How vision begins: An odyssey

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Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved May 15, 2008 (received for review April 1, 2008)

Retinal rods and cones, which are the front-end light detectors in the eye, achieve wonders together by being able to signal single-photon absorption and yet also able to adjust their function to brightness changes spanning 10^9-fold. How these cells detect light is now quite well understood. Not surprising for almost any biological process, the intial step of seeing reveals a rich complexity as the probing goes deeper. The odyssey continues, but the knowledge gained so far is already nothing short of remarkable in qualitative and quantitative detail. It has also indirectly opened up the mystery of odorant sensing. Basic science aside, clinical ophthalmology has benefited tremendously from this endeavor as well. This article begins by recapitulating the key developments in this understanding from the mid-1960s to the late 1980s, during which period the advances were particularly rapid and fit for an intricate detective story. It then highlights some details discovered more recently, followed by a comparison between rods and cones.

Vision begins with the absorption of light by visual pigments in the retinal rod and cone photoreceptors and its conversion into an electrical signal, a process called phototransduction. This electrical signal propagates to higher-order retinal neurons (the bipolar and ganglion cells) and eventually to the brain via the optic nerve. The study of phototransduction has a long history, starting with the discovery of the rod pigment, rhodopsin, approximately 130 years ago. Rod and cone pigments are prototypical G protein-coupled receptors (GPCRs). Indeed, phototransduction is at present the most quantitatively studied and arguably the best understood GPCR-signaling process in the body. Also, most phototransduction proteins, when mutated, are found to cause diseases affecting eyesight. These overall advances have come from a wonderful synergy of approaches involving biochemistry, biophysics, physiology, molecular biology, cell biology, and genetics.

The Early Days

Visual pigments consist of an opsin, the protein moiety, covalently linked by a Schiff base to 11-*cis*-retinal (a derivative of vitamin A), the chromophore. Upon absorbing a photon, 11-*cis*-retinal isomerizes to all-*trans*-retinal, and the opsin then undergoes a series of spontaneous conformational changes to become active (in its metaII state). Eventually the pigment is hydrolyzed into opsin and all-*trans*-retinal, and is said to be "bleached". Functional pigment is regenerated when opsin recombines with another 11-*cis*-retinal molecule. Rhodopsin, abundant in most retinas, was first extracted and so named by W. Kühne in the 1870s. Knowledge about the visual pigments and their photo-intermediates rapidly expanded in the 1930s and thereafter, thanks largely to the work of G. Wald, R. Hubbard, and colleagues (1). Despite this foundation, the understanding of how the photoisomerized pigment triggers vision began only in the mid-1960s, when T. Tomita and coworkers, as well as others, discovered surprisingly with single-cell electrophysiology that light elicits a membrane hyperpolarization (instead of depolarization typical of neuronal excitation) in rods and cones, resulting from the *closure* of a cation conductance, the "light-sensitive conductance," on the plasma membrane (2). Soon afterward, W. Hagins and coworkers (3) found that, in darkness, a steady membrane current flows into the rod outer segment, the cell compartment containing rhodopsin and transducing light, and that light suppresses this "dark current", consistent with the hyperpolarizing voltage response. Because neurotransmitter is released by membrane depolarization, the notion was, therefore, that continuous neurotransmitter release occurs in darkness from the synaptic terminal of the photoreceptor, and this release is reduced by light. This was soon confirmed by several laboratories (e.g., refs. 4–7).

How is the light-sensitive conductance closed by light? By around 1970, the concept of an intracellular second messenger mediating signal transduction (8) was already popular. In rods, the pigment is predominantly in the membranes of completely internalized disks in the outer segment (9), whereas the light-sensitive conductance is in the plasma membrane, thus requiring a diffusible messenger to communicate between the two (10). In cones, where the disk and plasma membranes are continuous with each other (9), a second messenger was, in principle, unnecessary, but the quantitative form of the relation between light intensity and electrical response still argued for mediation by a second messenger (11).

Ca^{2+} and cGMP

One suggested mechanism of phototransduction, the Ca hypothesis, was proposed by Hagins in 1971 (10, 12). It postulated that photoisomerized rhodopsin triggers an increase in cytoplasmic free Ca^{2+} concentration in the outer segment, and the Ca^{2+} then blocks the light-sensitive conductance. In rods, the Ca^{2+} was conceived to come from the interior of the membranous discs, whereas in cones, it presumably came from the cell exterior, which is continuous with the discs' interior (12). This hypothesis, rather analogous to excitation-contraction coupling in skeletal muscle, is simple and attractive. Its main supporting evidence was that the dark current and associated membrane depolarization (therefore, the light response) increased when the extracellular or intracellular Ca^{2+} concentration was lowered, and decreased when the latter was raised, suggesting that internal Ca^{2+} inhibited the dark current (13, 14). Although not explicitly stated in the Ca hypothesis, one implication was that the visual pigment might serve as a light-activated pathway for Ca^{2+} entry into the cytoplasm. Soon, a report indeed suggested rhodopsin as a light-activated ion channel (15), although it was never verified. Efforts to detect a Ca^{2+} release from rod disks or cytoplasmic Ca^{2+} rise triggered by light also led to conflicting results (16).

Concurrently, it was discovered that light affects cyclic-nucleotide metabolism in rods, with the experiments presumably inspired by cAMP being a well

Author contributions: D.-G.L., T.X., and K.-W.Y. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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known second messenger (8). After some mis-starts, it became clear that the dark cGMP (not cAMP) content in rods is unusually high and that photoactivated pigment, via a GTP-binding protein later termed transducin (G_t), robustly activates a phosphodiesterase (PDE) specifically hydrolyzing cGMP (16). A number of biochemical laboratories contributed collectively to this emerging picture, notably those of M. Bitensky, M.D. Bownds, N. Virmaux, P. Liebman, W. Zimmerman, H. Kühn, L. Stryer, and others (16). Indeed, G_t was discovered soon after G_s, the first trimeric G protein identified (17). One thinking among the cGMP proponents, albeit tentative and by no means unanimous, was that cGMP in darkness somehow made the light-sensitive conductance open and that its hydrolysis in the light made the conductance close. A piece of supporting evidence was that the dark membrane depolarization and the light response varied with the cytoplasmic cGMP level in the expected manner (14, 18, 19). On the flip side, the light-induced decrease in cGMP content in rods appeared slow and negligible even with light bright enough to saturate the electrical response rapidly (20, 21). Also, how cGMP might activate the light-sensitive conductance remained nebulous.

The unrelenting debate on Ca^{2+} and cGMP continued in the late 1970s and early 1980s, with the pendulum of support swinging back and forth between them. In due course, alternative proposals also surfaced combining Ca^{2+} and cGMP in various modes of interaction (16, 22, 23), prompted by evidence of cross-talk between them. Thus, there was not so much a dearth of evidence implicating some roles for Ca^{2+} and cGMP in phototransduction, but a difficulty in defining their exact roles. It was a chicken-and-egg problem: Which substance mediates photoexcitation and which substance consequently modulates it?

Alongside the above confusion, there was another puzzle. An observed reversal potential of near 0 mV for the light response (2, 11, 24) suggested an underlying nonselective cation conductance, but the invariable disappearance of the light response upon replacement of external Na^+ by other monovalent alkali cations would suggest a Na^+-selective conductance (2, 25, 26). This peculiar behavior even prompted some speculation that the conductance might be an ion carrier rather than ion channel, a notion that became more plausible when the unitary light-sensitive conductance was estimated with noise analysis to be in the femto-Siemen range (27, 28), much smaller than those of familiar ion channels. An alternative proposal, put forth after a Na^+-dependent Ca^{2+} efflux was discovered in rods (refs. 29 and 30; also see below), was that the light-sensitive conductance was nonselective among cations but required external Na^+ to stay open (31).

The Rapid Advances and the Ironies

Progress took a turn for the fast track in 1984–1985. In retrospect, some key elements pointing to the final truth had already existed, but were either misinterpreted or overlooked. On the Ca^{2+} side, an important observation in 1980 was that light triggered a Ca^{2+} efflux from the rod outer segment (29, 30). This Ca^{2+} efflux, via a Na/Ca exchange mechanism (i.e., Na^+ entering the cell in exchange for Ca^{2+} exiting), was interpreted by its discoverers to reflect—in line with the Ca hypothesis—a light-triggered increase in intracellular Ca^{2+} which consequently was extruded from the cell (29, 30). The Na/Ca exchanger is an important and ubiquitous Ca^{2+}-extrusion pathway first discovered in nerve and cardiac muscle in the 1960s (32). From flux measurements in these tissues, the exchange stoichiometry was found to be in the uncertain range of 2–6 Na^+ for one Ca^{2+} (32–34). A stoichiometry of >2, of course, would mean electrogenicity (i.e., the generation of an inward membrane current when the exchanger is running.) In 1984, such a current was indeed detected in single rods—in fact, the first "Na/Ca exchange current" ever observed in any tissue—with a stoichiometry consistent with one net positive charge moving inward for each Ca^{2+} moving outward (35), or three Na^+ exchanging for one Ca^{2+} if no other ions are involved. This exchange current conveniently provided an instantaneous and precise measurement of the Ca^{2+} efflux from a rod during illumination. It was quickly found that the exchange current, and therefore the Ca^{2+} efflux, already existed in darkness and was not enhanced by light, inconsistent with the Ca hypothesis (36, 37). Quite in contrast, the exchange current declined with an $\approx$1-sec time constant (for amphibian rods at room temperature) after bright-light onset (36, 37). Also surprisingly, the initial size of the exchange current and its subsequent decline time course were both stereotyped, being independent of the intensity and duration of the light stimulus once the dark current was completely suppressed, again unexpected from the Ca hypothesis (36, 37). Complementing these surprising findings was convincing evidence showing that the light-sensitive conductance was indeed not Na^+-selective. The experiment was simple (38, 39): When external Na^+ was replaced rapidly enough (within 0.2 sec) by, say, Li^+, the dark current did not decrease instantaneously, but, rather, decayed afterward over several seconds, consistent with a previous speculation that the conductance specifically required external Na^+ to stay open (ref. 31; see previous section). More importantly, the conductance was found permeable to monovalent as well as divalent alkali cations, including Ca^{2+} (40, 41). Thus, the physiological dark current has a Ca^{2+} component. Moreover, it was quickly shown that this steady dark Ca^{2+} influx through the conductance matched the initial Ca^{2+} efflux through the Na/Ca exchanger at the onset of bright light (36, 37). These findings suggested the picture of a steady Ca^{2+} influx balanced by a steady Ca^{2+} efflux in darkness (36, 37). In the light, the Ca^{2+} influx decreases or stops due to conductance closure, but the Ca^{2+} efflux continues unabated [the exchanger was separately found to have no intrinsic photosensitivity (36, 37)], thereby producing a net Ca^{2+} efflux as first observed by others. This net efflux drains the cytosolic Ca^{2+}, explaining why the efflux decreases rapidly in the light (36, 37). This picture is opposite to the tenet of the Ca hypothesis. The light-induced Ca^{2+} decrease was later confirmed directly with Ca-dye signals (42, 43). The Na/Ca exchange later was found also to have a K^+-efflux component, so it is really a Na/Ca,K exchange (44, 45) with a stoichiometry presumably of four Na^+ inward in exchange for one Ca^{2+} and one K^+ outward to produce the net entry of one positive charge per duty cycle (35). This K^+ involvement now appears to be quite unique to the Na/Ca exchanger in rods and cones, presumably designed for pumping intracellular Ca^{2+} to a very low level (theoretically to 2 nM in steady state) by using the outward K^+ electrochemical gradient as an additional driving force (44).

Concurrent with the discoveries on the Ca^{2+} dynamics, there was a major, surprising finding in 1985 about cGMP; namely, a cation channel directly gated by cGMP exists on the rod outer segment. Before this discovery, cyclic nucleotides were generally thought to act only through kinases and, therefore, phosphorylation of target proteins, including ion channels. This ingrained belief no doubt contributed to earlier resistance against cGMP being the direct mediator of phototransduction, because protein phosphorylation is "slow." Perhaps aptly, the cGMP-gated channel in rods was discovered by E. Fesenko and coworkers in the former Soviet Union

(46), who were perhaps less biased by scientific dogmas of the West owing to geopolitical isolation. The experiment, which was conceptually simple by employing the patch-clamp technique (47) to record from an excised, inside-out patch of rod-outer-segment plasma membrane, demonstrated the presence of a cation conductance opened by cGMP without requiring ATP. Interestingly, even before this observation, A. Cavaggioni and coworkers in Italy based on flux measurements had actually reported in 1979 that cGMP opened, probably directly, a cation conductance of unknown function in purified rod disk membranes (48, 49). Even as the work by Fesenko *et al.* appeared, Kaupp *et al.* in Germany (50) and others (51) affirmed with more detailed experiments the presence of a cGMP-gated, Ca^{2+}-permeable conductance in rod disk membrane. Given that the plasma and disk membranes are in close apposition and with signs of cytoskeletal links (52), the question arose whether the observation of Fesenko *et al.* might be an artifact resulting from disk-membrane fragments containing the cGMP-gated conductance being fused with the plasma-membrane patch during excision (53). This concern, however, was soon dispelled when an essentially identical cGMP-gated cation conductance was found on a truncated, open-ended rod outer segment, which resembled an excised patch (a "macropatch," so to speak) but had an intact plasma membrane (54, 55). Most importantly, this conductance was suppressible by light, provided that the light-triggered cGMP hydrolysis was allowed to proceed (i.e., in the presence of GTP to permit GTP/GDP exchange at G_t, hence PDE activation) (54, 55). Thus, the light-sensitive conductance and the cGMP-gated conductance are one and the same entity. As a further irony, subsequent immunocytochemistry demonstrated that the cGMP-gated conductance is present *only* on the plasma membrane (56); thus, the "purified" disk membranes in the biochemical experiments were contaminated by plasma membrane, not the other way around!

One surprise from the truncated-rod experiments was that the cGMP-gated current, when fully activated by high cGMP, was tens of times larger than the physiological dark current (54, 55; see also ref. 57). Thus, only ≈1% of the conductance is open in the intact rod in darkness, and this percentage never increases because light only closes the conductance. It would seem wasteful for the cell to make use of only a tiny fraction of the available conductance. However, if the cell chose to have a much smaller overall conductance and make full use of it, there would be only two alternatives, neither too desirable (58, 59). The first would be to keep the dark free cGMP level high enough for fully activating the conductance, in which case a substantial light-induced cGMP hydrolysis would be required for any conductance decrease to occur. Moreover, with constitutive PDE activity in darkness (see below), a high steady cGMP concentration would elicit high futile cGMP hydrolysis and, thus, be also wasteful. The second alternative would be to increase the affinity between the conductance and cGMP so that even a low cGMP concentration would fully activate the conductance. In this case, the cGMP already bound to the open conductance in darkness would unbind too slowly even as cytosolic cGMP was hydrolyzed, rendering vision slow and ineffective. Incidentally, unlike almost all ligand-gated conductances, this conductance does not show desensitization to its ligand, cGMP (46, 50, 54, 55). This unusual property is imperative for phototransduction by sustaining a steady dark current suppressible only by light.

From the ≈1% open conductance in darkness and the measured dose–response relation between cGMP concentration and conductance activation, the free cGMP concentration in the rod outer segment was estimated to be approximately a few micromolar in darkness (54, 55), compared to a total cGMP concentration of ≈60 μM (20). This small percentage of free cGMP nicely explained the puzzle mentioned earlier that the total cellular cGMP content changed little even with light bright enough to saturate the electrical response (corresponding to near-zero free cGMP). It is now clear that the bulk of cGMP resides at none other than the PDE, which has noncatalytic, high-affinity binding sites for cGMP (60, 61) apparently serving a modulatory function (62, 63). There is just enough PDE (30 μM of the $PDE_{\alpha\beta}$·2PDEγ heterotetramer, with one noncatalytic, cGMP-binding site on each of the catalytic subunits, PDE_α and PDE_β) to take up most of the cGMP.

Another irony is that Ca^{2+} turns out to block the light-sensitive conductance after all (64–66), although this action does not partake in phototransduction as postulated by the Ca hypothesis. Ca^{2+} permeates the conductance well, accounting for ≈15% of the dark current despite being 100-fold less concentrated extracellularly than Na^+ (36, 37), but also partially blocks it, a property readily observable in excised rod-membrane patches (65, 66). Although Ca^{2+} (and Mg^{2+}) is capable of conductance blockage from both extracellular and intracellular sides, the steady block is predominantly from the extracellular side under physiological ionic and voltage conditions. Thus, there would be little consequence (65) even if cytoplasmic free Ca^{2+} were to increase in the light. Indeed, it is possible to load a rod with a large amount of Ca^{2+} without the light-sensitive conductance closing immediately (35, 39). Likewise, the channel remains light-suppressible even when the free Ca concentration in the rod is buffered (67). The steady, fast block by extracellular divalent cations translates into a smooth reduction of the conductance after low-pass filtering by the membrane time constant of the cell, explaining the low apparent unitary conductance (in the femto-Siemen range; see previous section). Upon removal of divalent cations from both sides of an excised membrane patch, single-channel openings were detected with a unitary conductance in the pico-Siemen range (≈250-fold larger) (66, 68). Thus, the light-sensitive conductance is clearly made up of ion channels rather than carriers. Henceforth, we shall call it the "light-sensitive channel." The divalent-cation block allows a much larger number (≈10,000) of effectively tiny channels to participate in sustaining the dark current, thus reducing the channel-quantization noise in darkness otherwise detrimental to dim-light detection (59).

If intracellular Ca^{2+} does not block the channel physiologically, why does high internal Ca^{2+} reduce the dark current as found earlier by others? This question also became understood. As mentioned in the previous section, experiments in the late 1970s found cross-talk between Ca^{2+} and cGMP in rods, with the cGMP level varying inversely with the imposed high or low Ca^{2+} concentration (16, 69, 70). This apparently was due to Ca^{2+} inhibiting cGMP synthesis (71). Thus, high internal Ca^{2+} closes the channel indirectly by affecting cGMP metabolism. The same reciprocal relation between Ca^{2+} and cGMP explained the previously puzzling disappearance of the light response upon replacement of external Na^+ by other monovalent alkali cations, as follows. The Na^+-dependent Ca^{2+} extrusion cannot be driven by external cations other than Na^+ (35), without which the steady Ca^{2+} influx through the channel rapidly elevates Ca^{2+} intracellularly, thus lowering cGMP and closing channels. Indeed, the dark current persisted when external Na^+ replacement and Ca^{2+} removal occurred simultaneously (37, 72, 73).

A coherent picture of phototransduction emerged (65). In darkness, a steady

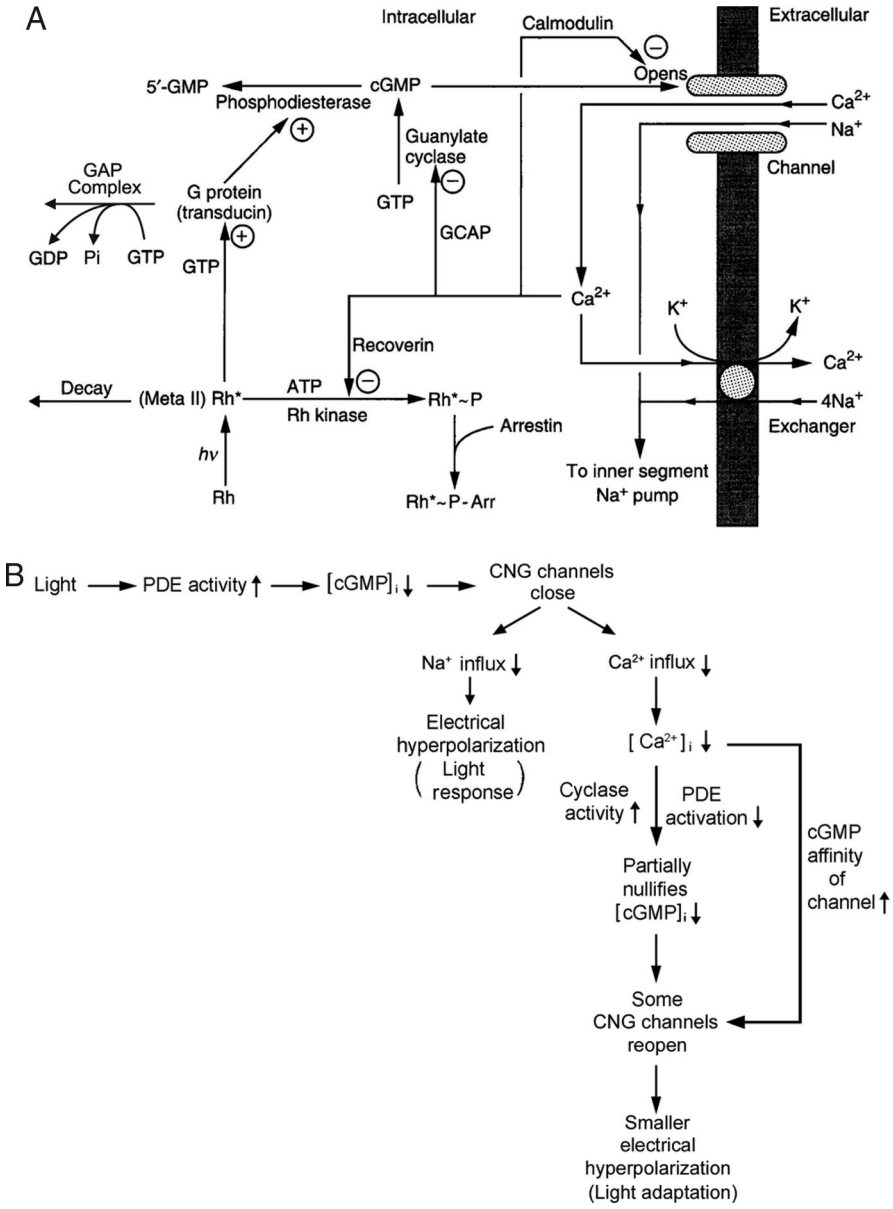

Fig. 1. Phototransduction mechanism in rods. (*A*) Schema showing the reaction pathways in the outer segment. GCAP, guanylate–cyclase-activating protein; *hν*, photon; Rh, rhodopsin; Rh*, photoactivated rhodopsin; Rh*~P, phosphorylated form of rhodopsin; GAP (GTPase-activating protein) complex, composed of (not shown) RGS-9 (regulator of G protein-signaling isoform 9), PDEγ (inhibitory subunit of the phosphodiesterase), R9AP (RGS-9 anchoring protein), and $G_{\beta 5}$ (an orphan G_{β} subunit). +, stimulation or positive modulation; −, inhibition or negative modulation. (Modified with permission from refs. 77 and 140, based on an original figure in ref. 65.) (*B*) Flow chart showing the sequence of events triggered by light in phototransduction. (Modified from ref. 77, based on an original figure in ref. 65.)

cytosolic cGMP concentration keeps some cGMP-gated channels open. Light stimulates PDE to heighten cGMP hydrolysis. The resulting decrease in cGMP level closes the cGMP-gated channels to produce a membrane hyperpolarization as the light response. This channel closure upsets the on-going balance between steady Ca^{2+} influx and efflux, causing cytosolic Ca^{2+} to decline. This Ca^{2+} decline disinhibits cGMP synthesis as negative feedback to produce a partial recovery in the cGMP level, effectively causing adaptation to light. Low Ca^{2+} also appears to reduce PDE activity (74), likewise producing negative feedback and light adaptation. In short, cGMP mediates photoexcitation, whereas Ca^{2+} mediates light adaptation. Supporting this picture, the active adaptation by rods to light essentially disappeared when intracellular Ca^{2+} was clamped at the dark level (72, 73).

The Ca^{2+} feedback not only underlies adaptation to light but is also expected to dampen the dark fluctuations in cGMP level present in darkness (75, 76), thereby improving dim-light detection (77). This dampening function has indeed been found recently (78). Hence, Ca^{2+} reduces background noise directly by channel block and indirectly by dampening cGMP fluctuations. Considering that ≈99% of the cGMP-gated channels are closed in darkness but will open with an accidental spike in cGMP, tight cGMP regulation is an important safeguard against any detrimental, excess influx of cations (77).

The Current Picture

Fig. 1*A* summarizes the key components of rod phototransduction as currently known. Fig. 1*B* shows the sequence of light-triggered events. This picture, also applicable to cones (refs. 65 and 77; also see next section), has remained largely unchanged since 1985 (65), but a large number of new details have been added over the years. In particular, mouse genetics together with suction-pipette recording (79, 80) from single mouse rods has allowed detailed and quantitative characterization of each step, permitting determination of physiological rate constants (81). Some new details, meant to be illustrative rather than comprehensive, are highlighted below.

One defining feature of rod phototransduction is the large number of G_t molecules activated by a single photoisomerized rhodopsin molecule, partly accounting for the exceptional sensitivity of rods, which can signal single-photon absorption (82, 83). The number of G_t activated per rhodopsin was initially thought to be as high as $\approx 10^3$ (84) but has since been revised downwards, recently to ≈20 in mouse assuming ≈300 activated G_t molecules per second per rhodopsin at 37°C (85, 86) and an ≈80-msec active rhodopsin lifetime *in situ* (87). The widely accepted concept of high amplification in GPCR signaling has also arisen partly from this multiplicity of G_t molecules activated per rhodopsin molecule (but see *Epilogue*). The rhodopsin-G_t interaction is by random collision via two-dimensional diffusion on the disk membrane (88). The active G_t α-subunit ($G_{t\alpha}$.GTP) dissociates from $G_{t\beta\gamma}$ and binds to PDE_{γ} to remove its inhibition on the catalytic $PDE_{\alpha\beta}$, thus activating PDE. With the PDE complex being $PDE_{\alpha\beta}$·$2PDE_{\gamma}$, two bound $G_{t\alpha}$ molecules are presumably required for full enzyme activation, but whether one bound $G_{t\alpha}$ causes half-activation is unclear. A highly useful theory, the Lamb–Pugh model, has been developed that succinctly describes the overall activation phase of phototransduction with a single parameter, the "amplification factor" (89). Constitutive

PDE activity exists in darkness to balance constitutive guanylate–cyclase (GC) activity (90), maintaining a steady free cGMP level and some open cGMP-gated channels. This dark PDE activity comes from PDE_{γ}"rocking" on $PDE_{\alpha\beta}$, causing intermittent spontaneous activation and consequently a continuous background noise (refs. 75 and 76; see previous section) to be dampened by the Ca^{2+} feedback. The steady level of PDE activity is an important determinant of photoreceptor sensitivity and response kinetics, because with higher steady PDE activity, the fractional increase in PDE activity per photon becomes smaller (i.e., lower sensitivity), and the recovery rate of cGMP after light becomes faster owing to a correspondingly higher GC activity. These properties contribute to adaptation to steady light (91).

The closure of the cGMP-gated channel constitutes the final step in phototransduction. The rod-channel protein was purified and its cDNA cloned soon after its discovery (92). These advances inspired the subsequent discovery (93) and cloning (94) of a homologous channel mediating olfactory transduction, which involves a rise in cAMP instead of a drop in cGMP (95). These two channels, together with a homologous channel mediating cone phototransduction (64, 96, 97), compose the small family of cyclic-nucleotide-gated (CNG) channels. Their prominent roles are in sensory transduction, but not exclusively (98). The originally cloned rod-, cone- and olfactory-channel proteins, which all form functional homomeric CNG channels when heterologously expressed, later turned out to be only the A (or α)-subunits of the respective native channels (see 99, 100 for nomenclature). B (or β)-subunits are also present, which are homologous to the A-subunits and serve modulatory, structural, and channel-targeting functions (98, 101, 102). The CNG channels are distant relatives of the Shaker superfamily of voltage-gated potassium channels, with also six transmembrane domains but an added cyclic-nucleotide-binding domain on the cytoplasmic C terminus (98). Like these potassium channels, the CNG channels form tetrameric complexes, with a 3CNGA1:1CNGB1 stoichiometry for the native rod channel (103–105) and a supposedly 2CNGA3:2CNGB3 stoichiometry for the native cone channel (106). The understanding of the structure-function relationships for these channels is quite advanced (98, 100–102, 107) and has spawned knowledge about another important, related ion-channel family called hyperpolarization- and cyclic-nucleotide-gated (HCN) channels (108). Two modulations of the rod cGMP-gated channel are known: a direct modulation by Ca^{2+}-calmodulin on the B1-subunit (109, 110) and a modulation by tyrosine phosphorylation on the A1-subunit (111). The first modulation participates in background-light adaptation, albeit very weakly (see below). The physiological significance of the second modulation remains unclear.

The deactivation of phototransduction is quite complex. The active conformation of rhodopsin (metarhodopsin II) decays very slowly (in minutes). However, long before this decay, rhodopsin is already partially inactivated by phosphorylation due to rhodopsin kinase (also called GRK1, or G protein-coupled-receptor-kinase 1), followed by complete inactivation upon binding of the protein, arrestin, to phosphorylated metaII (112). The effect of rhodopsin phosphorylation can be detected in mouse rods by ≈80 msec after a dim flash (113, 114), followed fairly quickly by arrestin binding (115). The single-photon response (i.e., electrical response triggered by a photoactivated rhodopsin molecule) is quite stereotyped in amplitude and time course (83). This was a long-standing puzzle because single-molecule deactivation should be stochastic, with an exponentially distributed decay time course if the inactivation is single-step. One proposal is that rhodopsin goes through multiple, small inactivation steps so that the stochastic nature becomes smeared over these steps (116–118). This smearing can be effected by the multiple phosphorylation sites (six to seven sites, depending on animal species) on the C terminus of rhodopsin (and the subsequent arrestin binding). Indeed, the response decay is more prolonged and variable for rhodopsin mutants lacking one or more of the phosphorylation sites (119, 120). Although multiple-phosphorylation is certainly important for reproducible rhodopsin shut-off, the constancy in response decay now turns out to have more to do with the averaging over the deactivation of multiple $G_{t\alpha}$ molecules (87; see next paragraph).

The timely deactivation of $G_{t\alpha}$ by intrinsic hydrolysis of the bound GTP to GDP requires a GTPase-activating-protein (GAP) complex consisting of RGS9 (a member of the RGS, or "regulator of G protein signaling," family), RGS9-anchoring protein (R9AP), an orphan G_{β} subunit ($G_{\beta 5}$), and PDE_{γ}, which is the effector itself (62, 121–128). The involvement of PDE_{γ} is thought to ensure that $G_{t\alpha}$.GTP has already bound PDE_{γ} and activated PDE before GAP-catalyzed GTP hydrolysis occurs. Without the GAP complex, the deactivation is much slower. However, even with the GAP complex, $G_{t\alpha}$ deactivation remains the slowest and rate-limiting step in response termination, with a time constant of ≈200 msec in mouse (87). Upon deactivation, $G_{t\alpha}$.GDP unbinds from PDE_{γ}, and the latter resumes its inhibition of $PDE_{\alpha\beta}$. It was mentioned above that ≈20 $G_{t\alpha}$ molecules are activated in the single-photon response (87). With this multiplicity, although individual $G_{t\alpha}$ molecules decay stochastically, this randomness becomes smeared by averaging over many $G_{t\alpha}$ molecules, thus contributing to the decay constancy of the single-photon response.

The Ca^{2+} feedback during light adaptation is still incompletely understood. In mouse rods, intracellular free Ca^{2+} decreases from ≈250 nM in darkness to ≈20 nM in bright light (129). The resulting feedback regulation of GC is fast and cooperative (78, 130), involving a Ca^{2+}-binding protein called guanylate–cyclase-activating protein (GCAP) (131–133). GC activity is facilitated by GCAP, but Ca^{2+} inhibits this facilitation (134). There are two GCs, Ret-GC1 and Ret-GC2, and multiple GCAPs comingled in both rods and cones (134). Ret-GC1 and Ret-GC2 (also called GC-E and GC-F) belong to the family of membrane guanylate–cyclase receptors, comprising GC-A through GC-G (135), except that these two members do not appear to sense extracellular ligands. The Ca^{2+} feedback on the light-activated PDE activity is via an inhibition of GRK1 (rhodopsin kinase), mediated by another Ca^{2+}-binding protein called recoverin or S-modulin (136–139). The Ca^{2+} decrease in the light disinhibits GRK1 and allows rhodopsin phosphorylation to proceed more rapidly; thus, less PDE is activated. Finally, the third Ca^{2+} feedback consists of a reduction, via Ca^{2+}-calmodulin, of the cGMP affinity for the cGMP-gated channel, as mentioned above (109, 110). Quantitative measurements and analysis have indicated that the Ca^{2+} feedback on GC is the most important at low and intermediate light intensities (78, 140). At higher intensities, the feedback on GRK1 kicks in (140). The feedback on the channel is insignificant at all light intensities (140). The Na/Ca,K exchanger extruding Ca^{2+} has also been cloned, permitting structure-function studies (141, 142). Interestingly, the exchanger and the channel appear to be stoichiometrically associated (143).

Where is the field heading? One direction is toward more domain mappings on the phototransduction proteins and also crystal structures, for finer details of the protein interactions. The visual pigments have long been cloned (144). Recently, the crystal structure of

rhodopsin has been solved (145, 146), the first GPCR with this success. An emerging but still unsettled question is whether rhodopsin in its native state is a dimer (147), as many other GPCRs are now believed to be. Another hotly pursued topic is the targeting of phototransduction proteins to the outer segment after synthesis (148), which requires passing through the checkpoint at the ciliary neck between the inner and outer segments. Somewhat related, some phototransduction proteins are now known to translocate between the outer segment and the rest of the cell depending on light conditions. For example, $G_{t\alpha}$ and $G_{t\beta\gamma}$ in rods both translocate away from the outer segment after many minutes in bright light, returning upon dark adaptation; arrestin translocates in the opposite direction (149). The relative importance of this translocation with respect to photosensitivity regulation versus cell protection from excess light remains to be examined. Additional translocating components may well exist.

A major recent advance not yet mentioned is the regeneration of 11-*cis*-retinal from all-*trans*-retinal (photoisomerized chromophore), an elaborate and chemically interesting process occurring in the retinal pigment epithelium (RPE) adjoining the rods and cones (150). The elusive key enzyme catalyzing this process has recently been identified (151, 152), as has a long-hypothesized receptor for the uptake of vitamin A (all-*trans*-retinol) into the RPE (153). The RPE and the shuttling of chromophore between the RPE and rods and cones are receiving increasing attention (154).

The list goes on. With the exceptionally quantitative information available, the hope is that ultimately the dynamics of the entire phototransduction process can be accurately described by a system of mathematical equations.

Rods Versus Cones

Without rods, we are merely night-blind. Without cones, we are legally blind. Thus, for humans, cones are far more important for daily functions. Cones have lower sensitivity (≈25–100 times less) than rods under dark-adapted conditions, and they adapt to light much more effectively. Cones also have faster response kinetics (typically by several fold), which provides higher temporal resolution. Most phototransduction studies so far have been on rods, helped by their abundance for biochemical studies. For cell electrophysiology, tissue abundance is not necessary, but synergy from biochemistry is. In recent years, there is increasing focus on cones, helped by the wealth of knowledge about rods. Although rods and cones use a similar phototransduction mechanism (65, 77), most proteins involved have different rod and cone isoforms (155). With heterologous expressions of cloned cone genes and the use of transgenic animals, insights are rapidly being gained about the functional differences between the rod and cone isoforms.

Much is known about the rod/cone differences at the pigment level. Rhodopsin and cone pigments (at least the red and blue cone pigments) appear to signal essentially identically downstream (156–158; but see ref. 159). Thus, the downstream components (G_t, pigment kinase, and arrestin) possibly in conjunction with specific rod- and cone-environments dictate the sensitivity and response kinetics. Cone pigment metaII decays >10-fold faster than metarhodopsin II (160). However, their identical signaling suggests that phosphorylation and arrestin-binding precede the cone pigment metaII decay and, thus, dictate its true active lifetime (156). Presumably, the faster decay is designed for the rapid regeneration of cone pigment. The chromophore-binding pocket in cone pigment is more exposed (161)—to the extent that the holo-cone-pigment, unlike holorhodopsin, has some tendency to dissociate into opsin and 11-*cis*-retinal in darkness (161–163)—a feature presumably also intended for rapid regeneration, which requires the departure of all-*trans*-retinal from the binding pocket and replacement by another 11-*cis*-retinal. The more open chromophore-binding pocket results in a small fraction of cone pigment without chromophore even in darkness (163). Opsin is now known to constitutively activate phototransduction, albeit weakly (164), so, in aggregate, it will trigger enough transduction to activate some Ca^{2+}-feedback, contributing (by a factor of approximately 2) to the cones' lower sensitivity compared to rods (163). More importantly, after a bright bleaching light, rod opsin will out-compete cone opsin in acquiring 11-*cis*-retinal because of cone pigment's redissociation. This is perhaps why, in addition to the chromophore supply/regeneration pathway in the RPE common to rods and cones, a second, dedicated pathway for cones appears to exist in the retina (165), possibly in Müller glial cells (166). Finally, rod and cone pigments have different thermal isomerization rates. Rhodopsin is extremely quiet (i.e., rarely giving a false signal), with an *in situ* half-life of ≈1,000 years at room temperature (75). Red and green cone pigments, however, are orders-of-magnitude more prone to spontaneous isomerization, although blue pigment seems very stable (156–159, 167). The rate of spontaneous isomerization appears to be correlated with the wavelength of maximum absorption (λ_{max}) of a pigment, presumably through the activation energy of isomerization (168).

There are also recent advances in understanding the steps downstream from the pigment. Biochemical experiments have suggested that the low sensitivity of cones stems from several factors. First, the rate of activation of cone G_t molecules by a cone pigment molecule is ≈10-fold lower than that of rod G_t by rhodopsin (169, 170). Second, the cone pigment kinase (GRK7) has a much higher specific activity and is also much more abundant than rhodopsin kinase (GRK1) (169–172), rendering cone pigment inactivation much more rapid. Cones also express a different arrestin (173), but its detailed significance remains unclear. Third, RGS9, in the GAP complex, is much more abundant in cones than in rods, making the deactivation of cone G_t more effective (174, 175). Finally, the much larger surface-to-volume ratio of the cone outer segment makes the Ca^{2+} decline and, therefore, the Ca^{2+} feedback proceed more rapidly in cones than in rods during illumination (176–179). The Ca^{2+} feedback on the cGMP-gated channel is also more severe in cones, possibly via a Ca^{2+}-binding protein other than calmodulin (180). All of the above differences reduce sensitivity and accelerate response kinetics in cones.

Many details about cone phototransduction nonetheless remain to be worked out. For many years, the combined efforts from mouse genetics and cell electrophysiology on cones have been hampered by the fragility of mouse cones and their rarity (≈3% of all photoreceptors) in the retina. A mouse line ($Nrl^{-/-}$) now exists in which all photoreceptors become cones by default during development (181), making cones much more abundant for study. Also, a variant of suction-pipette recording has recently been developed for mouse cones that should push the frontier forward (182).

Epilogue

The understanding of rod and cone phototransduction has advanced by leaps and bounds in the past four decades. With complementary evidence from human genetics, a large number of diseases associated with mutated phototransduction proteins have become known (183, 184). Human trials in gene therapy based on this knowledge are already in progress.

Rods and cones are ciliary photoreceptors, i.e., their light-sensitive struc-

ture is a modified cilium. It now appears that all ciliary photoreceptors, whether hyperpolarizing or depolarizing and whether vertebrate or invertebrate, use a cGMP-mediated signaling pathway for phototransduction, although details can vary (185–187). Interestingly, the scallop hyperpolarizing photoreceptor, an exemplary invertebrate ciliary photoreceptor, uses G_o instead of G_t for phototransduction (188, 189). Recently, a possible missing link between this presumably ancient ciliary photoreceptor and our rods and cones has been identified. This entity, the lizard parietal-eye photoreceptor, possesses within a given cell chromatically antagonistic signaling pathways mediated respectively by G_o and gustducin (G_{gust}), a close relative of G_t (186, 190, 191). The other major class of photoreceptors in the animal kingdom is the microvillous (rhabdomeric) photoreceptor, exemplified by the famously studied *Drosophila* and *Limulus* photoreceptors (192–196). Microvillous photoreceptors may all use a PLC- instead of cGMP-mediated phototransduction pathway. Also recently, a small subset of ganglion cells in the vertebrate retina have, surprisingly, been found to express a pigment, melanopsin, and to be intrinsically photosensitive (197–199). These cells offer no overt indication of being ciliary or microvillous, but evidence so far suggests that they may be more related to the latter than the former in phototransduction mechanism (200).

Finally, a comment is in order about GPCR signaling in general. The multiplicity of G_t molecules activated by a single photoactivated rhodopsin molecule has given rise to the textbook dogma that a gain $\gg 1$ at the GPCR-G protein interaction step is a key signature of G protein signaling. This generalization may not be valid. In olfactory transduction, an odorant was recently found to stay on the receptor so briefly, i.e., for a millisecond or less, that the complex has a very low probability of activating even just one downstream G protein (G_{olf}) molecule, i.e., the gain is $\ll 1$ (201). By the same token, the activated receptor appears to be inactivated by simple odorant-unbinding (201) rather than by phosphorylation and arrestin-binding as might have been supposed. This new picture may be the norm in ligand-activated GPCR signaling, granted that exceptions exist in which some ligands functioning at exceedingly low concentrations (*e.g.*, pheromones) may bind tightly to their cognate GPCRs. Obviously, despite a gain $\ll 1$, the cell may still signal effectively because the overall signal consists of the time-average of all binding events for the entire receptor population on the cell. Also, although ligand-unbinding rather than GPCR phosphorylation may be the standard termination step of a signaling pathway, the latter may still be important by serving as a safeguard against any unwanted prolonged or intense activation of the receptor.

ACKNOWLEDGMENTS. We thank Marie Burns, Vladimir Kefalov, and members of the Yau laboratory for comments on the manuscript.

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