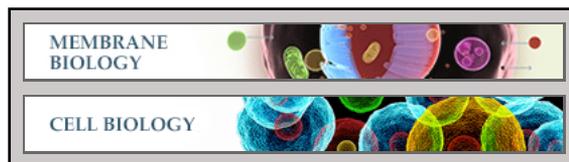


**Minireviews:**  
**Chemistry and Biology of Vision**

Krzysztof Palczewski

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# Chemistry and Biology of Vision\*

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Visual perception in humans occurs through absorption of electromagnetic radiation from 400 to 780 nm by photoreceptors in the retina. A photon of visible light carries a sufficient amount of energy to cause, when absorbed, a *cis,trans*-geometric isomerization of the 11-*cis*-retinal chromophore, a vitamin A derivative bound to rhodopsin and cone opsins of retinal photoreceptors. The unique biochemistry of these complexes allows us to reliably and reproducibly collect continuous visual information about our environment. Moreover, other nonconventional retinal opsins such as the circadian rhythm regulator melanopsin also initiate light-activated signaling based on similar photochemistry.

Our visual system operates over an extremely broad dynamic range, detecting variations in light intensity of over 8 orders of magnitude, from single photons to more than one-hundred million photons/s (1). This dynamic range is attributed to adaptation processes in rods and cones, with the remainder arising from pupil contractions, processes within inter-retinal neurons, and the production rate of visual chromophore. The rod cell saturates at several thousand photons/s, whereas cones continue to function at several millionfold higher light intensities (2). The central foundation of our vision is the photochemical isomerization of the vitamin A-derived visual chromophore (11-*cis*-retinal) from its *cis*- to *trans*-configuration. A single photon of light isomerizes a single 11-*cis*-retinal bound to rod or cone opsins. A photon carries ~2.5 eV energy (at 500 nm), but only a fraction (1.5 eV/opsin molecule) is utilized to elicit changes in retinal conformation and subsequently protein conformational changes, whereas the remaining energy is dissipated. The high excess of energy ensures that photoisomerization occurs with high fidelity (3). To renew a functional receptor after photoactivation, the chromophore must be regenerated metabolically through a series of enzymatic processes that include isomerization and oxidation of all-*trans*-retinyl ester to 11-*cis*-retinal. Enzymatic re-isomerization of all-*trans*-retinoid to 11-*cis*-retinoid requires only 3–4 kcal/mol energy (or 0.13–0.17 eV/molecule) (4).

The retina is a layered sensory organ containing all necessary functional and structural proteins to support human vision. How this remarkable tissue develops and operates over such an incredible dynamic range and how retinoids are recycled are some

of the most stirring questions in biology. Blindness is one of the most feared and debilitating illnesses affecting humans, and without a detailed understanding of the basic events in vision, rational approaches to treating blinding diseases will not be possible. With the current methodology, it is now possible to identify all components of the retina and trace mutations to retinopathies.

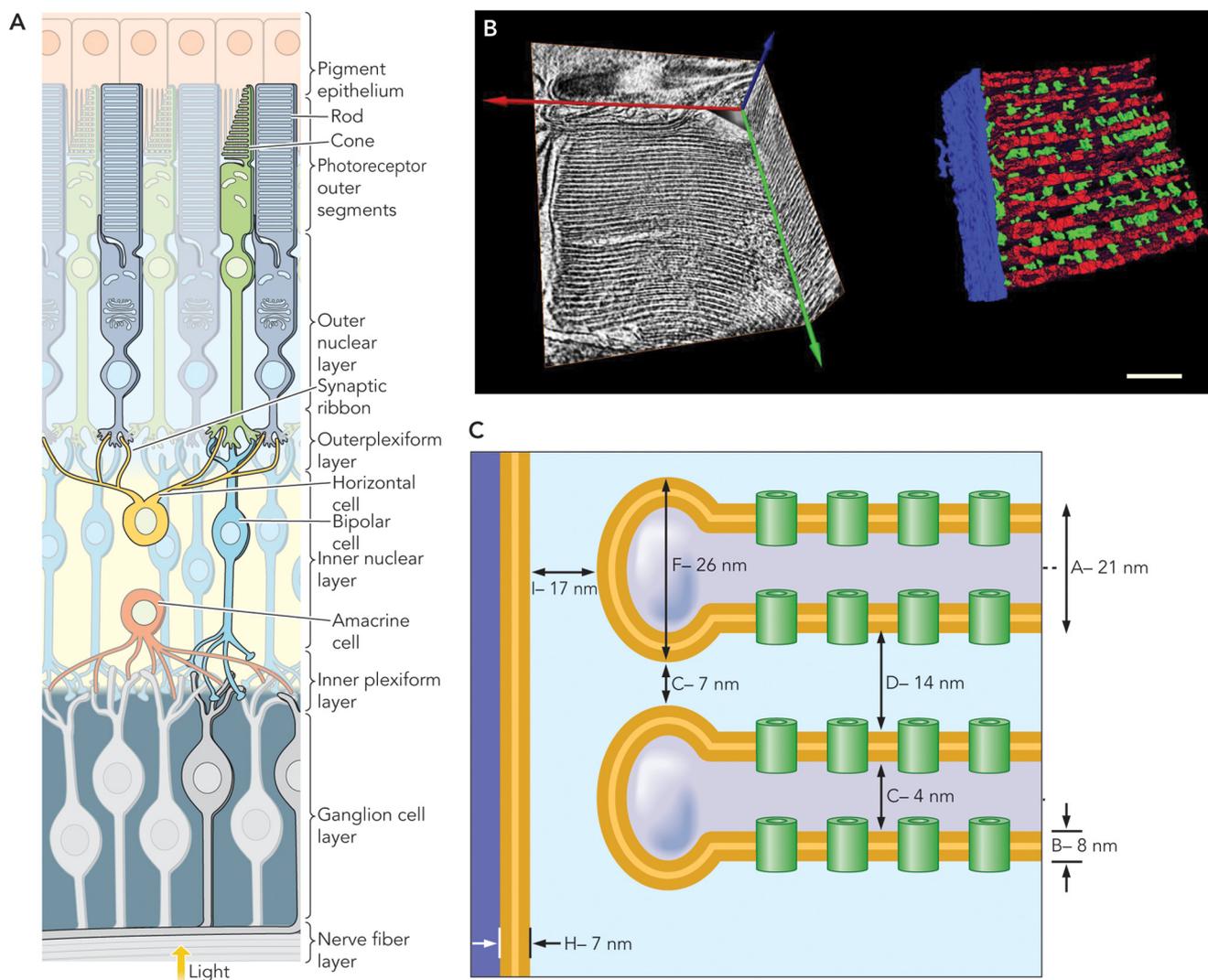
## Complete Mouse Transcriptome in Eye and Retina

The eye is a complex organ composed of specific tissues that carry out different functions to maintain continuous visual responsiveness. The main players are the cornea and lens in the front of the eye and the retina and retinal pigmented epithelium (RPE)<sup>2</sup> in the back. The primary light absorption events take place within the retina, a 0.24-mm thick tissue (mouse) composed of multiple cell layers (Fig. 1). Retinal development and maintenance, as well as light-sensitive visual functions, are highly regulated. Physical dissection of different ocular tissues followed by global analysis of their gene expression by massively parallel RNA sequencing (RNA-seq) allowed the assignment of a complete comprehensive transcriptome to the ocular tissue (5). The completeness of such analysis is an important prerequisite to understand the structure and physiology of the retina by identifying all players involved. Using RNA-seq of mature WT mouse ocular tissues (the retina and whole eye), we recently determined the complete composition of these transcriptomes (5). Retinal tissue yielded 13,406 unique transcripts, and as expected, many transcripts from WT retinal tissues had annotated functions that could be linked to specific metabolic processes or structural and regulatory functions (Fig. 2, upper) (5). In addition, analysis of WT whole eye tissues revealed a large number of genes with unknown functions that await further careful analysis (Fig. 2, lower). These studies complement and greatly expand earlier gene chip-based expression analyses in both accuracy and quantification (6, 7). This new depth of knowledge of the retinal transcriptome will facilitate large-scale analyses of the functional consequences of manipulating photoreceptor gene expression (*i.e.* using gene transfer by retinal electroporation) (8). In addition to protein-coding mRNAs, a large number of microRNAs (miRNAs) and other noncoding RNAs are expressed in the eye (9, 10). Together with many metabolites and dietary components, these RNAs regulate developmental and circadian control over the translation of proteins in each cell type. At least 78 miRNAs are preferentially expressed in the mouse retina from 689 identified miRNAs (miRBASE Sequence Database Release 13.0, March 10, 2009) (11), suggesting the importance of miRNAs in modulating gene expression profiles in retinal cells. Moreover, inactivation of Dicer (an essential RNase III endonuclease required for miRNA maturation) leads to progressive functional and structural degeneration of the mouse retina (12). Regulating miRNA levels could be an important approach to treat human retinal dis-

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<sup>2</sup> The abbreviations used are: RPE, retinal pigmented epithelium; miRNA, microRNA; OS, outer segment(s); ROS, rod outer segment(s); GPCR, G protein-coupled receptor.



**FIGURE 1. Structures of rod and cone OS and ROS internal membranes.** *A*, neuronal organization of a typical mammalian retina. A cross-sectional representation of rod and cone photoreceptors is presented, illustrating their connections to the RPE distally and to relaying cells (bipolar, horizontal, amacrine, and ganglion cells) proximally. The rod structure has a longer OS with membrane-enclosed disks tightly packed without connections to the plasma membrane. Cone disks are continuously connected with the plasma membrane. This figure was reprinted from Ref. 101 with permission. *B*, electron tomogram of vitrified ROS. The electron tomogram is represented in three orthogonal slices through the ROS volume. An *x-y* slice (*right*) and a *y-z* slice (*left*) display the high order and regular arrangement of stacked disks. *Red* represents the high concentrations of rhodopsin found in disk membranes; spacer structures (pillars) are colored *green*. Scale bar = 200 nm. *C*, blueprint of ROS. A schematic of a plasma membrane and two disks with measured distances between membrane components is shown. *Green cylinders* represent monomeric rhodopsin, which forms a larger cluster in native ROS. *B* and *C* were reprinted from Ref. 19 with permission.

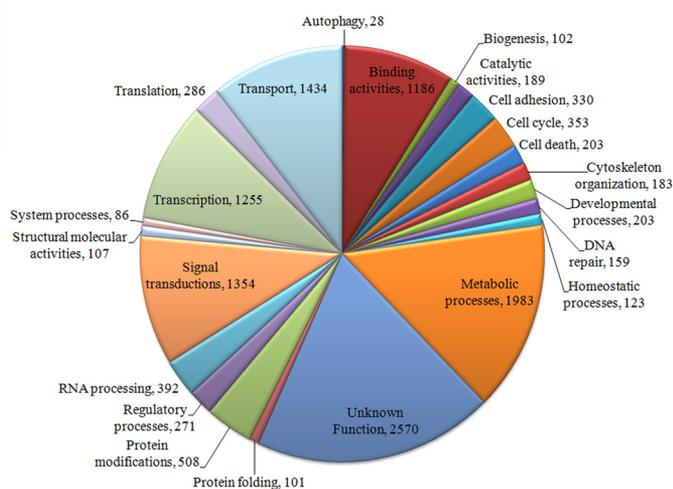
eases, as demonstrated in a mouse model of light-induced retinal degeneration (13).

### Photoreceptor Structure

Proteins involved in visual phototransduction are located predominantly in the photoreceptor outer segments (OS) of rods and cones. Photoreceptor OS of these highly differentiated neurons are actually specialized cilia (Fig. 1A). Structural studies of these cilia were first carried out with guinea pig and frog rod outer segments (ROS) (14, 15). More recently, mouse tissue has been favored because of the ease of genetic manipulation (5, 16).

The average mouse ROS length and diameter were estimated to be  $23.8 \pm 1.0 \mu\text{m}$  and  $1.22\text{--}1.32 \pm 0.12 \mu\text{m}$ , respectively (17). A mouse ROS contains  $\sim 800$  membranous disks stacked on top of each other (Fig. 1A). These internal disk membranes increase the total membrane surface area by  $\sim 1500$ -fold compared with

the plasma membrane surface alone (18), promoting a high density of the rod visual pigment rhodopsin. Cryo-electron tomography of vitrified mouse retina provided reliable three-dimensional morphological information about this structure (Fig. 1B) (19). Fig. 1C presents a diagram of this ROS structure with distances between different membrane components obtained from cryo-electron tomograms. Based on these and the abovementioned electron microscopy data, the ROS interior volume, including both the intradiskal and cytoplasmic space, is  $32 \times 10^{-12}$  ml, and the cytoplasm occupies  $10 \times 10^{-12}$  ml in the ROS (19). Thus, it is amazing that the cytoplasmic space used for phototransduction represents only  $\sim 30\%$  of the space inside a ROS, underscoring the importance of internal membrane structures in phototransduction. This phototransduction cascade occurs as catalytic processes on the interface of disk membranes and the cytoplasm (interfacial catalysis). Cryo-



GO term	Genes detected in WT eye
Signal Transductions	1,354
Phototransductions	23
Metabolic Processes	1,983
Retinoid Metabolism	24
Retina Cytoskeleton	237
Transport	1,434
Phagocytosis	43
Transcription/Translation	1,541
Unknown Function	2,570
Total Transcripts	13,406

**FIGURE 2. Transcriptome analysis of wild-type mouse eye.** RNA sequencing of WT mouse eye reveals the transcriptional landscape of this tissue and the precise quantification of transcripts present. A breakdown of assigned transcripts is presented along with the number of transcripts in each category. The *table* highlights key Gene Ontology (GO) term categories and sub-categories that relate to different aspects of visual processing. Notable are 2570 transcripts of unknown function of a total of 13,406 transcripts detected in WT eye, prospects for new avenues of vision research. Data shown are reprinted from Ref. 5 with permission.

electron tomograms also show spacers that keep the disks separate from one another and maintain appropriate distances between adjacent disks and the plasma membrane (19). Spacers consist of complexes of proteins with estimated molecular masses of  $\sim 500$  kDa distributed at a mean density of  $\sim 500$  molecules/ $\mu\text{m}^2$  throughout the disks (19). Intuitively, the presence of proteins responsible for maintaining this structure could be predicted because structural components are essential for maintaining the complex architecture of these fluid internal membranes. The intervening spacers are likely occupied solely or in part by glutamic acid-rich proteins and a membrane-bound retinal tetraspanin protein called peripherin/RDS (20).

Rhodopsin occupies  $\sim 50\%$  of the membrane volume within the disks of ROS (21). This high density of photoreceptor opsin could be needed to increase the probability of photon absorption. In addition, it appears that rhodopsin could play a critical structural role in establishing ROS morphology, as opsin knock-out mice form only small ROS appendices early in life, before the cells degenerate (22). The size of the ROS is dictated by the expression level of rhodopsin (17), as heterozygous knock-out mice for the opsin gene possess  $\sim 50\%$  smaller ROS (23), and overexpression of this protein leads to rod cell degeneration (24). Rhodopsin is not uniformly distributed throughout disks (25). For example, cryo-electron microscopy images

of vitrified unstained native mouse ROS reveal high density regions on the disk surface. This difference in density could arise only from an uneven distribution of rhodopsin, which is the main protein in these disks, representing  $>90\%$  of all disk proteins. Moreover, patches of disk membrane containing rows of rhodopsin dimers have been observed by atomic force microscopy, a finding supported by other biochemical methods summarized previously (3). Paracrystalline patches within carefully isolated fresh disks from photoreceptors of mouse retina, wherein the building blocks consist of rhodopsin dimers (26), imply functional significance in rhodopsin biosynthesis or function (27) and remain a topic of considerable interest (reviewed in Ref. 28). Interestingly, Corless *et al.* (29) found that crystalline structure is formed from visual pigments in cone cells when frog retinas are exposed to light.

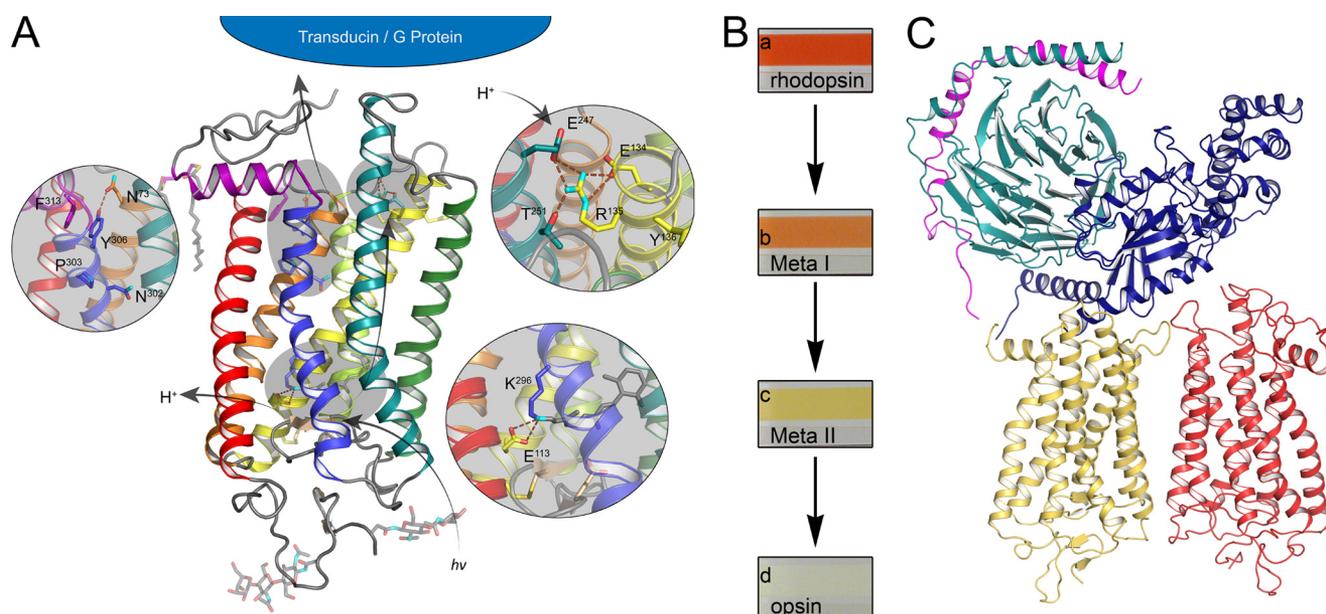
Because both the mouse rhodopsin level ( $\sim 520$  pmol/eye) (16, 17) and total cell number ( $6.4 \times 10^6$  rods) (30) can be measured precisely, rhodopsin is calculated to have a concentration of 4.62 mM in disk membranes and 8.23 mM with respect to the ROS cytoplasm. The density of rhodopsin in the disk membrane is estimated to be  $2.4 \times 10^4$  molecules/ $\mu\text{m}^2$  on average or up to  $\sim 3.4 \times 10^4$  molecules/ $\mu\text{m}^2$  in high density patches. Atomic force microscopy measurements yielded a density of 30,000–55,000 rhodopsin molecules/ $\mu\text{m}^2$  and  $\sim 10^8$  rhodopsin molecules/rod, partially organized in paracrystalline arrays (16, 26). As the whole retinal transcriptome has now been analyzed and the majority of the ROS proteome has been identified by mass spectrometry, attention is now focused on the interactions of these proteins, their effects on function, and their regulation. More structural studies are required to answer these questions.

### Structures of Phototransduction and Visual Cycle Components

Further molecular understanding of phototransduction inevitably focuses on the structures of phototransduction and retinoid cycle components and their complexes because the spatial organization of photoreceptor proteins underlies their functional ability to harvest light and generate a neuronal signal. Great progress has already been made by defining structures of a number of full-length proteins or fragments, either alone or in complex with effector proteins (see Ref. 31). A few interesting examples are listed below, but it is likely that more will be known in the near future about the structures of different components involved in this G protein-mediated process than about most other signal transduction systems in nature.

Structures of multiple forms of the G protein-coupled receptor (GPCR) rhodopsin (Fig. 3) (32–37), as well as various forms of G proteins (38–40), the receptor-capping protein arrestin (41), or likely G protein partners involved in intracellular translocation between photoreceptor compartments (42), have been determined (43). Rhodopsin has been extensively studied as a prototypical GPCR (3), and insights derived from comprehensive biochemical and biophysical studies of rhodopsin and its cognate G protein, transducin, have significantly improved our understanding of GPCR signaling in general (44).

An enhanced insight into the dynamics of rhodopsin activation and interaction with ligand and G protein has been obtained more recently by NMR techniques that show confor-



**FIGURE 3. Visualization of photoactivation and subsequent G protein activation.** *A*, structural representation of the photoactivation process. Upon adsorption of a photon of light, the bound inverse agonist 11-*cis*-retinal chromophore isomerizes to the all-*trans*-state. Through a series of small-scale changes in protein side chains and their interactions with bound water molecules, this initial signal is transmitted to the cytoplasmic surface 40 Å away, where it triggers nucleotide exchange on the heterotrimeric G protein transducin. Upon nucleotide exchange, transducin dissociates and activates downstream signaling events. *B*, photographic documentation of spectral changes in rhodopsin upon activation. Once rhodopsin in its dark 11-*cis*-retinal-bound state (*a*) is exposed to light, it immediately goes through a series of photointermediate states, including metarhodopsin I (*Meta I*; *b*), and eventually progressing to the Rho\* (metarhodopsin II (*Meta II*)) activated state (*c*). All images shown were taken upon exposure with standard room lighting (10 and 40 s). Upon treatment with hydroxylamine, the chromophore is hydrolyzed, resulting in a largely colorless solution (*d*). *C*, model of the G protein rhodopsin complex based on single-particle reconstruction of the negatively stained native entity. A model based on solved x-ray structures was built into constraints imposed by the map provided from single-particle analysis (62). This orientation of a G protein and its N and C termini is recapitulated only to the same degree by the  $\beta_2$ -adrenergic receptor-G<sub>s</sub>-nanobody structure (100).

mational flexibility of this receptor and the G protein upon activation (45–48). Several methods demonstrated that membrane proteins (49), including rhodopsin (50), contain integral ordered water molecules that play important roles in both structure and function. These water molecules could be key to the initial folding of these proteins as they insert into membranes, facilitating their assembly into functional entities, as well as playing roles in the activation process. Using radiolytic footprinting techniques, we found that water molecules are associated with highly conserved and functionally important residues (50). In all sub-3 Å resolution GPCR crystal structures determined to date, the observation of “conserved” waters in similar locations supports the notion that these waters are likely to be as important to receptor function as the conserved amino acid residues (32, 37, 51).

Key myristoylated Ca<sup>2+</sup>-binding proteins involved in phototransduction, namely guanylate cyclase-activating proteins (52), have been visualized at high resolution by NMR and crystallographic methods to reveal their internal architecture, but only in their Ca<sup>2+</sup>-bound forms (53–55). More advanced studies have been performed on another myristoylated photoreceptor protein called recoverin. In recoverin, Ca<sup>2+</sup> induces the N-terminal extrusion of a myristoyl group that interacts with a lipid membrane bilayer (56, 57). This transition, termed a calcium-myristoyl switch, could allow a protein to translocate from the cytoplasm to membranes in a calcium-dependent manner (56). In contrast, GCAP1 has its myristoylated group bound within a cavity formed by the polypeptide chain, but this

does not exclude the possibility that this acyl group is mobilized in complexes with targeted guanylate cyclases.

Other important structures of phototransduction proteins include rhodopsin kinase (GRK1) (58) and RGS-9 (regulator of G protein signaling 9), the latter alone or in complex with the activated  $\alpha$ -subunit of the photoreceptor G protein transducin and/or an inhibitory subunit of phosphodiesterase 6 (59, 60). These studies provide specific information about the termination of signal transduction on photoactivated rhodopsin and the activated G protein transducin.

In addition to high resolution crystal structures, complementary methods have proven to be informative about complex proteins that are not yet amenable to crystallographic approaches. Among these methods are cryo-electron microscopy and single-particle analysis. For example, single-particle analysis and modeling provided the first views of phosphodiesterase organization (60, 61) and of the complex of dimeric rhodopsin and heterotrimeric transducin (Fig. 3)(62). However, many additional proteins whose atomic level structural details are critical to understanding the regulation and precise mechanism of phototransduction continue to escape structural interrogation.

In addition to these functional receptors, enzymes, and structural proteins, the chemical transformation of retinoid metabolites, *i.e.* the retinoid cycle, is critical for proper visual function. The structure of retinoid isomerase RPE65, the key enzyme of this metabolic pathway, has been determined (63). This crystal structure reveals a seven-bladed  $\beta$ -propeller motif with single-strand extensions on blades VI and VII and a two-

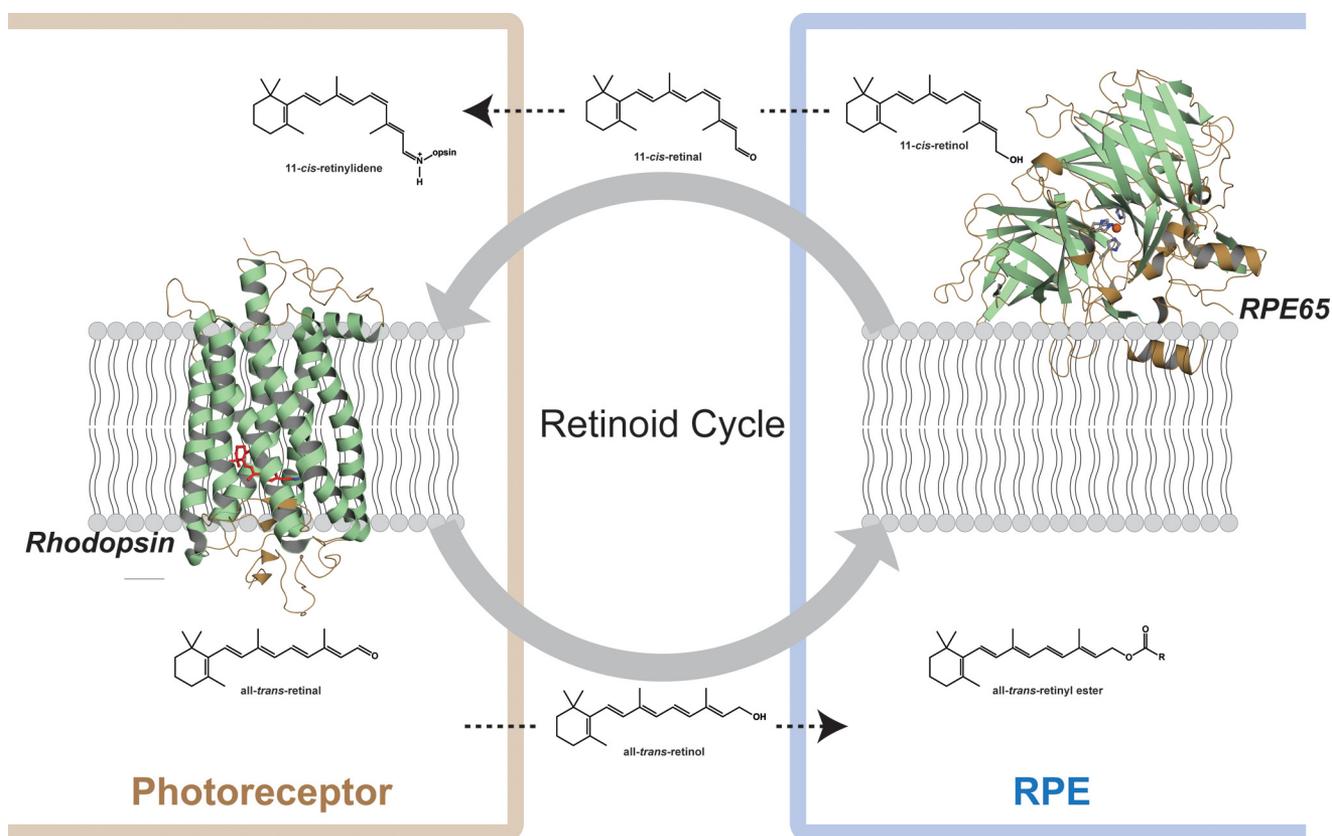


FIGURE 4. **Retinoid cycle regenerates visual pigment chromophore 11-*cis*-retinal.** In ROS, 11-*cis*-retinal is bound to opsin, forming rhodopsin (structure taken from Ref. 32). Absorption of a photon of light by rhodopsin causes photoisomerization of 11-*cis*-retinal to all-*trans*-retinal and productive signaling, eventually leading to release of all-*trans*-retinal from the chromophore-binding pocket of this opsin. All-*trans*-retinal is reduced to all-*trans*-retinol in a reaction catalyzed by NADPH-dependent all-*trans*-retinol dehydrogenases. Then, all-*trans*-retinol must diffuse into the adjacent RPE cell layer. This process is enabled by esterification of retinol with fatty acids in a reaction catalyzed by lecithin:retinol acyltransferase. In the RPE, these all-*trans*-retinyl esters tend to form intracellular structures called retinosomes. These esters serve as substrates for the RPE65 retinoid isomerase, which converts them to 11-*cis*-retinal (structure taken from Ref. 63), which is further oxidized back to 11-*cis*-retinal by retinol dehydrogenases. 11-*cis*-Retinal formed in the RPE diffuses back into the ROS because this reaction is virtually irreversible. This last step also completes the cycle by recombining 11-*cis*-retinal with opsin to form rhodopsin. The concept embodied in this figure was taken from Ref. 102.

strand extension on blade III (Fig. 4). This crystal structure provided a basis for understanding RPE65 membrane binding and enzyme-catalyzed retinoid isomerization. The structure of an important 11-*cis*-retinal-binding protein called cellular retinaldehyde-binding protein has a defined hydrophobic core that is responsible for sequestering 11-*cis*-retinal (64). Additionally, the structure of the R234W mutant of cellular retinaldehyde-binding protein, which is associated with Bothnia dystrophy and compromises visual pigment regeneration, identified the structural basis of that disease (4). Despite these advances, many questions remain with regard to the chemistry of the retinoid cycle.

### Regenerating Spent Chromophore: Retinoid Cycle

For the retina to remain responsive to light and maintain vision, 11-*cis*-retinal, which is isomerized to all-*trans*-retinal, must be continuously and efficiently regenerated (65). The time constant for rhodopsin regeneration is  $\sim 400$  s, and that for cone pigment regeneration is  $\sim 100$  s (66). The pioneering work of Kühne and Wald (67–69) laid the foundation for our current understanding of the photochemistry of vision. This process takes place in two cellular systems, retinal photoreceptors and the adjacent RPE (Fig. 4). From a chemical perspective, enzymatic isomerization of the chromophore appears to be a formi-

dable problem in regioselectivity. What regulates the specificity of the conversion of an all-*trans*-retinol to a specific 11-*cis*-isomer, when this molecule has only one functional group ( $-\text{OH}$ ) and several possibilities for single or multiple *cis*-isomerizations? This reaction also must occur continuously in a membranous/aqueous environment at body temperature. Moreover, the chromophore has other chemical properties that must be cleverly utilized. First, it contains five or six conjugated double bonds that allow light absorption in the visible range of the spectrum when conjugated with protein via a Schiff base. Second, as predicted by Pauling (70), the repulsion between two methyl groups makes 11-*cis*-retinal an unstable isomer, which encourages its isomerization to all-*trans*-retinal. Third, retinol easily forms one of the most stable carbocations in biology (71), allowing reshuffling of double bonds. Fourth, the isomerization of retinol has a relatively low activation energy (72). Three chemical mechanisms for isomerization of conjugated double-bond polyisoprenoids in biological systems have been identified. (a) A transition state carbocation product is formed from retinyl esters by alkyl cleavage; this carbocation then adjusts to an 11-*cis*-retinyl-like conformation to fit the active site of the enzyme, and double bonds are re-established when water is added (reviewed in Ref. 73). (b) A specific double bond is satu-

rated; the resulting transition state intermediate rotates, and the double bond is re-established by desaturation (74), as observed in tomato and *Arabidopsis* carotenoid isomerase, CRTISO (75). (c) An oxidative cleavage of carotenoids generates two retinal molecules in *cis*- and *trans*-forms, as in the case of NinaB (76). The structural explanation of these disparate dioxygenase and isomerase activities is critical to understanding the molecular mechanisms employed by this class of enzymes.

Remarkable progress has increased our knowledge of the retinoid cycle, expanding the work so brilliantly started over a century ago (Fig. 4). Several extensive reviews have provided a current update of this progress (4, 65, 66, 73). Although the cycle's unique photochemistry maintains vision, a high flux of photons by light exposure can lead to elevated levels of toxic retinal metabolites that accumulate throughout life and induce photoreceptor degeneration (77). Blocking the accumulation and action of these toxic intermediates and preventing such photoreceptor degeneration can alleviate major human visual diseases such as Stargardt disease and age-related macular degeneration.

As mentioned before, the broad dynamic range of our vision also raises the intriguing question of how much chromophore is consumed during one's lifetime. This estimate requires several assumptions (see, for example, Ref. 78), but the high sensitivity of the visual system, the large Avogadro number, and the low molecular mass of the chromophore suggest that a realistic exposure to light would equate to consumption of  $\sim 1$  mmol or only 284 mg of 11-*cis*-retinal during a life span!

Although it is unclear why cell types other than photoreceptors are employed for chromophore regeneration *per se*, the adjacent RPE is vital for maintaining photoreceptor architecture and function. Thus, two cellular compartments are primarily associated with the retinoid cycle, the photoreceptor OS of rods and cones and the closely associated RPE (65). RPE cells are essential for chromophore regeneration in both rods and cones (79, 80). In addition, cones appear to be supplemented with 11-*cis*-retinol by Müller cells (81, 82).

The outflow of retinoids from photoreceptors to the RPE requires RPE-expressed lecithin:retinol acyltransferase, which esterifies retinol with fatty acid to form retinyl esters (Fig. 4) (83). Because retinyl esters have a propensity to self-aggregate and they form oil droplet-like structures (84) called retinosomes in the RPE (85, 86), a flow of retinol out of rods and cones to the RPE would be expected based on thermodynamic considerations. The flow of 11-*cis*-retinal back from the RPE to rods and cones is governed by diffusion facilitated by an opsin "sink", *i.e.* the virtually irreversible reaction of opsins, especially rod opsin, with the chromophore that re-establishes the protonated Schiff base (21). The chromophore undergoes cyclic regeneration for each absorbed photon that causes isomerization of visual pigments, but occasionally retinoids condense with lipids or between themselves to form harmful byproducts of the retinoid cycle (87) that require photoreceptor cell regeneration.

### Photoreceptor Renewal

Rods and cones are extensively exposed to light in the presence of high oxygen levels throughout the life of an animal. This environment would inevitably lead to rapid retinal degeneration if this damaging process was not countered by protective

biochemical mechanisms and continuous renewal of these cells. Photoreceptor OS are particularly vulnerable to damage, as they contain highly reactive retinoids and high levels of unsaturated phospholipids such as esters of docosahexaenoic acid (88). However, as terminally differentiated post-mitotic cells, rods and cones do not divide. Thus, they have developed a unique mechanism of renewing photoreceptor OS content by shedding OS tips (Fig. 1A), which are then phagocytosed by the RPE. The apical processes of RPE cells encircle the distal 1/3–2/3 ends of photoreceptor OS (89). In the case of mammalian rods,  $\sim 10\%$  of ROS disks are shed every day, and the same amounts of membrane and protein components are produced at the base of ROS (89). This process necessitates the synthesis of up to  $10^7$  new rhodopsins/ROS/day, or a half-million rhodopsins/cell/h. In addition, the membrane support must also be synthesized at a rate of  $\sim 77$  cm<sup>2</sup>/day (18). This incredible load of GPCR and membrane synthesis strains the capacity of this system such that a minimal aberration could lead to disruption of photoreceptor OS disk renewal and related rod degeneration. When photoreceptor OS disk morphology and renewal are affected by mutations in the opsin genes, degeneration ensues, as is the case for the P23H mutation in the opsin gene (90) and over 100 other documented defects in production and transport of rhodopsin caused by rhodopsin gene mutations associated with retinitis pigmentosa (3).

Interestingly, photoreceptor OS disk recycling occurs in a circadian manner, with the peak of rod shedding in the morning and cone shedding after dark (91). The components involved in this recycling process are only partially known (Fig. 2). When ingested by the RPE, a photoreceptor OS is surrounded by the plasma membrane, producing a "phagosome." This structure undergoes a series of fusion events with endosomes and lysosomes, where several elements such as unsaturated lipids and retinoids are recycled back to photoreceptors and incorporated into new photoreceptor OS disks. Perhaps a number of genes with unknown function found in the total retina/RPE transcriptome will be shown to play roles in this process and its regulation (92).

Thus, photoreceptor cells absolutely require an extremely metabolically active RPE for their maintenance and survival. Genetic and age-related degenerative processes in RPE cells subsequently lead to degeneration of photoreceptors. For example, at the most metabolically active region of the retina around the fovea, each RPE cell must engulf  $4 \times 10^8$  rhodopsin molecules/day. It is likely that photoreceptors around the fovea place the greatest demand on the RPE, and as a consequence, this region is the first to degenerate during age-related macular degeneration, initially sparing the fovea.

### Melanopsin: An Invertebrate-like Opsin in Retina

Patients with inherited retinal degeneration retain light-dependent sleep pattern regulation even when almost all of their photoreceptors have degenerated, but this is not the case when eyes are missing or in advanced stages of glaucoma when the optic nerve that connects the retina to the brain is severed (93). Two possible explanations of this phenomenon are that (i) only a small number of surviving photoreceptors are needed to regulate the sleep cycle, and (ii) the retina contains other types of light-sensitive cells. Using physiological and molecular tech-

niques with the help of mouse genetics, it was unequivocally established that the retina contains a small subset of ganglion cells that are sensitive to light (94–96). These ganglion cells (intrinsically photosensitive retinal ganglion cells) express a rhodopsin-like molecule, melanopsin, with characteristics of an invertebrate opsin. Intrinsically photosensitive retinal ganglion cells consist of distinct subpopulations that innervate the hypothalamus to control circadian photoentrainment, and the olivary pretectal nucleus and other brain targets involved, e.g. pupillary, produce other specific light-induced functions (97). Use of melanopsin, which has a stably associated chromophore, rather than a member of the opsin subfamily that recycles the chromophore enzymatically is likely dictated by the need to avoid the canonical retinoid cycle. Ganglion cells are located too far from the RPE to be readily supplied with new chromophore. Thus, a bistable pigment evolutionarily conserved from amphioxus (protochordate) would represent a useful solution.

We do not yet have structural information on melanopsin, but it is similar to other invertebrate rhodopsins. Significant insight into the function of invertebrate rhodopsin has been derived from crystallographic studies. The 2.5 Å resolution crystal structure of an invertebrate rhodopsin (squid *Todarodes pacificus*) displays a prototypical seven-helical bundle structure with the chromophore located about two-thirds away from the cytoplasmic surface (98). Notably, invertebrate phototransduction uses a G<sub>q</sub>-type G protein that is involved in regulating inositol 1,4,5-trisphosphate production. In contrast to bovine rhodopsin, however, helices V and VI extend into the cytoplasmic medium and comprise part of the G protein recognition surface. It has been suggested that invertebrate rhodopsin can oscillate between *cis*- and *trans*-retinal conformations upon photon absorption by one of these forms (99). In physiological native membranes, invertebrate rhodopsin is organized in hexagonally packed microvillar membranes of photoreceptors, and in crystals, it is tightly associated in a dimeric form (98).

Extraordinary progress made over the last 2 decades has allowed the development of multiple approaches targeted at understanding blinding diseases. This marriage of basic and translational investigation exemplifies the highest standard of current progress in biology.

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