ELSEVIER

Regulation of cyclic nucleotide-gated channels

Jonathan Bradley[1], Johannes Reisert[1] and Stephan Frings[2]

Cyclic nucleotide-gated (CNG) channels are found in several cell types, and are best studied in photoreceptors and olfactory sensory neurons. There, CNG channels are gated by the second messengers of the visual and olfactory signalling cascades, cGMP and cAMP respectively, and operate as transduction channels generating the stimulus-induced receptor potentials. In visual and olfactory sensory cells CNG channels conduct cationic currents. Calcium can contribute a large fraction of this current, and calcium influx serves a modulatory role in CNG-channel mediated signal transduction. There have been recent developments in our understanding of how the regulation of CNG channels contributes to the physiological properties of photoreceptors and olfactory sensory cells, and in particular on the role of calcium-mediated feedback.

Addresses
[1] Department of Neuroscience, Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, USA
[2] Abteilung für Molekulare Physiologie, Universität Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

Corresponding author: Frings, Stephan (s.frings@zoo.uni-heidelberg.de)

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Introduction

Channels directly gated by cyclic nucleotides (CNG channels) were first identified in vertebrate rod and cone photoreceptors, and were soon found in olfactory sensory neurons (OSNs) and other neuronal and nonneuronal cell types. In visual and olfactory sensory cells, CNG channels convert stimuli into electrical signals for the nervous system to interpret (for review see Kaupp and Seifert [1]). In mammals, a family of six genes codes for four 'A' subunits (CNGA1–4) and two 'B' subunits (CNGB1 and CNGB3) [2]. When expressed heterologously, CNGA1, A2 and A3 can form functional homomeric channels. However, the native transduction CNG channels of rods, cones and OSNs are heterotetramers of A and B subunits (see Figure 1).

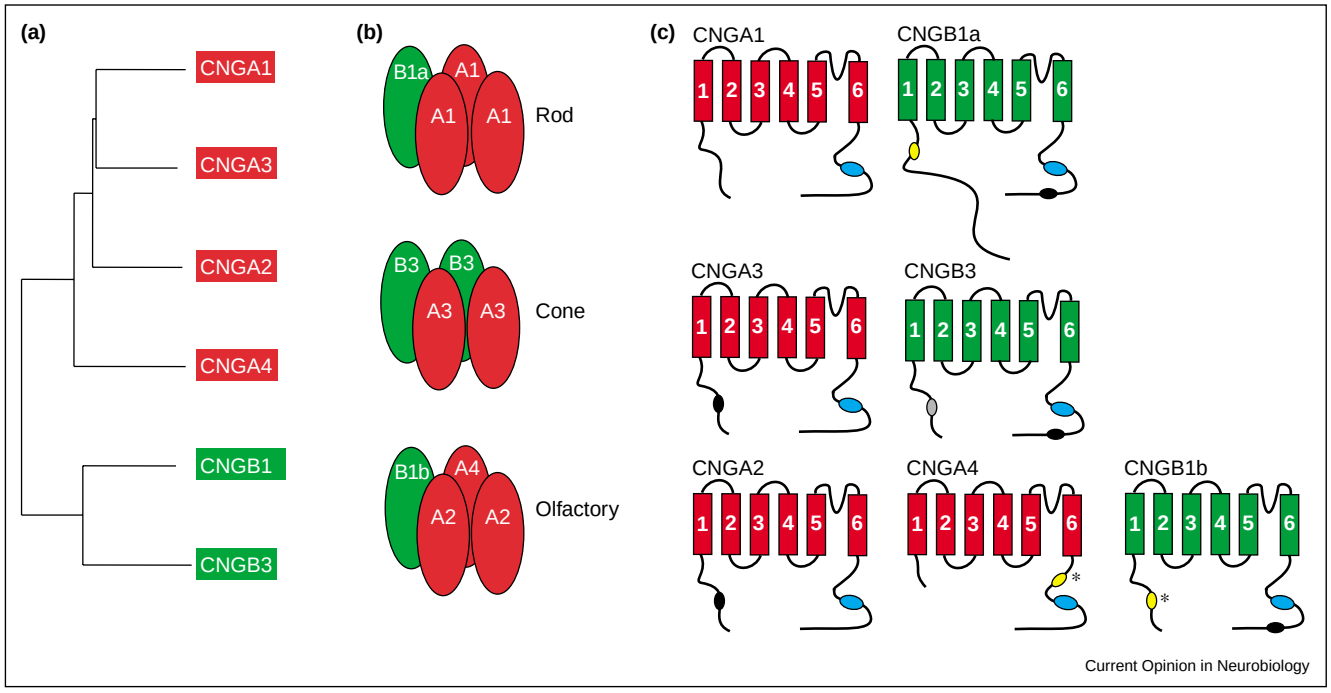
In rods, cones and OSNs, fundamental channel properties, such as open probability, ligand sensitivity and ion permeability, vary according to the subunits expressed (A and B types) and their stoichiometry. The correct physiological function of the cell, thus, depends on a specific channel composition. Despite these differences essential features are shared among all CNG channels. All are nonselective cation channels that have substantial Ca^{2+} permeability under physiological conditions. CNG channels also do not desensitize or inactivate when exposed to cyclic nucleotides. They are, however, subject to feedback regulation, particularly via Ca^{2+}-mediated mechanisms, similar to other Ca^{2+}-permeable ion channels. Ca^{2+}-dependent regulation of CNG channels can distinctly influence the stimulus–response relationships of sensory neurons. This brief article does not review the post-translational modifications that occur on a slow timescale (many seconds to minutes) and that determine the steady-state properties of CNG channels [3–5]. Instead, we focus on several recent insights into the dynamic CNG-channel regulation relevant for the rapid adaptive properties of photoreceptors and olfactory neurons (milliseconds to seconds).

Cyclic nucleotide-gated channel regulation in photoreceptors

In the vertebrate retina, day vision (in bright light) and night vision are mediated by cone and rod photoreceptors, respectively. Cones have the remarkable ability to adapt to a wide range of background light intensities. The cone system is able to perceive contrast when the number of photons absorbed ranges from about 20 to 1 million per second [6]. Among the various types of sensory neurons, cones probably have the widest dynamic range of operation. By contrast, rod photoreceptors show limited adaptation and, consequently, display a narrow dynamic range.

Adaptation is controlled to a large extent by the free Ca^{2+} concentration within the photoreceptor outer segment (for review see Fain *et al.* [7]). cGMP-sensitive CNG channels are open in darkness, which enables Ca^{2+} to enter the outer segment. A Na^+–Ca^{2+}–K^+ exchange mechanism extrudes Ca^{2+} to establish a steady Ca^{2+} level near 600 nM in the dark. Upon illumination, phosphodiesterase activity is stimulated, CNG channels close and Ca^{2+} influx decreases. Because Ca^{2+} extrusion continues the Ca^{2+} concentration falls to about 30–50 nM in rods and 5 nM in cones [8–10]. This light-dependent Ca^{2+} decrease controls various components of the phototransduction cascade; in particular the guanylyl cyclase, which has a higher rate of cGMP synthesis at low Ca^{2+} levels in both rods and cones. But does the Ca^{2+} signal actually modulate the CNG channels in both types of photoreceptors (see Figure 2a)?

Figure 1



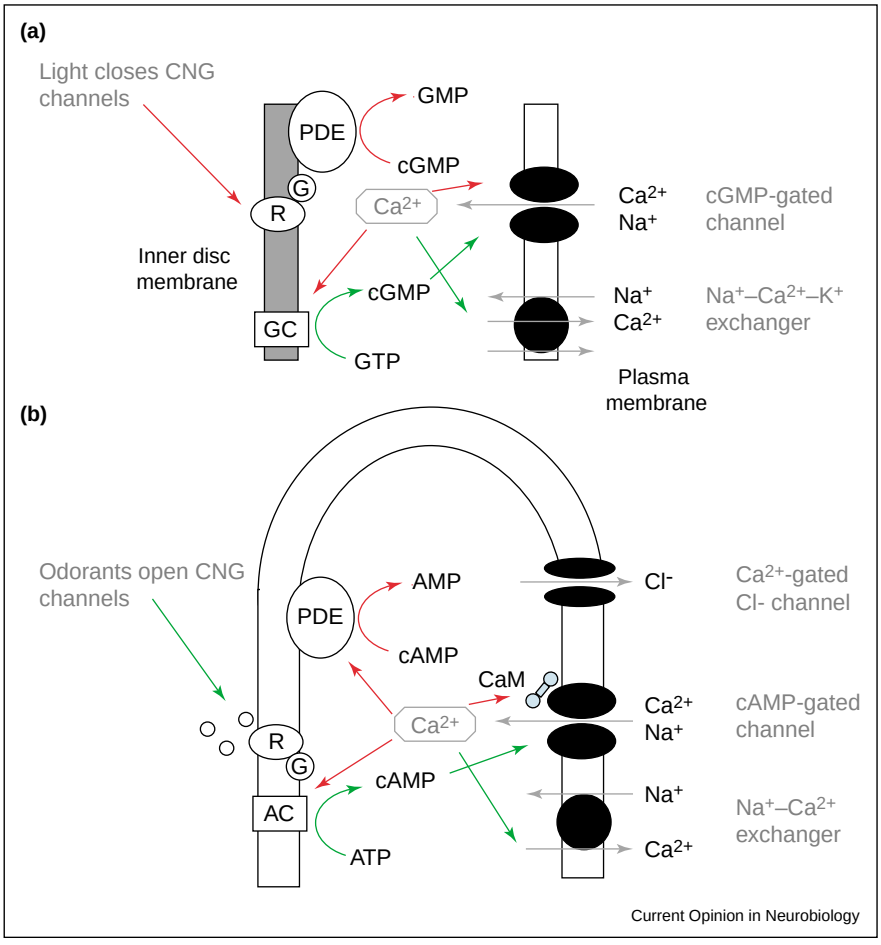
Subunits and stoichiometry of CNG channels in photoreceptors and OSNs. **(a)** Phylogenetic tree of the human A and B type CNG channel subunits. **(b)** Stoichiometry and subunit composition of rod, cone and olfactory CNG transduction channels. **(c)** Topological models of the CNG channel subunits of rod, cone and olfactory sensory cells. Six transmembrane domains (TMDs) are indicated by numbers, the pore loop is located between TMD 5 and 6. Both N- and C-termini are intracellular and contain functional regions for channel regulation: the cyclic nucleotide-binding sites (blue), the calmodulin-binding sites of the calcium independent 'IQ-type' and calcium-dependent 'Baa type' (yellow and black respectively). A site in CNGB3 lacking a critical signature residue of an IQ sequence is depicted in grey. Asterisks indicate the sites most likely, to date, to support physiological channel regulation (see text for details).

A recent examination of mammalian cones and rods has revealed that the sensitivity of the CNG channels is modulated by Ca^{2+} in cones but not in rods [11••]. A sudden drop of free intracellular Ca^{2+} from 600 to <30 nM in the outer segment, induced by photo-liberating the Ca^{2+} chelator Diazo-2 (see Figure 3), increased channel activity in cones but not rods. Taken together with results from previous studies [12,13], the authors conclude that the ligand sensitivity of cone CNG channels is controlled by intracellular Ca^{2+}. Decreasing Ca^{2+} levels increase ligand sensitivity — a mechanism that promotes channel opening (and hence adaptation) as the intensity of the ambient light increases.

In a well studied example of fish cones, the Ca^{2+} effect is substantial, raising the effective concentration for half maximal channel activation (EC_{50}) by cGMP from 84 to 335 μM [13]. CNG channels in these cells have an EC_{50} for Ca^{2+} regulation of 857 nM that enables responsiveness over much of the physiological range of Ca^{2+} concentration (5–600 nM in cone outer segments). This is well suited for a role of Ca^{2+} as a modulator of cone channel sensitivity. The molecular basis of this Ca^{2+}-dependent regulation is not, however, understood. Although calmodulin (CaM), a ubiquitous mediator of Ca^{2+} signaling, is able to reduce cGMP sensitivity when added to excised patches from the cone plasma membrane, Ca^{2+}–CaM appears not to be responsible for channel regulation in intact cones. The Ca^{2+}–CaM mediated decrease in sensitivity is far too small when compared with the Ca^{2+}-dependent desensitization observed in intact cones [14,15]. Furthermore, the heterologously expressed cone channel, a tetrameric protein formed from two copies each of CNGA3 and CNGB3 [16•], is only subject to subtle modulation by Ca^{2+}–CaM [15,17], which is inconsistent with the regulation of native channels.

Native CNG channels of rod photoreceptors, or heterologously expressed mammalian rod channels that have the native $CNG(A1)_3$-B1a stoichiometry [18–20], can display a modest Ca^{2+}–CaM mediated reduction in cGMP sensitivity in excised inside-out patches [15,21,22]. Native rod CNG channels bind Ca^{2+}–CaM with high affinity. The $K_{1/2}$ for binding of Ca^{2+}–CaM is near 1 nM, and CaM dissociates from the channels only when Ca^{2+} concentrations fall below 100 nM [23]. Studies on amphibian rods have revealed a half-maximal binding of Ca^{2+}–CaM at a Ca^{2+} concentration of 48 nM [24], which only slightly overlaps with the physiological range of intracellular Ca^{2+} concentrations at which Ca^{2+}–CaM

Figure 2



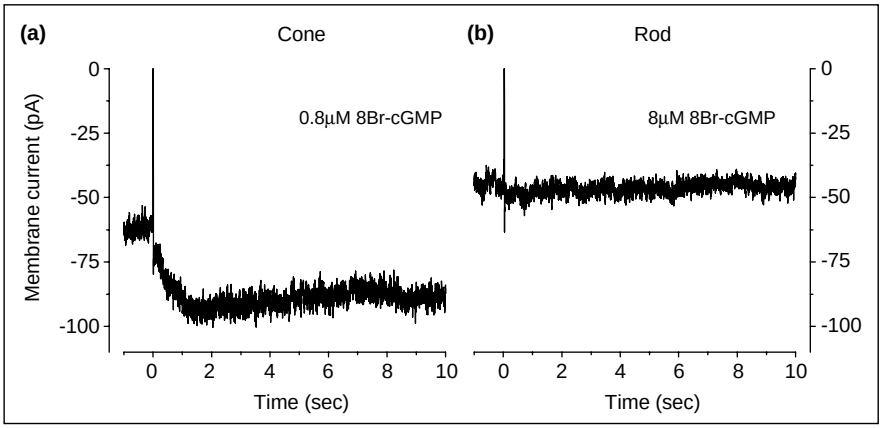
Signal transduction pathways in photoreceptors and OSNs. Color conforms to processes that act to promote opening (green) or closing (red) of the CNG transduction channels. **(a)** During phototransduction in the outer segment of rod and cone photoreceptors light is absorbed by a seven-transmembrane receptor (opsin bound to a chromophore, R) on the inner disk membranes of photoreceptors. Activation of a G protein (transducin, G) stimulates phosphodiesterase (PDE) activity. A reduction in cGMP concentration closes the CNG channels in the photoreceptor plasma membrane, reducing calcium influx. Calcium continues to be extruded by a Na^+–Ca^{2+}–K^+ exchanger and, as the intracellular calcium concentration drops, guanylyl cyclase (GC) in the disk membrane becomes disinhibited. A drop in calcium can also potentiate the CNG channel response to cGMP. **(b)** During olfactory transduction on the ciliary membrane of OSNs odorants bind to odorant receptors (R) on the cilia of OSNs, which in turn activate a G protein (G) that stimulates adenylyl cyclase (AC). The increase in cAMP concentration opens CNG channels, which conduct calcium into the cilia. The consequent increase in calcium acts to stimulate phosphodiesterase (PDE) activity and inhibit adenylyl cyclase, both processes leading to a reduced cAMP concentration. Calcium binds to calmodulin (CaM), which is associated with the CNG channel, to inhibit the action of cAMP. A calcium-activated chloride channel opens to enable chloride efflux, and a Na^+–Ca^{2+} exchanger extrudes calcium.

regulation can be effective. These results suggest that channel regulation is limited to physiological conditions at which intracellular Ca^{2+} is extremely low, that is, close to light saturation. Ca^{2+}-dependent adaptation is well documented in amphibian rods, but a possible regulation of the cGMP-gated transduction channels is considered to be of minor importance (for review see Fain *et al.* [7]).

Cyclic nucleotide-gated channel regulation in olfactory sensory neurons

In OSNs, the odor-induced receptor current desensitizes to constant stimuli [25,26], and OSNs have a very limited ability to maintain sensitivity under prolonged stimulation. Within a 10-fold increase in background odor concentration OSNs lose their ability to respond dynamically to odor [27]. During short odor exposures OSNs can adapt. Adaptation appears to be downstream of cAMP production and might be mediated by Ca^{2+}–CaM dependent inhibition of the CNG channel [28] (see Figure 2b). However, the Ca^{2+}–CaM modulation of CNG channel activity is not the only Ca^{2+}–CaM dependent adaptation mechanism. Ca^{2+}–CaM also reduces cAMP levels by activating a phosphodiesterase (PDE1C2; [29,30]) and by inhibiting adenylyl cyclase through CaMKII-mediated

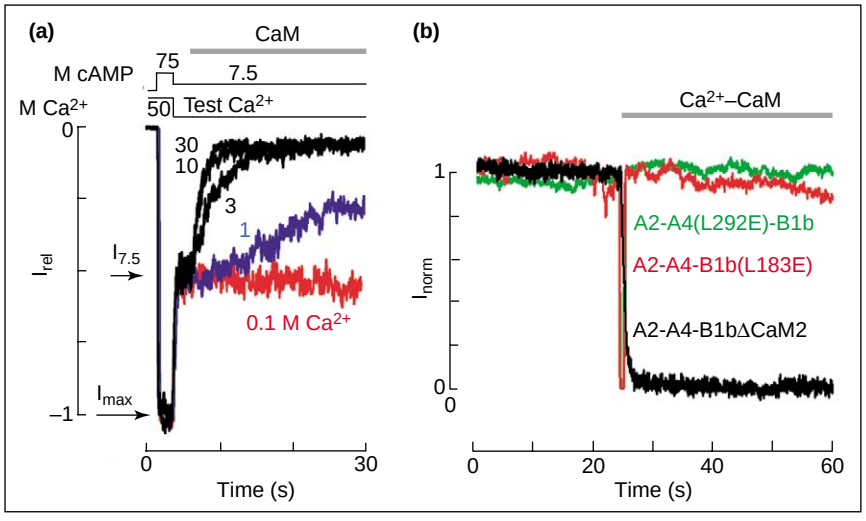
Figure 3



The effect of lowering intracellular calcium on the CNG current of rod or cone photoreceptors. The cyclic nucleotide analog 8-Br-cGMP was introduced via a whole-cell recording pipette with a photoactivatable calcium chelator and 600 nM free calcium. Two and a half minutes after attaining whole-cell configuration at a holding potential of −40 mV, Diazo-2 was uncaged (artefact at time 0), rapidly (50 ms) lowering calcium. **(a)** In cones, an inward current induced by the uncaging of Diazo-2 peaks by 2 sec and slowly drifts back to its starting steady-state level (not shown). **(b)** In rods, the current activated by 8-Br-cGMP is not affected by the uncaging. Modified from [11••] with permission of Rockefeller Press.

phosphorylation [31,32]. The olfactory CNG channel, unlike the rod and cone channels, comprises three different types of subunits [33,34] that have a CNG$(A2)_2$-A4-B1b stoichiometry [35•]. The roles of the modulatory subunits A4 and B1b (a B1 splice variant) are to increase the sensitivity to cAMP [33,34,36] and to make the feedback inhibition by Ca^{2+}–CaM rapid and state independent [37]. An unexpected finding was that Ca^{2+}-free calmodulin, called apocalmodulin, is constitutively bound to the olfactory CNG channel even at resting (low) Ca^{2+} levels [38••]. For Ca^{2+}–CaM mediated inhibition of the olfactory CNG channel to occur, intracellular Ca^{2+} has to reach at least 100 nM [38••] (see Figure 4a). The resting Ca^{2+} level in olfactory cilia is reported to be 40 nM [39]

Figure 4



Analysis of heteromeric OSN channel inhibition by Ca^{2+}–CaM in excised inside-out patches. **(a)** Heteromeric $(CNGA2)_2$-A4-B1b channels were expressed in HEK 293 cells and then exposed, in excised patches, to 200 nM calmodulin in different concentrations of Ca^{2+} (30, 10, 3, 1 or 0.1 μM) while the sensitivity of the channels to 7.5 μM cAMP was continually monitored. With decreasing Ca^{2+} concentration the current declined less rapidly, until at 0.1 μM Ca^{2+} there was essentially no modulation. Thus, a Ca^{2+} concentration greater than 0.1 μM is necessary for Ca^{2+}–CaM modulation of these channels. **(b)** The IQ-type CaM binding sites in CNGA4 and CNGB1b are necessary for Ca^{2+}–CaM inhibition of heteromeric channels. Deletion of the classic basic amphiphilic alpha-helix (Baa-motif) Ca^{2+}–CaM binding site, CaM2, in CNGB1b has no effect on heteromeric channel inhibition by Ca^{2+}–CaM (black trace). Single point mutations in the IQ-type CaM binding sites in CNGA4 or CNGB1b completely abolish heteromeric channel inhibition by Ca^{2+}–CaM (green and red traces, respectively). Modified from [38] (a) and [47] (b) with permission of http://www.nature.com/ and Wiley-VCH.

and odors can at least trigger micromolar levels needed for activation of the Ca^{2+}-activated Cl^- channel [40,41] (see Figure 2b). Hence, Ca^{2+} concentrations high enough to induce Ca^{2+}–CaM mediated inhibition are easily achieved during the odor response, especially close to the apocalmodulin-bound CNG channel itself, because about half of its current is carried by Ca^{2+} [42]. At Ca^{2+} concentrations exceeding 1 μM, Ca^{2+}–CaM reduces the sensitivity of the CNG channels by shifting the EC_{50} for activation by cAMP from 7 μM to 25 μM (a regulatory effect that promotes channel closure at cAMP concentrations <100 μM) [38••]. This desensitization of CNG channels is thought to cause fast adaptation in OSNs [28], and might contribute to the oscillatory response pattern observed during prolonged odorant stimulation [43], but has yet to be directly tested in intact OSNs. An additional twist to Ca^{2+}-mediated effects in olfactory signal transduction lies in the Ca^{2+}-activated Cl^- conductance, which amplifies the CNG current and carries most of the receptor current. Its EC_{50} for activation by Ca^{2+} is 2–5 μM [40,41], a concentration at which the CNG channel is already substantially inactivated [38••]. Therefore, large receptor currents might be observed only once the CNG channel is already desensitized by bound Ca^{2+}–CaM.

The molecular basis of channel regulation

In all known CNG channels, the ligand sensitivity seems to be influenced by the intracellular Ca^{2+} concentration. In recent years, considerable progress has been made in elucidating the molecular events that underlie this control mechanism. A common motif seems to be that the regulation is mediated by the modulatory subunits, A4, B1 and B3. The subunit CNGB1 is absolutely necessary for CaM-mediated feedback inhibition in both rod and olfactory CNG channels. The N-terminus of CNGB1 contains an IQ-type CaM-binding site (an 11 amino acid sequence that binds apocalmodulin) [21] that is present in both the CNGB1a (rod) and the CNGB1b (OSN) splice variants. Deletion of this site renders both heteromeric channels insensitive to Ca^{2+}–CaM [21,38••,44]. Interestingly, although CNGB1a seems to be sufficient for CaM-inhibition in rod channels, a second modulatory subunit, CNGA4, is needed in olfactory channels [38••]. CNGA4 also possesses an IQ-type CaM-binding site located within a C-terminal region called the 'C linker' (see 'C-linker' chapter in Kaupp and Seifert [1]), which connects the ligand-binding domain with the gating machinery of the channel. The fact that two CaM-binding sites on two different modulatory subunits (see Figure 4b) are necessary for feedback control points to a theme that has been extensively examined in rod CNG channels: the interaction of intracellular channel domains (for review see Trudeau and Zagotta [45]). It appears that the direct interaction of the CNGA1 C-terminus with the CNGB1a N-terminus is essential to obtain high ligand sensitivity in the rod channel, and that Ca^{2+}–CaM, by interfering with this interdomain interaction, reduces ligand sensitivity [46]. In olfactory channels, ligand sensitivity is reduced when Ca^{2+}–CaM interacts with both modulatory subunits [38••], but the nature of this interaction is not yet understood. Finally, heterologously expressed cone CNG channels exhibit a modest CaM-sensitivity conferred by the CNGB3 subunits. There is, however, no evidence yet that CNGB3 is also responsible for the dramatic Ca^{2+}-dependent regulation of native cone channels.

Taken together, recent data suggest that the ligand sensitivity of CNG channels depends on the interaction of intracellular channel domains residing on the modulatory subunits, and that cytosolic 'Ca^{2+} sensors', such as calmodulin, might control the channels by interfering with these interactions.

Conclusions

The different subunit compositions of rod, cone and OSN CNG channels confer different regulatory properties. In the evolutionarily older sensory neurons, OSNs and cones, the choice of subunits conveys a Ca^{2+} control over the ligand sensitivity of their CNG channels. This control appears to be mediated by CaM in OSNs and by a different Ca^{2+}-binding factor in cones. Rods have evolved later than cones as specialized light detectors with extreme sensitivity, and only appear to employ Ca^{2+}–CaM mediated regulation of CNG channels at light levels near saturation, which might not be related to their adaptation. The selective pressure in the evolution of rods was obviously directed toward their ability to respond robustly (with high gain) to as few photons as possible, and adaptation became a less important task. By contrast, the regulation of CNG channels in cones and OSNs is a major component of adaptation, with their CNG channels being tailored via distinct subunit compositions for the regulation of ligand sensitivity.

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