Commentary

The Calculus of Rod Phototransduction

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In this issue, two articles present major advances in the quantitative analysis of the molecular mechanisms underlying rod phototransduction. One is by Nikonov et al. (1998), the other by Calvert et al. (1998). These two papers are complimentary, but with substantial areas of intersection.

At the present time, the activation cascade in rod phototransduction that leads to the hydrolysis of the internal transmitter, cyclic GMP (cGMP) and to the closure of light-sensitive channels is fairly well understood. The inactivation steps responsible for the termination of the photoresponse and the feedback mechanisms, which modulate sensitivity and kinetics and also contribute to response termination, are not understood nearly as well. The field of phototransduction has always been fraught with controversy: for every point, there has been a counterpoint. However, one can argue, with little fear of inciting controversy, that a complete understanding of phototransduction must include an understanding of the steps by which the photoresponse is initiated and the steps by which it is terminated. For this reason, the Nikonov et al. paper, "The Kinetics of Recovery of the Dark-adapted Salamander Rod Photoresponse" is especially significant. This paper moves us closer to a definitive answer to an old, controversial question: What is the rate-limiting biochemical reaction that determines the time course of recovery of the photocurrent from a flash bright enough to temporarily shut off all light-sensitive current? Two main contenders have been the inactivation of rhodopsin and the inactivation of the activated phosphodiesterase–G-protein complex. The authors present a case for the latter. A highlight of the paper is a new approach to quantify the extent of guanylyl cyclase activation in a feedback pathway mediated by calcium. The role of cyclase in determining the time at which photocurrent recovery begins and its role in sculpting the waveform of recovery are quantified. This analysis supports the existence of at least one more significant target for calcium feedback.

A notable feature of the paper is that the authors make stunning progress largely through powerful new theoretical analysis applied to data gathered with state-of-the-art techniques. A number of important observations and conclusions are stated in a formal manner in mathematical language, which includes theorems, lemmas, and proofs. The rigorous approach in this paper has the advantages of clarity and completeness. The aspects of phototransduction that can now be well understood are highlighted by a mathematical model, and the gaps in our knowledge are set off in stark contrast. Fortunately for the reader with less mathematical background, sufficient explanatory discussion surrounds the mathematical statements, such that a reader may even choose to skip the theorems entirely without major loss of information.

A Phototransduction Primer

The following brief and somewhat simplified summary of the molecular and biophysical mechanisms underlying phototransduction may aid those readers lacking familiarity with phototransduction. For sake of clarity, the notation of Nikonov et al. (1998) will be used here, and the reader is referred to their Fig. 1 for a reaction diagram that includes most of the processes discussed below.

In darkness, there is a circulating current that is carried inward through light-sensitive channels in the rod outer-segment membrane. These channels are kept open by the cooperative binding of two to three cGMP molecules to the channel protein complex in a reaction with extremely fast kinetics. Consequently, the light-sensitive current (photocurrent) at any moment serves as an instantaneous measure of the free cGMP concentration (cG), because the photocurrent is given by an invertible function of cG. The photocurrent is carried inward in large part by Na^+, with a minor contribution by Ca^{2+}. This current returns outward largely through K^+ channels in the inner-segment membrane. The absorption of light by rhodopsin in the disk membranes initiates a cascade of reactions leading, in the feed-forward pathway, to the conversion of phosphodiesterase from its inactive form (E) to its active form (E*), the hydrolysis of cGMP by E*, and the closure of light-sensitive channels. Thus, an inward, depolarizing current is shut off and the rod membrane potential moves in the hyperpolarizing direction. The rod is an approximately isopotential compartment, so that light hyperpolarizes the rod

synaptic terminal as well. This diminishes the influx of Ca^{2+} into the synaptic terminal and diminishes the release of synaptic transmitter. A surprising feature of this sensory system is that transmitter is released at the highest rate in darkness, when the rod might be considered to be at rest, as it is not processing an input light signal. Light, which might be thought of as an excitatory signal, shuts off the release of transmitter!

The steps between the absorption of light and the hydrolysis of cGMP begin with the conversion of rhodopsin to activated rhodopsin, R*. Each molecule of R* executes a random walk in the disk membrane. When R* encounters an inactive, membrane-bound G-protein (G), R* catalyzes the exchange of GDP for GTP on the α-subunit of G to create an activated enzyme, G*. Each G* molecule, in turn, executes a random walk on the disk membrane, rapidly encounters an inactive, membrane-bound cGMP-phosphodiesterase enzyme, and binds to its inhibitory subunit. This frees phosphodiesterase (PDE) from inhibition and creates an active PDE–G-protein complex, E* (also referred to as the E*/G* complex). It is important to note that, even after the light is shut off, R* will continue to produce E* until it is inactivated. Furthermore, even after R* is inactivated, E* will continue to hydrolyze cGMP and keep the light-sensitive channels closed until E* is inactivated. It is known that the inactivation of R* involves phosphorylation by rhodopsin kinase followed by the binding of arrestin, but a detailed kinetic picture is lacking. The inactivation of the E*/G* complex is thought to occur as soon as G* carries out its GTPase activity on its bound GTP, but, again, a detailed kinetic picture is lacking.

At any moment, the concentration of cGMP changes at a rate given by the difference between its rate of synthesis by activated guanylyl cyclase, GC*, and its rate of hydrolysis by E*. The activity of guanylyl cyclase is modulated, in a feedback pathway, by the intracellular concentration of free Ca^{2+} (Ca^{2+}_i), which covaries with the photocurrent, as indicated below. The Na/Ca,K exchange pump, located in the inner-segment membrane, establishes and maintains Ca^{2+}_i low by pumping out Ca^{2+} at a rate that is roughly proportional to Ca^{2+}_i. Similarly, the total Ca^{2+} concentration of Ca^{2+} changes at a rate given by the difference between the rate at which Ca^{2+} enters the cell via the light-sensitive channels (proportional to the photocurrent) and the rate at which Ca^{2+} is pumped out by the exchanger. Consequently, Ca^{2+}_i always moves towards a steady state value in which the ratio of Ca^{2+}_i to its value in the dark is equal to the ratio of the photocurrent to its value in the dark. When the photocurrent is completely shut off by a saturating flash, for example, Ca^{2+}_i declines towards zero with an approximately exponential time course.

The rate at which activated guanylyl cyclase (GC*) synthesizes cGMP from the substrate GTP is controlled by Ca^{2+}_i in a cooperative manner, with a Hill coefficient that in vivo seems to be ∼2. In the mathematical model for guanylyl cyclase activity used by Nikonov et al., it is assumed that cyclase activity at any moment can be approximated by its steady state value for the momentary value of Ca^{2+}_i. Thus, the rate of cGMP synthesis is an instantaneous nonlinear function of Ca^{2+}_i. This function has a maximum value when Ca^{2+}_i is equal to zero, and the function declines sigmoidally towards zero as Ca^{2+}_i increases. The direct effect of light is to lower cG and close the cGMP-gated channels, but as Ca^{2+}_i decreases, guanylyl cyclase is released from inhibition and cGMP is synthesized at a higher rate. This causes cG to increase. This negative feedback pathway seems to be the major one underlying the light adaptation over a range of low to intermediate background light levels (Koutatols et al., 1995a, 1995b). In addition to this feedback pathway, there is at least one other important feedback pathway in which Ca^{2+}_i modulates the gain or inactivation time constant of an early intermediate in the transduction cascade. The evidence that this intermediate is R* is discussed by Nikonov et al. In addition to Ca^{2+}_i, the internal transmitter itself may play a role in adaptation. The binding of cGMP to noncatalytic sites on PDE can regulate the rate of the G-protein (transducin) GTPase reaction (see Calvert et al., 1998, in this issue).

New Insights

Pepperberg et al. (1992) showed that the time taken for the salamander rod photoresponse to recover by a criterion amount, say 10%, increased a characteristic amount, τ_C (roughly 2 s), per e-fold increment in flash intensity for a range of super-saturating intensities spanning several decades. The same behavior is illustrated in Fig. 5 of Nikonov et al. (1998). Pepperberg et al. (1992) hypothesized that this behavior resulted from a biochemical intermediate whose concentration reached a peak value proportional to the number of photoisomerizations (Φ) and decayed with an exponential time course characterized by an exponential time constant τ_C. They tentatively hypothesized that this putative intermediate was activated rhodopsin (R*), and they presented a quantitative argument that seemed to make G* and E* unlikely candidates for this intermediate.

Nikonov et al. demonstrate a closely related phenomenon: the rod photocurrent response recovery curves translate on the time axis (with fixed shape) by a characteristic amount (the same τ_C as above) per e-fold increment in flash intensity over a range (100-fold or more) of super-saturating flash intensities. This so-called recovery translation invariance (RTI) is a feature shared by photoresponse recovery curves measured in Ringer's solution in which Ca^{2+}_i is free to vary and by recovery

curves measured under conditions that maintain Ca^{2+}_i near its dark, resting level (their Fig. 3). The difference is that the photocurrent remains in saturation (i.e., shut off) for 5–7 s longer under clamped Ca^{2+}_i conditions. As shown in a previous report (Lyubarsky et al., 1996), the dominant time constant of photocurrent recovery is not dependent on Ca^{2+}_i. Nikonov et al. make an important contribution in formalizing the notion of RTI and its implications with respect to the idea that there is an underlying biochemical entity whose concentration decays with an exponential time course. This analysis creates a powerful tool that the authors use to great effect to retrieve hidden information about signal termination stages in the transduction cascade.

To proceed further, the authors apply a sophisticated mathematical model for phototransduction. This model summarizes and formalizes current hypotheses concerning activation, inactivation, and feedback. If either R* or E* were to decay with an exponential time course characterized by time constant τ_C, would the kinetics of cGMP hydrolysis or the kinetics of the feedback activation of guanylyl cyclase disturb or preclude RTI? The resounding answer, no, tidies up a hitherto messy area of phototransduction.

The same mathematical model serves as a tool to answer the question of whether the feedback activation of guanylyl cyclase alone is enough to account for the 5–7-s difference in recovery time between photoresponses measured under physiological conditions in Ringer's and photoresponses measured under Ca^{2+}_i-clamp conditions. This impressive analysis makes use of photocurrent recovery curves measured in the same cell both in Ringer's and under Ca^{2+}_i-clamp conditions. A very clear answer is obtained. The 5–7-s difference is accounted for largely by the activation of guanylyl cyclase, but a residual 1–2 s appears to be mediated by calcium feedback, which modulates a nondominant time constant or the gain of a feed-forward stage in the transduction cascade.

An unexpected "fringe benefit" of the analysis is a new estimate of the calcium-buffering capacity of the rod at rest. This estimate is an order of magnitude lower than the previous estimate of Lagnado et al. (1992). A remaining challenge is to develop an independent method for resolving the question of the true buffering capacity.

The experimental and analytical results of Nikonov et al. enable them to argue strongly that there is reason to rethink the Pepperberg et al. (1992) hypothesis that R* is the long-lived intermediate with the dominant time constant. Nikonov et al. make a good case for E* (a.k.a., the G*/E* complex) as this intermediate, and they show that a number of pieces of the phototransduction puzzle fit together rather nicely if one makes this assumption. A point not emphasized by Nikonov et al. is that it may be difficult to reconcile the idea that

R* decays slowly with an exponential time course (time constant $\tau_R = \tau_C = 2$ s) with the stereotypical (low noise) nature of the single-photon response. The problem is that a simple exponential decay of R* implies a rate-limiting first-order reaction. This implies that on a stochastic, molecular level, a single slow step with exponentially distributed waiting time determines the life time of a single R* molecule. It is well known that the coefficient of variation (standard deviation divided by the mean) of an exponentially distributed random variable is equal to one. The coefficient of variation of the single-photon response waveform is typically ≤ 0.2, and there is very little dispersion in the single-photon response waveform (Baylor et al., 1979, 1984; Schneeweis and Schnapf, 1995).

A puzzling result of Nikonov et al. provides food for thought: the dimmest flash responses could not be fit by their mathematical model with the value of τ_R (R* inactivation time constant) or τ_E (E* inactivation time constant) set equal to the value of τ_C that was estimated by analyzing the photoresponse recovery from super-saturating flashes. The best-fitting value of τ_R or τ_E for the dim-flash responses were systematically lower by $\sim 25\%$. Thus, there is a possibility that one or more of the transduction enzymes is, in a way not yet understood, in a different state in the low and high light regimes.

Concluding Remarks

A number of gaps remain in the field of phototransduction. A notable few, relevant to the paper under discussion, are: the detailed kinetics of R* and E* inactivation, the mechanisms that lead to the breakdown of RTI at very high flash intensities and the emergence of even slower processes, a detailed account of all the sites and molecular mechanisms by which gain and kinetics of the photoresponse are modulated, and the development of a comprehensive model for phototransduction that accounts for the responses to the full range of physiological light intensities, from a single photon to super-saturating intensities. It is safe to say that careful and thoughtful experimental and analytical approaches along the lines of those taken by Nikonov et al. and by Calvert et al. would go a long way towards addressing these outstanding problems.

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