Commentary

Shedding Light on Adaptation

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One of the most remarkable features of the vertebrate retina is its ability to adapt to ambient illumination. One can read from this page by candlelight or one can read from it on a sunny afternoon, but the page always appears white and the letters black. It is the molecular trickery of light adaptation that enables photoreceptors to evade saturation and accurately report relative contrast even under extreme illumination conditions. Photoreceptors reduce their sensitivity in direct proportion to the level of background illumination, a relationship known as Weber's Law (for review see Dowling, 1987). Without adaptation, even the black ink in these letters would reflect enough sunlight to drive photoreceptors into saturation. Outdoor activities, such as reading on a park bench, would be impossible.

Thirty years of thorough investigations into the biochemistry and physiology of phototransduction have shown how flashes or step increments of light can be detected by a cascade of enzymatic reactions that rapidly closes channels in the photoreceptor's plasma membrane (Ebrey and Koutalos, 2001). Recent reflections on the implications of that mechanism (Pugh et al., 1999; Fain et al., 2001) have led investigators to recognize two requirements for effective light adaptation. First, when background light is so intense that it initially closes all of the channels, some channels must be reopened for there to be any subsequent responses. Second, the sensitivity to further stimuli must be adjusted so that responses are appropriate for the level of background illumination.

In the early 1970s, advances in electroretinography and intracellular recording methods made it possible to reliably isolate electrical responses from photoreceptors. Studies at that time suggested that tens of seconds to minutes are required for full development of light adaptation (Dowling and Ripps, 1972). Later, as recording methods became more sophisticated, a more rapid phase of light adaptation was identified. The rapid phase requires light-stimulated changes in intracellular Ca^{2+} (Matthews et al., 1988; Nakatani and Yau, 1988) and it depends on regulation of enzymes that control the synthesis and degradation of cGMP (Koch and Stryer, 1988; Kawamura and Murakami, 1991). Until now, the relationships between the rapid and slow phases of light adaptation have been largely unexplored. In this issue, Calvert et al. (2002) describe experiments that begin to resolve the two phases of light adaptation. They report that the rapid form of light adaptation predominates under dim illumination but with brighter backgrounds both forms contribute substantially. Calvert et al. (2002) found that the rapid phase of light adaptation reduces the sensitivity of bullfrog rods by 80-fold and the slow phase by 40-fold. The effects are multiplicative, so when both are in operation the outcome is an overall ~3,000-fold reduction of sensitivity.

Two independent strategies were used by Calvert et al. (2002) to quantify the contributions of each phase of light adaptation. The first strategy was based on comparisons of actual step responses with step responses calculated from a model for phototransduction in which adaptation is disabled. Recent advances in mathematical modeling of phototransduction have produced accurate simulations of the photoresponses of rod photoreceptors. The models are based on evaluations of the synthesis and degradation of cGMP, and they have provided new insights into the physiology of vision (Hamer, 2000; Nikonov et al., 2000). Degradation of cGMP in rods is stimulated by photoactivation of rhodopsin and an ensuing cascade of biochemical reactions. Each photoactivated rhodopsin stimulates hundreds of transducins per second to bind GTP (Leskov et al., 2000). Each transducin–GTP complex counters the action of an inhibitory subunit of a phosphodiesterase, thereby initiating cGMP hydrolysis at a rate of hundreds per second per activated phosphodiesterase. This highly amplified response to light rapidly closes cGMP-gated cation channels in the rod outer segment plasma membrane. Calvert et al. (2002) used a model based on this cascade to calculate what the level of phosphodiesterase activity would be without adaptation. They first recorded dim flash responses under fully dark adapted conditions. Next, they integrated those responses with time, and used their model to calculate the degree of phosphodiesterase activation and the degree of channel closure that would occur in the absence of light adaptation. By comparing these calculated responses to actual responses recorded during steady illumination, Calvert et al. (2002) were able to evaluate the extent and time courses of desensitization caused by the two phases of light adaptation.

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Calvert et al. (2002) also used another strategy to evaluate the slow phase of light adaptation. Previously, Pepperberg had analyzed responses to saturating flashes and found that the length of time rods spend in saturation is directly proportional to the natural log of the intensity of the flash stimulus (Pepperberg et al., 1992). This relationship derives directly from the exponential decay of phototransduction after a flash; the proportionality constant is simply the time constant of the rate-limiting step for inactivation. Pepperberg et al. (1992) also compared responses to saturating flashes recorded under dark and light-adapted conditions. They found that light-adapted flash responses recovered from saturation faster than dark-adapted responses. But the time constant of the rate-limiting step of inactivation turned out to be unaffected by light adaptation (Pepperberg et al., 1992). What Calvert et al. (2002) did was to extend this type of analysis to steps of light rather than flashes. They recorded responses to steps lasting from 2.5 to 80 s, and found that the same relationship that holds for flash responses also holds for step responses. The response to a 2.5-s step required 10 s to recover from saturation after the light was extinguished, whereas the response to an 80-s step took only ~1 s. But even though saturation times shortened as light adaptation progressed, the duration of exposure did not alter the time constant of inactivation. Since this rate was unaltered, Calvert et al. (2002) concluded that the shortened saturation times they observed could only be caused by desensitization. They quantified this desensitization and found that it develops with the same time course as the slow phase of light adaptation they quantified in their first analysis. Altogether, these results fit together nicely to show that the slow form of light adaptation is caused by desensitization of phototransduction in rods.

Previous studies have established a molecular basis for the more rapid phase of light adaptation. It depends on the loss of cytoplasmic Ca^{2+} that occurs when rods and cones are exposed to light (Matthews et al., 1988; Nakatani and Yau, 1988). Lowered Ca^{2+} influences rod photoreceptor biochemistry in two ways that contribute to light adaptation: (1) it stimulates synthesis of cGMP by guanylyl cyclase (Koch and Stryer, 1988); and (2) it indirectly reduces light-stimulated phosphodiesterase activity (Kawamura and Murakami, 1991). Each of these returns cGMP-gated channels to their open state, helping to restore the photoreceptor's ability to respond to light.

The molecular basis of the slow phase of light adaptation previously had not been investigated. So Calvert et al. (2002) initiated a biochemical investigation along two lines. Their approach was constrained by the limits of current knowledge of phototransduction biochemistry and their main criterion was that whatever the mechanism may be, it should be slow. They first considered release of cGMP from high affinity sites, a process which occurs slowly when phototransduction is stimulated (Arshavsky et al., 1991; Cote et al., 1994; Calvert et al., 1998). These sites were discovered many years ago (Yamazaki et al., 1980), and they have been characterized as noncatalytic GAF homology domains on the phosphodiesterase catalytic subunits (Aravind and Ponting, 1997; Ho et al., 2000; Mou and Cote, 2001). (The name, GAF, comes from the presence of these types of domains in cGMP-regulated cyclic nucleotide phosphodiesterases, adenylyl cyclases, and the bacterial transcription factor FhlA.) Occupancy of the sites determines the rate of phosphodiesterase inactivation via a complex mechanism involving transducin, the PDE inhibitor subunit, and RGS9–1, a GTPase accelerating protein (He et al., 2000; Witherow et al., 2000; Skiba et al., 2001; Slep et al., 2001). Dissociation of cGMP from the sites accelerates GTP hydrolysis by transducin, thereby shortening the lifetime of each activated phosphodiesterase. Arshavsky and his colleagues previously have put forward (Arshavsky et al., 1991; Calvert et al., 1998) the hypothesis that the light-induced dissociation of cGMP could be a slow feedback mechanism that causes light adaptation. In the current study, Calvert et al. (2002) tested this idea by measuring cGMP release under conditions similar to those they used for light adaptation measurements. cGMP release and the slow phase of light adaptation did have similar time courses. But Calvert et al. (2002) had to exclude cGMP release as a mechanism for light adaptation because they also found that the intensity of illumination required to stimulate cGMP release was much greater than the intensity required for light adaptation.

The other possibility Calvert et al. (2002) considered was that slow changes in intracellular Ca^{2+} levels might determine the slow phase of adaptation. They evaluated intracellular Ca^{2+} levels by monitoring $Na^{+}/K^{+},Ca^{2+}$ exchange current in the rod outer segment plasma membrane. This electrogenic exchange produces a current that is directly dependent on intracellular free Ca^{2+}, so its activity is an accurate reporter of the free cytoplasmic Ca^{2+} concentration (Gray-Keller and Detwiler, 1994). The decline in Ca^{2+} during steady illumination turned out to have rapid ($\tau = 0.6$ s) and slow ($\tau = 6.0$ s) phases in the Calvert et al. study. The time constant of the slow phase was similar to the time constant of the slow phase of light adaptation. Calvert et al. (2002) concluded that a change in Ca^{2+} may contribute to the slow phase but further experimentation will be necessary to establish causality.

Despite these well designed strategies, Calvert et al. (2002) were not able to divulge the molecular basis of slow light adaptation in the current study. But their findings bring the issue into sharper focus and serve as a starting point in the search for molecular explanations. The cGMP cascade of phototransduction is common knowledge, but less appreciated is the "orphan

biochemistry" associated with phototransduction. Many protein–protein interactions and biochemical reactions involving photoreceptor proteins have been discovered experimentally, but have not been assigned functions that fit neatly into the commonly accepted model of phototransduction. For example, the inhibitory subunit of the phosphodiesterase can be phosphorylated and ADP-ribosylated (Hayashi et al., 2000; Matsuura et al., 2000; Paglia et al., 2001), but the roles of these modifications are not addressed in most models of phototransduction. Recent reports have suggested that RGS9–1 is modified in multiple ways (Balasubramanian et al., 2001; Hu et al., 2001), but the extent and consequences of these modifications are unknown. There is evidence that complexes of phototransduction proteins exist in rods (Korschen et al., 1999; Poetsch et al., 2001; Seno et al., 2001), but the ways in which they form and regulate phototransduction are unknown. Discrepancies between biochemical and physiological analyses of the Ca^{2+}-binding protein, recoverin, have not been resolved (Otto-Bruc et al., 1998; Burns and Baylor, 2001; Hurley and Chen, 2001), and the recently reported release of Ca^{2+} into the cytoplasm of light-stimulated photoreceptors (Matthews and Fain, 2001) has not been assigned a function. The importance of these and other biochemical activities may eventually become clear as more of the diverse functions of photoreceptors become apparent through physiological analyses such as the Calvert et al. study and others (Gray-Keller and Detwiler, 1996).

Rod photoreceptors from bullfrog retinas were used in these studies. That was not an arbitrary choice, but it was influenced by practicality. Suction electrode recordings from rod photoreceptors are straightforward and bullfrog retinas are amenable to the types of biochemical analyses that Calvert et al. (2002) used to quantify cGMP. Rod photoreceptors are highly specialized to detect dim illumination, even single photons. But their ability to adapt is limited to, at most, three log units of background intensity before they saturate and become unresponsive. Although studies of rod photoreceptors are informative about basic mechanisms of light adaptation, one must keep in mind that it is the cone photoreceptors, not the rods, that are the true masters of light adaptation. Cones have the remarkable ability to desensitize according to Weber's Law over eight log units of background intensity (Burkhardt, 1994) without saturating. The important findings that Calvert et al. (2002) have made using rod photoreceptors raise obvious questions about light adaptation in general. For example, do both forms of light adaptation also occur in cones or does one of them predominate? More detailed physiological studies of light adaptation in cones, complemented by biochemical (Tachibanaki et al., 2001) and genetic analyses (Lyubarsky et al., 2001), may ultimately explain how cones elude saturation even under conditions of intense illumination that bleach >99% of their visual pigment (Burkhardt, 1994, 2001).

What is particularly unique about the study by Calvert et al. (2002) is that it touches on an important aspect of photoreceptor function that previously has received surprisingly little attention. The majority of laboratory analyses of phototransduction has focused on how photoreceptors respond to flashes or increments of light; but, in real life, that is only half the story. Photoreceptors in our eyes spend just as much time responding to decrements of light. For example, our photoreceptors are detecting frequent "flashes of darkness" as they scan the letters on this page. Light adaptation is essential for photoreceptors to evade saturation, but it is also essential for efficient detection of darkness. In fact, responses of light-adapted cones to decrements of illumination may be as quick as their responses to increments (Burkhardt, 1994). The importance of this is obvious when one considers that swift detection of a predator's shadow is essential for survival. The experiments reported by Calvert et al. (2002) reveal at least one mechanism that enhances detection of darkness, desensitization of transduction. They showed that desensitization reduces the delay between the onset of darkness and the electrical response of the cell. Many questions about the dark response remain unresolved, but again the study by Calvert et al. (2002) helps bring them into focus. How does desensitization occur at the molecular level? Is desensitization the only process that accelerates the dark response? Do additional dark detection mechanisms exist and are they present in both rods and cones? Further studies that effectively integrate physiological and biochemical strategies, as Calvert et al. (2002) have done in their study, should reveal how photoreceptors adjust their chemistry to respond most effectively both to increments as well as to decrements of photon flux.

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