normal goat serum, stained with affinity-purified rabbit anti-peropsin in the same solution overnight at 4°C, and then stained with goat anti-rabbit antibodies conjugated to 18 nm gold particles in 10 mM Tris (pH 7.5)/150 mM NaCl and five washes in water, the grids were stained with 0.2\% uranyl acetate in 0.1 M maleate (pH 6.2) for 15 min at room temperature. Some sections were also counterstained with lead acetate.

For immunostaining of frozen sections, eye cups from CD1 mice were fixed at 4°C in PBS/4\% paraformaldehyde for 3 hr, cryoprotected in 30\% sucrose overnight, and embedded in OCT. Ten \mu m frozen sections were preincubated for 1 hr at room temperature in PBS containing 5\% normal goat serum/0.3\% Triton X-100 and then incubated in the same buffer overnight at 4°C in affinity-purified primary antibody, Biotinylated goat anti-rabbit secondary antibody and Texas Red conjugated streptavidin (Vector Laboratories) or avidin-horseradish peroxidase (ExtrAvidin peroxidase; Sigma) were used to visualize immunostaining.

\textbf{RESULTS}

\textbf{Identification of a Novel Visual Pigment Homologue.} To efficiently identify novel genes expressed in ocular tissues, we have determined partial sequences from cDNA clones derived from these tissues (J. P. Macke, P. Smallwood, A. Rattner, J. Williams, and J.N., unpublished). In an initial group of 5,000 partial sequences derived from human retina cDNAs, one clone showed significant homology to visual pigments but was

\textbf{FIG. 1.} Amino acid sequences of human and mouse peropsin aligned with human rhodopsin, octopus rhodopsin, bovine RGR opsin, and squid retinochrome. The positions of the retinylidene Schiff base (Lys-296 in rhodopsin) and its counterion (Glu-113 in rhodopsin) are indicated by arrowheads. The region encompassing the seventh putative transmembrane segment of squid retinochrome has been manually aligned to bring the seven transmembrane region into register with Lys-296 in human rhodopsin. h, human; m, mouse; o, octopus; b, bovine; s, squid.
clearly distinct from known visual pigments. As described below, subsequent experiments revealed that the corresponding gene is expressed in the RPE but not in the retina. The presence of these sequences in the human retina cDNA library presumably reflects contamination by RPE of the human retina sample used to prepare the library. To indicate its RPE localization, this protein has been named peropsin, the first three letters of which are a rearrangement of RPE.

![Diagram](image)

**Fig. 2.** (Left) Quantitative analysis of homologies between protein sequences in GenBank (May 1997 release) and human peropsin, bovine RGR, and squid retinochrome as determined by the BLAST algorithm (27). The \( P \) value is the probability of finding a comparable match by chance in a random sequence database of equivalent size. Horizontal bars indicate the range of \( P \) values for the indicated classes of sequences. Redundant database entries have not been eliminated for this analysis. (Right) Dendrogram of visual pigments and visual pigment-related proteins calculated using GeneWorks software with gap penalties set at 5 for each gap and 25 for lengthening a gap. In the dendrogram, the length of each line is proportional to the degree of amino acid sequence divergence. The rat 5HT-7 and human alpha 1B adrenergic receptors are two of the five nonvisual pigment G protein-coupled receptors in the GenBank database that are most homologous to peropsin.

**Fig. 3.** RNase protection, Northern blot analysis, and in situ hybridization localize peropsin transcripts to the RPE. (A) Total RNA from the indicated adult mouse tissues was used for RNase protection with a mouse peropsin probe. Peropsin transcripts are detected only in the eye. A control reaction with an RNA polymerase II probe is shown at bottom. (B) Northern blot of total RNA from the indicated bovine tissues was hybridized to a probe encompassing the human peropsin coding region. Peropsin transcripts are detected only in the RPE. Arrowheads indicate the mobilities of the ribosomal RNAs. (C) In situ hybridization using a digoxigenin-labeled mouse peropsin probe localizes peropsin transcripts in the adult rat to the RPE (arrowhead). (Left) Antisense probe. (Right) Sense probe. Albino rats were used for this experiment so that RPE melanin would not obscure the signal. At long substrate incubation times, a faint hybridization signal is seen in the inner nuclear layer; the origin of this signal is not known. Arrowhead, nucleus of RPE cell. OS, outer segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer.
than the BLAST algorithm, peropsin falls within the RGR
orogies among visual pigment family members are calculated
retinochrome. We note, however, that when sequence homol-
sin is overall more similar to visual pigments than are RGR or
pigments is greater than that between peropsin and RGR or
that the similarity between peropsin and the majority of visual
carboxyl-terminus of mouse peropsin (Right). The molecular masses of
protein size standards, indicated at the left, are, from top to bottom,
192, 127, 73, 43, 32, and 17 kDa.
that presumably correspond to membrane-spanning α-helices, a
(glu/asp)-arg-tyr triplet at the beginning of the second cytosoli-
cloop, and two (mouse) or five (human) serine and threonine residues in the carboxyl-terminal tail that are potential sites for
regulation by receptor kinases (Fig. 1). The human and mouse
orthologues share 80% amino acid identity, with the regions of
greatest divergence occurring in the amino- and the carboxyl-
terminal tails. A potential site for asn-linked glycosylation is
present within the first 10 residues of both human and mouse
peropsin. In the discussion that follows, we will refer to amino
acid sequence comparisons with human peropsin, but similar
results were obtained with mouse peropsin.
When the human peropsin amino acid sequence is compared
with all GenBank protein sequences (May 1997 release) using
the BLAST algorithm (27), the top 124 matches are with vertebrate and invertebrate visual pigments (Fig. 2). A large
variety of hormones, neurotransmitter, and neuropeptide G
protein-coupled receptors comprise the next most homologous
group of sequences, of which the most homologous is a
putative tachykinin receptor. Significantly, peropsin contains
a lysine at the position corresponding to Lys-296 in bovine
rhodopsin, the site of covalent attachment of 11-cis retinal in
all visual pigments. To our knowledge, no G protein-coupled
receptors other than members of the visual pigment family
have a lysine at this position. The glutamate that serves in all
vertebrate rod and cone pigments as the retinylidene Schiff
base counterion (corresponding to Glu-113 in bovine rhodop-
sin) is occupied by tyrosine in peropsin, as it is in many
invertebrate visual pigments.
The peropsin sequence also shows significant homology to
bovine RGR and squid retinochrome that rank, respectively,
99th and 121st in a BLAST search of the GenBank protein
database (May 1997 release). The BLAST alignment indicates
that the similarity between peropsin and the majority of visual
pigments is greater than that between peropsin and RGR or
retinochrome (Fig. 2). This analysis also indicates that perop-
sin is overall more similar to visual pigments than are RGR or
retinochrome. We note, however, that when sequence homol-
gogies among visual pigment family members are calculated
using an algorithm (GENEWORKS), which penalizes gaps less
than the BLAST algorithm, peropsin falls within the RGR/
retinochrome branch of the visual pigment family (Fig. 2).
Whole genome Southern blot hybridization at 42°C in the
presence of 27% formamide/5× SSC/10% dextran sulfate
using a probe containing the human peropsin coding and 5’
untranslated regions shows one or several discrete hybridizing
fragments in DNA from human, cow, mouse, chicken, Xeno-
pus, catfish, and zebrafish (data not shown), suggesting that
peropsin orthologues are present within many vertebrates.
Under these conditions, the known visual pigment genes would
not be expected to hybridize to the peropsin probe.
**Localization of Peropsin mRNA and Protein.** In the adult
duck, RNase protection with samples from brain, eye, heart,
kidney, liver, lung, spleen, and testis shows peropsin transcripts
only in the eye, and in situ hybridization to the adult rat eye
localizes peropsin transcripts to the RPE (Fig. 3). The absence
of peropsin from the retina and nonocular tissues, and its
presence in the RPE, was also seen by Northern blot hybrid-
ization to total bovine RNA (Fig. 3).
To localize the peropsin protein, antibodies were raised
against a synthetic peptide corresponding to the carboxyl-
terminal 20 residues of mouse peropsin. Immunoblots of total
protein from mouse brain or eye (minus the lens) shows that
the affinity-purified anti-peropsin recognizes a single eye-
specific protein with an apparent molecular mass of approxi-
ately 38 kDa, close to the predicted molecular mass of 37 kDa
for unglycosylated mouse peropsin (Fig. 4). Immunofluores-
cent and immunoperoxidase staining with affinity-purified anti-
peropsin antibodies shows immunoreactivity exclusively
on the apical face of the RPE (Fig. 5). Immunofluores-
cent anti-peropsin signal is seen within numerous microvilli
projecting from the apical surface of the RPE. Occasional
larger immunostained structures are observed projecting be-
Beyond the array of microvilli. Immunogold staining of 1 μm plastic sections also indicates a microvillar localization of peropsin, and shows that the immunostained region encompasses the distal one-third of the outer segment zone. To further assess the subcellular distribution of peropsin, ultrathin sections were examined by postembedding immunoelectron microscopy (Fig. 6). This analysis confirms that peropsin is localized to the apical plasma membrane of the RPE, including the microvilli that occupy the space between rod outer segments, and that it is absent from rod outer segments.

Chromosomal Location of the Mouse Peropsin Gene. The peropsin gene will be referred to as Rrh, an abbreviation for “RPE-derived rhodopsin homologue.” The mouse chromosomal location of Rrh was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × M. spretus) F1 × C57BL/6J] mice. The mapping panel has been typed for over 2,400 loci that are well distributed along all the autosomes and the X chromosome (21). Rrh is located in the distal region of mouse chromosome 3, linked to Egf and Nfkb1. Although 180 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 7), up to 187 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for each recombination frequency using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere - Egf - 2/185 - Rrh - 7/187 - Nfkb1. The recombination frequencies (expressed as genetic distances in centimorgans ± SEM) are: Egf - 1.1 ± 0.8 - Rrh - 3.7 ± 1.4 - Nfkb1. The distal region of mouse chromosome 3 shares a region of homology with human chromosome 4q (summarized in Fig. 7). The placement of Rrh in this interval in the mouse suggests that the human homologue will map to 4q.

**DISCUSSION**

This paper describes the discovery and localization of peropsin, a novel visual pigment-like G protein-coupled receptor. Peropsin is localized to the apical microvilli of the RPE, where it is in close proximity to photoreceptor outer segments. Electron microscopic studies in many vertebrate retinas have demonstrated an intimate association between RPE microvilli and photoreceptor outer segments. This association is presumed to be important for adhesion of the retina to the RPE, transport of small molecules between the RPE and the photoreceptors, and orderly phagocytosis of photoreceptor outer segments by the RPE. The localization of peropsin to the microvilli suggests that this close association may also play a
role in regulating RPE physiology via cell-surface G protein-coupled receptors. In contrast, RGR opsin is localized in the RPE to internal rather than surface membranes.

Based on its homology to the visual pigments, it seems reasonable to speculate that peropsin binds to a retinoid ligand. The presence of a lysine at a position homologous to Lys-296 in bovine rhodopsin further suggests that, like the visual pigments, peropsin may form a complex with an isomer of retinal via a Schiff base with the amino group of lysine. If retinal is the ligand, peropsin could have either of two distinct modes of action. First, peropsin could be a light receptor that signals in response to photoisomerization of the bound chromophore. In this context, the location of peropsin in the apical microvilli would permit efficient light absorption even in the presence of high concentrations of RPE melanin, because the vast majority of melanosomes are sequestered within the RPE cell bodies. Second, peropsin could be activated (or inactivated) by binding to one or more retinal isomers in a reaction that does not depend on illumination. In this model, peropsin might act as a sensor of retinal that is released by photobleaching of visual pigments in the adjacent outer segments or it might monitor retinal metabolism in the RPE. Although we favor retinal as the ligand, at present there is no experimental data to support this hypothesis. In preliminary experiments, we have attempted to reconstitute recombinant peropsin produced in transfected 293 cells or in baculovirus-infected Sf9 cells by incubation with a variety of retinal isomers. Using protocols that were developed for mammalian visual pigment reconstitution in vitro (28), we have not observed a photolabile absorption band in the visible region of the spectrum. However, it is possible that retinal may only bind to peropsin at a particular stage in its biosynthesis, as suggested for Drosophila visual pigments (29), that binding requires cofactors that are present in the RPE but absent in the tissue culture expression system, or that retinal binding produces a photostable or UV-absorbing pigment.

In an alternate model of peropsin action, peropsin might recognize a nonretinoid ligand, but share sequence similarity with visual pigments based on its evolutionary origin as a visual pigment gene that was recruited for RPE-specific expression. The possibility that the RPE and retina express divergent copies of genes that were recently derived from a common ancestor is consistent with the close embryological relationship between these two tissues. Both tissues are derived from the neuroepithelium of the optic vesicle, and in many species the RPE retains the ability to transdifferentiate into neural retina (30). In this model, the peropsin binding pocket would have diverged to generate specificity for a nonretinoid ligand that would presumably be present in the subretinal space.

All of the models described above envision a role for peropsin in regulating RPE function or activity. It will be of interest to determine which intracellular signal transduction pathway mediates peropsin action and how the transduced signal modulates RPE physiology. Whether regulation is based directly on light absorption or is via detection of a molecule that is released into the subretinal space, these findings further reinforce the idea that the RPE and retina function together as a coordinated unit.

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