

## CHAPTER

## 51

## Molecular Biology of Vision

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## OUTLINE

<b>Structure and Development of the Visual System</b>	
The visual system is composed of unique structures optimized for collection, detection and processing of visual information	
The retina is composed of highly organized neuronal sublayers	
The ganglion cell axons of the optic nerve carry visual signals from the retina to the brain	
The eye develops as an outcropping of the developing brain	
<b>Photoreceptors and Phototransduction</b>	
Photoreceptors are polarized cells, with specialized primary cilia, outer segments, devoted to phototransduction	
Phototransduction consists of a highly amplified cascade of light-triggered changes in protein conformation, and changes in interactions of proteins with one another and with guanine nucleotides	
Recovery of the dark current after light stimulation is a multistep process mediated by $\text{Ca}^{2+}$ and proteins exerting negative regulation	
Cone phototransduction uses mechanisms and molecules similar to those in rods, but is optimized for speed rather than sensitivity	

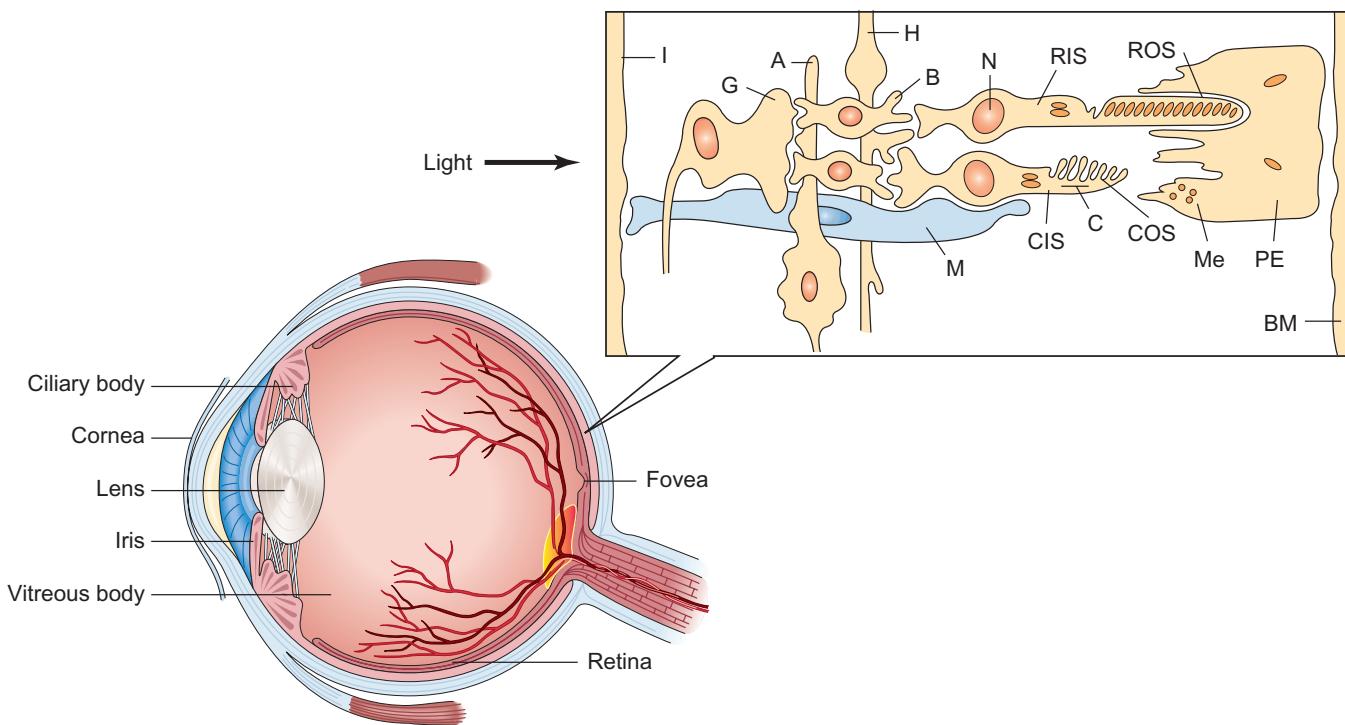
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## STRUCTURE AND DEVELOPMENT OF THE VISUAL SYSTEM

**The visual system is composed of unique structures optimized for collection, detection and processing of visual information**

Vision results from the detection and processing of information inherent in light reflected from the external world into the eye, a remarkable structure devoted to this unique function. The basic structure and major components of the eye are shown in Fig. 51-1. Most of the globe of the eye is

encased in a tough and opaque tissue composed largely of collagen-rich extracellular matrix, known as the sclera. The sclera is replaced by the transparent cornea at the front of the eye. Light passes through the cornea, then through the pupil, whose variable diameter is controlled by the iris; next through the aqueous humor of the anterior chamber; and then into the crystalline lens. The cornea and lens together act as a compound lens to focus the light through the gel-like vitreous humor which makes up most of the volume of the eye, onto the retina, which extends circumferentially from the back of the eye to the ciliary margin near the front.



**FIGURE 51-1** The vertebrate retina: A, amacrine cell; B, bipolar cell; BM, Bruch's membrane; C, ciliary process (cilium); CIS, cone inner segment; COS, cone outer segment; G, ganglion cell; H, horizontal cell; I, inner limiting membrane; M, Müller cell (glial cell); Me, melanin granule; MC, mitochondrion; N, nucleus; PE, retinal pigmented epithelium; RIS, rod inner segment; ROS, rod outer segment. (from H. Shichi, 2006).

### The retina is composed of highly organized neuronal sublayers

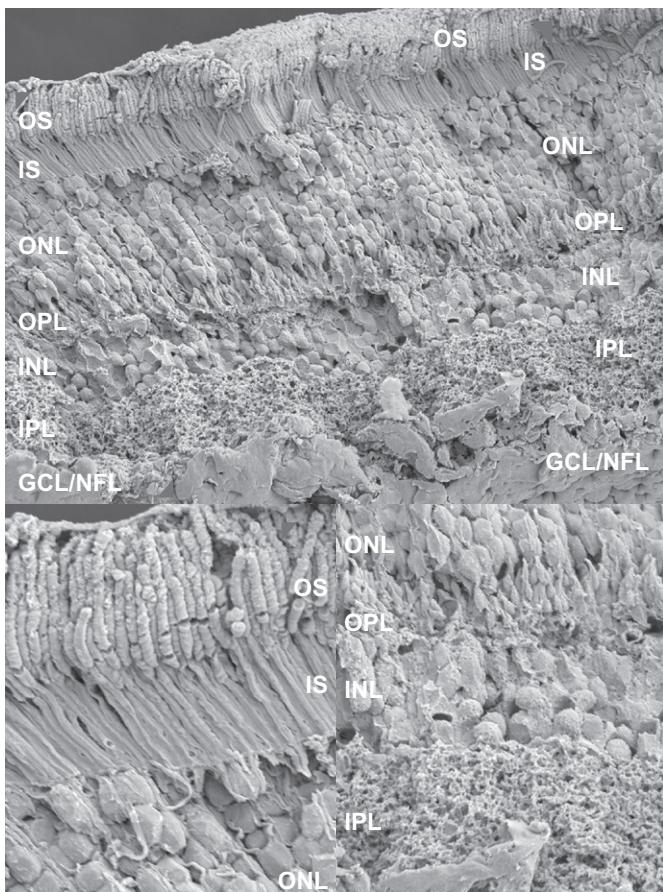
The retina is a thin layer of neurons divided into sublamina into which different subtypes of neural cells and glia are segregated. At the very back of the retina is the photoreceptor layer containing the rods and cones, the cells directly responsible for sensing light. The outer segments (OS) of the photoreceptors are embedded in the apical processes of the cells of the retinal-pigmented epithelium (RPE). There are two vascular systems providing a flow of blood for the retina: (1) the central retinal vasculature and (2) the choriocapillaris, whose vessels extend between the sclera, to which it is attached by a suprachoroid lamina, and the RPE, from which it is separated by a basement membrane known as Bruch's membrane.

Histologically, the retina appears as a series of layers arranged radially from back to front (see Fig. 51-2): Most distal are the outer segment layer (OSL), the inner segment layer (ISL), and the outer nuclear layer (ONL), containing the subcellular compartments of the photoreceptors. Next is the outer plexiform layer containing the synapse-forming processes from the photoreceptors and the dendrites of secondary neurons known as bipolar cells and amacrine cells, as well as the processes of horizontal cells. The nuclei of these secondary neurons are found in the next histological layer, the inner nuclear layer (INL), and their axons make up much of the next layer, the inner plexiform layer. This layer also contains the receptive dendrites of the ganglion cells, the final integrators and

transmitters of the information about the visual scene initially detected by the photoreceptors. The cell bodies of the ganglion cells, along with a subset of amacrine cells known as displaced amacrine cells, make up the ganglion cell layer (GCL). Finally, the myelinated axons of the ganglion cells bundle together to form the nerve fiber layer (NFL), whose fibers meet at the optic nerve head and pass outward through all the other layers to form the optic nerve which carries signals from the retina to the visual areas of the brain. The retina is bounded in the front by the inner limiting membrane, and at the back by the outer limiting membrane, formed by tight junctions between photoreceptors and long radial glial cells known as Müller cells. These glia extend processes all the way from the inner limiting membrane, formed by their processes and those of astrocytes, to the outer limiting membrane; their nuclei are found in the inner nuclear layer.

### The ganglion cell axons of the optic nerve carry visual signals from the retina to the brain

Ganglion cells receive inputs from bipolar cells and amacrine cells. The location, timing, strength, and sign (inhibitory or excitatory) of these signals, as well as correlations among them, encode complex information about the visual scene and its dynamics. Different types of ganglion cells respond differentially to different types of stimuli, such as onset of light, onset of darkness, motion, direction of motion, color, contrast,



**FIGURE 51-2** The layers of the retina. Scanning electron micrograph of a mouse retina. OS, photoreceptor outer segments; IS, photoreceptor inner segments; ONL, outer nuclear layer, containing photoreceptor nuclei; OPL, outer plexiform layer, containing axonal termini of rods and cones, dendrites of bipolar cells and horizontal cells, and the synapses formed between them. INL, inner nuclear layer, containing nuclei of bipolar cells, horizontal cells, most amacrine cells, and Müller glial cells. Courtesy of Drs. Ivan Anastassov and Alecia K. Gross and the 2010 class of the “Fundamental Issues in Vision Research” course of the Marine Biological Laboratory, Woods Hole, MA.

and others. This information is encoded in patterns of firing, i.e., depolarization and action potential generation, in the ganglion cells. These action potentials are actively propagated along the myelinated ganglion cell axons of the optic nerve through the optic chiasm and optic tract to their targets in the brain. There are six target regions for ganglion cell axons: the suprachiasmatic nucleus; the accessory optic system, consisting of the medial terminal nucleus, the lateral terminal nucleus and the dorsal terminal nucleus; the superior colliculus, the pretectum; the pregeniculate; and the multiple layers and sublayers of the lateral geniculate nucleus. Within the mammalian brain, the main targets for the axons of the optic nerve are in the lateral geniculate nucleus (LGN) and superior colliculus. In these areas signals are processed and transmitted to other areas of the brain, including the visual areas of the cortex, and to motor neurons controlling eye movement.

## The eye develops as an outcropping of the developing brain

Eye development begins (for excellent reviews of visual system development see [Chow & Lang, 2001](#); [Dreher & Robinson, 1991](#)) with the evagination of the developing brain to form the optic vesicle. When the outer layer of this structure contacts the surface ectoderm of the head, a program of gene expression is induced that leads to formation of the lens placode. This lens placode thickens and then invaginates, together with the outer layer of the optic vesicle, which will give rise to the retina, to form the optic cup. The inner layer of the optic vesicle, which becomes immediately apposed to the presumptive retinal layer, eventually gives rise to the retinal-pigmented epithelium. The corneal epithelium develops from the ectoderm remaining after lens placode formation, and the corneal endothelium is produced from neural crest cells that migrate into the space between corneal epithelium and lens. Other neural crest cells migrate to the region between epithelium and endothelium to become keratoblasts, and eventually these keratocytes secrete the extensive extracellular matrix of the corneal stroma.

A program of gene expression in the optic cup is initiated that gives rise to retinal progenitor cells, which continue to proliferate and are competent to form neurons and glia ([Turner & Cepko, 1987](#)). Likewise, in the lens placode, another set of intercellular signals and transcription factors specify the differentiation of lens fiber cells ([Beebe, 2008](#); [Lang, 2004](#)).

Retinal development proceeds unevenly, with different cell types emerging at different stages (reviewed in [Livesey & Cepko, 2001](#)). At the earlier stages, ganglion cells, cones, many amacrine cells, and horizontal cells differentiate. Later, primarily after birth in the mouse, and in the last weeks before birth in humans, most rods, Müller glial cells and bipolar cells differentiate.

A number of extracellular signaling proteins and intracellular transcription factors have been identified as critical for different stages of eye development. The homeodomain protein Otx2 is widely expressed in developing anterior brain, and mice homozygous for deletion of the *Otx2* gene display microphthalmia (small eyes) or anophthalmia (no eyes). The homeobox transcription factor, Pax6, has been identified as a master regulatory element for eye development in vertebrates and invertebrates alike, and ectopic expression of Pax6 can cause formation of ectopic eyes ([Gehring, 1996](#)). However, additional transcription factors, such as Rx ([Mathers et al., 1997](#)), Lhx2 ([Yun et al., 2009](#)) and Six3 ([Liu et al., 2010](#)), have been shown to be required for eye formation, and in some instances independent ([Zhang et al., 2000](#)) of Pax6. FGF (fibroblast growth factor) signaling has been implicated in various stages of eye development ([Pittack et al., 1997](#)), and signaling by Wnt and Frizzled family members is important as well ([Chen et al., 2008](#); [de Jongh et al., 2006](#); [Fuhrmann, 2008](#); [Liu et al., 2008](#)). Mutations or knockouts of *Vax1*, *Pax2*, *Gli1* and *BF-1* all lead to defects in formation of the retina.

Additional factors control cell-fate determination and expression of cell-type specific genes. The transcription factor Crx ([Chen et al., 1997](#); [Furukawa et al., 1997](#)) is important for rhodopsin gene expression, but not absolutely required. Crx deficiency leads to cone-rod dystrophy in humans ([Freund et al., 1997](#)). Additional transcription factors important for

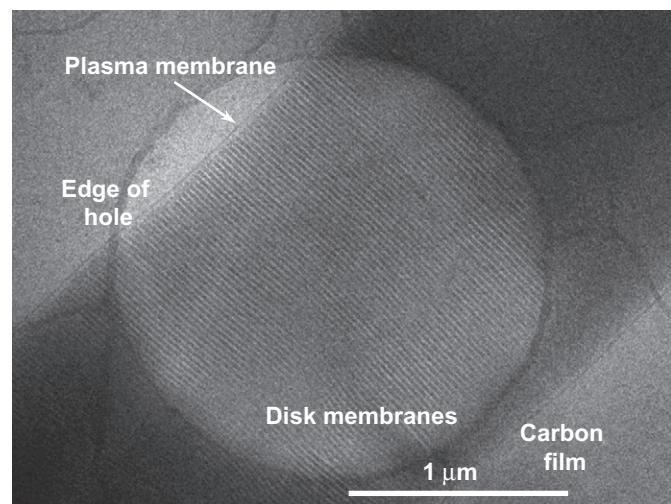
rod development are Nrl and NR2E3 (Cheng et al., 2004). These proteins are thought to act as heterodimers to suppress expression of cone-specific genes, and to promote a rod cell fate in nascent photoreceptors. Mice deficient in these factors have no rods and excessive numbers of cones, and humans with genetic deficiencies in NR2E3 are afflicted with enhanced S-cone syndrome (Haider et al., 2000), where S stands for short-wavelength or blue-sensitive cones. Thyroid hormone has also been reported to be important for photoreceptor development (Trimarchi et al., 2008). The transcription factor Brn-3b is important for ganglion cell differentiation (Gan et al., 1999; Gan et al., 1996), and deletion of Chx10 leads to reduced proliferation of retinal progenitors and absence of bipolar cells (Burmeister et al., 1996).

## PHOTORECEPTORS AND PHOTOTRANSDUCTION

### Photoreceptors are polarized cells, with specialized primary cilia, outer segments, devoted to phototransduction

Photoreceptors are highly polarized neurons, divided morphologically into distinct compartments with distinct biochemical compositions and functions. Detection of light takes place in highly specialized structures known as *outer segments* (OS), which are modified primary cilia. As with other primary cilia, the base of the outer segment extends from two cylindrical bundles of nine microtubule triplets each, known as the basal bodies. These are derived from the centrioles, which migrate to the distal tip of the cell following the last cell division. Extending from the basal bodies is an axoneme formed of a bundle of 9 doublet microtubules with no central pair (9 + 0). Close to the basal bodies, the cilium is surrounded by a thin sheath of cytoplasm and a cylindrical plasma membrane, but beyond this transition zone, known as the *connecting cilium*, large flattened vesicles known as disk membranes (Fig. 51-3) extend to a diameter of about 1.5 μm in mammals, and up to several microns in cold-blooded vertebrates. Disk membranes contain a high density, ~25,000 μm<sup>2</sup>, of the visual pigment (rhodopsin in rods, and cone pigments in cones). They have a high content of phospholipids with highly unsaturated fatty acyl side chains, with 40% of the side chains consisting of docosahexenoic acid, or 22:6, ω-3, an acid with 22 carbons and six double bonds. At the rims of the disks are found the tetraspanin proteins rds/peripherin and ROM-1 (Molday et al., 1999), which appear to hold the two bilayers of the disk together, and form a complex with plasma membrane proteins including the cyclic nucleotide-gated (CNG) channel (see below).

Biosynthesis of the molecular components of the outer segments primarily takes place in the region of the cell on the proximal side of the connecting cilium, known as the *inner segment* (IS). The region immediately adjacent to the connecting cilium, the *ellipsoid*, is packed with mitochondria, and contains the distal portion of a large filamentous structure known as the ciliary rootlet, composed largely of the protein rootletin, which in photoreceptors extends all the way from the axon to the basal body. Closer to the nucleus, the *myoid* region contains ribosomes and rough endoplasmic reticulum.



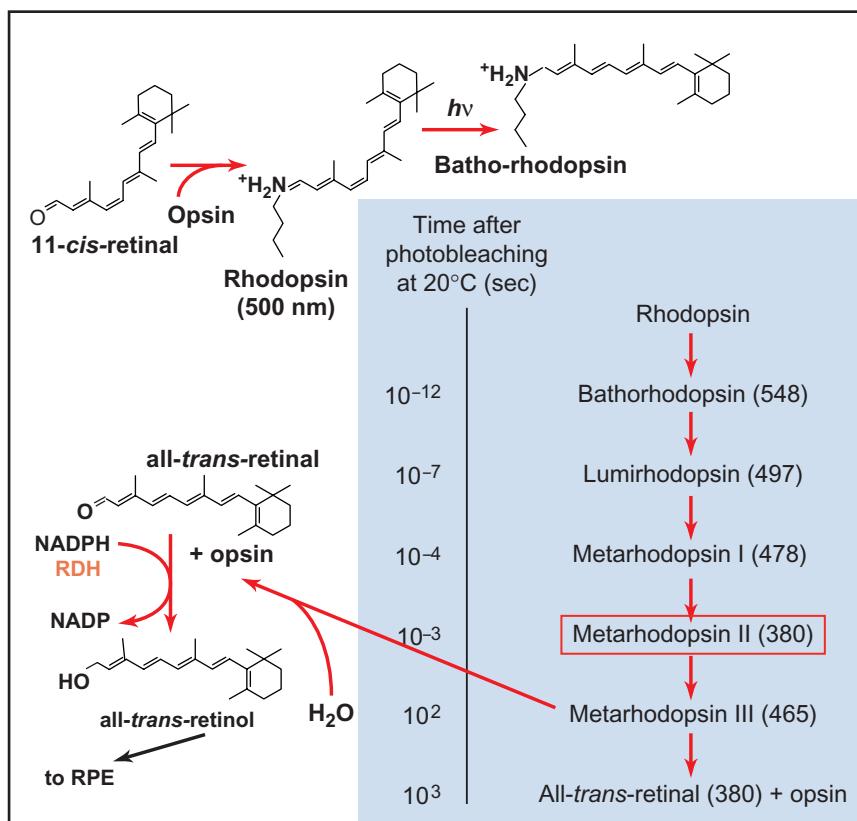
**FIGURE 51-3** Membrane organization of rod outer segments. Electron micrograph of an isolated mouse rod outer segment, flash-frozen in liquid ethane and imaged in vitreous ice, without stains or fixatives. The outer segment is suspended over a hole in a carbon film. The highly regular arrangement of the stacked disks and their relationship to the plasma membrane can be seen clearly. Courtesy of Drs. Jared C. Gilliam and Juan T. Chang and the National Center for Macromolecular Imaging.

**Phototransduction consists of a highly amplified cascade of light-triggered changes in protein conformation, and changes in interactions of proteins with one another and with guanine nucleotides**

Phototransduction is the generation of an electrochemical signal in response to absorbance of light (Baylor, 1996; Burns & Arshavsky, 2005; Gross & Burns, 2011; Stryer, 1986). Photoreceptors are relatively depolarized in the dark, due to Na<sup>+</sup> ions entering the outer segment through light-sensitive channels, and as a result they continuously release the neurotransmitter glutamate. Light induces a biochemical cascade that causes a graded hyperpolarization through closing of the channels, with a resultant graded slowing of glutamate release.

Whereas most excitable cells are in a hyperpolarized state in the absence of stimulation, with a membrane potential of somewhere between -90 mV (inside more negative than outside) and -60 mV, photoreceptors in the dark have membrane potentials close to -40 mV, so they are considered relatively depolarized. The molecules promoting hyperpolarization are Na,K-ATPase, which uses the energy derived from hydrolysis of ATP to pump Na<sup>+</sup> out of the cell and K<sup>+</sup> into the cytoplasm, and potassium-selective channels in the inner segment, which allow K<sup>+</sup> ions to diffuse out to a lower concentration in the extracellular space, thereby generating a deficit of positive charge inside the cell and a negative membrane potential. Balancing this outward positive current in the inner segment (where the K<sup>+</sup> channels are located) is the large inward current carried by Na<sup>+</sup> in the outer segment (see Chs. 3, 4 for further discussion).

The channel catalyzing this inward current is a cyclic nucleotide-gated channel selective for cGMP. This channel



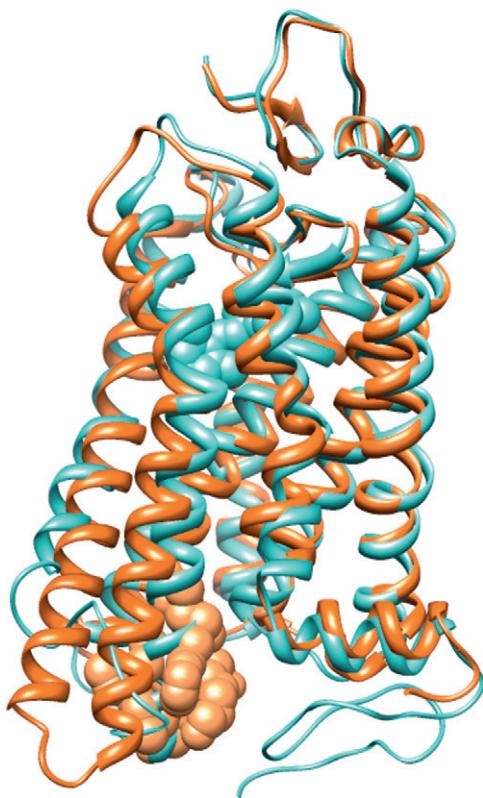
**FIGURE 51-4** Molecular transformations of retinoids in photoreceptors during the primary events of vision and the photoreceptor portion of the visual cycle. Rhodopsin is formed when the aldehyde moiety of 11-cis-retinal forms a protonated Schiff's base with lysine 296 of the *apo*-receptor opsin. Its absorbance spectrum shifts dramatically to the red, from a maximum absorbance in the ultraviolet (380 nm) to the visible (500 nm). Absorption by rhodopsin leads to a photoisomerization from all-trans to 11-cis, forming bathorhodopsin. In a series of protein conformational changes and deprotonation and protonation steps, bathorhodopsin relaxes to the form responsible for activating the G protein, metarhodopsin II. Ultimately, metarhodopsin II decays to metarhodopsin III, from which all-trans-retinal is hydrolyzed, generating a transient pool of free all-trans-retinal and opsin. Free all-trans-retinal is converted to all-trans-retinol by the action of a class of enzymes known as retinol de-hydrogenases (RDH), which use NADPH as the reductant. The all-trans-retinol can diffuse through the cell membrane and make its way to retinal pigmented epithelium, where further transformations of the visual cycle take place (see Fig. 51-10), eventually regenerating 11-cis-retinal.

is relatively nonspecific for physiological cations, but primarily conducts  $\text{Na}^+$  ions from the extracellular space to the cytoplasm, due to the high extracellular  $\text{Na}^+$  concentration. There is a complete circuit consisting of the extrusion of  $\text{Na}^+$  ions and the outward diffusion of  $\text{K}^+$  ions from the inner segment, and the diffusion primarily of  $\text{Na}^+$  ions (partly balanced by outward diffusion of  $\text{K}^+$  ions, leading to a reversal potential much lower than that expected for  $\text{Na}^+$ -selective channels (Yau & Nakatani, 1984) into the outer segment, known as the *circulating dark current* (Hagins & Yoshikami, 1970). There is also a minor, but physiologically very important, component of the dark current carried by  $\text{Ca}^{2+}$  ions.  $\text{Ca}^{2+}$  ions are extruded by an electrogenic  $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$  exchanger (Schnetkamp, 2004) and leak back into the cell through the CNG channel.

Why are these currents so important for phototransduction? The essence of phototransduction is a molecular mechanism whereby light impinging on the photoreceptor cells can be detected and communicated to the downstream neurons, the ON and OFF bipolar cells, and horizontal cells. Photoreceptors communicate with these downstream cells through their release of the excitatory neurotransmitter glutamate, and the release

of glutamate is controlled by membrane potential through the action of voltage-gated  $\text{Ca}^+$  channels, whose opening floods the presynaptic space with  $\text{Ca}^{2+}$ , triggering synaptic vesicle fusion and glutamate release (Heidelberger et al., 2005). In both rods and cones, glutamate release occurs in a graded fashion, controlled by the membrane potential, and therefore, ultimately, by light-dependent changes in current passing through the CNG channel. The current through this channel controls the membrane potential, and that current is controlled by the cytoplasmic concentration of cGMP, which in turn is controlled by light.

How does light control the cGMP concentration? The answer begins with photoactivation of rhodopsin. Rhodopsin is in many ways a prototypical G-protein-coupled receptor (GPCR). In the dark it has an inverse agonist (a compound whose binding lowers the ability of a GPCR to activate G proteins), 11-cis retinaldehyde, covalently coupled (as a Schiff's base) to a lysine residue in its transmembrane domain (Fig. 51-4). In this state, rhodopsin has essentially no ability to catalyze the exchange of GTP for GDP on the  $\alpha$  subunit of the heterotrimeric G protein of vision, transducin ( $\text{G}_{\alpha}\beta\gamma$ ). Absorption of light induces photoisomerization of retinal from 11-cis to



**FIGURE 51-5** Conformational activation of rhodopsin. Ribbon diagram of structure of dark, inactive state of rhodopsin (cyan ribbons) with the covalently coupled chromophore, 11-cis retinaldehyde shown in space-filling mode (cyan) (pdb file 1U19, Okada et al., 2004) superimposed on the structure of an active conformation of opsin (orange ribbons; pdb file 3DQB; Scheer et al., 2008) with a C-terminal peptide from the visual G protein  $\text{G}\alpha\text{t}$  bound (orange space-filling model) at its cytoplasmic face. All molecular graphics in this chapter were rendered with UCSF Chimera (Pettersen et al., 2004).

all-trans with an unusually high quantum yield of 0.65 (Kim et al., 2001). The quantum efficiency of rhodopsin combined with the amplification of the G-protein cascade allows rods to respond to very low levels of light, at the level of individual photons. The cis-trans isomerization induces a conformational change in rhodopsin (Figs. 51-4, 51-5). All-trans-retinal is a potent agonist, and converts rhodopsin to the activated form, metarhodopsin II (MII), which in turn catalyzes the conversion of  $\text{G}_{\alpha\beta\gamma}\text{-GDP}$  to  $\text{G}_{\alpha\beta}\text{-GTP} + \text{G}_{\beta\gamma}$  at a rate of a few hundred per second (Fig. 51-6).

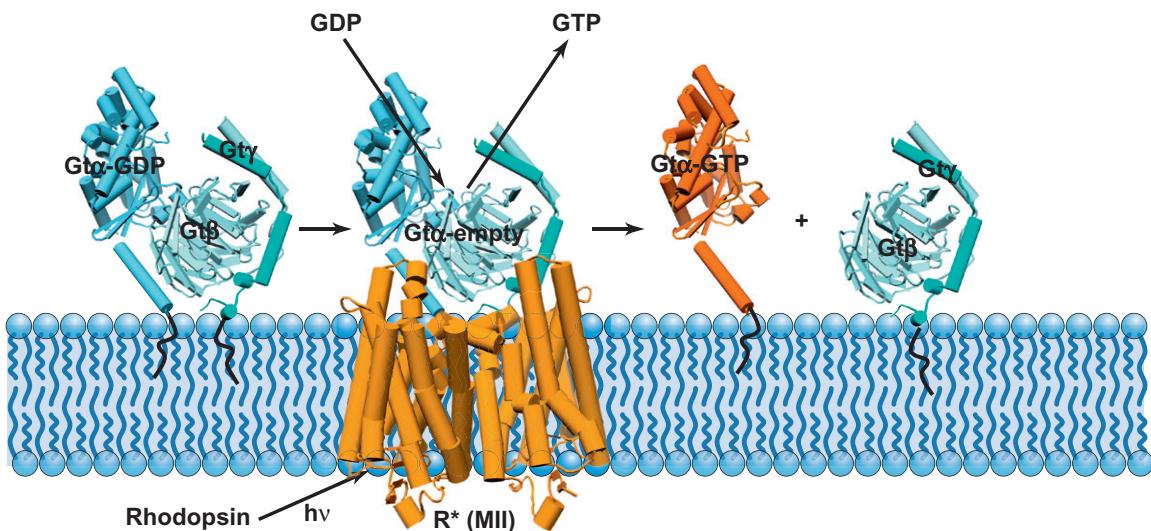
The significance of  $\text{G}_{\alpha\beta}\text{-GTP}$  formation is that this complex controls the activity of a catalytically potent cGMP-specific phosphodiesterase, PDE6 (Wensel, 1993). PDE6 is a heterotetrameric protein consisting of two large (nearly 100 kDa each) catalytic subunits, PDE6 $_{\alpha\beta}$ , and two identical small inhibitory subunits, PDE6 $_\gamma$ . The dark GDP-bound state of  $\text{G}_{\alpha\beta}$  has very low affinity for PDE6, but GTP triggers a conformational change that dramatically enhances its ability to activate PDE6 (Figs. 51-7, 51-8). When PDE6 $_\gamma$  is bound to the catalytic subunits and  $\text{G}_{\alpha\beta}\text{-GTP}$  is absent, then the activity of PDE6 is about one one-thousandth of its maximal activity.  $\text{G}_{\alpha\beta}\text{-GTP}$  binds

to this complex, and apparently pushes PDE6 $_\gamma$  into a position that no longer blocks catalytic activity. In this state, with  $\text{G}_{\alpha\beta}\text{-GTP}$  bound, PDE6 hydrolyzes cGMP with a catalytic efficiency on the order of  $10^8 \text{ M}^{-1}\text{s}^{-1}$ , near the diffusion limit. As the cytoplasmic concentration of cGMP rapidly falls, cGMP dissociates from the CNG channel, channels close, the dark current is reduced or abolished, and the membrane potential becomes hyperpolarized. The dark current is reduced in a graded fashion over a wide range of light intensities, ranging from one-photon excitation of individual dark-adapted rods, to a rain of hundreds of thousands of photons per cell, required to approach saturation of light-adapted cones. The activation of PDE6 by  $\text{G}_{\alpha\beta}\text{-GTP}$  is greatly enhanced by the lipid surface of the disk membrane (Malinski & Wensel, 1992; Melia et al., 2000), to which both are tethered by covalently attached lipid groups, N-terminal fatty acyl modifications for  $\text{G}_{\alpha\beta}$  (Kokame et al., 1992; Neubert et al., 1992; Z. Yang & Wensel, 1992b), and both farnesyl and geranylgeranyl isoprenoids attached to the C-termini of PDE6 $_{\alpha\beta}$  catalytic subunits (Anant et al., 1992; Qin et al., 1992).

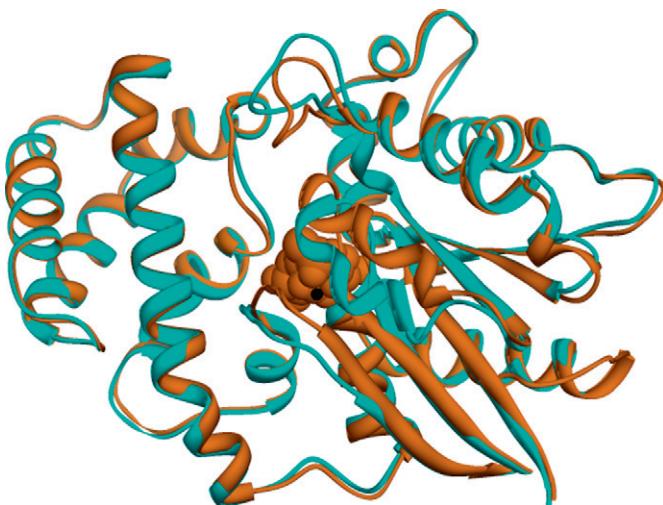
### Recovery of the dark current after light stimulation is a multistep process mediated by $\text{Ca}^{2+}$ and proteins exerting negative regulation

The lifetime of active, photoexcited rhodopsin is not long; current estimates suggest about 40 ms (Gross & Wensel, 2010). In dim light, the main mechanism for inactivating MII is phosphorylation by rhodopsin kinase, GRK1, an enzyme whose activity is triggered by the conformational changes accompanying formation of MII. There are multiple sites in the carboxyl-terminal region of rhodopsin that can be phosphorylated by rhodopsin kinase. Phosphorylation of these sites greatly enhances the affinity of MII for the 48 kDa protein arrestin, which acts as a negative regulator of MII. Arrestin binds tightly to phosphorylated MII, thereby blocking access of both the G protein transducin and the phosphatase, which process ultimately removes the phosphates from the carboxyl terminus. In this state MII loses its ability to activate the G protein, thereby setting the stage for additional events that restore the dark resting state of the cascade.

The cytoplasmic concentration of cGMP is controlled not only by PDE6, but also by the enzyme that catalyzes synthesis of cGMP from GTP (see Fig. 51-9), with the concomitant release of inorganic pyrophosphate, guanylate cyclase. Even in the dark, there is a high turnover of cGMP, requiring a higher concentration of inorganic pyrophosphatase in rod outer segments than in any other tissue examined (Yang & Wensel, 1992a). Photoreceptor guanylate cyclase (Koch et al., 2002) consists of two transmembrane catalytic subunits and two  $\text{Ca}^{2+}$ -sensing subunits from the calmodulin superfamily, known as guanylate cyclase activating proteins (GCAPs) (Gorczyca et al., 1995). In the dark,  $\text{Ca}^{2+}$  concentrations in the cytoplasm of the rod outer segment are relatively high, on the order of a few hundred nanomolar, because, as noted above, the action of the  $\text{Na}^+/\text{K}^+$ / $\text{Ca}^{2+}$  exchanger, which extrudes  $\text{Ca}^{2+}$  from the cell using the energy stored in the  $\text{Na}^+$  gradient to drive this energetically unfavorable transport, is balanced by the entry of  $\text{Ca}^{2+}$  through the CNG channel. Closure of the channels in response to light



**FIGURE 51-6** G protein activation by metarhodopsin II. In the dark, the visual G protein transducin exists primarily as a heterotrimer,  $\text{G}\alpha\beta\gamma$ , with GDP bound to the  $\text{G}\alpha$  subunit. This is the inactive state of the G protein, and it interacts only weakly with rhodopsin. GDP dissociation is extremely slow, occurring with a time constant of 10,000s. Upon light activation and formation of metarhodopsin II (MII), the heterotrimer binds MII, which induces a conformational change that allows rapid GDP dissociation. In the absence of GTP, this complex of MII (shown here as a dimer of MII and rhodopsin), and nucleotide-free  $\text{G}\alpha\beta\gamma$  is very stable. Within the rod outer segment GTP concentration is on the order of  $10^{-3}$  mol/L, so that GDP binds very rapidly to the nucleotide-free  $\text{G}\alpha$  subunit. GTP binding induces a conformational change (see Fig. 51-7) that causes  $\text{G}\alpha\beta\gamma$  to dissociate both from  $\text{G}\alpha\beta\gamma$  and MII. Structures are based on the following pdb files: 1GOT (Lambright et al., 1996); 3DQ (Pettersen et al., 2004); and 1TND (Noel et al., 1993).



**FIGURE 51-7** Conformational activation of transducin,  $\text{G}\alpha_t$ . Ribbon diagram of structure of dark, inactive state of  $\text{G}\alpha_t$  (cyan ribbons) with bound GDP shown in space-filling mode (cyan) (pdb file 1TAG, (Lambright et al., 1994) superimposed on the structure of an active conformation of  $\text{G}\alpha_t$  (orange ribbons; pdb file 1TNDb (Noel et al., 1993); with a non-hydrolyzable GTP analogue bound (orange space-filling model; coordinated  $\text{Mg}^{2+}$  in black).

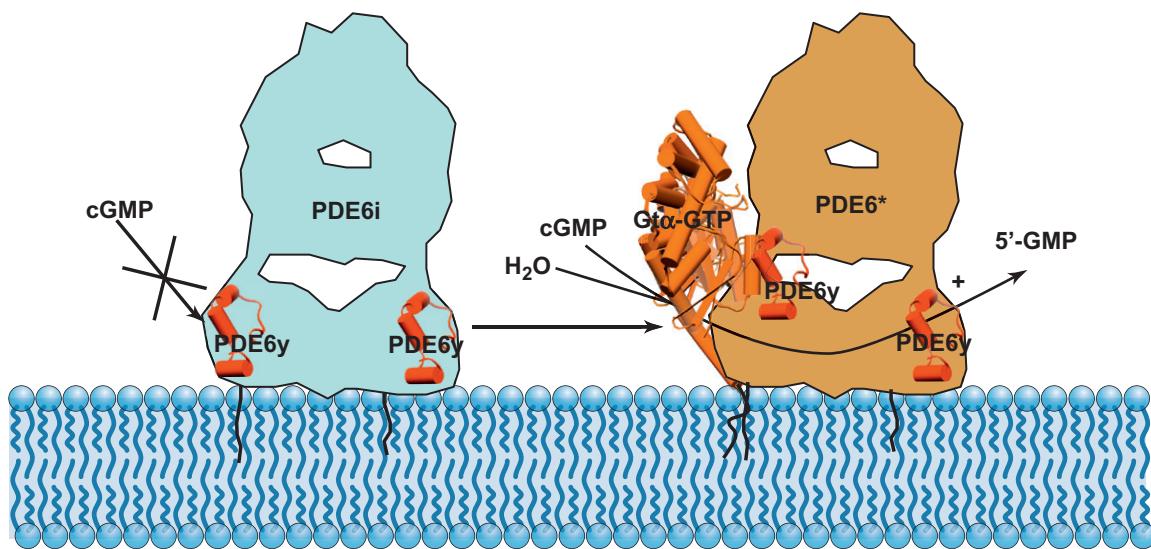
leads to rapid depletion of intracellular  $\text{Ca}^{2+}$  as the exchanger continues to operate. At low ( $\sim 100 \text{nM}$ )  $\text{Ca}^{2+}$ ,  $\text{Ca}^{2+}$  dissociates from GCAP, converting it into an activator, rather than an inhibitor of guanylate cyclase. This calcium feedback mechanism appears to occur with very little lag compared to PDE6 activation (Burns et al., 2002).

Inactivation of MII and activation of guanylate cyclase are not sufficient for termination on the light response. As long as the G protein remains in the GTP state and activates PDE6, the dark current will continue to be suppressed. A mechanism that is ubiquitous in G-protein signaling, GTPase acceleration by a GTPase accelerating protein, or GAP, is used to ensure timely recovery (Chen et al., 2000). The GAP is a heterotrimeric complex consisting of RGS9-1, a photoreceptor-specific variant of the RGS9 gene (He & Wensel, 1998; Rahman et al., 1999; Zhang et al., 1999); R9AP, a single-pass transmembrane anchor protein related to syntaxins (Hu & Wensel, 2002); and G protein  $\beta$  subunit variant  $\text{G}\beta_5$  (Chen et al., 2003; He et al., 2000; Makino et al., 1999). The level of R9AP expression determines the level of the entire GAP complex, which in turn controls the rate-limiting step in rod vision (Krispel et al., 2006).

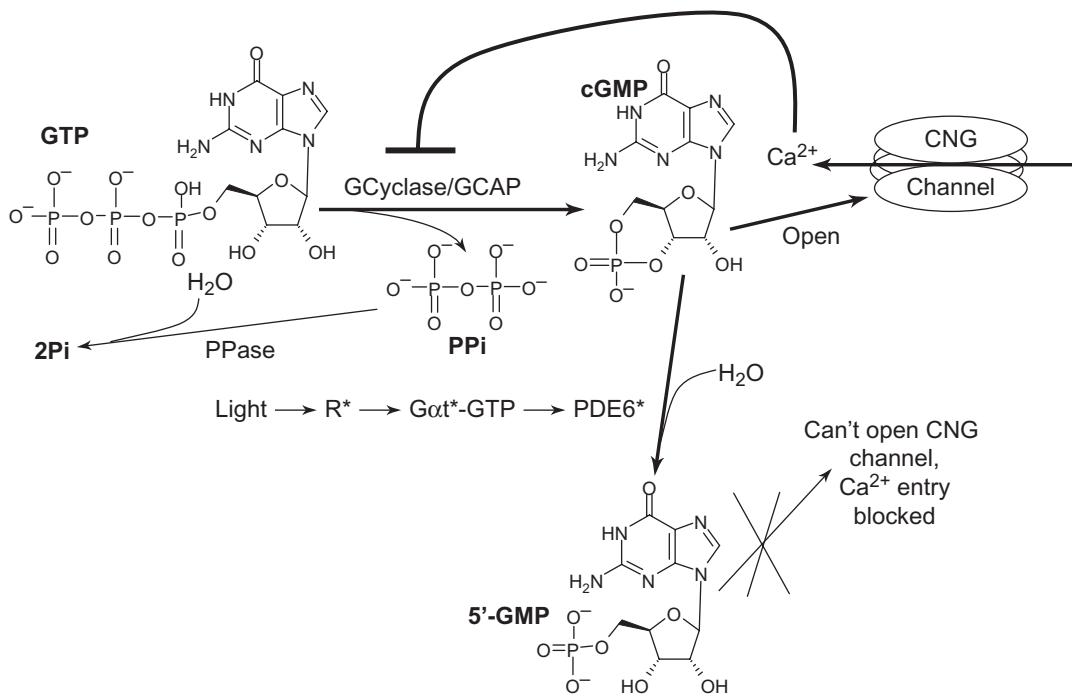
### Cone phototransduction uses mechanisms and molecules similar to those in rods, but is optimized for speed rather than sensitivity

Whereas rod cells are capable of providing robust responses to individual quantum events, i.e., the capture of individual photons by individual rhodopsins, cone cells are nearly two orders of magnitude less sensitive. However, the cone system provides a much richer information content than that detected and conveyed by rods, providing color discrimination and the ability to discern fine details and rapid changes in the visual scene.

The photoreceptor proteins, or pigments, in cone cells are very similar to rhodopsin in their structures. Changes in the atomic-scale environment of the chromophore 11-cis-retinal



**FIGURE 51-8** Activation of PDE6 by activated transducin, G $\alpha$ t-GTP. PDE6 is kept at a very low level of activity (PDE6i) in the dark by its inhibitory PDE6 $\gamma$  subunits. In its GTP-bound form, G $\alpha$ t binds tightly to PDE6 and relieves the inhibitory constraint imposed by PDE6 $\gamma$ , forming the active form PDE6\*, and allowing rapid catalysis of cGMP hydrolysis. Structures taken from pdb file 1FQJ, Slep et al., 2001 (G $\alpha$ t and C-terminal fragment of PDE6 $\gamma$ ), and from unpublished electron microscopy data, courtesy of Dr. Zhixian Zhang (PDE6).



**FIGURE 51-9** cGMP and CNG channel activity are regulated by PDE6, guanylate cyclase, GCAP and Ca $^{2+}$  feedback. cGMP is synthesized from GTP in a reaction that is catalyzed by guanylate cyclase, GCYclase, and releases inorganic pyrophosphate (PPi). PPi is rapidly hydrolyzed by inorganic pyrophosphatase (PPase), which drives the equilibrium toward cGMP formation. GCYclase is a transmembrane enzyme, bound constitutively to Ca $^{2+}$  sensors known as guanylate cyclase activating proteins (GCAP), which inhibit its activity in the moderate concentrations of intracellular Ca $^{2+}$  found in the dark. Thus GCYclase activity is low in the dark. Basal GCYclase activity in the dark is balanced by relatively low dark activity of PDE6, a cGMP-specific phosphodiesterase, which catalyzes hydrolysis of cGMP. A steady state [cGMP] of a few micromolar is established, and maintains enough CNG channels in the open state to produce a dark current of 10–20 pA, and a resting membrane potential near –40 mV. When PDE6 is converted to its active form, PDE6\*, by binding G $\alpha$ t-GTP generated by light activation of R\*, PDE6\* rapidly hydrolyzes cGMP leading to channel closure, blockage of the dark current, and membrane hyperpolarization. CNG channel closure also blocks Ca $^{2+}$  from leaking into the outer segment, whereas the Na $^+$ /K $^+$ /Ca $^{2+}$  exchanger continues to extrude Ca $^{2+}$  from the cytoplasm. Thus, in the light, cytosolic [Ca $^{2+}$ ] falls to very low levels, and the lowered Ca $^{2+}$  concentration leads to stimulation of the GCYclase/GCAP complex. Homeostasis is restored as cGMP is synthesized to balance its hydrolysis by PDE6\*, and a new steady state is achieved. This Ca $^{2+}$  feedback mechanism ensures rapid recovery from light stimulation, and provides a mechanism contributing to light adaptation.

provides a range of wavelength sensitivities ranging from the blue or near ultraviolet to the red edge of the visible spectrum in the different cone pigments. Photoactivated cone pigments tend to become inactivated more rapidly than MII does (Shi et al., 2007; Tachibanaki et al., 2007).

Whereas most mammals are dichromats with only short-wavelength-sensitive (S-) cones and mid-wavelength-sensitive (M-) cones, old-world primates (including humans), birds, and many cold-blooded species have three or more types of cones with differing wavelength sensitivities. Gene structures and locations suggest that old-world primates gained trichromacy through duplication and mutation of the M pigment encoded on the X chromosome. The proximity (Vollrath et al., 1988) and sequence similarity of the M- and L- (long wavelength-, or red-sensitive) cone pigment genes apparently facilitates intrachromosomal homologous recombination, accounting for the high frequency of X-linked color blindness in human males (Nathans, 1987).

Many phototransduction proteins in cones are distinct from, but closely related to, the rod isoforms. These include cone-specific G proteins, PDE6 isoforms, arrestin (Craft & Whitmore, 1995), and CNG channel subunits (Kaupp & Seifert, 2002). Cones have identical RGS proteins and R9AP, but a mixture of long and short splice variants of  $G_{\beta\gamma}$  (Zhang et al., 2003). Their levels of the RGS9-1 complex are an order of magnitude higher than those in rods (Cowan et al., 1998), consistent with the faster kinetics of cones. Cones of some species contain one or more alternative rhodopsin kinase isoforms, e.g., human GRK7 (Chen et al., 2001; Wada, et al., 2006; Weiss et al., 1998), but many cones also contain GRK1.

in response to light, whereas OFF bipolar cells, which form synapses only with cones and not rods, hyperpolarize or turn off in response to light. The OFF cells can fire action potentials upon the cessation of a light signal.

## ON and OFF bipolar cells use different types of receptors and response mechanisms

Neurons respond to neurotransmitters such as glutamate, or  $\gamma$ -amino butyric acid (GABA) through the activation of two types of receptors: ionotropic and metabotropic (reviewed by Yang, 2004) (Chapter 17). Ionotropic glutamate receptors are ion channels with extracellular domains that bind glutamate and allosterically regulate channel opening. These are the major receptors in the postsynaptic membranes of OFF bipolar cells. These cells express glutamate receptor subunits GluR1-7, KA1 and KA2. It has been reported that OFF bipolar cells also express metabotropic glutamate receptors, but these likely play modulatory roles. When glutamate from cones binds to these receptors, they open, allowing entry of  $\text{Na}^+$  into the cell, and triggering depolarization. In contrast, the major glutamate sensing mechanism of ON bipolar cells relies on the metabotropic receptor mGluR6 (Masu et al., 1995). Metabotropic glutamate receptors are Class C G-protein-coupled receptors. In ON bipolar cells, mGluR6 activates G proteins containing the  $G_{\alpha\omega}$  subunit (Dhingra et al., 2000). Activation of  $G_{\alpha\omega}$  through mGluR6-catalyzed nucleotide exchange leads to closure of a cation-selective channel, TRPM1 (Koike et al., 2010; Morgans et al., 2009). As with photoreceptor cells, termination of the response is accelerated by a GAP complex containing either RGS7 or RGS11 (Mojumder et al., 2009) and  $G_{\beta\gamma}$ .

## SIGNALING DOWNSTREAM OF PHOTORECEPTORS

### Secondary neurons respond to changes in glutamate release by rods and cones

Rod synaptic termini, known as spherules, and cone synaptic termini, known as pedicles, contain structures known as ribbon synapses (reviewed by Matthews & Fuchs, 2010). These consist of long, thin ribbon structures containing the protein Ribeye, with associated synaptic vesicles. Extracellular release of the content of the synaptic vesicles, the excitatory neurotransmitter glutamate, is controlled by local concentrations of  $\text{Ca}^{2+}$ . Voltage-gated L-type  $\text{Ca}^{2+}$  channels, containing Cav1.4alpha1F subunits in rods, and Cav1.4alpha1D subunits in cones (Morgans et al., 2005), mediate the control by voltage, and thus by light, of presynaptic  $\text{Ca}^{2+}$  concentration and the rate of glutamate release (Barnes & Kelly, 2002).

Two broad types of secondary neurons, horizontal cells and bipolar cells, are the direct recipients of the glutamate released by photoreceptors. There are multiple functional types of bipolar cells, but they can be divided into two classes based on whether they depolarize (OFF bipolar cells) or hyperpolarize (ON bipolar cells) in response to glutamate. The terminology is based on their opposite responses to light; because light causes slowing or cessation of glutamate release, ON bipolar cells depolarize, i.e., they turn on and can fire action potentials,

### Cone bipolar cells signal to ganglion cells, and rod bipolar cells signal to AII amacrine cells

The synaptic termini of bipolar cells, like those of photoreceptors, contain ribbon synapses and release glutamate. Cone bipolar cells communicate directly with ganglion cells, which have their own set of postsynaptic ionotropic glutamate receptors. In contrast, the major pathway for signaling by rod ON bipolar cells is through synaptic contacts with AII amacrine cells. These cells contain ionotropic receptors that trigger depolarization in response to glutamate, and this response is communicated in two different ways to cone bipolar cells. AII amacrine cells form direct electrical connections through gap junctions on cone ON bipolar cells, so rod bipolar cell depolarization contributes to depolarization of cone ON bipolar cells. AII amacrine cells also form inhibitory chemical synapses with cone OFF bipolar cells. Upon depolarization, they release glycine, an inhibitory neurotransmitter, which causes opening of chloride channels on the postsynaptic membranes of the OFF bipolar cells. Acceleration of chloride entry into the cell promotes hyperpolarization, and thus inhibits glutamate release by OFF bipolar cells. There are multiple additional types of amacrine cells, which, along with horizontal cells, mediate lateral communication and signal modulation among neurons of the retina.

## RECYCLING OF PHOTOTRANSDUCTION MOLECULES

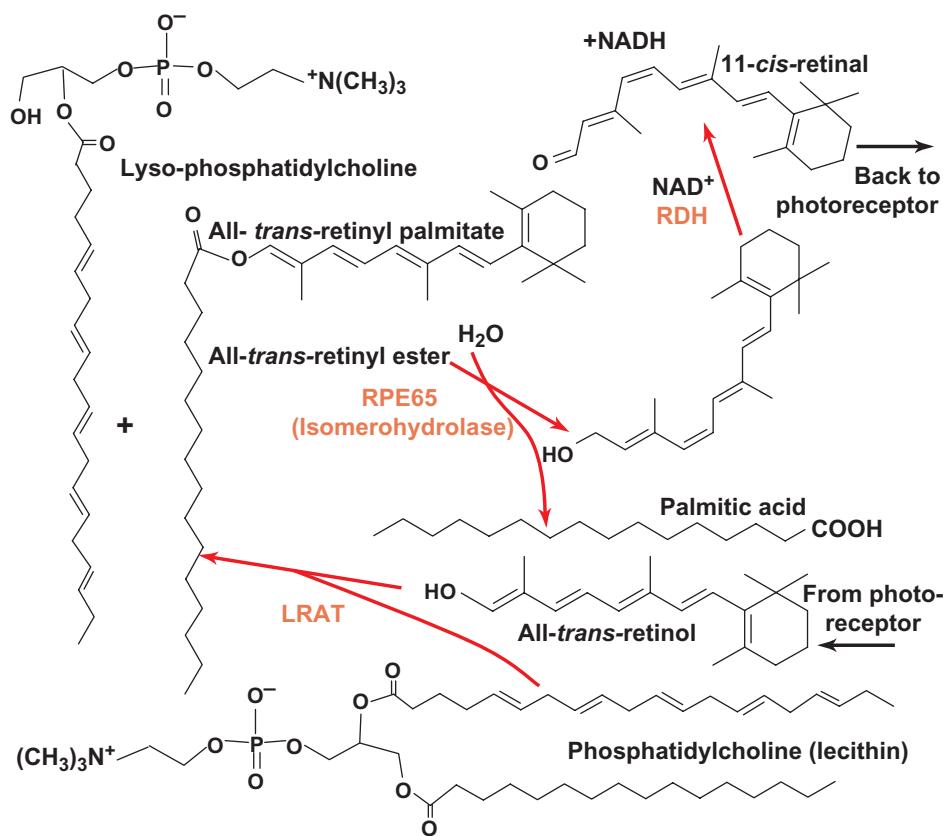
Rhodopsin regeneration requires a complex series of enzyme-catalyzed reactions in photoreceptors and RPE

The all-trans-retinal covalently linked to photoexcited rhodopsin does not remain attached indefinitely after photoisomerization. The Schiff's base linkage to lysine 296 is accessible to water and becomes hydrolyzed, leading to the formation of all-trans-retinal and opsin. At this point, the opsin is phosphorylated and has arrestin bound, but dissociation of all-trans-retinal lowers its susceptibility to phosphorylation by rhodopsin kinase and its affinity for arrestin, so it becomes dephosphorylated by the action of protein phosphatase 2A. Opsin can be regenerated to form rhodopsin by binding 11-cis-retinal, but formation of 11-cis-retinal from all-trans-retinal requires a series of enzyme-catalyzed reactions known as the visual cycle (McBee et al., 2001).

As an aldehyde, all-trans-retinal is capable of reacting with lysyl and amino terminal groups of proteins, as well as with phosphatidylethanolamine, an abundant phospholipid in outer

segment membranes, potentially generating toxic by-products. It is rapidly converted to a more inert alcohol form, all-trans-retinol, by the action of one or more oxidoreductase enzymes in the photoreceptor known as retinol dehydrogenases, or RDH. Defects in genes encoding RDH8 and RDH12, lead to visual defects, but additional RDH isoforms can reduce all-trans-retinal in their absence (Maeda et al., 2007; Parker & Crouch, 2010).

Subsequent events in the rod visual cycle take place in the RPE (retinal pigmented epithelium; see Fig. 51-10). There, all-trans-retinol is coupled in an ester linkage to fatty acids transferred from phosphatidylcholine by the action of the enzyme lecithin:retinol acyl transferase (LRAT). Fatty acyl esters are the major forms of all-trans-retinol in the retinal. The isomerization of retinol from all-trans to the 11-cis isomer is catalyzed in a remarkable reaction that couples energy released by hydrolysis of the ester bond to the energy-requiring isomerization (Deigner et al., 1989; Gollapalli & Rando, 2003; Rando, 1991). This reaction is catalyzed by an isomerohydrolase enzyme known as RPE65, because of its 65 kDa size (Hamel et al., 1993; Moiseyev et al., 2006). Finally, 11-cis-retinol is oxidized, using NAD as oxidant, to form 11-cis-retinal, in a reaction catalyzed by another RDH



**FIGURE 51-10** Recycling of retinoids by the enzymes of the visual cycle in the retinal pigmented epithelium (RPE). The alcohol all-trans-retinol moves from the photoreceptors to the RPE, where it is conjugated to fatty acids to produce retinyl esters in a reaction catalyzed by lecithin:retinol acyl transferase (LRAT), which transfers the fatty acyl group from phosphatidylcholine (lecithin). The retinyl esters then serve as substrates for a reaction catalyzed by an isomerohydrolase enzyme known as RPE65, in which the energy released by ester hydrolysis is harnessed to effect a *trans*-to-*cis* isomerization at the 11-position to form 11-cis-retinol. The 11-cis retinol is then oxidized in a reaction catalyzed by another retinol dehydrogenase (RDH) enzyme to form the aldehyde 11-cis-retinal, which returns to photoreceptors for regeneration of rhodopsin and cone pigments.

isoform, primarily RDH5, and facilitated by cellular retinaldehyde binding protein (CRALBP) (Saari & Crabb, 2005). The visual cycle is completed when 11-cis-retinal returns to the rod and reacts with opsin to regenerate rhodopsin.

### Cones use a visual cycle distinct from that of rods to regenerate pigments

Cones have additional mechanisms available for processing photoisomerized retinoids (Wang & Kefalov, 2010). These likely involve Müller cells, and include enzymes that catalyze isomerization of all-trans-retinol, rather than all-trans-retinyl esters, transfer of fatty acid to retinol from acyl coenzyme A instead of from phosphatidylcholine, and hydrolysis of retinyl esters, as well as a retinol dehydrogenase that uses NADP and has a preference for 11-cis-retinol.

### Retinal pigmented epithelial (RPE) cells promote disk membrane turnover by phagocytosis

In addition to their important role in recycling retinoids, RPE cells are important in facilitating turnover of disk membranes and their constituents. Every day about 10% of the disks at the distal end of the outer segment are engulfed and internalized through phagocytosis by the processes of RPE cells in which they are embedded. This process of destruction is balanced by a renewal of disk membranes at the base of the outer segments. A genetic disruption of a gene essential for this process, MERTK, leads to retinal degeneration in rodents and humans (Strick & Vollrath, 2010).

## RETINAL NEURODEGENERATION

### Defects in genes essential for functions of photoreceptors cause retinal degeneration

There are a large number of inherited disorders (Wright et al., 2010) leading to neurodegeneration of the retina (tabulated at <http://www.sph.uth.tmc.edu/retnet/>). Identification of the molecular bases for these conditions has provided insights into functions of normal retina and RPE, and has led to development of animal models useful for testing both gene-specific and more generic therapies for retinal neurodegeneration (see Box 51-1). The significance of these studies goes well beyond the very important goal of identifying new therapeutic approaches to blinding diseases. As an exceptionally accessible part of the brain, the retina serves as an excellent test bed for developing concepts and novel therapeutics for neurodegeneration in general.

Retinitis pigmentosa, or RP, is an unscientific term used to identify a heterogeneous group of disorders having certain common features, including night blindness and loss of peripheral vision preceding a decline in central vision and cone function; accumulation of pigment deposits and so-called “bony spicule” appearance visible in fundus examinations; and a general decline of visual function with age. Inheritance patterns include autosomal dominant, autosomal recessive, and X-linked forms. The first gene associated with RP was the rhodopsin gene

(Dryja et al., 1990), and in the last 20 years mutations at over 100 different positions within the rhodopsin gene have been associated with this disease (McAlear et al., 2010). Defects in genes encoding multiple additional phototransduction proteins cause either RP or night blindness, including those encoding PDE6 subunits, CNG channel subunits, G<sub>ατ</sub>, rhodopsin kinase and arrestin. Genes encoding transcription factors, including CRX, NRL, NR2E3, as well as genes important for the visual cycle, including those encoding LRAT, RDH12, and RPE65, have also been associated with RP. RPE65, along with guanylate cyclase, are also associated with an early-onset blinding disease, Leber’s congenital amaurosis. Mutations in dozens of other genes, including some implicated in functions specific for photoreceptors or RPE, as well as a number involved in more generic functions, can also cause RP, while numerous others give rise to retinopathies presenting with symptoms distinct from RP.

A number of syndromic diseases termed ciliopathies, in which retinal degeneration is found in conjunction with pathologies in other organs, have been identified. The genes involved in ciliopathies encode proteins important for structure and function of primary cilia (Badano et al., 2006; Veland et al., 2009). These include the Bardet-Biedel genes, BBS1–BBS14, some of which form the “BBSome,” a membrane vesicle coat (Jin et al., 2010), and others of which are important for assembly of the BBSome or transport. Some of these conditions display digenic or more complex inheritance patterns, resulting from additive effects of defects in more than one component of the cilium-associated molecular machinery.

### Age-related macular degeneration is emerging as the most common blinding disease of the developed world

A more common form of retinal neurodegeneration than these rare genetic disorders is a multifactorial disease known as age-related macular degeneration (AMD) (Gehrs et al., 2006; Gehrs et al., 2010; Stone, 2007). AMD is characterized by a progressive loss of central vision as a result of degenerative and neovascular changes affecting the photoreceptors, RPE, choriocapillaris and Bruch’s membrane. In contrast to RP, which first afflicts the peripheral retina, the pathology of AMD is first noticed in a more central region of the retina known as the macula, which in primates contains a higher density of cones than the peripheral retina. Typically these changes are accompanied by the appearance of deposits of yellow or white material in Bruch’s membrane, known as drusen, seen upon fundus examination. AMD is usually classified as being of a “dry” or nonexudative form, or of a “wet,” exudative form, in which neovascularization, or growth of new blood vessels, leads to severe loss of vision. Large population and genome-wide association studies have implicated smoking and a number of specific alleles of certain genes as contributing risk factors. Most important of these risk- (or protection-, depending on the allele) associated genes are those encoding complement factor H (CFH) and other genes encoding complement pathway components. In addition, a clear association has been demonstrated for a locus within the chromosomal region 10q2 containing the two genes LOC387715/ARMS2 and PRS11/HTRA1; the roles of each of these are the subject of ongoing studies.

## MOLECULE-DIRECTED THERAPIES FOR RETINAL DISEASE

**Theodore G. Wensel**

Recent progress in understanding of molecular mechanisms underlying disease progression has led to the development of new therapeutic approaches to treating degenerative diseases of the retina based on identified molecular targets (Jacobson & Cideciyan, 2010). These include drugs that target specific molecules and gene replacement or supplementation strategies that use virus-derived vectors or encapsulated cells to provide missing gene products or neuroprotective factors.

Therapies targeting vascular endothelial growth factor, VEGF, or its receptor, including DNA aptamers (pegaptanib) and monoclonal antibodies (ranibizumab and, as an off-label use, bevacizumab), have been widely and successfully used for treatment of AMD (Folk & Stone, 2010; Gehrs et al., 2010). These drugs were designed to inhibit the neovascularization that accompanies the “wet” form of AMD. These treatments have been developed on the basis of many years of basic research into the molecular mechanisms of angiogenesis and its regulation. Sustained delivery of anti-angiogenic molecules using viral vectors (see below) has also been tested in animal models, with some promising results.

Extensive basic research and work with animal models have also set the stage for new molecular therapies targeting inherited retinal degeneration. The greatest progress in treatment of recessive disease using a gene replacement strategy has been achieved with vectors derived from recombinant adeno-associated virus, rAAV (Alexander & Hauswirth, 2008). The only components of the parent virus, which even in native form is nonpathogenic, contained in these vectors are the protein capsids surrounding the viral genome, and inverted repeat sequences at the termini of the single-strand recombinant DNA inserted for therapeutic purposes. Over 60 clinical trials have been conducted using rAAV (Mitchell et al., 2010) in a range of tissues, but one of most successful outcomes has been achieved in the treatment of the blinding disease Leber’s congenital amaurosis (LCA). A clinical trial (one

of three that are ongoing) using rAAV encoding RPE65 to treat patients with LCA (Simonelli et al., 2010) has demonstrated both safety and efficacy. This work was preceded by extensive studies of rAAV gene replacement in rodent and dog models of RPE65 deficiency. Recruitment is under way for a clinical trial using rAAV to treat Leber hereditary optic neuropathy, caused by a defect in the mitochondrial gene LHON gene (Lam et al., 2010). Successful treatment of animal models of achromatopsia, a disease featuring lack of cone-mediated vision, with rAAV supplying a functional gene encoding one of the cone CNG channel subunits, sets the stage for clinical trials of this approach to treating the most common cause of achromatopsia in humans (Komaromy et al., 2010). The use of lentiviral and adenoviral vectors for retinal therapy has also been proposed and tested in some animal models. A clinical trial was conducted using adenovirus for treatment of retinoblastoma (Ildefonso et al., 2010).

Whereas gene replacement therapies require a unique viral construct for each rare disease gene, neuroprotective strategies could potentially be more widely efficacious. For example, rAAV-mediated delivery of the ER resident chaperone Grp78/BiP in a rat model of retinal degeneration produced a neuroprotective effect, apparently through reducing apoptosis driven by the unfolded protein response (Gorbatyuk et al., 2010). Another chaperone-type protein, HSP90, has been used to treat an animal model of RP (Tam et al., 2010). Yet another molecular therapy that has been tested in animals is the use of light-regulated ion channels to restore light sensitivity to retinal neurons (Lagali et al., 2008). Transplantation of cells that can replace missing retinal photoreceptors or RPE cells is yet another area of active study (Lamba et al., 2010; Liao et al., 2010). Finally, induced pluripotent stem cells have been reported to be capable of producing cells with RPE and photoreceptor phenotypes (Parameswaran et al., 2010).

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