

ACTIVATION, DEACTIVATION, AND ADAPTATION IN VERTEBRATE PHOTORECEPTOR CELLS

Marie E Burns and Denis A Baylor

*Department of Neurobiology, Stanford University Medical Center, Stanford,
California 94305; e-mail: mburns@stanford.edu, dbaylor@stanford.edu*

Key Words rods, cones, vision, G protein, rhodopsin

■ **Abstract** Visual transduction captures widespread interest because its G-protein signaling motif recurs throughout nature yet is uniquely accessible for study in the photoreceptor cells. The light-activated currents generated at the photoreceptor outer segment provide an easily observed real-time measure of the output of the signaling cascade, and the ease of obtaining pure samples of outer segments in reasonable quantity facilitates biochemical experiments. A quiet revolution in the study of the mechanism has occurred during the past decade with the advent of gene-targeting techniques. These have made it possible to observe how transduction is perturbed by the deletion, overexpression, or mutation of specific components of the transduction apparatus.

INTRODUCTION

The purpose of this review is to assess current progress in our understanding of visual transduction. This step initiates the visual process and thus directly impacts our perception of the outside world. Whereas notable advances have been made in understanding transduction, much remains to be done, particularly in understanding the kinetics and structural basis of the underlying molecular interactions as well as their relation to pathological changes in the photoreceptor cells.

The absolute necessity of transduction for vision has important consequences. Failure of transduction, resulting from degeneration of the photoreceptor cells, occurs in the blinding diseases macular degeneration and retinitis pigmentosa, which leave the visual system without input signals to analyze. Given the intricate nature of transduction and the large amount of a photoreceptor cell's total protein content devoted to it, it is perhaps not surprising that mutations in the genes that encode transduction proteins are now known to underlie an appreciable fraction of cases of these dread diseases (reviewed in Shastry 1997, Phelan & Bok 2000).

The manner in which transduction is accomplished sets important limits on visual perception. Thus, only photons that are transduced can be seen, and the wavelength dependence of vision as well as the trichromacy of color vision result

directly from the wavelength dependence of absorption by the visual pigments. The absolute sensitivity of vision seems to be determined by the requirement that the number of transduced photons exceed the number of photon-like noise signals generated within the photoreceptor cells. Similarly, the range of light intensities in which rod and cone vision operate is set by the range over which the photoreceptor cells can generate output signals in response to incident photons. The dynamics of the light-induced signals in the retinal photoreceptors set the persistence time of visual sensations as well as our visual sensitivity to flickering lights of different frequencies. Finally, recovery of visual sensitivity after exposure to bright light is limited by the recovery of sensitivity in the transduction apparatus.

Our review is topical rather than comprehensive; interested readers may consult several other recent reviews (see e.g. *Methods in Enzymology*, Vols 315–316, *Novartis Foundation Symposium*, Vol. 224, *Handbook of Biological Physics*, Vol. 3; see also Rieke & Baylor 1998b, Sakmar 1998, Pugh et al 1999). Somewhat arbitrarily, we have divided our presentation into sections treating activation, deactivation, and adaptation (sensitivity control). Much of our review focuses on rods, which are better understood than cones.

ACTIVATION

Elucidation of the activation steps in rod phototransduction is a great success story of modern biochemistry (see Stryer 1995). In the activation process light excites rhodopsin, which activates the G protein transducin, which in turn activates the cGMP phosphodiesterase (PDE), lowering the intracellular concentration of cGMP and hyperpolarizing the cell.

Rhodopsin Activation by Light

Activation of rhodopsin begins when the absorption of a photon isomerizes rhodopsin's 11-cis retinal chromophore to the all-trans configuration (Wald 1968). The ensuing changes in the protein portion of the molecule are still not well understood. How is isomerization of the deeply buried chromophore communicated to the surrounding transmembrane helices and thence to the cytoplasmic surface of the molecule? What changes at the cytoplasmic surface confer catalytic activity?

Whereas most G protein-coupled receptors detect the presence of extracellular ligands, in the visual pigments the light-absorbing chromophore, the analog of a diffusible activating ligand, is covalently attached to the protein moiety at Lys296. The covalent attachment is advantageous because it abolishes temporal constraints imposed by ligand diffusion. Fast spectroscopic measurements indicate that photoisomerization of the chromophore occurs on a subpicosecond time scale (Hayward et al 1981, Schoenlein et al 1991). Isomerization of the chromophore then leads to series of changes in the configuration of the protein, and the form (Metarhodopsin II) competent to activate the G protein appears within a few milliseconds (Baumann 1976, Dickopf et al 1998).

The chromophore is attached to the amino group of Lys296 by a protonated Schiff base linkage. The apparent pK of this linkage is unusually high (pK > 16) (Steinberg et al 1993, Deng et al 1994) as a result of stabilization by the negatively charged Glu113 counterion (Sakmar et al 1989, Zhukovsky & Oprian 1989, Nathans 1990) and a tightly packed water molecule (Deng et al 1994, Nagata et al 1997, Creemers et al 1999, Eilers et al 1999). Biophysical studies have indicated that one of the early steps leading to the formation of Metarhodopsin II is deprotonation of the Schiff base linkage (Matthews et al 1963, Doukas et al 1978, Longstaff et al 1986).

It has been proposed that deprotonation of the Schiff base linkage leads to activation by permitting rearrangement of the helix bundle. Indeed, mutations that deconstrain the helices, particularly mutations in helix 3 and helix 6, result in constitutive activation of the mutant receptor (Han et al 1998). Constraining the helices via disulfide linkages reduces rhodopsin's ability to activate transducin (Farrens et al 1996, Sheikh et al 1996, Struthers et al 2000). Definitive understanding of the structural basis for rhodopsin activation will require crystallization and diffraction studies. A giant step toward this goal was recently achieved with the X-ray determination of bovine rhodopsin's three-dimensional structure at 2.8 Å resolution (Palczewski et al 2000). The new structure (PDB ID number 1f88) can be viewed at the website of the RCSB Protein Data Bank, www.rcsb.org/pdb. The crystal structure confirmed and extended the helical arrangement previously determined by cryo-EM on frog rhodopsin (Unger et al 1997), revealing the residues that surround the chromophore and redshift its absorption spectrum. In addition, the configuration of the chromophore itself was found to be 6s-cis, 11-cis, 12s-trans, anti C=N, settling a source of some controversy (see Grobner et al 2000). Next on the "wish list" is the structure of the catalytically active intermediate Metarhodopsin II.

Spontaneous Activation of Visual Pigments

In darkness rod cells display spontaneous noise fluctuations, one component of which resembles responses to single absorbed photons and arises from thermal activation of rhodopsin (Baylor et al 1980). In a toad rod near 20°C the rate constant for thermal activation of rhodopsin was estimated as 10^{-11} s^{-1} per molecule, corresponding to an average wait to isomerization of 2000 years. The great thermal stability of rhodopsin is highly advantageous, for it allows many molecules to be packed into a single rod cell, giving high light-catching ability, while keeping noise events confusable with single photons very rare, allowing dim light to be reliably detected. The basis for rhodopsin's thermal stability, as well as the reaction mechanism that leads to thermal activation, are still not well understood.

The temperature dependence of thermal activation of rhodopsin in toad rods gave an apparent activation energy of about 22 kcal/mole (Baylor et al 1980). This is similar to the activation energy for thermal isomerization of 11-cis retinal in solution (Hubbard 1966), suggesting that thermal activation resulted

from spontaneous isomerization of the retinal chromophore. However, the activation energy of both processes is much smaller than the energy stored in an early intermediate (bathorhodopsin) of photoexcited rhodopsin (36 kcal/mole, Cooper 1979), suggesting that photoexcitation of rhodopsin proceeds over a much larger energy barrier than thermal isomerization. Furthermore, the apparent rate constant for thermal activation of a rhodopsin molecule is two to three orders of magnitude lower than that for thermal isomerization of 11-cis retinal in solution (Hubbard 1966, Baylor et al 1980). Assuming that thermal activation of rhodopsin is triggered by cis-trans isomerization of the retinal chromophore, the discrepancies might be explained by assuming that thermal activation of rhodopsin proceeds by a different mechanism than photoexcitation, or that thermal activation only takes place in rhodopsin molecules that have a very improbable structural state.

A specific hypothesis along the latter lines has been put forward by Birge & Barlow (1995), who propose that only chromophores with unprotonated Schiff base linkages to lysine 296 will undergo thermal isomerization at an appreciable rate. Because of the high pK of the protonated Schiff base (see above), few rhodopsins would be capable of undergoing isomerization in any brief interval. This model is based upon theoretical calculations as well as experimental observations on the pH dependence of the thermal event rate in *Limulus* photoreceptors (Barlow et al 1993). The behavior of the E113Q rhodopsin mutant may be consistent with the Birge-Barlow model. In this mutant, glutamate 113, the counterion to the protonated Schiff base, is changed to a neutral glutamine, reducing the pK of the Schiff base nitrogen to about 7 (Sakmar et al 1991, Lin et al 1992). This mutation causes E113Q rhodopsin to activate the G protein without light exposure (Robinson et al 1992). It is not yet clear whether the mutant rhodopsin's constitutive catalytic activity represents sudden impulsive bursts resulting from thermal isomerization of rhodopsins with unprotonated Schiff base linkages, or instead a continuous low level activity.

One problem with the Birge-Barlow model is that changes in external pH altered the relative rate of thermal events in *Limulus* photoreceptors considerably more than the expected change in the relative number of unprotonated rhodopsins (Barlow et al 1993). Furthermore, changes in internal pH changed the rate of thermal events in the opposite direction to changes in external pH (Corson & Fein 1980); the direction of the change ought to be the same if internal and external pH affect only the protonation state of the Schiff base linkage. Thus, it will be important to test the Birge-Barlow model further and extend the tests to vertebrate photoreceptors.

Rhodopsin's dark stability may also depend on interaction of the 11-cis retinal chromophore with the amino acid residues that spectrally tune the pigment. A possible connection between a pigment's wavelength of maximal absorption and its rate of spontaneous thermal activation was suggested many years ago by Barlow (1957), who proposed that the energy of the photons to which the pigment is most sensitive was the same as the energy barrier for thermal activation. Recently, it was

found that the visual pigment in long wavelength-sensitive cones of the salamander retina undergoes thermal activation at a high rate (about 600 s^{-1} per cone at room temperature). This pigment, which is maximally excited by relatively low energy photons ($\lambda_{\text{max}} 600 \text{ nm}$), is at least 300 times less stable than the pigment of short wavelength ($\lambda_{\text{max}} 430 \text{ nm}$)–sensitive cones (Rieke & Baylor 2000). However, the apparent barrier to spontaneous activation of a long wavelength pigment molecule is far less than the energy of photons at the optimal wavelength (Baylor et al 1980, Koskelainen et al 2000, Rieke & Baylor 2000). Thus, it seems that the activation energy may be related to, but certainly does not equal, the energy of the photon that the pigment maximally absorbs. The specific relation between spectral tuning and thermal stability of pigments remains to be quantitatively defined and its physical basis determined.

Amplification in the Excitation Cascade

Once activated by light, photoexcited rhodopsin (MII) generates a macroscopic electrical signal in which each photoisomerization interrupts the flow of 10^5 or more cations into the cell. This particle amplification arises primarily from three processes: (a) activation of many copies of the G protein by a single photoexcited rhodopsin; (b) hydrolysis of a large number of cGMP molecules by an activated subunit of PDE; and (c) the response of multiple channels to the drop in cGMP concentration, each channel allowing cations to enter at a high rate in the open state. One goal of work on the transduction mechanism is to understand in quantitative terms the contribution of each process to the observed amplification.

Because particle amplification is achieved during the relatively brief duration of the elementary response, a photoexcited rhodopsin molecule must increase PDE activity at a very high rate. Pugh & Lamb (1993) provided an elegant quantitative picture of this process. Using the known cubic dependence of the rod membrane current on cGMP concentration, they converted the rate of rise of the flash response into the rate of appearance of PDE activity. Assuming that the ratio $k_{\text{cat}}/K_{\text{m}}$ (in which k_{cat} is the maximal catalytic rate at saturating substrate concentration and K_{m} is the substrate concentration that gives half-maximal reaction rate) for an active subunit of PDE was $7 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, they deduced that the rate of activation of PDE subunits was about 5000 s^{-1} .

This estimate can be compared with rates obtained by physical and biochemical techniques. Measurements of the rate of transducin activation have been made by light scattering studies (e.g. Kuhn et al 1981, Vuong et al 1984, Bruckert et al 1988). This method (reviewed in Uhl et al 1990) detects a change in the scattering of infrared light following photoactivation of rhodopsin. Scattering measurements have suggested that the rate of activation of transducin by photoexcited rhodopsin could proceed as rapidly as $700\text{--}1000 \text{ s}^{-1}$ (Vuong et al 1984, Kahlert & Hofmann 1991, Bruckert et al 1992) under conditions of saturating guanosine triphosphate (GTP).

In contrast, estimates of the rate of transducin activation based on biochemical methods, such as measurement of $\text{GTP}\gamma\text{S}$ binding to nitrocellulose filters, have

repeatedly yielded much lower values (summarized in Pugh & Lamb 1993; see also Dumke et al 1994, Leskov et al 2000). Initially, the lower rates of activation were thought to be due to disruption of the rod outer segment structure and dilution of cellular components. However, in a recent study, the degree of outer segment disruption did not affect the concentration of membrane-associated transducin or the observed rate of transducin activation by rhodopsin (120 s^{-1}) (Leskov et al 2000). The large discrepancy between the rates of activation determined by light scattering and biochemical methods remains puzzling.

Additional new evidence supports the idea that transducin activation may indeed be slower than previously thought. As mentioned above, Pugh & Lamb's (1993) estimate of the rate of PDE activation depended on the catalytic power (kcat/Km) of a single activated PDE subunit. Estimated Km's for PDE have varied widely because the Km observed *in vitro* depends both on the amount of light activation and the extent of the remaining rod outer segment structure (reviewed in Pugh & Lamb 1993, Dumke et al 1994). Because it is nearly impossible to completely disrupt all disc structure *in vitro*, Leskov and colleagues (2000) measured the effective Km of PDE under conditions of minimal PDE activation using very low concentrations of GTP γ S. They found that the Km of PDE systematically decreased with the extent of PDE activation and finally reached a minimum at about $10 \mu\text{M}$. This Km, with a kcat per subunit of 2200 s^{-1} , gave a kcat/Km of $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ per subunit. The implication is that an activated subunit of PDE is roughly 30-fold more adept at hydrolyzing cGMP than previously thought. Therefore, the rate of PDE activation needed to account for the rising phase of the electrical response is 30-fold lower, or about 150 s^{-1} . These biochemical results paint a coherent new picture of the light-induced rise in PDE activity, namely a somewhat slower rate of activation and a higher catalytic activity per subunit than previously supposed.

Generation of the Electrical Signal at the cGMP-Activated Channel

The final step in activation is closure of cGMP-sensitive cation channels at the plasma membrane. In excised patches of rod outer segment membrane or internally dialyzed rod outer segments there is a cubic dependence of the membrane conductance on cGMP (e.g. Yau & Nakatani 1985; Haynes et al 1986; Zimmerman & Baylor 1986; Rieke & Baylor 1996; Ruiz & Karpen 1997, 1999). Because the value of $K_{1/2}$, the concentration of cGMP necessary to half-maximally activate the channel, is higher (ca $10\text{--}40 \mu\text{M}$) (Fesenko et al 1985, Yau & Nakatani 1985, Nakatani & Yau 1988c, Karpen & Brown 1996) than the free intracellular cGMP concentration (perhaps $3\text{--}10 \mu\text{M}$) (Nakatani & Yau 1988c, Pugh & Lamb 1993), a small relative change in the cGMP concentration produces a threefold larger relative change in the current through the channels under physiological conditions. Because currents through rod channels have little voltage dependence (Baylor & Nunn 1986), light-dependent changes in the membrane current can easily be converted to changes in cGMP concentration.

Technological advances in recent years have improved our understanding of the structure and function of the rod channel. Expression of tandemly linked channel subunit dimers suggests that native channels possess a symmetrical diagonal arrangement ($\alpha\beta\alpha\beta$) of subunits (He et al 2000b). Mutagenesis and chimera studies have enriched understanding of the conformational changes that occur during channel activation by cGMP (Matulef et al 1999, Paoletti et al 1999, Sunderman & Zagotta 1999). Modification of tryptophan residues by UV light has also provided new insights into the energetics of channel gating (Middendorf & Aldrich 2000, Middendorf et al 2000).

The sensitivity of the rod channel for cGMP varies widely in different preparations. For example, homomeric expressed channels show a lower sensitivity for cGMP (Karpen & Brown 1996, Ruiz & Karpen 1997) than native channels. Furthermore, there also appears to be significant heterogeneity even in native channels (e.g. Haynes et al 1986, Nakatani & Yau 1988c). These variations may also explain the low apparent cooperativities in some studies (Ruiz et al 1999). The sensitivity of the channel may be modulated by kinases/phosphatases endogenous to the rod (Gordon et al 1992; Molokanova et al 1997, 1999) or the oocyte expression system (Brown et al 2000).

It has recently been discovered that the alpha subunit of the cGMP-gated channel binds to the $\text{Na}^+/\text{Ca}^{2+}\text{-K}^+$ exchanger in the plasma membrane, forming a stable complex (Schwarzer et al 2000). Does this represent a mechanism for inserting channels and exchangers into the membrane in equal numbers, or does it ensure specific spatial interactions between the ions being processed? The N-terminus of the channel's beta subunit contains a glutamic acid-rich protein-like (GARP) domain that has likewise been found in other GARP proteins in rod photoreceptors (GARP-1 and GARP-2) (Korschen et al 1999). GARP-2 has been shown to bind to and inhibit light-activated PDE, but the physiological significance of this action is unknown. Furthermore, GARP-1 can bind guanylate cyclase. The landscape of vertebrate phototransduction is beginning to resemble that of *Drosophila* phototransduction, with its "transducisome" microdomains of signaling molecules (Tsunoda et al 1997). Similar microdomains of intracellular signaling have been implicated in a number of other systems (reviewed in Pawson & Scott 1997, Garner et al 2000, Sheng & Pak 2000). Future work should help to define how such domains shape the specificity and temporal characteristics of intracellular signaling.

The cone channel has a higher $K_{1/2}$ for cGMP than the rod channel (Haynes & Stotz 1997, Rebrik & Korenbrot 1998). The $K_{1/2}$ of the cone channel is also more strongly dependent on calcium (Ca) concentration in the physiological range (see adaptation section, below). Recent cloning of the channel from cones has confirmed that it is indeed a different protein than the rod channel (Bonigk et al 1996, Gerstner et al 2000).

Spontaneous Activation of PDE

Spontaneous activation of rhodopsin generates about half of the total noise variance of a rod's dark current (Baylor et al 1980). The other half of the noise variance,

termed the continuous noise, can be attributed to the spontaneous activation of individual catalytic subunits of PDE (Rieke & Baylor 1996). Analysis of the continuous noise fluctuations indicated that during the 0.5 s average lifetime of an activated PDE subunit, the hydrolytic activity was $1.6 \times 10^{-5} \text{ s}^{-1}$. This is an order of magnitude lower than the transducin-mediated PDE activity measured biochemically (Leskov et al 2000). Although each figure is an estimate, the difference suggests that spontaneous and G protein-mediated PDE activation may involve different mechanisms. In an intact rod the spontaneous activation of PDE has the functional effect of increasing the rate of cGMP turnover, thereby shortening the duration of the dim flash response (Rieke & Baylor 1996). As was recently shown by Nikonov et al (2000), background light further shortens the response to an incremental flash by increasing the steady level of PDE activity.

Despite the fact that there is nearly an order of magnitude more transducin than PDE in the outer segment, no detectable component of the continuous noise variance could be attributed to the spontaneous activation of transducin (Rieke & Baylor 1996). This fits with the low *in vitro* estimates for the spontaneous rate of nucleotide exchange in the absence of photoexcited rhodopsin, namely $1 \times 10^{-4} \text{ s}^{-1}$ (Ramdas et al 1991). This latter figure indicates that the average wait to spontaneous activation of a transducin molecule is about 8 days. Whereas this stability pales in comparison with that of rhodopsin, it is still much higher than that of PDE. Likewise, although guanylate cyclase is active in the dark, spontaneous bursts of guanylate cyclase activity, which could also produce impulsive changes in cGMP concentration, do not appear to contribute significantly to the dark noise variance.

Rate-Limiting Step of Activation

The rod channel responds within a few milliseconds to changes in cGMP concentration (Cobbs & Pugh 1987, Karpen et al 1988). Therefore, the rise of the single photon response is not limited by the response time of the channel, but by the rate of fall of the cGMP concentration. What is the rate-limiting step for the rise in PDE activity after photon absorption? In biochemical experiments, the rates of transducin and PDE activation were very similar ($\sim 120 \text{ s}^{-1}$) (Leskov et al 2000), suggesting that G protein-activation by rhodopsin rate-limits the rise in PDE activity. The rate of G protein-activation depends upon the rate of its binding to photoexcited rhodopsin as well as the rate of exchange of GTP for GDP (guanosine diphosphate) on the rhodopsin-transducin complex. A clue about which step is rate-limiting in transducin activation has recently been provided by physiological recordings from mouse rods expressing half the normal level of rhodopsin (Rh +/- rods). Photoresponses from these rods rose at twice the normal rate (PD Calvert, VI Govardovskii, N Krasnoperova, RE Anderson, J Lem, CL Makino, submitted). The authors suggest that a lower rhodopsin concentration reduces protein crowding on the disc membrane, thereby increasing rhodopsin's diffusion coefficient and its rate of encounter of transducin. This finding points to the diffusional

encounter of transducin by photoexcited rhodopsin as the rate-limiting step in PDE activation.

DEACTIVATION

Just as amplification is essential for the transduction of light into a macroscopic response, timely deactivation of the response is important for good temporal resolution. Furthermore, deactivation should be reproducible if the timing of photon absorption is to be accurately encoded. In recent years good progress has been made in understanding deactivation mechanisms in rods (see Figure 1), but important problems remain.

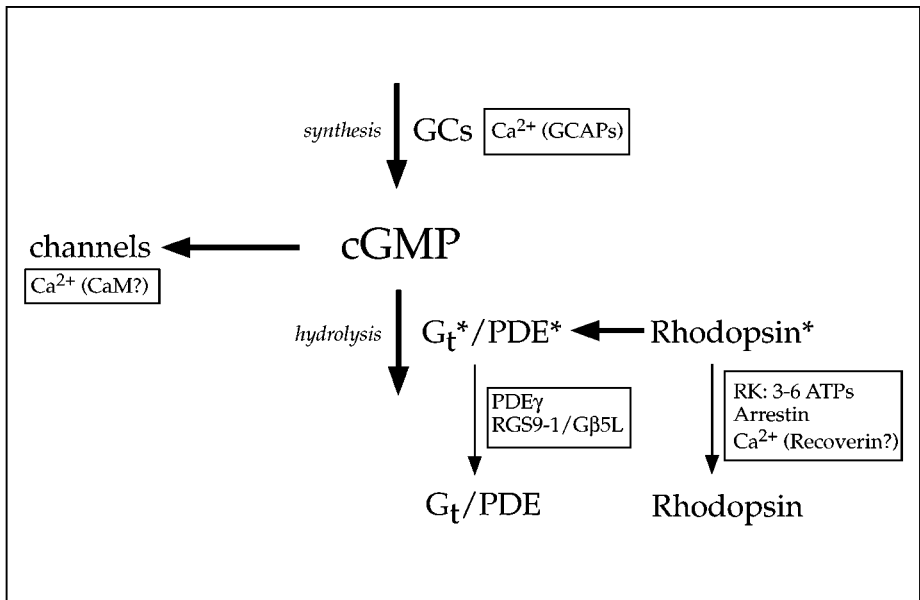


Figure 1 Scheme for regulation of visual transduction. Cyclic GMP (cGMP) controls the ion channels that generate the electrical response. Channel activation by cGMP is regulated by calcium-sensitive proteins related to calmodulin (CaM?). Synthesis of cGMP is performed by guanylate cyclases (GCs), whose activity is regulated by the calcium-dependent proteins GCAP1 and GCAP2. Hydrolysis of cGMP is accomplished by catalytically active cGMP phosphodiesterase (PDE*), which is complexed with the activated subunit of the G protein transducin (G_t*). This complex deactivates by hydrolysis of the GTP on transducin, forming inactive G_t/PDE. This deactivation requires the formation of a complex between the gamma subunit of PDE, the accelerator protein RGS9-1, and the protein Gβ5L. Photoexcited rhodopsin, which catalytically activates the G protein and PDE, is deactivated by phosphorylation catalyzed by rhodopsin kinase, followed by the binding of arrestin. The calcium-dependent protein recoverin may control the time course of rhodopsin deactivation by regulating the activity of rhodopsin kinase.

Rhodopsin Deactivation and the Reproducibility of the Single Photon Response

The amplitude and duration of the rod cell's single photon response are highly reproducible from trial to trial (Baylor et al 1979, Rieke & Baylor 1998a). This behavior is unexpected because the response is triggered by a single photoexcited rhodopsin molecule; typically, the activity of a single molecule varies widely from trial to trial. Statistical fluctuations in the duration of rhodopsin's catalytic activity ought to give rise to corresponding fluctuations in the single photon responses. Stages downstream from rhodopsin do not make the electrical response reproducible in the face of wide fluctuations in rhodopsin's activity, because rhodopsin continues to generate an amplified electrical response as long as it remains catalytically active. This is demonstrated by the elementary responses of rods in which rhodopsin deactivation was perturbed by removal (Chen et al 1995b) or substitution (Mendez et al 2000) of the phosphorylation sites, removal of adenosine triphosphate (ATP) (Nakatani & Yau 1988c), or deletion of rhodopsin kinase (Chen et al 1999). In these modified rods, abnormally large single photon responses could persist for up to 10 s. The behavior of the anomalous responses shows that reproducibility does not arise from a saturation (depletion of channels available to respond or depletion of an essential intermediate such as transducin, PDE, or cGMP) or from a nonlinear feedback loop that selectively attenuates responses produced by unusually large or long-lasting PDE activity. Thus, in the normal single photon response, rhodopsin's catalytic activity is apparently extinguished along a similar time course in every trial.

What makes rhodopsin's catalytic activity reproducible? Two candidate mechanisms have been suggested (Rieke & Baylor 1998a, Whitlock & Lamb 1999). One idea is that rhodopsin is shut off by a series of steps, each individually stochastic. If the catalytic activity in each trial depends on the cumulative behavior of these steps, reproducibility of any degree can theoretically be achieved (Figure 2). Another idea is that rhodopsin's catalytic activity may be terminated by a nonlinear feedback signal such as the fall in intracellular Ca concentration that occurs during the single photon response. Rieke & Baylor (1998a) argued that Ca feedback could not explain the reproducibility because reproducibility was substantially preserved when the intracellular Ca was clamped. In contrast, Whitlock & Lamb (1999) suggested that Ca might play an important role because incorporation of Ca buffers impaired reproducibility. However, Matthews (1995) has shown that in salamander rods the critical period for sensitivity to Ca concentration lasts only about 0.5 s after a flash. Furthermore, the change in Ca concentration caused by a bright flash caused only about a 30% change in the light-activated PDE activity (Matthews 1995). During a single photon response, the Ca concentration will hardly have changed within 0.5 s, and any possible effect on rhodopsin activity would be small.

If reproducibility depends on multiple steps in rhodopsin deactivation, how many steps are required? From an analysis of the variations in the single photon

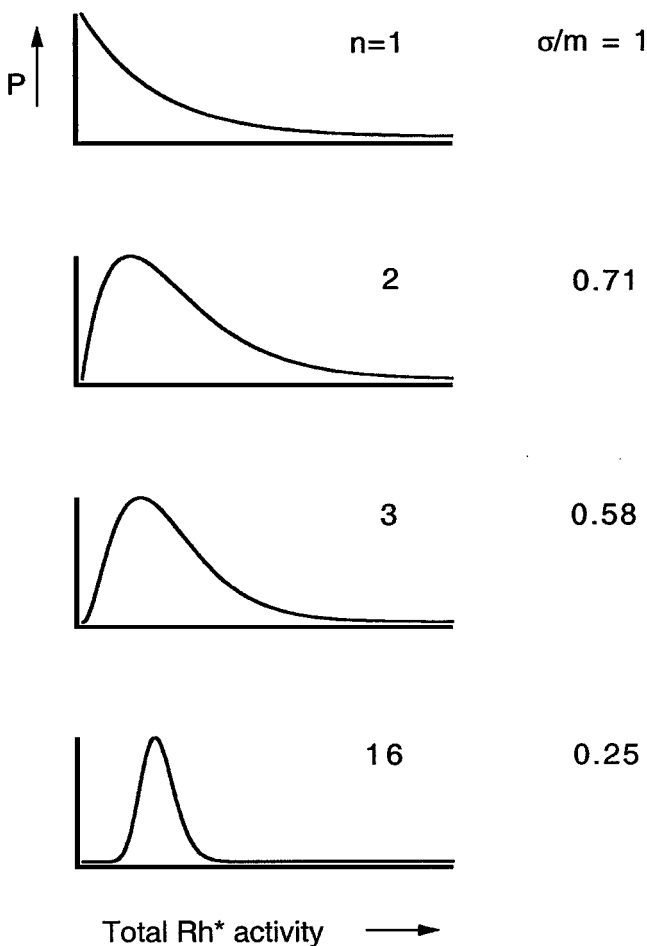


Figure 2 Multi-step mechanism for reproducibility of rhodopsin's catalytic activity. Theoretical plots of the probability (P , ordinate scale) that a molecule's total catalytic activity in a given trial will have the magnitude plotted on the abscissa. For a single step deactivation ($n = 1$) governed by first-order kinetics, the probability curve is exponential and the total activity has poor reproducibility across trials, with a ratio of standard deviation to mean (σ/m) of 1. As the number of steps in deactivation increases, the distributions become narrower and reproducibility improves. With $n = 16$, the ratio σ/m becomes 0.25, which is similar to that observed for the time integral of the single photon response (F Rieke & DA Baylor, unpublished observations). In this example it is assumed that the steps are independent, make identical contributions to lowering the catalytic activity, and have the same microscopic rate constants; the distributions have been scaled to have the same mean.

response, Rieke & Baylor (1998a) suggested that the number was 10–20. Whitlock & Lamb (1999) reported slightly more variation in the elementary response and, on the assumption that reproducibility depended on multiple steps of deactivation rather than Ca feedback, suggested that about 6 steps would suffice. For the reasons outlined, it appears that multiple-step deactivation is the more likely mechanism, and the challenge now is to identify these steps.

Biochemical studies over the years have suggested a two-step process for rhodopsin deactivation, namely phosphorylation of Metarhodopsin II followed by arrestin binding (Kuhn & Wilden 1987). Within rhodopsin's C-terminal domain there are multiple potential phosphorylation sites, six in mouse and seven in bovine. The essential role of phosphorylation in deactivation has been confirmed by the physiological experiments mentioned above (Nakatani & Yau 1988c, Chen et al 1995b, Mendez et al 2000). Mass spectrometry of purified C-terminal peptides of rhodopsin has indicated that monophosphorylated rhodopsin predominates after a light exposure (Ohguro et al 1995). However, the number and sequence of phosphorylation events that normally quench rhodopsin's catalytic activity during the single photon response have remained unclear.

Recent physiological studies on transgenic mouse rods suggest that deactivation of photoexcited rhodopsin requires multiple phosphorylation of rhodopsin (Mendez et al 2000). Single-cell recordings were made from rods expressing mutant rhodopsins whose phosphorylation sites were manipulated by site-directed mutagenesis. Mutant rhodopsins with fewer than three phosphorylation sites gave greatly prolonged single photon responses with poor reproducibility. Mutant rhodopsins with three or more phosphorylation sites gave responses that were reproducible, but all six phosphorylation sites were required for normal recovery kinetics. These results suggest that photoexcited rhodopsin may be phosphorylated at all 6 sites during normal deactivation. Whether multiple phosphorylation contributes directly to the reproducibility of rhodopsin shutoff or is merely a prerequisite for it remains to be determined.

Following phosphorylation of rhodopsin, the protein arrestin binds to rhodopsin, quenching its remaining catalytic activity (Kuhn & Wilden 1987). The essential role of arrestin binding has been demonstrated by electrical recordings from mouse rods lacking the arrestin gene; in these cells recovery of the single photon response was grossly abnormal (Xu et al 1997). Two splice-variant products of the arrestin gene, full-length arrestin (p48) and its truncated form, p44, are present in rod outer segments (Smith et al 1994). Although p44 is less abundant than full-length arrestin, p44 binds with somewhat higher affinity to phosphorylated rhodopsin (Pulvermuller et al 1997), and *in vitro* studies have suggested that p44 may be more efficient at turning off photoexcited rhodopsin (Langlois et al 1996). Which form of arrestin mediates rhodopsin deactivation in intact cells is not yet known.

Deactivation of Transducin and Phosphodiesterase

Although rhodopsin deactivation is essential for timely and reproducible deactivation of the light response, the downstream players, transducin and PDE, must

also turn off for prompt recovery of the light response. As in other G-protein cascades, the rate limiting step for deactivation of transducin and its effector is the rate of GTP hydrolysis by transducin. As long as transducin is bound to GTP it continues to bind the gamma subunit of PDE, thus relieving gamma's inhibitory constraint on the catalytic subunits of PDE (Hurley & Stryer 1982, Wensel & Stryer 1986). The hydrolysis of GTP to GDP causes transducin to dissociate from the gamma subunit of the PDE, allowing gamma to re-inhibit PDE's catalytic subunits.

What sets the rate of GTP hydrolysis by transducin? In rod photoreceptors normal transducin deactivation requires the gamma subunit of PDE (Arshavsky & Bownds 1992, Antonny et al 1993, Tsang et al 1998) as well as the GTPase accelerator protein, RGS9-1 (Angleson & Wensel 1993, He et al 1998, Chen et al 2000). RGS9-1 is a member of the family of RGS proteins (Regulators of G-protein Signaling), which accelerate GTP hydrolysis by heterotrimeric G proteins (for review see Koelle 1997). Acceleration of transducin's GTPase activity is enhanced by PDE γ (Angleson & Wensel 1994, He et al 1998), but PDE γ exerts no effect on transducin's GTPase activity in the absence of RGS9-1 (Chen et al 2000). Furthermore, a point mutation in PDE γ that impairs the binding of transducin to PDE also reduces the rate of GTP hydrolysis and the rate of recovery of the flash response (Tsang et al 1998). Together, these results suggest that RGS9-1 stimulates GTP hydrolysis by transducin only when transducin is bound to PDE. This provides an elegant mechanism for ensuring that the activation signal from photoexcited rhodopsin is received by PDE before deactivation occurs. Deactivation of a few other G proteins also appears to be regulated by the target (Berstein et al 1992, Cook et al 2000), suggesting that effector-dependent deactivation may be a general mechanism for providing efficient activation.

Recently, another player in the deactivation of transducin/PDE has emerged. In rod outer segments RGS9-1 is tightly associated (Makino et al 1999) with an unusual isoform of the beta subunit of the G protein termed G β 5L (Watson et al 1996). RGS9-1 and G β 5L appear to be coexpressed, and strictly depend upon one another for proper structure and function (He et al 2000a). Binding of transducin to PDE γ increases the affinity of the RGS9-1/G β 5L complex for transducin at least 15-fold and accelerates GTP hydrolysis by transducin to rates of 100 s⁻¹ (Skiba et al 2000). The structural aspects of this association are beginning to be understood (He et al 2000a).

Restoration of cGMP Concentration by Guanylate Cyclase

Recovery of the light response requires restoration of the cGMP concentration to the dark level. In photoreceptors this is accomplished by guanylate cyclase enzymes, GC-1 and GC-2 (or GC-E and GC-F in mice), which synthesize cGMP from GTP (for review see Pugh et al 1997). Retinal GCs belong to a family of transmembrane cyclases, many of which are regulated by extracellular peptides (Yang et al 1995). Although retinal GCs possess an extracellular domain that resembles that of their cousin enzymes, no ligand is known to regulate GC activity

in photoreceptor cells. If such a ligand existed it might provide a pathway over which higher-order neurons could influence photoreceptor signaling.

The activity of GCs is controlled by guanylate cyclase activating proteins, or GCAPs (reviewed in Dizhoor & Hurley 1999). There are at least two GCAPs in photoreceptors, GCAP-1 and GCAP-2, both of which appear to present in rods (Kachi et al 1999). Recently, a third member of the GCAP family, GCAP3, was also discovered (Haeseleer et al 1999). GCAPs activate GCs when Ca falls during the light response; they inhibit GCs when Ca is high. This calcium feedback regulation of GCs is one of several Ca-dependent mechanisms that oppose the light response (see below). Slowing or abolishing changes in intracellular calcium increases the amplitude and duration of the dark-adapted dim flash response (Korenbrodt & Miller 1986, Lamb et al 1986; Matthews et al 1988). This indicates that one or more Ca feedback mechanisms attenuate the flash response. How much of the attenuation can be attributed to increased GC activity per se remains to be determined. However, biochemical experiments have shown that the dependence of cyclase activity on calcium is cooperative, and that GCs are half-maximally activated at a calcium concentration ($K_{1/2}$) of about 200 nM (reviewed in Dizhoor & Hurley 1999). This $K_{1/2}$ is situated at about the midpoint of the rod's physiological range of calcium levels. Thus, one would expect the rod to amplify small fluctuations in calcium into larger changes in GC activity throughout the physiological range of calcium concentrations.

The role of the multiple GCAPs is unclear. Are they redundant or do they serve distinct functions? In vitro, GCAP1 activates primarily GC1, whereas GCAP2 and GCAP3 activate both GC1 and GC2 with similar potencies (Haeseleer et al 1999). Selective expression of one or more GCAPs in a GCAP knockout background is required to determine the specific functions of each GCAP.

What Rate Limits the Recovery of the Light Response?

The "dominant time constant" of recovery in rods is about 2 s in amphibian rods (Pepperberg et al 1992, Lyubarsky et al 1996) and about 0.2 s in mouse rods (Lyubarsky & Pugh 1996, Chen et al 2000). The underlying rate-limiting process is not cGMP resynthesis, because the dominant time constant is invariant for a range of flash strengths over which GC activity varies about 10-fold (Hodgkin & Nunn 1988, Koutalos et al 1995a). Instead, the rate-limiting step is almost certainly the deactivation of rhodopsin or transducin/PDE.

Experiments in truncated toad rods suggest that rhodopsin deactivation rate-limits response recovery (Rieke & Baylor 1998a). In these experiments rhodopsin's catalytic activity was assessed at various times after a flash by observing the increase in the light response produced by a sudden increase in the internal GTP concentration. These measurements showed that the catalytic activity of rhodopsin decayed with a time constant of 2 s, identical to the dominant time constant in intact toad rods.

Experiments on truncated salamander rods have been interpreted to indicate instead that rhodopsin deactivation is fast (Sagoo & Lagnado 1997). Slowing

transducin deactivation by substituting GTP γ S for GTP slowed the falling phase of the flash response but did not prolong the rising phase or increase the response amplitude, indicating that significant GTP hydrolysis did not occur during the rising phase of the response. The authors concluded that transducin deactivation is slow and that rhodopsin's catalytic activity has largely ceased by the peak of the response.

Recent studies indicate that whatever the identity of the rate-limiting step, it is governed by the rate of diffusion of one or more proteins on the disc membrane. Rods with half the normal rhodopsin content in the disc membranes generated flash responses that recovered twofold faster than normal (PD Calvert, VI Govardovskii, N Krasnoperova, RE Anderson, J Lem, CL Makino, submitted). Rods that over-express membrane-associated proteins such as RGS9-1/G β 5L and/or rhodopsin kinase may help to identify the crucial diffusing species.

Much less is known about the deactivation mechanisms of cones. Although cones use a G-protein cascade similar to that of rods, their elementary responses are much smaller and faster than those of rods. Several mechanisms may contribute to this difference. First, cones may possess unique deactivation proteins tailored for speed of operation. For example, cone photoreceptors of the ground squirrel express a specific opsin kinase (Weiss et al 1998).

A second possibility is that cones simply express higher concentrations of the proteins that mediate deactivation. Indeed, cones express more RGS9-1 than rod cells (Cowan et al 1998). Therefore, if GTP hydrolysis rate-limits recovery of the photoresponse, briefer responses may have been achieved by speeding the rate of G-protein shutoff.

A third possibility is suggested by the unique membrane topology of cones. Because the transduction machinery of cones is housed in deep invaginations of the plasma membrane rather than intracellular discs, cones have a higher ratio of membrane surface area to volume. This may permit faster changes in Ca concentration during the light response (Korenbrodt 1995). Faster changes in intracellular Ca would allow Ca feedback to attenuate the elementary response earlier (e.g. Miller & Korenbrot 1994).

Finally, the faster responses of cones may reflect the behavior of an effectively light-adapted cell. The high dark noise of cone photoreceptors (Lamb & Simon 1977), particularly that of long wavelength-sensitive cones (Rieke & Baylor 2000), mimics the effect of a steady background light of low intensity. This "equivalent background" will increase the cGMP turnover time and shorten the time required to restore the cGMP to its initial level (see below).

MECHANISMS OF BACKGROUND ADAPTATION

Changing light levels confront photoreceptor cells with a formidable problem. The transduction cascade possesses high sensitivity at low light levels so that every photoisomerization produces a detectable output response. However, incident photons must continue to generate useful signals when the mean light level

increases by many orders of magnitude. If a cell's steady-state sensitivity (ratio of response amplitude to light intensity) remained constant, the response amplitude would reach the maximal level at relatively modest light levels. Nature's solution is a nonlinear relation between response amplitude and steady light intensity: The slope is steep near the origin but progressively decreases with increasing intensity, so the response increases approximately with the log of intensity. In the steady state, small changes around the background level of the input produce small changes around the steady level of response up to very high background light levels. These incremental responses vary linearly with the incremental stimuli that produce them, and indeed under natural conditions transduction continues to operate linearly in this sense (Vu et al 1997).

The steady-state relation between response amplitude and light intensity may be viewed as the resultant of two factors: (a) a compressive nonlinearity that effectively operates instantaneously, conferring a dependence of response amplitude on light intensity that takes the form of a saturating exponential (Lamb et al 1981) and (b) time-dependent mechanisms that act over periods of seconds to minutes to selectively reduce the response to brighter lights, flattening the relation into its final form (Figure 3). In rods the time-dependent mechanisms involve the light-induced fall in intracellular Ca that results from the reduction in Ca influx through the cGMP-activated channels and continued efflux through the Na/Ca-K exchanger (Nakatani & Yau 1988b, Lagnado et al 1992).

Similar mechanisms probably also operate in cones, but in addition, pigment bleaching plays an important role in bright light. Bleaching makes fewer cone pigment molecules available to absorb photons, thus producing an effect comparable to reducing the incident light intensity (Rushton 1977). Unlike the rods, which become overloaded by the presence of bleached pigment (Lamb 1980), the cones seem designed to ignore it. They continue to transduce successfully when only a small fraction of their photopigment remains unbleached (Burkhardt 1994).

As background light reduces the amplitude of the incremental flash response, it shortens the effective duration, improving the time resolution of transduction (e.g. Baylor & Hodgkin 1974). Over a considerable range, the incremental flash sensitivity varies inversely with background intensity, as described by the Weber-Fechner relation (see Pugh et al 1999). Background adaptation is a necessary prerequisite for good function of the visual system, because if the output of the photoreceptors saturates, no amount of postreceptor processing can restore useful signaling.

Ca-Dependent Mechanisms of Adaptation

The fall in intracellular Ca concentration that accompanies the light response helps to orchestrate light adaptation. Thus, when Ca is prevented from falling the time-dependent component of background adaptation largely disappears (Matthews et al 1988, Nakatani & Yau 1988a). Lowered intracellular Ca acts upon the cascade to

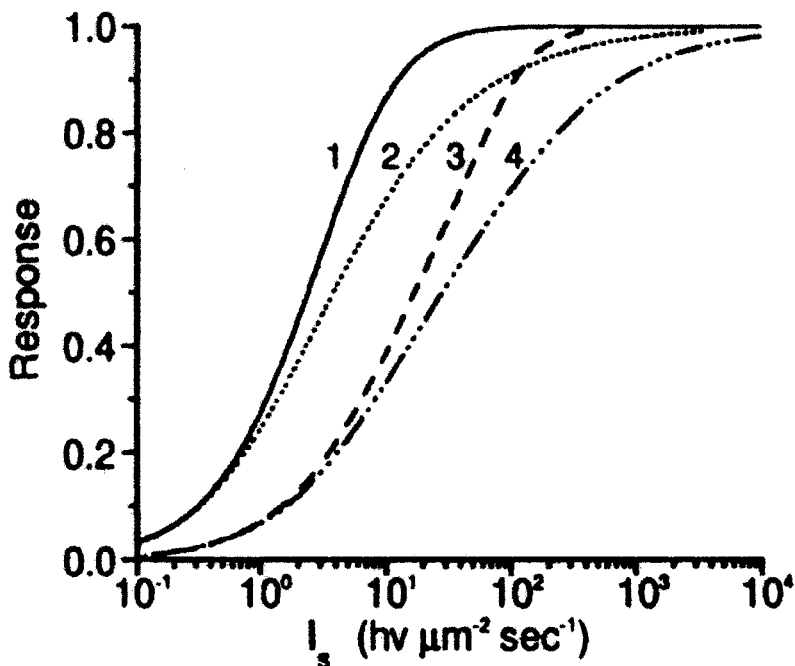


Figure 3 Ca-dependent contributions to background adaptation in a salamander rod. Calculated relations between steady-state response amplitude (ordinate) and log steady light intensity (abscissa) in a rod with intracellular Ca clamped (curve 1), with only Ca-dependent control of phosphodiesterase activity (curve 2), with only Ca-dependent control of guanylate cyclase activity (curve 3), or with both mechanisms (curve 4). The Ca-dependent reduction in phosphodiesterase activity accounts for most of the adaptation in bright light, whereas activation of cyclase activity accounts for nearly all of the sensitivity adjustment in dim light. [From Koutalos et al (1995b) by copyright permission of The Rockefeller University Press.]

oppose the effect of light, selectively reducing steady-state responses at higher stimulus intensities. Three known Ca-dependent mechanisms are thought to contribute to background adaptation: (a) regulation of light-dependent PDE activity, probably via regulation of the catalytic activity of rhodopsin, (b) regulation of guanylate cyclase activity, and (c) regulation of the cGMP-activated channel's sensitivity to cGMP.

Regulation of PDE Activity Initial indications that PDE activity might be controlled by Ca were obtained by Kawamura and colleagues in biochemical experiments on frog rod outer segment membranes. They isolated from rods a factor termed S-modulin (now known as recoverin), which prolonged the lifetime of light-activated PDE in the presence of high Ca (Kawamura & Murakami 1991).

The effect required the presence of ATP (Kawamura et al 1993), which suggests that it depended on inhibition of rhodopsin phosphorylation. Indeed, subsequent work has shown that Ca-recoverin can inhibit rhodopsin kinase (Gorodovikova et al 1994, Chen et al 1995a, Klenchin et al 1995, Sanada et al 1996, Sato & Kawamura 1997, Senin et al 1997). The binding of recoverin to rhodopsin kinase appears to be inhibited when the kinase is autophosphorylated (Satpaev et al 1998).

Physiological experiments have further tested recoverin's function in rods. Incorporation of recoverin into transducing Gecko rods prolonged the time to peak and duration of the flash response (Gray-Keller et al 1993). Similarly, in truncated salamander rods recombinant myristoylated recoverin prolonged the flash response (Erickson et al 1998). Prolongation was more pronounced after bright flashes (Erickson et al 1998, Gray-Keller et al 1993). In experiments on intact rods Matthews (1997) used an ingenious strategy to separate effects of Ca on "early" components of the cascade (light-dependent PDE activity) from "late" components (guanylate cyclase activity). His work showed that the effect of Ca on the light-dependent PDE activity depended on the Ca concentration only near the time of the flash. Furthermore, experiments in truncated salamander rods have shown that the total PDE activity is reduced when the Ca concentration is low (Sagoo & Lagnado 1997). Together, these results show that Ca-recoverin may effectively prolong the flash response in the dark-adapted state. Lagnado & Baylor (1994) observed a related effect of Ca in truncated rods and suggested that Ca might control the formation of catalytically active rhodopsin. Several lines of evidence suggest that rhodopsin's initial catalytic activity is not Ca dependent (summarized in Pugh et al 1999), and therefore it seems that Ca almost certainly controls the deactivation of rhodopsin or PDE rather than the formation of the active species.

Ca regulation of PDE activity appears to be sensitive to the prevailing Ca concentration only near the time of the flash (see above). Thus, a photon absorbed during steady background adaptation would therefore elicit a smaller, briefer response. Ca-dependent PDE adaptation makes little contribution to overall light adaptation in dim backgrounds but is particularly important in bright light (Koutalos et al 1995b) (see Figure 3 above). This mechanism is also likely to be responsible for the shortened duration of responses to saturating flashes in the presence of strong background light (Matthews 1995). To summarize, it seems that the Ca-dependent regulation of PDE, which may be mediated by recoverin, requires fairly large changes in calcium and is only sensitive to the steady-state Ca concentration at the time of the flash.

Regulation of Guanylate Cyclase Activity Activation of GC activity by the light-evoked fall in Ca increases the rate of synthesis of cGMP, opposing the light-induced drop in cGMP. Ca regulation of GC is essential for normal light adaptation (e.g. Koutalos et al 1995b). The activation of GC by steady light increases the steady state cGMP concentration and therefore reduces the steady-state response.

In bright light this fall in the steady state response rescues the cell from response saturation. Ca feedback to GCs can account for about half the total range extension (Figure 3) (Koutalos et al 1995b), accounting for roughly an order of magnitude.

Do both GCAP1 and GCAP2 contribute to GC activation during adaptation? Because both GC1 and GC2 appear to be present in rods (Yang & Garbers 1997), GCAP2 could be more important for maximal GC activation, because it can activate both GCs. Further experiments are needed to clarify the specific role of each GCAP in adaptation.

Regulation of the Channel Sensitivity Biochemical experiments on bovine ROS by Hsu & Molday (1993) demonstrated that Ca regulates the nucleotide sensitivity of the cGMP-gated channel. Low physiological Ca concentrations were found to increase the channels' affinity for cGMP, an effect that would reduce the response to bright steady light. The Ca dependence appeared to be mediated by calmodulin (CaM) (Hsu & Molday 1993, Gordon et al 1995, Bauer 1996, Haynes & Stotz 1997), which bound to the beta subunit of the channel (Chen et al 1994, Grunwald et al 1998, Weitz et al 1998) at high Ca. The association of Ca-CaM with the rod channel increases the channel's $K_{1/2}$ for cGMP from about 30 to 40 μM without affecting the Hill coefficient (Hsu & Molday 1994). Thus, when Ca falls during the steady-state response to light CaM releases its Ca and unbinds from the channel, increasing the channel's sensitivity for cGMP, opening more channels, and extending the rod's operating range. More recent experiments suggest that an additional protein may be involved in mediating the Ca dependence of channel activation by cGMP (Gordon et al 1995, Sagoo & Lagnado 1996). The cooperativity of the Ca dependence is 1.5–2 (Nakatani et al 1995, Bauer 1996, Sagoo & Lagnado 1996), and the $K_{1/2}$ of the effect is about 50 nM Ca (Hsu & Molday 1993, Nakatani et al 1995, Sagoo & Lagnado 1996). Thus, in rods it seems that the increase of the channel's sensitivity by lowered Ca will occur primarily at relatively high background intensities.

Experiments on truncated salamander rods suggested initially that the contribution of channel modulation to light adaptation in rods is smaller than that of the other Ca-dependent mechanisms (Koutalos et al 1995a). More recently, however, Sagoo & Lagnado (1996) reported that the effect could be sizable—changing the inward current by up to an order of magnitude. The magnitude and speed of the channel effect, its specific role in adaptation, and the molecular identity of the protein(s) that mediate the Ca dependence deserve further study.

In cones a more potent effect of Ca on the channel's sensitivity for cGMP has been observed (Rebrik & Korenbrot 1998, Rebrik et al 2000). The Ca modulation of the cone channels' sensitivity occurs throughout the physiological range of Ca concentrations (Rebrik et al 2000). This effect does not seem to be mediated by CaM (Hackos & Korenbrot 1997, Haynes & Stotz 1997) but by an as yet unidentified soluble factor (Hackos & Korenbrot 1997, Rebrik & Korenbrot 1998).

ACKNOWLEDGMENTS

Work from this laboratory was supported by NIH grants EY01543 and EY05750.

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED

- Angleon JK, Wensel TG. 1993. A GTPase-accelerating factor for transducin, distinct from its effector cGMP phosphodiesterase, in rod outer segment membranes. *Neuron* 11:939–49
- Angleon JK, Wensel TG. 1994. Enhancement of rod outer segment GTPase accelerating protein activity by the inhibitory subunit of cGMP phosphodiesterase. *J. Biol. Chem.* 269:16290–96
- Antonny B, Otto-Bruc A, Chabre M, Vuong TM. 1993. GTP hydrolysis by purified alpha-subunit of transducin and its complex with the cyclic GMP phosphodiesterase inhibitor. *Biochemistry* 32:8646–53
- Arshavsky V, Bownds MD. 1992. Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. *Nature* 357:416–17
- Barlow HB. 1957. Purkinje shift and retinal noise. *Nature* 179:255–56
- Barlow RB, Birge RR, Kaplan E, Tallent JR. 1993. On the molecular origin of photoreceptor noise. *Nature* 366:64–66
- Bauer PJ. 1996. Cyclic GMP-gated channels of bovine rod photoreceptors: affinity, density and stoichiometry of Ca(2+)-calmodulin binding sites. *J. Physiol.* 494:675–85
- Baumann C. 1976. The formation of metarhodopsin380 in the retinal rods of the frog. *J. Physiol.* 259:357–66
- Baylor DA, Hodgkin AL. 1974. Changes in time scale and sensitivity in turtle photoreceptors. *J. Physiol.* 242:729–58
- Baylor DA, Lamb TD, Yau KW. 1979. Responses of retinal rods to single photons. *J. Physiol.* 288:613–34
- Baylor DA, Matthews G, Yau KW. 1980. Two components of electrical dark noise in toad retinal rod outer segments. *J. Physiol.* 309:591–621
- Baylor DA, Nunn BJ. 1986. Electrical properties of the light-sensitive conductance of rods of the salamander *Ambystoma tigrinum*. *J. Physiol.* 371:115–45
- Berstein G, Blank JL, Jhon DY, Exton JH, Rhee SG, Ross EM. 1992. Phospholipase C-beta 1 is a GTPase-activating protein for Gq/11, its physiologic regulator. *Cell* 70:411–18
- Birge RR, Barlow RB. 1995. On the molecular origins of thermal noise in vertebrate and invertebrate photoreceptors. *Biophys. Chem.* 55:115–26
- Bonigk W, Muller F, Middendorff R, Weyand I, Kaupp UB. 1996. Two alternatively spliced forms of the cGMP-gated channel alpha-subunit from cone photoreceptor are expressed in the chick pineal organ. *J. Neurosci.* 16:7458–68
- Brown RL, Haley TL, Snow SD. 2000. Irreversible activation of cyclic nucleotide-gated ion channels by sulfhydryl-reactive derivatives of cyclic GMP. *Biochemistry* 39:432–41
- Bruckert F, Chabre M, Vuong TM. 1992. Kinetic analysis of the activation of transducin by photoexcited rhodopsin. Influence of the lateral diffusion of transducin and competition of guanosine diphosphate and guanosine triphosphate for the nucleotide site. *Biophys. J.* 63:616–29
- Bruckert F, Vuong TM, Chabre M. 1988. Light and GTP dependence of transducin solubility in retinal rods. Further analysis by near infrared light scattering. *Eur. Biophys. J.* 16:207–18
- Burkhardt DA. 1994. Light adaptation and photopigment bleaching in cone photoreceptors

- in situ in the retina of the turtle. *J. Neurosci.* 14:1091–105
- Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI. 2000. Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature* 403:557–60
- Chen CK, Inglese J, Lefkowitz RJ, Hurley JB. 1995. Ca(2+)-dependent interaction of recoverin with rhodopsin kinase. *J. Biol. Chem.* 270:18060–66
- Chen CK, Burns ME, Spencer M, Niemi GA, Chen J, et al. 1999. Abnormal photoreponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc. Natl. Acad. Sci. USA* 96:3718–22
- Chen J, Makino CL, Peachey NS, Baylor DA, Simon MI. 1995. Mechanisms of rhodopsin inactivation in vivo as revealed by a COOH-terminal truncation mutant. *Science* 267:374–77
- Chen TY, Illing M, Molday LL, Hsu YT, Yau KW, Molday RS. 1994. Subunit 2 (or beta) of retinal rod cGMP-gated cation channel is a component of the 240-kDa channel-associated protein and mediates Ca(2+)-calmodulin modulation. *Proc. Natl. Acad. Sci. USA* 91:11757–61
- Cobbs WH, Pugh EN Jr. 1987. Kinetics and components of the flash photocurrent of isolated retinal rods of the larval salamander, *Ambystoma tigrinum*. *J. Physiol.* 394:529–72
- Cook B, Bar-Yaacov M, Cohen Ben-Ami H, Goldstein RE, Paroush Z, et al. 2000. Phospholipase C and termination of G-protein-mediated signalling in vivo. *Nat. Cell Biol.* 2:296–301
- Cooper A. 1979. Energy uptake in the first step of visual excitation. *Nature* 282:531–33
- Corson DW, Fein A. 1980. The pH dependence of discrete wave frequency in *Limulus* ventral photoreceptors. *Brain Res.* 193:558–61
- Cowan CW, Fariss RN, Sokal I, Palczewski K, Wensel TG. 1998. High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. *Proc. Natl. Acad. Sci. USA* 95:5351–56
- Creemers AF, Klaassen CH, Bovee-Geurts PH, Kelle R, Kragl U, et al. 1999. Solid state 15N NMR evidence for a complex Schiff base counterion in the visual G-protein-coupled receptor rhodopsin. *Biochemistry* 38:7195–99
- Deng H, Huang L, Callender R, Ebrey T. 1994. Evidence for a bound water molecule next to the retinal Schiff base in bacteriorhodopsin and rhodopsin: a resonance Raman study of the Schiff base hydrogen/deuterium exchange. *Biophys. J.* 66:1129–36
- Dickopf S, Mielke T, Heyn MP. 1998. Kinetics of the light-induced proton translocation associated with the pH-dependent formation of the metarhodopsin I/II equilibrium of bovine rhodopsin. *Biochemistry* 37:16888–97
- Dizhoor AM, Hurley JB. 1999. Regulation of photoreceptor membrane guanylyl cyclases by guanylyl cyclase activator proteins. *Methods* 19:521–31
- Doukas AG, Aton B, Callender RH, Ebrey TG. 1978. Resonance Raman studies of bovine metarhodopsin I and metarhodopsin II. *Biochemistry* 17:2430–35
- Dumke CL, Arshavsky VY, Calvert PD, Bownds MD, Pugh EN Jr. 1994. Rod outer segment structure influences the apparent kinetic parameters of cyclic GMP phosphodiesterase. *J. Gen. Physiol.* 103:1071–98
- Eilers M, Reeves PJ, Ying W, Khorana HG, Smith SO. 1999. Magic angle spinning NMR of the protonated retinylidene Schiff base nitrogen in rhodopsin: expression of 15N-lysine- and 13C-glycine-labeled opsin in a stable cell line. *Proc. Natl. Acad. Sci. USA* 96:487–92
- Erickson MA, Lagnado L, Zozulya S, Neubert TA, Stryer L, Baylor DA. 1998. The effect of recombinant recoverin on the photoresponse of truncated rod photoreceptors. *Proc. Natl. Acad. Sci. USA* 95:6474–79
- Farrens DL, Altenbach C, Yang K, Hubbell WL, Khorana HG. 1996. Requirement of rigid-body motion of transmembrane helices

- for light activation of rhodopsin. *Science* 274:768–70
- Fesenko EE, Kolesnikov SS, Lyubarsky AL. 1985. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* 313:310–13
- Garner CC, Nash J, Haganir RL. 2000. PDZ domains in synapse assembly and signalling. *Trends Cell Biol.* 10:274–80
- Gerstner A, Zong X, Hofmann F, Biel M. 2000. Molecular cloning and functional characterization of a new modulatory cyclic nucleotide-gated channel subunit from mouse retina. *J. Neurosci.* 20:1324–32
- Gordon SE, Brautigan DL, Zimmerman AL. 1992. Protein phosphatases modulate the apparent agonist affinity of the light-regulated ion channel in retinal rods. *Neuron* 9:739–48
- Gordon SE, Downing-Park J, Zimmerman AL. 1995. Modulation of the cGMP-gated ion channel in frog rods by calmodulin and an endogenous inhibitory factor. *J. Physiol.* 486:533–46
- Gorodovikova EN, Gimelbrant AA, Senin II, Philippov PP. 1994. Recoverin mediates the calcium effect upon rhodopsin phosphorylation and cGMP hydrolysis in bovine retina rod cells. *FEBS Lett.* 349:187–90
- Gray-Keller MP, Polans AS, Palczewski K, Detwiler PB. 1993. The effect of recoverin-like calcium-binding proteins on the photoreceptor response of retinal rods. *Neuron* 10:523–31
- Grobner G, Burnett II, Glaubitz C, Choi G, Mason AJ, Watts A. 2000. Observations of light-induced structural changes of retinal within rhodopsin. *Nature* 405:810–13
- Grunwald ME, Yu WP, Yu HH, Yau KW. 1998. Identification of a domain on the beta-subunit of the rod cGMP-gated cation channel that mediates inhibition by calcium-calmodulin. *J. Biol. Chem.* 273:9148–57
- Hackos DH, Korenbrot JJ. 1997. Calcium modulation of ligand affinity in the cyclic GMP-gated ion channels of cone photoreceptors. *J. Gen. Physiol.* 110:515–28
- Haeseleer F, Sokal I, Li N, Pettenati M, Rao N, et al. 1999. Molecular characterization of a third member of the guanylyl cyclase-activating protein subfamily. *J. Biol. Chem.* 274:6526–35
- Han M, Smith SO, Sakmar TP. 1998. Constitutive activation of opsin by mutation of methionine 257 on transmembrane helix 6. *Biochemistry* 37:8253–61
- Haynes LW, Kay AR, Yau KW. 1986. Single cyclic GMP-activated channel activity in excised patches of rod outer segment membrane. *Nature* 321:66–70
- Haynes LW, Stotz SC. 1997. Modulation of rod, but not cone, cGMP-gated photoreceptor channels by calcium-calmodulin. *Vis. Neurosci.* 14:233–39
- Hayward G, Carlsen W, Siegman A, Stryer L. 1981. Retinal chromophore of rhodopsin photoisomerizes within picoseconds. *Science* 211:942–44
- He W, Cowan CW, Wensel TG. 1998. RGS9, a GTPase accelerator for phototransduction. *Neuron* 20:95–102
- He W, Lu L, Zhang X, El-Hodiri HM, Chen CK, et al. 2000a. Modules in the photoreceptor RGS9-1/Gbeta5L GAP complex control effector coupling, GTPase acceleration, protein folding, and stability. *J. Biol. Chem.* 275:37093–100
- He Y, Ruiz M, Karpen JW. 2000b. Constraining the subunit order of rod cyclic nucleotide-gated channels reveals a diagonal arrangement of like subunits. *Proc. Natl. Acad. Sci. USA* 97:895–900
- Hodgkin AL, Nunn BJ. 1988. Control of light-sensitive current in salamander rods. *J. Physiol.* 403:439–71
- Hsu YT, Molday RS. 1993. Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. *Nature* 361:76–79
- Hsu YT, Molday RS. 1994. Interaction of calmodulin with the cyclic GMP-gated channel of rod photoreceptor cells. Modulation of activity, affinity purification, and localization. *J. Biol. Chem.* 269:29765–70
- Hubbard R. 1966. The stereoisomerization of 11-cis-retinal. *J. Biol. Chem.* 241:1814–18
- Hurley JB, Stryer L. 1982. Purification and

- characterization of the gamma regulatory subunit of the cyclic GMP phosphodiesterase from retinal rod outer segments. *J. Biol. Chem.* 257:11094–99
- Kachi S, Nishizawa Y, Olshevskaya E, Yamazaki A, Miyake Y, et al. 1999. Detailed localization of photoreceptor guanylate cyclase activating protein-1 and -2 in mammalian retinas using light and electron microscopy. *Exp. Eye Res.* 68:465–73
- Kahlert M, Hofmann KP. 1991. Reaction rate and collisional efficiency of the rhodopsin-transducin system in intact retinal rods. *Biophys. J.* 59:375–86
- Karpen JW, Brown RL. 1996. Covalent activation of retinal rod cGMP-gated channels reveals a functional heterogeneity in the ligand binding sites. *J. Gen. Physiol.* 107:169–81
- Karpen JW, Zimmerman AL, Stryer L, Baylor DA. 1988. Gating kinetics of the cyclic-GMP-activated channel of retinal rods: flash photolysis and voltage-jump studies. *Proc. Natl. Acad. Sci. USA* 85:1287–91
- Kawamura S, Hisatomi O, Kayada S, Tokunaga F, Kuo CH. 1993. Recoverin has S-modulin activity in frog rods. *J. Biol. Chem.* 268:14579–82
- Kawamura S, Murakami M. 1991. Calcium-dependent regulation of cyclic GMP phosphodiesterase by a protein from frog retinal rods. *Nature* 349:420–23
- Klenchin VA, Calvert PD, Bownds MD. 1995. Inhibition of rhodopsin kinase by recoverin. Further evidence for a negative feedback system in phototransduction. *J. Biol. Chem.* 270:16147–52
- Koelle MR. 1997. A new family of G-protein regulators—the RGS proteins. *Curr. Opin. Cell Biol.* 9:143–47
- Korenbrot JI. 1995. Ca²⁺ flux in retinal rod and cone outer segments: differences in Ca²⁺ selectivity of the cGMP-gated ion channels and Ca²⁺ clearance rates. *Cell Calcium* 18:285–300
- Korenbrot JI, Miller DL. 1986. Calcium ions act as modulators of intracellular information flow in retinal rod phototransduction. *Neurosci. Res. Suppl.* 4:S11–34
- Korschen HG, Beyermann M, Muller F, Heck M, Vantler M, et al. 1999. Interaction of glutamic-acid-rich proteins with the cGMP signalling pathway in rod photoreceptors. *Nature* 400:761–66
- Koskelainen A, Ala-Laurila P, Fyhrquist N, Donner K. 2000. Measurement of thermal contribution to photoreceptor sensitivity. *Nature* 403:220–23
- Koutalos Y, Nakatani K, Tamura T, Yau KW. 1995a. Characterization of guanylate cyclase activity in single retinal rod outer segments. *J. Gen. Physiol.* 106:863–90
- Koutalos Y, Nakatani K, Yau KW. 1995b. The cGMP-phosphodiesterase and its contribution to sensitivity regulation in retinal rods. *J. Gen. Physiol.* 106:891–921
- Kuhn H, Bennett N, Michel-Villaz M, Chabre M. 1981. Interactions between photoexcited rhodopsin and GTP-binding protein: kinetic and stoichiometric analyses from light-scattering changes. *Proc. Natl. Acad. Sci. USA* 78:6873–77
- Kuhn H, Wilden U. 1987. Deactivation of photoactivated rhodopsin by rhodopsin-kinase and arrestin. *J. Recept. Res.* 7:283–98
- Lagnado L, Baylor DA. 1994. Calcium controls light-triggered formation of catalytically active rhodopsin. *Nature* 367:273–77
- Lagnado L, Cervetto L, McNaughton PA. 1992. Calcium homeostasis in the outer segments of retinal rods from the tiger salamander. *J. Physiol.* 455:111–42
- Lamb TD. 1980. Spontaneous quantal events induced in toad rods by pigment bleaching. *Nature* 287:349–51
- Lamb TD, Matthews HR, Torre V. 1986. Incorporation of calcium buffers into salamander retinal rods: a rejection of the calcium hypothesis of phototransduction. *J. Physiol.* 372:315–49
- Lamb TD, McNaughton PA, Yau KW. 1981. Spatial spread of activation and background desensitization in toad rod outer segments. *J. Physiol.* 319:463–96

- Lamb TD, Simon EJ. 1977. Analysis of electrical noise in turtle cones. *J. Physiol.* 272:435–68
- Langlois G, Chen CK, Palczewski K, Hurley JB, Vuong TM. 1996. Responses of the phototransduction cascade to dim light. *Proc. Natl. Acad. Sci. USA* 93:4677–82
- Leskov IB, Klenchin VA, Handy JW, Whitlock GG, Govardovskii VI, et al. 2000. The gain of rod phototransduction: reconciliation of biochemical and electrophysiological measurements. *Neuron* 27:525–37
- Lin SW, Sakmar TP, Franke RR, Khorana HG, Mathies RA. 1992. Resonance Raman microprobe spectroscopy of rhodopsin mutants: effect of substitutions in the third transmembrane helix. *Biochemistry* 31:5105–11
- Longstaff C, Calhoon RD, Rando RR. 1986. Deprotonation of the Schiff base of rhodopsin is obligate in the activation of the G protein. *Proc. Natl. Acad. Sci. USA* 83:4209–13
- Lyubarsky A, Nikonov S, Pugh EN Jr. 1996. The kinetics of inactivation of the rod phototransduction cascade with constant Ca^{2+} . *J. Gen. Physiol.* 107:19–34
- Lyubarsky AL, Pugh EN Jr. 1996. Recovery phase of the murine rod photoresponse reconstructed from electroretinographic recordings. *J. Neurosci.* 16:563–71
- Makino ER, Handy JW, Li T, Arshavsky VY. 1999. The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein beta subunit. *Proc. Natl. Acad. Sci. USA* 96:1947–52
- Matthews HR. 1995. Effects of lowered cytoplasmic calcium concentration and light on the responses of salamander rod photoreceptors. *J. Physiol.* 484:267–86
- Matthews HR. 1997. Actions of Ca^{2+} on an early stage in phototransduction revealed by the dynamic fall in Ca^{2+} concentration during the bright flash response. *J. Gen. Physiol.* 109:141–46
- Matthews HR, Murphy RL, Fain GL, Lamb TD. 1988. Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration. *Nature* 334:67–69
- Matthews RG, Hubbard R, Brown PK, Wald G. 1963. Tautomeric forms of metarhodopsin. *J. Gen. Physiol.* 47:215–40
- Matulef K, Flynn GE, Zagotta WN. 1999. Molecular rearrangements in the ligand-binding domain of cyclic nucleotide-gated channels. *Neuron* 24:443–52
- Mendez A, Burns ME, Roca A, Lem J, Wu L-W, et al. 2000. Rapid and reproducible deactivation of rhodopsin requires multiple phosphorylation sites. *Neuron* 28:153–64
- Middendorf TR, Aldrich RW. 2000. Effects of ultraviolet modification on the gating energetics of cyclic nucleotide-gated channels. *J. Gen. Physiol.* 116:253–82
- Middendorf TR, Aldrich RW, Baylor DA. 2000. Modification of cyclic nucleotide-gated ion channels by ultraviolet light. *J. Gen. Physiol.* 116:227–52
- Miller JL, Korenbrot JI. 1994. Differences in calcium homeostasis between retinal rod and cone photoreceptors revealed by the effects of voltage on the cGMP-gated conductance in intact cells. *J. Gen. Physiol.* 104:909–40
- Molokanova E, Maddox F, Luetje CW, Kramer RH. 1999. Activity-dependent modulation of rod photoreceptor cyclic nucleotide-gated channels mediated by phosphorylation of a specific tyrosine residue. *J. Neurosci.* 19:4786–95
- Molokanova E, Trivedi B, Savchenko A, Kramer RH. 1997. Modulation of rod photoreceptor cyclic nucleotide-gated channels by tyrosine phosphorylation. *J. Neurosci.* 17:9068–76
- Nagata T, Terakita A, Kandori H, Kojima D, Shichida Y, Maeda A. 1997. Water and peptide backbone structure in the active center of bovine rhodopsin. *Biochemistry* 36:6164–70
- Nakatani K, Koutalos Y, Yau KW. 1995. Ca^{2+} modulation of the cGMP-gated channel of bullfrog retinal rod photoreceptors. *J. Physiol.* 484:69–76
- Nakatani K, Yau KW. 1988a. Calcium and light adaptation in retinal rods and cones. *Nature* 334:69–71
- Nakatani K, Yau KW. 1988b. Calcium and

- magnesium fluxes across the plasma membrane of the toad rod outer segment. *J. Physiol.* 395:695–729
- Nakatani K, Yau KW. 1988c. Guanosine 3',5'-cyclic monophosphate-activated conductance studied in a truncated rod outer segment of the toad. *J. Physiol.* 395:731–53
- Nathans J. 1990. Determinants of visual pigment absorbance: identification of the retinylidene Schiff's base counterion in bovine rhodopsin. *Biochemistry* 29:9746–52
- Nikonov S, Lamb TD, Pugh EN Jr. 2000. The role of steady phosphodiesterase activity in the kinetics and sensitivity of the light-adapted salamander rod photoresponse. *J. Gen. Physiol.* 116:795–824
- Ohguro H, Van Hooser JP, Milam AH, Palczewski K. 1995. Rhodopsin phosphorylation and dephosphorylation in vivo. *J. Biol. Chem.* 270:14259–62
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, et al. 2000. Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 289:739–45
- Paoletti P, Young EC, Siegelbaum SA. 1999. C-linker of cyclic nucleotide-gated channels controls coupling of ligand binding to channel gating. *J. Gen. Physiol.* 113:17–34
- Pawson T, Scott JD. 1997. Signaling through scaffold, anchoring, and adaptor proteins. *Science* 278:2075–80
- Pepperberg DR, Cornwall MC, Kahlert M, Hofmann KP, Jin J, et al. 1992. Light-dependent delay in the falling phase of the retinal rod photoresponse. *Vis. Neurosci.* 8:9–18
- Phelan JK, Bok D. 2000. A brief review of retinitis pigmentosa and the identified retinitis pigmentosa genes. *Mol. Vis.* 6:116–24
- Pugh EN Jr, Duda T, Sitaramayya A, Sharma RK. 1997. Photoreceptor guanylate cyclases: a review. *Biosci. Rep.* 17:429–73
- Pugh EN Jr, Lamb TD. 1993. Amplification and kinetics of the activation steps in phototransduction. *Biochim. Biophys. Acta* 1141:111–49
- Pugh EN Jr, Nikonov S, Lamb TD. 1999. Molecular mechanisms of vertebrate photoreceptor light adaptation. *Curr. Opin. Neurobiol.* 9:410–18
- Pulvermuller A, Maretzki D, Rudnicka-Nawrot M, Smith WC, Palczewski K, Hofmann KP. 1997. Functional differences in the interaction of arrestin and its splice variant, p44, with rhodopsin. *Biochemistry* 36:9253–60
- Ramdas L, Disher RM, Wensel TG. 1991. Nucleotide exchange and cGMP phosphodiesterase activation by pertussis toxin inactivated transducin. *Biochemistry* 30:11637–45
- Rebrik TI, Korenbrot JI. 1998. In intact cone photoreceptors, a Ca²⁺-dependent, diffusible factor modulates the cGMP-gated ion channels differently than in rods. *J. Gen. Physiol.* 112:537–48
- Rebrik TI, Kotelnikova EA, Korenbrot JI. 2000. Time course and Ca²⁺ dependence of sensitivity modulation in cyclic GMP-gated currents of intact cone photoreceptors. *J. Gen. Physiol.* 116:521–34
- Rieke F, Baylor DA. 1996. Molecular origin of continuous dark noise in rod photoreceptors. *Biophys. J.* 71:2553–72
- Rieke F, Baylor DA. 1998a. Origin of reproducibility in the responses of retinal rods to single photons. *Biophys. J.* 75:1836–57
- Rieke F, Baylor DA. 1998b. Single-photon detection by rod cells of the retina. *Rev. Mod. Phys.* 70:1027–36
- Rieke F, Baylor DA. 2000. Origin and functional impact of dark noise in retinal cones. *Neuron* 26:181–86
- Robinson PR, Cohen GB, Zhukovsky EA, Oprian DD. 1992. Constitutively active mutants of rhodopsin. *Neuron* 9:719–25
- Ruiz M, Brown RL, He Y, Haley TL, Karpen JW. 1999. The single-channel dose-response relation is consistently steep for rod cyclic nucleotide-gated channels: implications for the interpretation of macroscopic dose-response relations. *Biochemistry* 38:10642–48
- Ruiz M, Karpen JW. 1999. Opening mechanism of a cyclic nucleotide-gated channel based on analysis of single channels locked in each liganded state. *J. Gen. Physiol.* 113:873–95
- Ruiz ML, Karpen JW. 1997. Single cyclic

- nucleotide-gated channels locked in different ligand-bound states. *Nature* 389:389–92
- Rushton WA. 1977. Visual adaptation. *Biophys. Struct. Mech.* 3:159–62
- Sagoo MS, Lagnado L. 1996. The action of cytoplasmic calcium on the cGMP-activated channel in salamander rod photoreceptors. *J. Physiol.* 497:309–19
- Sagoo MS, Lagnado L. 1997. G-protein deactivation is rate-limiting for shut-off of the phototransduction cascade. *Nature* 389:392–95
- Sakmar TP. 1998. Rhodopsin: a prototypical G protein-coupled receptor. *Prog. Nucleic Acid Res. Mol. Biol.* 59:1–34
- Sakmar TP, Franke RR, Khorana HG. 1989. Glutamic acid-113 serves as the retinylidene Schiff base counterion in bovine rhodopsin. *Proc. Natl. Acad. Sci. USA* 86:8309–13
- Sakmar TP, Franke RR, Khorana HG. 1991. The role of the retinylidene Schiff base counterion in rhodopsin in determining wavelength absorbance and Schiff base pKa. *Proc. Natl. Acad. Sci. USA* 88:3079–83
- Sanada K, Shimizu F, Kameyama K, Haga K, Haga T, Fukada Y. 1996. Calcium-bound recoverin targets rhodopsin kinase to membranes to inhibit rhodopsin phosphorylation. *FEBS Lett.* 384:227–30
- Sato N, Kawamura S. 1997. Molecular mechanism of S-modulin action: binding target and effect of ATP. *J. Biochem.* 122:1139–45
- Satpaev DK, Chen CK, Scotti A, Simon MI, Hurley JB, Slepak VZ. 1998. Autophosphorylation and ADP regulate the Ca²⁺-dependent interaction of recoverin with rhodopsin kinase. *Biochemistry* 37:10256–62
- Schoenlein RW, Peteanu LA, Mathies RA, Shank CV. 1991. The first step in vision: femtosecond isomerization of rhodopsin. *Science* 254:412–15
- Schwarzer A, Schauf H, Bauer PJ. 2000. Binding of the cGMP-gated channel to the Na/Ca-K exchanger in rod photoreceptors. *J. Biol. Chem.* 275:13448–54
- Senin II, Zargarov AA, Akhtar M, Philippov PP. 1997. Rhodopsin phosphorylation in bovine rod outer segments is more sensitive to the inhibitory action of recoverin at the low rhodopsin bleaching than it is at the high bleaching. *FEBS Lett.* 408:251–54
- Shastry BS. 1997. Signal transduction in the retina and inherited retinopathies. *Cell. Mol. Life Sci.* 53:419–29
- Sheikh SP, Zvyaga TA, Lichtarge O, Sakmar TP, Bourne HR. 1996. Rhodopsin activation blocked by metal-ion-binding sites linking transmembrane helices C and F. *Nature* 383:347–50
- Sheng M, Pak DT. 2000. Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. *Annu. Rev. Physiol.* 62:755–78
- Skiba NP, Hopp JA, Arshavsky VY. 2000. The effector enzyme regulates the duration of G protein signaling in vertebrate photoreceptors by increasing the affinity between transducin and RGS protein. *J. Biol. Chem.* 275:32716–20
- Smith WC, Milam AH, Dugger D, Arendt A, Hargrave PA, Palczewski K. 1994. A splice variant of arrestin. Molecular cloning and localization in bovine retina. *J. Biol. Chem.* 269:15407–10
- Steinberg G, Ottolenghi M, Sheves M. 1993. pKa of the protonated Schiff base of bovine rhodopsin. A study with artificial pigments. *Biophys. J.* 64:1499–502
- Struthers M, Yu H, Oprrian DD. 2000. G protein-coupled receptor activation: analysis of a highly constrained, “straitjacketed” rhodopsin. *Biochemistry* 39:7938–42
- Stryer L. 1995. *Biochemistry*. New York: Freeman
- Sunderman ER, Zagotta WN. 1999. Sequence of events underlying the allosteric transition of rod cyclic nucleotide-gated channels. *J. Gen. Physiol.* 113:621–40
- Tsang SH, Burns ME, Calvert PD, Gouras P, Baylor DA, et al. 1998. Role for the target enzyme in deactivation of photoreceptor G protein in vivo. *Science* 282:117–21
- Tsunoda S, Sierralta J, Sun Y, Bodner R, Suzuki

- E, et al. 1997. A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. *Nature* 388:243–49
- Uhl R, Wagner R, Ryba N. 1990. Watching G proteins at work. *Trends Neurosci.* 13:64–70
- Unger VM, Hargrave PA, Baldwin JM, Schertler GF. 1997. Arrangement of rhodopsin transmembrane alpha-helices. *Nature* 389:203–6
- Vu TQ, McCarthy ST, Owen WG. 1997. Linear transduction of natural stimuli by dark-adapted and light-adapted rods of the salamander, *Ambystoma tigrinum*. *J. Physiol.* 505:193–204
- Vuong TM, Chabre M, Stryer L. 1984. Millisecond activation of transducin in the cyclic nucleotide cascade of vision. *Nature* 311:659–61
- Wald G. 1968. Molecular basis of visual excitation. *Science* 162:230–39
- Watson AJ, Aragay AM, Slepak VZ, Simon MI. 1996. A novel form of the G protein beta subunit Gbeta5 is specifically expressed in the vertebrate retina. *J. Biol. Chem.* 271:28154–60
- Weiss ER, Raman D, Shirakawa S, Ducceschi MH, Bertram PT, et al. 1998. The cloning of GRK7, a candidate cone opsin kinase, from cone- and rod-dominant mammalian retinas. *Mol. Vis.* 4:27
- Weitz D, Zoche M, Muller F, Beyermann M, Korschen HG, et al. 1998. Calmodulin controls the rod photoreceptor CNG channel through an unconventional binding site in the N-terminus of the beta-subunit. *EMBO J.* 17:2273–84
- Wensel TG, Stryer L. 1986. Reciprocal control of retinal rod cyclic GMP phosphodiesterase by its gamma subunit and transducin. *Proteins* 1:90–99
- Whitlock GG, Lamb TD. 1999. Variability in the time course of single photon responses from toad rods: termination of rhodopsin's activity. *Neuron* 23:337–51
- Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J. 1997. Prolonged photoreponses in transgenic mouse rods lacking arrestin. *Nature* 389:505–9
- Yang RB, Foster DC, Garbers DL, Fulle HJ. 1995. Two membrane forms of guanylyl cyclase found in the eye. *Proc. Natl. Acad. Sci. USA* 92:602–6
- Yang RB, Garbers DL. 1997. Two eye guanylyl cyclases are expressed in the same photoreceptor cells and form homomers in preference to heteromers. *J. Biol. Chem.* 272:13738–42
- Yau KW, Nakatani K. 1985. Light-suppressible, cyclic GMP-sensitive conductance in the plasma membrane of a truncated rod outer segment. *Nature* 317:252–55
- Zhukovsky EA, Oprian DD. 1989. Effect of carboxylic acid side chains on the absorption maximum of visual pigments. *Science* 246:928–30
- Zimmerman AL, Baylor DA. 1986. Cyclic GMP-sensitive conductance of retinal rods consists of aqueous pores. *Nature* 321:70–72