Cell. Mol. Life Sci. 64 (2007) 2917 – 2932
1420-682X/07/222917-16
DOI 10.1007/s00018-007-7253-1

Cellular and Molecular Life Sciences

Review

The opsins of the vertebrate retina: insights from structural, biochemical, and evolutionary studies

B. Nickle and P. R. Robinson*

Biological Sciences Department, University of Maryland, Baltimore County, Baltimore, Maryland 21250 (USA), Fax: +410 455 3875, e-mail: probinso@umbc.edu

Received 31 May 2007; received after revision 10 July 2007; accepted 25 July 2007
Online First 30 August 2007

Abstract. The vertebrate retina contains several classes of visual pigments responsible for such diverse functions as image- and nonimage-forming vision, the entrainment of circadian cycles, and the pupilary light response. With vision being vital to the survival of many species, the elucidation of the structural and biochemical properties of visual pigments has been the focus of a large body of research that has led to rapid advances in the field of photoreception. In this review, the current understanding of the structure, function, biochemistry, and evolution of the opsins that make up the photopigments in the vertebrate retina will be reviewed. These include the rod and cone opsins, melanopsin, RGR, peropsin, and VA-opsin. The goal is to highlight important questions that have been answered and to define some of the remaining questions in the field that will provide future directions for research.

Keywords. Opsin, photopigment, rod and cone, RGR, melanopsin, VA-opsin, peropsin/RRH.

Introduction

The retina is the primary, although not exclusive, site for light detection in vertebrates. It is in this specialized sensory organ that photopigment-expressing cells that give rise to image- and nonimage-forming vision are found. These light-dependent functions that are vital for the survival of many vertebrate species depend on light absorption by photopigments that consist of two parts, the protein called opsin and a retinal-derived chromophore. The chromophore confers light sensitivity to the photopigment; its absorption of light triggers a transition in the photopigment from the inactive dark state to an active state. This conformational change in the visual pigment initiates a cascade of molecular events that result in the transduction of a signal through the optic nerve to the brain for higher-order processing. By gaining a complete understanding of the photopigments in the retina, we can increase our knowledge of light signaling and gain insight into mechanisms that may be shared by similar signal transduction systems.

Photopigments are found in both vertebrate and invertebrate species; however, this review will concentrate specifically on the vertebrate photopigments in the retina. The topic of invertebrate photopigments is a separate field of inquiry and has been addressed in other recent reviews [1]. This review will discuss what is currently known about the photopigment structure and activation properties, as well as the evolutionary relationships of the opsins with one another.

Opsins are members of the protein superfamily known as G-protein-coupled receptors (GPCRs). GPCRs are defined by their heptahelical transmembrane structure and their ability to activate a GTP-binding

* Corresponding author.

protein (G-protein). In vertebrates, the family of GPCRs is one of the largest and most diverse protein families, with thousands of members predicted in several mammalian genomes [2, 3]. GPCR functions range from hormone and neurotransmitter detection, to sensory system receptors such as visual, gustatory, and olfactory receptors. The wide range of functions is largely responsible for the high level of interest in GPCRs by the pharmaceutical industry, with a quarter to half of all therapeutic drugs targeting GPCRs [4, 5]. Although many GPCRs are commonly studied, much of what is known about GPCRs has come from the knowledge gained from the study of opsins.

The opsins, as a subclass of GPCRs, are defined by their ability to bind a retinal-based chromophore to form a light-sensitive photopigment. In the vertebrate retina, several families of opsins can be found with functions involved in regulating image- and non-image-forming vision, coordination of circadian cycles, and other as yet unidentified functions. These classes of opsins include rod and cone opsins, melanopsin, peropsin, vertebrate-ancient opsin (VA-opsin), and retinal G-protein-coupled receptor (RGR). Most of our knowledge about opsins is derived from the study of rod and cone opsins. For this reason, the rod and cone opsins will be the primary focus of this review. However, the study of melanopsin has emerged in the past few years to provide a new way of thinking about photoreception in the vertebrate retina. This topic, as well as a brief discussion of other opsins found in the vertebrate retina, will also be discussed.

Nomenclature

As a preamble to this article, it is helpful to discuss briefly the nomenclature used when referring to different opsins. In the literature, the term rhodopsin can often have different meanings depending on the published work. Historically, the term rhodopsin has been used to signify any photopigment with an 11-*cis*-retinal (A1) chromophore bound in its binding pocket. In contrast, a photopigment with an 11-*cis*-3,4-dehydroretinal (A2) chromophore bound in its binding pocket is known as a porphyropsin. However, in recent literature, the term rhodopsin has been used to specifically refer to a rod opsin with an A1 chromophore. In this paper, we will keep with the current trend in the literature and use the term rhodopsin to refer to an A1-based rod photopigment. Unless otherwise noted, the term rhodopsin by itself will specifically mean bovine rhodopsin since this is the photopigment that is the most heavily studied in vision science.

Structure of opsins

GPCRs as a family have proven to be difficult proteins for structural studies due to the requirements for large amounts of protein that remain stable upon removal from native membranes. As a result, bovine rhodopsin, hereafter referred to simply as rhodopsin, has emerged as an ideal model for studying GPCRs, and much of what we know about the structure of GPCRs comes from such studies. This is due to the abundance of rhodopsin in rod outer segments (ROS) and the ease of isolation from bovine retinas. Rhodopsin is considered the prototypical opsin member, and it was the first GPCR to be sequenced [6, 7] and cloned [8, 9]. Additionally, rhodopsin was the first, and to date the only GPCR with its crystal structure solved [10]. This structure has undergone several refinements since it was originally reported [11–13], providing us with a detailed understanding of the dark-state structure of rhodopsin as well as a starting template for homology modeling of other opsins [14].

Like all GPCRs, opsins contain seven transmembrane helices (Fig. 1); however, they are unique among GPCRs in that opsins covalently bind their ligand, a retinal-based chromophore, to form a light-sensitive photopigment. The site of retinal attachment is highly conserved in all opsins and resides at K296 in the seventh transmembrane helix [15, 16]. For simplicity, all amino acid residues referenced in this paper will use the rhodopsin numbering system. The aldehyde group on the chromophore undergoes a condensation reaction with the ε-amino group from K296 to form a covalent Schiff base attachment. In most vertebrate pigments, an A1 chromophore is used; however an A2 chromophore is used in some species of amphibians, fish, and reptiles.

In rhodopsin the Schiff base is protonated, producing a charged complex in the photopigment-binding pocket. The highly conserved E113 residue in transmembrane helix III serves as the counterion to the protonated Schiff base [17–19]. Growing evidence suggests that the Schiff base is only protonated for photopigments that absorb maximally in the visible range. For photopigments that have a maximal absorbance in the UV range, the Schiff base is unprotonated in the dark state [20–22].

When the crystal structure of rhodopsin was solved, several startling discoveries were revealed about the three-dimensional shape of the molecule. First, the seven transmembrane α helices form a bundle of irregular helices crossing the membrane at angles (Fig. 2). Helices I, IV, VI, and VII are all bent at proline residues in the helix. The bend in helix I is minimal and the bend in helix IV only results in distortion around the extracellular end. Helix V also

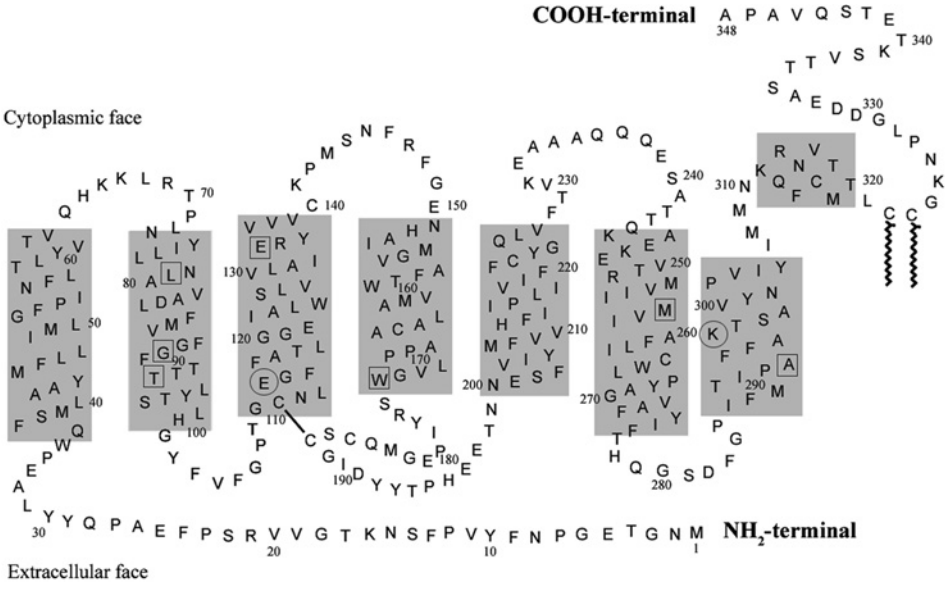

Figure 1. Secondary structure of bovine rhodopsin. Residues in the transmembrane helices that have been shown to confer constitutive activity to the opsin have been boxed or circled. The two sites shown in circles represent the site of chromophore attachment in helix VII (K296) and its counterion in helix III (E113). Palmitylation sites are shown on C322 and C323 and the disulfide bond linking C110 and C187 is represented. The model is based upon the rhodopsin crystal structure [10].

has a proline residue in the center but this does not affect the regularity of the helix. Helices II and III both have double glycine residues in the helix; this causes a bend in helix II but helix III has minimal distortion. Second, the crystal structure revealed that extracellular loop II folds into the helical bundle creating a plug that comes in close proximity to the retinal-binding pocket. The presence of this plug has led to the question of what pathway the chromophore moves through to enter and leave the binding pocket during pigment regeneration. This question has still not been answered and understanding this mechanism may lead to an understanding of why rod and cone photopigments regenerate at different rates. Finally, the crystallization also revealed a previously unrecognized eighth α helix located on the cytoplasmic face of the opsin running along the membrane surface [10]. Regarding the higher-order structure of vertebrate photopigments, we still do not know if they exist in a monomeric, dimeric, or multimeric state in native rod and cone outer segments. In the 1970s and 1980s, many biophysical studies concluded that rod photopigments exist in a monomeric state in the native disc membrane. These studies included research using techniques such as small-angle neutron scattering, analytical ultracentrifugation, and light scattering techniques [reviewed in ref. 23]. However, in 2003, atomic-force microscopy (AFM) experiments challenged the traditional view of a monomeric rod photopigment by suggesting the native state of the pigments to be dimeric [24]. This study has launched a renewed

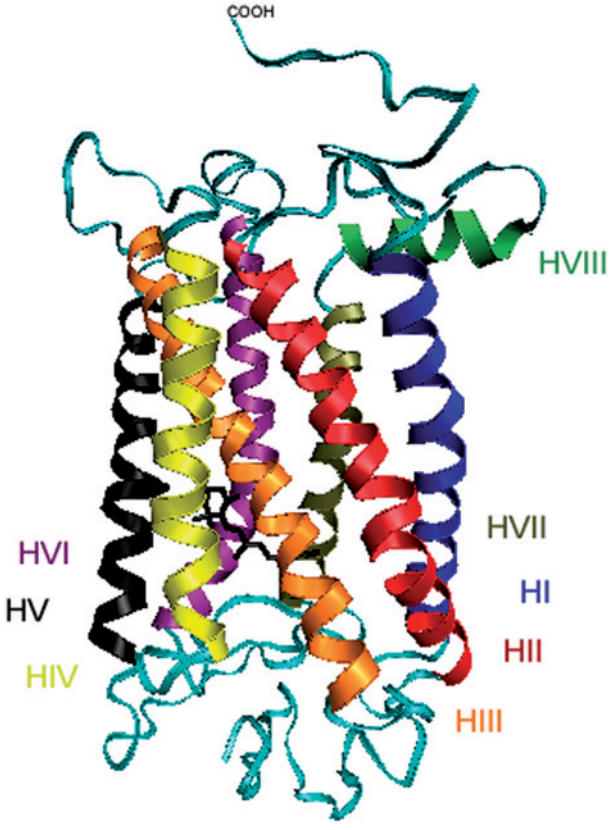

Figure 2. Three-dimensional structure of bovine rhodopsin based on the PDB data file 1U19. Different helices are shown in different colors with the 11-*cis* retinal chromophore located in the binding pocket. The carboxy terminus is localized on the cytoplasmic side of the membrane; the amino terminus is localized on the extracellular side of the membrane.

interest into the higher-order structure of photopigments *in vivo*.

Recent attempts to examine the higher-order structure of rhodopsin have produced a large amount of data to support the dimerization model. First, rhodopsin expressed in COS-1 cells was shown through cross-linking to have the ability to form dimers and possibly multimers [25]. Fluorescence resonance energy transfer (FRET) analysis in reconstituted liposomes has also revealed the dimerization of rhodopsin [26]. Finally, dimerization has been observed in detergent-solubilized rhodopsin, with the dimeric state having a greater ability than the monomeric state to activate transducin [27, 28]. Together these data have supported dimerization as a model for rhodopsin, and as such the model has begun to be generalized to all GPCRs [29].

In fact, since the idea of GPCRs forming oligomeric structures was first introduced through work with the glucagon receptor [30], several class A GPCRs like the histamine H1 receptor and α_{1b}-adrenoreceptor have been shown to dimerize [31]. Additionally, dimerization in the β_2-adrenergic receptor has been shown to occur in HEK-293 cells [32] and is necessary for export of the protein from the endoplasmic reticulum (ER) and cell surface targeting [33]. However, research using reconstituted monomers in high-density lipoproteins has shown that dimerization is not required for the β_2-adrenergic receptor to functionally activate its cognate G-protein [34]. Additionally, research on the M2 muscarinic cholinergic receptor has shown that receptor purified from sf9 cells can exist as a monomer or dimer, but only the monomeric form is active [35], suggesting that dimerization is not universal among the class A GPCRs.

This leaves open the question as to the endogenous arrangement of rhodopsin in rod photoreceptors. Does the rod photopigment exist *in vivo* in a monomeric state as suggested by studies from the 1970s and 1980s, or in a higher-order arrangement as suggested by recent studies? While it is still premature to conclude that rhodopsin and other opsins dimerize *in vivo*, future work may provide valuable insight into mechanisms of protein-protein interactions involved in photopigment signaling.

Classification and general evolution of rod and cone opsins

With so much information emerging on the structure of opsins based on studies using rhodopsin, it is important to note that rhodopsin falls into just one of the five classes of vertebrate visual pigments. The rod and cone opsins are divided into classes based on amino acid sequence similarity and spectral tuning properties [36, 37]. These classes consist of two rhodopsin-like classes (RH1 and RH2), two short-wavelength-sensitive classes (SWS1 and SWS2), and a medium/long-wavelength-sensitive class (M/LWS) (Fig. 3). Each class operates within a given range of maximal absorbance; however, there is some overlap in absorbance between classes (Table 1). Phylogenetic analysis shows that the ancestral visual opsin class is the M/LWS class, with the SWS and rhodopsin-like classes evolving more recently [36–38]. In mammals two of these classes, the RH2 and SWS2 class, have been lost, leaving only the RH1, SWS1, and M/LWS classes represented.

Table 1. Maximal absorbance range for the five classes of vertebrate visual pigments.

Pigment class	Maximal absorbance range [36]
RH1	cluster around 500 nm
RH2	466–511 nm
SWS1	358–425 nm
SWS2	437–455 nm
M/LWS	521–575 nm

Significantly, the opsin class we know the most about is the most derived class. Through the evolutionary process, the RH1 class, to which rhodopsin belongs, has gained increased stability over the other opsin classes and the ability to respond reliably to a single photon of light [39]. Additionally, the lifetime of the meta-II state has increased for the RH1 pigments versus the other four pigment classes to which the cone opsins belong. The change in the meta-II lifetime has been traced to substitutions at sites 122 and 189. Position 122 is conserved as an acidic residue in the RH1 and RH2 classes but not in the rest of the cone classes, while P189 is conserved in the cone classes but not the RH1 class (the exception to this rule is chicken rhodopsin) [40, 41]. Together, the increased stability and single-photon response of RH1 photopigments allows for the high degree of sensitivity needed for scotopic vision.

Activation of opsins

With only the dark-state crystal structure of rhodopsin solved, the question remains as to what process occurs when the photopigment moves from an inactive state to an active state. Although we still do not have a high-resolution crystal structure for the active state of rhodopsin, various biophysical techniques have help-

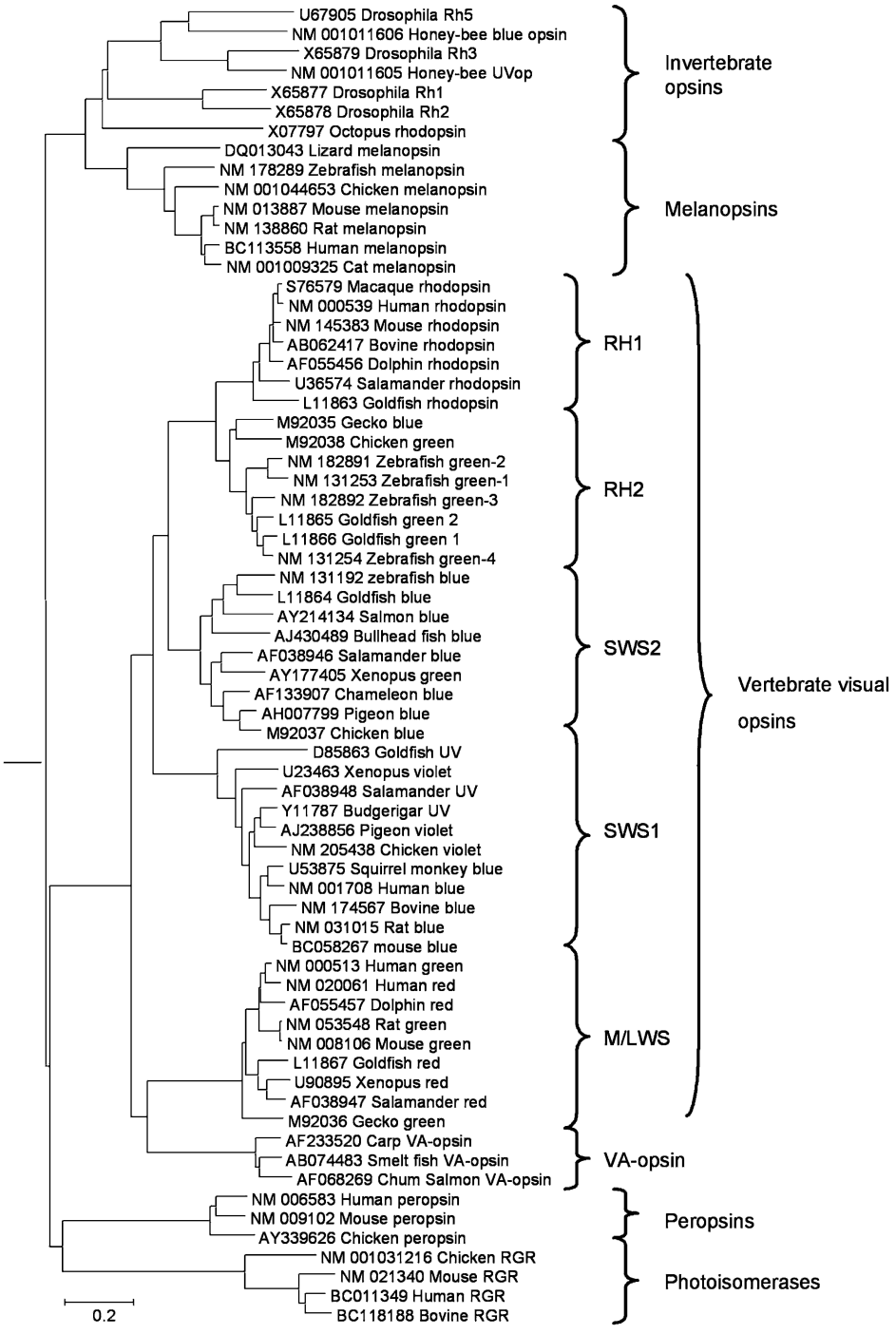

Figure 3. Phylogenetic tree depicting the different classes of opsin found in the vertebrate retina. For each branch, the NCBI accession number is listed and followed by the opsin name. The tree was constructed using the neighbor-joining method. During tree construction, the following sequences were used as out-groups to root the tree, human beta-2-adrenergic receptor (NM_000024) and human GPR119 receptor (BC095502).

ed create a working model of the activated form. It has long been known that light activation of photopigments begins with the isomerization of the chromophore from 11-*cis* retinal to all-*trans* retinal. The isomerization, which occurs in ~200 fs [42], starts a transition from the inactive dark state of rhodopsin through a series of spectrally distinct photointermediates to reach the active meta-II conformation of the photopigment (Fig. 4). Site-directed spin labeling (SDSL) and fluorescence quenching assays have revealed that the transition from the dark state to the meta-II state is accompanied by a gross helical

movement of transmembrane helix VI away from helix III [43, 44]. The helical rearrangement exposes a hydrophobic patch on the inner face of helix VI that has been implicated in G-protein binding and activetion [45]. Recent crystallographic structures of the batho [46], lumi [47], and a low- (5.5 Å) resolution meta-I [48] state of rhodopsin reveal little change in the helical arrangement from the dark state. This suggests that the gross helical changes observed in meta-II must occur during the meta-I to meta-II transition.

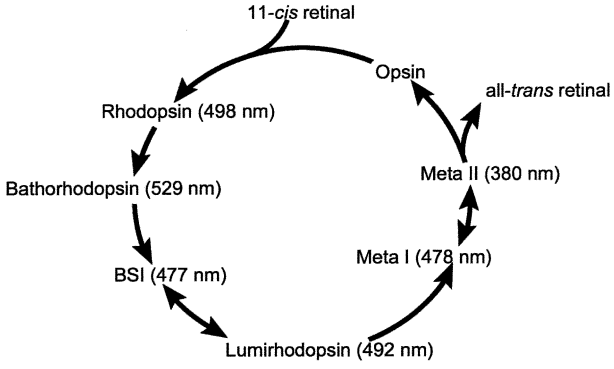

Figure 4. Sequence of rhodopsin states during photoactivation and photopigment regeneration.

Recently low-resolution light-stable crystals have provided a structure for a deprotonated active form of rhodopsin at 4.15 Å. Salom et al. [49] observed in their crystal structure a movement of the cytoplasmic loops in relation to the dark state, but they failed to see any gross helical displacement described by SDSL experiments [43, 44]. The crystals yielded a dark pigment with a λ_{max}= 500 nm, matching that of the rhodopsin dark state. Upon light activation, the crystallized pigments blue-shifted to a λ_{max} of 390–400 nm. This maximal absorbance value does not match any of the previously identified photointermediates of rhodopsin, suggesting that the crystallized active form may not be the true active state of rhodopsin. However, Salom *et al.* [49] did show, using fluorescent assays, that their photoactive structure could activate transducin.

Accompanying the activation of rhodopsin is a proton rearrangement in the chromophore-binding pocket. The Schiff base becomes deprotonated and E113 becomes protonated [50]. This deprotonation occurs during the meta-I to meta-II transition. Deprotonation breaks the ionic interaction between the E113 and the Schiff base that locks the protein in an inactive state [51]. Recently Yan *et al.* [52] have suggested a counterion switch model that hypothesizes that E181 on the extracellular loop II acts as the primary proton acceptor from the Schiff base during photoactivation. E181 transfers a proton through a H-bond network involving two water molecules, S186, and E113 as the final proton acceptor. The counterion switch model was challenged by Fourier transform infrared (FTIR) studies that suggest E181 and E113 are both in a deprotonated state during the inactive conformation, thus preventing a counterion switch [53]. Instead Ludeke et al. [53] proposed that both sites operate in a 'complex-counterion' with E113 acting as the primary counterion in the inactive state and early photointermediate states, while during meta-I, E181 becomes the primary counterion with some contribution by E113. This is due to a rearrangement of internal hydrogen bond networks that occurs during photoactivation.

It is of importance to note that in both the counterion switch model and the complex-counterion model, E181 plays an important role in the transition from the dark state to the active conformation. This residue is highly conserved in all but the M/LWS opsin class, where in most members it is replaced by a histidine residue. In the M/LWS class, H181 has been implicated as a Cl^- binding site [54]. To date the only known M/LWS opsins that do not have an H181 are found in the mouse [55] and rat, which have a tyrosine substitution. The presence of a Cl^- binding site at residue 181 suggests that the M/LWS opsin class may have a different sequence leading to Schiff base deprotonation. While such a sequence would need to be further investigated, it is possible that E113 may act as the exclusive proton acceptor, gaining its proton directly from the protonated Schiff base during photoactivation.

Spectral tuning in the vertebrate visual opsins

Color vision is based upon the ability of multiple photopigments to discriminate between different wavelengths of light. Modulation of photopigment wavelength sensitivity can be accomplished in two ways: either the chromophore can be exchanged, as occurs in some fish and other vertebrates, where the A1 retinal is exchanged for an A2 retinal [56], or more commonly, there can be substitutions of amino acids that come in close contact with the chromophore to affect spectral modulation. Changes in the polarity and charge of the amino acids that come in close contact with the conjugated double-bond system of the chromophore alter the amount of energy needed to trigger the chromophore isomerization, leading to an active pigment. The more energy needed to accomplish this conversion, the more blue-shifted the spectral sensitivity will be. The specific residues

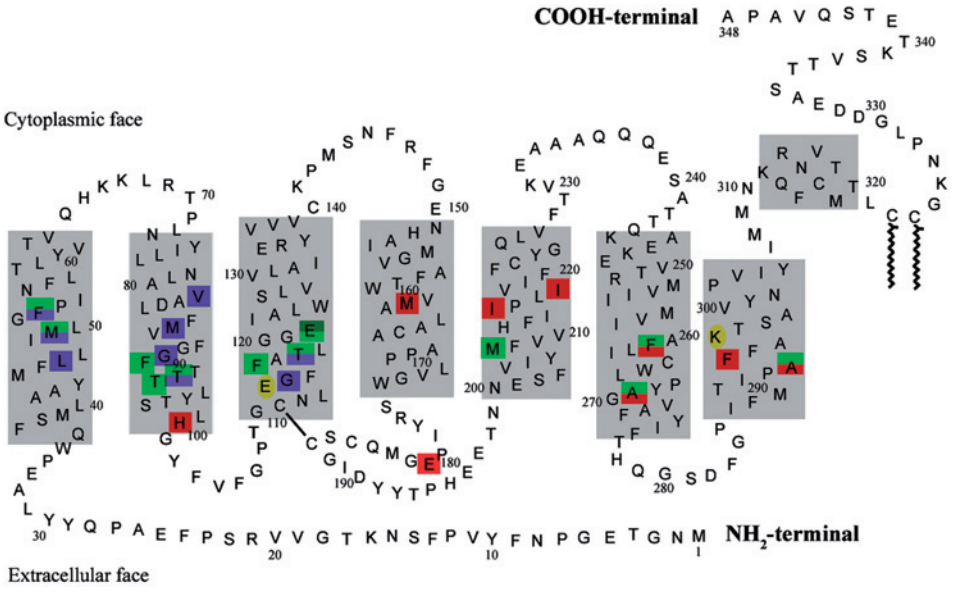

Figure 5. Cone opsin spectral tuning sites overlaid on the secondary structure of bovine rhodopsin. Red indicates sites involved in tuning M/LWS photopigments, blue indicates sites involved in tuning SWS1 photopigments, green indicates sites involved in tuning SWS2 photopigments, and the dark shadowing at site 122 indicates a site involved in tuning RH2 photopigments. The yellow ovals indicate the chromophore binding site and its counterion.

that accomplish this spectral tuning have been well researched and found to vary between the different classes of opsin (Fig. 5).

Using the SWS1 class as a starting point for discussion, we can begin to look at the spectral tuning sites. The SWS1 photopigments, which span the largest naturally occurring range of wavelength sensitivities of any of the classes of vertebrate opsin, have been extensively studied and have served as an interesting model for examining the evolution of opsins and UV sensitivity [57]. This class includes UV-sensitive pigments as well as violet-sensitive pigments. The sites involved in tuning the photopigment between UV and violet sensitivities have been shown to differ between avian and nonavian species. Avian species have a single amino acid substitution at position 90 that accounts for this shift, with C90 pigments being UV sensitive while S90 pigments are violet sensitive [58, 59]. In nonavian species, the UV-sensitive pigments retain S90 [20], suggesting that the spectral tuning properties have evolved differently, and there is no common consensus for a single mode of SWS1 spectral tuning.

Position 86 has been demonstrated to induce a shift of ~70 nm in mouse UV and bovine blue SWS1 opsins. Where pigments with F86 are UV sensitive, those that have Y86 are violet sensitive [60]. Primates and *Xenopus* have neither a Phe nor Tyr at position 86, rather they have a Leu and Met, respectively. Substitutions of Leu or Met into goldfish UV-sensitive pigment at position 86 produced no effect on wavelength absorption [20], suggesting that residue 86 by itself is not involved in the tuning of all SWS1 pigments and that spectral tuning is rarely as simple as changing a single residue. In humans, it was shown that a more complicated spectral tuning system evolved that involves up to eight tuning sites at positions 46, 49, 52, 81, 86, 93, 114, and 118. While once again site 86 is found to be important, changes at all eight sites are required for a full shift between UV and the normal violet sensitivity in the human blue pigment [22, 61]. Since these tuning mechanisms vary so drastically, they must have evolved separately [60]. This is reinforced by phylogenetic studies showing that the ancestral SWS1 opsin was UV sensitive, with violet sensitivity evolving at least three separate times [20, 57].

A different set of spectral tuning sites has evolved in the SWS2 class. In cottoid fish from Lake Baikal, T269A and T118G substitutions create significant blue-shifts [62]. SWS2 pigments from other vertebrates have much more complex spectral tuning patterns. The newt (*Cynops pyrrhogaster*) has five sites that cause spectral shifts of 5 nm or more at positions 91, 94, 122, 261, and 292 [63]. Research with the chameleon (*Anolis carolinensis*) added sites 49, 52, 93, 207, and 269 to that list, with the spectral shifts shown to occur in an additive fashion [64]. To further show how complicated spectral tuning can be, site 116 was added to the list by observing the absorption difference in goldfish (λ_{max}= 443 nm) and zebrafish (λ_{max}= 416 nm) SWS2 pigments [65].

For studies of the spectral tuning of the M/LWS class, researchers have mainly used the human red and green opsins as examples. Both maintain a high degree of homology, yet they differ in absorbance maxima by 31 nm. The high degree of homology is a result of these two distinct opsins evolving from an X-linked gene duplication event in Old World primates [66]. In these opsins, sites 164, 261, and 269 have been shown to produce the majority of the shift between the two pigments [67], with sites 100, 214, 217, and 293 contributing the remainder of the difference [68]. Additional tuning sites were discovered by looking at the difference between human red and mouse green M/LWS pigments, where substitutions at sites 181 and 292 combined to produce a shift of 44 nm [55]. S292A substitutions were also shown to produce a 28-nm red-shift in M/LWS pigments of the bottlenose dolphin [69].

With sites 261, 269, and 292 being common tuning sites in both the M/LWS and SWS2 classes, and sites 49, 52, 93, and 118 being common to two SWS classes, we see a pattern emerging of overlapping spectral tuning mechanisms between some opsin classes. However, the overall pattern is that all tuning sites, with the single exception of 181, are located in the transmembrane region of the opsin. This allows the sites to influence the electron distribution in the chromophore to modulate tuning. Site 181 can exert a similar influence because it lies in extracellular loop 2, which folds into the helical bundle to create an extracellular plug.

The same pattern of tuning sites localized in the transmembrane helices extends to the least-studied opsin class, the RH2 class. Researchers studying the RH2 class have turned to the zebrafish as a model due to duplication events that created four separate RH2 genes in its genome, with the expressed pigments having absorption maxima spread over a 38-nm range. Using this model system, changes at site 122, a site shared with the SWS2 class, have been implicated in producing a major shift of approximately 15 nm [70], with the rest of the difference being an additive effect of multiple substitutions in the RH2 genes.

Biochemical and structural differences between rod and cone opsin classes

To date there are no crystallographic models for any opsins other than bovine rhodopsin. Instead, all attempts to understand other opsin classes have used the crystal structure of rhodopsin as a template for modeling the various pigments. However, over the years, mounting evidence has developed to show that the vertebrate opsin classes that make up the rod and cone opsins may differ both biochemically and structurally from rhodopsin (Table 2), leaving the question open as to how much information we can infer about the other opsin classes from the rhodopsin structure.

Before discussing the evidence suggestive of biochemical and structural differences among the opsin classes, it must be noted that many studies fail to discriminate among the five vertebrate rod and cone opsin classes, and instead draw the differentiation line simply as rod and cone photopigments. As a general rule, the RH1 class is localized to rod photoreceptors, and the RH2, SWS1, SWS2, and M/LWS classes are localized to the cone photoreceptors. While this generalization normally holds true, it is not absolute. For example, gecko (*Gecko gecko*) M/LWS-p521 opsin is expressed in rods [71], salamander (*Ambystoma tigrinum*) SWS2 opsin is expressed in both rod and cone photoreceptors [72], while chameleon (*A. carolinensis*) cones express an RH1 pigment [73].

To begin looking at some of the properties that differ among the various opsin classes, studies first turned to the well-documented susceptibility of the different opsin classes to hydroxylamine bleaching. Photopigments belonging to the M/LWS [74], SWS1 [75–77], SWS2 [75, 78], and RH2 [75, 79] classes all appear to be sensitive to hydroxylamine bleaching in the dark state, whereas RH1 photopigments are resistant [75, 79]. This suggests that the cone opsin classes may have a relatively open conformation in the dark state that allows the hydroxylamine to compete with the opsin for binding to the retinal. A recent exception to this rule has emerged with studies of the salamander (*A. tigrinum)* SWS1 pigment that showed that the photopigment was stable in the dark in the presence of hydroxylamine [80]. The idea of the cone opsins having an accessible binding pocket is supported by work showing that attachment of the chromophore to the opsin in cone photopigments has a greater degree of instability than the rod photopigment. In cones, a random dissociation of chromophore from the opsin can occur in the absence of light, but in the rod photopigments, the covalent attachment of chromophore to opsin is virtually irreversible absent light activation of the photopigment and meta-II decay [81]. This may be the result of a binding pocket that is tighter in the RH1 photopigments and therefore more protected from random hydrolysis of the chromophore.

Other studies using site-directed mutagenesis have shown that the opsin classes do not respond identically to mutations at the same sites. In the RH1 opsins, a critical salt bridge exists between residues K296 and E113 that acts to hold the opsin in an inactive state [51]. Mutations at E113 that neutralize its charge

Table 2. Areas in which vertebrate opsin classes exhibit different properties.

	Property	RH1	RH2	SWS1	SWS2	M/LWS	References
1)	Use of retinal analogs: 11-*cis*-13-demethyl retinal or β-ionone	increased transducin activation	?	increased transducin activation	increased transducin activation	decreased transducin activation	89–92
	11-*cis*-9-demethyl retinal	decreased transducin activation rate	?	decreased transducin activation rate	no affect on transducin activation rate	no effect on transducin activation rate but increased stability of the meta-II state	80, 93
2)	Susceptibility to hydroxylamine bleaching in the dark	resistant to bleaching	susceptible to bleaching	susceptible to bleaching	susceptible to bleaching	susceptible to bleaching	74–79
3)	Stability of dark-adapted photopigment	high stability	?	susceptible to random dissociation into opsin and chromophore	susceptible to random dissociation into opsin and chromophore	susceptible to random dissociation into opsin and chromophore	81
4)	Affects of counterion mutations	high level of constitutive activity	high level of constitutive activity	high level of constitutive activity that is not quenched by chromophore addition	high level of constitutive activity that is not quenched by chromophore addition	low level of constitutive activity	82
5)	Posttranslational palmitylation	palmitylated at C322 and C323	?	not palmitylated	equal ratio of nonpalmitylated and single palmitylated opsin at C323	not palmitylated	94
6)	Spontaneous activation rate in intact salamander rods and cones	~0.03 s^{-1}	?	~2000 times that of rhodopsin (RH1)	?	~61×10^5 times that of rhodopsin (RH1)	95

result in opsins that can activate transducin in the absence of light. Homologous mutations have been shown in an *in vitro* transducin activation assay to differentially affect the opsins of different classes in their degree of activation [82]. These assays suggest that each class of opsin may have different residues that affect its overall stability, as well as the possibility that a break in the salt bridge between K296 and E113 in classes other than the RH classes may be insufficient to fully achieve activation. In addition to E113 and K296, other sites have been linked to constitutive activation in rhodopsin (Fig. 1). These include mutations at G90D [83], A292E [84], T94I [85], L79A [86], W175A[86], M257Y [87], and E134Q [88]. By exploring how homologous mutations in opsins from the other classes affect the protein, a determination may be achieved of class-specific residues that are involved in either activation or deactivation of their photopigments.

Other studies have used retinal analogs to study their effect on rod and cone activation in the tiger salamander (*A. tigrinum*). Studies using 11-*cis*-13-demethyl retinal or β-ionone have revealed that both analogs act as agonists in bleach-adapted rods (rods bleached prior to addition of chromophore) [89–91], but they act as antagonists in bleach-adapted cones [90, 92]. Further work shows that of the cone classes, it is only the M/LWS class that shows an antagonistic effect with β-ionone; the SWS1 and SWS2 classes behave the same as the RH1 [89]. These results are explained by a slower rate of Schiff base formation between the 11-*cis*-13-demethyl retinal and opsin, resulting in a transient activation due to an initial noncovalent association in the binding pocket of opsin. The activation level decreased gradually as a covalent association was formed to create a pigment [90]. These results are at odds with the effect of 11-*cis*-9-demethyl retinal on the various opsins. In the RH1 and SWS1 classes, the transducin activation rate is decreased in pigments reconstituted with 11-*cis*-9-demethyl compared to 11-*cis* retinal. In contrast, 11-*cis*-9-demethyl had no effect on the transducin activation rate in SWS2 and M/LWS pigments but caused an overall increase in the total amount of transducin activation in M/LWS opsins [80]. The increase in transducin activation is attributed to a prolonged meta-II state. The 9-methyl group of retinal interacts with the M/LWS opsin to promote efficient meta-II decay, in its absence, the meta-II state is stabilized allowing greater transducin activation [93].

Structurally, several differences are known to exist between the different opsin classes. First, the palmi-

tylation state of the C terminus of the opsin varies from class to class. The RH1 class, which has two palmitylation sites (C322 and C323), is doubly palmitylated forming a fourth cytoplasmic loop that contains α helix VIII. The SWS2 class also contains a palmitylation site, but exists in roughly equal amounts of palmitylated versus nonpalmitylated photopigments. SWS1 and M/LWS are not palmitylated, and the RH2 class has not yet been experimentally determined [94].

Other structural differences can be found by sequence alignment. In rhodopsin, helix II exhibits a distortion around the double glycine residues, G89 and G90. The RH1 class is the only class of vertebrate opsin that has a double glycine conserved in helix II; in the RH2 and SWS2 classes, only G90 is conserved, while in the SWS1 and M/LWS classes, neither Gly residue is conserved. Since this bend in helix II serves to bring G90 close to E113 in rhodopsin, removal of the helix II kink may explain the apparently looser conformation of the retinal-binding pocket in cone photopigments versus rod photopigments.

Finally, the rate of spontaneous activation of the photopigments varies among the different vertebrate opsin classes. Using electrophysiological recordings from salamander (*A. tigrinum*) cones, it was demonstrated that the M/LWS photopigments have a spontaneous activation rate $\sim 6 \times 10^5$ times that of rhodopsin [95]. While this is much higher than estimates derived from recordings of *Xenopus* rods expressing salamander M/LWS pigments [96], both studies showed a drastic decrease in the stability of the M/LWS pigments compared to the RH1 pigments. Additionally, electrophysiological recordings have shown that the salamander SWS cones contain photopigments that are also much less stable than their RH1 photopigments but with a spontaneous activation rate much lower than the M/LWS photopigments [95]. While the spontaneous activation rate of an RH2 pigment has not been shown, the current information confirms that the stability rates of the different opsin classes vary greatly.

Melanopsin

Outside of the traditional rod and cone opsins, melanopsin is the opsin that has generated the most attention in vertebrates, with extensive recent reviews covering the rapidly growing field [97–99]. Melanopsin activation has been shown to lead to physiological responses such as circadian photoentrainment and the pupilary light response [100]. Having been first discovered in dermal melanophores of *Xenopus*, melanopsin was subsequently found to localize to the *Xenopus* inner retina [101]. In humans, it has since been shown to be expressed in the ganglion cell layer of the inner retina [102] and its expression has been further described in a number of different vertebrates [102–106]. In the retina, the melanopsin-expressing retinal ganglion cells make up only a small subset of the total retinal ganglion cells, with estimates of approximately 1% in mouse and 2.5% in rat [100]. Genomic analysis has shown that excluding mammals, most vertebrates express two orthologs of melanopsin, termed Opn4 m and Opn4x. In placental and marsupial mammals, only Opn4 m is expressed and is restricted to the retinal ganglion cells. However, in nonmammalian vertebrates, both Opn4 m and Opn4x are expressed in a wide range of tissues, including the retina, brain, and skin. It is still uncertain if these two orthologs have independent functions in the tissues in which they are expressed [107].

One of the most intriguing features of melanopsin is that it shows greater sequence homology with invertebrate opsins than to vertebrate opsins. Most notably, the site of the vertebrate Schiff base counterion has an E113Y substitution in melanopsin [101] as has been described in invertebrate opsins. With the tyrosine residue being an unlikely counterion, the melanopsin counterion is most likely to be E181. This would correspond with the invertebrate opsins, where E181 has been shown to be the counterion for amphioxus rhodopsin [108]. Phylogenetic analysis also places melanopsin in its own clade closer to the invertebrate than the vertebrate opsins (Fig. 3).

Other aspects of melanopsin that make it seem more invertebrate-like than vertebrate-like have come from studies that concentrate on the molecular characterization of melanopsin and its signaling pathway. Early studies using heterologous expression showed that melanopsin could functionally bind retinal and activate a G-protein [109]. However, that study made no attempt to identify the native G-protein that is activated by melanopsin. Subsequent studies also done in heterologous systems have since pointed toward a G_q class G-protein pathway by demonstrating that activation of G_q can trigger a transmembrane current [110, 111]. *In vivo* data obtained through the use of cultured *Xenopus* dermal melanophores have also pointed toward a G_q signaling cascade. In culture, the use of phospholipase C (PLC) and protein kinase C inhibitors, as well as intracellular calcium chelators, blocked the melanophore light responses [112]. However, before accepting that the same pathway exists in the retinal ganglion cells, it should be noted that the melanophore light response is a redistribution of melanosomes which could be the result of a different signaling cascade than the membrane depolarization found in retinal ganglion cells [112]. For this reason, *in*

vivo data are needed to confirm the identity of the cognate G-protein in the melanopsin-expressing retinal ganglion cells. However, the demonstration that melanopsin can activate a G_q G-protein is interesting, since many invertebrate photopigments signal through a G_q signaling pathway that uses PLC as an effector. In contrast, the more established vertebrate opsins signal through a $G_{i/o}$ class, including transducin, signaling pathway that uses cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase (PDE) as its effector.

Finally, additional evidence of a close evolutionary tie of melanopsin to the invertebrate opsins comes from studies showing that melanopsin may act as a bistable pigment. In vertebrate opsins, upon reaching the meta-II state, the chromophore is hydrolyzed and dissociates from the opsin. The photopigment is reestablished by the introduction of a new 11-*cis* retinal molecule into the opsin-binding pocket. In contrast, invertebrates maintain a bistable pigment whose chromophore remains in the binding pocket after activation and is reisomerized back to the *cis* isoform upon absorption of a subsequent photon of light. Evidence that melanopsin may be a bistable pigment was obtained from heterologous expression in neuro-2a cells. In this expression system, 9-*cis* and 11-*cis* retinal activated a strong light-dependent cellular response, while the all-*trans* isoform produced a low level of light response. The all-*trans* response was enhanced after exposure to 540-nm light suggesting that in melanopsin, the chromophore can reversibly photoisomerize [113]. Additionally, all-*trans* retinal was found to restore photosensitivity to melanopsin-expressing cells in RPE65 knock-out mice [114]. Bistability may help explain how melanopsin can remain photosensitive while being expressed in the ganglion cells, which are far removed from the retinal pigment epithelium (RPE) where the known retinoid cycle takes place.

VA-opsin, RGR and peropsin

Aside from the rod and cone opsins and melanopsin, we know very little about the other opsin members found in vertebrate retinas. Three other opsin classes have been shown to exist in the retina, two of which, RGR and peropsin, are in the RPE while the third class, VA-opsin, is found in the amacrine cells. Of these, RGR has been studied the most extensively, but like VA-opsin and peropsin, we still do not understand the function it plays in the retina. RGR has been localized to the RPE and Müller cells [115], and the endogenous chromophore has been identified as all-*trans* retinal which forms 11-*cis* retinal upon light activation [116]. Although RGR was originally thought to act as a photoisomerase playing a role in the retinoid cycle by regenerating 11-*cis* retinal pools for use by the rod and cone photoreceptors, it has been shown that the isomerized 11-*cis* retinal does not readily dissociate from RGR [116], suggesting that its photoisomerase activity is not a significant contributor to the 11-*cis* retinal pools. Instead, it was found that the major isomerohydrolase in the RPE is Rpe65 [117], although RGR is necessary for maintaining a regular rate of 11-*cis* retinal synthesis [118, 119]. Since RGR has been shown to interact with proteins involved in the retinoid cycle [120], and it was found that 11-*cis* retinal pools can regenerate independent of RGR [119], it is currently believed that RGR does not act as an isomerohydrolase, rather it behaves as a cofactor in the retinoid cycle that modulates isomerohydrolase activity [119]. Some of the questions that still surround RGR concern its specific mode of isomerohydrolase activity modulation.

Peropsin, also called retinal pigment epithelium-derived rhodopsin homolog (RRH), makes up a second class of opsins localized to the RPE [121]. Studies comparing its gene structure with other opsins have shown that peropsin contains two common introns with RGR, suggesting that they are derived from a common lineage. As a result, peropsin may also function *in vivo* as a retinal isomerase [122]. Support for this theory comes from phylogenetic analysis which clusters the peropsins close to the RGRs (Fig. 3). Additionally, a heterologously expressed amphioxus homolog in HEK293S cells was found to be capable of photopigment formation with both 11-*cis* and all-*trans* retinal and of being able to photoisomerize between the two [123]. The study of peropsin is very immature, and there have been no studies showing that the photoisomerase activity is necessary for any physiological function.

The final class of opsins, the VA-opsin, has only been described in teleost fish. VA-opsin was first identified in the eyes of Atlantic salmon [124]. *In situ* hybridization assays localized VA-opsin to horizontal and amacrine cells of the inner retina [125]. VA-opsin was also found to be expressed outside the retina in the pineal organ and central brain of the Atlantic salmon [126]. Homologs have been described in carp [127] and smelt fish [128]. The smelt fish has two isoforms, where expression involves alternate spicing of intron 4 that affects carboxy-terminal length and sequence [127, 128]. To date very little is known about VA-opsin other than that it can form a pigment when reconstituted *in vitro* with 11-*cis* retinal [125]. It will be interesting to find out if any of these final three opsin classes have the ability to activate a G-protein, and if so,

what class of G-protein they interact with. To date there have been no data to suggest this other than their sequence homology to other opsins. If they do act in a signal transduction pathway, there is still a large amount of research needed to illuminate their roles as well as the downstream targets of such signaling pathways.

Conclusions and future directions

As we have come to see, the retina plays host to many light-dependent processes. Our understanding of these processes at a molecular and biochemical level has been rapidly advance by studies of the photopigments. Although the field is relatively mature, many interesting questions remain. Some of the most pressing questions surround the active meta-II structure of rhodopsin. The crystallization of the dark state provided an invaluable tool in the study of photopigments and GPCRs as a whole; however, we will not fully understand the activation processes occurring on a molecular level until an active crystal structure is solved. This structure is needed to provide an understanding of what conformational changes ultimately occur. Those changes can be used to further increase our understanding of the interactions that occur between the photopigment and other interacting partners. In addition, such a structure would be of great value to the pharmaceutical industry for use in drug design for a multitude of GPCRs.

The question of the higher-order structure of vertebrate photopigments is also of interest to provide insight into intermolecular mechanisms of interactions with arrestins, G-proteins, G-protein receptor kinases, and between neighboring receptors. This same question applies not only to the traditional rod and cone photopigments, but extends to studies of melanopsin, RGR, and peropsin. Do these other opsins dimerize or oligomerize? These questions are still hot topics, and advances in biophysical assays that can detect intermolecular binding such as florescence quenching assays may lead to greater understanding in this field.

Turning to the cone opsin classes, the evidence suggestive of rod and cone opsin structural differences is driving the need for crystal structures for members of the various cone opsin classes. Many studies on the cone opsin classes have been hampered by the lack of sufficient amounts of protein. To compound this problem, the cone opsin classes are less stable than rhodopsin and do not have as high yields when heterologously expressed. To reach the goal of a crystal structure of some of the cone opsins, advances must first be made in expression systems that will allow for isolation of sufficient supplies of protein.

Outside the traditional rod and cone opsins, biochemical analysis of melanopsin has become an active field. There have been many biochemical studies in heterologous systems, but the real interest lies in the *in vivo* signaling pathway through which melanopsin acts. In the retina, this work has been hampered by the low abundance of melanopsin-expressing cells in the ganglion cell layer and difficulty in isolating these cells without contamination from surrounding cells. Electrophysiological recordings coupled with pharmacological agents have started to provide putative components of the melanopsin-signaling cascade, but further work needs to be completed to find other interacting proteins in the signaling cascade. These types of study can answer questions on the similarity of the melanopsin signaling cascade to known invertebrate opsin cascades and may show a conserved pathway that has existed since the evolutionary split of the invertebrates and vertebrates.

Finally, the study of RGR, peropsin, and VA-opsin leaves many questions unanswered, since investigation of all of these opsins is still in its infancy. As RGR is the best studied of these three opsin classes, the suggestion that it may be involved as a regulator of the retinoid cycle provides the basis for several pressing questions. If this is true, at what level does the regulation occur and what is the biochemistry behind that regulation? Do the peropsins, which are coexpressed in the RPE, have a similar function as RGR or are they involved in other cellular physiology?

Seeking answers to questions like these will take us a long way toward understanding the light driven processes in the vertebrate retina. Such an understanding should provide valuable insight into the molecular basis of vision and vision-related disorders. It may also open the door to an understanding of other GPCR-mediated signal transduction systems by providing a model for the identification of common mechanisms in such systems. Together these questions can pave the way for the future of research in vision and phototransduction.

Acknowledgements. The authors thank, T. W. Cronin, M. L. Porter, M. T. Walker, and, J. R. Blasic for helpful discussions and editing assistance. This work was supported by NSF grant 0615569.

1 Gartner, W. (2000) Invertebrate visual pigments. In: Molecular Mechanisms in Visual Transduction, Stavenga, D. G., Degrip, W. J. and Pugh, E. N., Jr. Eds., vol. 3, pp. 297 – 388. Elsevier, Amsterdam.

2 Hur, E. M. and Kim, K. T. (2002) G protein-coupled receptor signalling and cross-talk: achieving rapidity and specificity. Cell Signal. 14, 397 – 405.

3 Fredriksson, R. and Schioth, H. B. (2005) The repertoire of G-protein-coupled receptors in fully sequenced genomes. Mol. Pharmacol. 67, 1414 – 1425.
4 Dahl, S. G. and Sylte, I. (2005) Molecular modelling of drug targets: the past, the present and the future. Basic Clin. Pharmacol. Toxicol. 96, 151 – 155.
5 Hopkins, A. L. and Groom, C. R. (2002) The druggable genome. Nat. Rev. Drug. Discov. 1, 727 – 730.
6 Ovchinnikov Yu, A. (1982) Rhodopsin and bacteriorhodopsin: structure-function relationships. FEBS Lett. 148, 179 – 191.
7 Hargrave, P. A., McDowell, J. H., Curtis, D. R., Wang, J. K., Juszczak, E., Fong, S. L., Rao, J. K. and Argos, P. (1983) The structure of bovine rhodopsin. Biophys. Struct. Mech. 9, 235 – 244.
8 Nathans, J. and Hogness, D. S. (1983) Isolation, sequence analysis, and intron-exon arrangement of the gene encoding bovine rhodopsin. Cell 34, 807 – 814.
9 Nathans, J. and Hogness, D. S. (1984) Isolation and nucleotide sequence of the gene encoding human rhodopsin. Proc. Natl. Acad. Sci. USA 81, 4851 – 4855.
10 Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M. and Miyano, M. (2000) Crystal structure of rhodopsin: G protein-coupled receptor. Science 289, 739 – 745.
11 Teller, D. C., Okada, T., Behnke, C. A., Palczewski, K. and Stenkamp, R. E. (2001) Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs). Biochemistry 40, 7761 – 7772.
12 Okada, T., Fujiyoshi, Y., Silow, M., Navarro, J., Landau, E. M. and Shichida, Y. (2002) Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. Proc. Natl. Acad. Sci. USA 99, 5982 – 5987.
13 Okada, T., Sugihara, M., Bondar, A. N., Elstner, M., Entel, P. and Buss, V. (2004) The retinal conformation and its environment in rhodopsin in light of a new 2.2 A crystal structure. J. Mol. Biol. 342, 571 – 583.
14 Stenkamp, R. E., Filipek, S., Driessen, C. A., Teller, D. C. and Palczewski, K. (2002) Crystal structure of rhodopsin: a template for cone visual pigments and other G protein-coupled receptors. Biochim. Biophys. Acta 1565, 168 – 182.
15 Bownds, D. (1967) Site of attachment of retinal in rhodopsin. Nature 216, 1178 – 1181.
16 Wang, J. K., McDowell, J. H. and Hargrave, P. A. (1980) Site of attachment of 11-cis-retinal in bovine rhodopsin. Biochemistry 19, 5111 – 5117.
17 Zhukovsky, E. A. and Oprian, D. D. (1989) Effect of carboxylic acid side chains on the absorption maximum of visual pigments. Science 246, 928 – 930.
18 Sakmar, T. P., Franke, R. R. and Khorana, H. G. (1989) Glutamic acid-113 serves as the retinylidene Schiff base counterion in bovine rhodopsin. Proc. Natl. Acad. Sci. USA 86, 8309 – 8313.
19 Nathans, J. (1990) Determinants of visual pigment absorbance: identification of the retinylidene Schiff's base counterion in bovine rhodopsin. Biochemistry 29, 9746 – 9752.
20 Cowing, J. A., Poopalasundaram, S., Wilkie, S. E., Robinson, P. R., Bowmaker, J. K. and Hunt, D. M. (2002) The molecular mechanism for the spectral shifts between vertebrate ultraviolet- and violet-sensitive cone visual pigments. Biochem. J. 367, 129 – 135.
21 Kusnetzow, A. K., Dukkipati, A., Babu, K. R., Ramos, L., Knox, B. E. and Birge, R. R. (2004) Vertebrate ultraviolet visual pigments: protonation of the retinylidene Schiff base and a counterion switch during photoactivation. Proc. Natl. Acad. Sci. USA 101, 941 – 946.
22 Shi, Y., Radlwimmer, F. B. and Yokoyama, S. (2001) Molecular genetics and the evolution of ultraviolet vision in vertebrates. Proc. Natl. Acad. Sci. USA 98, 11731 – 11736.
23 Chabre, M. and le Maire, M. (2005) Monomeric G-protein-coupled receptor as a functional unit. Biochemistry 44, 9395 – 9403.
24 Fotiadis, D., Liang, Y., Filipek, S., Saperstein, D. A., Engel, A. and Palczewski, K. (2003) Atomic-force microscopy: Rhodopsin dimers in native disc membranes. Nature 421, 127 – 128.
25 Kota, P., Reeves, P. J., Rajbhandary, U. L. and Khorana, H. G. (2006) Opsin is present as dimers in COS1 cells: identification of amino acids at the dimeric interface. Proc. Natl. Acad. Sci. USA 103, 3054 – 3059.
26 Mansoor, S. E., Palczewski, K. and Farrens, D. L. (2006) Rhodopsin self-associates in asolectin liposomes. Proc. Natl. Acad. Sci. USA 103, 3060 – 3065.
27 Jastrzebska, B., Fotiadis, D., Jang, G. F., Stenkamp, R. E., Engel, A. and Palczewski, K. (2006) Functional and structural characterization of rhodopsin oligomers. J. Biol. Chem. 281, 11917 – 11922.
28 Jastrzebska, B., Maeda, T., Zhu, L., Fotiadis, D., Filipek, S., Engel, A., Stenkamp, R. E. and Palczewski, K. (2004) Functional characterization of rhodopsin monomers and dimers in detergents. J. Biol. Chem. 279, 54663 – 54675.
29 Fotiadis, D., Jastrzebska, B., Philippsen, A., Muller, D. J., Palczewski, K. and Engel, A. (2006) Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. Curr. Opin. Struct. Biol. 16, 252 – 259.
30 Rodbell, M. (1980) The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284, 17 – 22.
31 Carrillo, J. J., Pediani, J. and Milligan, G. (2003) Dimers of class A G protein-coupled receptors function via agonist-mediated trans-activation of associated G proteins. J. Biol. Chem. 278, 42578 – 42587.
32 Angers, S., Salahpour, A. and Bouvier, M. (2001) Biochemical and biophysical demonstration of GPCR oligomerization in mammalian cells. Life Sci. 68, 2243 – 2250.
33 Salahpour, A., Angers, S., Mercier, J. F., Lagace, M., Marullo, S. and Bouvier, M. (2004) Homodimerization of the beta2-adrenergic receptor as a prerequisite for cell surface targeting. J. Biol. Chem. 279, 33390 – 33397.
34 Whorton, M. R., Bokoch, M. P., Rasmussen, S. G., Huang, B., Zare, R. N., Kobilka, B. and Sunahara, R. K. (2007) A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. Proc. Natl. Acad. Sci. USA 104, 7682 – 7687.
35 Park, P. S. and Wells, J. W. (2003) Monomers and oligomers of the M2 muscarinic cholinergic receptor purified from Sf9 cells. Biochemistry 42, 12960 – 12971.
36 Ebrey, T. and Koutalos, Y. (2001) Vertebrate photoreceptors. Prog. Retin. Eye Res. 20, 49 – 94.
37 Hisatomi, O. and Tokunaga, F. (2002) Molecular evolution of proteins involved in vertebrate phototransduction. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 133, 509 – 522.
38 Okano, T., Kojima, D., Fukada, Y., Shichida, Y. and Yoshizawa, T. (1992) Primary structures of chicken cone visual pigments: vertebrate rhodopsins have evolved out of cone visual pigments. Proc. Natl. Acad. Sci. USA 89, 5932 – 5936.
39 Baylor, D. A., Lamb, T. D. and Yau, K. W. (1979) Responses of retinal rods to single photons. J. Physiol. 288, 613 – 634.
40 Kuwayama, S., Imai, H., Hirano, T., Terakita, A. and Shichida, Y. (2002) Conserved proline residue at position 189 in cone visual pigments as a determinant of molecular properties different from rhodopsins. Biochemistry 41, 15245 – 15252.
41 Imai, H., Kojima, D., Oura, T., Tachibanaki, S., Terakita, A. and Shichida, Y. (1997) Single amino acid residue as a functional determinant of rod and cone visual pigments. Proc. Natl. Acad. Sci. USA 94, 2322 – 2326.
42 Wang, Q., Schoenlein, R. W., Peteanu, L. A., Mathies, R. A. and Shank, C. V. (1994) Vibrationally coherent photochem-

istry in the femtosecond primary event of vision. Science 266, 422 – 424.
43 Altenbach, C., Yang, K., Farrens, D. L., Farahbakhsh, Z. T., Khorana, H. G. and Hubbell, W. L. (1996) Structural features and light-dependent changes in the cytoplasmic interhelical E-F loop region of rhodopsin: a site-directed spin-labeling study. Biochemistry 35, 12470 – 12478.
44 Farrens, D. L., Altenbach, C., Yang, K., Hubbell, W. L. and Khorana, H. G. (1996) Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 274, 768 – 770.
45 Janz, J. M. and Farrens, D. L. (2004) Rhodopsin activation exposes a key hydrophobic binding site for the transducin alpha-subunit C terminus. J. Biol. Chem. 279, 29767 – 29773.
46 Nakamichi, H. and Okada, T. (2006) Crystallographic analysis of primary visual photochemistry. Angew. Chem. Int. Ed. Engl. 45, 4270 – 4273.
47 Nakamichi, H. and Okada, T. (2006) Local peptide movement in the photoreaction intermediate of rhodopsin. Proc. Natl. Acad. Sci. USA 103, 12729 – 12734.
48 Ruprecht, J. J., Mielke, T., Vogel, R., Villa, C. and Schertler, G. F. (2004) Electron crystallography reveals the structure of metarhodopsin, I. EMBO, J. 23, 3609 – 3620.
49 Salom, D., Lodowski, D. T., Stenkamp, R. E., Le Trong, I., Golczak, M., Jastrzebska, B., Harris, T., Ballesteros, J. A. and Palczewski, K. (2006) Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. Proc. Natl. Acad. Sci. USA 103, 16123 – 16128.
50 Jager, F., Fahmy, K., Sakmar, T. P. and Siebert, F. (1994) Identification of glutamic acid 113 as the Schiff base proton acceptor in the metarhodopsin II photointermediate of rhodopsin. Biochemistry 33, 10878 – 10882.
51 Robinson, P. R., Cohen, G. B., Zhukovsky, E. A. and Oprian, D. D. (1992) Constitutively active mutants of rhodopsin. Neuron 9, 719 – 725.
52 Yan, E. C., Kazmi, M. A., Ganim, Z., Hou, J. M., Pan, D., Chang, B. S., Sakmar, T. P. and Mathies, R. A. (2003) Retinal counterion switch in the photoactivation of the G protein-coupled receptor rhodopsin. Proc. Natl. Acad. Sci. USA 100, 9262 – 9267.
53 Ludeke, S., Beck, M., Yan, E. C., Sakmar, T. P., Siebert, F. and Vogel, R. (2005) The role of Glu181 in the photoactivation of rhodopsin. J. Mol. Biol. 353, 345 – 356.
54 Wang, Z., Asenjo, A. B. and Oprian, D. D. (1993) Identification of the Cl(–)-binding site in the human red and green color vision pigments. Biochemistry 32, 2125 – 2130.
55 Sun, H., Macke, J. P. and Nathans, J. (1997) Mechanisms of spectral tuning in the mouse green cone pigment. Proc. Natl. Acad. Sci. USA 94, 8860 – 8865.
56 Flamarique, I. N. (2005) Temporal shifts in visual pigment absorbance in the retina of Pacific salmon. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. 191, 37 – 49.
57 Hunt, D. M., Carvalho, L. D., Cowing, J. A., Parry, J. W., Wilkie, S. E., Davies, W. L. and Bowmaker, J. K. (2006) Spectral tuning of shortwave-sensitive visual pigments in vertebrates. Photochem. Photobiol. 832, 3035 – 310.
58 Yokoyama, S., Radlwimmer, F. B. and Blow, N. S. (2000) Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change. Proc. Natl. Acad. Sci. USA 97, 7366 – 7371.
59 Wilkie, S. E., Robinson, P. R., Cronin, T. W., Poopalasundaram, S., Bowmaker, J. K. and Hunt, D. M. (2000) Spectral tuning of avian violet- and ultraviolet-sensitive visual pigments. Biochemistry 39, 7895 – 7901.
60 Fasick, J. I., Applebury, M. L. and Oprian, D. D. (2002) Spectral tuning in the mammalian short-wavelength sensitive cone pigments. Biochemistry 41, 6860 – 6865.
61 Yokoyama, S. and Shi, Y. (2000) Genetics and evolution of ultraviolet vision in vertebrates. FEBS Lett. 486, 167 – 172.
62 Cowing, J. A., Poopalasundaram, S., Wilkie, S. E., Bowmaker, J. K. and Hunt, D. M. (2002) Spectral tuning and evolution of short wave-sensitive cone pigments in cottoid fish from Lake Baikal. Biochemistry 41, 6019 – 6025.
63 Takahashi, Y. and Ebrey, T. G. (2003) Molecular basis of spectral tuning in the newt short wavelength sensitive visual pigment. Biochemistry 42, 6025 – 6034.
64 Yokoyama, S. and Tada, T. (2003) The spectral tuning in the short wavelength-sensitive type 2 pigments. Gene 306, 91 – 98.
65 Chinen, A., Matsumoto, Y. and Kawamura, S. (2005) Spectral differentiation of blue opsins between phylogenetically close but ecologically distant goldfish and zebrafish. J. Biol. Chem. 280, 9460 – 9466.
66 Nathans, J., Thomas, D. and Hogness, D. S. (1986) Molecular genetics of human color vision: the genes encoding blue, green, and red pigments. Science 232, 193 – 202.
67 Neitz, M., Neitz, J. and Jacobs, G. H. (1991) Spectral tuning of pigments underlying red-green color vision. Science 252, 971 – 974.
68 Asenjo, A. B., Rim, J. and Oprian, D. D. (1994) Molecular determinants of human red/green color discrimination. Neuron 12, 1131 – 1138.
69 Fasick, J. I., Cronin, T. W., Hunt, D. M. and Robinson, P. R. (1998) The visual pigments of the bottlenose dolphin (*Tursiops truncatus*). Vis. Neurosci. 15, 643 – 651.
70 Chinen, A., Matsumoto, Y. and Kawamura, S. (2005) Reconstitution of ancestral green visual pigments of zebrafish and molecular mechanism of their spectral differentiation. Mol. Biol. Evol. 22, 1001 – 1010.
71 Kojima, D., Okano, T., Fukada, Y., Shichida, Y., Yoshizawa, T. and Ebrey, T. G. (1992) Cone visual pigments are present in gecko rod cells. Proc. Natl. Acad. Sci. USA 89, 6841 – 6845.
72 Ma, J., Znoiko, S., Othersen, K. L., Ryan, J. C., Das, J., Isayama, T., Kono, M., Oprian, D. D., Corson, D. W., Cornwall, M. C., Cameron, D. A., Harosi, F. I., Makino, C. L. and Crouch, R. K. (2001) A visual pigment expressed in both rod and cone photoreceptors. Neuron 32, 451 – 461.
73 Kawamura, S. and Yokoyama, S. (1997) Expression of visual and nonvisual opsins in American chameleon. Vis. Res. 37, 1867 – 1871.
74 Wald, G., Brown, P. K. and Smith, P. H. (1955) Iodopsin. J. Gen. Physiol. 38, 623 – 681.
75 Kawamura, S. and Yokoyama, S. (1998) Functional characterization of visual and nonvisual pigments of American chameleon (*Anolis carolinensis*). Vis. Res. 38, 37 – 44.
76 Oprian, D. D., Asenjo, A. B., Lee, N. and Pelletier, S. L. (1991) Design, chemical synthesis, and expression of genes for the three human color vision pigments. Biochemistry 30, 11367 – 11372.
77 Parry, J. W., Poopalasundaram, S., Bowmaker, J. K. and Hunt, D. M. (2004) A novel amino acid substitution is responsible for spectral tuning in a rodent violet-sensitive visual pigment. Biochemistry 43, 8014 – 8020.
78 Darden, A. G., Wu, B. X., Znoiko, S. L., Hazard, E. S., 3rd, Kono, M., Crouch, R. K. and Ma, J. X. (2003) A novel *Xenopus* SWS2, P434 visual pigment: structure, cellular location, and spectral analyses. Mol. Vis. 9, 191 – 199.
79 Okano, T., Fukada, Y., Artamonov, I. D. and Yoshizawa, T. (1989) Purification of cone visual pigments from chicken retina. Biochemistry 28, 8848 – 8856.
80 Das, J., Crouch, R. K., Ma, J. X., Oprian, D. D. and Kono, M. (2004) Role of the 9-methyl group of retinal in cone visual pigments. Biochemistry 43, 5532 – 5538.
81 Kefalov, V. J., Estevez, M. E., Kono, M., Goletz, P. W., Crouch, R. K., Cornwall, M. C. and Yau, K. W. (2005) Breaking the covalent bond – a pigment property that contributes to desensitization in cones. Neuron 46, 879 – 890.
82 Nickle, B., Wilkie, S. E., Cowing, J. A., Hunt, D. M. and Robinson, P. R. (2006) Vertebrate opsins belonging to different classes vary in constitutively active properties resulting from salt-bridge mutations. Biochemistry 45, 7307 – 7313.

83 Rao, V. R., Cohen, G. B. and Oprian, D. D. (1994) Rhodopsin mutation G90D and a molecular mechanism for congenital night blindness. Nature 367, 639 – 642.

84 Dryja, T. P., Berson, E. L., Rao, V. R. and Oprian, D. D. (1993) Heterozygous missense mutation in the rhodopsin gene as a cause of congenital stationary night blindness. Nat. Genet. 4, 280 – 283.

85 Gross, A. K., Rao, V. R. and Oprian, D. D. (2003) Characterization of rhodopsin congenital night blindness mutant T94I. Biochemistry 42, 2009 – 2015.

86 Madabushi, S., Gross, A. K., Philippi, A., Meng, E. C., Wensel, T. G. and Lichtarge, O. (2004) Evolutionary trace of G protein-coupled receptors reveals clusters of residues that determine global and class-specific functions. J. Biol. Chem. 279, 8126 – 8132.

87 Han, M., Smith, S. O. and Sakmar, T. P. (1998) Constitutive activation of opsin by mutation of methionine 257 on transmembrane helix 6. Biochemistry 37, 8253 – 8261.

88 Cohen, G. B., Yang, T., Robinson, P. R. and Oprian, D. D. (1993) Constitutive activation of opsin: influence of charge at position 134 and size at position 296. Biochemistry 32, 6111 – 6115.

89 Isayama, T., Chen, Y., Kono, M., Degrip, W. J., Ma, J. X., Crouch, R. K. and Makino, C. L. (2006) Differences in the pharmacological activation of visual opsins. Vis. Neurosci. 23, 899 – 908.

90 Corson, D. W., Kefalov, V. J., Cornwall, M. C. and Crouch, R. K. (2000) Effect of 11-cis 13-demethylretinal on phototransduction in bleach-adapted rod and cone photoreceptors. J. Gen. Physiol. 116, 283 – 297.

91 Kefalov, V. J., Carter Cornwall, M. and Crouch, R. K. (1999) Occupancy of the chromophore binding site of opsin activates visual transduction in rod photoreceptors. J. Gen. Physiol. 113, 491 – 503.

92 Jin, J., Crouch, R. K., Corson, D. W., Katz, B. M., MacNichol, E. F. and Cornwall, M. C. (1993) Noncovalent occupancy of the retinal-binding pocket of opsin diminishes bleaching adaptation of retinal cones. Neuron 11, 513 – 522.

93 Estevez, M. E., Ala-Laurila, P., Crouch, R. K. and Cornwall, M. C. (2006) Turning cones off: the role of the 9-methyl group of retinal in red cones. J. Gen. Physiol. 128, 671 – 685.

94 Ablonczy, Z., Kono, M., Knapp, D. R. and Crouch, R. K. (2006) Palmitylation of cone opsins. Vis. Res. 46, 4493 – 4501.

95 Rieke, F. and Baylor, D. A. (2000) Origin and functional impact of dark noise in retinal cones. Neuron 26, 181 – 186.

96 Kefalov, V., Fu, Y., Marsh-Armstrong, N. and Yau, K. W. (2003) Role of visual pigment properties in rod and cone phototransduction. Nature 425, 526 – 531.

97 Berson, D. M. (2007) Phototransduction in ganglion-cell photoreceptors. Pflugers Arch. A 54, 849 – 855.

98 Peirson, S. and Foster, R. G. (2006) Melanopsin: another way of signaling light. Neuron 49, 331 – 339.

99 Nayak, S. K., Jegla, T. and Panda, S. (2007) Role of a novel photopigment, melanopsin, in behavioral adaptation to light. Cell. Mol. Life Sci. 64, 144 – 154.

100 Hattar, S., Liao, H. W., Takao, M., Berson, D. M. and Yau, K. W. (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295, 1065 – 1070.

101 Provencio, I., Jiang, G., De Grip, W. J., Hayes, W. P. and Rollag, M. D. (1998) Melanopsin: an opsin in melanophores, brain, and eye. Proc. Natl. Acad. Sci. USA 95, 340 – 345.

102 Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F. and Rollag, M. D. (2000) A novel human opsin in the inner retina. J. Neurosci. 20, 600 – 605.

103 Semo, M., Munoz Llamosas, M., Foster, R. G. and Jeffery, G. (2005) Melanopsin (Opn4) positive cells in the cat retina are randomly distributed across the ganglion cell layer. Vis. Neurosci. 22, 111 – 116.

104 Frigato, E., Vallone, D., Bertolucci, C. and Foulkes, N. S. (2006) Isolation and characterization of melanopsin and pinopsin expression within photoreceptive sites of reptiles. Naturwissenschaften 93, 379 – 385.

105 Chaurasia, S. S., Rollag, M. D., Jiang, G., Hayes, W. P., Haque, R., Natesan, A., Zatz, M., Tosini, G., Liu, C., Korf, H. W., Iuvone, P. M. and Provencio, I. (2005) Molecular cloning, localization and circadian expression of chicken melanopsin (Opn4): differential regulation of expression in pineal and retinal cell types. J. Neurochem. 92, 158 – 170.

106 Hannibal, J., Hindersson, P., Nevo, E. and Fahrenkrug, J. (2002) The circadian photopigment melanopsin is expressed in the blind subterranean mole rat, *Spalax*. Neuroreport 13, 1411 – 1414.

107 Bellingham, J., Chaurasia, S. S., Melyan, Z., Liu, C., Cameron, M. A., Tarttelin, E. E., Iuvone, P. M., Hankins, M. W., Tosini, G. and Lucas, R. J. (2006) Evolution of melanopsin photoreceptors: discovery and characterization of a new melanopsin in nonmammalian vertebrates. PLoS Biol. 4, e254.

108 Terakita, A., Koyanagi, M., Tsukamoto, H., Yamashita, T., Miyata, T. and Shichida, Y. (2004) Counterion displacement in the molecular evolution of the rhodopsin family. Nat Struct. Mol. Biol. 11, 284 – 289.

109 Newman, L. A., Walker, M. T., Brown, R. L., Cronin, T. W. and Robinson, P. R. (2003) Melanopsin forms a functional short-wavelength photopigment. Biochemistry 42, 12734 – 12738.

110 Panda, S., Nayak, S. K., Campo, B., Walker, J. R., Hogenesch, J. B. and Jegla, T. (2005) Illumination of the melanopsin signaling pathway. Science 307, 600 – 604.

111 Qiu, X., Kumbalasiri, T., Carlson, S. M., Wong, K. Y., Krishna, V., Provencio, I. and Berson, D. M. (2005) Induction of photosensitivity by heterologous expression of melanopsin. Nature 433, 745 – 749.

112 Isoldi, M. C., Rollag, M. D., Castrucci, A. M. and Provencio, I. (2005) Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. Proc. Natl. Acad. Sci. USA 102, 1217 – 1221.

113 Melyan, Z., Tarttelin, E. E., Bellingham, J., Lucas, R. J. and Hankins, M. W. (2005) Addition of human melanopsin renders mammalian cells photoresponsive. Nature 433, 741 – 745.

114 Fu, Y., Zhong, H., Wang, M. H., Luo, D. G., Liao, H. W., Maeda, H., Hattar, S., Frishman, L. J. and Yau, K. W. (2005) Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. Proc. Natl. Acad. Sci. USA 102, 10339 – 10344.

115 Jiang, M., Pandey, S. and Fong, H. K. (1993) An opsin homologue in the retina and pigment epithelium. Invest. Ophthalmol. Vis. Sci. 34, 3669 – 3678.

116 Hao, W. and Fong, H. K. (1999) The endogenous chromophore of retinal G protein-coupled receptor opsin from the pigment epithelium. J. Biol. Chem. 274, 6085 – 6090.

117 Moiseyev, G., Chen, Y., Takahashi, Y., Wu, B. X. and Ma, J. X. (2005) RPE65 is the isomerohydrolase in the retinoid visual cycle. Proc. Natl. Acad. Sci. USA 102, 12413 – 12418.

118 Chen, P., Hao, W., Rife, L., Wang, X. P., Shen, D., Chen, J., Ogden, T., Van Boemel, G. B., Wu, L., Yang, M. and Fong, H. K. (2001) A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat. Genet. 28, 256 – 260.

119 Wenzel, A., Oberhauser, V., Pugh, E. N., Jr., Lamb, T. D., Grimm, C., Samardzija, M., Fahl, E., Seeliger, M. W., Reme, C. E. and von Lintig, J. (2005) The retinal G protein-coupled receptor (RGR) enhances isomerohydrolase activity independent of light. J. Biol. Chem. 280, 29874 – 29884.

120 Chen, P., Lee, T. D. and Fong, H. K. (2001) Interaction of 11-cis-retinol dehydrogenase with the chromophore of retinal G protein-coupled receptor opsin. J. Biol. Chem. 276, 21098 – 21104.

121 Sun, H., Gilbert, D. J., Copeland, N. G., Jenkins, N. A. and Nathans, J. (1997) Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. Proc. Natl. Acad. Sci. USA 94, 9893 – 9898.

122 Bellingham, J., Wells, D. J. and Foster, R. G. (2003) In silico characterisation and chromosomal localisation of human RRH (peropsin) – implications for opsin evolution. BMC Genom. 4, 3.
123 Koyanagi, M., Terakita, A., Kubokawa, K. and Shichida, Y. (2002) Amphioxus homologs of Go-coupled rhodopsin and peropsin having 11-cis- and all-trans-retinals as their chromophores. FEBS Lett. 531, 525 – 528.
124 Soni, B. G. and Foster, R. G. (1997) A novel and ancient vertebrate opsin. FEBS Lett. 406, 279 – 283.
125 Soni, B. G., Philp, A. R., Foster, R. G. and Knox, B. E. (1998) Novel retinal photoreceptors. Nature 394, 27 – 28.
126 Philp, A. R., Garcia-Fernandez, J. M., Soni, B. G., Lucas, R. J., Bellingham, J. and Foster, R. G. (2000) Vertebrate ancient (VA) opsin and extraretinal photoreception in the Atlantic salmon (*Salmo salar*). J. Exp. Biol. 203, 1925 – 1936.
127 Moutsaki, P., Bellingham, J., Soni, B. G., David-Gray, Z. K. and Foster, R. G. (2000) Sequence, genomic structure and tissue expression of carp (*Cyprinus carpio,* L.) vertebrate ancient (VA) opsin. FEBS Lett. 473, 316 – 322.
128 Minamoto, T. and Shimizu, I. (2002) A novel isoform of vertebrate ancient opsin in a smelt fish, *Plecoglossus altivelis.* Biochem. Biophys. Res. Commun. 290, 280 – 286.