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MINIREVIEW

Vertebrate Phototransduction: Activation, Recovery, and Adaptation

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Summary

Vision is a fascinating example of the interaction of a biological system with the outside world. The first step of translating electromagnetic energy into a biologically recognizable signal involves the phototransduction cascade in retinal photoreceptor cells. Phototransduction is the best studied example of a GTP binding protein (G protein)-coupled signal transduction pathway. A great body of knowledge about phototransduction has been established in the past several decades but there are still many unanswered questions, particularly about photoresponse recovery and adaptation. The purpose of this review is to outline the events following photon absorption by vertebrate photoreceptors, to demonstrate the great complexity of the phototransduction cascade mechanisms, and to point out some of the controversies arising from recent findings in the field of visual transduction.

Key words

Vertebrate phototransduction – Signal transduction – G protein – Photoresponse recovery – Light adaptation

Abbreviations

ATP	adenosine 5'-triphosphate
cGMP	guanosine 3'5'-cyclic monophosphate
G protein	GTP-binding protein
GAP	GTPase activity protein
GCAP	guanylyl cyclase accelerating protein
GRKs	G protein coupled receptor kinases
GTP	guanosine 5'-triphosphate
H-8	N-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide, protein kinase C inhibitor
HPLC	high pressure liquid chromatography
PDE	cGMP specific phosphodiesterase
PDE*	an activated form of PDE
R*	metarhodopsin II, an active photolyzed form of rhodopsin
RGS	regulator of G protein signalling
RIS	rod inner segment
ROS	rod outer segment
$T\alpha^*$	GTP-bound α subunit of G protein transducin, an active form of transducin

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Introduction

Visual perception gives us the vast majority of our information about the outside world. This review gives a brief summary of the molecular mechanisms of the phototransduction process that initiates the chain of events that ultimately result in vision.

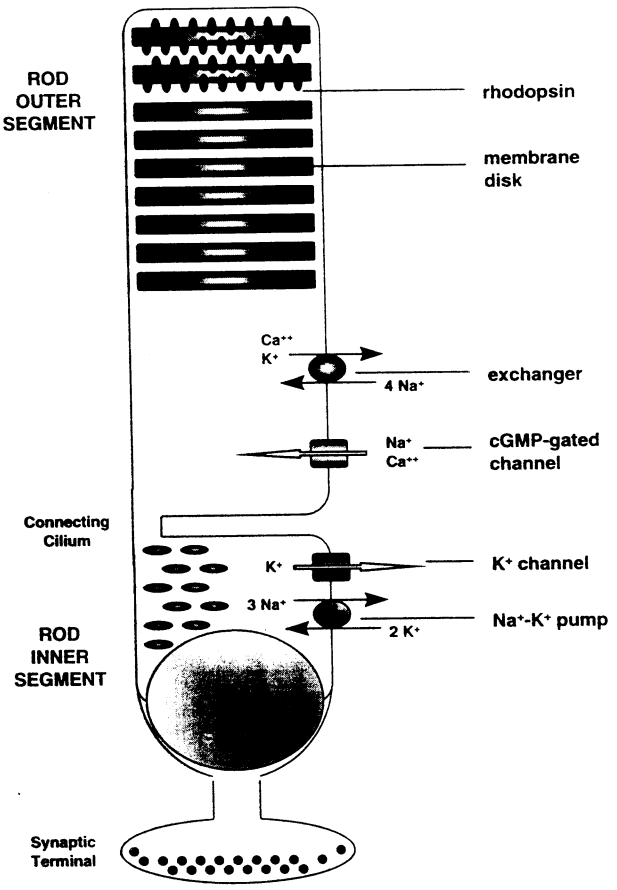

Fig. 1. *Vertebrate retinal rod. Rod photoreceptors have three main compartments, outer segment, inner segment, and synaptic terminal. The outer segment contains the phototransduction machinery. It is filled with a stack of membrane disks which contain rhodopsin and other proteins of the phototransduction cascade. The inner segment is a metabolic center of the cell and contains the nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus and lysosomes. The synaptic terminal is the site of the synthesis, storage and release of the neurotransmitter glutamate. The dark current, carried by sodium and calcium ions, enters the outer segment through cGMP-gated channels in the plasma membrane. The calcium is pumped out of the outer segment via Na^+:Ca^{2+},K^+ exchange. The principal current carrier in the inner segment is potassium which leaves the cell through K^+ channels. The ionic gradients for sodium and potassium ions are maintained by ATP-driven Na^+:K^+ pumps in the inner segment.*

1. Photoreceptor structure

The visual process begins with the absorption of a photon in the highly specialized retinal photoreceptor cells, rods and cones. In most vertebrates rods outnumber cones. They are also larger and more sensitive to light, being able to detect single photons reliably. For these reasons more is known about rods than cones and my minireview deals exclusively with the former.

Rods are modified neurons and have three main compartments, outer segment, inner segment, and synaptic terminal (Fig. 1). The cylindrical rod outer segment (ROS) contains all the molecular components of the phototransduction cascade. ROS is filled with a stack of about 1000 flattened membrane disks carrying rhodopsin molecules and other transduction proteins. The compact structure of ROS helps to limit diffusion times for messengers involved in the transduction pathway. The outer segment is connected to the rod inner segment (RIS) by a short connecting cilium. Metabolic processes are located in the RIS which contains the cell nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus and other cell organelles. The inner segment is joined to the synaptic terminal which transmits the light signal to second order neurons in the retina, bipolar and horizontal cells. Light, by hyperpolarizing the photoreceptor cell, terminates the continuous release of a neurotransmitter, glutamate, which occurs in darkness. The signal is then further processed in the retina and sent on to visual centers in the brain.

2. Basic electrophysiology of a rod cell

The rod photoreceptor is a nonspiking cell which uses light evoked changes in a second messenger, 3',5'-cyclic guanosine monophosphate (cGMP), to generate an electrical signal. In the darkness, cGMP is high and a standing dark current circulates through the receptor (Fig. 1) keeping the cell in a slightly depolarized state. Current flows into the outer segment through cGMP-gated channels and out of the inner segment through potassium channels. Light activates an enzyme cascade that hydrolyzes cGMP causing the cGMP-gated cation channels to close and the cell to hyperpolarize as potassium continues to leave the inner segment. The hyperpolarization causes voltage-gated calcium channels in the synaptic terminal to close decreasing the release of glutamate.

The cGMP-gated channels are cation-selective with sodium and calcium ions carrying about 80 and 15 % of the current, respectively (Detwiler and Gray-Keller 1992). There are probably three cGMP binding sites on the channel which undergoes allosteric regulation following cGMP binding. This makes the

channel very sensitive to small changes in ligand concentration. The channel is composed of α and β subunits that are thought to form a tetrameric complex of two α and two β subunits. The exact stoichiometry of the native channel has yet to be determined (Molday 1996). There has been speculation about the influence of channel modulation in shaping the light response. For example, calmodulin-mediated decrease in channel affinity for cGMP in high calcium has been described but the contribution of this modulation to photoresponse kinetics or light adaptation is estimated to be minimal in rod cells (Gray-Keller and Detwiler 1995, 1996, Molday 1996, Koutalos and Yau 1996, Younger *et al.* 1996). The ROS channel is a special case among ligand-gated channels because it does not undergo desensitization in the constant presence of cGMP (Zimmerman and Baylor 1986).

The flow of dark current out of the rod is carried by potassium ions flowing through light-insensitive potassium channels in the RIS. Two ion transport mechanisms are necessary to maintain the ion gradients needed to support the dark current. Calcium is removed from the ROS by a powerful electrogenic Na^+:K^+,Ca^{2+} exchanger (4:1,1) (Cervetto *et al.* 1989) and sodium is extruded by Na^+:K^+ ATPase in the RIS.

The retinal rod exchanger differs from the more common Na^+:Ca^{2+} exchangers in other tissues by cotransport of potassium with calcium (Schnetkamp *et al.* 1989). The stoichiometry of the exchanger is such that there is one net inward positive charge for each extruded calcium ion. The resulting inward exchange current makes up about 8 % of the circulating dark current (Gray-Keller and Detwiler 1994). An exchanger that uses the energy stored in both the sodium and potassium electrochemical gradients to extrude calcium should be able to pump internal calcium to lower than 1 nM intracellular concentration. In the rod, however, the minimum calcium level is about 50 nM, indicating that the exchanger inactivates before it reaches its thermodynamic limit as measured in functionally intact ROS (Gray-Keller and Detwiler 1994) or in purified ROS (Schnetkamp 1995). This finding suggests that the operation of the exchanger is regulated by a mechanism that is currently not understood.

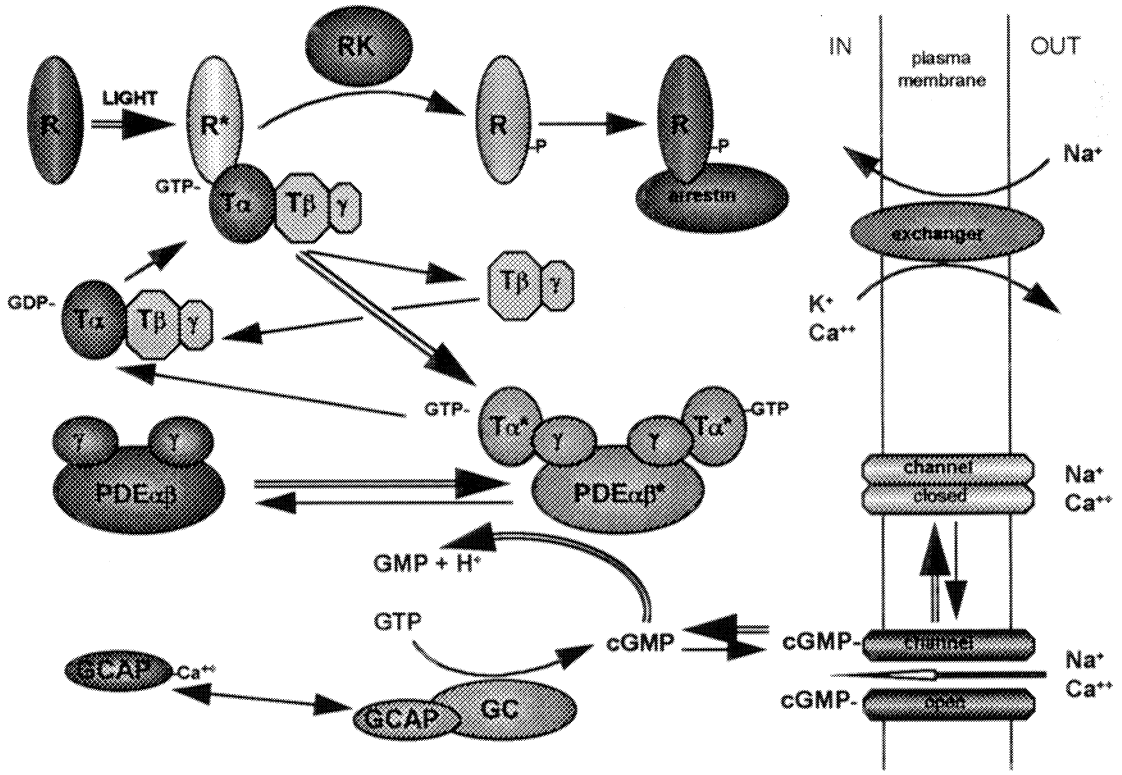

Fig. 2. *Phototransduction in vertebrate ROS. The scheme shows the standard view of the phototransduction cascade. Triple line arrows – activation steps, dotted line arrows – recovery steps. R – rhodopsin, RK – rhodopsin kinase, T – transducin, PDE – cGMP phosphodiesterase, GC – guanylyl cyclase, GCAP – guanylyl cyclase activating protein, channel – cGMP-gated channel. Further comment in the text.*

When light triggers the transduction cascade, the channels close and the intracellular calcium concentration declines because the exchanger keeps pumping calcium out of the cell. The resulting fall in calcium concentration is generally known as the calcium feedback signal and plays an important role in response recovery and some aspects of adaptation (reviewed by Detwiler and Gray-Keller 1992, Hurley 1994, Koutalos and Yau 1996).

3. Photoresponse activation

The activation phase of the phototransduction cascade is well established (Fig. 2) (Lamb and Pugh 1992, Pugh and Lamb 1993, Lamb 1996). Robust models exist that can reproduce the activation kinetics of the rod electrical light response under physiological conditions (Lamb and Pugh 1992, Pugh and Lamb 1993, Kraft *et al.* 1993). Simulation programs based on these successful models are available on the Internet (Lamb, program Walk on the World Wide Web at the site http//classic.physiol.cam.ac.uk).

The activation phase of the photoresponse begins when a photon is absorbed by rhodopsin, a membrane protein belonging to the superfamily of G protein-coupled receptors. Rhodopsin was the first member of this important class of receptor proteins to be sequenced at the amino acid level (Ovchinnikov *et al.* 1982, Hargrave *et al.* 1983). It is a 40 kDa protein consisting of a protein, opsin and a chromophore, 11-*cis*-retinal which is bound by a protonated Schiff base linkage to opsin Lys^{296} (Hargrave and McDowell 1992a,b). The schematic molecular structure is shown in Figure 3. Rhodopsin crosses the rod disk membrane seven times with its carboxy-terminus (C-terminus) and four loops facing the cytoplasm. Rhodopsin's seven transmembrane helices form three cytoplasmic loops, C-1, C-2 and C-3. The two adjacent cysteines Cys^{322} and Cys^{323} of the rhodopsin C-terminus are palmitoylated. The palmitates dip into the lipid bilayer forming the fourth rhodopsin loop, C-4 (Fig. 3).

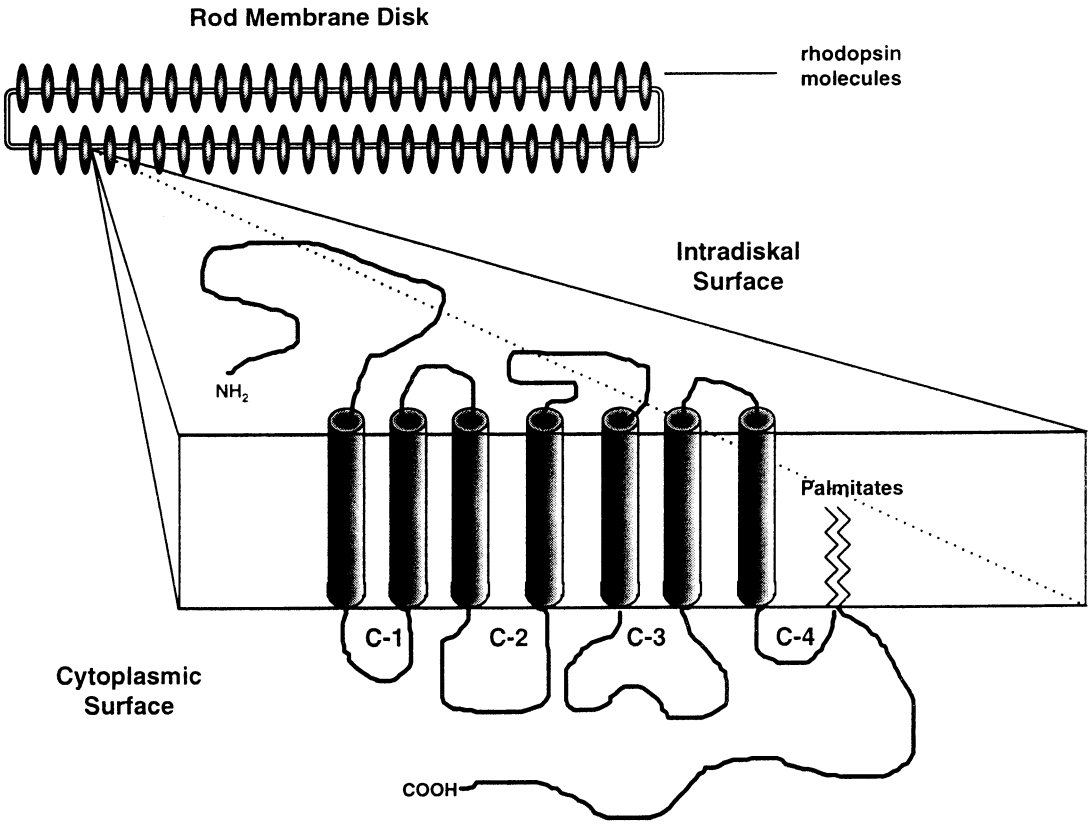

Fig. 3. *Model of rhodopsin topography. Rhodopsin molecules are densely packed in the lipid bilayer of both surfaces of outer segment disks. The rhodopsin polypeptide chain traverses the membrane seven times as shown in the expanded view. The N-terminal region faces the intradiskal space and the C-terminal region is exposed to the cytoplasm. The three cytoplasmic loops, C-1, C-2 and C-3, are formed by the polypeptide chain crossing the membrane. The fourth loop, C-4, is formed by anchoring part of the C-terminal region to the lipid bilayer via two neighbouring palmitoyl-cysteines. The C-terminus and the cytoplasmic loop regions comprise the surface that is accessible to proteins participating in phototransduction, such as transducin, rhodopsin kinase, and arrestin.*

Light induces *cis-trans* isomerisation of the chromophore changing it from 11-*cis*-retinal to all-*trans*-retinal in less than 200 femtoseconds. This initial event triggers a series of conformational changes which generate a number of rhodopsin intermediates that can be distinguished by their spectral characteristics. Metarhodopsin II (R*) is the active form of rhodopsin which catalytically stimulates the heterotrimeric G protein transducin (Hargrave *et al.* 1993). R* formation takes about 1 ms. The rhodopsin cytoplasmic surface undergoes the conformational change when R* is formed allowing it to interact with transducin.

Transducin mediates signal transmission between the light receptor rhodopsin and the effector enzyme, cGMP specific phosphodiesterase (PDE). Transducin was the first GTP binding protein that was characterized. G proteins are heterotrimeric proteins with GTPase activity that couple sensory, hormonal and neurotransmitter receptors to a variety of effector enzymes and channels (reviewed by Birnbaumer *et al.* 1990, Bourne *et al.* 1991, Neer 1995, Clapham 1996, Bourne 1997). The crystal structures of transducin T$\alpha\beta\gamma$ trimer (Lambright *et al.* 1996) and T$\beta\gamma$ dimer (Sondek *et al.* 1996) have been determined. The T$\beta\gamma$ dimer forms a functional monomer since the Tβ and Tγ subunits can dissociate only when they are denatured. The Tα subunit in its inactive GDP-bound form is associated with T$\beta\gamma$ dimer. R* binds to transducin and then activates Tα by triggering the exchange of GDP for GTP. This active form of Tα (Tα*) then dissociates both from the receptor and T$\beta\gamma$ dimer. Transducin activation is a greatly amplified step. One R* activates several hundred transducin molecules at a rate about one per ms. The Tα* then stimulates PDE *via* a one-to-one binding reaction.

Rod PDE belongs to PDE6 family of phosphodiesterase isozymes and is a tetrameric membrane associated protein. The holoenzyme consists of two equally active catalytic subunits, α and β, and two identical inhibitory γ subunits. Rod photoreceptors also possess a soluble form of PDE that contains two δ subunits along with α, β and two γ subunits. The PDEδ subunits are thought to be responsible for the enzyme's solubility (reviewed by Beavo 1995). The inhibitory PDEγ subunit is the site of interaction with transducin. The Tα* activates PDE by releasing the inhibitory activity of one of the PDEγ subunits (Yamazaki *et al.* 1983). It takes two Tα* molecules to fully activate the holoenzyme. The activated PDE α and β subunits (PDE*) hydrolyze cGMP to 5'-GMP.

Decrease in cGMP concentration closes cGMP-gated channels in the plasma membrane and hyperpolarizes the cell. Hyperpolarization finishes the photoresponse activation phase causing closure of voltage-gated calcium channels in the synaptic terminal. The resulting drop in calcium reduces the synaptic release of glutamate.

4. Recovery of the photoresponse

The photoresponse recovery consists of two parts, cascade shut-off and cGMP resynthesis. The standard pathway is presented in Figure 2. The underlying molecular mechanisms are much less certain than in the case of response activation. The following discussion presents the generally accepted scheme of the response recovery process.

a) Transduction cascade shut-off

The shut-off of the phototransduction cascade requires that the three activated intermediates, R*, T* and PDE*, be inactivated. The following discussion treats the quenching mechanisms of each intermediate separately.

1. R*

The lifetime of R* determines the gain of the first step of the phototransduction cascade and the quenching of R* is the first necessary process in shutting off the cascade. R* shut-off is a two step mechanism that begins with the phosphorylation of the C-terminus of R* by rhodopsin kinase and ends with the binding of a 48 kDa accessory protein called arrestin.

The importance of the rhodopsin C-terminus for rhodopsin shut-off *in vivo* has been demonstrated by work of Chen *et al.* (1995b). Their experiments used transgenic mice that expressed rhodopsin with the C-terminus truncated by 15 amino acids ending with amino acid residue 333 before Ser^{334}. Rods containing the truncated rhodopsin gave light responses that were prolonged and had increased sensitivity consistent with disrupted R* shut-off by rhodopsin kinase phosphorylation.

The rhodopsin C-terminus has several potential serine/threonine phosphorylation sites. Growing evidence suggests, however, that only some of them play a major role in rhodopsin shut-off. Wilden (1995) found that the first phosphate bound to a bleached rhodopsin markedly shortens R* lifetime as monitored by the ability of R* to activate PDE. Ohguro *et al.* (1995) used HPLC and mass spectrometry to determine the number and location of phosphate groups in rhodopsin C-terminal peptides purified from mice that were exposed to different levels of illumination. Their results suggest that phosphorylation sites Ser^{334}, Ser^{338} and Ser^{343} are critical residues for R* shut-off.

Rhodopsin phosphorylation by rhodopsin kinase is the first step in R* shut-off. Rhodopsin kinase

belongs to a family of serine/threonine protein kinases called G protein-coupled receptor kinases (GRKs) (reviewed by Lefkowitz 1993, Premont *et al.* 1995, Palczewski 1997). There have been six GRKs identified so far and they are expressed in a wide variety of mammalian tissues. Rhodopsin kinase is a 63 kDa protein found exclusively in rods, cones and, in low levels, in the pineal gland. It is a cytosolic enzyme that translocates to the membrane upon receptor activation (Kelleher and Johnson 1990, Lefkowitz 1993). The association of rhodopsin kinase with membrane is mediated by a posttranslational modification in which an isoprenoid farnesyl is attached to the C-terminal cysteine residue (Inglese *et al.* 1992a,b). The mechanism for the light-dependent translocation of rhodopsin kinase is not known.

Rhodopsin kinase has two major (Ser^{488}, Thr^{489}) and one minor (Ser^{21}) autophosphorylation sites (Palczewski *et al.* 1992, 1995). The authors speculate that the autophosphorylation of rhodopsin kinase during the photoresponse may lower the affinity of this enzyme for R* which might promote its dissociation from rhodopsin and therefore facilitate arrestin binding. Another possibility is that autophosphorylation affects the selectivity of rhodopsin kinase phosphorylation for R* residues.

Rhodopsin kinase seems to be regulated by a 23 kDa calcium-binding protein called recoverin. Recent papers report direct inhibition of rhodopsin kinase by recoverin in a calcium-dependent manner *in vitro* (Chen *et al.* 1995a, Klenchin *et al.* 1995, Calvert *et al.* 1995). This suggests that rhodopsin kinase is inhibited in darkness when intracellular calcium is high and becomes disinhibited by the fall in calcium concentration during the light response. This negative feedback is thought to play a role in light adaptation and recovery.

The second step in R* inactivation is thought to be arrestin binding to phosphorylated rhodopsin (Wilden *et al.* 1986, Wilden 1995). Arrestin binds to phosphorylated R* and prevents further activation of transducin by steric hindrance (Wilden *et al.* 1986, Wilden 1995, Krupnick *et al.* 1997). Arrestin belongs to a family of inhibitory proteins participating in signal transduction (reviewed by Palczewski 1994). Langlois *et al.* (1996) used microcalorimetry to monitor PDE activity in a reconstituted system and showed that exogenous arrestin and rhodopsin kinase speeded the inactivation of PDE following light stimulation. More recent work by Xu *et al.* (1997) addressed questions about arrestin function in rods in transgenic mice in which arrestin expression was either absent or half its normal level. Photoresponse recovery was found to be normal when expression was halved, which indicates that arrestin binding is not rate-limiting for recovery of the flash response. Photoresponses from rods completely lacking arrestin showed initial fast partial recovery followed by a prolonged final phase, suggesting that there are both arrestin independent and dependent mechanisms involved in rhodopsin inactivation.

Visual arrestin, located predominantly in rods, and cone arrestin are restricted to the retina whereas other members of the arrestin family are relatively ubiquitous. There have been four mammalian arrestins identified to date with visual arrestin being the most selective one (Gurevich *et al.* 1995). There is also a 44 kDa splice variant of arrestin that binds to both phosphorylated and nonphosphorylated R* (Palczewski *et al.* 1994, Smith *et al.* 1994). The splice variant is truncated at the C-terminus and is membrane-associated which suggests that the C-terminal region of arrestin is responsible for its solubility (Palczewski *et al.* 1994). The precise role of the two arrestin variants is currently under intense investigation (Pulvermuller *et al.* 1997). Immunocytochemical localization of these proteins in the bovine retina shows different distributions. Arrestin is found throughout the whole rod cell and the splice variant of arrestin seems to be localized in the outer segment only (Smith *et al.* 1994).

2.Tα*

The second step in shutting off the phototransduction cascade is to terminate the activity of Tα*. Like the alpha subunit of all heterotrimeric G proteins, Tα* has an intrinsic GTPase activity and turns itself off by hydrolyzing GTP to GDP. GDP-bound Tα is unable to stimulate PDE and reassociates with T$\beta\gamma$ ending the T$\beta\gamma$ lifetime as well. Time-resolved microcalorimetry of heat released by GTP hydrolysis demonstrated that activated transducin hydrolyzes GTP in less than one second (Vuong and Chabre 1990). When the GTPase rate was measured using purified transducin, it has been found that the hydrolysis of GTP is much slower, lasting many seconds (Arshavsky and Bownds 1992). The addition of PDE accelerated the reaction and the effect has been attributed to the PDEγ subunit (amphibian – Arshavsky and Bownds 1992, bovine – Arshavsky *et al.* 1994, Slepak *et al.* 1995). GTPase acceleration by PDEγ exhibited a strong dependence on membrane concentration (Arshavsky *et al.* 1994). Higher membrane concentrations significantly enhanced the PDEγ effect which suggests that membranes contain additional essential factor for GTPase acceleration. Other researchers maintain that PDEγ acts along with an unidentified membrane factor (Angleson and Wensel 1993). This proposal is supported by Otto-Bruc *et al.* (1994) who showed that PDE itself does not accelerate GTPase in the absence of membranes and the evidence points to a membrane-bound protein with GAP-like (GTPase activating protein) properties.

A good candidate for the photoreceptor GAP protein appears to be a recently identified membrane protein (Chen *et al.* 1996, Faurobert and Hurley 1997,

Wieland *et al.* 1997) that belongs to the RGS family of proteins (Regulator of G protein signaling) (Roush 1996, reviewed by Koelle 1997). RGS proteins are thought to regulate negatively G protein coupled signalling pathways (reviewed by Roush 1996). Recent *in vitro* experiments with expressed human RGS10 protein demonstrate potent and selective acceleration of GTPase hydrolytic activity in activated Gα subunits by the RGS protein (Hunt *et al.* 1996). Transducin GTPase acceleration by expressed retinal RGS protein *in vitro* has also been reported (Natochin *et al.* 1997). The mechanism of RGS regulation is unknown.

In general, it is still not clear if GTPase acceleration is mediated by both PDEγ and some regulatory membrane factor, like the RGS protein, or if only the membrane factor is necessary. Work by Arshavsky *et al.* (1994) and Otto-Bruc *et al.* (1994) directly contradict each other in this respect. Further investigation is necessary to resolve this issue.

For transducin recycling it is necessary that the GDP-bound Tα reassociates with T$\beta\gamma$. T$\beta\gamma$ availability seems to be downregulated by phosphoprotein phosducin (Lee *et al.* 1992, Yoshida *et al.* 1994, Wilkins *et al.* 1996). Phosducin is a soluble 28 kDa phosphoprotein found in mammalian retinas (Lee *et al.* 1984). This protein or its analogs seem to be present in many other tissues (Bauer *et al.* 1992). Brain rat and bovine phosducin homologues and their inhibiting effect on G protein activity have been described (Bauer *et al.* 1992, Miles *et al.* 1993).

The phosducin content in ROS is the same as the transducin content (Wilkins *et al.* 1996) so the downregulation of T$\beta\gamma$ can occur stoichiometrically. Phosducin has been found to form a specific complex with T$\beta\gamma$ in its dephosphorylated state (Lee *et al.* 1987, Yoshida *et al.* 1994). From the point of view of phototransduction, it is very interesting that the phosducin dephosphorylation was shown to be light-dependent (Lee *et al.* 1984). The functional significance and regulation of this process are not fully understood. It is suggested that phosducin light-dependent dephosphorylation plays a role in light adaptation as discussed in Section 6.

Phosducin is phosphorylated on Ser^{73} which is located within the consensus phosphorylation sequence for protein kinase A (Lee *et al.* 1990, Wilkins *et al.* 1996). Phosducin has been shown to be phosphorylated by exogenous protein kinase A (Lee *et al.* 1990, Bauer *et al.* 1992, Yoshida *et al.* 1994). Wilkins *et al.* (1996) identify the ROS endogenous kinase responsible for phosducin phosphorylation as protein kinase A, based on their *in vitro* experiments in bovine ROS preparations. There is no *in vivo* evidence of protein kinase A activity in ROS during phototransduction.

Phosphorylation of phosducin prevents G protein inactivation (Bauer *et al.* 1992, Yoshida *et al.* 1994) and has been found to be 3 times faster in high calcium than in low calcium (Willardson *et al.* 1996). The calcium sensitivity of the process is ascribed to calcium/calmodulin-dependent adenylyl cyclase but it is not known if this cyclase is actually localized in ROS (Willardson *et al.* 1996).

Amphibian ROS do not have a phosducin protein but they contain two soluble low molecular mass phosphoproteins that seem to be equivalent to phosducin in mammalian tissues (Polans *et al.* 1979, Suh and Hamm 1996). These proteins, called Component I and II, are also phosphorylated in the dark and undergo light-dependent dephosphorylation (Polans *et al.* 1979). Suh and Hamm (1996) also demonstrated direct interaction of Component I and II with T$\beta\gamma$. Krapivinsky *et al.* (1992) claim that Component I and II form an oligomeric complex. The kinase responsible for phosphorylation of Component I and II was reported to be protein kinase A (Hamm 1990). The stoichiometry of phosphorylation is not known (Suh and Hamm 1996).

3. PDE*

The final step in photoresponse shut-off is quenching PDE*. The reassociation of the inhibitory PDEγ subunits with PDEα and β catalytic subunits, when Tα hydrolyzes GTP to GDP, prevents further cGMP hydrolysis. The PDEγ binding has been localized to the catalytic sites of PDEα and β subunits (Artemyev *et al.* 1996). The release of PDEγ from Tα seems to require interaction of the PDEγ/Tα complex with T$\beta\gamma$. PDEγ was found to be bound to Tα even in its GDP-bound form but it was released upon interaction with T$\beta\gamma$ *in vitro* (Yamazaki *et al.* 1990).

The catalytic subunits of photoreceptor PDE have two classes of noncatalytic cGMP binding sites on the catalytic subunits, one high affinity and one low affinity (Yamazaki *et al.* 1980, Cote and Brunnock 1993). Occupation of these sites on PDE by cGMP inhibits the acceleration of transducin GTPase by PDEγ (Arshavsky *et al.* 1992). The noncatalytic cGMP binding sites on PDE catalytic subunits are claimed to be major cGMP binding sites in ROS with more than 90 % of total cGMP bound to these sites (Cote and Brunnock 1993). Yamazaki *et al.* (1996) studied the possible involvement of these sites in photoresponse recovery *in vitro*. Their results suggest that release of cGMP from PDE noncatalytic sites can participate in recovery of the photoresponse. This speculation is based on estimates that the free cGMP level in the dark is 5 μM and PDE concentration is around 30 μM, thus release of cGMP from PDE, when cGMP levels are reduced after illumination, can significantly increase cGMP concentration during the recovery process. There is nothing known at this point about the possible contribution of this mechanism versus resynthesis of cGMP by guanylyl cyclase on the increase of cGMP level *in vivo*.

There are reports suggesting that GTP hydrolysis by Tα is not required for PDE* shut-off (Erickson *et al.* 1992, Tsuboi *et al.* 1994a,b). Some of these studies indicate the possibility that PDEγ phosphorylation is responsible for PDE* shut-off (Tsuboi *et al.* 1994a,b). PDEγ was found to be phosphorylated on a single Thr^{22} by a novel specific kinase in amphibian ROS. Phosphorylated PDEγ inhibited active PDE more effectively than nonphosphorylated PDEγ. In addition, PDEγ specific kinase was found to be inhibited by cGMP. This suggests that the phosphorylation would be enhanced when cGMP levels drop during the light response, which may offer a possible mechanism of PDE shut-off that is independent of GTP hydrolysis.

b) cGMP resynthesis

Guanylyl cyclase is responsible for the resynthesis of cGMP in ROS. The drop in cGMP concentration caused by PDE was found to be insufficient for stimulating *via* mass action sufficiently rapid resynthesis of cGMP by the cyclase. Recently, two novel proteins called guanylyl cyclase accelerating proteins I and II (GCAP I and II) have been identified (Dizhoor *et al.* 1994, 1995 Gorczyca *et al.* 1994a, 1995, Palczewski *et al.* 1994). GCAP detects a drop in calcium level caused by light exposure and stimulates the guanylyl cyclase restoring cGMP concentration to the original resting dark level. As the cGMP levels are restored and the channels reopen, the resulting increase in calcium influx shuts off the cyclase, reestablishing the dark state.

Initially, recoverin was postulated to be the soluble activator of guanylyl cyclase (Dizhoor *et al.* 1991). The recoverin effects on phototransduction, however, are in a direction opposite to what would be expected if recoverin accelerated guanylyl cyclase. The evidence for this conclusion came from two independent studies. The frog analog of recoverin, S-modulin, enhanced the effect of light and inhibited rhodopsin phosphorylation *in vitro* (Kawamura 1993). Bovine recoverin and its chicken and gecko homologues were found to prolong photoresponses when internally dialyzed to ROS (Gray-Keller *et al.* 1993).

A retina specific guanylyl cyclase has been purified (Horio and Murad 1991), cloned and sequenced (Shyjan *et al.* 1992). The enzyme is considered a novel isoform of membrane-associated guanylyl cyclases. Laura *et al.* (1996), using deletion mutants of retinal guanylyl cyclase, demonstrated that GCAP II functions through the intracellular domain of the cyclase.

The photoreceptor guanylyl cyclase molecule contains a kinase-like domain. Maximal activation of other particulate guanylyl cyclases requires ATP (Kurose *et al.* 1987, Chang *et al.* 1990, Duda and Sharma 1995). Experiments with retinal guanylyl cyclase indicate similar regulation. ATP enhances the photoreceptor guanylyl cyclase two-fold and the observed stimulation is calcium-independent (Gorczyca *et al.* 1994b, Aparicio and Applebury 1996). Aparicio and Applebury (1996) went on to show that photoreceptor guanylyl cyclase possesses intrinsic kinase activity and autophosphorylates serine residues. The significance and regulation of such activity is unclear. Up to this time the only regulative light-dependent connection between photoreceptor guanylyl cyclase and the rest of the phototransduction cascade seems to be the calcium sensing GCAP proteins.

5. Role of calcium in phototransduction

Until 1985 calcium was thought to be one of two possible "activation" messengers in vertebrate phototransduction. The "calcium hypothesis" proposed that calcium ions released from the disks by photoactivation of rhodopsin closed the channels in the plasma membrane by binding to them. This view was abandoned when Fesenko *et al.* (1985) showed that light-sensitive channels in excised inside-out patches from ROS were opened by cGMP binding. Even though the "calcium hypothesis" has been rejected, calcium has remained in the spotlight as a key regulator of several steps in the phototransduction process (reviewed by McNaughton 1995, Koutalos and Yau 1996, Polans *et al.* 1996). The calcium feedback signal is considered as one of the main causes of light adaptation and it is also thought to play a role in photoresponse recovery (reviewed by Polans *et al.* 1996, Detwiler and Gray-Keller 1996).

Calcium participates in the regulation of cGMP synthesis through the action of GCAPs discussed above. Another site of calcium action seems to be rhodopsin kinase, acting through calcium-recoverin mediated inhibition as discussed in the R* shut-off section. Gray-Keller and Detwiler (1996) have shown that calcium-sensitive gain-control operates in both dark and light adapted ROS. Lagnado and Baylor (1994) suggest the existence of a novel calcium-dependent regulatory mechanism affecting the catalytic activity of rhodopsin. They showed that lowering intracellular calcium in truncated rods reduced the initial slope of the rising phase of the light response, consistent with a reduction of the catalytic activity of R*. The effect of low calcium is observed only when calcium is decreased at the beginning of the photoresponse, suggesting that calcium acts on one of the earliest steps in the transduction cascade.

6. Light adaptation

The dark-adapted vertebrate retinal rod exhibits the ultimate sensitivity to light in that it is capable of reliably detecting single photons. The

process of light adaptation reduces the sensitivity of the receptor and thus expands the dynamic range of the rod allowing it to detect changes in light intensity over nearly five orders of magnitude (McNaughton 1990). The operating range of photoreceptors is quite remarkable but the temporal resolution is not outstanding. The time lag between the absorption of a photon and the response peak in dark-adapted rods is around 1 s. Even light-adapted rods, with improved time resolution, have a delay of around 20 ms caused by the reaction times of the enzyme intermediates in the cascade (McNaughton 1990).

The adapted flash responses in vertebrate photoreceptors are desensitized, reach peak earlier and recover sooner than responses in the dark (Baylor and Hodgkin 1974). Changes in intracellular calcium level are thought to underlie the adaptational changes and there is a great volume of literature dealing with the precise role of calcium in adaptation. The question of whether the decline of calcium is the sole cause of changes in photoresponse sensitivity and kinetics during adaptation is controversial. Nakatani and Yau (1988), Matthews *et al.* (1988), Matthews (1995) and Fain *et al.* (1996) argue that light adaptation is mediated by changes in calcium level only. Other researches clearly demonstrate that it is impossible to reproduce all photoresponse adaptational phenomena by manipulating intracellular calcium levels in the dark in functionally intact ROS (Gray-Keller and Detwiler 1996). Their results show that calcium regulates the gain of the photoresponse equally in dark and steady light, but the characteristic changes in the kinetics of the adapted response described earlier are only seen when low calcium is combined with light.

As mentioned above, when the two noncatalytic cGMP binding sites on PDEα and PDEβ catalytic subunits are occupied (Yamazaki *et al.* 1980) GTPase acceleration of transducin by PDEγ is inhibited (Arshavsky *et al.* 1992). This feedback might also play a role in adaptation by delaying PDE shut off when cGMP levels are high as in the dark-adapted state (Detwiler and Gray-Keller 1996). During background illumination, when cGMP concentration is lowered, the GTPase would be accelerated and PDE activity would shut-off earlier. This would accelerate response recovery as observed during light adaptation.

Another suggested feedback in adaptation is downregulation of T$\beta\gamma$ by dephosphorylated phosducin (Lee *et al.* 1992, Yoshida *et al.* 1994, Wilkins *et al.* 1996). This process is reported to be regulated by calcium levels through calcium/calmodulin-dependent adenylyl cyclase (Willardson *et al.* 1996). Phosducin phosphorylation is slower in low calcium which tends to promote the formation of dephosphorylated phosducin. This would decrease the gain of phototransduction cascade by blocking the formation of the functional transducin trimer by binding to T$\beta\gamma$.

An interesting putative explanation for adaptation in low light levels involves a special phenomenon, "high gain" phosphorylation of rhodopsin. An activation of one rhodopsin molecule causes phosphorylation of many molecules of non-bleached rhodopsin at low light levels. This "high gain" phosphorylation of rhodopsin has been described in bovine ROS (Aton 1986) and in electropermeabilized frog ROS (Binder *et al.* 1990). Some researchers attribute this phosphorylation to rhodopsin kinase activity (Dean and Akhtar 1993, 1996) and evidence from experiments conducted in the reconstituted ROS system shows that rhodopsin kinase itself is able to cause this effect (Chen *et al.* 1995a).

If large portions of non-bleached rhodopsin were phosphorylated during dim background light, one could explain the decreased amplitude and the shorter duration of the adapted responses by faster arrestin binding to the pre-phosphorylated rhodopsin. A recent paper by Binder *et al.* (1996) excludes this model for adaptation by showing that the maximal amount of phosphorylated nonbleached rhodopsin molecules under optimal conditions does not exceed 3 % of the total rhodopsin content which is not enough to account for light adaptation. The researchers also show that phosphorylation of nonbleached rhodopsin molecules does occur in photoreceptors of living animals but the significance of this phenomenon remains unclear.

Conclusion

The main pieces of the phototransduction puzzle seem to be on the table. The crucial task in the future is to fit them together and to determine if there are some pieces missing and, if so, to identify them. The preceding discussion reflects the immense complexity of the molecular mechanisms involved in the phototransduction process and the difficulty of integrating observations obtained from various experimental approaches. Many findings from biochemical experiments are still to be verified by tests in functionally intact system.

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