HHS Public Access

Author manuscript

Prog Retin Eye Res. Author manuscript; available in PMC 2018 November 14.

Published in final edited form as:

Prog Retin Eye Res. 2018 November ; 67: 87–101. doi:10.1016/j.preteyeres.2018.06.001.

Regulation of calcium homeostasis in the outer segments of rod and cone photoreceptors

Frans Vinberg[1,2], **Jeannie Chen**[3], and **Vladimir J. Kefalov**[1]

[1]Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri, USA

[2]Current address: John A. Moran Eye Center, University of Utah, Salt Lake City, Utah, USA

[3]Zilkha Neurogenetic Institute, Department of Physiology and Neuroscience, University of Southern California Keck School of Medicine, Los Angeles, California, USA

Abstract

Calcium plays important roles in the function and survival of rod and cone photoreceptor cells. Rapid regulation of calcium in the outer segments of photoreceptors is required for the modulation of phototransduction that drives the termination of the flash response as well as light adaptation in rods and cones. On a slower time scale, maintaining proper calcium homeostasis is critical for the health and survival of photoreceptors. Decades of work have established that the level of calcium in the outer segments of rods and cones is regulated by a dynamic equilibrium between influx via the transduction cGMP-gated channels and extrusion via rod- and cone-specific Na^+/Ca^{2+}, K^+ exchangers (NCKXs). It had been widely accepted that the only mechanism for extrusion of calcium from rod outer segments is via the rod-specific NCKX1, while extrusion from cone outer segments is driven exclusively by the cone-specific NCKX2. However, recent evidence from mice lacking NCKX1 and NCKX2 have challenged that notion and have revealed a more complex picture, including a NCKX-independent mechanism in rods and two separate NCKX-dependent mechanisms in cones. This review will focus on recent findings on the molecular mechanisms of extrusion of calcium from the outer segments of rod and cone photoreceptors, and the functional and structural changes in photoreceptors when normal extrusion is disrupted.

Keywords

Photoreceptors; Calcium; Light Adaptation; NCKX1; NCKX2; NCKX4

1. Introduction

The description and discussion of the mechanisms that regulate the extrusion of calcium in the outer segments of rods and cones, and how they affect the function and survival of photoreceptor cells requires a brief introduction of the structure of photoreceptors and of their functional properties. The differences in the functional properties of rods and cones

Correspondence to: Vladimir J Kefalov, Department of Ophthalmology & Visual Sciences, Washington University in St. Louis, 660 S. Euclid Ave, Campus Box 8096, St. Louis, MO 63110, USA., Tel: 1-314-747-9041; Fax: 1-314-747-9046; kefalov@wustl.edu.

originate in differences in their respective transduction cascades, which in turn are affected by the dynamics of the calcium homeostasis in each photoreceptor type.

1.1. Rod and cone photoreceptors in the retina

The perception of vision in vertebrate animals is initiated in rod and cone photoreceptors. These photoreceptors reside at the distal layer of the retina and the signals that they generate upon photoactivation are processed and then relayed to other parts of the brain via the optic nerve. Vertebrate rods and cones have similar overall structures, with outer segments (OS), inner segments (IS), nuclear regions, and synaptic terminals (Carter-Dawson and LaVail, 1979). The detection of light and its conversion into an electric signal, a process known as phototransduction, takes place in the outer segments of rods and cones. The outer segments in both rods and cones consist of stacks of several hundreds to thousands of membrane discs, depending on species. In rods, these discs are separated from the plasma membrane which envelops them. In contrast, in cones, discs are formed from stacked invaginations of the plasma membrane (Mustafi et al., 2009). However, this rule is not absolute and vertebrate cones with closed discs have been observed (Crescitelli, 1959; Morshedian and Fain, 2015). It is believed that the greatly increased ratio of membrane surface area to cytosolic outer segment volume of cones, produced by the invaginations of their plasma membrane and/or small outer segment, facilitate the flow of metabolites in and out of the cells, potentially contributing to the fast reactions of phototransduction and metabolism in cones (Yau, 1994).

1.2. Phototransduction

The detection of light in both rods and cones is initiated when light triggers the isomerization of their visual chromophore from 11-cis to all-trans retinal (Figure 1). This results in conformational rearrangement of the helices of opsin, a G protein-coupled receptor protein, which is covalently attached to the chromophore to form the visual pigment (reviewed in (Ebrey and Koutalos, 2001)). Once activated, the visual pigment (R*) binds to and activates multiple copies of the heteromeric G protein transducing (T), causing exchange of GTP for GDP on its alpha subunit, followed by the dissociation of the transducin trimer into Tα-GTP and Tβγ. In turn, Tα-GTP binds to the γ-subunit of the effector enzyme phosphodiesterase (PDE), relieving its inhibition and causing upregulation in the hydrolysis of cGMP (reviewed in (Arshavsky et al., 2002; Lamb and Pugh, 1992; Pugh and Lamb, 1993)). The resulting decrease in the level of free cGMP in the outer segments of photoreceptors leads to the closure of some or all of the cGMP-gated channels (CNG channels, reviewed in (Yau, 1994)), and reduction or full suppression of the current normally flowing into photoreceptor outer segments (Lamb and Pugh, 2006; Yau and Hardie, 2009). The reduction in the current depends on the number of PDE molecules activated, which in turn is determined by the intensity of the stimulating light. Ultimately, this leads to hyperpolarization of the photoreceptors and reduction of the rate of release of neurotransmitter from their synapse. This change in signal is detected by the second order bipolar cells in the retina, and eventually relayed to ganglion cells and then further into the brain to drive visual perception (Martemyanov and Sampath, 2017). Maintenance of vision under continuous illumination and re-opening of the CNG channels after transient light stimuli require efficient inactivation of R* (Chen et al., 1995b) and the Tα-GTP-PDE complex (Chen et al., 2000), as well as restoration of cGMP levels. The activity of pigments

is quenched by phosphorylations by rhodopsin kinase (GRK) (Chen et al., 1999a; Kennedy et al., 2004; Mendez et al., 2000; Mendez et al., 2001; Sakurai et al., 2015) and arrestin binding (Gurevich et al., 2011; Nikonov et al., 2008; Xu et al., 1997) whereas Tα-GTP-PDEs are deactivated by GTPase activating GAP proteins that accelerate the hydrolysis of GTP (Krispel et al., 2006). The synthesis of cGMP is achieved by retinal membrane guanylyl cyclases (RetGCs), which together with phosphodiesterase, maintain a proper cGMP homeostasis in the photoreceptor outer segments (Dizhoor et al., 1994; Koch, 1991). Many of the steps in the phototransduction, including inactivation of the visual pigment and the rate of cGMP synthesis, are modulated by calcium (see below).

1.3. Functional properties of rods and cones

Rod and cone photoreceptors have distinct functional properties that make them perfectly suited for their function in dim-light and bright light conditions, respectively. Rods are exquisitely sensitive and able to detect single photons of light (Hecht et al., 1942; Tinsley et al., 2016). This makes rods excellent at detecting photostimuli in dim nighttime light conditions (Figure 2A, C). In contrast, cones are 30-1000 fold less sensitive (reviewed in (Ingram et al., 2016)) and require simultaneous activation by about ~10-100 photons for the generation of a detectable signal (Figure 2B, C) (Koenig and Hofer, 2011; Korenbrot, 2012; Naarendorp et al., 2010; Nikonov et al., 2006). This makes cones unable to signal in very dim light but perfectly suitable for their function as daytime photoreceptors. The difference in sensitivity between rods and cones (Figure 2C) originates from different levels of amplification of the signal at multiple steps of their respective transduction cascades (Ingram et al., 2016; Mao et al., 2013; Shi et al., 2007). The amplification of the signal, in turn, is affected by different kinetics of the phototransduction activation and inactivation reactions as well as the outer segment volume of rods and cones. Activation of a single pigment causes closure of more CNG channels in rods as compared to cones due to the higher gain of the rod transduction activation reactions (Chen et al., 2010a; Majumder et al., 2015; Tachibanaki et al., 2012). On the other hand, faster phototransduction inactivation reactions and higher baseline rate of cGMP synthesis/hydrolysis in cones contribute to their faster photoresponse shut-off as compared to rods (Figure 2A, B) (Cowan et al., 1998; Majumder et al., 2015; Tachibanaki et al., 2005; Tachibanaki et al., 2012; Takemoto et al., 2009; Tomizuka et al., 2015). Further, a typically smaller outer segment volume of the cones accelerates their cGMP turnover and, consequently, change of the CNG channel current when compared to rods. As a result, the temporal resolution of cone-mediated daytime vision is substantially higher than that of rod-mediated dim light vision (Hess and Nordby, 1986; Umino et al., 2008). As discussed in detail below, the kinetics of the photoresponse, in particular the speed of its inactivation, is greatly affected by the dynamics of calcium and its modulation of the phototransduction cascade.

A third important functional difference between rods and cones is their ability to adapt to background light (reviewed in (Perlman and Normann, 1998)). Rods saturate under moderately bright light and are mostly unable to mediate vision in bright daytime conditions (Figure 3) (Baylor et al., 1984; Thomas and Lamb, 1999) but see (Tikidji-Hamburyan et al., 2017)). In contrast, cones are able to respond to light of practically any natural illumination level that exists on Earth or under experimental conditions (Burkhardt, 1994; Jones et al.,

1993; Schnapf et al., 1990). Strikingly, cones, exposed to continuous illumination expected to bleach >90% of their pigment content, can retain more than 50% of their maximal dark-adapted response amplitude (Jones et al., 1993; Kenkre et al., 2005; Schnapf et al., 1990). The mechanisms that produce the difference in adaptation capacity between rods and cones are still poorly understood and are an area of active research (Sakurai et al., 2011; Sakurai et al., 2015). Adaptation in the short time scale of seconds is driven primarily by calcium-mediated feedback on their phototransduction cascades in both rods and cones (Matthews et al., 1988; Nakatani and Yau, 1988a). Thus, it can be expected that differences in the calcium modulation of their respective transduction cascades, as well as differences in the calcium homeostasis in their outer segments, contribute to the different light adaptation kinetics and capacity of rods and cones.

1.4. Light adaptation in rods and cones

As stated above, the sensitivity of rod and cone photoreceptors changes as they are exposed to background light. In electrophysiological recordings, this adaptation to a step of background light can be observed in three distinct ways. Initially, after the onset of the background light, the photoreceptors produce a rapid response with amplitude dependent on the intensity of the background light (Figure 3A and 3B). The first manifestation of adaptation of the photoreceptors is the subsequent partial relaxation in the response after it reaches its initial peak. As the intensity of the background light is steady, this partial reduction in the response reflects the reduction in the photoreceptors sensitivity as well as acceleration of the cGMP synthesis rate by RetGCs as the transduction cascade is adapting to the background light (Chen et al., 2010b; Sakurai et al., 2011). This partial relaxation can be blocked completely in both rods and cones by preventing changes in calcium in the outer segments (Matthews et al., 1988; Nakatani and Yau, 1988a). Notably, the relaxation of the response in steady background light occurs several times faster in cones compared to rods (Figure 3), indicative of the underlying faster cone light adaptation compared to that in rods.

A second manifestation of light adaptation can be observed in the responses to a test flash after the photoreceptors reach the steady state. Experiments with both rods and cones have shown that the amplitude of the response to a flash of the same intensity gradually declines with increasing background light intensity. This result also demonstrates the gradual adaptation and desensitization of the rod and cone phototransduction cascade. The decreasing sensitivity of photoreceptors in gradually increasing background light over a wide range of background light intensities can be described well by the Weber-Fechner function (Figure 3C), i.e. test flashes of identical contrast between the flash and background produce identical flash response amplitudes (Baylor and Hodgkin, 1974; Baylor et al., 1980; Fain, 1976; Fain et al., 2001; Kraft et al., 1993; Nakatani et al., 1991; Schneeweis and Schnapf, 1999; Tamura et al., 1989, 1991).

A third manifestation of the adaptation of photoreceptors is the gradual acceleration of their response kinetics with increasing background light intensity (Baylor et al., 1979; Matthews et al., 1990; Nikonov et al., 2000; Nymark et al., 2012; Woodruff et al., 2008). The faster decay of the flash response in background light reflects the accelerated turnover of cGMP driven by the upregulation of both its hydrolysis by phosphodiesterase, and its upregulated

synthesis by guanylyl cyclase as well as accelerated inactivation of visual pigment and/or phosphodiesterase (Chen et al., 2015; Koutalos et al., 1995a; Koutalos et al., 1995b; Makino et al., 2004; Mendez et al., 2001; Nikonov et al., 2000). The net result of this acceleration is a reduction in the amplitude of the response, effectively desensitizing the photoreceptors.

2. Calcium in rods and cones

As in other neurons, calcium in photoreceptors is important for sustaining their function. Abnormal calcium homeostasis resulting from mutations in phototransduction genes has been suggested to be one of the dominant mechanisms causing photoreceptor degeneration and blinding disorders (see below). In addition, calcium is intricately involved in controlling the functional properties of photoreceptors (Matthews et al., 1988; Nakatani and Yau, 1988a). Our understanding of the functional significance of light adaptation and the mechanisms driving this process in photoreceptors has been driven by combination of electrophysiological/behavioral experiments and genetic manipulations of the photoreceptors transduction cascades as discussed in details below.

2.1. Modulation of calcium in rods and cones

The rapid light adaptation in rods and cones is mediated primarily by the light-driven change in the level of calcium in their outer segments (Matthews et al., 1988; Nakatani and Yau, 1988a). Calcium enters the outer segments of photoreceptors via their cGMP-gated (CNG) transduction channels (Figure 1). These channels are nonselective cation channels and the bulk of their inward current (also called dark current) is carried by Na^+. However, calcium also represents a substantial fraction of their current. In rods, 15% of the dark current is carried by calcium whereas in cones this fraction can be as high as 30% (Ohyama et al., 2000). In darkness, when bound to cGMP, a fraction of the CNG channels are open, allowing the steady influx of Na^+ and Ca^{2+} driven by the electrochemical gradient across the plasma membrane of the outer segment (Fesenko et al., 1985; Yau and Baylor, 1989). This inward current keeps the photoreceptors depolarized at rest, contrary to most other neurons, and drives the release of glutamate neurotransmitter from their synaptic terminals onto rod and cone bipolar cells (Heidelberger et al., 2005). At rest, in darkness or in a constant background light, the influx of Ca^{2+} through the CNG channels must be matched with efflux in the outer segments of rods and cones in order to maintain a steady state Ca^{2+} concentration. When the cells are exposed to light, the transduction cascade is activated and the level of cGMP declines, leading to reduction in the dark current and hyperpolarization, as well as in reduction in the influx of Ca^{2+} in the outer segment (Hodgkin et al., 1985; Yau and Nakatani, 1984a). As the extrusion of Ca^{2+} from the outer segments lags behind and remains active at least for some time after the closure of the CNG channels, the level of Ca^{2+} in the outer segments declines rapidly (Cervetto et al., 1989; Hodgkin et al., 1987; Yau and Nakatani, 1984b). In amphibian photoreceptors, this light-driven decline in outer segment Ca^{2+} occurs substantially faster in cones compared to rods. In addition, the dynamic range of Ca^{2+} in cones is several times wider than that in rods (Sampath et al., 1999; Sampath et al., 1998). Although measurements of outer segment Ca^{2+} in mammalian species have been done only in rods (Woodruff et al., 2002), it is likely that the differences in kinetics of Ca^{2+} are preserved there as well. The faster decline of Ca^{2+} in cones is likely driven in part by the

smaller volume of cone outer segments compared to these of rods. Another possible contributing factor is the larger fraction of Ca^{2+}-driven current in cones compared to rods (Ohyama et al., 2000), which implies also more efficient extrusion of Ca^{2+} in cones. This conclusion is supported by Ca^{2+} measurements in zebrafish UV cones where the kinetics of the light-induced change in Ca^{2+} convolved with a single exponential with the time constant representing Ca^{2+} extrusion by Na^+/Ca^{2+}, K^+ exchanger was similar to that of CNG channel current (Leung et al., 2007). The differences in the mechanisms of extrusion of Ca^{2+} are discussed below.

2.2. Role of calcium in photoreceptor function

For both rods and cones, the dynamic range from threshold to saturation under dark-adapted conditions is only about 100-fold (Figure 2C). Yet, rods are able to detect single photons in darkness and function up to ambient light levels producing ~10^5 R* rod^{-1} s^{-1} corresponding to natural scenes from cloudy moonless night to about sunrise (Aguilar and Stiles, 1954; Hess et al., 1989; Naarendorp et al., 2010; Sharpe et al., 1992; Sharpe and Nordby, 1990) (but see also (Tikidji-Hamburyan et al., 2017)). The functional range of cones is even wider and they are able to detect light from starry night to bright sunny day (Boynton and Whitten, 1970; Naarendorp et al., 2010; Stiles, 1939; Valeton and van Norren, 1983).

It is now widely accepted that calcium modulates the phototransduction cascades of rods and cones to shape their photoresponses and also to mediate their adaptation to background light. The timely feedback on the transduction cascade drives the prompt termination of the flash responses of rods and cones. In addition, this feedback is largely responsible for the shift in sensitivity of photoreceptors which allows rods and cones to function over a wide dynamic range. The molecular mechanisms by which calcium modulates phototransduction are well understood in rods and significant progress has been made in elucidating these mechanisms in cones (Figure 1). In both photoreceptor types, the dominant mechanism by which calcium affects signaling is via a pair of calcium-binding proteins, called guanylyl cyclase activating protein 1 and 2 (GCAP1/2), that modulate the synthesis of cGMP by retinal membrane guanylyl cyclases (RetGC1 and RetGC2). Another calcium-binding protein, recoverin, also modulates phototransduction in rods and cones, by accelerating the inactivation of their visual pigments by rhodopsin kinase. Finally, the CNG channels can also be modulated directly by calcium, a mechanism that seems to be insignificant in rods, but more prominent in cones.

2.2.1. Modulation of GCAPs by calcium—The most powerful mechanism by which Ca^{2+} modulates phototransduction in rods and cones involves the synthesis of cGMP by RetGCs, a reaction regulated by the Ca^{2+}-binding proteins GCAP1 and GCAP2 (Burns et al., 2002; Dizhoor et al., 1994; Dizhoor et al., 1995; Gorczyca et al., 1994; Gorczyca et al., 1995; Koch and Stryer, 1988; Palczewski et al., 1994). In darkness, when the level of calcium in the outer segments of photoreceptors is high, these calcium sensors are bound to calcium and unable to activate RetGC. When the photoreceptors are exposed to light and the level of calcium in their outer segments declines, calcium is released from GCAPs and replaced by Mg^{2+} (Dizhoor et al., 2010). Magnesium-bound GCAPs activate RetGC to accelerate the synthesis of cGMP. This, in turn, promotes the rapid reopening of CNG

channels that terminates the photoresponse and drives the recovery of the dark current after a transient light stimulation (Burns et al., 2002; Mendez et al., 2001). During prolonged exposure to background light, that same mechanism helps to prevent the closure of all CNG channels, extending in that way the functional range of photoreceptors. GCAPs modulate RetGC up to 20-fold, inhibiting it at high Ca^{2+} and activating it at low Ca^{2+} levels (Palczewski et al., 2000).

The deletion of GCAPs in mouse rods abolishes the Ca^{2+} feedback on RetGC and results in slowed photoresponse shutoff and impaired light adaptation (Burns et al., 2002; Mendez et al., 2001). The functional significance of GCAPs has been demonstrated most clearly in studies with mice lacking both genes (GCAPs$^{-/-}$). The simultaneous deletion of GCAP1 and GCAP2 in mouse rods results in a dramatic delay in the recovery phase of photoresponses. As a result, the single-photon responses of GCAPs$^{-/-}$ rods become 5-fold larger than these in wild type controls. Adaptation is also severely compromised in rods lacking GCAPs (Mendez et al., 2001). This phenotype is largely repeated in GCAPs-deficient cones (Sakurai et al., 2011). Finally, in humans, mutations in GCAPs, particularly in GCAP1, have also been linked to severe hereditary blinding diseases, including Leber Congenital Amaurosis (LCA), Macular Dystrophy (MD) and cone-rod dystrophies (CRDs) (Dizhoor et al., 1998; Downes et al., 2001; Jiang et al., 2005; Nishiguchi et al., 2004; Payne et al., 1998; Sokal et al., 2005; Sokal et al., 1998; Wilkie et al., 2001).

Both GCAP1 and GCAP2 are expressed in rod photoreceptors where they exert distinct modulation of cGMP synthesis. As the light-induced decline in calcium develops, GCAP1, which has a lower affinity to Ca^{2+}, is activated first, making it more important in determining the peak amplitude of the flash response. GCAP2, which has higher affinity to Ca^{2+}, activates RetGCs at lower calcium, and shapes the response recovery kinetics after the peak amplitude and/or in brighter light (Makino et al., 2012). The functional contribution of GCAP1 and GCAP2 has not been previously tested in cones. However, previous studies in normal or cone-dominant *Nrl*$^{-/-}$ mice have suggested that GCAP2 may not be substantially present in cone outer segments (Boye et al., 2015; Howes et al., 1998; Xu et al., 2013). Thus, the general dogma has been that only GCAP1 and not GCAP2 mediates the Ca^{2+} dependent modulation of RetGC activity in the mouse cones. However, we recently found that GCAP2 is expressed in mouse cones and is able to modulate cGMP synthesis in cones lacking GCAP1 (Vinberg et al., 2018). Thus, GCAP2 can contribute significantly to the activation of RetGC1 in low Ca^{2+} when GCAP1 is not present. However, in wild-type mouse cones the regulation of cGMP synthesis seems to be dominated by GCAP1. Comparison of the effects of GCAPs deletion in mammalian rods and cones reveals that, surprisingly, considering the larger adaptation capacity of cones compared to rods, the Ca^{2+} modulation on guanylyl cyclase contributes less to the sensitivity modulation of cones as compared to rods both in darkness and during light adaptation (Sakurai et al., 2011). Thus, differences in GCAPs-mediated modulation of cGMP synthesis are unlikely to contribute to the functional differences between rods and cones.

2.2.2. Modulation of recoverin by calcium—Another mechanism by which calcium regulates phototransduction in rods and cones involves modulation of the lifetime of the photoactivated visual pigment. Fish and amphibian photoreceptors use two separate calcium

modulators of rhodopsin kinase: recoverin (or S-modulin) in rods (Dizhoor et al., 1991; Kawamura, 1993) and visinin in cones (Kawamura et al., 1996). In the case of mammals, both rods and cones use recoverin to modulate the activity of rhodopsin kinase (Chen et al., 1995a; Dizhoor et al., 1993; Kawamura, 1993; Klenchin et al., 1995; Milam et al., 1993). In darkness, when the calcium level in the outer segments is high, recoverin inhibits rhodopsin kinase which results in delayed visual pigment phosphorylation (Palczewski et al., 2000). Upon exposure to light, the calcium level in photoreceptors declines and that relieves the inhibition of rhodopsin kinase by recoverin, effectively accelerating the phosphorylation and inactivation of visual pigment. In rods, the recoverin-mediated suppression of pigment inactivation delays the flash response recovery under dim light when $[Ca^{2+}]_i$ is high, but has little effect on sensitivity during light adaptation when $[Ca^{2+}]_i$ declines (Makino et al., 2004). Notably, the expression of the corresponding calcium modulator of rhodopsin kinase is estimated to be 20 times higher in cones compared to rods (Arinobu et al., 2010). In addition, calcium had been shown to modulate the sites and extent of pigment phosphorylation in zebrafish cones but not in rods (Kennedy et al., 2004). Finally, unlike in rods where the photoresponse recovery is rate-limited by inactivation of PDE (Krispel et al., 2006), in cones the inactivation of visual pigment appears to be the rate-limiting step of the photoransduction shut-off (Matthews and Sampath, 2010). As a result, it had been suggested that the inhibition of rhodopsin kinase by recoverin might produce a more substantial modulation of the transduction cascade in cones compared to rods. This issue was examined directly by testing the function of cones in recoverin-deficient mice. As in rods, deletion of recoverin in cones was found to accelerate the response recovery in darkness and in dim background light. However, unlike the case in rods, the deletion of recoverin also caused reduction of the estimated single-photon response amplitude and decrease in cone sensitivity (Sakurai et al., 2015). Thus, by slowing pigment inactivation, recoverin in cones boosts their sensitivity in dim background light, possibly enhancing the ability of cones to detect light near threshold. Consistent with that notion, the deletion of recoverin affected largely the sensitivity in darkness and in dim background light but this effect gradually disappeared with increasing background light so that the functional range of cones in bright background light was unchanged. Thus, despite its more potent modulation of the transduction cascade of cones compared to rods, recoverin does not seem to contribute to the great adaptation capacity of cones in bright light. Another interesting conclusion from these studies is that for cones in bright light, phototransduction inactivation is likely to be dominated not by phosphorylation of their visual pigment but rather by its spontaneous decay to apo-opsin and all-*trans* retinal. However, it is worth noting that, although inactivation of visual pigment was shown to be the rate-limiting step of the photoresponse recovery in amphibian cones, this has not been yet demonstrated in mouse or other mammalian cones. Finally, in rods, recoverin has been also recently suggested to modulate PDE directly (Chen et al., 2015; Morshedian et al., 2018).

2.2.3. Modulation of CNG channels by calcium—A third known mechanism by which calcium modulates the function of rods and cones involves the conductance of the CNG channels. In rods, the CNG channels form a tetramer composed of three CNGA1 and one CNGB1 subunits (Weitz et al., 2002; Zheng et al., 2002; Zhong et al., 2002). Notably, the CNGB1 subunit contains a binding site for Ca^{2+}-calmodulin (Grunwald et al., 1998;

Weitz et al., 1998) that is occupied in the dark, when outer segment calcium is high (Gordon and Zagotta, 1995; Hsu and Molday, 1993; Koutalos et al., 1995b; Nakatani et al., 1995). When calmodulin is released from the channel in low Ca^{2+}, the affinity of the channel for cGMP increases, creating the potential for increasing the dark current. Although this mechanism could modulate phototransduction and contribute to the adaptation of rod photoreceptors, functional studies with rods indicate that its controbution is minor. Electrophysiological recordings from amphibian rods indicate that light adaptation in dim backgrounds is mediated primarily by modulation of cGMP synthesis, with modulation of its hydrolysis by phosphodiesterase as the light intensity increases, and only a small contribution by direct modulation of the channels at relatively brigh light (Koutalos et al., 1995a; Koutalos et al., 1995b). In addition, experiments with rods expressing mutant CNGB1 that lacks the calmodulin binding site also revealed no effect on response kinetics or on light adaptation (Chen et al., 2010b).

The modulation of the CNG channel by calcium appears to play a more prominent role in cones. The cone photoreceptor channel is also a tetramer, consistsing of two CNGA3 and two CNGB3 subunits (Peng et al., 2004). Recordings from fish cones have shown that their CNG channels are modulated effectively by calcium via a diffusible factor different from calmodulin (Rebrik and Korenbrot, 1998). Subsequent recordings from intact mammalian photoreceptors confirmed the robust calcium-dependent modulation of CNG channels in cones but not in rods (Rebrik and Korenbrot, 2004). Eventually, a novel calcium regulator, CNG-modulin, was identified in striped bass cones and shown to modulate the ligand sensitivity of the channels, as well as the kinetics of their light responses and light adaptation (Rebrik et al., 2012). Subsequently, the homolog of CNG-modulin was identified as EML1 (E chinoderm microtubule-associated protein like-1) in zebrafish (Korenbrot et al., 2013), a microtubule-binding protein that is required for properly orienting the cleavage plane of neuronal progenitors in the developing brain, and which is present also in mammals (Kielar et al., 2014). However, the expression of EML1 in mammalian photoreceptors or its potential functional role in mammalian cone signaling have not been examined.

3. Extrusion of calcium from rod outer segments

Maintaining calcium homeostasis in rods requires that the continuing influx of calcium into their outer segments is matched with equal efflux. The extrusion of calcium is believed to be mediated by the Na^+/Ca^{2+}, K^+ exchanger NCKX1 (for recent review, see (Schnetkamp et al., 2014)). Using the electrochemical gradients for Na^+ and K^+, NCKX1 removes Ca^{2+} by exchanging four Na^+ for one Ca^{2+} and one K^+ ions. NCKX1 is the only known mechanism for extruding calcium from rod outer segments, suggesting that the function of NCKX1 would be critical for the timely recovery of the rod photoresponse and for light adaptation. In addition, as disrupting Ca^{2+} homeostasis is implicated in photoreceptor cell death (Fain, 2006; Paquet-Durand et al., 2011; Woodruff et al., 2007), NCKX1 also would be expected to play a role in supporting rod survival. Indeed, mutant human NCKX1 has been linked to autosomal-recessive night blindness (CSNB, (Riazuddin et al., 2010)). However, the human disease is stationary, indicating that, contrary to expectations, rods lacking functional NCKX1 do not degenerate rapidly.

3.1. NCKX1-dependent calcium extrusion from rods

Extensive biochemical studies with bovine rod outer segments have suggested that NCKX1 is the rod-specific Ca^{2+} extrusion mechanism (Cook and Kaupp, 1988; Reid et al., 1990; Reilander et al., 1992; Schnetkamp, 1986). However, these studies could not provide insights into the role of NCKX1 in regulating the physiology of intact mammalian rods. It was also not known whether NCKX1 is the only mechanism responsible for Ca^{2+} extrusion from rod outer segments. To investigate the role of NCKX1 in rod function and survival, we recently generated NCKX1-deficient (*Nckx1*$^{-/-}$) mice (Vinberg et al., 2015). This allowed us to evaluate the role of NCKX1-dependent as well as NCKX1-independent calcium extrusion mechanisms for the survival and function of mammalian rods. Consistent with the hypothesized role of NCKX1 in the extrusion of calcium from rods, the exchanger was found to be expressed exclusively in their outer segments (Figure 4A) (Vinberg et al., 2015). Rods were viable and contributed to mouse dim light vision and rod-mediated synaptic transmission. However, the absolute rod response amplitudes as well as rod sensitivity were dramatically decreased by the deletion of NCKX1, most likely due to a combination of reduced CNG channel expression and reduced cGMP. Consistent with the expected suppression of the calcium feedback on the rod transduction cascade in the absence of NCKX1, the responses of *Nckx1*$^{-/-}$ rods had delayed recovery and increased fraction of channels closed by a single rhodopsin isomerization. These effects are reminiscent of those from *GCAPs*$^{-/-}$ mice that lack the dominant Ca^{2+}-feedback mechanism (Mendez et al., 2001). Thus, consistent with its hypothesized role in extruding calcium from rod outer segments, NCKX1 was found to play a key role in mediating the fast reduction of Ca^{2+} concentration upon light stimulation. In this way NCKX1 helps to set the sensitivity and temporal properties of rod signaling and drives the timely recovery of the rod response.

3.2. NCKX1-independent calcium extrusion from rods

The studies of NCKX1-deficient rods confirmed the important role of this protein in extruding calcium and regulating the kinetics, gain, and adaptation of the transduction cascade in rods. However, they also revealed the existence of a previously unknown NCKX1-independent mechanism for removing calcium from rod outer segments. Thus, contrary to the expected rapid degeneration of rods without NCKX1, the retinas of *Nckx1*$^{-/-}$ mice appeared normal at early age and underwent only a slow progressive degeneration. This finding is consistent with the stationary nature of the human NCKX1-linked visual disorder and suggested that additional, NCKX1-independent, mechanisms could also be contributing to the extrusion of calcium from rod outer segments. Also consistent with this notion, the rods in *Nckx1*$^{-/-}$ mice exhibited normal steady state background light adaptation. These findings indicate that Ca^{2+} can somehow be extruded from the outer segments of rods by an unknown NCKX1-independent mechanism within several seconds after the onset of background light. This mechanism appears to be too slow to provide normal feedback to the phototransduction cascade in the time scale of dim flash responses but is sufficient to mediate steady state light adaptation and, in the long run, protect *Nckx1*$^{-/-}$ rods from degeneration by maintaining their calcium homeostasis. The nature of this NCKX1-independent mechanism for Ca^{2+} extrusion from rod outer segments is currently unknown. However, recent work in zebrafish photoreceptors suggests that, contrary to current beliefs, calcium in the outer segment might not be independent of the rest of the cells, so that

calcium uptake by mitochondria in the inner segments might affect calcium in the outer segments (Giarmarco et al., 2017). Thus, it is possible that calcium might be released from the outer segments of rods via their connecting cilium (Figure 8), providing a relatively slow "safety-valve" mechanism for sustaining normal outer segment calcium even in the absence of functional NCKX1. The interplay between inner and outer segment calcium mechanisms and their relative role in controlling the function and survival of mammalian rods are yet to be established.

4. Extrusion of calcium from cone outer segments

Until recently, the mechanisms of extrusion of Ca^{2+} from mammalian cones were less clear than these in rods. The dominant view had been that the cone-specific isoform of the Na^+/Ca^{2+}, K^+ exchanger, NCKX2 is responsible for the Ca^{2+} extrusion from cones (Lytton, 2007). However, using photopic b-wave measurements from *in vivo* electroretinogram (ERG) recordings to indirectly evaluate the function of mouse cones, a previous study found no cone phenotype in NCKX2-deficient (*Nckx2*$^{-/-}$) mice (Li et al., 2006). This result raised the possibility that NCKX2 may not be involved in the Ca^{2+} homeostasis of mammalian cones, and that an alternative mechanism is responsible for its extrusion. Recent studies have helped elucidate the role of NCKX2 in regulating cone phototransduction and have also revealed that the dominant mechanism for extruding calcium from cones involves another member of the NCKX family, namely NCKX4.

4.1. NCKX2-dependent calcium extrusion from cones

Past evidence from *in vitro* studies had suggested that the only mechanism of extrusion of Ca^{2+} out of the outer segments of vertebrate cone photoreceptors is by NCKX2 (Prinsen et al., 2002; Prinsen et al., 2000; Schnetkamp, 2013). However, NCKX2-deficient (*Nckx2*$^{-/-}$) mice did not show retinal degeneration or changes in cone-driven bipolar cell responses assayed by photopic b-wave measurements from *in vivo* electroretinogram (ERG) recordings (Li et al., 2006). This unexpected result suggested that NCKX2 may not be involved in regulating the Ca^{2+} homeostasis of mammalian cones, leaving the mechanism of its extrusion from cone outer segments unknown. We addressed this question recently by directly analyzing the physiological properties of NCKX2-deficient mouse cones in dark- and light-adapted conditions. Our results demonstrated that NCKX2 is expressed selectively in the outer segments of mouse cone photoreceptors (Figure 4B) and that its deletion in *Nckx2*$^{-/-}$ mice does not cause detectable cone degeneration (Sakurai et al., 2016). The better resolution of *ex vivo* recordings used in our study, compared to the previously published *in vivo* ERG experiments (Li et al., 2006), allowed us to show clearly that the deletion of NCKX2 affects the flash responses of dark-adapted mouse cones as well as their light adaptation. The deletion of NCKX2 from mouse cones slowed down the recovery of both dim and saturating light responses (Figure 5A), demonstrating that NCKX2-mediated Ca^{2+} extrusion is an integral component of the rapid feedback to the cone phototransduction cascade and its timely inactivation. The onset of light adaptation in NCKX2-deficient cones was also delayed, indicative of their slower calcium extrusion and supporting a role for NCKX2 in the regulation of cone calcium homeostasis.

As discussed above, blocking the major Ca^{2+}-mediated negative feedback on cone phototransduction by deleting GCAPs results in several-fold larger cone single photon response and a dramatic delay in the recovery of cone responses (Sakurai et al., 2011). If NCKX2 was the dominant mechanism for extruding Ca^{2+} from cone outer segments, it would be expected that its deletion would largely delay the onset of the Ca^{2+}-mediated feedback so that the physiological phenotype in *Nckx2*$^{-/-}$ cones would be comparable to that in GCAPs-deficient cones or NCKX1-deficient rods. However, the deletion of NCKX2 in mouse cones produced only a slight delay in the response recovery without affecting substantially the amplitude of the single photon response. Similarly, given sufficient time (~2 s), the background light response amplitude of *Nckx2*$^{-/-}$ cones reached steady state comparable to that of control cones. These results indicate that the negative feedback on cone phototransduction is still largely functioning in NCKX2-deficient cones. The normal cone survival in *Nckx2*$^{-/-}$ retina is not consistent with their hypothesized high and toxic Ca^{2+} levels (Fain and Lisman, 1999; Lisman and Fain, 1995). Together, these findings demonstrated that the deletion of NCKX2 does not affect dramatically the extrusion of Ca^{2+} from cone outer segments, and pointed to the existence of additional, NCKX2-independent, Ca^{2+} extrusion pathway(s) for the maintenance of Ca^{2+} homeostasis in cones.

4.2. NCKX4-dependent calcium extrusion from cones

An initial clue about the nature of the additional Ca^{2+} extrusion mechanism in cones came from comparing microarray data from rod-dominant wild type (WT) and cone-dominant NRL-deficient (*Nrl*$^{-/-}$) mouse retinas (Corbo et al., 2007). This analysis revealed that the Na^{+}/Ca^{2+}, K^{+} exchanger *Nckx4* (*Slc24a4*) is strongly upregulated in the retina of *Nrl*$^{-/-}$ mice, suggesting that NCKX4 could potentially be present in cone photoreceptors. Subsequent *in situ* hybridization and immunocytochemistry experiments demonstrated the expression of Nckx4 in mouse cones and the presence of NCKX4 protein in fish, mouse (Figure 4D), and primate (Figure 4C) cones (Vinberg et al., 2017). Functional analysis of NCKX4-deficient mouse cones revealed larger than normal single photon response and slower light response recovery (Figure 5B). The properties of dark-adapted cones lacking NCKX4 resembled those of GCAPs-deficient cones in which the Ca^{2+} feedback that accelerates cGMP synthesis is absent (Sakurai et al., 2011). Thus, NCKX4 is important for rapidly lowering [Ca^{2+}] in cones following light-induced closure of CNG channels to mediate the Ca^{2+} feedbacks in the time scale of flash responses (~500 ms). As could be expected from the presence of NCKX2 in NCKX4-deficient cones, they had normal cone light response amplitudes, lacked detectable degeneration, and were able to adapt according to Weber-Fechner law to steady background light (Figure 6). Comparison of the kinetics of responses from wild type, NCKX2-deficient, and NCKX4-deficient cones revealed that the two exchangers have distinct roles in tuning the cone transduction cascade: the extrusion of Ca^{2+} that modulates phototransduction after photoactivation is dominated initially by NCKX4, whereas NCKX2 contributes mostly during the late phase of the flash response. The molecular mechanism for such temporal separation is not clear as NCKX2 and NCKX4 appear to have similar molecular properties (Jalloul et al., 2016). One possibility is that they have different kinetics or ionic equilibrium of inactivation enabling them to differentially modulate Ca^{2+} extrusion. Regardless of the exact mechanism, the emerging picture is that in cones, the combined activity of NCKX2 and NCKX4 is required for achieving the rapid and

efficient extrusion of Ca^{2+} that enables these cells to adapt rapidly and remain functional in a wide range of light backgrounds throughout the day (Figure 8). Consistent with that notion, the simultaneous deletion of NCKX2 and NCKX4 in cones resulted in largely suppressed cone sensitivity, further delay in response kinetics and a reduced maximal amplitude (Figure 7)(Vinberg et al., 2017). It is still unclear whether additional, NCKX-independent mechanisms similar to that identified in rods, are also at play in cones.

5. Role of calcium in photoreceptor degeneration

Abnormal calcium homeostasis has adverse effects on cell function and survival. High intracellular calcium concentration activates calpains (Nakazawa, 2011), which are calcium-dependent cysteine proteases. In addition, calcium influx can influence calcium dynamics from intracellular stores, both of which can lead to apoptosis. Photoreceptors are among the most vulnerable cells of the body and their function and survival can be severely affected by mutations or environmental factors (reviewed in (Stone et al., 1999)). Calcium enters the photoreceptor outer segment through the CNG channels, and the cell body/synaptic terminal through the L-type calcium channels. The role of calcium influx through the L-type channels in photoreceptor apoptosis is controversial, inasmuch as pharmacologic blockage of these channels or their genetic ablation have limited efficacy in delaying retinal degeneration caused by elevated calcium (Barabas et al., 2010). Here we focus on the role of calcium entry through the CNG channels.

5.1. Excessive calcium entry through the CNG channels as a mechanism of photoreceptor cell death

The CNG gated channel is the only route of calcium entry into the outer segment. The probability of the opening of the CNG channel, which in turn determines the size of the circulating current, depends on the amount of free [cGMP], which in the dark-adapted rod is estimated to be 3-4 μM (Pugh and Lamb, 1993). At this concentration, the probability of channel opening is estimated to be only 0.1-0.2 (Nakatani and Yau, 1988b). Due to the high cooperativity of cGMP binding (EC50 ≈ 30 μM, Hill coefficient = 3; (Eismann et al., 1994; Hsu and Molday, 1994; Ruiz et al., 1999)), even a slight increase in [cGMP] can have a profound effect on the number of open channels. In addition, mutations that increase the channel's affinity for cGMP can affect the number of open channels which could also result in toxic calcium influx into the cell. Such mutations have been found in CNGA3 and CNGB3 genes that form the cone's CNG channel (Liu and Varnum, 2005; Peng et al., 2003, 2004).

The concentration of cGMP depends on its synthesis by retinal guanylyl cyclases (retGCs) and its degradation by phosphodiesterase, PDE6. In the dark-adapted state, the intracellular [cGMP] is determined by the basal activities of RetGCs and PDE6. GCAPs form a molecular complex with RetGCs in rods and cones which enables rapid modulation of RetGC activity in response to calcium fluctuations in rod and cone outer segments (Wen et al., 2014). Following light exposure, GCAPs sense the drop in $[Ca^{2+}]$ by replacing the bound calcium with magnesium to stimulate RetGCs (Fig. 1). Mutations in GCAP1 and RetGC1 have been linked to inherited autosomal dominant cone and cone-rod retinopathies in

humans (Newbold et al., 2002; Olshevskaya et al., 2002), as have GCAP2 (Sato et al., 2005). Many of these mutations affect calcium binding domains in GCAPs or affect GCAP/retGC interaction so that GCAPs will stimulate RetGC even under higher calcium concentrations (Sato et al., 2018). This in turn leads to elevated [cGMP] synthesis and cell death.

Mutations in PDE6 also have a profound effect on intracellular [cGMP]: its concentration can increase dramatically as a consequence of defective cGMP hydrolysis (Iribarne and Masai, 2017). The *rd1* mouse, an early mouse model of retinal degeneration, harbors a loss-of-function mutation in the *Pde6b* gene (Bowes et al., 1990; McLaughlin et al., 1993; Pittler and Baehr, 1991). Since that discovery, numerous PDE6 mutations have been identified in human patients diagnosed with retinitis pigmentosa (McLaughlin et al., 1993). The severity of retinal degeneration correlates with the degree of impairment of PDE6 activity. In the case of complete loss-of-function in *rd1*, retinal degeneration is rapid and complete within days of eye-opening (Farber and Lolley, 1974). If an increase in intracellular [cGMP] causes toxic calcium influx through CNG channels, then retinal degeneration should be delayed by preventing CNG channel expression. Indeed, retinal degeneration in *rd1* is slowed in the *Cngb1* knockout background wherein expression of functional CNG channels is greatly reduced (Paquet-Durand et al., 2011; Tosi et al., 2011). Together, these findings support an important role of [cGMP] in photoreceptor function and survival and a mechanism of cell death caused by excess calcium entry through CNG channels gated open by elevated [cGMP].

5.2. Absence of CNG channel expression leads to increased intracellular cGMP concentration and photoreceptor cell death

Although preventing CNG channel expressing has a rescuing effect on the *rd1* retina, rods lacking CNG channel expression gradually die, albeit at a much slower time course than that of *rd1* (Huttl et al., 2005; Zhang et al., 2009). Importantly, mutations in rod and cone CNG channels have been found in human patients diagnosed with retinitis pigmentosa and achromatopsia (Schon et al., 2013). In both rods and cones, cGMP level is dramatically elevated in the absence of the CNG channels (Biel et al., 1999). What could be the cause of this cGMP buildup? Because of the lack of calcium entry through CNG channels and its continuous efflux through the NCKX exchangers, it can be expected that calcium levels in the CNG knockout photoreceptor would be abnormally low, and this would then lead to GCAPs-mediated stimulation of RetGCs. Prolonged elevation of cGMP, in turn, appears to activate protein kinase G (Ma et al., 2015; Paquet-Durand et al., 2009; Xu et al., 2013). In support of this mechanism, preventing protein kinase 1 expression had a rescuing effect on *Cngb1* knockout rods (Wang et al., 2017). These results highlight the relationship between calcium concentration, cGMP accumulation, and identification of protein kinase G activity as a mechanism of cell death caused by elevated [cGMP].

5.3. Low intracellular [Ca^{2+}] and photoreceptor cell death

In addition to CNG channel loss-of-function mutations, other mutations affecting genes in the phototransduction cascade are also expected to lower intracellular [Ca^{2+}]. For example, null-mutations in RetGC1 or RD3, a protein that inhibits basal GC activity and aids in its transport (Azadi et al., 2010), would be expected to reduce intracellular [cGMP], close CNG

channels and lower [Ca^{2+}]. In addition, mutations of RPE65 that prevent formation of visual pigments are expected to lower [Ca^{2+}] due to the constitutive activity of free opsin proteins which continuously stimulate the phototransduction cascade (Woodruff et al., 2003). Other examples of mutations leading to uncontrolled phototransduction and photoreceptor cell death include the rod arrestin knockout (Chen et al., 1999b; Wang and Chen, 2014) and rhodopsin kinase knockout (Chen et al., 1999a) mouse models. Indeed, an "equivalent light" hypothesis has been put forward to explain how the dominantly active mutations in the phototransduction cascade lead to cell death (Fain, 2006; Fain and Lisman, 1993). Despite these advances, the underlying mechanism by which low calcium leads to cell death remains unclear.

5.4. Intracellular [Ca^{2+}] and photoreceptor cell death: future challenges

In most instances, how mutations in phototransduction genes may affect intracellular [Ca^{2+}] is assumed based on our knowledge and understanding of the phototransduction cascade. Because phototransduction is confined to the outer segment compartment and the cell death machinery is located in the inner segment, it is not known how alteration in calcium levels in the outer segment is propagated to the inner segment. This is particularly baffling given that calcium might be regulated independently in different photoreceptor cell compartments (Krizaj and Copenhagen, 1998), and that the presence of the abundant mitochondria in the photoreceptor ellipsoid regions would act as a calcium "sink" between the two compartments. Additionally, how calcium entry to the outer segment affect internal calcium stores (such as mitochondria and endoplasmic reticulum) in the photoreceptors is also not known, but an important area of investigation because perturbation of calcium homeostasis within these organelles can lead to execution of cell death pathways (Barabas et al., 2010; Krizaj, 2012). Direct measurement of calcium concentration, and how it is affected by various mutations, would greatly increase our understanding of the relationship between calcium dynamics in the different photoreceptor cell compartments and cell death. While genetically encoded fluorescent calcium indicators have provided much insight into calcium dynamics in other tissues, their fluorescent signal requires light stimulation, which confound their usage in the light-sensitive retina. However, advances in multi-photon microscopy and sensitive detectors that requires little excitation may render calcium imaging in all compartments of the photoreceptors possible in the near future.

6. Conclusions

Decline of Ca^{2+} concentration in the rod and cone outer segments mediate important feedback mechanisms that allow both rods and cones to modulate their response kinetics and sensitivity when ambient light level increases. This widens the dynamic range of photoreceptors and contributes importantly to the "contrast constancy" of our vision. Ca^{2+} feedback mechanisms on the phototransduction components, including visual pigment phosphorylation and cGMP synthesis, have been studied in amphibian and mammalian rods and cones. These mechanisms appear not to explain the functional differences between rods and cones. Calcium-dependent modulation of the CNG channels, on the other hand, has been shown to be more prominent in cones as compared to rods. However, the mechanism of the channel modulation in mammalian photoreceptors is not known. In this review, we

focused on the role of Ca^{2+} extrusion mechanisms in regulating phototransduction and light adaptation in the rod and cone outer segments. It has been a dogma for decades that the extrusion of Ca^{2+} from the rod outer segments is exclusively mediated by NCKX1, and from cone outer segments by NCKX2. However, our recent work discovered a novel cone Ca^{2+} extrusion mechanism via NCKX4, expressed in the cone outer segment, and contributing importantly to the cone phototransduction shut-off and temporal resolution of cone vision. In addition, our work showed that even in the absence of NCKX1, mouse rods degenerate only very slowly and light adapt normally, indicating an NCKX-independent pathway of clearing Ca^{2+} from the rod outer segments. Unlike rods, cones express two NCKX isoforms, NCKX2 and NCKX4, which both contribute to Ca^{2+} extrusion and modulation of the phototransduction of cones. Only after deleting both of these isoforms, a slowly progressing cone degeneration and severely compromised phototransduction is observed. It is unclear, though, why rods and cones express different NCKX isoforms that have very similar biophysical properties.

7. Future directions

Despite decades of research elucidating the phototransduction and light adaptation mechanisms in vertebrate rod and cone photoreceptors it is still not fully understood why rods are more sensitive than cones. Furthermore, it is not known why cones practically never saturate whereas rods saturate in ambient light producing ~10^5 R* rod^{-1} s^{-1}. One plausible explanation is that Ca^{2+} feedbacks, e.g. via channel modulation, can modulate sensitivity of cones stronger and faster as compared to rods. These differences can be due to differences in the feedback proteins and/or their expression levels. The faster and larger light-induced change of Ca^{2+} in cones compared to rods is also likely to contribute. Although some of the differences in the rod vs. cone phototransduction proteins and their expression levels are known (reviewed in (Ingram et al., 2016)), it is still not well understood why mammalian cones can light adapt over wider range and faster as compared to rods. This difference becomes even more puzzling if one considers that the dominant mechanisms of calcium-dependent modulation in rods and cones are mediated by the same set of proteins, GCAPs and recoverin. In the future, it will be important to determine the range and kinetics of light-induced change of Ca^{2+} in the mammalian cone outer segments, and compare it to that of mammalian rods. Further, studying how different Ca^{2+} transport mechanisms contribute to the Ca^{2+} homeostasis by combining Ca^{2+} imaging and electrophysiology with e.g. *Nckx1*$^{-/-}$, *Nckx2*$^{-/-}$ and *Nckx4*$^{-/-}$ mouse models will advance our mechanistic understanding of the functional differences between rods and cones. Another tempting approach would be to replace NCKX1 with either NCKX4 and/or NCKX2 in the rods, and study how this would affect phototransduction, light adaptation and Ca^{2+} homeostasis in rods.

Our studies using NCKX knockout models as well as recent studies demonstrating that mutations that initially compromise Ca^{2+} homeostasis in the outer segment of photoreceptors also lead to abnormal Ca^{2+} in endoplasmic reticulum (Butler et al., 2017) and potentially also in mitochondria (Giarmarco et al., 2017) indicate that transport of Ca^{2+} between photoreceptor outer and inner segments may also play a role in phototransduction, light adaptation, and photoreceptor death/survival. Future Ca^{2+} imaging and

electrophysiology studies in rods and cones lacking either the outer segment plasma membrane Ca^{2+} extrusion mechanisms and/or Ca^{2+} transport mechanisms in mitochondria and endoplasmic reticulum (ER) would shed light on how the complex interplay between various plasma membrane and intracellular Ca^{2+} transport mechanisms in the outer and inner segment contribute to the Ca^{2+} homeostasis and function of rod and cone photoreceptors.

Acknowledgements

This work was supported by National Institutes of Health grants, EY027387 (V.J.K. and J.C.), EY019312 and EY025696 (V.J.K.), EY012155 (J.C.), EY026651 (F.V.), and EY002687 (Washington University, Department Ophthalmology), Research to Prevent Blindness, and the Ella & Georg Ehrnrooth Foundation (F.V.).

Abbreviations:

cGMP	cyclic guanosine monophosphate
CNG	channel cGMP-gated channels
GCAP	guanylyl cyclase activating protein
ER	endoplasmic reticulum
ERG:	electroretinogram
MCU	mitochondrial calcium uniporter
NCX	Na^+/Ca^{2+} exchanger
NCKX	Na^+/Ca^{2+}, K^+ exchanger
PDE	phosphodiesterase
R*	photoactivated visual pigment
rd1	retinal degeneration 1
RD3	retinal degeneration 3 protein
RetGC (GC)	retinal membrane guanylyl cyclase.

References

Aguilar M, Stiles WS, 1954 Saturation of the rod mechanism of the retina at high levels of illumination. Optica Acta 1, 7.

Arinobu D, Tachibanaki S, Kawamura S, 2010 Larger inhibition of visual pigment kinase in cones than in rods. J Neurochem 115, 259–268. [PubMed: 20649847]

Arshavsky VY, Lamb TD, Pugh EN, Jr., 2002 G proteins and phototransduction. Annu Rev Physiol 64, 153–187. [PubMed: 11826267]

Azadi S, Molday LL, Molday RS, 2010 RD3, the protein associated with Leber congenital amaurosis type 12, is required for guanylate cyclase trafficking in photoreceptor cells. Proc Natl Acad Sci U S A 107, 21158–21163. [PubMed: 21078983]

Barabas P, Cutler Peck C, Krizaj D, 2010 Do calcium channel blockers rescue dying photoreceptors in the Pde6b (rd1) mouse? Adv Exp Med Biol 664, 491–499. [PubMed: 20238051]

Bauer PJ, Drechsler M, 1992 Association of cyclic GMP-gated channels and Na(+)-Ca(2+)-K+ exchangers in bovine retinal rod outer segment plasma membranes. J Physiol 451, 109–131. [PubMed: 1328615]

Baylor DA, Hodgkin AL, 1974 Changes in time scale and sensitivity in turtle photoreceptors. J Physiol 242, 729–758. [PubMed: 4449053]

Baylor DA, Lamb TD, Yau KW, 1979 The membrane current of single rod outer segments. J Physiol 288, 589–611. [PubMed: 112242]

Baylor DA, Matthews G, Yau KW, 1980 Two components of electrical dark noise in toad retinal rod outer segments. J Physiol 309, 591–621. [PubMed: 6788941]

Baylor DA, Nunn BJ, Schnapf JL, 1984 The photocurrent, noise and spectral sensitivity of rods of the monkey Macaca fascicularis. J Physiol 357, 575–607. [PubMed: 6512705]

Biel M, Seeliger M, Pfeifer A, Kohler K, Gerstner A, Ludwig A, Jaissle G, Fauser S, Zrenner E, Hofmann F, 1999 Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. Proc Natl Acad Sci U S A 96, 7553–7557. [PubMed: 10377453]

Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB, 1990 Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. Nature 347, 677–680. [PubMed: 1977087]

Boye SL, Peterson JJ, Choudhury S, Min SH, Ruan Q, McCullough KT, Zhang Z, Olshevskaya EV, Peshenko IV, Hauswirth WW, Ding XQ, Dizhoor AM, Boye SE, 2015 Gene Therapy Fully Restores Vision to the All-Cone Nrl(−/−) Gucy2e(−/−) Mouse Model of Leber Congenital Amaurosis-1. Hum Gene Ther 26, 575–592. [PubMed: 26247368]

Boynton RM, Whitten DN, 1970 Visual adaptation in monkey cones: recordings of late receptor potentials. Science 170, 1423–1426. [PubMed: 4991522]

Burkhardt DA, 1994 Light adaptation and photopigment bleaching in cone photoreceptors in situ in the retina of the turtle. J Neurosci 14, 1091–1105. [PubMed: 8120614]

Burns ME, Mendez A, Chen J, Baylor DA, 2002 Dynamics of cyclic GMP synthesis in retinal rods. Neuron 36, 81–91. [PubMed: 12367508]

Butler MR, Ma H, Yang F, Belcher J, Le YZ, Mikoshiba K, Biel M, Michalakis S, Iuso A, Krizaj D, Ding XQ, 2017 Endoplasmic reticulum (ER) Ca(2+)-channel activity contributes to ER stress and cone death in cyclic nucleotide-gated channel deficiency. J Biol Chem 292, 11189–11205. [PubMed: 28495882]

Carter-Dawson LD, LaVail MM, 1979 Rods and cones in the mouse retina. I. Structural analysis using light and electron microscopy. J Comp Neurol 188, 245–262. [PubMed: 500858]

Cervetto L, Lagnado L, Perry RJ, Robinson DW, McNaughton PA, 1989 Extrusion of calcium from rod outer segments is driven by both sodium and potassium gradients. Nature 337, 740–743. [PubMed: 2537471]

Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI, 2000 Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9–1. Nature 403, 557–560. [PubMed: 10676965]

Chen CK, Burns ME, Spencer M, Niemi GA, Chen J, Hurley JB, Baylor DA, Simon MI, 1999a Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. Proc Natl Acad Sci U S A 96, 3718–3722. [PubMed: 10097103]

Chen CK, Inglese J, Lefkowitz RJ, Hurley JB, 1995a Ca(2+)-dependent interaction of recoverin with rhodopsin kinase. J Biol Chem 270, 18060–18066. [PubMed: 7629115]

Chen CK, Woodruff ML, Chen FS, Shim H, Cilluffo MC, Fain GL, 2010a Replacing the rod with the cone transducin subunit decreases sensitivity and accelerates response decay. J Physiol 588, 3231–3241. [PubMed: 20603337]

Chen CK, Woodruff ML, Fain GL, 2015 Rhodopsin kinase and recoverin modulate phosphodiesterase during mouse photoreceptor light adaptation. J Gen Physiol 145, 213–224 [PubMed: 25667411]

Chen J, Makino CL, Peachey NS, Baylor DA, Simon MI, 1995b Mechanisms of rhodopsin inactivation in vivo as revealed by a COOH-terminal truncation mutant. Science (New York, N Y) 267, 374–377. [PubMed: 7824934]

Chen J, Simon MI, Matthes MT, Yasumura D, LaVail MM, 1999b Increased susceptibility to light damage in an arrestin knockout mouse model of Oguchi disease (stationary night blindness). Invest Ophthalmol Vis Sci 40, 2978–2982. [PubMed: 10549660]

Chen J, Woodruff ML, Wang T, Concepcion FA, Tranchina D, Fain GL, 2010b Channel modulation and the mechanism of light adaptation in mouse rods. J Neurosci 30, 16232–16240. [PubMed: 21123569]

Cook NJ, Kaupp UB, 1988 Solubilization, purification, and reconstitution of the sodium-calcium exchanger from bovine retinal rod outer segments. J Biol Chem 263, 11382–11388. [PubMed: 2841326]

Corbo JC, Myers CA, Lawrence KA, Jadhav AP, Cepko CL, 2007 A typology of photoreceptor gene expression patterns in the mouse. Proc Natl Acad Sci U S A 104, 12069–12074. [PubMed: 17620597]

Cowan CW, Fariss RN, Sokal I, Palczewski K, Wensel TG, 1998 High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. Proc Natl Acad Sci U S A 95, 5351–5356. [PubMed: 9560279]

Crescitelli F, 1959 The natural history of visual pigments. Ann N Y Acad Sci 74, 230–255. [PubMed: 13627855]

Dizhoor AM, Boikov SG, Olshevskaya EV, 1998 Constitutive activation of photoreceptor guanylate cyclase by Y99C mutant of GCAP-1. Possible role in causing human autosomal dominant cone degeneration. J Biol Chem 273, 17311–17314. [PubMed: 9651312]

Dizhoor AM, Chen CK, Olshevskaya E, Sinelnikova VV, Phillipov P, Hurley JB, 1993 Role of the acylated amino terminus of recoverin in Ca(2+)-dependent membrane interaction. Science 259, 829–832. [PubMed: 8430337]

Dizhoor AM, Lowe DG, Olshevskaya EV, Laura RP, Hurley JB, 1994 The human photoreceptor membrane guanylyl cyclase, RetGC, is present in outer segments and is regulated by calcium and a soluble activator. Neuron 12, 1345–1352. [PubMed: 7912093]

Dizhoor AM, Olshevskaya EV, Henzel WJ, Wong SC, Stults JT, Ankoudinova I, Hurley JB, 1995 Cloning, sequencing, and expression of a 24-kDa Ca(2+)-binding protein activating photoreceptor guanylyl cyclase. J Biol Chem 270, 25200–25206. [PubMed: 7559656]

Dizhoor AM, Olshevskaya EV, Peshenko IV, 2010 Mg2+/Ca2+ cation binding cycle of guanylyl cyclase activating proteins (GCAPs): role in regulation of photoreceptor guanylyl cyclase. Mol Cell Biochem 334, 117–124. [PubMed: 19953307]

Dizhoor AM, Ray S, Kumar S, Niemi G, Spencer M, Brolley D, Walsh KA, Philipov PP, Hurley JB, Stryer L, 1991 Recoverin: a calcium sensitive activator of retinal rod guanylate cyclase. Science 251, 915–918. [PubMed: 1672047]

Downes SM, Holder GE, Fitzke FW, Payne AM, Warren MJ, Bhattacharya SS, Bird AC, 2001 Autosomal dominant cone and cone-rod dystrophy with mutations in the guanylate cyclase activator 1A gene-encoding guanylate cyclase activating protein-1. Arch Ophthalmol 119, 96–105. [PubMed: 11146732]

Ebrey T, Koutalos Y, 2001 Vertebrate photoreceptors. Prog Retin Eye Res 20, 49–94. [PubMed: 11070368]

Eismann E, Muller F, Heinemann SH, Kaupp UB, 1994 A single negative charge within the pore region of a cGMP-gated channel controls rectification, Ca2+ blockage, and ionic selectivity. Proc Natl Acad Sci U S A 91, 1109–1113. [PubMed: 7508120]

Fain GL, 1976 Sensitivity of toad rods: Dependence on wave-length and background illumination. J Physiol 261, 71–101. [PubMed: 825637]

Fain GL, 2006 Why photoreceptors die (and why they don't). Bioessays 28, 344–354. [PubMed: 16547945]

Fain GL, Lisman JE, 1993 Photoreceptor degeneration in vitamin A deprivation and retinitis pigmentosa: the equivalent light hypothesis. Exp. Eye Res. 57, 335–340. [PubMed: 8224021]

Fain GL, Lisman JE, 1999 Light, Ca2+, and photoreceptor death: new evidence for the equivalent-light hypothesis from arrestin knockout mice. Invest Ophthalmol Vis Sci 40, 2770–2772. [PubMed: 10549634]

Fain GL, Matthews HR, Cornwall MC, Koutalos Y, 2001 Adaptation in vertebrate photoreceptors. Physiol Rev 81, 117–151. [PubMed: 11152756]

Farber DB, Lolley RN, 1974 Cyclic guanosine monophosphate: elevation in degenerating photoreceptor cells of the C3H mouse retina. Science 186, 449–451. [PubMed: 4369896]

Fesenko EE, Kolesnikov SS, Lyubarsky AL, 1985 Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. Nature 313, 310–313. [PubMed: 2578616]

Giarmarco MM, Cleghorn WM, Sloat SR, Hurley JB, Brockerhoff SE, 2017 Mitochondria Maintain Distinct Ca(2+) Pools in Cone Photoreceptors. J Neurosci 37, 2061–2072. [PubMed: 28115482]

Gorczyca WA, Gray-Keller MP, Detwiler PB, Palczewski K, 1994 Purification and physiological evaluation of a guanylate cyclase activating protein from retinal rods. Proc Natl Acad Sci U S A 91, 4014–4018. [PubMed: 7909609]

Gorczyca WA, Polans AS, Surgucheva IG, Subbaraya I, Baehr W, Palczewski K, 1995 Guanylyl cyclase activating protein. A calcium-sensitive regulator of phototransduction. J Biol Chem 270, 22029–22036. [PubMed: 7665624]

Gordon SE, Zagotta WN, 1995 Subunit interactions in coordination of Ni2+ in cyclic nucleotide-gated channels. Proc Natl Acad Sci U S A 92, 10222–10226. [PubMed: 7479756]

Grunwald ME, Yu WP, Yu HH, Yau KW, 1998 Identification of a domain on the beta-subunit of the rod cGMP-gated cation channel that mediates inhibition by calcium-calmodulin. J Biol Chem 273, 9148–9157. [PubMed: 9535905]

Gurevich VV, Hanson SM, Song X, Vishnivetskiy SA, Gurevich EV, 2011 The functional cycle of visual arrestins in photoreceptor cells. Prog Retin Eye Res 30, 405–430. [PubMed: 21824527]

Hecht S, Shlaer S, Pirenne MH, 1942 Energy, Quanta, and Vision. J Gen Physiol 25, 819–840. [PubMed: 19873316]

Heidelberger R, Thoreson WB, Witkovsky P, 2005 Synaptic transmission at retinal ribbon synapses. Prog Retin Eye Res 24, 682–720. [PubMed: 16027025]

Hess RF, Mullen KT, Sharpe LT, Zrenner E, 1989 The photoreceptors in atypical achromatopsia. J Physiol 417, 123–149. [PubMed: 2621588]

Hess RF, Nordby K, 1986 Spatial and temporal properties of human rod vision in the achromat. J Physiol 371, 387–406. [PubMed: 3486272]

Hodgkin AL, McNaughton PA, Nunn BJ, 1985 The ionic selectivity and calcium dependence of the light-sensitive pathway in toad rods. J Physiol 358, 447–468. [PubMed: 2580087]

Hodgkin AL, McNaughton PA, Nunn BJ, 1987 Measurement of sodium-calcium exchange in salamander rods. J Physiol 391, 347–370. [PubMed: 2451008]

Howes K, Bronson JD, Dang YL, Li N, Zhang K, Ruiz C, Helekar B, Lee M, Subbaraya I, Kolb H, Chen J, Baehr W, 1998 Gene array and expression of mouse retina guanylate cyclase activating proteins 1 and 2. Invest Ophthalmol Vis Sci 39, 867–875. [PubMed: 9579466]

Hsu YT, Molday RS, 1993 Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin. Nature 361, 76–79. [PubMed: 7678445]

Hsu YT, Molday RS, 1994 Interaction of calmodulin with the cyclic GMP-gated channel of rod photoreceptor cells. Modulation of activity, affinity purification, and localization. J Biol Chem 269, 29765–29770. [PubMed: 7525588]

Huttl S, Michalakis S, Seeliger M, Luo DG, Acar N, Geiger H, Hudl K, Mader R, Haverkamp S, Moser M, Pfeifer A, Gerstner A, Yau KW, Biel M, 2005 Impaired channel targeting and retinal degeneration in mice lacking the cyclic nucleotide-gated channel subunit CNGB1. J Neurosci 25, 130–138. [PubMed: 15634774]

Ingram NT, Sampath AP, Fain GL, 2016 Why are rods more sensitive than cones? J Physiol 594, 5415–5426. [PubMed: 27218707]

Iribarne M, Masai I, 2017 Neurotoxicity of cGMP in the vertebrate retina: from the initial research on rd mutant mice to zebrafish genetic approaches. J Neurogenet 31, 88–101. [PubMed: 28812418]

Jalloul AH, Szerencsei RT, Schnetkamp PP, 2016 Cation dependencies and turnover rates of the human K(+)-dependent Na(+)-Ca(2)(+) exchangers NCKX1, NCKX2, NCKX3 and NCKX4. Cell Calcium 59, 1–11. [PubMed: 26631410]

Jiang L, Katz BJ, Yang Z, Zhao Y, Faulkner N, Hu J, Baird J, Baehr W, Creel DJ, Zhang K, 2005 Autosomal dominant cone dystrophy caused by a novel mutation in the GCAP1 gene (GUCA1A). Mol Vis 11, 143–151. [PubMed: 15735604]

Jones GJ, Fein A, MacNichol EF, Jr., Cornwall MC, 1993 Visual pigment bleaching in isolated salamander retinal cones. Microspectrophotometry and light adaptation. J Gen Physiol 102, 483–502. [PubMed: 8245820]

Kawamura S, 1993 Rhodopsin phosphorylation as a mechanism of cyclic GMP phosphodiesterase regulation by S-modulin. Nature 362, 855–857. [PubMed: 8386803]

Kawamura S, Kuwata O, Yamada M, Matsuda S, Hisatomi O, Tokunaga F, 1996 Photoreceptor protein s26, a cone homologue of S-modulin in frog retina. J Biol Chem 271, 21359–21364. [PubMed: 8702916]

Kenkre JS, Moran NA, Lamb TD, Mahroo OA, 2005 Extremely rapid recovery of human cone circulating current at the extinction of bleaching exposures. J Physiol 567, 95–112. [PubMed: 15932890]

Kennedy MJ, Dunn FA, Hurley JB, 2004 Visual pigment phosphorylation but not transducin translocation can contribute to light adaptation in zebrafish cones. Neuron 41, 915–928. [PubMed: 15046724]

Kielar M, Tuy FP, Bizzotto S, Lebrand C, de Juan Romero C, Poirier K, Oegema R, Mancini GM, Bahi-Buisson N, Olaso R, Le Moing AG, Boutourlinsky K, Boucher D, Carpentier W, Berquin P, Deleuze JF, Belvindrah R, Borrell V, Welker E, Chelly J, Croquelois A, Francis F, 2014 Mutations in Eml1 lead to ectopic progenitors and neuronal heterotopia in mouse and human. Nat Neurosci 17, 923–933. [PubMed: 24859200]

Klenchin VA, Calvert PD, Bownds MD, 1995 Inhibition of rhodopsin kinase by recoverin. Further evidence for a negative feedback system in phototransduction. J Biol Chem 270, 16147–16152. [PubMed: 7608179]

Koch KW, 1991 Purification and identification of photoreceptor guanylate cyclase. J Biol Chem 266, 8634–8637. [PubMed: 1673683]

Koch KW, Stryer L, 1988 Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. Nature 334, 64–66. [PubMed: 2455233]

Koenig D, Hofer H, 2011 The absolute threshold of cone vision. J Vis 11.

Korenbrot JI, 2012 Speed, adaptation, and stability of the response to light in cone photoreceptors: the functional role of Ca-dependent modulation of ligand sensitivity in cGMP-gated ion channels. J Gen Physiol 139, 31–56. [PubMed: 22200947]

Korenbrot JI, Mehta M, Tserentsoodol N, Postlethwait JH, Rebrik TI, 2013 EML1 (CNG-modulin) controls light sensitivity in darkness and under continuous illumination in zebrafish retinal cone photoreceptors. J Neurosci 33, 17763–17776. [PubMed: 24198367]

Koutalos Y, Nakatani K, Tamura T, Yau KW, 1995a Characterization of guanylate cyclase activity in single retinal rod outer segments. J Gen Physiol 106, 863–890. [PubMed: 8648296]

Koutalos Y, Nakatani K, Yau KW, 1995b The cGMP-phosphodiesterase and its contribution to sensitivity regulation in retinal rods. J Gen Physiol 106, 891–921. [PubMed: 8648297]

Kraft TW, Schneeweis DM, Schnapf JL, 1993 Visual transduction in human rod photoreceptors. J Physiol 464, 747–765. [PubMed: 8229828]

Krispel CM, Chen D, Melling N, Chen YJ, Martemyanov KA, Quillinan N, Arshavsky VY, Wensel TG, Chen CK, Burns ME, 2006 RGS expression rate-limits recovery of rod photoresponses. Neuron 51, 409–416. [PubMed: 16908407]

Krizaj D, 2012 Calcium stores in vertebrate photoreceptors. Adv. Exp. Med. Biol. 740, 873–889. [PubMed: 22453974]

Krizaj D, Copenhagen DR, 1998 Compartmentalization of calcium extrusion mechanisms in the outer and inner segments of photoreceptors. Neuron 21, 249–256. [PubMed: 9697868]

Lamb TD, Pugh EN, Jr., 1992 A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. J Physiol 449, 719–758. [PubMed: 1326052]

Lamb TD, Pugh EN, Jr., 2006 Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. Invest Ophthalmol Vis Sci 47, 5137–5152. [PubMed: 17122096]

Lem J, Krasnoperova NV, Calvert PD, Kosaras B, Cameron DA, Nicolo M, Makino CL, Sidman RL, 1999 Morphological, physiological, and biochemical changes in rhodopsin knockout mice. Proc Natl Acad Sci U S A 96, 736–741. [PubMed: 9892703]

Leung YT, Fain GL, Matthews HR, 2007 Simultaneous measurement of current and calcium in the ultraviolet-sensitive cones of zebrafish. J Physiol 579, 15–27. [PubMed: 17124271]

Li X-F, Kiedrowski L, Tremblay F, Fernandez FR, Perizzolo M, Winkfein RJ, Turner RW, Bains JS, Rancourt DE, Lytton J, 2006 Importance of K+-dependent Na+/Ca2+-exchanger 2, NCKX2, in motor learning and memory. J Biol Chem 281, 6273–6282. [PubMed: 16407245]

Lisman J, Fain G, 1995 Support for the equivalent light hypothesis for RP. Nat Med 1, 1254–1255. [PubMed: 7489404]

Liu C, Varnum MD, 2005 Functional consequences of progressive cone dystrophy-associated mutations in the human cone photoreceptor cyclic nucleotide-gated channel CNGA3 subunit. Am J Physiol Cell Physiol 289, C187–198. [PubMed: 15743887]

Lytton J, 2007 Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport. The Biochemical journal 406, 365–382. [PubMed: 17716241]

Ma H, Butler MR, Thapa A, Belcher J, Yang F, Baehr W, Biel M, Michalakis S, Ding XQ, 2015 cGMP/Protein Kinase G Signaling Suppresses Inositol 1,4,5-Trisphosphate Receptor Phosphorylation and Promotes Endoplasmic Reticulum Stress in Photoreceptors of Cyclic Nucleotide-gated Channel-deficient Mice. J Biol Chem 290, 20880–20892. [PubMed: 26124274]

Majumder A, Pahlberg J, Muradov H, Boyd KK, Sampath AP, Artemyev NO, 2015 Exchange of Cone for Rod Phosphodiesterase 6 Catalytic Subunits in Rod Photoreceptors Mimics in Part Features of Light Adaptation. J Neurosci 35, 9225–9235. [PubMed: 26085644]

Makino CL, Dodd RL, Chen J, Burns ME, Roca A, Simon MI, Baylor DA, 2004 Recoverin regulates light-dependent phosphodiesterase activity in retinal rods. J Gen Physiol 123, 729–741. [PubMed: 15173221]

Makino CL, Wen XH, Olshevskaya EV, Peshenko IV, Savchenko AB, Dizhoor AM, 2012 Enzymatic relay mechanism stimulates cyclic GMP synthesis in rod photoresponse: biochemical and physiological study in guanylyl cyclase activating protein 1 knockout mice. PLoS One 7, e47637. [PubMed: 23082185]

Mao W, Miyagishima KJ, Yao Y, Soreghan B, Sampath AP, Chen J, 2013 Functional comparison of rod and cone Galpha(t) on the regulation of light sensitivity. J Biol Chem 288, 5257–5267. [PubMed: 23288843]

Martemyanov KA, Sampath AP, 2017 The Transduction Cascade in Retinal ON-Bipolar Cells: Signal Processing and Disease. Annu Rev Vis Sci 3, 25–51. [PubMed: 28715957]

Matthews HR, Fain GL, Murphy RL, Lamb TD, 1990 Light adaptation in cone photoreceptors of the salamander: a role for cytoplasmic calcium. J Physiol 420, 447–469. [PubMed: 2109062]

Matthews HR, Murphy RL, Fain GL, Lamb TD, 1988 Photoreceptor light adaptation is mediated by cytoplasmic calcium concentration. Nature 334, 67–69. [PubMed: 2455234]

Matthews HR, Sampath AP, 2010 Photopigment quenching is Ca2+ dependent and controls response duration in salamander L-cone photoreceptors. J Gen Physiol 135, 355–366. [PubMed: 20231373]

Matveev AV, Quiambao AB, Browning Fitzgerald J, Ding X-Q, 2008 Native cone photoreceptor cyclic nucleotide-gated channel is a heterotetrameric complex comprising both CNGA3 and CNGB3: a study using the cone-dominant retina of Nrl−/− mice. J. Neurochem. 106, 2042–2055. [PubMed: 18665891]

McLaughlin ME, Sandberg MA, Berson EL, Dryja TP, 1993 Recessive mutations in the gene encoding the beta-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. Nat Genet 4, 130–134. [PubMed: 8394174]

Mendez A, Burns ME, Roca A, Lem J, Wu LW, Simon MI, Baylor DA, Chen J, 2000 Rapid and reproducible deactivation of rhodopsin requires multiple phosphorylation sites. Neuron 28, 153–164. [PubMed: 11086991]

Mendez A, Burns ME, Sokal I, Dizhoor AM, Baehr W, Palczewski K, Baylor DA, Chen J, 2001 Role of guanylate cyclase-activating proteins (GCAPs) in setting the flash sensitivity of rod photoreceptors. Proc Natl Acad Sci U S A 98, 9948–9953. [PubMed: 11493703]

Milam AH, Dacey DM, Dizhoor AM, 1993 Recoverin immunoreactivity in mammalian cone bipolar cells. Vis Neurosci 10, 1–12. [PubMed: 8424920]

Morshedian A, Fain GL, 2015 Single-photon sensitivity of lamprey rods with cone-like outer segments. Curr Biol 25, 484–487. [PubMed: 25660538]

Morshedian A, Woodruff ML, Fain GL, 2018 Role of recoverin in rod photoreceptor light adaptation. J Physiol.

Mustafi D, Engel AH, Palczewski K, 2009 Structure of cone photoreceptors. Prog Retin Eye Res 28, 289–302. [PubMed: 19501669]

Naarendorp F, Esdaille TM, Banden SM, Andrews-Labenski J, Gross OP, Pugh EN, Jr., 2010 Dark light, rod saturation, and the absolute and incremental sensitivity of mouse cone vision. J Neurosci 30, 12495–12507. [PubMed: 20844144]

Nakatani K, Koutalos Y, Yau KW, 1995 Ca2+ modulation of the cGMP-gated channel of bullfrog retinal rod photoreceptors. J Physiol 484 (Pt 1), 69–76. [PubMed: 7541463]

Nakatani K, Tamura T, Yau KW, 1991 Light adaptation in retinal rods of the rabbit and two other nonprimate mammals. J Gen Physiol 97, 413–435. [PubMed: 2037836]

Nakatani K, Yau KW, 1988a Calcium and light adaptation in retinal rods and cones. Nature 334, 69–71. [PubMed: 3386743]

Nakatani K, Yau KW, 1988b Guanosine 3',5'-cyclic monophosphate-activated conductance studied in a truncated rod outer segment of the toad. J Physiol 395, 731–753. [PubMed: 2457686]

Nakazawa M, 2011 Effects of calcium ion, calpains, and calcium channel blockers on retinitis pigmentosa. J Ophthalmol 2011, 292040. [PubMed: 21253505]

Newbold RJ, Deery EC, Payne AM, Wilkie SE, Hunt DM, Warren MJ, 2002 Guanylate cyclase activating proteins, guanylate cyclase and disease. Adv Exp Med Biol 514, 411–438. [PubMed: 12596936]

Nikonov S, Lamb TD, Pugh EN, Jr., 2000 The role of steady phosphodiesterase activity in the kinetics and sensitivity of the light-adapted salamander rod photoresponse. J Gen Physiol 116, 795–824. [PubMed: 11099349]

Nikonov SS, Brown BM, Davis JA, Zuniga FI, Bragin A, Pugh EN, Jr., Craft CM, 2008 Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 59, 462–474. [PubMed: 18701071]

Nikonov SS, Kholodenko R, Lem J, Pugh EN, Jr., 2006 Physiological features of the S- and M-cone photoreceptors of wild-type mice from single-cell recordings. J Gen Physiol 127, 359–374. [PubMed: 16567464]

Nishiguchi KM, Sokal I, Yang L, Roychowdhury N, Palczewski K, Berson EL, Dryja TP, Baehr W, 2004 A novel mutation (I143NT) in guanylate cyclase-activating protein 1 (GCAP1) associated with autosomal dominant cone degeneration. Invest Ophthalmol Vis Sci 45, 3863–3870. [PubMed: 15505030]

Nymark S, Frederiksen R, Woodruff ML, Cornwall MC, Fain GL, 2012 Bleaching of mouse rods: microspectrophotometry and suction-electrode recording. J Physiol 590, 2353–2364. [PubMed: 22451436]

Ohyama T, Hackos DH, Frings S, Hagen V, Kaupp UB, Korenbrot JI, 2000 Fraction of the dark current carried by Ca(2+) through cGMP-gated ion channels of intact rod and cone photoreceptors. J Gen Physiol 116, 735–754. [PubMed: 11099344]

Olshevskaya EV, Ermilov AN, Dizhoor AM, 2002 Factors that affect regulation of cGMP synthesis in vertebrate photoreceptors and their genetic link to human retinal degeneration. Mol Cell Biochem 230, 139–147. [PubMed: 11952089]

Palczewski K, Polans AS, Baehr W, Ames JB, 2000 Ca(2+)-binding proteins in the retina: structure, function, and the etiology of human visual diseases. Bioessays 22, 337–350. [PubMed: 10723031]

Palczewski K, Subbaraya I, Gorczyca WA, Helekar BS, Ruiz CC, Ohguro H, Huang J, Zhao X, Crabb JW, Johnson RS, et al., 1994 Molecular cloning and characterization of retinal photoreceptor guanylyl cyclase-activating protein. Neuron 13, 395–404. [PubMed: 7520254]

Paquet-Durand F, Beck S, Michalakis S, Goldmann T, Huber G, Muhlfriedel R, Trifunovic D, Fischer MD, Fahl E, Duetsch G, Becirovic E, Wolfrum U, van Veen T, Biel M, Tanimoto N, Seeliger

MW, 2011 A key role for cyclic nucleotide gated (CNG) channels in cGMP-related retinitis pigmentosa. Hum Mol Genet 20, 941–947. [PubMed: 21149284]

Paquet-Durand F, Hauck SM, van Veen T, Ueffing M, Ekstrom P, 2009 PKG activity causes photoreceptor cell death in two retinitis pigmentosa models. J. Neurochem. 108, 796–810. [PubMed: 19187097]

Payne AM, Downes SM, Bessant DA, Taylor R, Holder GE, Warren MJ, Bird AC, Bhattacharya SS, 1998 A mutation in guanylate cyclase activator 1A (GUCA1A) in an autosomal dominant cone dystrophy pedigree mapping to a new locus on chromosome 6p21.1. Hum Mol Genet 7, 273–277. [PubMed: 9425234]

Peng C, Rich ED, Varnum MD, 2003 Achromatopsia-associated mutation in the human cone photoreceptor cyclic nucleotide-gated channel CNGB3 subunit alters the ligand sensitivity and pore properties of heteromeric channels. J Biol Chem 278, 34533–34540. [PubMed: 12815043]

Peng C, Rich ED, Varnum MD, 2004 Subunit configuration of heteromeric cone cyclic nucleotide-gated channels. Neuron 42, 401–410. [PubMed: 15134637]

Perlman I, Normann RA, 1998 Light adaptation and sensitivity controlling mechanisms in vertebrate photoreceptors. Prog Retin Eye Res 17, 523–563. [PubMed: 9777649]

Pittler SJ, Baehr W, 1991 Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. Proc Natl Acad Sci U S A 88, 8322–8326. [PubMed: 1656438]

Prinsen CF, Cooper CB, Szerencsei RT, Murthy SK, Demetrick DJ, Schnetkamp PP, 2002 The retinal rod and cone Na+/Ca2+-K+ exchangers. Adv Exp Med Biol 514, 237–251. [PubMed: 12596925]

Prinsen CF, Szerencsei RT, Schnetkamp PP, 2000 Molecular cloning and functional expression of the potassium-dependent sodium-calcium exchanger from human and chicken retinal cone photoreceptors. J Neurosci 20, 1424–1434. [PubMed: 10662833]

Pugh EN, Jr., Lamb TD, 1993 Amplification and kinetics of the activation steps in phototransduction. Biochimica et biophysica acta 1141, 111–149. [PubMed: 8382952]

Rebrik TI, Botchkina I, Arshavsky VY, Craft CM, Korenbrot JI, 2012 CNG-modulin: a novel Ca-dependent modulator of ligand sensitivity in cone photoreceptor cGMP-gated ion channels. J Neurosci 32, 3142–3153. [PubMed: 22378887]

Rebrik TI, Korenbrot JI, 1998 In intact cone photoreceptors, a Ca2+-dependent, diffusible factor modulates the cGMP-gated ion channels differently than in rods. J Gen Physiol 112, 537–548. [PubMed: 9806963]

Rebrik TI, Korenbrot JI, 2004 In intact mammalian photoreceptors, Ca2+-dependent modulation of cGMP-gated ion channels is detectable in cones but not in rods. J Gen Physiol 123, 63–75. [PubMed: 14699078]

Reid DM, Friedel U, Molday RS, Cook NJ, 1990 Identification of the sodium-calcium exchanger as the major ricin-binding glycoprotein of bovine rod outer segments and its localization to the plasma membrane. Biochemistry 29, 1601–1607. [PubMed: 2334719]

Reilander H, Achilles A, Friedel U, Maul G, Lottspeich F, Cook NJ, 1992 Primary structure and functional expression of the Na/Ca,K-exchanger from bovine rod photoreceptors. EMBO J 11, 1689–1695. [PubMed: 1582405]

Riazuddin SA, Shahzadi A, Zeitz C, Ahmed ZM, Ayyagari R, Chavali VR, Ponferrada VG, Audo I, Michiels C, Lancelot ME, Nasir IA, Zafar AU, Khan SN, Husnain T, Jiao X, MacDonald IM, Riazuddin S, Sieving PA, Katsanis N, Hejtmancik JF, 2010 A mutation in SLC24A1 implicated in autosomal-recessive congenital stationary night blindness. Am J Hum Genet 87, 523–531. [PubMed: 20850105]

Ruiz M, Brown RL, He Y, Haley TL, Karpen JW, 1999 The single-channel dose-response relation is consistently steep for rod cyclic nucleotide-gated channels: implications for the interpretation of macroscopic dose-response relations. Biochemistry 38, 10642–10648. [PubMed: 10451358]

Sakurai K, Chen J, Kefalov VJ, 2011 Role of guanylyl cyclase modulation in mouse cone phototransduction. J Neurosci 31, 7991–8000. [PubMed: 21632921]

Sakurai K, Chen J, Khani SC, Kefalov VJ, 2015 Regulation of mammalian cone phototransduction by recoverin and rhodopsin kinase. J Biol Chem 290, 9239–9250. [PubMed: 25673692]

Sakurai K, Vinberg F, Wang T, Chen J, Kefalov VJ, 2016 The Na(+)/Ca(2+), K(+) exchanger 2 modulates mammalian cone phototransduction. Sci Rep 6, 32521. [PubMed: 27580676]

Sampath AP, Matthews HR, Cornwall MC, Bandarchi J, Fain GL, 1999 Light-dependent changes in outer segment free-Ca2+ concentration in salamander cone photoreceptors. J Gen Physiol 113, 267–277. [PubMed: 9925824]

Sampath AP, Matthews HR, Cornwall MC, Fain GL, 1998 Bleached pigment produces a maintained decrease in outer segment Ca2+ in salamander rods. J Gen Physiol 111, 53–64. [PubMed: 9417134]

Sato M, Nakazawa M, Usui T, Tanimoto N, Abe H, Ohguro H, 2005 Mutations in the gene coding for guanylate cyclase-activating protein 2 (GUCA1B gene) in patients with autosomal dominant retinal dystrophies. Graefes Arch Clin Exp Ophthalmol 243, 235–242. [PubMed: 15452722]

Sato S, Peshenko IV, Olshevskaya EV, Kefalov VJ, Dizhoor AM, 2018 GUCY2D Cone-Rod Dystrophy-6 is a 'Phototransduction Disease' Triggered by Abnormal Calcium Feedback on Retinal Membrane Guanylyl Cyclase 1. J. Neurosci.

Schnapf JL, Nunn BJ, Meister M, Baylor DA, 1990 Visual transduction in cones of the monkey Macaca fascicularis. J Physiol 427, 681–713. [PubMed: 2100987]

Schneeweis DM, Schnapf JL, 1999 The photovoltage of macaque cone photoreceptors: adaptation, noise, and kinetics. J Neurosci 19, 1203–1216. [PubMed: 9952398]

Schnetkamp PP, 1986 Sodium-calcium exchange in the outer segments of bovine rod photoreceptors. J Physiol 373, 25–45. [PubMed: 2427696]

Schnetkamp PP, 2013 The SLC24 gene family of Na(+)/Ca(2)(+)-K(+) exchangers: from sight and smell to memory consolidation and skin pigmentation. Mol Aspects Med 34, 455–464. [PubMed: 23506883]

Schnetkamp PP, Jalloul AH, Liu G, Szerencsei RT, 2014 The SLC24 family of K(+)-dependent Na(+)-Ca(2)(+) exchangers: structure-function relationships. Curr Top Membr 73, 263–287. [PubMed: 24745986]

Schon C, Biel M, Michalakis S, 2013 Gene replacement therapy for retinal CNG channelopathies. Mol Genet Genomics 288, 459–467. [PubMed: 23861024]

Sharpe LT, Fach CC, Stockman A, 1992 The field adaptation of the human rod visual system. J Physiol 445, 319–343. [PubMed: 1501137]

Sharpe LT, Nordby K, 1990 The photoreceptors in the achromat. Cambridge UP, Cambridge, UK.

Shi G, Yau K-W, Chen J, Kefalov VJ, 2007 Signaling properties of a short-wave cone visual pigment and its role in phototransduction. J. Neurosci. 27, 10084–10093. [PubMed: 17881515]

Sokal I, Dupps WJ, Grassi MA, Brown J, Jr., Affatigato LM, Roychowdhury N, Yang L, Filipek S, Palczewski K, Stone EM, Baehr W, 2005 A novel GCAP1 missense mutation (L151F) in a large family with autosomal dominant cone-rod dystrophy (adCORD). Invest Ophthalmol Vis Sci 46, 1124–1132. [PubMed: 15790869]

Sokal I, Li N, Surgucheva I, Warren MJ, Payne AM, Bhattacharya SS, Baehr W, Palczewski K, 1998 GCAP1 (Y99C) mutant is constitutively active in autosomal dominant cone dystrophy. Mol Cell 2, 129–133. [PubMed: 9702199]

Stiles WS, 1939 The directional sensitivity of the retina and the spectral sensitivities of the rods and cones. Proc R Soc B 127, 428–450.

Stone J, Maslim J, Valter-Kocsi K, Mervin K, Bowers F, Chu Y, Barnett N, Provis J, Lewis G, Fisher SK, Bisti S, Gargini C, Cervetto L, Merin S, Peer J, 1999 Mechanisms of photoreceptor death and survival in mammalian retina. Prog Retin Eye Res 18, 689–735. [PubMed: 10530749]

Tachibanaki S, Arinobu D, Shimauchi-Matsukawa Y, Tsushima S, Kawamura S, 2005 Highly effective phosphorylation by G protein-coupled receptor kinase 7 of light-activated visual pigment in cones. Proc Natl Acad Sci U S A 102, 9329–9334. [PubMed: 15958532]

Tachibanaki S, Yonetsu S, Fukaya S, Koshitani Y, Kawamura S, 2012 Low activation and fast inactivation of transducin in carp cones. J Biol Chem 287, 41186–41194. [PubMed: 23045532]

Takemoto N, Tachibanaki S, Kawamura S, 2009 High cGMP synthetic activity in carp cones. Proc Natl Acad Sci U S A 106, 11788–11793. [PubMed: 19556550]

Tamura T, Nakatani K, Yau KW, 1989 Light adaptation in cat retinal rods. Science 245, 755–758. [PubMed: 2772634]

Tamura T, Nakatani K, Yau KW, 1991 Calcium feedback and sensitivity regulation in primate rods. J Gen Physiol 98, 95–130. [PubMed: 1719127]

Thomas MM, Lamb TD, 1999 Light adaptation and dark adaptation of human rod photoreceptors measured from the a-wave of the electroretinogram. J Physiol 518 (Pt 2), 479–496. [PubMed: 10381594]

Tikidji-Hamburyan A, Reinhard K, Storchi R, Dietter J, Seitter H, Davis KE, Idrees S, Mutter M, Walmsley L, Bedford RA, Ueffing M, Ala-Laurila P, Brown TM, Lucas RJ, Munch TA, 2017 Rods progressively escape saturation to drive visual responses in daylight conditions. Nat Commun 8, 1813. [PubMed: 29180667]

Tinsley JN, Molodtsov MI, Prevedel R, Wartmann D, Espigule-Pons J, Lauwers M, Vaziri A, 2016 Direct detection of a single photon by humans. Nat Commun 7, 12172. [PubMed: 27434854]

Tomizuka J, Tachibanaki S, Kawamura S, 2015 Phosphorylation-independent suppression of light-activated visual pigment by arrestin in carp rods and cones. J Biol Chem 290, 9399–9411. [PubMed: 25713141]

Tosi J, Davis RJ, Wang NK, Naumann M, Lin CS, Tsang SH, 2011 shRNA knockdown of guanylate cyclase 2e or cyclic nucleotide gated channel alpha 1 increases photoreceptor survival in a cGMP phosphodiesterase mouse model of retinitis pigmentosa. J Cell Mol Med 15, 1778–1787. [PubMed: 20950332]

Umino Y, Solessio E, Barlow RB, 2008 Speed, spatial, and temporal tuning of rod and cone vision in mouse. J Neurosci 28, 189–198. [PubMed: 18171936]

Valeton JM, van Norren D, 1983 Light adaptation of primate cones: an analysis based on extracellular data. Vision Res 23, 1539–1547. [PubMed: 6666056]

Vinberg F, Peshenko IV, Chen J, Dizhoor AM, Kefalov VJ, 2018 Guanylate Cyclase Activating Protein 2 Contributes to Phototransduction and Light Adaptation in Mouse Cone Photoreceptors. J Biol Chem [Epub ahead of print].

Vinberg F, Wang T, De Maria A, Zhao H, Bassnett S, Chen J, Kefalov VJ, 2017 The Na+/Ca2+, K+ exchanger NCKX4 is required for efficient cone-mediated vision. Elife 6, DOI 10.7554/eLife.24550.

Vinberg F, Wang T, Molday RS, Chen J, Kefalov VJ, 2015 A new mouse model for stationary night blindness with mutant Slc24a1 explains the pathophysiology of the associated human disease. Hum Mol Genet 24, 5915–5929. [PubMed: 26246500]

Wang T, Chen J, 2014 Induction of the unfolded protein response by constitutive G-protein signaling in rod photoreceptor cells. J Biol Chem 289, 29310–29321. [PubMed: 25183010]

Wang T, Tsang SH, Chen J, 2017 Two pathways of rod photoreceptor cell death induced by elevated cGMP. Hum Mol Genet 26, 2299–2306. [PubMed: 28379353]

Weitz D, Ficek N, Kremmer E, Bauer PJ, Kaupp UB, 2002 Subunit stoichiometry of the CNG channel of rod photoreceptors. Neuron 36, 881–889. [PubMed: 12467591]

Weitz D, Zoche M, Muller F, Beyermann M, Korschen HG, Kaupp UB, Koch KW, 1998 Calmodulin controls the rod photoreceptor CNG channel through an unconventional binding site in the N-terminus of the beta-subunit. EMBO J 17, 2273–2284. [PubMed: 9545240]

Wen XH, Dizhoor AM, Makino CL, 2014 Membrane guanylyl cyclase complexes shape the photoresponses of retinal rods and cones. Front Mol Neurosci 7, 45. [PubMed: 24917784]

Wilkie SE, Li Y, Deery EC, Newbold RJ, Garibaldi D, Bateman JB, Zhang H, Lin W, Zack DJ, Bhattacharya SS, Warren MJ, Hunt DM, Zhang K, 2001 Identification and functional consequences of a new mutation (E155G) in the gene for GCAP1 that causes autosomal dominant cone dystrophy. Am J Hum Genet 69, 471–480. [PubMed: 11484154]

Woodruff ML, Janisch KM, Peshenko IV, Dizhoor AM, Tsang SH, Fain GL, 2008 Modulation of phosphodiesterase6 turnoff during background illumination in mouse rod photoreceptors. J Neurosci 28, 2064–2074. [PubMed: 18305241]

Woodruff ML, Olshevskaya EV, Savchenko AB, Peshenko IV, Barrett R, Bush RA, Sieving PA, Fain GL, Dizhoor AM, 2007 Constitutive excitation by Gly90Asp rhodopsin rescues rods from degeneration caused by elevated production of cGMP in the dark. J Neurosci 27, 8805–8815. [PubMed: 17699662]

Woodruff ML, Sampath AP, Matthews HR, Krasnoperova NV, Lem J, Fain GL, 2002 Measurement of cytoplasmic calcium concentration in the rods of wild-type and transducin knock-out mice. J Physiol 542, 843–854. [PubMed: 12154183]

Woodruff ML, Wang Z, Chung HY, Redmond TM, Fain GL, Lem J, 2003 Spontaneous activity of opsin apoprotein is a cause of Leber congenital amaurosis. Nat. Genet. 35, 158–164. [PubMed: 14517541]

Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J, 1997 Prolonged photoresponses in transgenic mouse rods lacking arrestin. Nature 389, 505–509. [PubMed: 9333241]

Xu J, Morris L, Thapa A, Ma H, Michalakis S, Biel M, Baehr W, Peshenko IV, Dizhoor AM, Ding XQ, 2013 cGMP accumulation causes photoreceptor degeneration in CNG channel deficiency: evidence of cGMP cytotoxicity independently of enhanced CNG channel function. J Neurosci 33, 14939–14948. [PubMed: 24027293]

Yau KW, 1994 Phototransduction mechanism in retinal rods and cones. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 35, 9–32. [PubMed: 7507907]

Yau KW, Baylor DA, 1989 Cyclic GMP-activated conductance of retinal photoreceptor cells. Annu Rev Neurosci 12, 289–327. [PubMed: 2467600]

Yau KW, Hardie RC, 2009 Phototransduction motifs and variations. Cell 139, 246–264. [PubMed: 19837030]

Yau KW, Nakatani K, 1984a Cation selectivity of light-sensitive conductance in retinal rods. Nature 309, 352–354. [PubMed: 6427629]

Yau KW, Nakatani K, 1984b Electrogenic Na-Ca exchange in retinal rod outer segment. Nature 311, 661–663. [PubMed: 6434995]

Zhang Y, Molday LL, Molday RS, Sarfare SS, Woodruff ML, Fain GL, Kraft TW, Pittler SJ, 2009 Knockout of GARPs and the beta-subunit of the rod cGMP-gated channel disrupts disk morphogenesis and rod outer segment structural integrity. J Cell Sci 122, 1192–1200. [PubMed: 19339551]

Zheng J, Trudeau MC, Zagotta WN, 2002 Rod cyclic nucleotide-gated channels have a stoichiometry of three CNGA1 subunits and one CNGB1 subunit. Neuron 36, 891–896. [PubMed: 12467592]

Zhong H, Molday LL, Molday RS, Yau KW, 2002 The heteromeric cyclic nucleotide-gated channel adopts a 3A:1B stoichiometry. Nature 420, 193–198. [PubMed: 12432397]

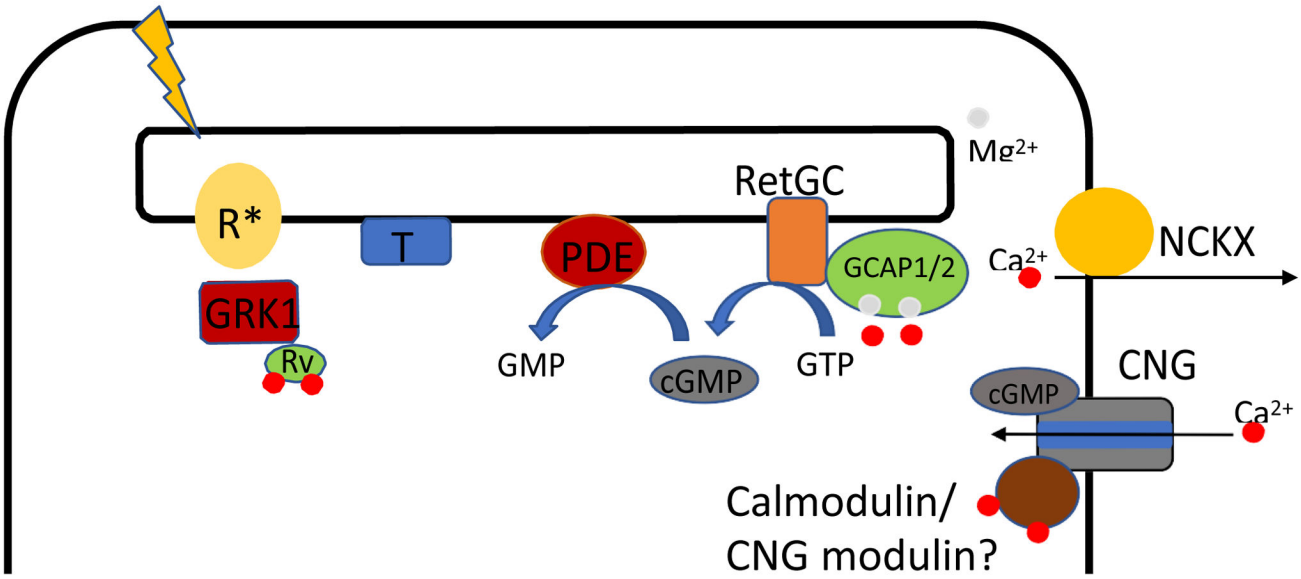

Figure 1. Mechanisms of modulation of the phototranstuction in rods and cones by calcium.

The activation of the light-sensitive G-protein coupled receptor (R*) results in the activation of the G protein transducin (T) that, in turn, activates the effector enzyme phosphodiesterase (PDE), leasing to increased hydrolysis of cGMP and the closure of CNG channels. This results in reduction in the influx of Ca^{2+} which combined with its continuous extrusion via cell-specific NCKX drives down the level Ca^{2+} in the outer segments of photoreceptors. The reduction in Ca^{2+} modulates several steps in the phototransduction cascade, including the synthesis of cGMP by guanylyl cycles (RetGC), regulated by guanylyl cyclase activating proteins 1/2 (GCAP1/2); the inactivation of the visual pigment by rhodopsin kinase (GRK1), regulated by recoverin (Rv); and the conductance of the CNG channel, regulated by calmodulin in rods and possibly CNG modulin in cones.

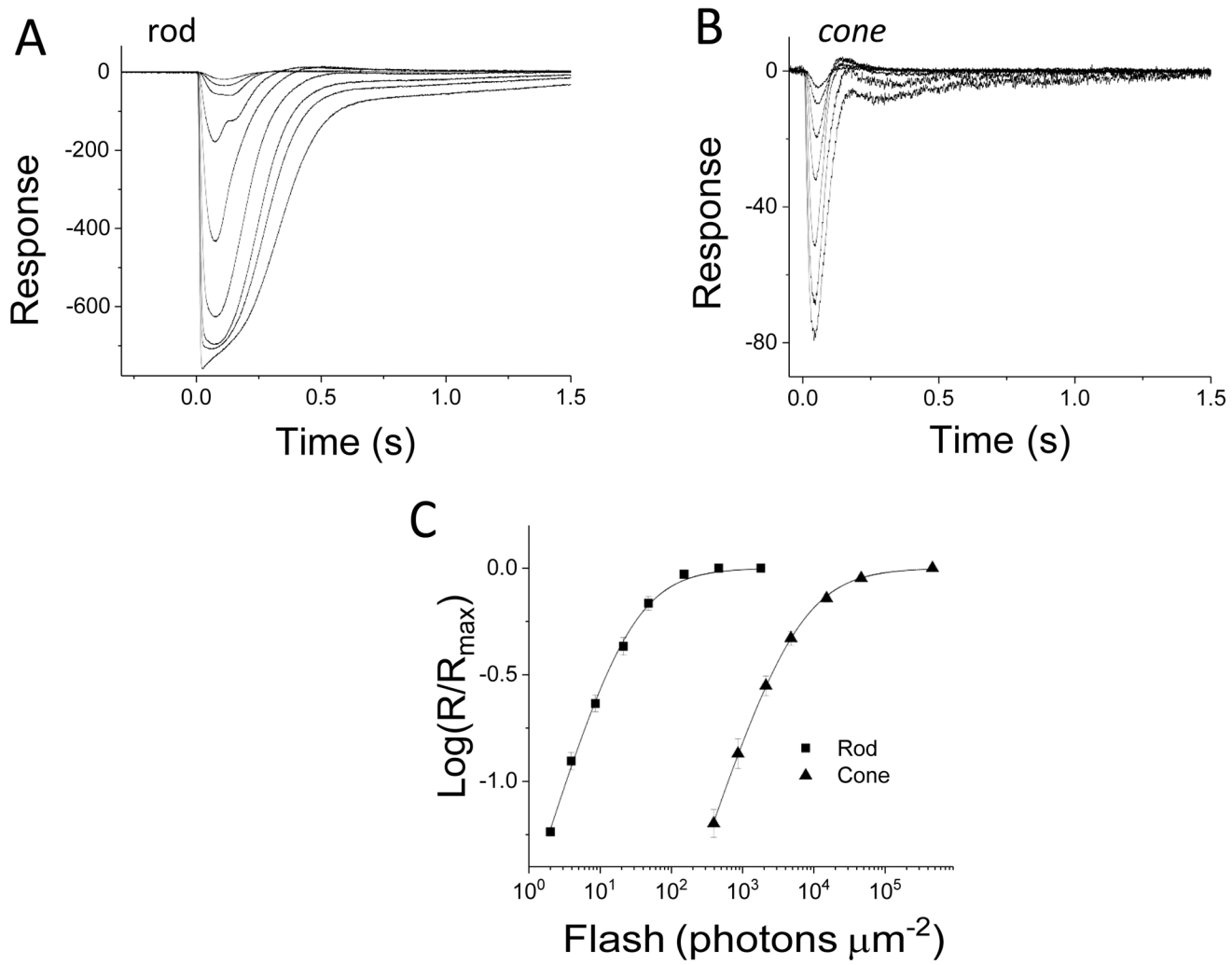

Figure 2. Phototransduction and light adaptation in mouse rods and cones.

Photoreceptor responses to 1 ms flashes presented at time zero of mouserods (A) and cones (B) recorded using the *ex vivo* ERG technique. Rod responses were recorded from retinas of wild-type mice while cone responses were recorded from retinas of *Gnat1*$^{-/-}$ mice that lack expression of rod transducin (Lem et al., 1999). (C) Normalized response amplitudes plotted as a function of flash intensity in photons μm^{-2} for rods (squares) and cones (triangles). The smooth traces plot $Q^n/(Q^n + Q_{1/2}{}^n)$, where Q is flash intensity, $Q_{1/2}$ half-saturating flash intensity, and n steepness constant, with $Q_{1/2}$ = 25 photons μm^{-2}, n = 1.1 and $Q_{1/2}$ = 5,400 photons μm^{-2}, n = 1.0 for rods and cones, respectively. Data is from Vinberg et al., 2015 and Vinberg et al., 2017.

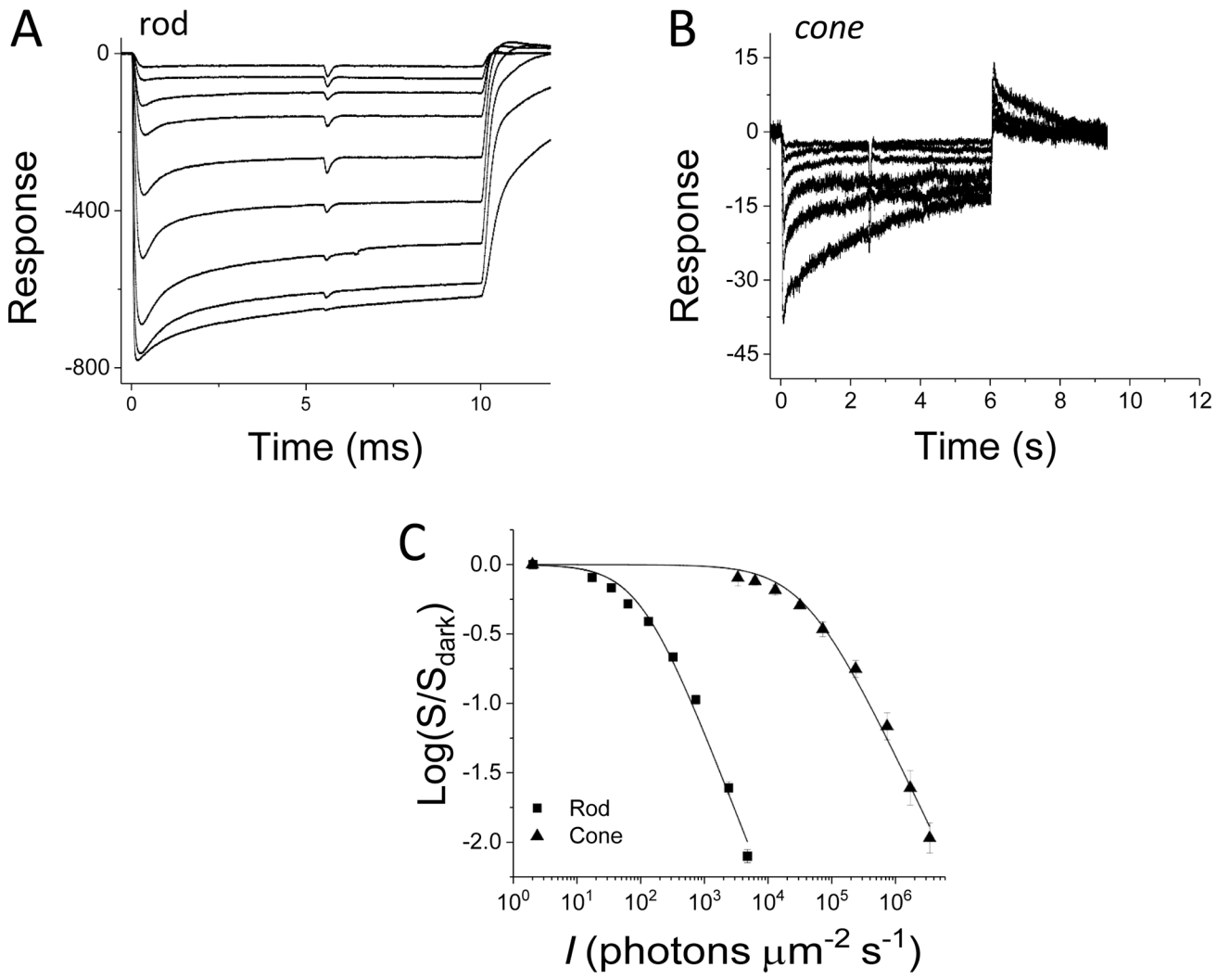

Figure 3. Light adaptation in mouse rods and cones.

Responses to steps of light recorded from rods in wild-type (A) and cones in *Gnat1*$^{-/-}$ (B) mouse retinas using *ex vivo* ERG technique. Step intensities ranged from 9 to 5,100 photons μm^{-2} s^{-1} for rods and from 3,300 to 236,300 photons μm^{-2} s^{-1} for cones. Sensitivity of photoreceptors was probed by delivering 1 ms flashes of light during background light illumination. Starting from the dimmest background, the flash intensities were 2, 2, 2, 5.7, 16, 16, 39, 124 and 370 photons μm^{-2} for rods, and 859, 859, 859, 859, 2,100 and 2,100 photons μm^{-2} for cones. (C) Sensitivity (S) of rods (squares) and cones (triangles) normalized to their sensitivity in darkness (S_{dark}) plotted as a function of background light intensity. The smooth traces plot $I^n/(I^n + I_0^n)$, where I is background light intensity, I_0 background intensity at which $S/S_{dark} = 0.5$, and n is steepness factor, with I_0 = 99 photons μm^{-2} s^{-1}, n = 1.2 and I_0 =39,600 photons μm^{-2} s^{-1}, n = 1.0 for rods and cones, respectively. Data is from Vinberg et al., 2015 and Vinberg et al., 2017.

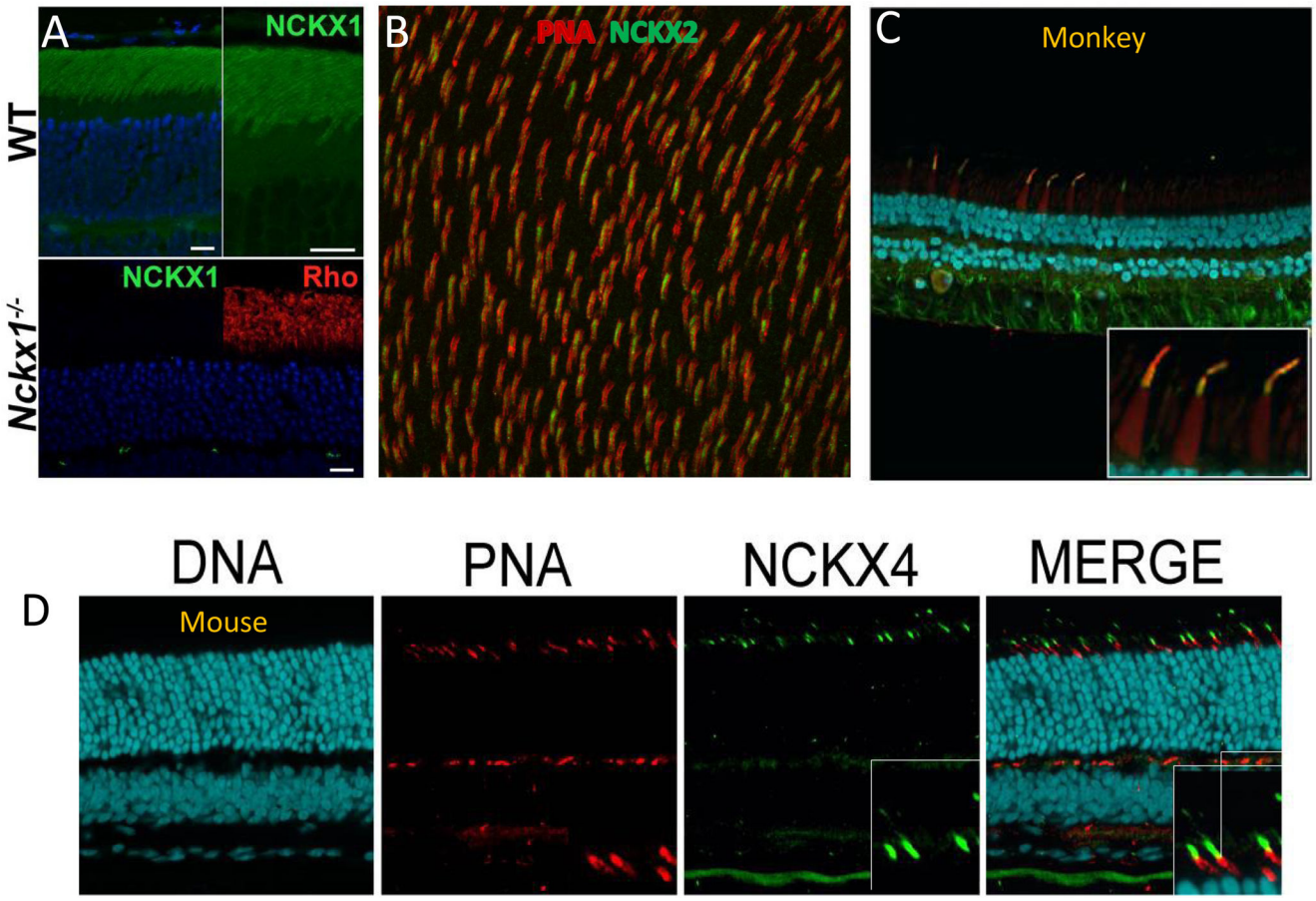

Figure 4. Expression of NCKX1, NCKX2, and NCKX4 in the retina.

(A) Immunostaining of NCKX1 (green) in the retinal sections from WT and *Nckx1*$^{-/-}$ mouse retinas. DAPI (blue) and rhodopsin (red) show the location of nuclei and rod outer segments, respectively. Scale bar = 25 μm. (B) Whole mount staining of cones (PNA, red) and NCKX2 (green) showing cone-specific NCKX2 expression in the mouse retina. (C) Co-staining of NCKX4 (green) and cones (PNA, red) in a macaque retinal section. (D) Immunostaining of NCKX4 (green) in a mouse retinal section. Cones are labeled using PNA (red) and nuclei (DNA) are labeled using methyl green (cyan). Data is from Vinberg et al., 2015 and Vinberg et al., 2017.

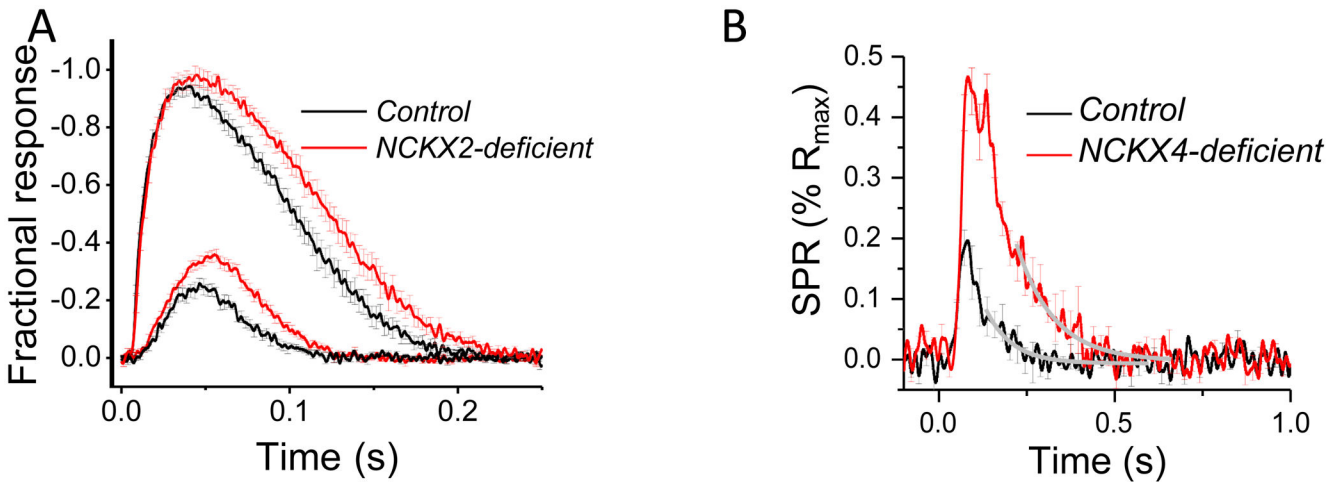

Figure 5. Role of NCKX2 and NCKX4 in the cone phototransduction.

(A) Cone responses normalized by the maximal saturated response amplitude to dim and bright light flashes recorded from WT (black) and *Nckx2*$^{-/-}$ (red) mouse retinas using *ex vivo* ERG. (B) Estimated fractional single photon responses (SPR) from control and NCKX4-deficienc cones. Cone responses were normalized by the maximal saturated response amplitude and flash intensity (in estimated cone pigment photoisomerizations calculated based on a cone collection area of 0.2 μm^2 from (Nikonov et al., 2006)). Responses from WT (black) and NCKX4-deficient (red) mouse cones were recorded using the single-cell suction electrode method. Grey smooth traces plot single exponential function with time constants of 75 and 106 ms in WT and NCKX4-deficient cones, respectively. Data is from Sakurai et al., 2016 and Vinberg et al., 2017.

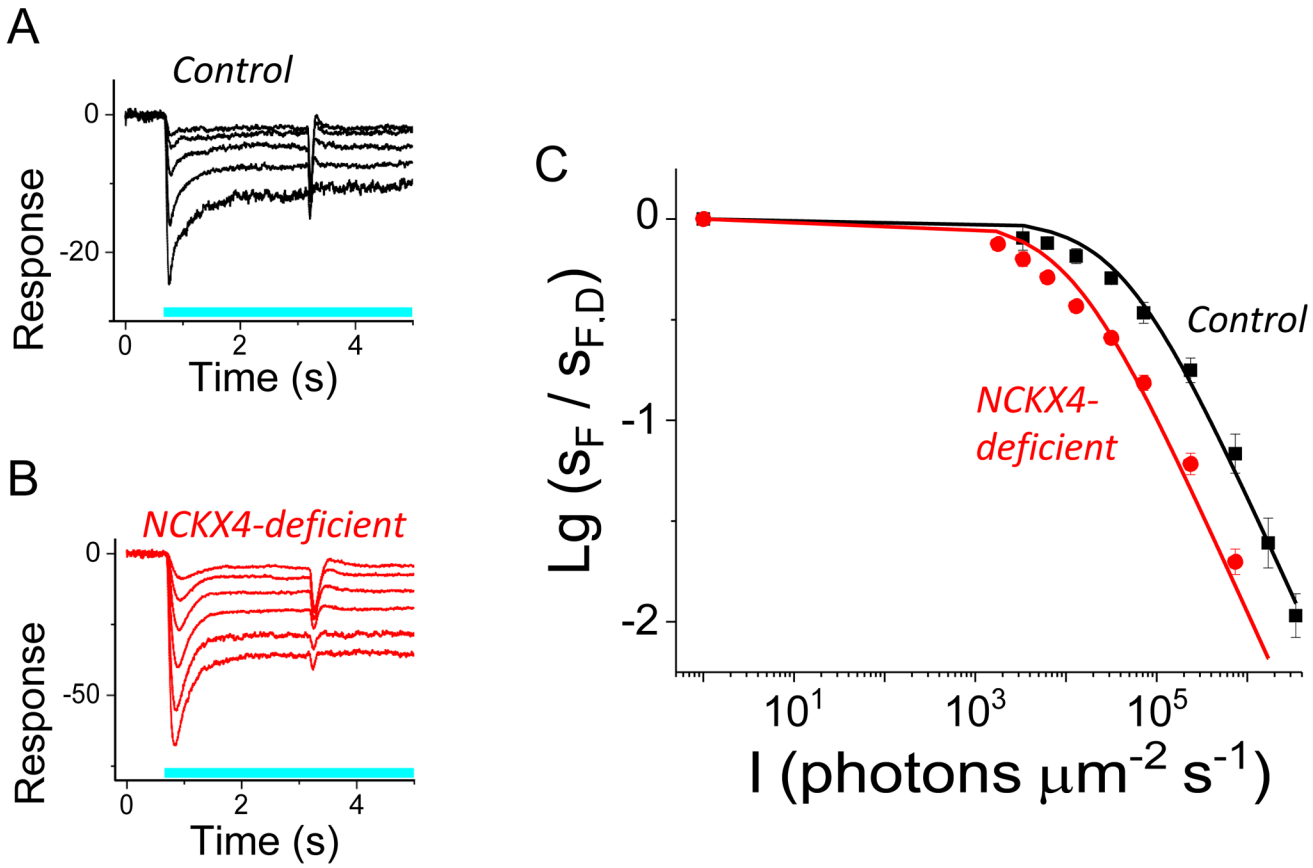

Figure 6. Role of NCKX4 in the light adaptation of cones.

Sensitivity of control (A) and NCKX4-deficient (B) cones was probed by delivering a flash of light at 2.5 s after the light step onset. (C) Sensitivity of cones ($S_F = r(t_p)/Q_F$, where $r(t_p)$ is peak response to flashes in the linear range and Q_F is flash intensity in photons μm^{-2}) normalized to the sensitivity in darkness is plotted as function of background light intensity for control (black) and NCKX4-deficient (red) mice. Smooth traces plot Weber-Fechner function with I_0 = 43,000 and 11,300 photons μm^{-2} s^{-1} for control and NCKX4-deficient cones, respectively. Data is from Vinberg et al., 2017.

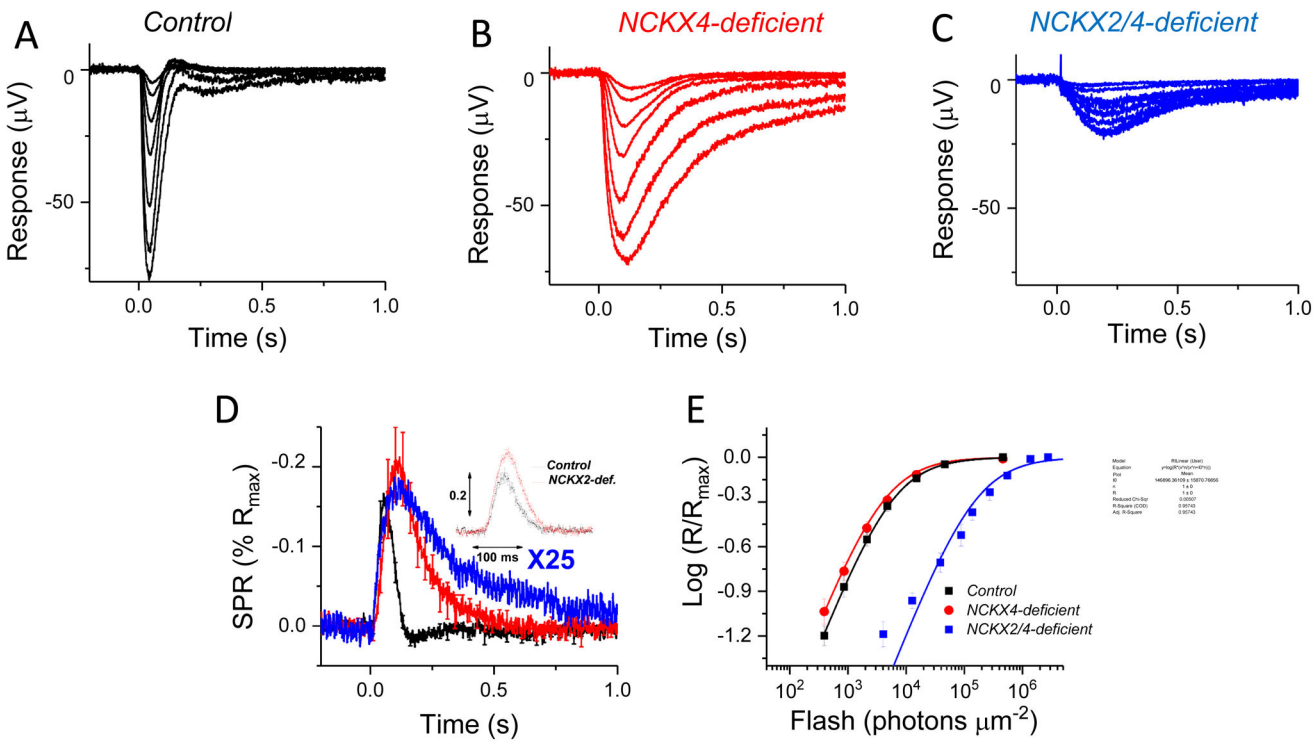

Figure 7. Role of NCKX2 and NCKX4 in the cone phototransduction.

Light responses of control (A), NCKX4-deficient (B), and NCKX2/4 double knockout (C) cones recorded from isolated *Gnat1*$^{-/-}$ mouse retinas using *ex vivo* ERG technique. (D) Responses of control (black), NCKX4-deficient (red), and NCKX2/4 DKO (blue) cones to a dim flash normalized with the maximal response amplitude and flash intensity (in estimated cone pigment photoisomerizations based on collection area of 0.12 μm^2 from (Sakurai et al., 2011)). Note that the response from NCKX2/4 DKO cones has been multiplied by 25 to facilitate comparison of the response kinetics. Inset plots dim flash responses to identical stimulus normalized with the maximal response amplitudes from control (black) and *Nckx2*$^{-/-}$ (red) cones. (E) Amplitudes of flash responses (R) normalized with the maximal response amplitude (R_{max}) are plotted as a function of flash intensity for control (black), NCKX4-deficient (red), and NCKX2/4 double knockout (blue) mice. Smooth traces plot Naka-Rushton function with half-saturating flash intensity of 5,200,3,900, and 146,900 photons μm^{-2} for control (black),NCKX4-deficient (red), NCKX2/4 double knockout (blue, dimmest flash data point excluded from fitting) cones, respectively. Modified from Vinberg *et al.* 2017.

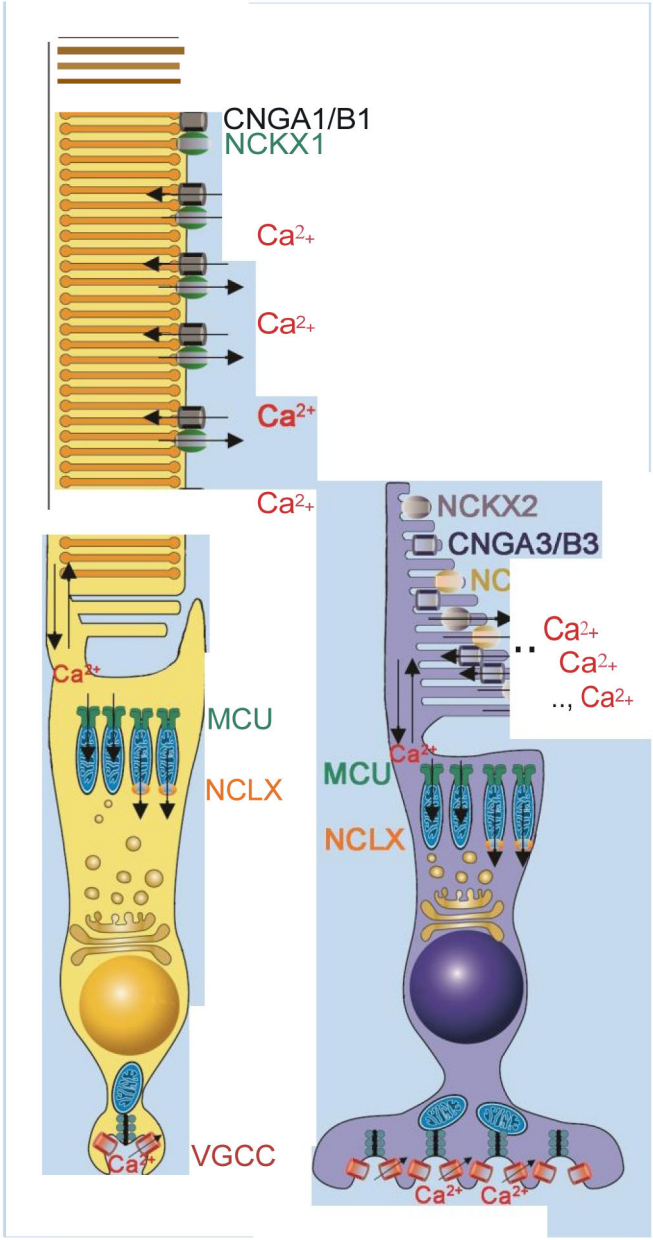

Figure 8. Summary of the mechanisms regulating calcium in the outer segments of rods and cones.

(A) Calcium enters the outer segments of rods via the transduction CNGA1/CNGB1 heteromeric channels and is rapidly extruded by NCKX1. A slower mechanism for extruding calcium from rod outer segments likely involves outflow through the connecting cilium, possibly combined with uptake by mitochondria via the mitochondrial calcium uniporter (MCU) and release via Na^+/Ca^{2+} transporters (NCLX). (B) Calcium enters the outer segments of cones via the transduction CNGA3/CNGB3 heteromeric channels and is rapidly extruded by the combined action of NCKX2 and NCKX4. Outflow through the connecting cilium, possibly combined with uptake by mitochondria, could also play a role in regulating cone calcium. Biochemical studies have suggested direct interaction between NCKX1 and

the rod CNG channel (Bauer and Drechsler, 1992) but not between NCKX2 and the cone CNG channel (Matveev et al., 2008). The possible interaction of NCKX4 and the cone CNG channel has not been examined.