The Chemistry of Vision

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Summary. The visual response is initiated by light reception and transduction into chemical and electrical energy in the outer-segment membranes of rod and cone cells. Recent research on the molecular events controlled by light has clarified the roles of some of the rod outer-segment biomolecules. These developments and the current unresolved questions are described.

Many will remember textbook comparisons of the eye and the camera with analogies between the pupil and the *f*-stop and between the retina and the photographic film. In a sense the inventors of the camera designed it to conform to a 19th-century concept of the functioning of the eye. It is now known that these systems function by completely different mechanisms. The photographer regulates the luminance level of the scene to match the sensitivity characteristics of the photographic film by varying the time of exposure and the size of the aperture. The retinal sensitivity of the eye adjusts to correspond to the luminance level of the scene, a process known as adaptation. To adapt successfully over the several orders of magnitude of light level that we encounter in our lives, the rod and cone cells of the retina must be exceedingly sensitive to light.

A properly dark-adapted rod cell can detect a single photon (*1*). Rods (Fig. 1) are elongated cells with a light-sensitive outer segment at one end and a synaptic body at the opposite terminus. Illumination of the outer segment alters the plasma membrane conductance with hyperpolarization of the cell membrane and subsequent modulation of synaptic transmitter release. In the dark, the sodium ion permeability of outer-segment plasma membranes is high, 10^9 sodium ions per second. At low light, the sodium ion flux is reduced by about 10^7 Na^+ per second per light-excited rhodopsin molecule (*2, 3*). It is generally accepted that an internal transmitter is essential to communicate between light-sensitive rhodopsin molecules in the disc membranes of the rod outer segment (ROS) and the sodium ion permeability sites of the plasma membrane (*4*). This central concept poses the issue of how the large change in sodium ion permeability is controlled by a few photostimulated rhodopsin molecules. Two general approaches have advanced the understanding of vertebrate vision; electrophysiologists have delineated the electrical response of intact photoreceptor cells, and chemists have isolated and characterized components of the ROS. In this article, the biochemistry of the photoreceptor cell outer segments, the site of light reception, and transduction of light energy into chemical and electrical energy are emphasized.

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Rhodopsin in Membranes

The greater size and availability of rods compared to cones dictate the use of ROS membranes for many biochemical studies. Intact ROS membranes with enclosed cytoplasmic proteins and small molecules can be released from the retina by gentle homogenization and purified by flotation on solutions of sucrose or Ficoll polymer (*5*). For some purposes, it is useful to isolate the disc membranes by osmotically shocking the plasma membrane, with subsequent flotation of the sealed discs (*5*).

The composition of the isolated disc membranes is dependent on the osmolarity of the isolation buffer. Hypotonic conditions yield purified ROS disc membranes that are composed primarily of phospholipids and rhodopsin, about 70 lipids per rhodopsin (*6*), and are free of the soluble species of the cytoplasm and the enzymes that associate with the disc surface. The ROS lipids are known for their high degree of unsaturation (*7*); consequently the lipid bilayer is very fluid, as shown by the rapid rotational (*8*) and lateral (*9*) diffusion of rhodopsin in ROS membranes.

The primary structure of bovine rhodopsin, a single polypeptide with a molecular weight of about 38,000 (Fig. 2), has been partially determined. Initial evidence for the elongated nature of rhodopsin was provided by fluorescence energy transfer (*10*). Data from x-ray diffraction (*11, 12, 13*), neutron diffraction (*14*), freeze-fracture electron microscopy (*15*), and nuclear magnetic resonance spectroscopy (*16*) demonstrated that rhodopsin penetrates into and probably through the disc membrane. The transmembrane character of rhodopsin was convincingly confirmed by chemical labeling (*17*). The carboxyl terminal that bears seven phosphorylation sites is accessible from the cytoplasmic side of the disc to water-soluble proteolytic enzymes (*18*), and rhodopsin is cleaved into two major fragments by thermolysin (*18*). The peptide spans the disc membrane an odd number of times with 7 ± 2 α-helical segments. The α-helical content was estimated from the circular dichroism (*19*), and the perpendicular orientation of the α helices to the membrane plane was deduced from the infrared linear dichroism (*20*).

The transmembrane protein rhodopsin can be solubilized and purified in selected detergents (*5*). Mixed micelles of rhodopsin, lipid, and detergent are photochemically sensitive. Light exposure causes the chromophore, 11-*cis*-retinal, to undergo a series of transformations that result in the dissociation of *trans*-retinal from the protein to yield bleached rhodopsin (opsin). This bleaching sequence is marked by a series of spectral intermediates (*21*) whose relaxation characteristics have been established for whole cells, ROS membranes, and rhodopsin-detergent micelles. Relaxation rates are faster for rhodopsin in micelles than in natural membranes. Furthermore, the addition of 11-*cis*-retinal to opsin in most detergents does not produce rhodopsin; therefore, although rhodopsin is stable in the dark in detergent micelles, it is no longer in a native configuration after light exposure.

These difficulties are circumvented by incorporation of purified rhodopsin into phospholipid bilayers. Hubbell and his associates introduced the preparation of rhodopsin-phospholipid membranes by detergent dialysis and demonstrated the bilayer nature of the synthetic membranes by freeze-fracture electron microscopy (*22*). The lipid bilayer main-

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tains bleached rhodopsin in a native, regenerable configuration. Rhodopsin-lipid bilayers are light-sensitive and show the normal bleaching intermediates. Membranes prepared from unsaturated phospholipids display kinetics of relaxation of the spectral intermediates comparable to that of ROS membranes (*23*). The availability of rhodopsin-lipid membranes has been especially useful in the study of rhodopsin interaction with other components of the visual system.

Many investigators now assume that rhodopsin is monomeric in the membrane. The best available evidence from equatorial x-ray diffraction of intact ROS provides estimates of the nearest approach of rhodopsin molecules of 34 to 45 Å with an average spacing of rhodopsin molecules of 56 Å (*13, 24*). These data are consistent with monomeric rhodopsin, but the broadness of the diffraction profiles makes it difficult to exclude some portion of the rhodopsin as multimers. Although rotational and lateral diffusion measurements are readily interpretable on the basis of a monomeric rhodopsin (*8, 9*), the degree of self-association of rhodopsin in membranes remains to be established.

Light-Activated Enzymes of the ROS

The focus of research on visual excitation has shifted from rhodopsin to include other recently detected components of the ROS. Since the early 1970's, an increasing number of light-activated enzymes have been studied (*25*). These include a cyclic nucleotide phosphodiesterase (PDE) (*26*), a complex of polypeptides with guanosine triphosphatase (GTPase) activity (*27, 28*), and a kinase that phosphorylates bleached rhodopsin (opsin kinase) (*29*). Each enzyme is transparent to visible light, with an action spectrum that corresponds to the absorption spectrum of rhodopsin; thus some mode of interaction between the enzymes and light-exposed rhodopsin is essential.

The enzymes responsible for the light-initiated PDE and GTPase activity of the ROS are peripheral proteins associated with the cytoplasmic surface of the ROS disc membranes. Preferential binding of the enzymes to disc membranes under dark isotonic conditions permits the preparation of disc membranes that consist of the integral protein, predominantly rhodopsin, and these two peripheral proteins (Fig. 3). The peripheral enzymes may be extracted in the dark with hypotonic solutions and separated by gel permeation chromatography (*30*). Kühn found that light induces a binding of the enzymes with GTPase activity to the disc membrane even under hypotonic conditions (*31*). This phenomenon can be used to separate and purify these enzymes (*31*). If the membranes are exposed to light and then washed with hypotonic buffer, only the PDE is removed, as the GTPase activity is still associated with the ROS. Subsequent treatment of the ROS-GTPase with a buffer containing guanosine triphosphate (GTP) results in the dissociation of the GTPase from the rhodopsin-rich disc membranes (*31*).

The PDE (bovine) is a holoenzyme that shows three subunits (at estimated molecular weights of 88,000, 84,000, and 13,000) upon sodium dodecyl sulfate (SDS) discontinuous gel electrophoresis

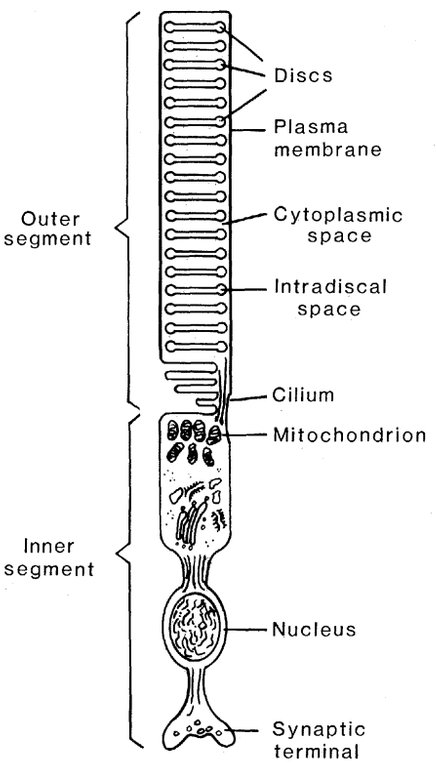

Fig. 1. Schematic diagram of a vertebrate rod cell. Approximately 10^8 rod cells are closely packed in a retina and are aligned with the incoming light. The cell consists of the synaptic terminal for communication with the secondary neurons of the retina, the inner segment concerned with cellular metabolism, and the specialized outer segment. The inner and outer segments are connected by a narrow ciliary stalk. The outer segment is composed of several hundred to a few thousand flattened, stacked disc membranes, which are enclosed by the plasma membrane. The repeat distance of the disc membranes is about 300 Å (*12*). The disc membranes are formed by evagination and sealing off of the plasma membrane near the base of the outer segment (*63*). Thus, the intradiscal space is originally contiguous with the extracellular fluid and is distinct in composition from the cytoplasm.

(*30*) (Fig. 3). The preferred substrate is cyclic guanosine monophosphate (cyclic GMP) (*26*), which is estimated to have a cytoplasmic concentration of 30 to 60 μM (*32*). The enzyme Michaelis constant, K_m, is 70 ± 20 μM, with a turnover number of ~ 10^3 cyclic GMP molecules per second at 25°C (*33*).

Electrophoresis of the material with GTPase activity shows three bands with estimated molecular weights of 37,000, 35,000, and 6,000 (*31*). The low turnover rate, 1 mole of GTP hydrolyzed per mole of GTPase per minute at 30°C (*31*), indicates that this heterogeneous complex of three polypeptides regulates other enzymes in the ROS. The higher molecular weight (α) polypeptide can be isolated by detergent-free high-pressure liquid chromatography (*34*) or by native gel electrophoresis (*35*). In addition to showing GTPase activity, these polypeptides, or the α unit alone (*34*), bind GTP upon light activation of the ROS (*36, 37*). These key observations clarified the role of these three polypeptides, which have been termed guanine nucleotide-binding protein (*36*), transducin (*34*), and G protein for GTP-binding protein (*35*).

Activation of the Phosphodiesterase

Light-induced PDE activation produces a remarkable amplification. Yee and Liebman introduced a real-time method of assaying PDE activity and found a rate of 4×10^5 molecules of cyclic GMP hydrolyzed per second per light-activated rhodopsin (Rh*) in isolated frog or bovine ROS disc suspensions (*33*). Woodruff and Bownds (*38*) observed a decrease in cyclic GMP content of isolated intact whole frog ROS of 10^4 per Rh* molecule in 100 milliseconds. Liebman and Pugh (*39*) deduced that the amplification occurs in two stages, first the activation of several PDE per Rh*, then the rapid hydrolysis of cyclic GMP by each activated PDE. A current hypothesis for the activation sequence is given by the equations below, where G_{GDP} represents G protein with bound guanosine diphosphate (GDP), G^*_{GTP} symbolizes G protein with bound GTP, and the asterisk indicates an activated species (see scheme 1). Light-activated rhodopsin (Rh*) catalyzes the binding of GTP to the G protein, and as many as a few hundred GTP molecules are bound in exchange for GDP (one per G protein) for each Rh* (*37*). The G^*_{GTP} activates PDE by reducing inhibitory constraint (*40*). The activated PDE hydrolyzes about 10^3 molecules of cyclic GMP per second until the γ phosphate of the

bound GTP is removed by the GTPase activity to inactivate the system. The overall in vitro amplification is therefore $> 10^5$ cyclic GMP molecules hydrolyzed per second per Rh*. The cyclic nature of the relations is represented by the scheme shown in Fig. 4. Several authors (*27, 34, 41*) have noted the similarities between this sequence and the hormonally activated adenylate cyclase in the plasma membrane of other cells. In both systems, the exchange of cytoplasmic GTP for bound GDP is mediated by an activator (hormone-receptor complex or Rh*), and the activity is terminated by GTP hydrolysis. Functional exchange of the components of these systems was reported recently (*42*).

The modes of interaction between a transmembrane protein, such as rhodopsin, and membrane surface–associated enzymes, such as G protein and PDE, are of general interest for the understanding of membrane structure and function. First it is necessary to establish the location of the surface-associated enzymes on the disc membranes in the dark. Recombination of the extracted surface-associated enzymes, G protein and PDE, with rhodopsin-lipid membrane vesicles in isotonic media yields preparations that exhibit light-induced enzymatic activity comparable to that of ROS discs isolated under isotonic conditions (*43*). The observed activity does not depend strongly on the lipid head group composition of the rhodopsin-lipid vesicles (PC, PC-PE, PC-PE-PS, as explained in the legend to Fig. 2). This shows that either the peripheral enzymes bind to the constant factor in each membrane, the rhodopsin, or the membrane association is nonselective in the dark, and the G protein and PDE adhere well to various phospholipid surfaces. The latter explanation is supported by the following observations. Gel electrophoresis patterns of extracted ROS enzymes combined with PC-PE bilayers are shown in Fig. 3. The PDE and G protein are associated with the lipid membrane in the samples obtained by pelleting the lipid bilayers under isotonic conditions but are not associated with the membranes under hypotonic conditions. Either both peripheral enzymes associate with phospholipid surfaces under isotonic conditions or one binds to the lipid surface while the second adheres to the first. The PDE strongly associates with phospholipid membranes, whereas the G protein prefers to associate with PDE or rhodopsin.

Light acts to alter the random distribution of transmembrane rhodopsin molecules and the peripheral enzymes nonspecifically associated with the lipid-membrane surface to initiate enzyme activity. A light-activated rhodopsin (Rh*) could diffuse laterally and integrate (sweep out) the disc membrane surface in ~ 1 second (*39*). Thus the Rh* would serially interact with the G proteins on the surface and catalyze the GTP binding. Subsequently the G^*_{GTP} must find and interact with a PDE. The rapid

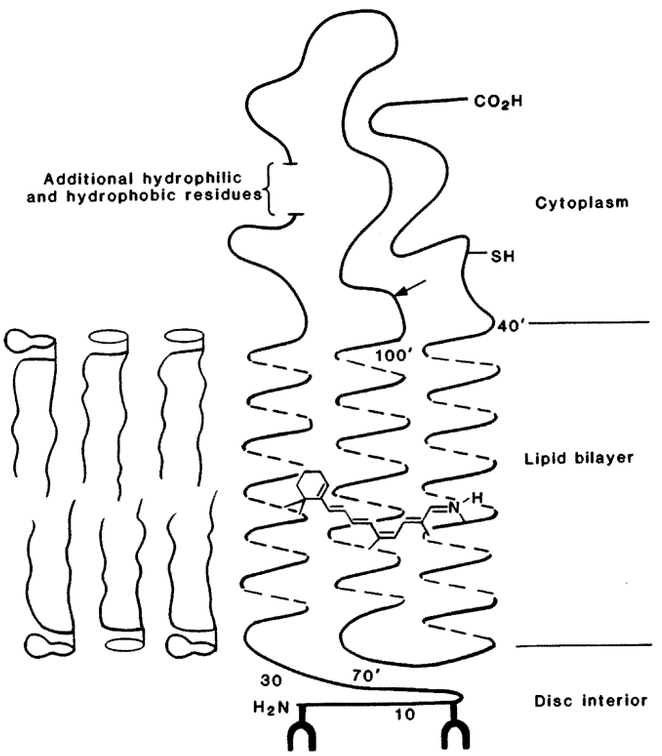

Fig. 2. Schematic representation of rhodopsin and phospholipids in the disc membrane bilayer. Three of the 7 ± 2 α helices of rhodopsin are shown. The amino acid sequence is established from the carboxyl terminus to the thermolysin cleavage site indicated by the arrow. The first 40 residues are predominantly hydrophilic and include several phosphorylation sites (residues 6′, 7′, 9′, 11′, and 12′ to 14′, where the prime sign indicates the numbering is from the carboxyl terminus) and three cysteines, one of which is very reactive (33′). The peptide then has two hydrophobic sequences (residues 40′ to 66′ and 73′ to 96′), each of which is ascribed to an α helix. Halfway down the first helix is the retinal binding site (lysine 53′). The 11-*cis*-retinylidene chromophore is parallel to the membrane surface and is presumed to lie between some of the α helices. The amino-terminal sequence has two oligosaccharides (indicated by the dark lines) at the 2 and 15 positions. This model of rhodopsin is adopted from Fung and Hubbell (*17*) and Hargrave *et al.* (*18*). The disc membrane lipids, 40 percent phosphatidylcholine (PC), 40 percent phosphatidylethanolamine (PE), and 13 percent phosphatidylserine, are represented with a preference for PE (small head group) facing the cytoplasm and PC (large head group) facing the disc interior (*6, 64*). While this article was in preparation, two groups reported the complete amino acid sequence of bovine rhodopsin (*65*).

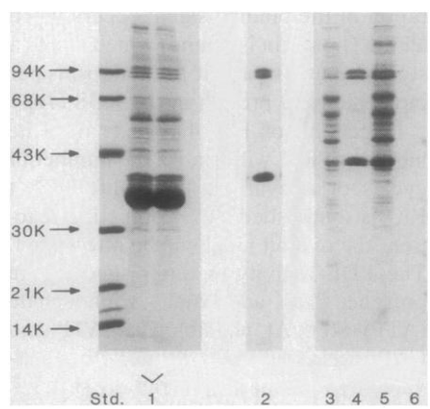

Fig. 3. Polypeptide composition of samples of ROS and extracts analyzed by electrophoresis on discontinuous 15 percent polyacrylamide gels in the presence of 0.1 percent SDS. The numbers in the left margin are the molecular weights of the standards (*Std.*). The slots labeled 1 contain partially purified bovine ROS from a 38 percent sucrose flotation under isotonic conditions. The major band is due to rhodopsin, and the two doublets, near 40K and 90K (K = 1000), are due to the G protein and PDE, respectively. Many of the unidentified bands are due to soluble polypeptides which may be removed from the ROS by isotonic washes (*30*). The G-protein doublet appears at 37K and 35K on continuous gels (*31*). Slot 2 shows the polypeptides extracted from purified ROS membranes by a hypotonic wash. The ROS were previously washed with isotonic buffer to remove soluble polypeptides. The predominant pair of doublets are due to the G protein and PDE. The lower molecular weight γ subunits of these enzymes run at the buffer front and are not distinguished on these gels. Slots 3 to 6 show polypeptides from hypotonic extracts of partially purified ROS in combination with PC-PE lipid bilayers. Two experiments are shown. In the first (slots 3 and 4), the extract of polypeptides is mixed with the lipid bilayers in isotonic media then centrifuged, and the supernatant (slot 3) and the pellet (slot 4) are separated. The two doublets in slot 4 are due to the G protein and PDE associated with the lipid bilayer pellet. The second experiment (slots 5 and 6) was performed under hypotonic conditions. The G protein and PDE remain with the supernatant (slot 5) and are not observed with the lipid membrane pellet (slot 6).

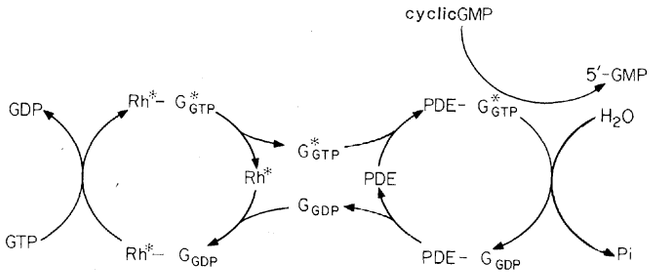

Fig. 4. Cyclic scheme for the activation of cyclic GMP–phosphodiesterase in ROS membranes; Rh*, light-activated rhodopsin; G_{GDP}, G protein with bound GDP; G^*_{GTP}, G protein with bound GTP; PDE, phosphodiesterase. The Rh* and G_{GDP} transiently associate until GTP is exchanged for GDP. The G^*_{GTP} dissociates from Rh*, freeing it to catalyze additional exchanges. The G^*_{GTP} interacts with PDE to activate it, until the bound GTP is hydrolyzed to GDP by the GTPase. The G_{GDP} is then available to bind with the same or another Rh*.

$Rh \xrightarrow{h\upsilon} Rh^*$	Light absorption	
$G_{GDP} + GTP \xrightarrow{Rh^*} G^*_{GTP} + GDP$	GTP binding	
$PDE + G^*_{GTP} \longrightarrow PDE{-}G^*_{GTP}$	PDE activation	Scheme 1
$\text{Cyclic GMP} + H_2O \xrightarrow{PDE-G^*_{GTP}} GMP + H^+$	Hydrolysis	
$PDE-G^*_{GTP} \xrightarrow{\text{GTP hydrolysis}} PDE + G_{GDP}$	Inactivation	

movement of the peripheral enzymes on the surface may complicate this picture. We have observed that a bleached rhodopsin in one membrane vesicle can activate, within a few seconds, enzymes that originate on another membrane (*43*); for example, rhodopsin-lipid vesicles devoid of enzyme activity were exposed to a light flash, then mixed in the dark with unilluminated ROS membrane that had associated peripheral enzymes. Phosphodiesterase activity was observed within 2 seconds after mixing. The rhodopsin-lipid vesicles and ROS membranes were separated by their different sedimentation behavior, and both PDE and G protein were found on the vesicle fraction as well as the ROS membranes. These experiments demonstrate that the peripheral enzymes are in rapid equilibrium between binding sites. Further research is necessary to evaluate the roles of lateral diffusion and enzyme hopping during light activation of PDE and deactivation of the PDE:G-protein complex.

As was noted earlier, light in the absence of GTP increases the binding of the G protein to the membranes, and GTP will displace the enzyme from the membrane surface if PDE is not present (*31*). In terms of the cyclic scheme (Fig. 4), the G_{GDP} associates temporarily with the Rh*, until GTP replaces GDP. In the absence of GTP, the association may last several minutes. The G^*_{GTP} preferentially binds to PDE to activate the PDE. In the absence of PDE, the G^*_{GTP} is not tightly associated with the membrane and can be extracted easily and isolated (*31*). Therefore, G_{GDP} prefers to bind to Rh*, and G^*_{GTP} binds to PDE. Kühn *et al.* (*44*) have evaluated the speed of these binding phenomena by light-scattering measurements. The binding of the G_{GDP} to ROS membranes causes a light-scattering phenomenon with a latency of ~ 100 msec at 20°C. A second scattering change in the presence of GTP is ascribed to the dissociation of G^*_{GTP} from Rh*. Both of these signals are faster than the electrical response of the rod. Saturation of the binding signal occurs when all the G protein is bound, which yields a stoichiometry of about 10 rhodopsins per molecule of G protein in the ROS (*44*).

Inspection of Fig. 4 reveals that the lifetime of Rh* will control the number of cycles of activation. If the lifetime of Rh* is diminished by a competing reaction, the overall yield will be attenuated. The PDE activity is diminished in the presence of adenosine triphosphate (ATP) (*41*). At physiological ATP and GTP levels (millimolar) the PDE is activated by Rh* and then is rapidly shut off. The K_m for the deactivation process was estimated to be 4 μM ATP (*41*), which is similar to that reported for the phosphorylation of opsin (*45*). In the presence of ATP and opsin kinase, Rh* is phosphorylated (*29*) in a manner independent of cyclic GMP.

$$Rh^* + n\ ATP \xrightarrow{\text{kinase}} Rh(PO_4)_n + n\ ADP$$

Isolated ROS membranes with partly phosphorylated rhodopsin (two phosphates per rhodopsin) show reduced PDE activity (*46*). However, ATP-mediated phosphorylations in the ROS other than rhodopsin phosphorylation may also serve to regulate the GTP-based PDE activity (*47*).

The function of the activation of PDE awaits definition. The speed and gain of the enzyme chemistry data obtained in vitro with isolated ROS or reconstituted systems of rhodopsin-lipid and enzymes suggest that the sequence communicates the light excitation from Rh* into the cytoplasm and partly fulfills the requirements for a transmitter from Rh* to the site of the plasma membrane sodium ion permeability. It remains to be shown whether the reactions are fast enough in vivo to precede the electrical response to the cell. Further, it is necessary to demonstrate how the cytoplasmic cyclic GMP concentration might regulate the sodium ion permeability. The potential of cyclic GMP–dependent protein phosphorylations for this role is the subject of current research (*47–49*).

Regulation of ROS Calcium

Experiments with intact isolated ROS demonstrate a parallelism between the cyclic GMP concentration in the ROS and the sodium ion permeability (*38*). Chemical agents that reduce the cytoplasmic cyclic GMP in intact ROS also diminish the sodium ion permeability. The injection of cyclic GMP into rod cells via intracellular electrodes depolarizes and increases the latency of the response of the membrane (*50*). Other experiments with perfused retinas revealed that the effects of cyclic GMP and Ca^{2+} are antiparallel. A decrease in cyclic GMP concentration or an increase in Ca^{2+} concentration in the rod cells of the retina causes the cells to hyperpolarize (*51*). Both types of cellular messengers, ionic (Ca^{2+}) and nucleotide (cyclic GMP), are important in the ROS system (*48*). The interaction between them and their respective functions in the outer-segment membranes of photoreceptor cells is a major unsolved question.

The role of Ca^{2+} in ROS has been a topic of active investigation over the past decade. In 1971 Yoshikami and Hagins (*52*) proposed that the excitatory transmitter is Ca^{2+}. Evidence in favor of a Ca^{2+} transmitter comes from electrophysiological experiments, where the addition of Ca^{2+} to the retina mimics the effect of light (*51, 53, 54*) and the addition of EGTA to retinas attenuates the effect

of light (*54, 55*). Osmotic measurements on isolated ROS demonstrate that Ca^{2+} in the buffer reduces the sodium ion permeability of the plasma membrane (*56*) and depresses the cyclic GMP levels in the ROS (*38*). Sufficient Ca^{2+} is present in the ROS and in the discs for it to be available as a transmitter (*57*). Recently two groups of investigators have observed light-induced Ca^{2+} release from the outer segments of photoreceptor cells in isolated retinas (*58*). The measured release of > 500 Ca^{2+} per Rh* implies that light absorption by rhodopsin leads to an increase in the Ca^{2+} activity of the ROS cytoplasm. The Ca^{2+} release precedes the receptor potential, which strongly suggests that the increase in Ca^{2+} activity regulates the sodium ion permeability of the plasma membrane (*58*) (Fig. 5). In the dark-adapted rod, there is a high sodium ion permeability at the plasma membrane of the outer segment of the cell. Light absorption dramatically decreases the sodium ion permeability, and a calcium ion efflux is observed at the outer-segment plasma membrane.

The source of the light-regulated Ca^{2+} pool may be either the interior of the disc membranes or cytoplasmic binding sites. The transmembrane nature of rhodopsin suggested that it might function as a light-activated ion gate for release of Ca^{2+} from the intradiscal space to the cytoplasm. Several attempts to measure the photoinduced release of Ca^{2+} from disc membranes have failed to show large or fast Ca^{2+} release (or both) upon rhodopsin bleaching (*59*). Many of these attempts were summarized by Smith *et al.* (*60*), who found that the photorelease of $^{45}Ca^{2+}$ from sonicated ROS discs occurred with a yield of 0.75 Ca^{2+} per Rh* at high levels of bleaching. Larger photoreleases of Ca^{2+} ($\sim 10^2$ Ca^{2+} per Rh*) were observed on light exposure of rhodopsin-phospholipid membrane vesicles (*59, 61*). Light exposure of a single rhodopsin per vesicle increases the membrane permeability without destroying the closed unilamellar vesicles (*61*). Although high yields of photoreleased Ca^{2+} were found, the flux is low, ~ 1 Ca^{2+} per Rh* per second (*61*). The inability to demonstrate large light-induced Ca^{2+} fluxes may be a result of current techniques of preparing rhodopsin-lipid vesicles, although the same membranes were successfully used for the reconstitution of the PDE chemistry (*43*). Therefore, the rhodopsin is not likely to have been significantly perturbed by the preparation of the vesicles. The lack of firm evidence for light-induced channel formation in rhodopsin membranes leaves cytoplasmic binding sites as a source of the light-regulated calcium in the ROS (*62*), a hypothesis under study at several laboratories.

Concluding Remarks

Light absorption by the 11-*cis*-retinal chromophore of the transmembrane rhodopsin produces an intermediate (Rh*) which catalyzes the binding of GTP to the membrane surface–associated G protein. Interaction between the G^*_{GTP} protein and the phosphodiesterase on the membrane surface activates the PDE, which catalyzes the hydrolysis of cyclic GMP and thereby decreases the cytoplasmic cyclic GMP concentration. This reaction sequence may be modulated by the phosphorylation of Rh*, and it is terminated by the ROS GTPase activity. The in vivo function of the light regulation of the cytoplasmic cyclic GMP concentration is uncertain, although in vitro experiments suggest that it precedes the light-induced change in receptor potential of the cell. Light absorption also increases the Ca^{2+} activity of the ROS cytoplasm. The relationship between the cyclic GMP chemistry and the cytoplasmic Ca^{2+} activity is at present undefined. The increase in Ca^{2+} activity precedes and may control the decrease in ROS plasma membrane sodium ion permeability. The hyperpolarization of the cell modulates synaptic transmitter release and thereby controls cellular communication and information transmission.

Progress in the past decade has been rapid and was aided by the introduction of new techniques and the discovery of several ROS proteins, especially the peripheral enzymes. A clearer understanding of the chemical processes of the photoreceptor cell that regulate visual excitation and adaptation is likely over the next decade as many of the other constituents of the ROS cytoplasm are identified and studied.

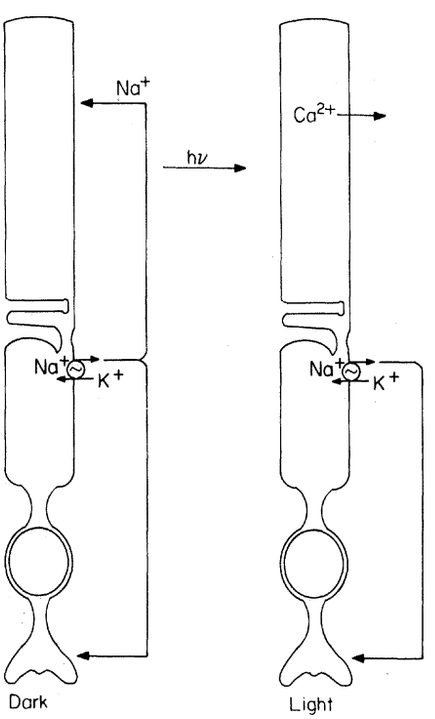

Fig. 5. Representation of a rod cell and the associated current in the dark and with light exposure. The plasma membrane of the rod cell outer segment has a high sodium ion permeability in the dark (left), which is reduced by light. The light-induced decrease in sodium ion permeability is accompanied by calcium ion fluxes from the interior of the outer segment to the interstitial space.

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