

Photoreceptor Signaling: Supporting Vision across a Wide Range of Light Intensities*

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For decades, photoreceptors have been an outstanding model system for elucidating basic principles in sensory transduction and biochemistry and for understanding many facets of neuronal cell biology. In recent years, new knowledge of the kinetics of signaling and the large-scale movements of proteins underlying signaling has led to a deeper appreciation of the photoreceptor's unique challenge in mediating the first steps in vision over a wide range of light intensities.

First Steps in Vision Occur in Photoreceptor Outer Segments

Retinal photoreceptors transduce information obtained in the form of absorbed photons into an electrical response that can be relayed across synapses to other neurons in the retina. In vertebrate photoreceptors, photon absorption and visual signaling take place in the outer segment (Fig. 1), a sensory cilium tightly packed with stacks of membranous discs containing extremely high densities of visual pigments and other signaling proteins. This morphological arrangement allows photons to be efficiently absorbed as they pass through the outer segment. The signal from activated visual pigment (rhodopsin in rods or cone opsins in cones) must then be sufficiently amplified to generate an electrical response that overcomes intrinsic noise. Transduction from the light-absorbing visual pigment into an electrical response utilizes a G protein signaling pathway termed the phototransduction cascade, which leads to a decrease in the second messenger cGMP and the closure of cGMP-sensitive cation channels. The resulting hyperpolarization transiently decreases the release of glutamate from the photoreceptor synaptic terminals, signaling the number of absorbed photons to the rest of the visual system. Remarkably, photoreceptors both detect low light levels (single photons in the case of rods) and continue to rapidly and reliably signal changes in light intensity as illuminance increases over 10 orders of magnitude during the course of a typical day.

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Phototransduction: Rhodopsin Activation, Amplification, and Deactivation

Phototransduction has been the subject of many comprehensive reviews (1–4). Here, we provide a framework introduction and briefly summarize the latest findings that are shaping our understanding of this first step in vision.

Phototransduction begins when a photon causes *cis-trans*-isomerization of the chromophore 11-*cis*-retinal, which induces a rapid conformational change to the protein's fully active form, R*. R* activates molecules of the G protein transducin by catalyzing GDP/GTP exchange on the transducin α -subunit, $G\alpha_t$ (Fig. 1). $G\alpha_t$ ·GTP separates from the transducin $\beta\gamma$ -subunits and binds to the γ -subunit of its effector, cGMP phosphodiesterase (PDE),³ which releases this subunit's inhibitory constraint on the catalytic α - and β -subunits of PDE. Activated PDE rapidly hydrolyzes cGMP, thereby reducing its concentration in the cytoplasm and causing cGMP-sensitive cation channels in the plasma membrane to close. The closure of channels reduces the inward cation current, resulting in a transient photoresponse generated within milliseconds (Fig. 2).

The photoresponse persists until each phototransduction protein becomes deactivated through the action of one or more regulatory enzymes (Fig. 1). Specifically, each R* in rods must be phosphorylated at multiple C-terminal sites by rhodopsin kinase, with each added phosphate partially reducing the rate with which R* can activate transducin (5). After the addition of three phosphates (6–8), arrestin (Arr1 in rods and Arr1 and Arr4 in cones) binds to R* with high affinity, completely blocking subsequent transducin activation. Likewise, transducin and PDE remain active until transducin hydrolyzes GTP. This hydrolysis is catalyzed by a triumvirate complex of proteins consisting of RGS9-1, G β 5-L, and R9AP (the "RGS9 complex") (9). Finally, cGMP is restored through the action of guanylate cyclase (GC) (10).

In normal mouse rods, the expression level of the RGS9 complex rate-limits the recovery of the rod's response to both single photons and bright flashes of light (11). Altering the expression level of R9AP changes the cellular content of the entire RGS9 complex (11, 12). Underexpression of R9AP reduces the level of the RGS9 complex and makes rod responses much slower to recover (11, 13), whereas R9AP overexpression increases the RGS9 content and makes photoresponses recover much faster (11, 14, 15). Interestingly, the rod responses of every mammalian species examined so far recover with approximately the same time constant of ~200 ms. This similarity suggests that the expression level of the RGS9 complex is tightly regulated and that there is some evolutionary pressure that sets this relatively slow time constant for rod vision. Notably, cones express more RGS9 than rods (16, 17), which is likely to contribute to their faster recovery. Indeed, recent physiology experiments with salamander cones suggest that the rate-limiting step for

³ The abbreviations used are: PDE, phosphodiesterase; GC, guanylate cyclase; GCAP, GC-activating protein.

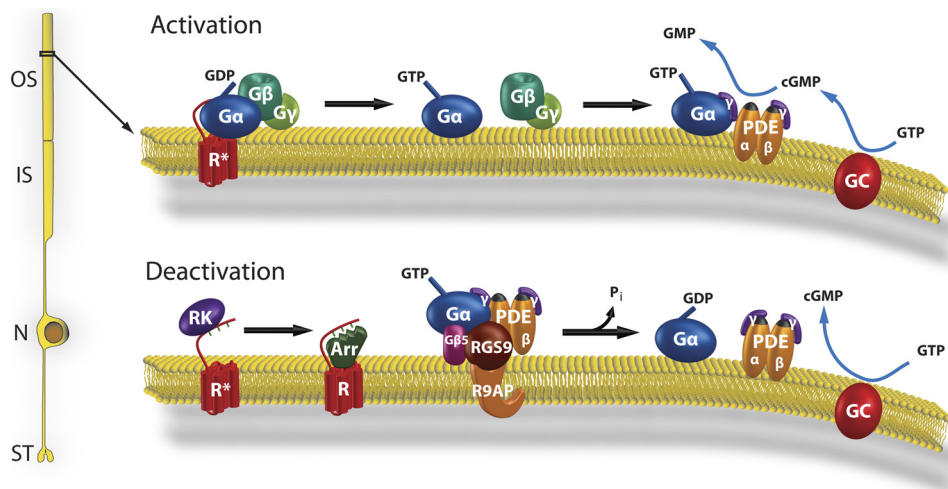


FIGURE 1. **Rod phototransduction.** *Left panel*, schematic of the compartmentalization of a rod cell, including the outer segment (OS), inner segment (IS), nuclear region (N), and synaptic terminal (ST). *Right panel*, phototransduction activation and deactivation reactions. The *upper disc* illustrates photoexcited rhodopsin (R^*) activating transducin ($G\alpha$, $G\beta$, and $G\gamma$ subunits) and PDE (α -, β -, and γ -subunits). cGMP synthesized by GC is hydrolyzed by activated PDE. The reactions in the *lower disc* represent cascade deactivation. R^* is quenched by phosphorylation by rhodopsin kinase (RK; GRK1), followed by arrestin (Arr) binding. Transducin and PDE are deactivated by the RGS9-1-G β 5-L-R9AP complex, which accelerates the rate of GTP hydrolysis on $G\alpha$. cGMP synthesis by GC restores cGMP to its dark level. The *right panel* was modified from Ref. 58 with permission.

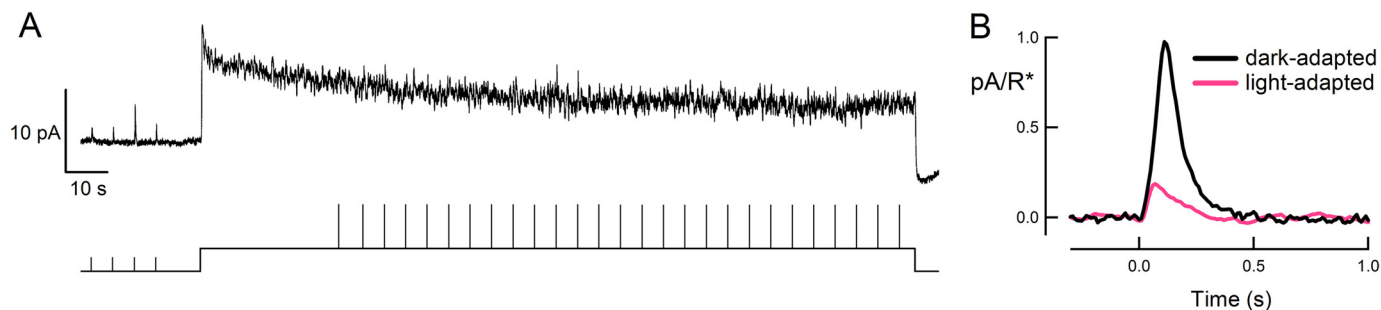


FIGURE 2. **Adaptation of rod photoresponses.** *A*, outer segment membrane current (*upper trace*) in response to flashes presented in the dark or in the presence of background light activating $150 R^*/s$. Ticks on the *lower trace* represent flashes, and the *step* represents background light. The onset of background light evoked a large and rapid decrease in inward current (upward deflection) that subsequently relaxed to a steady-state level over tens of seconds, reflecting the engagement of adaptation mechanisms. Flashes delivered on this background yielded small responses that were largely obscured by the current fluctuations produced by the background light, despite the flashes on the background being >3 times brighter than the flashes delivered in the dark (8.5 versus 2.5 R^*/flash). *B*, comparison of averaged dark- and light-adapted flash responses of the cell in *A*. The *black trace* is the average of 28 flash responses given in the dark; the *red trace* is the average of 26 flash responses given on the background. The dark current was 15 pA, and the temperature was 36 °C.

the recovery of cone responses is the deactivation of cone opsin (18).

The ability of rods to generate sizable responses to the absorption of single photons arises in part from the signal amplification conferred by the activation of many transducin molecules by a single R^* . The rate of transducin activation in rods is $\sim 150 \text{ s}^{-1}$ in cold-blooded vertebrates (19) and 2–3-fold faster in mammals (20). This rate is far higher than those measured in other G protein signaling pathways, undoubtedly because of the unusually high density of transducin on the disc membrane ($\sim 1:8$ molar ratio with rhodopsin in mice). However, despite the high rate of transducin activation, the number of activated $G\alpha_t$ molecules produced during the single photon response is actually surprisingly small, ~ 10 –15 in the mouse. This is because the effective lifetime of R^* is relatively brief, ~ 40 ms (13, 14), and even during the brief time before arrestin binds, the rate of transducin activation is being gradually diminished by sequential phosphorylations. In mouse rods, the brief effective lifetime of R^* is set by the rate of its phosphorylation and by the concentration of arrestin (14). Remarkably, the concentra-

tion of arrestin available for R^* binding is continuously buffered by its self-association into dimers and tetramers, neither of which can bind R^* (21, 22). Arrestin oligomerization not only regulates R^* lifetime but helps to minimize the adverse consequences of high arrestin levels on photoreceptor viability (23).

Light Adaptation: Role of Calcium

Rods and cones employ many mechanisms to avoid saturation by bright light and to adjust the amplitude and time course of their photoresponses to ever-changing ambient illumination, a process collectively known as light adaptation (Fig. 2) (2, 24). Historically, most photoreceptor adaptation was thought to be mediated by the decline in intracellular Ca^{2+} that accompanies the photoresponse. The levels of Ca^{2+} fall in light because its influx is reduced when cGMP-gated channels close, whereas Ca^{2+} efflux via the $\text{Ca}^{2+}/\text{K}^+/\text{Na}^+$ exchanger continues. The reduction in intracellular Ca^{2+} is sensed by several different Ca^{2+} -binding proteins, including GC-activating proteins (GCAPs), which stimulate cGMP synthesis by GC when Ca^{2+} falls (10). The Ca^{2+} /GCAP-dependent regulation of GC

activity forms a powerful feedback mechanism in which the rate of cGMP synthesis increases as Ca^{2+} falls during the response to light, helping to restore cGMP levels rapidly and allowing the cGMP channels to reopen.

There are two GC isoforms in photoreceptors, RetGC1 and RetGC2, and also several different GCAPs, with all vertebrates expressing at least two, GCAP1 and GCAP2; the relative expression levels of GC1/GC2 and GCAP1/GCAP2 are not equivalent in rods and cones (10). Likewise, the Ca^{2+} dependence of GC1 and GC2 regulation by GCAP1 and GCAP2 is different. However, the maximal ranges of each GC activity regulation by each GCAP are comparable (see discussion in Ref. 25).

These properties of GC regulation by Ca^{2+} have important functional consequences, as revealed in recent experiments that utilized knock-out mice lacking one or both GCs and one or both GCAPs and crossed each of these strains for biochemical characterization and electrophysiological recordings. Although both pairs, GC1/GC2 and GCAP1/GCAP2, function in normal rods (26–28), GCAP1 appears to respond to the fall in Ca^{2+} more rapidly because it has lower affinity for Ca^{2+} , whereas GCAP2 releases Ca^{2+} a bit more slowly at lower Ca^{2+} concentrations (25). As a result, GC activation by GCAP1 affects earlier stages of the photoresponse, whereas GC activation by GCAP2 affects photoresponse recovery later (28–30). Overall, the GC activity in light-adapted rods can be ~10-fold higher than the activity in darkness (28, 31–33), comparable with the activity change measured biochemically over the physiological range of Ca^{2+} (25, 34–36).

In cones, the maximal extent of the Ca^{2+} -dependent GC regulation is thought to be comparable with that in rods. However, the dark Ca^{2+} level in cones is lower than in rods (37, 38), suggesting that the degree to which the light-evoked change of Ca^{2+} could activate GCs is smaller in cones than in rods. This was experimentally confirmed in a recent direct comparison of rod and cone light responses in double GCAP1/GCAP2 knock-out mice (39).

The fall in intracellular Ca^{2+} also affects other calcium-binding proteins, including recoverin. Ca^{2+} -bound recoverin inhibits the ability of GRK1 to phosphorylate R^* (40, 41). Experiments using rods of recoverin knock-out mice indicate that this regulation by recoverin has a relatively minor effect on dim flash responses but a larger effect on bright light responses and responses to steady light (15, 42). Importantly, there is no *dynamic* regulation of R^* deactivation during the small brief changes in Ca^{2+} that accompany the single photon response in rods or the flash response in cones; all of the dynamic regulation appears to be conferred by GCAP-dependent GC modulation alone (33, 39). Instead, it seems that inhibition of the actions of recoverin, which requires sequestration of its myristoyl tail and extrusion from the disc membrane, requires larger and longer changes in intracellular free Ca^{2+} , like those occurring during steady moderate light. The dynamics of the Ca^{2+} -myristoyl switch has been recently described (43) and presumably confers the light dependence of recoverin translocation to the inner segment upon steady illumination (see below).

A third Ca^{2+} -dependent adaptation mechanism is the regulation of the sensitivity of the cGMP-gated channels by calmod-

ulin or calmodulin-like proteins (44). When Ca^{2+} falls in light, calmodulin dissociates from the channel, increasing the channel's sensitivity to cGMP. This allows the channel in a light-adapted cell to operate at a lower cGMP concentration range than in a dark-adapted photoreceptor. Recent work in intact mammalian rods indicates that the overall effect of this sensitivity modulation is relatively modest and has a slow onset (45). In cones, however, the effect is more rapid and has greater magnitude (46, 47).

Taken together, these Ca^{2+} feedback mechanisms account for most adaptation that occurs at low-to-moderate levels of light intensity, developing over a period of seconds. Ca^{2+} -independent mechanisms also contribute to adaptation in photoreceptors, most notably the increased cGMP turnover in constant illumination (48). When the PDE activity is high in steady light, the activation of the same amount of PDE by a photon results in a smaller fractional change in the overall PDE activity. This produces a smaller and briefer change in cGMP, resulting in a smaller response that recovers more quickly. These contributions of the steady PDE activity to the amplitude and time course of photoresponses may not be intuitive and so have been reviewed using various physical analogies (48, 49). Finally, light adaptation has also been documented to occur at longer time scales of tens of seconds in both lower and higher vertebrates (50, 51), although the underlying mechanisms remain unknown.

Several Signaling Proteins Translocate between Subcellular Compartments in Response to Light

A different type of adaptation mechanism induced by sustained bright light involves massive translocation of several phototransduction proteins between the outer segment and the rest of the photoreceptor cell (52–55). Illumination causes significant fractions of transducin and recoverin to exit rod outer segments, whereas arrestin translocates in the opposite direction. These processes take place over the course of several minutes.

The adaptive nature of transducin translocation in rods was demonstrated by experiments that correlated the loss of transducin from outer segments with a nearly 10-fold reduction in signal amplification in the phototransduction cascade (56). This effect is likely mediated by the reduction in the rate of transducin activation by R^* because this rate is dependent on the transducin concentration (19, 20). Although transducin translocation takes place at light intensities saturating rod light responses (see below), this reduction in signal amplification may be adaptive after the bright light is dimmed or extinguished, *e.g.* as dusk approaches, and vision is gradually switching from being cone-dominant to rod-dominant.

Although still awaiting experimental validation, the functional role of translocation of other proteins is thought to be adaptive as well. Outer segments contain only a small fraction of total cellular arrestin in the dark (23, 57). As a result, phosphorylated R^* produced by fairly low light levels could rapidly deplete the outer segment of free arrestin, slowing subsequent R^* quenching. Thus, arrestin translocation provides a means to supply additional protein as needs arise upon illumination. Recoverin translocation from outer segments may also play an

adaptive role by increasing the amount of rhodopsin kinase available to phosphorylate R*. This could contribute to light adaptation by speeding R* deactivation and reducing photoreceptor sensitivity in a deeply light-adapted rod.

A complementary role of protein translocation may be to protect rods from adverse effects of persistent light exposure (58, 59). In bright light, rods contribute little to vision, and instead of transducin running fruitlessly through the activation/deactivation cycle, it is stored away in a different cellular compartment to reduce energy consumption (60, 61). This energy-saving mechanism can reduce the metabolic demand in the rods (see Ref. 58 for a specific calculation of associated energy savings), which can protect cells from death and dysfunction (60).

Another neuroprotective aspect of protein translocation may arise from reduced cellular signaling caused by the redistributions of transducin, arrestin, and recoverin. This is because apoptosis of rods, particularly in rodents, is often associated with excessive signaling of the phototransduction cascade (59, 62). Also neuroprotective could be the light-dependent translocation of another signaling protein, Grb14, which moves to rod outer segments in response to light (63), thereby facilitating insulin receptor signaling in these cells (64). Stimulation of the insulin-mTOR (mammalian target of rapamycin) pathway was recently shown to protect photoreceptors against stress-induced apoptosis (64) and delay cone death in a mouse model of retinitis pigmentosa (65).

Mechanistic Insights into Arrestin and Transducin Translocation

At this point, the field has reached the consensus that protein translocation, at least in the light-driven direction, occurs by diffusion (Refs. 52–54 and 66–68, but see Refs. 69 and 70). Although diffusion may underlie the movement of proteins, the light-dependent changes in their distribution patterns are explained by the appearance or disappearance of specific protein-binding sites in individual subcellular compartments.

For example, phosphorylation of large amounts of R* in bright light generates binding sites for arrestin, which serves as at least one major driving force for its outer segment accumulation in light (*e.g.* Ref. 66). However, at the light intensity triggering arrestin movement, the number of arrestin molecules entering the outer segment was estimated to exceed the number of produced R* by ~30-fold, and this stoichiometry was dependent on the extent of phototransduction activation (57). This suggests that additional light-dependent binding sites control arrestin distribution and release. One candidate for binding arrestin is the microtubular cytoskeleton (66, 71), which is by far more abundant in the inner than outer segment (72). However, it remains unknown whether this interaction with microtubules could be regulated by light. It has been recently hypothesized that arrestin translocation is triggered by phosphoinositide signaling downstream from rhodopsin and that both phospholipase C and PKC are involved (73). Likewise, the return of arrestin to the inner segment in the dark can also be achieved by diffusion following its release from rhodopsin, as the latter becomes regenerated, and the putative binding sites in the inner segment are restored.

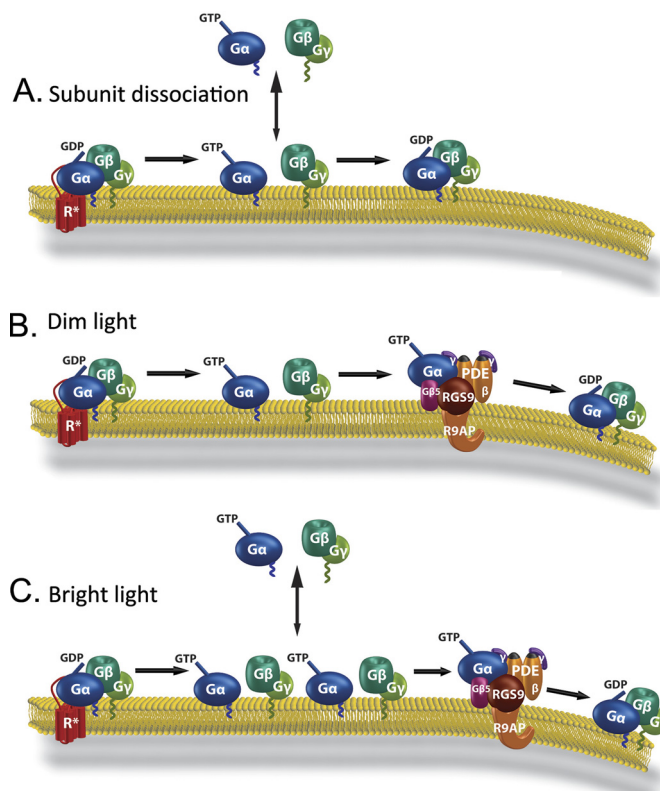


FIGURE 3. **Mechanism of transducin translocation.** *A*, the translocation of transducin requires the activation and separation of its functional subunits. The heterotrimer is tightly associated with the membrane as a result of the cooperative action of its two lipid moieties. Individual subunits have a lower membrane affinity, allowing their dissociation from the disc membranes while transducin remains activated. *B*, in dim light, activated transducin binds to PDE and is rapidly deactivated by the RGS9 complex before it can dissociate from the membrane. *C*, in bright light, translocation occurs when there is more activated transducin than PDE. This excess transducin, neither retained on the membrane by PDE nor rapidly deactivated by RGS9, stays activated sufficiently long to dissociate from the membrane to the cytosol and ultimately diffuse out of the rod outer segment. This figure was modified from Ref. 88 with permission.

Notably, the passive redistribution of arrestin caused by its binding to phosphorylated R* would fail to rapidly replenish the outer segment pool of free arrestin after a bright flash because arrestin diffusion from the inner segment takes minutes to be completed (54, 57). In contrast, a pre-emptive release of arrestin from the inner segment-binding sites at the onset of illumination should better compensate for its outer segment depletion to support normal R* deactivation during photoreceptor exposure to bright light.

The mechanism of transducin translocation in rods is understood in greater detail (52–54) and is based on the difference in membrane affinities between the $\alpha\beta\gamma$ -heterotrimer of transducin and its individual $G\alpha_t$ and $G\beta_1\gamma_1$ subunits. In dark-adapted rods, the heterotrimer is tightly associated with the outer segment disc membranes due to the combined action of two lipid modifications on the $G\alpha_t$ and $G\gamma_1$ subunits (Fig. 3A) (74, 75). Upon transducin activation by R*, $G\alpha_t$ binds GTP and separates from $G\beta_1\gamma_1$. Because each separated subunit has only one lipid modification, their membrane affinities become significantly reduced, allowing their dissociation from the disc membranes and subsequent diffusion from the outer segment through the

rod cytoplasm. Indeed, mutations of either subunit enhancing their membrane affinity caused a marked reduction in transducin translocation efficiency (68, 76), and the efficiency of $G\alpha_t$ translocation is inversely proportional to the hydrophobicity of its acyl group (77).

This basic mechanism illustrated in Fig. 3A suggests that the extent of transducin translocation should be simply proportional to the number of activated transducin molecules produced by light. However, in real rods, translocation is observed only after light intensity reaches a critical threshold, producing ~ 4000 $R^*/\text{rod}/s$ (77). This is likely because, in dimmer light, all of the activated $G\alpha_t$ rapidly binds its membrane-associated effector, PDE (Fig. 3B), which retains $G\alpha_t$ on the disc membranes. In addition, PDE serves as a cofactor for the GTPase-activating RGS9 complex (78) to allow rapid deactivation of $G\alpha_t$ and its return to the high membrane affinity state of the transducin heterotrimer. Thus, transducin translocation can occur only in light that is bright enough to activate transducin in excess of PDE (Fig. 3C), a condition that is achievable because photoreceptors have ~ 10 -fold more transducin than PDE in their disc membranes. Such excess activated $G\alpha_t$ can be neither retained on the discs by PDE nor rapidly deactivated by RGS9, and so it dissociates from disc membranes. As a result, transducin translocation occurs at precisely the light intensity at which phototransduction signaling is becoming saturated in the sense that no additional activated $G\alpha_t$ can successfully drive additional PDE activity.

We are just beginning to understand the transportation mode by which transducin returns to rod outer segments in the dark. Most likely, transducin relocated to the inner segment re-forms the $\alpha\beta\gamma$ -heterotrimer, whose membrane affinity is extremely high; thus, it is hard to imagine that it can return to the outer segment by diffusion. A motor-based mechanism (69, 70) is unlikely to help as well because molecular motors are not known to extract lipidated proteins from membranes. A solution to this problem was offered (79) by demonstrating that the knock-out of UNC119, a protein capping the acylated N terminus of $G\alpha_t$ (79, 80), significantly impairs transducin return to the outer segment in the dark. In this mechanism, UNC119 acts by keeping $G\alpha_t$ apart from $G\beta_1\gamma_1$ and by maintaining $G\alpha_t$ in the soluble form to allow its diffusion to the outer segment. On the basis of the finding that UNC119 elutes $G\alpha_t$ from membranes only in the presence of GTP, one study proposed that UNC119-dependent transducin return from the inner segment is initiated by the spontaneous activation of transducin (79). Indeed, the rate of spontaneous activation (81) is very close to that of transducin return (56). Another study found that UNC119 is able to pull $G\alpha_t$ from the membrane-bound transducin trimer, suggesting that no such spontaneous activation would be required (80). Once $G\alpha_t$ is bound to UNC119, solubilization of $G\beta_1\gamma_1$ could be subsequently facilitated by the isoprenoid-binding protein PrBP/ δ (82, 83) or by another $G\beta_1\gamma_1$ -interacting protein, phosducin, shown to reduce $G\beta_1\gamma_1$ membrane affinity (84) and assist $G\beta_1\gamma_1$ translocation, at least in the light-induced direction (85). What remains to be understood is the directionality of transducin movement, *i.e.* why, in the dark, transducin subunits solubilized by these proteins end up in the

outer segment rather than distributed evenly throughout the cell.

Why Transducin Does Not Translocate in Wild-type Cones

Cones are unique in their ability to adapt to light of essentially any intensity without saturating their photoresponses. It may appear that they could benefit from signal amplification control conferred by transducin translocation even more than rods. However, transducin does not translocate in cones under normal conditions (67, 86–89). It was first proposed that cone transducin does not translocate because its subunits ($G\alpha_{tc}$ and $G\beta_3\gamma_8$) do not separate upon activation (67). However, a recent study argued that there is no difference in the mechanism of transducin translocation in rods and cones and instead provided evidence that cone transducin does not translocate because it is not normally activated in excess of PDE (88). This is achieved because of cones' exquisite efficiency in rapid deactivation of all phototransduction proteins at virtually all light intensities. When the steady-state level of activated transducin in cones was experimentally increased by slowing phototransduction deactivation, robust translocation of $G\alpha_{tc}$ and $G\beta_3\gamma_8$ occurred (88). The minimal light intensity allowing transducin translocation in these experimental models rendered cones unresponsive to light, consistent with the idea that, in both rods and cones, transducin translocates only when the biochemical cascade is saturated.

Clearly, such biochemical saturation does not normally occur in cones, which can adapt to ambient illumination of the brightest intensity within a period of only a few seconds. On the other hand, rods are easily saturated in daylight, and the resulting transducin translocation does not impair overall visual function while likely providing neuroprotection. Thus, the presence or absence of transducin translocation can be viewed as an evolutionary adaptation of each photoreceptor type: translocation allows rods to transition into a deeply light-adapted, energy-saving mode, whereas its absence in cones allows signaling under all natural conditions.

What are the mechanisms employed by cones to avoid more than a momentary saturation of their phototransduction cascade in bright light? Particularly critical is the deactivation rate of cone R^* . Not only has phosphorylation of cone R^* been shown to be more rapid than that of rod R^* (87, 90), but also cone R^* has been long known to undergo a much more rapid thermal decay than rod R^* (*e.g.* Ref. 91). The latter is essential because photoreceptors have much less rhodopsin kinase than rhodopsin. Accordingly, rhodopsin kinase can be easily saturated by excess R^* , and the phosphorylation rate of individual R^* molecules falls drastically as the light intensity increases (*e.g.* Refs. 87 and 92).

Both phosphorylation- and arrestin-independent deactivation of R^* are much more efficient in cones than in rods (93–95). To illustrate this point, Fig. 4 shows a direct comparison of the flash response kinetics in rods and cones of wild-type mice and mice lacking visual arrestin(s). In arrestin knock-out rods, the response undergoes an initial partial recovery due to R^* phosphorylation, but a persistent plateau of remaining activity recovers very slowly with a time constant of ~ 1 min, reflecting

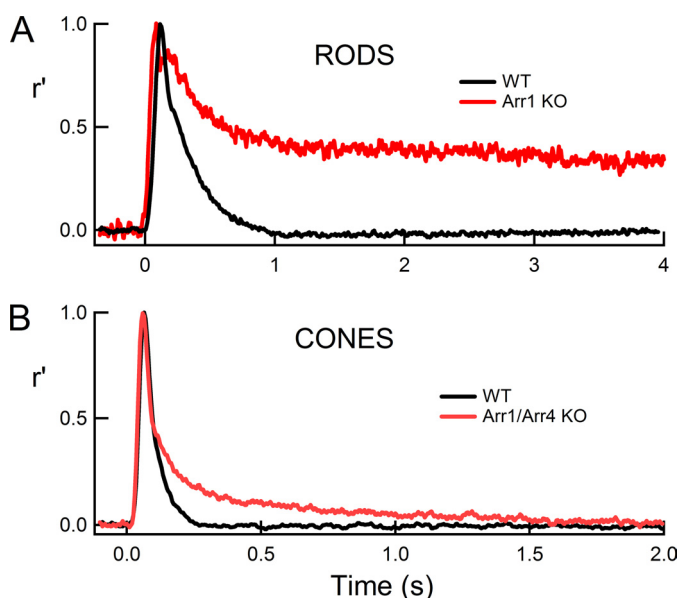


FIGURE 4. Faster arrestin-independent R^* deactivation in cones than in rods. *A*, comparison of average normalized dim flash responses of WT and arrestin-1 knock-out (*Arr1 KO*) rods. The final response recovery in the arrestin knock-out rod has a time constant comparable with that for the thermal decay of R^* , ~ 50 s. *B*, comparison of average normalized dim flash responses of a WT cone and a double knock-out cone lacking both arrestin-1 and arrestin-4 (*Arr1/Arr4 KO*). The recovery phases of the cone responses are much more similar with and without arrestins; the double knock-out response decays with a final time constant of ~ 750 ms. Traces in *A* were adapted from Ref. 100, and those in *B* from Ref. 94 with permission.

the thermal decay rate of rod R^* (96). Arrestin knock-out cones also display slower photoresponse recovery, but the effect is comparatively small. The time constant of the final recovery phase in this case is ~ 50 -fold faster than that of arrestin knock-out rods, consistent with more rapid thermal decay of cone R^* .

There are several additional mechanisms that contribute to the lack of phototransduction saturation in cones. First, cone R^* activates transducin at a slower rate than rod R^* . This effect is particularly prominent in fish (97), but the difference in the activation rate in mice does not exceed 2-fold (98). Second, transducin deactivation mechanisms by the RGS9 complex seem to be faster in cones than in rods (16, 17). Third, in persistent bright light, there is a steady-state depletion of available visual pigment, which decreases the efficiency of photon capture and thus decreases cascade activation (99). These mechanisms in cones prevent biochemical saturation of the cascade in bright light, and, combined with other adaptation mechanisms, prevent electrical saturation caused by the complete closure of the cGMP-gated channels. Altogether, these processes allow continued signaling of changes in illumination across a wide range of light intensities.

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REFERENCES

1. Burns, M. E., and Baylor, D. A. (2001) *Annu. Rev. Neurosci.* **24**, 779–805
2. Fain, G. L., Matthews, H. R., Cornwall, M. C., and Koutalos, Y. (2001) *Physiol. Rev.* **81**, 117–151
3. Arshavsky, V. Y., Lamb, T. D., and Pugh, E. N., Jr. (2002) *Annu. Rev.*

4. Fu, Y., and Yau, K. W. (2007) *Pflugers Arch.* **454**, 805–819
5. Maeda, T., Imanishi, Y., and Palczewski, K. (2003) *Prog. Retin. Eye Res.* **22**, 417–434
6. Vishnivetskiy, S. A., Raman, D., Wei, J., Kennedy, M. J., Hurley, J. B., and Gurevich, V. V. (2007) *J. Biol. Chem.* **282**, 32075–32083
7. Doan, T., Mendez, A., Detwiler, P. B., Chen, J., and Rieke, F. (2006) *Science* **313**, 530–533
8. Mendez, A., Burns, M. E., Roca, A., Lem, J., Wu, L. W., Simon, M. I., Baylor, D. A., and Chen, J. (2000) *Neuron* **28**, 153–164
9. Anderson, G. R., Posokhova, E., and Martemyanov, K. A. (2009) *Cell Biochem. Biophys.* **54**, 33–46
10. Stephen, R., Filipek, S., Palczewski, K., and Sousa, M. C. (2008) *Photochem. Photobiol.* **84**, 903–910
11. Krispel, C. M., Chen, D., Melling, N., Chen, Y. J., Martemyanov, K. A., Quillinan, N., Arshavsky, V. Y., Wensel, T. G., Chen, C. K., and Burns, M. E. (2006) *Neuron* **51**, 409–416
12. Keresztes, G., Martemyanov, K. A., Krispel, C. M., Mutai, H., Yoo, P. J., Maison, S. F., Burns, M. E., Arshavsky, V. Y., and Heller, S. (2004) *J. Biol. Chem.* **279**, 1581–1584
13. Burns, M. E., and Pugh, E. N., Jr. (2009) *Biophys. J.* **97**, 1538–1547
14. Gross, O. P., and Burns, M. E. (2010) *J. Neurosci.* **30**, 3450–3457
15. Chen, C. K., Woodruff, M. L., Chen, F. S., Chen, D., and Fain, G. L. (2010) *J. Neurosci.* **30**, 1213–1220
16. Cowan, C. W., Fariss, R. N., Sokal, I., Palczewski, K., and Wensel, T. G. (1998) *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5351–5356
17. Zhang, X., Wensel, T. G., and Kraft, T. W. (2003) *J. Neurosci.* **23**, 1287–1297
18. Matthews, H. R., and Sampath, A. P. (2010) *J. Gen. Physiol.* **135**, 355–366
19. Leskov, I. B., Klenchin, V. A., Handy, J. W., Whitlock, G. G., Govardovskii, V. I., Bownds, M. D., Lamb, T. D., Pugh, E. N., Jr., and Arshavsky, V. Y. (2000) *Neuron* **27**, 525–537
20. Heck, M., and Hofmann, K. P. (2001) *J. Biol. Chem.* **276**, 10000–10009
21. Hanson, S. M., Van Eps, N., Francis, D. J., Altenbach, C., Vishnivetskiy, S. A., Arshavsky, V. Y., Klug, C. S., Hubbell, W. L., and Gurevich, V. V. (2007) *EMBO J.* **26**, 1726–1736
22. Kim, M., Hanson, S. M., Vishnivetskiy, S. A., Song, X., Cleghorn, W. M., Hubbell, W. L., and Gurevich, V. V. (2011) *Biochemistry* **50**, 2235–2242
23. Song, X., Vishnivetskiy, S. A., Seo, J., Chen, J., Gurevich, E. V., and Gurevich, V. V. (2011) *Neuroscience* **174**, 37–49
24. Pugh, E. N., Jr., Nikonov, S., and Lamb, T. D. (1999) *Curr. Opin. Neurobiol.* **9**, 410–418
25. Peshenko, I. V., Olshevskaya, E. V., Savchenko, A. B., Karan, S., Palczewski, K., Baehr, W., and Dizhoor, A. M. (2011) *Biochemistry* **50**, 5590–5600
26. Yang, R. B., Robinson, S. W., Xiong, W. H., Yau, K. W., Birch, D. G., and Garbers, D. L. (1999) *J. Neurosci.* **19**, 5889–5897
27. Baehr, W., Karan, S., Maeda, T., Luo, D. G., Li, S., Bronson, J. D., Watt, C. B., Yau, K. W., Frederick, J. M., and Palczewski, K. (2007) *J. Biol. Chem.* **282**, 8837–8847
28. Makino, C. L., Peshenko, I. V., Wen, X. H., Olshevskaya, E. V., Barrett, R., and Dizhoor, A. M. (2008) *J. Biol. Chem.* **283**, 29135–29143
29. Howes, K. A., Pennesi, M. E., Sokal, I., Church-Kopish, J., Schmidt, B., Margolis, D., Frederick, J. M., Rieke, F., Palczewski, K., Wu, S. M., Detwiler, P. B., and Baehr, W. (2002) *EMBO J.* **21**, 1545–1554
30. Mendez, A., Burns, M. E., Sokal, I., Dizhoor, A. M., Baehr, W., Palczewski, K., Baylor, D. A., and Chen, J. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9948–9953
31. Koutalos, Y., Nakatani, K., Tamura, T., and Yau, K. W. (1995) *J. Gen. Physiol.* **106**, 863–890
32. Hodgkin, A. L., and Nunn, B. J. (1988) *J. Physiol.* **403**, 439–471
33. Burns, M. E., Mendez, A., Chen, J., and Baylor, D. A. (2002) *Neuron* **36**, 81–91
34. Calvert, P. D., Ho, T. W., LeFebvre, Y. M., and Arshavsky, V. Y. (1998) *J. Gen. Physiol.* **111**, 39–51
35. Dizhoor, A. M., Olshevskaya, E. V., and Peshenko, I. V. (2010) *Mol. Cell Biochem.* **334**, 117–124
36. Otto-Bruc, A., Buczylo, J., Surgucheva, I., Subbaraya, I., Rudnicka-

- Nawrot, M., Crabb, J. W., Arendt, A., Hargrave, P. A., Baehr, W., and Palczewski, K. (1997) *Biochemistry* **36**, 4295–4302
37. Sampath, A. P., Matthews, H. R., Cornwall, M. C., Bandarchi, J., and Fain, G. L. (1999) *J. Gen. Physiol.* **113**, 267–277
38. Sampath, A. P., Matthews, H. R., Cornwall, M. C., and Fain, G. L. (1998) *J. Gen. Physiol.* **111**, 53–64
39. Sakurai, K., Chen, J., and Kefalov, V. J. (2011) *J. Neurosci.* **31**, 7991–8000
40. Klenchin, V. A., Calvert, P. D., and Bownds, M. D. (1995) *J. Biol. Chem.* **270**, 16147–16152
41. Chen, C. K., Inglese, J., Lefkowitz, R. J., and Hurley, J. B. (1995) *J. Biol. Chem.* **270**, 18060–18066
42. Makino, C. L., Dodd, R. L., Chen, J., Burns, M. E., Roca, A., Simon, M. I., and Baylor, D. A. (2004) *J. Gen. Physiol.* **123**, 729–741
43. Xu, X., Ishima, R., and Ames, J. B. (2011) *Proteins* **79**, 1910–1922
44. Hsu, Y. T., and Molday, R. S. (1993) *Nature* **361**, 76–79
45. Chen, J., Woodruff, M. L., Wang, T., Concepcion, F. A., Tranchina, D., and Fain, G. L. (2010) *J. Neurosci.* **30**, 16232–16240
46. Rebrik, T. I., and Korenbrot, J. I. (1998) *J. Gen. Physiol.* **112**, 537–548
47. Rebrik, T. I., Kotelnikova, E. A., and Korenbrot, J. I. (2000) *J. Gen. Physiol.* **116**, 521–534
48. Nikonov, S., Lamb, T. D., and Pugh, E. N., Jr. (2000) *J. Gen. Physiol.* **116**, 795–824
49. Govardovskii, V. I., Calvert, P. D., and Arshavsky, V. Y. (2000) *J. Gen. Physiol.* **116**, 791–794
50. Krispel, C. M., Chen, C. K., Simon, M. I., and Burns, M. E. (2003) *J. Gen. Physiol.* **122**, 703–712
51. Calvert, P. D., Govardovskii, V. I., Arshavsky, V. Y., and Makino, C. L. (2002) *J. Gen. Physiol.* **119**, 129–145
52. Artemyev, N. O. (2008) *Mol. Neurobiol.* **37**, 44–51
53. Slepak, V. Z., and Hurley, J. B. (2008) *IUBMB Life* **60**, 2–9
54. Calvert, P. D., Strissel, K. J., Schiesser, W. E., Pugh, E. N., Jr., and Arshavsky, V. Y. (2006) *Trends Cell Biol.* **16**, 560–568
55. Gurevich, V. V., Hanson, S. M., Song, X., Vishnivetskiy, S. A., and Gurevich, E. V. (2011) *Prog. Retin. Eye Res.* **30**, 405–430
56. Sokolov, M., Lyubarsky, A. L., Strissel, K. J., Savchenko, A. B., Govardovskii, V. I., Pugh, E. N., Jr., and Arshavsky, V. Y. (2002) *Neuron* **34**, 95–106
57. Strissel, K. J., Sokolov, M., Trieu, L. H., and Arshavsky, V. Y. (2006) *J. Neurosci.* **26**, 1146–1153
58. Burns, M. E., and Arshavsky, V. Y. (2005) *Neuron* **48**, 387–401
59. Fain, G. L. (2006) *BioEssays* **28**, 344–354
60. Chertov, A. O., Holzhausen, L., Kuok, I. T., Couron, D., Parker, E., Linton, J. D., Sadilek, M., Sweet, I. R., and Hurley, J. B. (2011) *J. Biol. Chem.* **286**, 34700–34711
61. Okawa, H., Sampath, A. P., Laughlin, S. B., and Fain, G. L. (2008) *Curr. Biol.* **18**, 1917–1921
62. Organisciak, D. T., and Vaughan, D. K. (2010) *Prog. Retin. Eye Res.* **29**, 113–134
63. Rajala, A., Daly, R. J., Tanito, M., Allen, D. T., Holt, L. J., Lobanova, E. S., Arshavsky, V. Y., and Rajala, R. V. (2009) *Biochemistry* **48**, 5563–5572
64. Rajala, R. V., and Anderson, R. E. (2010) *Mol. Neurobiol.* **42**, 39–47
65. Punzo, C., Kornacker, K., and Cepko, C. L. (2009) *Nat. Neurosci.* **12**, 44–52
66. Nair, K. S., Hanson, S. M., Mendez, A., Gurevich, E. V., Kennedy, M. J., Shestopalov, V. I., Vishnivetskiy, S. A., Chen, J., Hurley, J. B., Gurevich, V. V., and Slepak, V. Z. (2005) *Neuron* **46**, 555–567
67. Rosenzweig, D. H., Nair, K. S., Wei, J., Wang, Q., Garwin, G., Saari, J. C., Chen, C. K., Smrcka, A. V., Swaroop, A., Lem, J., Hurley, J. B., and Slepak, V. Z. (2007) *J. Neurosci.* **27**, 5484–5494
68. Kerov, V., and Artemyev, N. O. (2011) *Mol. Cell. Neurosci.* **46**, 340–346
69. Peterson, J. J., Orisme, W., Fellows, J., McDowell, J. H., Shelamer, C. L., Dugger, D. R., and Smith, W. C. (2005) *Invest. Ophthalmol. Vis. Sci.* **46**, 3988–3998
70. Reidel, B., Orisme, W., Goldmann, T., Smith, W. C., and Wolfrum, U. (2006) *Vision Res.* **46**, 4464–4471
71. Hanson, S. M., Cleghorn, W. M., Francis, D. J., Vishnivetskiy, S. A., Rahman, D., Song, X., Nair, K. S., Slepak, V. Z., Klug, C. S., and Gurevich, V. V. (2007) *J. Mol. Biol.* **368**, 375–387
72. Eckmiller, M. S. (2000) *Vis. Neurosci.* **17**, 711–722
73. Orisme, W., Li, J., Goldmann, T., Bolch, S., Wolfrum, U., and Smith, W. C. (2010) *Cell. Signal.* **22**, 447–456
74. Bigay, J., Faurobert, E., Franco, M., and Chabre, M. (1994) *Biochemistry* **33**, 14081–14090
75. Kosloff, M., Alexov, E., Arshavsky, V. Y., and Honig, B. (2008) *J. Biol. Chem.* **283**, 31197–31207
76. Kassai, H., Aiba, A., Nakao, K., Nakamura, K., Katsuki, M., Xiong, W. H., Yau, K. W., Imai, H., Shichida, Y., Satomi, Y., Takao, T., Okano, T., and Fukada, Y. (2005) *Neuron* **47**, 529–539
77. Lobanova, E. S., Finkelstein, S., Song, H., Tsang, S. H., Chen, C. K., Sokolov, M., Skiba, N. P., and Arshavsky, V. Y. (2007) *J. Neurosci.* **27**, 1151–1160
78. Skiba, N. P., Hopp, J. A., and Arshavsky, V. Y. (2000) *J. Biol. Chem.* **275**, 32716–32720
79. Zhang, H., Constantine, R., Vorobiev, S., Chen, Y., Seetharaman, J., Huang, Y. J., Xiao, R., Montelione, G. T., Gerstner, C. D., Davis, M. W., Inana, G., Whitby, F. G., Jorgensen, E. M., Hill, C. P., Tong, L., and Baehr, W. (2011) *Nat. Neurosci.* **14**, 874–880
80. Gopalakrishna, K. N., Doddapuneni, K., Boyd, K. K., Masuho, I., Martemyanov, K. A., and Artemyev, N. O. (2011) *J. Biol. Chem.* **286**, 28954–28962
81. Ramdas, L., Disher, R. M., and Wensel, T. G. (1991) *Biochemistry* **30**, 11637–11645
82. Zhang, H., Li, S., Doan, T., Rieke, F., Detwiler, P. B., Frederick, J. M., and Baehr, W. (2007) *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8857–8862
83. Norton, A. W., Hosier, S., Terew, J. M., Li, N., Dhingra, A., Vardi, N., Baehr, W., and Cote, R. H. (2005) *J. Biol. Chem.* **280**, 1248–1256
84. Yoshida, T., Willardson, B. M., Wilkins, J. F., Jensen, G. J., Thornton, B. D., and Bitensky, M. W. (1994) *J. Biol. Chem.* **269**, 24050–24057
85. Sokolov, M., Strissel, K. J., Leskov, I. B., Michaud, N. A., Govardovskii, V. I., and Arshavsky, V. Y. (2004) *J. Biol. Chem.* **279**, 19149–19156
86. Elias, R. V., Sezate, S. S., Cao, W., and McGinnis, J. F. (2004) *Mol. Vis.* **10**, 672–681
87. Kennedy, M. J., Dunn, F. A., and Hurley, J. B. (2004) *Neuron* **41**, 915–928
88. Lobanova, E. S., Herrmann, R., Finkelstein, S., Reidel, B., Skiba, N. P., Deng, W. T., Jo, R., Weiss, E. R., Hauswirth, W. W., and Arshavsky, V. Y. (2010) *J. Neurosci.* **30**, 6815–6824
89. Coleman, J. E., and Semple-Rowland, S. L. (2005) *Invest. Ophthalmol. Vis. Sci.* **46**, 12–16
90. Tachibanaki, S., Arinobu, D., Shimauchi-Matsukawa, Y., Tsushima, S., and Kawamura, S. (2005) *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9329–9334
91. Imai, H., Kojima, D., Oura, T., Tachibanaki, S., Terakita, A., and Shichida, Y. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 2322–2326
92. Sitaramayya, A., and Liebman, P. A. (1983) *J. Biol. Chem.* **258**, 12106–12109
93. Lyubarsky, A. L., Chen, C., Simon, M. I., and Pugh, E. N., Jr. (2000) *J. Neurosci.* **20**, 2209–2217
94. Nikonov, S. S., Brown, B. M., Davis, J. A., Zuniga, F. I., Bragin, A., Pugh, E. N., Jr., and Craft, C. M. (2008) *Neuron* **59**, 462–474
95. Shi, G., Yau, K. W., Chen, J., and Kefalov, V. J. (2007) *J. Neurosci.* **27**, 10084–10093
96. Xu, J., Dodd, R. L., Makino, C. L., Simon, M. I., Baylor, D. A., and Chen, J. (1997) *Nature* **389**, 505–509
97. Kawamura, S., and Tachibanaki, S. (2008) *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **150**, 369–377
98. Nikonov, S. S., Kholodenko, R., Lem, J., and Pugh, E. N., Jr. (2006) *J. Gen. Physiol.* **127**, 359–374
99. Burkhardt, D. A. (1994) *J. Neurosci.* **14**, 1091–1105
100. Chan, S., Rubin, W. W., Mendez, A., Liu, X., Song, X., Hanson, S. M., Craft, C. M., Gurevich, V. V., Burns, M. E., and Chen, J. (2007) *Invest. Ophthalmol. Vis. Sci.* **48**, 1968–1975