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Beyond Counting Photons: Trials and Trends in Vertebrate Visual Transduction

Review

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For over 30 years, photoreceptors have been an outstanding model system for elucidating basic principles in sensory transduction and G protein signaling. Recently, photoreceptors have become an equally attractive model for studying many facets of neuronal cell biology. The primary goal of this review is to illustrate this rapidly growing trend. We will highlight the areas of active research in photoreceptor biology that reveal how different specialized compartments of the cell cooperate in fulfilling its overall function: converting photon absorption into changes in neurotransmitter release. The same trend brings us closer to understanding how defects in photoreceptor signaling can lead to cell death and retinal degeneration.

Introduction

Phototransduction is the process by which a photon of light generates an electrical response in a photoreceptor cell. The biochemical and electrophysiological bases of phototransduction have been intensely investigated for decades. Early studies in this field led to the discovery of the first G protein-coupled receptor (GPCR), rhodopsin (Kuhne, 1879). A century later, the solution of rhodopsin's crystal structure, the only so far for any GPCR, has greatly expanded our understanding of the molecular mechanisms underlying GPCR signaling (Palczewski et al., 2000). Functional studies of rhodopsin have resulted in the discoveries of its light-dependent phosphorylation (Bownds et al., 1972; Kuhn and Dreyer, 1972; Frank et al., 1973) and the first arrestin protein (Kuhn, 1978). Eventually, these studies led to the formulation of a general principle for GPCR inactivation in which full loss of its catalytic activity requires phosphorylation followed by arrestin binding (Wilden et al., 1986).

The analysis of visual signaling downstream of rhodopsin has also yielded many fundamental findings. Studies of the phototransduction cascade have been instrumental in the elucidation of the function (Fung et al., 1981) and structure of G proteins (Noel et al., 1993; Sondek et al., 1994; Lambright et al., 1996), the development of a complete theory of signal amplification in GPCR cascades (Lamb and Pugh, 1992), the understanding that the duration of heterotrimeric G protein signaling can be regulated by acceleration of G protein GTPase activity (Arshavsky and Bownds, 1992), and the identification of the first cyclic nucleotide-gated channel (Fesenko et al., 1985). Although not a subject of this review, studies of invertebrate phototransduction have also been highly productive, exemplified by the discovery of TRP channels in *Drosophila* rhabdomeric photoreceptors (Montell and Rubin, 1989; for reviews on invertebrate phototransduction, see Zuker, 1996; Montell, 1999; Minke and Hardie, 2000; Hardie and Raghu, 2001).

The vertebrate phototransduction cascade has been the subject of many reviews (for recent detailed reviews, see Pugh and Lamb, 2000; Burns and Baylor, 2001; Fain et al., 2001; Arshavsky et al., 2002). We will minimize the overlap by providing a relatively brief overview of phototransduction and instead concentrate on several new trends in the field. One is the transition from studying rod phototransduction under primarily dark-adapted conditions or brief periods of dim illumination to studying phototransduction under conditions of prolonged, bright illumination; this regime change has revealed important functional differences in both the gain and kinetics of photoreceptor signaling for acute and long-term light adaptation. Another transition is from focusing primarily on rods to understanding the differences between rod and cone signaling. Recent years have also been marked by renewed interest in the electrophysiological processes in photoreceptor inner segments and synaptic terminals that ultimately filter and transmit the visual signal to second-order neurons. Finally, photoreceptors are increasingly used as a model system for studying many cell biological questions, including cell polarity, protein sorting and intracellular trafficking, the assembly and targeting of multiprotein signaling complexes, and the mechanisms connecting the signaling events in the outer segments with cellular health and disease.

Overview of Phototransduction

The first step in encoding visual information requires the conversion of photon absorption into the modulation of electrical activity. In vertebrate photoreceptors, phototransduction takes place in the outer segment, a distal cellular organelle connected to the rest of the photoreceptor by a thin connecting cilium. Rod outer segments are tightly packed with stacks of intracellular disc membranes, whereas cone outer segments are formed by tight involutions of the plasma membrane, forming what are called sacs (Figure 1). These membranes contain high concentrations of visual pigment (rhodopsin in rods and cone opsins in cones), increasing the probability that a photon passing through the photoreceptor will be absorbed. Outer segments also contain high levels of other proteins that participate in phototransduction, whereas they lack many proteins responsible for other cellular functions. This high degree of compartmentalization makes the outer segment ideal for biochemical studies, since outer segments can be easily separated from the rest of the retina by gentle shaking and collected in large quantities with high purity. Moreover, the outer segment plasma membrane currents are easily recorded with suction electrode pipettes, providing a real-time readout of phototransduction.

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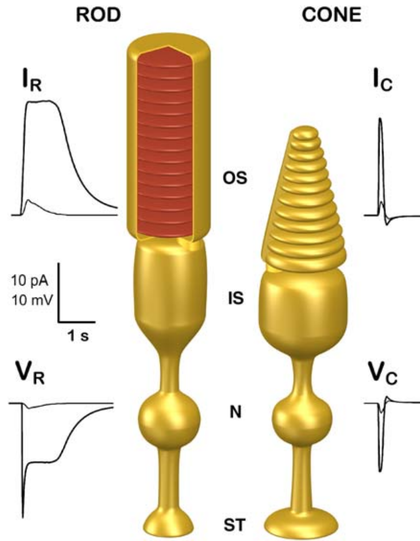

Figure 1. Schematics of Rod and Cone Photoreceptors

Photon absorption in the outer segments of a rod and a cone causes a decrease in cGMP-gated inward currents (top traces). The outer segment currents of cones are appreciably faster than those of rods and require flashes of higher intensity. The resulting membrane hyperpolarization (bottom traces) is filtered by the electrical properties of the photoreceptor membranes, including voltage-gated conductances located in the inner segments. This hyperpolarization slows neurotransmitter release from the synaptic terminals. All traces are schematic representations of responses to dim (thin) and bright (thick) brief flashes, and although generalizable to many different species, are shown here with scale bars that reflect the average properties of primate photoreceptors (Baylor et al., 1984b; Schneeweis and Schnapf, 1995, 1999), ignoring the effects of photoreceptor coupling. OS, outer segment; IS, inner segment; N, nucleus; ST, synaptic terminal.

The central molecule in phototransduction is the second messenger, cGMP. All aspects of visual signaling are dictated by the balance between its synthesis and degradation in the cytoplasm of the photoreceptor outer segment:

$$GTP \xrightarrow{cyclase} \underset{\underset{channels}{\upharpoonleft\downharpoonright}}{cGMP} \xrightarrow{PDE} GMP$$

cGMP synthesis is accomplished by guanylate cyclase, whereas cGMP hydrolysis is performed by cGMP phosphodiesterase (PDE, also known as PDE6). In the dark-adapted photoreceptor, the balance between cGMP synthesis and hydrolysis produces a steady-state level of cGMP concentration. The free cGMP concentration is constantly monitored by cGMP-gated cation channels located in the outer segment plasma membrane. The inward current through these channels keeps the cell partially depolarized. In the presence of light, cGMP levels decline as a result of PDE activation, which causes channels to close and the cell to hyperpolarize. This hyperpolarization slows neurotransmitter release from the synaptic terminal, signaling the presence of light to the secondary neurons in the retina.

Examples of photoresponses of mammalian rods and cones are shown in Figure 1. Although their light dependency and time course differ considerably, photoresponses of both photoreceptor types are produced by a transient decrease in cGMP concentration, mediated by three categories of molecular events: cascade activation, cascade inactivation, and cGMP restoration. The interplay among these reactions determines the response time course and the photoreceptor's sensitivity to light.

Phototransduction Cascade: Activation and Signal Amplification

The first event in vision is the absorption of a photon by rhodopsin (or cone opsin), which causes the *cis-trans* isomerization of its chromophore, 11-*cis*-retinal, and a conformational change to its active state (known as Metarhodopsin II or R*). Within a millisecond of photon capture, R* begins activating molecules of the G protein transducin by catalyzing GDP/GTP exchange on transducin's α subunit ($G\alpha_t$) (Figure 2). $G\alpha_t \cdot$GTP stimulates PDE activity by binding to the γ subunit of PDE, thereby releasing this subunit's inhibitory constraint on the catalytic α and β subunits of PDE. Activated PDE decreases the cGMP concentration in the photoreceptor cytoplasm, leading to channel closure. One hallmark of the phototransduction cascade is the high degree of signal amplification achieved at these steps.

Signal amplification at the first stage of the cascade is achieved by the activation of many transducins by a single R*. The rate of transducin activation approaches 150 turnovers per second in amphibian rods (Leskov et al., 2000) and is likely to be ~2-fold higher in warm-blooded animals (Heck and Hofmann, 2001). Thus, a few tens of $G\alpha_t \cdot$GTP molecules are produced in the course of a mammalian rod's response. This rate is much higher than those measured in other G protein signaling pathways (e.g., Mukhopadhyay and Ross, 1999; Bhandawat et al., 2005), which makes it a benchmark for the speed at which G proteins could be activated by GPCRs.

Equally important is the degree of signal amplification provided by activated PDE, which is one of a handful of enzymes whose catalytic activity is so high that it is limited primarily by the rate of cGMP diffusion into the catalytic site. The activation of just one of its catalytic subunits by transducin in a frog rod causes the hydrolysis of at least 600 cGMP molecules per second (Leskov et al., 2000), assuming dark-adapted amphibian rod outer segments contain ~4 μM cGMP (Pugh and Lamb, 2000).

Finally, additional signal amplification arises from the properties of the cGMP-gated channels. Because the Hill coefficient for channel opening by cGMP is ~3, a light-induced decrease in the inward current is ~3-fold larger than the decrease in cGMP concentration. Collectively, these amplification mechanisms ensure the high sensitivity of vertebrate vision, including the ability of rods to signal the absorption of single photons (Baylor et al., 1979).

Phototransduction Cascade: Inactivation

After the photoresponse reaches its peak, the inward current rapidly returns to the dark level (Figure 1). Timely recovery is essential for the photoreceptor to generate responses to subsequently absorbed photons, and to signal rapid changes in illumination. Recovery to the dark state requires efficient termination of each amplification step of the phototransduction cascade, and the rate at which these steps are inactivated sets the time course of the photoresponse.

In principle, termination of transducin activation could be achieved via the thermal decay of R*, but the rate of

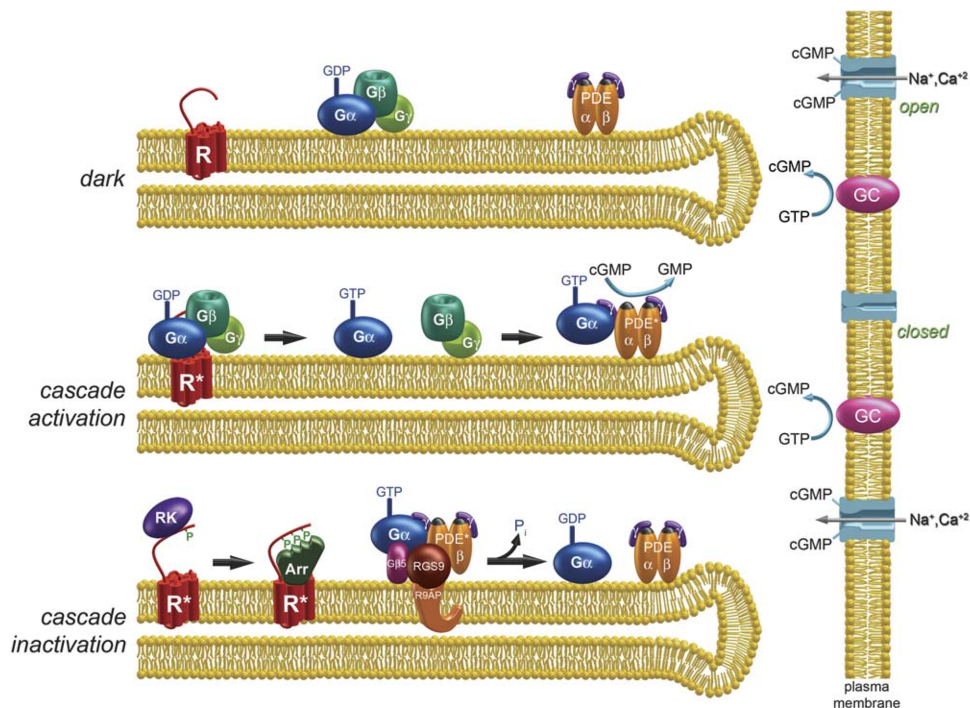

Figure 2. Schematic of the Phototransduction Cascade Activation and Inactivation

The upper disc illustrates inactive rhodopsin (R), transducin (Gα, Gβ, and Gγ subunits), and PDE (α, β, and γ subunits) in the dark. The reactions in the middle disc illustrate light-induced transducin and PDE activation. The reactions in the lower disc represent R* inactivation via phosphorylation by rhodopsin kinase (RK) followed by arrestin (Arr) binding and transducin/PDE inactivation by the RGS9-1·Gβ5·R9AP complex. Guanylate cyclase (GC) is shown as a component of the plasma membrane; in real cells it is likely to be present within the disc membranes as well.

this decay is ~1000-fold slower than the duration of a photoresponse (for a recent review, see Hofmann, 2000). Instead, rapid inactivation of R* during the photoresponse is accomplished through a specific mechanism involving phosphorylation of R* by rhodopsin kinase and subsequent arrestin binding (Figure 2). At least three phosphates are thought to be incorporated into a molecule of R* (Kennedy et al., 2001) for its timely inactivation and recovery of the photoresponse (Mendez et al., 2000). This multiple phosphorylation is necessary for high-affinity arrestin binding (Vishnivetskiy et al., 2000). It may also help to confer reproducibility to single-photon responses in rods. This is because sequential inactivation of R* by several independent molecular events, each partially reducing R* activity, produces a more uniform time course of R* inactivation than a single stochastic event, which would terminate R* activity abruptly (Rieke and Baylor, 1998; Whitlock and Lamb, 1999; Field and Rieke, 2002; Hamer et al., 2003).

The termination of PDE activation by $G\alpha_t$·GTP is achieved when the GTP bound to $G\alpha_t$ is hydrolyzed to GDP (Figure 2). Although $G\alpha_t$ is able to hydrolyze bound GTP by itself, the intrinsic rate of this GTPase activity is very slow compared to the time course of photoresponse, just as in the case of the thermal R* decay. This slow GTPase rate is significantly accelerated by a multiprotein complex containing the ninth member of the Regulators of G protein Signaling protein family (RGS9; He et al., 1998) bound to its obligatory Gβ5 subunit (Makino et al., 1999) and membrane anchor R9AP (Hu and Wensel, 2002).

cGMP Restoration: Guanylate Cyclase

Complete recovery of the photoresponse requires not only inactivation of cascade components, but also the restoration of cytoplasmic cGMP to the dark level. This is accomplished by guanylate cyclase, two isoforms of which are expressed in photoreceptors (reviewed in Pugh et al., 1997). Just as the rate of cGMP hydrolysis is regulated by light (via PDE activation), so is the rate of its synthesis, although indirectly through the changes in the intracellular Ca^{2+} concentration. Light causes a decline in the outer segment Ca^{2+} because Ca^{2+} continues to be extruded from the cell via the Ca^{2+}·K^+/Na^+ exchanger, while its entry through the cGMP-gated channels is reduced (Hodgkin et al., 1987; Nakatani and Yau, 1988). The fall in intracellular Ca^{2+} is sensed by Ca^{2+} binding proteins called guanylate cyclase-activating proteins, or GCAPs, which rapidly stimulate cGMP synthesis by guanylate cyclase (Palczewski et al., 1994; Dizhoor et al., 1995; Haeseleer et al., 1999; for a recent review, see Palczewski et al., 2004).

The Ca^{2+}/GCAP-dependent regulation of guanylate cyclase activity forms a powerful feedback mechanism in which the rate of cGMP synthesis increases as Ca^{2+} falls during the photoresponse. The abolishment of Ca^{2+} feedback to guanylate cyclase by knocking out GCAPs results in photoresponses that are much larger than normal and somewhat prolonged (Mendez et al., 2001). In addition, loss of GCAPs leads to a dramatic increase in the fluctuations in cGMP concentration in the dark (Burns et al., 2002), which arise primarily from spontaneous rhodopsin and PDE activations (Baylor

et al., 1980; Rieke and Baylor, 1996). Thus, Ca^{2+} feedback to the cyclase activity sets the photoresponse amplitude, enhances temporal response properties, and improves the signal-to-noise characteristics of the rod.

Visual Cycle

The biochemical reactions discussed so far take place on the rapid timescale of the photoresponse. However, the ability of photoreceptors to function during many hours of continuous illumination requires that inactivated visual pigment be continuously regenerated. The inactivation of R* by its phosphorylation and arrestin binding is followed by its thermal decay, ultimately producing opsin apoprotein and all-*trans*-retinal. The conversion of all-*trans*-retinal back to 11-*cis*-retinal requires a sequence of biochemical reactions, many of which take place outside the rods and cones, primarily in the retinal pigment epithelium. Collectively, these reactions are known as the retinoid cycle, or visual cycle. This cycle generates steady supply of new 11-*cis*-retinal, which recombines with free opsin within the rod and cone outer segments, reforming light-sensitive visual pigments.

Visual cycle not only maintains a pool of light-sensitive pigment available to absorb photons, but also has another important function. As a large fraction of visual pigment becomes bleached, some of the products of its thermal decay, including opsin, are incompletely inactivated and produce residual cascade activity (Cornwall and Fain, 1994). Until 11-*cis*-retinal rebinds, this activation of the phototransduction cascade produces a desensitizing “equivalent light” (Stiles and Crawford, 1932). In humans, this equivalent light fades as pigment regenerates, a process that requires many minutes and serves as the ultimate rate-limiting step in restoring sensitivity following bright light exposure (known as dark adaptation; reviewed in Lamb and Pugh (2004). In vitamin A deficiency and some forms of retinal disease resulting from mutations in visual cycle enzymes, dark adaptation is impaired due to the abundance of nonregenerated visual pigment.

Cones dark adapt more rapidly than rods (Hecht, 1937) because they regenerate their pigments more rapidly (reviewed in Lamb and Pugh, 2004). This allows them to maintain an appreciable fraction of regenerated pigment even at the highest light intensities. This is likely explained in part by the more rapid thermal decay of photoexcited cone pigment than rhodopsin (reviewed in DeGrip and Rothschild, 2000). It is also possible that cones utilize an additional regeneration pathway in which the retinal isomerization takes place in the Muller glial cells rather than in the pigment epithelium (Goldstein and Wolf, 1973; Das et al., 1992; Mata et al., 2002).

The past decade has been a time of tremendous progress in the identification of the enzymes participating in the visual cycle and the regulatory mechanisms that modulate their activity. The most recent breakthrough is the demonstration that RPE65, a protein whose mutations have been linked to a retinal dystrophy called Leber congenital amaurosis, is the retinoid isomerase acting in the pigment epithelium (Jin et al., 2005; Moiseyev et al., 2005; Redmond et al., 2005). A more detailed description of the molecular components of the visual cycle can be found in a series of recent reviews (Rando, 2001; McBee et al., 2001; Kuksa et al., 2003; Thompson and Gal, 2003; Lamb and Pugh, 2004; Saari, 2004).

Voltage-Sensitive Conductances in the Inner Segment and Synaptic Terminal Shape the Outer Segment Photoresponse for Synaptic Output

As described above, sophisticated biochemical mechanisms determine the time course of the cGMP-gated current in the outer segment, and this change in current drives the change in membrane potential that controls neurotransmitter release. However, the change in membrane potential is further shaped by voltage-sensitive conductances in the inner segment and synaptic terminals (Baylor et al., 1974; Detwiler et al., 1980; Attwell and Wilson, 1980). These conductances cause the photovoltage to reach a peak amplitude more quickly than the outer segment photocurrent (Baylor et al., 1984a), producing for bright flashes a prominent initial spike, which physiologists call the “nose” (Figure 1). The nose arises primarily from the hyperpolarization-activated cation current, I_h (Wollmuth and Hille, 1992), that rapidly depolarizes the membrane back toward its dark (resting) potential. Other voltage-dependent currents, including K^+ currents and Ca^{2+}-activated Cl^- currents (Barnes and Hille, 1989), also limit the extent of depolarization, thereby determining Ca^{2+} influx (Corey et al., 1984) and controlling the rate of neurotransmitter release from the synaptic terminal.

Unlike conventional synapses, the ribbon synapses of rods and cones release neurotransmitter continuously (reviewed in Heidelberger et al., 2005). In rods, the hyperpolarization caused by the absorption of a single photon (roughly 1–2 mV; Schneeweis and Schnapf, 1995) is sufficient to decrease neurotransmitter release and change the membrane potential of the postsynaptic bipolar cell. Not surprisingly, higher light intensities are needed to alter neurotransmitter release from cone terminals. This reduced sensitivity arises not only from differences in their phototransduction mechanisms (see below), but also because of inherent differences in the synaptic output of cones (Baylor and Fettiplace, 1977; Schnapf and Copenhagen, 1982). Recently, it has been discovered that the briefer synaptic output of cones arises from their >10-fold faster kinetics of neurotransmitter release (Rabl et al., 2005). We should also note that neurotransmitter release from both rods and cones is modulated by steady light, through regulation of the Ca^{2+} current by changes in extracellular ion concentrations (e.g., Barnes et al., 1993; Thoreson et al., 1997; DeVries, 2001; Xu and Slaughter, 2005), and by extrinsic factors like dopamine (e.g., Witkovsky et al., 1988).

Light Adaptation

The range of light intensities that we encounter daily spans more than ten orders of magnitude, and the two photoreceptor systems, rods and cones, mediate visual signaling throughout this entire range. This requires both rods and cones to constantly optimize their sensitivity as the level of illumination changes, a process known as light adaptation. Light adaptation causes two pronounced effects on the photoresponse. It decreases the response amplitude to incremental changes in illumination and speeds response kinetics. In doing

so, it rescues the cell from saturation that would otherwise occur at relatively low light intensities.

It is well known that rods contribute to vision under dim light conditions, whereas cones operate in bright light (Schultze, 1866). However, the light intensities over which rod- and cone-mediated vision overlap are not well defined. What is particularly unclear is the upper limit of light intensities over which rods remain functional. Psychophysical experiments on both normal subjects and rod monochromats estimate that rods continue to signal above 2000 scotopic trolands (reviewed in Makous, 2001), which corresponds to roughly 17,000 R*/rod/s (Kraft et al., 1993). However, single-cell recordings, traditionally performed on dark-adapted rod outer segments, show that rods saturate when presented with brief steady light of much lower intensities. For decades, this disparity has fueled interest in elucidating light adaptation from both physiological and biochemical perspectives.

Light adaptation is mediated by many mechanisms, and in general, the number of adaptational mechanisms invoked increases as the ambient light gets brighter. At least nine distinct mechanisms have well-described effects on regulating response sensitivity (summarized by Pugh et al., 1999), and several more are presently under intense investigation. The ultimate goal of adaptation on the molecular level is to keep a fraction of the cGMP-gated channels open, regardless of the extent of cascade activity. This can be accomplished by increasing the rate of cGMP synthesis, decreasing the rate of cGMP hydrolysis, and changing the sensitivity of the channels for cGMP. Here, we briefly describe the key molecular mechanisms conferring these adaptational changes.

Calcium-Dependent Adaptation

In rods, and likely also in cones, the most rapidly acting form of light adaptation, and one that contributes strongly at even the dimmest ambient light levels, is mediated by Ca^{2+} (reviewed in Fain et al., 2001). The light-induced fall in intracellular Ca^{2+}, described above, helps to maintain intracellular levels of cGMP by acting through at least three different targets. First, it persistently speeds the rate of cGMP synthesis via guanylate cyclase activation by GCAPs, thus maintaining the cytoplasmic cGMP concentration despite the high light-activated PDE activity. Thus, Ca^{2+}-dependent regulation of cyclase not only determines the shape of the flash response (see above), but also controls the steady-state level of the cGMP-gated current in continuous illumination.

A second calcium-dependent adaptation mechanism seems to require larger or longer-lasting changes in intracellular Ca^{2+} (at least in rods). It includes the regulation of rhodopsin activity by the Ca^{2+} binding protein recoverin. Ca^{2+} bound recoverin interacts with rhodopsin kinase and inhibits its ability to phosphorylate rhodopsin (Kawamura, 1993; Gorodovikova et al., 1994; Klenchin et al., 1995; Chen et al., 1995). Light-dependent reduction in intracellular Ca^{2+} relieves this inhibition and leads to a more rapid R* inactivation and ultimately to a lower level of PDE activity (Gray-Keller et al., 1993; Koutalos et al., 1995; Matthews, 1997). Experiments using rods of recoverin knockout mice indicate that this regulation by recoverin has a relatively minor effect on dim flash responses, but a larger effect on bright light responses and responses in steady light (Makino et al., 2004).

The third Ca^{2+}-dependent adaptation mechanism is the regulation of the sensitivity of the cGMP-gated channels by calmodulin or calmodulin-like proteins (Hsu and Molday, 1993; Nakatani et al., 1995; Bauer, 1996). When Ca^{2+} falls during the light response, calmodulin dissociates from the channel, increasing the channel's sensitivity to cGMP. This allows channels to report the changes in cGMP as the cGMP concentration becomes very low in a light-adapted cell. The overall effect of this sensitivity modulation in rods is thought to be relatively small. In cones, however, the effect is much more rapid and of greater magnitude (Rebrik and Korenbrot, 1998; Rebrik et al., 2000).

Adaptation by Increased Steady-State PDE Activity

In contrast to the calcium-dependent mechanisms that target specific regulatory proteins, another powerful mechanism that adjusts photoreceptor sensitivity to steady light is simply a direct consequence of transduction: the light-dependent increase in steady-state PDE activity (Nikonov et al., 2000). The fractional change in total cellular cGMP concentration caused by light is dependent on the overall level of PDE activity, both in darkness and in steady light (Hodgkin and Nunn, 1988). In the dark, when the PDE activity is low, the absorption of a photon produces a large relative change in the cGMP concentration, causing a sizable electrical response. In the light, when PDE activity is high, the activation of the same amount of PDE by a photon produces a smaller relative increase in the overall activity and a smaller relative cGMP decrease. This yields a response of substantially smaller amplitude. The effect of the light-dependent increase in PDE activity also tunes the time course of the flash response (Rieke and Baylor, 1996; Nikonov et al., 1998, 2000). These contributions of the steady PDE activity to the amplitude and time course of photoresponses may not be intuitive and so have been reviewed using various physical analogies (Pugh and Lamb, 2000; Govardovskii et al., 2000).

Slower Forms of Adaptation in Rods

Most studies addressing the mechanisms of light adaptation have been performed under conditions where light of relatively low intensity has been applied for relatively short times. Yet, it was noted long ago that further adaptational changes occur on longer timescales and in response to higher light intensities (e.g., Dowling and Ripps, 1972; Coles and Yamane, 1975). Recently, these slower forms of adaptation have been revisited in the context of the molecular mechanisms uncovered in the intervening decades. The temporal components of light adaptation were quantitatively described in intact frog rods (Calvert et al., 2002). After the rapid onset of the "classical" adaptation mechanisms described above, a slow phase of adaptation further reduced sensitivity by about 40-fold. The time constant of this phase of adaptation (~12 s) is similar to the slowest component of the light-dependent decline in intracellular Ca^{2+}, suggesting that it may be a Ca^{2+}-dependent process (Calvert et al., 2002). A robust adaptational mechanism with an even slower onset is evident in mouse rods,

where a few minutes of bright light bleaching ~2,000 R*/rod/s causes significant speeding of response recovery (Krispel et al., 2003). This acceleration of response recovery persisted long after the adapting stimulus was extinguished and the cGMP-gated current (and thus intracellular Ca^{2+} concentration) had returned to their dark levels. This suggests that this response acceleration operates via a Ca^{2+}-independent mechanism. Determining the molecular nature of these effects and their contribution to rod function at higher light intensities is an active area of current research.

Beyond Rods: Adaptation in the Photopic Range of Light Intensities

At higher light intensities, an additional form of light adaptation results from a cumulative loss of functional visual pigment due to its significant bleaching. This loss of pigment reduces the probability of successful photon absorption and thus the number of R* produced by stimulus of a given light intensity. This mechanism is particularly significant for cones, as they essentially do not electrically saturate at any steady light intensity (Barlow, 1972). Thus, cones continue to operate even when a substantial fraction of their visual pigment is bleached, with this loss of visual pigment contributing to their decrease in sensitivity (Burkhardt, 1994).

The striking ability of cones to function in very bright light likely arises from the numerous biochemical specializations that make cones less sensitive than rods. Cones appear to have reduced amplification in their phototransduction cascade (Tachibanaki et al., 2001; Nikonov et al., 2005), which may contribute to their lower light sensitivity. Cones also express much higher levels of RGS9-1 than rods (Cowan et al., 1998; Zhang et al., 2003b), which likely leads to a faster transducin inactivation and accelerated photoresponse recovery. In some species, cones also utilize a cone-specific opsin-kinase, GRK7 (Weiss et al., 2001), which has been suggested to contribute to faster and/or smaller responses via more efficient R* inactivation (Tachibanaki et al., 2005). However, this hypothesis is challenged by experiments suggesting that the rates of cone opsin and rhodopsin phosphorylation in intact photoreceptors are similar (Kennedy et al., 2004) and that the basic kinetic parameters of recombinant GRK1 and GRK7 may not be different (Horner et al., 2005).

In addition to differences in transduction machinery, intracellular free Ca^{2+} levels change much more rapidly in cones than in rods (reviewed in Korenbrot and Rebrik, 2002) and likely cause Ca^{2+} feedback mechanisms to engage more quickly. Furthermore, the magnitude of the effect of calcium feedback mechanisms may be more pronounced in cones than in rods, as exemplified by the more robust sensitivity modulation of cone channels discussed above. Elucidation of the relative contributions from these molecular differences to light adaptation in rods and cones has recently attracted significant interest. Studies in this direction are facilitated by new techniques and tools, like single-cell recordings from the cone-laden retinas of *nrl* knockout mice (Mears et al., 2001; Daniele et al., 2005) and utilization of cone-specific promoters for transgenic studies (Wang et al., 1992; Shaaban and Deeb, 1998).

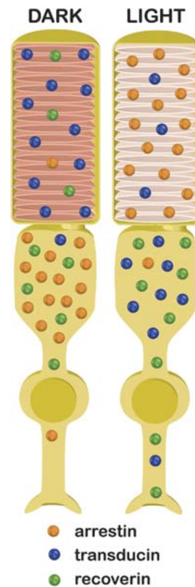

Figure 3. Schematic Representation of Transducin, Arrestin, and Recoverin Subcellular Distribution in the Dark- and Light-Adapted Rod

Protein Translocation: For Adaptation or Survival?

The adaptation mechanisms discussed so far are based on regulation of the activity of phototransduction proteins. However, bright light also causes significant changes in the amounts of some of these proteins in the outer segments. In rods, arrestin enters the outer segments upon illumination, whereas transducin and recoverin move in the opposite direction (Figure 3; Broekhuyse et al., 1985; Brann and Cohen, 1987; Philp et al., 1987; Whelan and McGinnis, 1988; Strissel et al., 2005). Arrestin also translocates in cones (Zhu et al., 2002; Zhang et al., 2003a). It is intuitive to expect that the light-induced translocation of each protein may contribute to either reduction of photoresponse sensitivity or shortening of response duration, both hallmarks of light adaptation. However, as yet there is only one report linking this phenomenon to adaptation: the extent of transducin translocation from rod outer segments correlates with a reduction in cascade amplification, with a nearly 10-fold effect occurring in saturating light (Sokolov et al., 2002).

The functional consequences of arrestin and recoverin translocation have not yet been tested, but it might be predicted that both would lead to an accelerated R* inactivation, and therefore lower light sensitivity. This effect would be additional to the amplification reduction caused by transducin translocation, because signal amplification determines the rate at which the photoresponse rises to a peak, whereas the timing of R* inactivation can only affect the time at which the peak occurs and thus the response amplitude.

While the precise relation between light-induced protein redistributions and photoreceptor light adaptation remains under active investigation, it is also possible that the functional consequences of translocation are even broader. For example, one might expect that protein translocation in rods may prevent excessive energy consumption by the phototransduction cascade during

the day when rods contribute little to vision. It is possible to estimate how much energy would be actually saved in human retinas through this mechanism. Assuming one human retina has $\sim 10^8$ rods, each containing $\sim 10^7$ transducin molecules (Rodieck, 1998), bright saturating light in the absence of translocation should maintain a constant cycle of transducin activation/inactivation at the rate of at least ~ 5 turnovers per second (the dominant rate constant of photoresponse recovery; Lyubarsky and Pugh, 1996). During a 12 hr period, this would consume a combined amount of $\sim 2 \cdot 10^{20}$ GTP molecules (or 0.33 mmol). Given that the free energy of GTP hydrolysis is $\sim$7.3 kcal/mol, the energy consumed through the hydrolysis of this GTP is $\sim$2.4 cal/retina. Thus, the translocation of 90% transducin from rod outer segments in both eyes for a period of 12 hr should save approximately 5 calories. The biological significance of such energy savings is entirely unknown, but it is widely appreciated that some of the pathological processes in the eye may arise from many years of continuous metabolic stress (c.f. Zarbin, 2004).

Another potential function of protein translocation could be to allow proofreading of key outer segment proteins by the ubiquitin proteasome system located in the inner segments. This hypothesis has been suggested by Obin and colleagues (Obin et al., 1996, 2002), who demonstrated that the γ subunit of transducin is ubiquitinylated, which is likely to target transducin for degradation by the 26S proteasome. Periodic proofreading of transducin (and perhaps other signaling proteins) may serve to minimize "molecular noise" in the phototransduction cascade, which could limit the rod's ability to reliably signal the absorption of single photons.

Finally, a reduced level of overall cellular signaling due to protein translocation may serve as a protective mechanism against apoptotic photoreceptor death caused by continuous bright illumination. This type of degeneration is particularly well documented in rodents (Chang et al., 1993) and is thought to arise, at least partially, from excessive phototransduction cascade activity, which is discussed in the next section.

A Connection between Phototransduction and Retinal Degeneration

The identification of many genes responsible for inherited retinal degeneration has demonstrated a clear link between phototransduction and the "decision" to undergo apoptotic cell death (see http://www.sph.uth.tmc.edu/RetNet/ for an updated list of mutations). A number of years ago, it was proposed that retinal degenerations caused by light, vitamin A deprivation, or certain mutations in phototransduction proteins arise from persistent activation of the phototransduction cascade (Fain and Lisman, 1993). Consistent with this "equivalent light" hypothesis, loss of functional cGMP-gated channels (Dryja et al., 1995; Bareil et al., 2001) or one of the guanylate cyclase isoforms (Perrault et al., 1996), both expected to decrease cGMP-gated currents in the dark, lead to photoreceptor degeneration in humans. Similar phenotypes were documented in rodents upon the loss of expression of homologous proteins (Leconte and Barnstable, 2000; Hüttl et al., 2005; Yang et al., 1999).

This hypothesis is also consistent with light-dependent photoreceptor degeneration in mice with impaired photoresponse turnoff due to the lack of arrestin and/or rhodopsin kinase (Chen et al., 1999a, 1999b; Hao et al., 2002). The light damage caused by the arrestin/rhodopsin kinase double knockout can be efficiently rescued by eliminating phototransduction through the knockout of transducin α subunit (Hao et al., 2002), in agreement with the idea that persistent cascade activation causes photoreceptor degeneration in this model. However, direct evidence that actual illumination that produces the same sustained cascade activity causes identical damage has not been reported. Thus, a direct causal link between equivalent light and degeneration remains to be established. In addition, light of higher intensities causes photoreceptor degeneration in wild-type mice, which could not be rescued by silencing phototransduction by knocking out transducin (Hao et al., 2002). This indicates that more than one apoptotic pathway may be stimulated by light (for a recent review, see also Reme, 2005). Additional complexity of the mechanisms governing photoreceptor degeneration is evident from the fact that the null mutations in rhodopsin kinase and arrestin that cause degeneration in mouse models cause congenital night blindness but not degeneration in humans (Yamamoto et al., 1997; Fuchs et al., 1995).

While the "equivalent light" hypothesis seems to explain the effects of mutations that produce a decrease in the photoreceptor dark current, another explanation is required to account for even more severe phenotypes caused by mutations in phototransduction proteins that apparently lead to an elevation in the photoreceptor dark current. For example, an elevated cGMP level is thought to cause photoreceptor degeneration in the classical model of the *rd* mouse where the β subunit of PDE is mutated, resulting in the loss of PDE activity (Farber and Lolley, 1974; Bowes et al., 1990; Pittler and Baehr, 1991). Similarly, the knockout of AIPL1, a chaperone required for PDE synthesis, leads to a drastic increase in the cGMP content of the mouse retina and rapid photoreceptor degeneration (Ramamurthy et al., 2004, see also Liu et al., 2004). Mutations in PDE are also associated with a subset of retinitis pigmentosa cases in humans (McLaughlin et al., 1993; Dryja et al., 1995). One more disease that might arise from an elevated dark current is a form of congenital dominant cone degeneration linked to the Y99C mutation in GCAP-1 (Payne et al., 1998). Biochemical studies indicate that this mutation results in constitutive activation of guanylate cyclase by GCAP-1 regardless of the Ca^{2+} concentration (Sokal et al., 1998; Dizhoor et al., 1998).

The cellular mechanisms connecting the defects in phototransduction with pathways involved in apoptotic cell death remain far from being understood. One curious aspect of these studies is that they reveal a rather unexpected connection between the survival of rods and cones. In many cases, mutations that lead to apoptotic death of rods eventually lead to the death of otherwise healthy cones. While the lack of rod vision does not dramatically change one's quality of life, the subsequent loss of cones leads to complete blindness. The reasons why cones do not survive without rods are unknown, but identification of rod-derived cone viability trophic factor(s) may facilitate progress in this direction (Leveillard et al., 2004). Interested readers are referred to recent reviews by Pacione et al. (2003), Delyfer et al. (2004), and

Lem and Fain (2004) for detailed discussions of the current status of this rapidly evolving field.

Although beyond the scope of this review, it is important to mention that defects in phototransduction cause only a subset of photoreceptor degenerations. Other causes include defects in cellular structure, intracellular trafficking, synaptic transmission, or deficiencies in the function of retinal pigment epithelium (reviewed in Rattner et al., 1999).

Protein Trafficking to the Outer Segment

One more area of photoreceptor biology where current success is marked by the synergy between the studies of signal transduction and photoreceptor cell biology concerns the mechanisms of outer segment compartmentalization. The protein composition of outer segments is optimized for conducting phototransduction. This compartmentalization is achieved through multiple processes regulating intracellular protein targeting and trafficking. The development of new imaging techniques and powerful transgenic animal models has greatly facilitated progress in this area during the last few years. Here, we will outline the major breakthroughs from recent studies. Readers interested in an in-depth analysis of this topic are referred to a recently published book that includes a number of excellent reviews (Williams, 2004).

The outer segment lacks the machinery required for protein synthesis and degradation, yet it undergoes constant renewal. Old discs are shed from the distal end of the outer segment, where they undergo phagocytosis by the adjacent retinal pigment epithelium. New discs are formed by the continuous delivery of lipids and proteins through the narrow connecting cilium that joins the inner and the outer segments (Young, 1967; Young and Bok, 1969). Active transport through the cilium is mediated by the microtubule-based mechanism called intraflagellar transport, or IFT (Pazour et al., 2002; reviewed in Baker et al., 2004), which utilizes two molecular motors, kinesin II (Marszalek et al., 2000) and cytoplasmic dynein 2 (Mikami et al., 2002) transporting the cargo into and out of the outer segment, respectively. The essential role of IFT in the normal morphogenesis and survival of the outer segment is evidenced by the severe defect in outer segment assembly caused by a reduced expression of one of the IFT proteins, IFT88 (Pazour et al., 2002). However, individual proteins carried by this transport system in photoreceptors have not yet been identified.

The actinomyosin transport system is also implicated in ciliary transport, with myosin VIIa likely to serve as the molecular motor (reviewed in Williams and Gibbs, 2004). It is thought to participate in rhodopsin delivery into the outer segment, since mutations in myosin VIIa result in abnormal opsin accumulation in the connecting cilium (Liu et al., 1999). A lot remains to be learned about any interplay between the IFT and actinomyosin transport systems, as well as their respective cargo molecules.

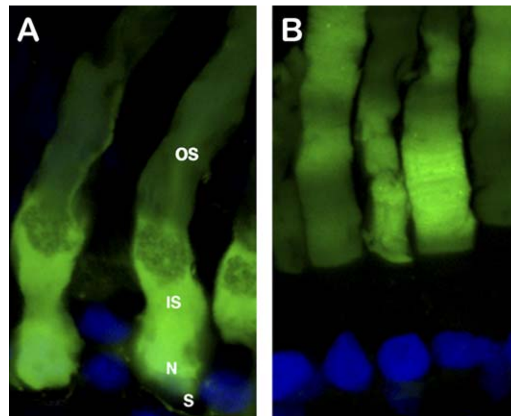

Figure 4. The Cytoplasmic Tail of Rhodopsin Redirects GFP Expression to Rod Outer Segments

(A) GFP transgenically expressed in *Xenopus* rods is distributed in the cytoplasm and enriched in the inner segment.

(B) GFP fused to the amino acid sequence corresponding to the 44 C-terminal residues of rhodopsin is expressed almost exclusively in rod outer segments. OS, outer segment; IS, inner segment; N, nucleus; S, synapse. Confocal micrographs are generously provided by D.S. Papermaster and reproduced from *The Journal of Cell Biology*, 2000, 151, pp. 1369–1380 by copyright permission of the Rockefeller University Press (Tam et al., 2000).

Protein Targeting to the Outer Segment

Proteins destined for outer segment delivery can be divided into three groups. The first are transmembrane proteins (e.g., rhodopsin, cGMP-gated channel, or guanylate cyclase), which are transported within post-Golgi vesicles from the site of their biosynthesis in the inner segment to the base of the connecting cilium. Once there, the vesicles fuse with the plasma membrane, which allows these proteins to move along the ciliary membrane into the outer segment. The second group includes proteins that are essentially soluble but are retained in the outer segment either by high-affinity protein-protein interactions (e.g., RGS9-1) or through their lipophilic posttranslational modifications (e.g., PDE). The third group includes cytoplasmic proteins, which are not solely confined to the outer segments. Some of them undergo light-driven redistribution (transducin, arrestin, and recoverin), whereas others retain their subcellular localization regardless of conditions of illumination (e.g., phosducin). Here, we will discuss a few of the targeting mechanisms revealed in recent studies.

Outer Segment Targeting of Membrane Proteins

Although it is well known that short amino acid sequences within many proteins often determine their intracellular destination (reviewed in Muth and Caplan, 2003), the identification of these sequences has proven difficult. In recent years, the sequence responsible for targeting rhodopsin to the outer segment has been identified within its C terminus (reviewed in Deretic, 2004; Sung and Chuang, 2004; Shi et al., 2004). This was accomplished by examining the subcellular localization of a series of rhodopsin C-terminal peptides fused to GFP in rods of transgenic frogs (Tam et al., 2000; Figure 4). Most recently, Deretic et al. (2005) demonstrated that this targeting sequence is recognized by a small GTP binding protein, ARF4. Based on the functions of other ARF proteins, they suggested that ARF4 plays a role in the budding of rhodopsin-enriched vesicles from the Golgi apparatus. Additional proteins are important for the trafficking of these vesicles toward the base of the

connecting cilium, where they fuse with the plasma membrane. These include the cytoplasmic dynein complex (Tai et al., 1999) and other small GTP binding proteins, such as Rab8, which are required for the docking of these vesicles at their destination (Deretic et al., 1995; Moritz et al., 2001).

The same targeting pattern was revealed in the study of photoreceptor-specific retinol dehydrogenase, although it is a peripheral rather than a transmembrane protein, involved in the visual cycle (Luo et al., 2004). A palmitoylated 16 amino acid C-terminal peptide from this protein fused to GFP was efficiently targeted to the outer segments of transgenic frog rods. A comparative analysis among rhodopsin, cone opsins, and retinol dehydrogenase suggests that the consensus outer segment targeting signal in all cases is lipid anchorage accompanied by the V/IXPX sequence.

This sequence, however, has not been found in other outer segment-specific proteins, suggesting the existence of additional targeting signals. For example, the rod outer segment targeting of peripherin 2, a protein residing at the edges of photoreceptor discs, requires 20 amino acid residues near the C terminus that are not homologous to the V/IXPX sequence (Tam et al., 2004). Furthermore, the intracellular trafficking pathways carrying rhodopsin and peripherin 2 also appear to be different, since these proteins display different patterns of abnormal localization in degenerating retinas (Fariss et al., 1997) and do not colocalize in the same vesicles (Tam et al., 2004).

Outer Segment Delivery of Multiprotein Complexes

Some proteins are delivered to the outer segment only as parts of larger multiprotein complexes. For example, RGS9-1·Gβ5 is enriched in the outer segment via its association with the transmembrane protein anchor R9AP (Hu and Wensel, 2002). R9AP binds to the N-terminal DEP domain of RGS9-1 (Lishko et al., 2002; Hu et al., 2003). Deletion of the DEP domain in mouse rods results in RGS9-1·Gβ5 mislocalization throughout the inner segment, nuclear region, and synapse. In contrast, the delivery of R9AP to the outer segment is not affected by either the DEP domain deletion or knockout of RGS9, suggesting that the targeting signal for the entire RGS9-1·Gβ5·R9AP complex is encoded within R9AP (Martemyanov et al., 2003).

The mechanism responsible for the outer segment targeting of R9AP itself remains a mystery. However, its homology to the SNARE family of proteins responsible for vesicular trafficking and fusion (Keresztes et al., 2003) suggests that common mechanisms may be utilized. Interest in R9AP and the mechanism of its intracellular targeting has been fueled by the recent discovery of its brain-specific homolog, R7BP, isolated as a protein interacting with four DEP-containing RGS proteins in the brain (Martemyanov et al., 2005). At least for the brain-specific isoform of RGS9, RGS9-2, this interaction is also mediated through the corresponding DEP domain. An independent identification of R7BP, based on its homology to R9AP, was reported by Drenan et al. (2005), who found that this protein can regulate subcellular localization of RGS proteins in cell culture through a mechanism dependent on R7BP palmitoylation. Furthermore, the role of the DEP domains, found in over 60 proteins in the human genome, in intracellular targeting of signaling proteins may extend beyond their interactions with R9AP and R7BP (for examples, see Burchett, 2000; Martemyanov et al., 2003).

Outer Segment Localization through Lipid Modifications

Another mechanism that allows a subset of signaling proteins to become enriched in the outer segment is based on their lipid modifications. Examples of phototransduction proteins containing such lipids include PDE, whose α and β subunits are modified by farnesyl and geranylgeranyl groups, respectively (Anant et al., 1992); Gα_t, which is acylated by a heterogeneous group of fatty acids (Neubert et al., 1992; Kokame et al., 1992); Gγ_t, which is farnesylated (Fukada et al., 1990); and the opsin kinase, which is farnesylated in rods (GRK1; Inglese et al., 1992; Anant and Fung, 1992) and geranylgeranylated in cones (GRK7; Hisatomi et al., 1998). While lipid modifications serve to concentrate signaling proteins on the disc membrane, they do not by themselves specify a protein for delivery to the outer segment. Recently, it has been suggested that this problem is ameliorated, at least in part, by proteins acting as "trafficking chaperones." It has been proposed that an isoprenoid binding protein, the δ subunit of PDE (Gillespie et al., 1989), serves as a chaperone for PDE delivery to the rod outer segment (Norton et al., 2005). Another "trafficking chaperone" could be phosducin (Lee et al., 1987), which is a protein that binds to the $\beta\gamma$ subunits of transducin, burying the farnesyl group into an intramolecular cleft, which makes the $\beta\gamma$ subunits more soluble (Yoshida et al., 1994; Gaudet et al., 1996). Consistent with this idea, a knockout of phosducin in mice results in almost one half of transducin $\beta\gamma$ not being delivered to the outer segments in dark-raised mice and in a reduced extent of transducin's light-driven translocation (Sokolov et al., 2004).

Some Phototransduction Proteins Are Not Confined to the Outer Segments

Surprisingly, some proteins involved in phototransduction reside primarily outside the outer segments. A striking example is recoverin; it regulates the lifetime of R* (see above), yet its outer segment fraction is only 12% in the dark and falls to under 2% in the light (Strissel et al., 2005). Presumably, the localization of most recoverin in other parts of the cell enables it to participate in other signaling pathways. A study of recoverin knockout mice demonstrated that the loss of recoverin leads to accelerated responses of rod bipolar and ganglion cells (Sampath et al., 2005). The effect on the rod bipolar response occurs before the loss of recoverin affects the outer segment photocurrent, consistent with a role for recoverin outside the outer segment.

The subcellular distribution of recoverin under different conditions of illumination may be regulated by a mechanism called the calcium-myristoyl switch (Zozulya and Stryer, 1992). Ca^{2+} binding to recoverin causes the exposure of the N-terminal myristoyl group, otherwise hidden within a hydrophobic pocket of the protein. Exposure of the myristoyl moiety promotes membrane association of recoverin. This mechanism was originally thought to regulate its activity in the outer segments

but may also be extrapolated to the entire cell, whereby the subcellular distribution of recoverin under various conditions of illumination may be determined by the combination of Ca^{2+} concentration and relative abundance of membranes in each individual compartment.

In some cases, the subcellular distribution of even closely related proteins is quite different. For example, one of the GCAP proteins, GCAP-1, is confined to the outer segments, whereas another, GCAP-2, resides predominantly in the inner segment (Dizhoor et al., 1995; Otto-Bruc et al., 1997; Cuenca et al., 1998). Both GCAPs have similar physicochemical properties and confer Ca^{2+} sensitivity to guanylate cyclase, and neither undergoes light-dependent translocation (Howes et al., 1998; Strissel et al., 2005). While the reason for their differential localization is entirely unknown, it suggests that, like recoverin, GCAP-2 also has additional signaling functions in the cell.

In summary, the last few years have marked a turning point for the field of photoreceptor biology. The tradition of quantitative rigor that gave rise to the field of phototransduction has slowly extended photoreceptors from being an experimental model for G protein signaling to serving also as a model for protein trafficking, cell polarity, neurotransmitter release, and other cell biological aspects of neuronal function. We believe that future progress in this field will be propelled by further conceptual integration between the studies of signal transduction and broader aspects of photoreceptor physiology and cell biology.

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