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*Minireview*

# Signal Transduction Enzymes of Vertebrate Photoreceptors

**J. B. Hurley**[1]

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A flash of light initiates a cascade of biochemical reactions inside vertebrate photoreceptor cells, culminating in hydrolysis of intracellular cyclic GMP and hyperpolarization of the cell. The cell recovers by shutting down this cascade and resynthesizing cGMP. Many of the reactions responsible for the excitation and recovery phases of the photoresponse have been identified. Here I review some characteristics of the proteins that participate in these reactions.

**KEY WORDS:** Phototransduction; vision; rods and cones; G-proteins; transducin; signal transduction; recoverin; kinases; channels; calcium.

## INTRODUCTION

Light initiates a cascade of biochemical reactions in vertebrate rod and cone photoreceptors. Ultimately, these reactions alter the photoreceptor outer segment plasma membrane conductance and stimulate postsynaptic bipolar cells by reducing neurotransmitter release from the photoreceptor. This review briefly summarizes biochemical processes that are known to participate in vertebrate phototransduction.

Many of the proteins involved in the phototransduction cascade have been identified and characterized. Before describing how they generate a photoresponse, I will briefly describe some of their properties.

## RHODOPSIN

Phototransduction is initiated by photon-induced isomerization of 11-*cis*-retinal. This light-triggered event occurs within 200 fs (Schoenlein *et al.*, 1991) and is catalyzed by opsin, the protein to which retinal is bound. 11-*cis*-Retinal bound to rhodopsin readily photoisomerizes with a temperature and wavelength independent efficiency of 70% (Hurley *et al.*, 1977). Details of the mechanisms by which opsin regulates the quantum efficiency and absorption spectrum of retinal are described in the accompanying review by Oprian (Oprian *et al.*, 1992).

The amino acid sequence of rhodopsin together with many of its biochemical properties predict that it forms seven membrane-spanning α helices within a phospholipid bilayer. This structure is a hallmark of G-protein coupled receptors. These make up a very large family of hormone and neurotransmitter receptors that may include more than 100 types of olfactory receptors (Buck and Axel, 1991). Rhodopsin has many characteristics common to this large family of receptor proteins (Dohlman *et al.*, 1991). Besides the ability to stimulate G-proteins, these include:

i. Multiple carboxy terminal phosphorylation sites for a receptor kinase that recognizes and inactivates stimulated receptors.
ii. High affinity of the phosphorylated receptor for arrestin or an arrestin-like protein. Arrestin is a 48-kDa protein from photoreceptors that binds to and blocks receptors from interacting with transducin.
iii. An amino terminal glycosylation site.
iv. A palmitoylation site that anchors a portion of the carboxy-terminal tail to the membrane.

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[1] Howard Hughes Medical Institute and Department of Biochemistry, University of Washington, Seattle, Washington 98195.

## TRANSDUCIN

The G-protein coupled to rhodopsin is transducin. The highly conserved structure and function of G-proteins and the extent of the G-protein gene family have been recently reviewed (Simon *et al.*, 1991). In general, seven-transmembrane $\alpha$-helix type receptors stimulate G-protein $\alpha$ subunits to bind GTP. The GTP-bound form of the G-protein then alters the activity of an enzyme or channel. Although transducin shares many highly conserved characteristics with other G-proteins, it is also strikingly unique in several aspects:

i. Rod and cone transducins are each expressed only in the appropriate photoreceptor cell (Lerea *et al.*, 1986). The only other G-protein $\alpha$ subunits reported to be expressed with such specificity are the olfactory epithelium specific $G_{olf}$ which may participate in olfactory signal transduction (Jones and Reed, 1989), the Drosophila $G_q$ $\alpha$ subunit (Y. J. Lee *et al.*, 1990), and a unique G-protein $\beta$ subunit (Yarfitz *et al.*, 1991) that are expressed only in the Drosophila compound eye.

ii. Transducin requires photolyzed rhodopsin to stimulate GTP binding to its $\alpha$ subunit (Wessling-Resnick and Johnson, 1987). Most other G-protein $\alpha$ subunits exchange GTP onto their $\alpha$ subunits even in the absence of stimulated receptor.

iii. Most of the transducin associates with photoreceptor membranes only weakly. The membrane affinity of most other G-proteins is quite high, so they are insoluble without detergents. However, most of the transducin from photoreceptor preparations readily elutes from membranes. There are, however, multiple forms of transducin. One is very soluble and typically washes away from rod outer segments when they are broken during purification (Lerea *et al.*, 1986). The second form associates with ROS but can be eluted with low ionic strength buffers particularly in the presence of GTP (Kuhn, 1980). The third form of transducin (Wensel and Stryer, 1988) associates tightly with ROS membranes and can be eluted only with detergents. Neither structural nor functional differences between these transducin forms have been reported. However, the tightly membrane associated form is a potent activator of cGMP phosphodiesterase.

## cGMP PHOSPHODIESTERASE

The major cyclic nucleotide phosphodiesterase in photoreceptors is cGMP-specific and is activated by the GTP-bound form of transducin $\alpha$ subunit ($T_\alpha$-GTP). It is a member of a large family of cyclic nucleotide phosphodiesterases (Beavo, 1988), but it has many unique features.

i. The photoreceptor phosphodiesterase consists of two nonidentical ~90-kDa catalytic subunits and two identical ~11-kDa inhibitor subunits (Deterre *et al.*, 1986). The inhibitor subunit, also referred to as $P_\gamma$, has an internal 18 amino acid long stretch of mostly basic amino acids that is very susceptible to proteolysis by trypsin. This basic region is essential for inhibition. Photoreceptor phosphodiesterases can be activated more than 100-fold by gentle treatment with trypsin (Hurley and Stryer, 1982). A carboxy-terminal domain of the inhibitor is also required for inhibition (Brown and Stryer, 1989). $P_\gamma$ has not been reported to inhibit any other known cGMP phosphodiesterases.

   The inhibitor subunit appears to be the site of interaction with transducin. A complex between $P_\gamma$ and activated transducin $\alpha$ subunit has been purified by ion-exchange chromatography (Deterre *et al.*, 1986). Complexes between $P_\gamma$ and $T_\alpha$ in both GTP and GDP states have also been purified by gel-filtration chromatography (Yamazaki *et al.*, 1990). The mechanism by which transducin binds the inhibitor subunit and releases it from the phosphodiesterase has not been reported.

ii. The carboxy termini of phosphodiesterase catalytic subunits are carboxymethylated and perhaps isoprenylated (Ong *et al.*, 1989). An amino acid sequence motif, referred to as a "CAAX box" (see Maltese, 1990), recognized by methylation and isoprenylation enzymes is present at the carboxy termini of the catalytic subunits of the photoreceptor phosphodiesterase. This site is particularly susceptible to proteolysis by trypsin and when it is removed the phosphodiesterase loses its affinity for rod outer segment membranes. A similar site at the carboxy-terminus of the transducin $\gamma$ subunit is farnesylated (Fukada *et al.*, 1990). Farnesylation is essential for the activity of this subunit.

iii. The photoreceptor cGMP phosphodiesterase has noncatalytic cGMP binding sites in addition to its catalytic sites. The affinity of these sites for cGMP is very high and requires the presence of the $P_\gamma$ subunit (Yamazaki *et al.*, 1983). It is possible that upon activation of the phosphodiesterase by transducin the bound cGMP is released. Any role that this possible sudden release of cGMP and subsequent hydrolysis might play in phototransduction is unclear (Pugh, 1987).

## RHODOPSIN KINASE

Following stimulation by light, rhodopsin is inactivated by several processes including (i) thermal decay of the protein conformation followed by hydrolysis and release of the *all-trans* retinal chromophore, (ii) phosphorylation of serines and threonines near the rhodopsin carboxyl terminus, and (iii) arrestin binding. The kinase that phosphorylates photolyzed rhodopsin is related to the $\beta$-adrenergic receptor kinase, $\beta$-ARK. Both these kinases are activated only by their substrates (Palczewski *et al.*, 1991a; Dohlman *et al.*, 1991). Functional domains of rhodopsin have been identified using specific proteolytic fragments and short synthetic peptides derived from the rhodopsin sequence (Onorato *et al.*, 1991). The carboxyl terminus of rhodopsin is the substrate domain recognized by rhodopsin kinase. A different portion of rhodopsin, intracellular loop V–VI, activates the kinase. Both rhodopsin kinase and $\beta$-ARK prefer acidic substrates, but unlike $\beta$-ARK, rhodopsin kinase prefers acidic residues on the carboxyl side of serine targets.

Rhodopsin kinase modifies up to eight to nine sites on rhodopsin (Wilden and Kuhn, 1982; Aton *et al.*, 1984). However, photolysis of only a few rhodopsin molecules can stimulate phosphorylation of other nonphotolyzed rhodopsin molecules (Binder *et al.*, 1990; Aton *et al.*, 1984). The mechanism for this might be similar to that described for photolyzed rhodopsin stimulated phosphorylation of synthetic peptides (Onorato *et al.*, 1991). Rhodopsin kinase also phosphorylates itself. Autophosphorylation inhibits kinase binding to phosphorylated rhodopsin without affecting its binding to unphosphorylated rhodopsin.

## ARRESTIN

Arrestin is a 48-kDa protein that binds with high affinity to phosphorylated rhodopsin. It is related to $\beta$-arrestin which binds to phosphorylated $\beta$-adrenergic receptors. Arrestin is abundant in photoreceptors and in the pineal gland. mRNA encoding it is also present at low levels in other tissues (Breitman *et al.*, 1991).

When arrestin binds phosphorylated rhodopsin it effectively shuts down transducin and phosphodiesterase activation (Wilden *et al.*, 1986). The carboxyl terminus of arrestin is required to discriminate between photoexcited and nonphotoexcited phosphorylated rhodopsin (Palczewski *et al.*, 1991b).

Blocking transducin activation may not be the only function of arrestin. Arrestin also prevents phosphatase 2A-catalyzed rhodopsin dephosphorylation (Palczewski *et al.*, 1989). However, reports of other activities of arrestin remain controversial. One report suggests that arrestin directly regulates phosphodiesterase activity (Zuckerman and Cheasty *et al.*, 1986), but several attempts by other laboratories to confirm this have failed. The presence of certain sequence motifs suggests that arrestin might bind nucleotide triphosphates or $Ca^{++}$. However, initial reports of such interactions (Huppertz *et al.*, 1990) have not been confirmed (Palczewski and Hargrave, 1991). Nevertheless, the possibility remains that arrestin may have activities beyond that of "capping" rhodopsin. Arrestin homologues have also been identified in Drosophila where there are two forms sometimes referred to as phosrestins because they are rapidly phosphorylated in response to light (see Smith *et al.*, 1992).

## PHOSPHATASE 2A

Phosphates can be removed from rhodopsin by a phosphatase present in the photoreceptor cell. This activity is inhibited by okadaic acid, a phosphatase 2A specific inhibitor, and it also has other characteristics of phosphatase 2A-catalyzed reactions (Palczewski *et al.*, 1989). Dephosphorylation can be inhibited by arrestin (Palczewski *et al.*, 1989).

## PHOSDUCIN

Phosducin is a 33-kDa photoreceptor protein which undergoes light-stimulated dephosphorylation (Lee *et al.*, 1984). It has been found only in bovine photoreceptors and pineal gland. Phosducin interacts with $T_{\beta\gamma}$ and effectively competes with $T_\alpha$ for $T_{\beta\gamma}$ (Lee *et al.*, 1987). Since phosducin appears to be present at a concentration approaching that of $T_\alpha$, it could potentially regulate activation of $T_\alpha$ by rhodopsin and $T_{\beta\gamma}$.

The amino acid sequence of phosducin is known from cDNA analyses (Lee *et al.*, 1990a; Kuo *et al.*, 1989). It includes a potential phosphorylation site favored by cAMP-dependent protein kinase. This site in phosducin is a substrate for this kinase *in vitro* (Lee *et al.*, 1990b). It also appears to be phosphorylated *in vivo*.

Might phosducin also perform other functions? Rhodopsin stimulation not only catalyzes GTP-binding to $T_\alpha$, but it also liberates free $T_{\beta\gamma}$. G-protein $\beta\gamma$ subunits are primary information-carrying intermediates in at least one signal transduction pathway, the yeast pheromone response (Whiteway *et al.*, 1989). $\beta\gamma$ subunits also transduce signals by potentiating activation of adenylate cyclase by the Gs $\alpha$ subunit (Tang and Gilman, 1991). $\beta\gamma$ subunits may generally mediate signal transduction pathways. A $T_{\beta\gamma}$ signalling branch in photoreceptors could be initiated through interactions between phosducin and $T_{\beta\gamma}$.

## GUANYLATE CYCLASE

Guanylate cyclase in photoreceptors provides cGMP to the outer segment. Two forms of guanylate cyclase in photoreceptors have been reported. One is soluble in Triton X-100 and is activated 20-fold by nitroprusside and nitric oxide (Horio and Murad, 1991). The other is regulated by $Ca^{++}$ and attaches to large particles of cytoskeleton (Fleischman and Denisevich, 1979; Hakki and Sitaramayu, 1990). The latter form has been solubilized using the nonionic detergent *n*-dodecyl-$\beta$-D-maltoside and purified (K.-W. Koch, 1991). It is a 112-kDa protein which is present at approximately the same concentration as the cGMP phosphodiesterase. The turnover number of this cyclase preparation is approximately 50-fold lower than the turnover number for unactivated phosphodiesterase and 3000-fold lower than that for activated phosphodiesterase (see Hurley, 1987). However, the phosphodiesterase probably functions far below its $V_{\max}$ *in vivo* (Pugh, 1987).

## RECOVERIN

The particulate form of guanylate cyclase is inhibited by concentrations of $Ca^{++}$ above a few hundred $\mu$M (Koch and Stryer, 1988). A soluble factor responsible for this $Ca^{++}$ dependence can be eluted from a particulate fraction of ROS with low ionic strength buffer. A 26-kDa protein was purified from this extract on the basis of its ability to activate photoreceptor guanylate cyclase at $Ca^{++}$ concentrations less than a few hundred $\mu$M (Lambrecht and Koch, 1991).

The same protein was also purified on the basis of its ability to bind to a column of immobilized rhodopsin (Dizhoor *et al.*, 1991). The protein was named recoverin for its ability to stimulate guanylate cyclase and promote recovery of cGMP following a flash of light. Its amino acid sequence includes three domains with homology to $Ca^{++}$ binding sites in proteins such as calmodulin and troponin C. Recoverin binds $Ca^{++}$, but its stoichiometry and affinity for $Ca^{++}$ have not been reported. The effect of $Ca^{++}$ on guanylate cyclase appears to be cooperative since initial estimates of the Hill coefficient for this effect were 2–3.

Recoverin is required for guanylate cyclase activation, but it fails to stimulate cyclase in the presence of significant amounts of $Ca^{++}$. This differs from the mechanism by which most other $Ca^{++}$ binding proteins like calmodulin activate their targets. In fact, the mechanism by which recoverin activates guanylate cyclase remains quite puzzling. Recoverin binds to ROS membranes only at high $Ca^{++}$ concentrations. Lowering the concentration of free $Ca^{++}$ releases recoverin from these membranes at physiological ionic strength (A. Dizhoor, personal communication). How then does it activate the guanylate cyclase remaining in the particulate fraction? One possible explanation, based on analogy with photoreceptor phosphodiesterase, is that another factor such as a dissociable inhibitor subunit participates in this process.

Recoverin is currently unique among photoreceptor proteins in that it does not yet have known counterparts in other tissues. Its closest relative is visinin, a $Ca^{++}$-binding protein from chicken retinas which might be recoverin from cones (Yamagata *et al.*, 1990). Since a recoverin cDNA clone is now available, it will be used to search for recoverin homologues in other tissues and organisms.

## Na/Ca EXCHANGER

The photoreceptor $Na^+/Ca^{++}$ exchanger plays a major role in determining internal $Ca^{++}$ concentrations in photoreceptors. It has been purified as a 215-kDa protein (Nicoll and Applebury, 1989; Cook and Kaupp, 1988) and has not been reported to be regulated in any way.

The photoreceptor $Na^{+}/Ca^{++}$ exchanger is similar to other known $Na^{+}/Ca^{++}$ exchangers (Nicoll and Applebury, 1989):

i. It is electrogenic.
ii. It is bidirectional.
iii. It has micromolar affinity for $Ca^{++}$ and millimolar affinity for $Na^{+}$.
iv. It is sensitive to the inhibitor dichloralbenzamil.

However, it is also unique among $Na^{+}/Ca^{++}$ exchangers (Nicoll and Applebury, 1989):

i. The molecular weight of the photoreceptor exchanger is two to three times that of $Na^{+}/Ca^{++}$ exchangers from other tissues.
ii. It does not cross-react with antibodies raised against other $Na^{+}/Ca^{++}$ exchangers.
iii. It requires $K^{+}$ ions on the $Ca^{++}$ side for exchange activity. $K^{+}$ may be co-transported with $Ca^{++}$ (Friedel *et al.*, 1991).
iv. The turnover number for exchange is lower than for most $Na^{+}/Ca^{++}$ exchangers.

$Na^{+}/Ca^{++}$ exchangers are estimated to be present in the photoreceptor plasma membrane at a density of 600–1400 $\mu m^{-2}$ based on the assumption that they are present only in the outer segment plasma membrane (Cook and Kaupp, 1988; Nicoll and Applebury, 1989). This is similar to the density of cGMP-gated cation channels estimated to be in photoreceptor plasma membranes.

## cGMP-GATED CATION CHANNEL

The cGMP sensor in the photoreceptor is the plasma membrane cGMP-gated cation channel (Cook *et al.*, 1989). It determines both the membrane potential of the cell and the internal $Ca^{++}$ concentration through its interaction with cGMP. Properties of the channel have been studied in intact cells (Fesenko *et al.*, 1985), reconstituted phospholipid vesicles (Cook *et al.*, 1987), and Xenopus oocytes in which the channels have been expressed (Kaupp *et al.*, 1989).

This channel is unique to photoreceptors, but other cyclic nucleotide-gated channels have also been identified using cDNA derived probes (Kaupp, 1991). One particularly intriguing relative is a channel that may participate in another type of sensory transduction mechanism, olfaction (Dhallan *et al.*, 1990; Ludwig *et al.*, 1990).

cDNA clones representing photoreceptor cGMP-gated channel transcripts encode an 80-kDa protein (Kaupp *et al.*, 1989), but the purified channel is a 63-kDa protein (Cook *et al.*, 1987). This discrepancy suggests that a precursor protein is processed to form the mature channel. Another possibility is that the channel is particularly sensitive to proteolysis during purification. Recently, a 78-kDa form of the channel was purified on a column of immobilized 8-bromo cGMP (Hurwitz and Holcombe, 1991). The identity of the 78-kDa protein as a larger form of the 63-kDa channel was confirmed by its activity and its immunoreactivity with antibodies that recognize the 63-kDa channel. Unlike the 63-kDa channel, the 78-kDa form is sensitive to *l-cis* diltiazem. This compound is an inhibitor of native cGMP-gated channels in ROS and those expressed in Xenopus oocytes using bovine mRNA derived from bovine cDNA (Kaupp *et al.*, 1989).

The photoreceptor cGMP-gated channel associates with a 240-kDa protein that is similar to spectrin and resembles proteins that anchor other channels. The channel density in the plasma membranes of bovine rods have been estimated to be 315 $\mu m^{-2}$ assuming that the channel is a tetramer of 63-kDa subunits (Cook *et al.*, 1987).

## HOW TO GENERATE A PHOTORESPONSE

The excitation phase of phototransduction is summarized in Fig. 1. Rhodopsin stimulated by light catalyzes GTP binding to $T_{\alpha}$. $T_{\alpha}$-GTP dissociates from $T_{\beta\gamma}$ and removes inhibitory constraints on the phosphodiesterase catalytic subunits imposed by $P_{\gamma}$. The catalytic subunits then hydrolyze cGMP. This reduces the activity of cGMP-gated channels in the plasma membrane and the cell hyperpolarizes. These reactions occur quickly. Transducin binds GTP within 1 ms (Vuong *et al.*, 1980) and channels begin to shut down within 30 ms of a bright flash (Baylor, 1987).

Processes that promote recovery of cGMP following a light flash are summarized in Table I. Phosphodiesterase activity drops as cGMP is hydrolyzed. This occurs because the $K_m$ of the phosphodiesterase for cGMP is approximately 100-fold higher than the intracellular cGMP concentration (Pugh, 1987).

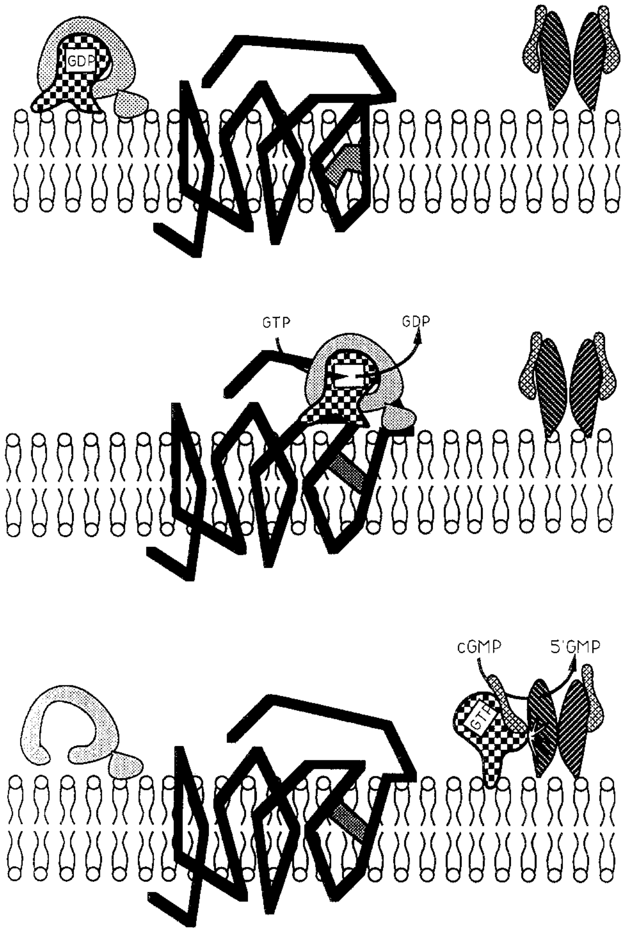

**Fig. 1.** Generation of the excitation phase of the photoresponse. Details are described in the text. The complex shown initially (top) on the left is transducin and on the right is the cGMP phosphodiesterase. Rhodopsin is shown in the center spanning the membrane seven times.

Phosphodiesterase activity therefore decreases proportionally to the cGMP levels.

Phosphodiesterase activation is also interrupted by other mechanisms. Rhodopsin kinase phosphorylates metarhodopsin II and reduces its ability to stimulate transducin. Arrestin further quenches the signal by binding to phosphorylated rhodopsin. Transducin auto-inactivates by hydrolyzing its bound GTP. This hydrolysis is rapid and may be stimulated by phosphodiesterase, $P_\gamma$, or some other protein of similar concentration in the outer segment (Arshavsky *et al.*, 1991). A burst of enthalpy release occurs within 1 s following a light flash (Vuong and Chabre, 1990). Characteristics of this burst suggest that its source is rapid GTP hydrolysis catalyzed by transducin.

**Table I.** Processes that Contribute to the Recovery Phase of the Photoresponse

1. Rhodopsin phosphorylation quenches its ability to activate transducin.
2. Arrestin binding to phosphorylated rhodopsin further quenches transducin activation.
3. Hydrolysis of GTP bound to $T_\alpha$ quenches $T_\alpha$.
4. $T_{\beta\gamma}$ reassociation with $T_\alpha$ releases $P_\gamma$.
5. $P_\gamma$ reassociation with phosphodiesterase quenches cGMP hydrolysis.
6. Low free $Ca^{++}$ concentrations stimulate resynthesis of cGMP by guanylate cyclase.

Following GTP hydrolysis, $T_\alpha$ releases its bound $P_\gamma$. This is enhanced by $T_{\beta\gamma}$ (Yamazaki *et al.*, 1990). Once $P_\gamma$ dissociates from transducin, it reassociates with and quenches the activity of the phosphodiesterase catalytic subunits.

The decrease in cGMP resulting from this burst of phosphodiesterase activity shuts down channels through which $Na^+$ and $Ca^{++}$ enter the cell. $Ca^{++}$ continues to exchange out of the cell through the $Na^+/Ca^{++}$ exchanger. This net outward $Ca^{++}$ flux lowers intracellular $Ca^{++}$ and $Ca^{++}$ dissociates from recoverin allowing it to stimulate resynthesis of cGMP by guanylate cyclase.

What determines the speed and sensitivity of the photoresponse? This is an important question because photoreceptors need to respond over a very wide range of light intensities. For example, it takes only 30 photons to produce a half-maximal response in a primate rod whereas 1200 photons are required to half-maximally stimulate a primate cone (Baylor, 1987). Primate rods can take as long as 200 ms to reach the peak of their response to a dim flash, whereas a peak of similar amplitude in cones occurs after only 50 ms.

What accounts for these differences in speed and sensitivity? The disc membranes of rods and infolded plasma membranes of cones create very different geometries which may affect the photoresponse. But there are even more fundamental differences between rods and cones. The opsins (Nathans, 1987), transducins (Lerea *et al.*, 1989), and phosphodiesterase catalytic (Hurwitz *et al.*, 1985) and inhibitory subunits (Hamilton and Hurley, 1990) from rods and cones are all known to be derived from rod- or cone-specific genes. Other components of the phototransduction pathway may also be derived from different genes in rods and cones. Kinetic differences between rod and cone phototransduction enzymes may contribute to differences in speed and sensitivity of these cells.

Mathematical modeling of the photoresponse (e.g., Forti *et al.*, 1989) has produced useful predictions and insights about phototransduction. However, these models are at the mercy of assumptions and preconceptions about the identities of phototransduction enzymes and their kinetic parameters. These kinetic parameters are estimates from *in vitro* experiments performed at enzyme and substrate concentrations 150-fold lower than *in vivo*. Recently, a rhodopsin promoter fragment has been used to express bacterial $\beta$-galactosidase in rod photoreceptors of transgenic mice (Zack *et al.*, 1991; Lem *et al.*, 1991). This creates new opportunites to test, *in vivo*, our notions of how the phototransduction cascade operates. Mutant forms of phototransduction enzymes can be expressed in rod photoreceptors *in vivo*. These types of experiments, like the genetic analyses of visual mutants in Drosophila (Smith *et al.*, 1992), will help bridge gaps between physiological and biochemical approaches to the understanding of phototransduction.

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