Mol Neurobiol (2007) 35:266–277
DOI 10.1007/s12035-007-0025-y

Function and Dysfunction of CNG Channels: Insights from Channelopathies and Mouse Models

Martin Biel · Stylianos Michalakis

Received: 21 September 2006 / Accepted: 5 January 2007 / Published online: 18 September 2007

Abstract Channels directly gated by cyclic nucleotides (CNG channels) are important cellular switches that mediate influx of Na^+ and Ca^{2+} in response to increases in the intracellular concentration of cAMP and cGMP. In photoreceptors and olfactory receptor neurons, these channels serve as final targets for cGMP and cAMP signaling pathways that are initiated by the absorption of photons and the binding of odorants, respectively. CNG channels have been also found in other types of neurons and in non-excitable cells. However, in most of these cells, the physiological role of CNG channels has yet to be determined. CNG channels have a complex heteromeric structure. The properties of individual subunits that assemble in specific stoichiometries to the native channels have been extensively investigated in heterologous expression systems. Recently, mutations in human CNG channel genes leading to inherited diseases (so-called channelopathies) have been functionally characterized. Moreover, mouse knockout models were generated to define the role of CNG channel proteins in vivo. In this review, we will summarize recent insights into the physiological and pathophysiological role of CNG channel proteins that have emerged from genetic studies in mice and humans.

Keywords CNG · Cyclic nucleotide-gated channel · Knockout · Channelopathies · Photoreceptor · Olfactory receptor neuron · Cyclic nucleotides · Retinitis pigmentosa · Achromatopsia · Vision · Olfaction

M. Biel (✉) · S. Michalakis
Center for Integrated Protein Science, CIPSM
and Department Pharmazie—Zentrum für Pharmaforschung,
Pharmakologie für Naturwissenschaften,
Ludwig-Maximilians-Universität München,
Butenandtstr. 7,
81377 München, Germany
e-mail: mbiel@cup.uni-muenchen.de

Role of CNG Channels in Vision and Olfaction

As the focus of this review is on the role of cyclic nucleotide-gated (CNG) channels in vision and olfaction, we will start with a brief outline of the sensory transduction pathways underlying these processes (for a more detailed description of visual and olfactory transduction, see [1–3]).

Vision

Vision in vertebrates is conferred by the concerted action of two phototransduction pathways, the rod and cone photoreceptor system. Rods are responsible for vision at low light levels (e.g., twilight), whereas vision at higher light levels (e.g., daylight) is provided by cones. The cone system also confers color vision, as cones exist in two (short- and medium-wave sensitive in most vertebrates) or three variants (short-, medium-, and long-wave sensitive in humans and simian primates) with different spectral sensitivities [4, 5]. In both rods and cones, signal transduction is conferred by the second messenger cGMP that controls the activity of a CNG channel present in the plasma membrane of the outer segments of these cells [3]. In the dark, the CNG channel is maintained in its open state by a high concentration of cGMP produced by a transmembrane guanylyl cyclase [6, 7]. The resulting influx of Na^+ and Ca^{2+} (dark current) depolarizes the photoreceptor and promotes synaptic glutamate release. Light absorption by visual pigments initiates a G protein (transducin)-mediated signaling cascade leading to activation of a cGMP phosphodiesterase, hydrolysis of cGMP, and closure of the CNG channel. As a result, the photoreceptor hyperpolarizes and shuts off synaptic transmission. CNG channels provide the only source for Ca^{2+} influx into rod and cone outer segments [3, 8, 9]. Ca^{2+} entry

is balanced by Ca^{2+} extrusion through the activity of a Na^+/Ca^{2+}–K^+ exchanger [9–12]. Thus, light also decreases the cellular calcium concentration because it shuts down Ca^{2+} entry, whereas the Na^+/Ca^{2+}–K^+ exchanger continues to clear Ca^{2+} from the cytosol [10]. The low Ca^{2+} concentration contributes to the recovery from light response which is achieved by several processes including inactivation of visual pigments and restoration of cGMP levels by the action of guanylate cyclase, activating proteins on retinal guanylyl cyclases [1]. In addition, low Ca^{2+} concentration also adjusts the sensitivity of the transduction machinery, a process known as light adaptation [13, 14].

Olfaction

Olfactory transduction takes place in the cilia of olfactory receptor neurons (ORNs). Cilia harbor all molecular components required for chemoelectrical transduction, and thus, represent the functional equivalent of the outer segments of photoreceptors. Binding of an odorant to its cognate receptor [15] initiates a G protein (G_{olf})-mediated signaling cascade that leads to activation of a ciliary adenylyl cyclase [16, 17]. The ensuing rise in the cAMP level opens the olfactory CNG channel that conducts mainly Ca^{2+} and little Na^+ under physiological ionic conditions [18, 19]. The resulting influx of Ca^{2+} slightly depolarizes the membrane and, at the same time, it activates a Ca^{2+}-activated Cl^- channel also present in the cilia [20–22]. There is recent evidence that this Ca^{2+}-activated Cl^- channel is most probably a member of the bestrophin family [23] and that gating of the channel involves calmodulin [24]. The intracellular Cl^- concentration in ORNs is maintained above electrochemical equilibrium by a Na^+–$2Cl^-$–K^+ cotransporter [25, 26]. Thus, activation of the Cl^- channel results in Cl^- efflux and strong membrane depolarization and acts as efficient amplifier of the primary CNG current [20–22]. Like in photoreceptors, Ca^{2+} also serves as negative feedback signal and modulator of signal transduction [27]. Fast odor adaptation is achieved by reduction of the sensitivity of the olfactory CNG channel through a Ca^{2+}-calmodulin-dependent mechanism [28]. In addition, Ca^{2+} also exerts an inhibitory effect on the ciliary adenylyl cyclase [29, 30] and stimulates cAMP hydrolysis by a Ca^{2+}-calmodulin-dependent ciliary isoform of the phosphodiesterase (PDE1C2) [31–33]. Both processes may contribute to adaptation to long odorant exposure. A recent report shows that odorant molecules itself might also contribute to adaptation by directly inhibiting the olfactory CNG channel [34].

While the vast majority of ORNs use the described cAMP pathway of signal transduction, a small subset of neurons seems to utilize cGMP as principal second messenger [35]. These neurons were shown to contain the cone-type CNG channel [36], a membrane-bound guanylyl cyclase [37], and a cGMP-stimulated phosphodiesterase [38]. In contrast, they lack the components typical of other ORNs [36, 38]. The "cone-type" ORNs project to a group of atypical glomeruli in the main olfactory bulb designated as "necklace" glomeruli [39]. The physiological role of these ORNs and their contribution to general olfaction remains to be determined.

A brief Overview on the Structure and Basic Properties of CNG Channels

CNG channels form a distinct branch within the superfamily of voltage-gated-like channels [40–42]. In mammals, the CNG channel family comprises six homologous members which are classified as A subunits (CNGA1-4) and B subunits (CNGB1 and CNGB3; CNGB2 does not exist). A and B subunits are also found in the genome of several invertebrates (e.g., *Caenorhabditis elegans* and *Drosophila melanogaster*), suggesting an early segregation of these two channel subunits in evolution. Diversity of mammalian CNG channels is further increased by alternative splicing (overview in [3]). Importantly, splice variants of CNGB1 containing different cytosolic N termini are specifically expressed in rod photoreceptors (CNGB1a) and ORNs (CNGB1b), respectively [43–46]. A and B subunits share the same principal membrane topology which is characterized by six transmembrane domains (S1–S6), a reentrant pore (P) loop between S5 and S6, and cytosolic N and C termini (Fig. 1a). Like the related voltage-gated K^+ channels whose crystal structure has been recently solved [47], CNG channels are tetramers, with the four subunits arranged around the centrally located pore [48]. The ion-conducting pore is lined by the P-loops and the S6 segments of the four subunits [49]. Three domains are involved in the activation process of CNG channels: (1) the channel gate which is localized in the distal portion of the S6 segment [50, 51], (2) the cyclic nucleotide-binding domain (CNBD) in the C terminus [52, 53] and (3) the C-linker, a domain that allosterically couples cyclic nucleotide-binding to the channel gate [54–56]. The principal elements of the gating machinery are also found in HCN channels, the closest relatives of CNG channels [57–60], and some voltage-dependent potassium channels including HERG [61] and KAT1 channels [62].

Although all six CNG channel subunits share significant sequence homology, only CNGA1, CNGA2, and CNGA3 can be functionally expressed in heterologous cell systems. However, the biophysical and pharmacological properties of the respective homomeric channels differ in several aspects (e.g., single channel current characteristics, affinity

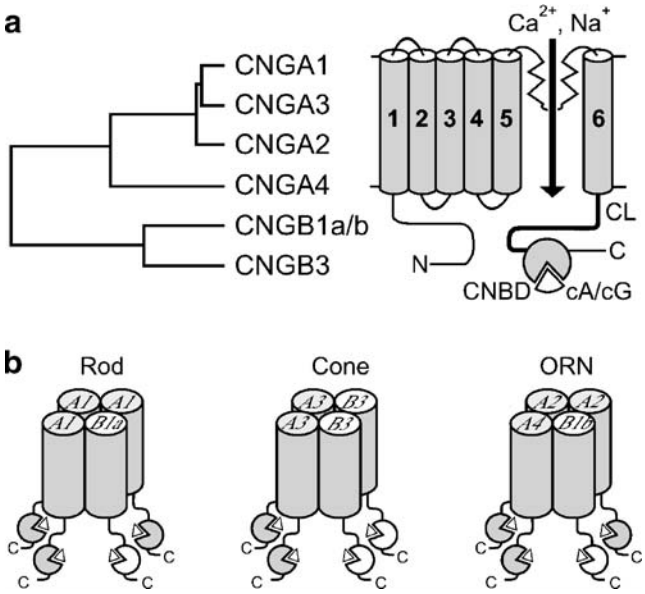

Fig. 1 **a** Phylogenetic tree of human CNG channel subunits (*left panel*). Membrane topology of CNG channel subunits (*right panel*). **b** Subunit composition and stoichiometry of the CNG channels from rods (*left*), cones (*middle*), and olfactory receptor neurons (*ORN*, *right*). *1–6*, transmembrane segments 1–6; *C* Carboxy terminus, *CNBD* cyclic nucleotide binding domain, *N* amino-terminus

for cyclic nucleotides, permeation properties, regulation by Ca^{2+}) from those of native CNG channels (for detailed review, see [3, 63]). This discrepancy could be solved in coexpression studies showing that distinct combinations of A and B subunits generated channels that almost perfectly matched the functional properties of native channels. This finding strongly suggested that native CNG channels are heterotetramers. This hypothesis has been confirmed by recent biochemical and fluorescence resonance energy transfer (FRET) studies. Using these methods, the exact subunit compositions and stoichiometries have been determined for the channels expressed in rod photoreceptors (3 CNGA1:1 CNGB1a) [64–66], cone photoreceptors (2 CNGA3:2 CNGB3) [67], and olfactory neurons (2 CNGA2:1 CNGA4:1 CNGB1b) [68] (Fig. 1b). It should be noted that the stoichiometry of the cone and olfactory channel has only been determined in heterologous expression systems. As knockout studies suggest that subunit assembly may differ between native and heterologous cells (see below), it will be necessary to confirm the results in purified native channels. Moreover, it remains to be determined whether homomeric CNG channels and CNG channels with subunit compositions different from those of photoreceptors and ORNs exist in other cell types.

CNG channels characterized so far share some common features. All pass Na^+ and K^+, but do not discriminate between them. Calcium is also permeable, but at the same time, acts as a voltage-dependent blocker of monovalent cation permeability [19, 69]. Moreover, CNG channels do not desensitize or inactivate when exposed to cyclic nucleotides. They are, however, subject to feedback regulation, particularly via Ca^{2+}-calmodulin-mediated mechanisms [70]. CNG channels reveal a higher sensitivity for cGMP than for cAMP. However, the extent of ligand discrimination varies significantly between the individual CNG channel types. Photoreceptor channels strongly discriminate between cGMP and cAMP [71], whereas the olfactory channel is almost equally sensitive to both ligands [72].

The CNGA1, CNGA2, and CNGA3 subunits are expressed in rod photoreceptors, ORNs, and cone photoreceptors, respectively. Because these three subunits were able to form functional homomeric channels in heterologous systems, they were considered as the principal subunits of the native channels. By contrast, the CNGA4 as well as the two B subunits were designated as "modulatory" subunits because they were obviously not required for principal channel formation but rather affected some biophysical properties of the respective channels. Recent genetic studies have modified this notion and shed new light on the physiological role of A and B subunits in vivo. In the next section, we will summarize the results of these studies. We will focus on vision and olfaction but will also shortly discuss other physiological systems CNG channels were found to be involved in.

Channelopathies and Knockout Models of the Rod Photoreceptor CNG Channel

CNGA1

Mutations in the CNGA1 gene have been found in patients suffering from retinitis pigmentosa (RP) [73]. RP comprises a genetically diverse group of progressive degenerative diseases effecting the photoreceptors of the retina [74]. The most common symptoms of RP include night blindness, progressive concentric reduction of the visual field, an abnormal accumulation of pigmentation in the retina [75]. In most cases, RP finally leads to legal blindness. At the cellular level, the disease is characterized by a primary impairment or total loss of rod function and structure followed by a secondary degeneration of the cones. So far, RP has been mapped to >30 genes (http://www.sph.uth.tmc.edu/Retnet) encoding proteins involved in the visual transduction pathway (e.g., rhodopsin) or required for the maintenance of rod architecture (e.g., peripherin). Mutations in CNGA1 lead to an autosomal recessive form of RP that accounts for about 1% of cases of RP [73]. Three of the five mutant alleles identified are null mutants (E76Stop, K139Stop, and deletion of most or all of the CNGA1 transcription units) because they would encode proteins that are lacking essential channel domains. The remaining two

other alleles (S316F and R654DStop) encode channels that fail to reach the plasma membrane in heterologous expression systems. Data of Mallouk et al. [76] in *Xenopus* oocytes suggest that the KLKQDStop sequence present at the C terminus of the R654DStop mutant represents an ER retention signal that effectively prevents CNGA1 targeting to the plasma membrane. Based on experiments in the same system, Trudeau and Zagotta [77] proposed an alternative model. These authors claimed that truncation of the last 32 amino acids in the R654DStop mutant exposes a normally buried region from the CNGB1a N-terminal domain harboring a retention signal, and, as a consequence, prevents membrane expression of the heteromeric channel.

To date, mice lacking the CNGA1 subunit are not available. However, transgenic mice revealing an about 50% reduction of CNGA1 transcript levels due to the overexpression of a CNGA1 antisense mRNA have been produced [78]. These mice show some histological features reminiscent of RP (e.g., reduced number of photoreceptors, apoptotic death of retinal cells). However, electroretinograms (ERGs) of these mice have not been reported so far. Thus, it remains an open issue to which extent the down-regulation of CNGA1 affects rod- and cone-mediated vision. Moreover, it cannot be excluded that the over-expression of the antisense mRNA exerts non-specific effects (e.g., toxic effects caused by suppression of other mRNAs) that may affect the phenotype.

Taken together, the genetic data imply that photoreceptors degenerate in response to the absence (or down-regulation) of functional CNG channels in outer segments. The molecular pathways leading to the initiation of cell death in CNGA1-deficient rods are unknown. One possible mechanism refers to the "equivalent-light" hypothesis [79]. According to this hypothesis, the absence of functional CNG channels is equivalent to the permanent closure of channels occurring under continuous bright light conditions. Continuous exposure of experimental animals to light has been shown to result in photoreceptor degeneration [80–82]. It is unclear, however, how constitutive activation of phototransduction leads to retinal degeneration. Low Ca^{2+} levels resulting from the loss of Ca^{2+} influx through CNG channels as well as toxicity induced by reactive oxygen species may play a role in this process [79–81, 83].

CNGB1a

The CNGB1 gene locus consists of at least 33 exons encoding several splice variants [84]. Rods express a 240-kD isoform (CNGB1a) containing a long cytosolic N terminus that is also translated as a separate cytosolic protein (glutamic acid-rich protein, GARP) [43, 44, 85]. Shorter variants of the subunit lacking the GARP-part are present in the native olfactory CNG channel (CNGB1b) [45, 46] and have been identified in sperm cells and other tissues [86, 87]. A CNGB1-deficient mouse model covering all potential CNGB1 splice variants was generated by deleting exon 26 which encodes the pore-forming region and the S6 segment [88]. These mice show an impairment of both vision and olfaction. In this section, we will focus on the retinal phenotype.

CNGB1-deficient mice show a slow-progressing degeneration of the retina caused by apoptotic death and concurred by retinal gliosis. The degeneration primarily affects rod photoreceptors and is detectable in these cells as early as postnatal day 15 (P15). Cones are quite unaffected up to an age of 6 months, but start to degenerate subsequently. At the age of about 1 year, the retina of CNGB1-deficient animals is essentially devoid of both rods and cones. The principal structure of the inner retina is unaffected by the deletion of CNGB1. However, rod bipolar cells and horizontal cells reveal some morphological alterations (e.g., sprouting extensions, retraction of

Table 1 Overview on diseases associated with CNG channels

Type	Subunit	Animal model	Human disease
Rod channel	CNGA1	CNGA1 antisense expressing mice: retinal degeneration [78]	Retinitis pigmentosa [73]
	CNGB1a	CNGB1-deficient mice: impaired rod function and retinal degeneration [88]	Retinitis pigmentosa [89]
Cone channel	CNGA3	CNGA3-deficient mice: loss of cone function [109], cone degeneration [112, 113]	Achromatopsia [90, 97, 99, 101], progressive cone dystrophy [97, 98, 100]
	CNGB3	Canine models (null-deletion or missense mutation): cone degeneration [123]	Achromatopsia [91, 92, 99, 101, 121], progressive cone dystrophy [151]
Olfactory channel	CNGA2	CNGA2-deficient mice: impaired olfaction [126–129, 132], impaired hippocampal LTP [141]	–
	CNGA4	CNGA4-deficient mice: impaired odor adaptation [142], impaired olfaction [143]	–
	CNGB1b	CNGB1-deficient mice: impaired odor adaptation and impaired olfaction [149]	–

processes, misplacement of cell bodies). CNGB1-deficient mice show no detectable rod-mediated responses in ERGs, whereas up to 6 months of age, cone responses are normal. Later on, the cone responses also decrease, and at the age of 12 months, knockout mice are legally blind. In agreement with the ERG measurements, the vast majority of isolated rod photoreceptors of young mice (P17) fails to respond to light. Only in a few cells can a tiny dark current be detected. The severe retinal phenotype of CNGB1-deficient mice was unexpected given that CNGB1 was considered to be a modulatory subunit that is not needed for principal channel formation. This conundrum could be solved by Western blots and immunohistochemistry showing that in CNGB1-deficient mice, only trace amounts of the CNGA1 subunit are present on the rod outer segment. Thus, in contrast to *Xenopus* oocytes or HEK293 cells, rod photoreceptors are not capable of producing substantial amounts of the homomeric CNGA1 channel and/or cannot target homomeric channels to the rod outer segment. The mechanism of cell death may be analogous to that discussed above for CNGA1-channelopathies. It is not known why photoreceptors unlike *Xenopus* oocytes or HEK293 cells cannot efficiently target homomeric CNGA1 channels to the plasma membrane. In any case, it is tempting to speculate that the requirement of CNGB1 for targeting CNGA1 to the outer segment may have evolved to ensure that only heteromeric channels reach the outer segment membrane.

The total loss of CNG channel expression explains why the retinal phenotype of CNGB1-deficent mice is so reminiscent of that found in RP patients with null mutations in the CNGA1 gene. Interestingly, RP patients have been recently identified who carry a point mutation in the CNBD of CNGB1 [89]. So far, the functional consequences of this mutation, G993V, which involves a highly conserved residue in the CNG channel family, have not been determined in expression systems. Importantly, it is not known whether the mutant protein is targeted to the plasma membrane of rod outer segments. If the mutant protein is not targeted, the disease mechanism in CNGB1-deficient mice would be equivalent to the one leading to human CNGB1-related RP. Alternatively, if the mutant CNGB1 is produced and assembled with CNGA1, it might impair the cGMP-dependent activation of the channel.

Channelopathies and Knockout Models of the Cone Photoreceptor CNG Channel

CNGA3

Mutations in the CNGA3 subunit cause achromatopsia (total colorblindness or rod monochromacy) [90]. CNGB3 (see below) and GNAT2 (encodes cone-specific transducin alpha subunit) have been identified as additional achromatopsia genes [91–94]. Achromatopsia is a rare autosomal recessive disorder (incidence about 1 in 30,000) in which functional cones are absent from the retina [95]. Affected individuals show a total loss of color discrimination, photophobia, nystagmus, and poor visual acuity. Electroretinography reveals absent photopic (cone) responses and normal scotopic (rod) responses. Individuals with incomplete achromatopsia retain some color vision [96]. About 50 mutations have been identified in the CNGA3 gene of patients suffering from achromatopsia [97–103]. Most of the mutations are amino acid substitutions compared to a few stop-codon mutations and deletions or insertions. The effect of several mutations on channel function was examined in heterologous expression systems. CNGA3 subunits carrying mutations in the S1 region (Y181C, N182Y, L186F, and C191Y) fail to be targeted to the plasma membrane, indicating that the folding and/or subunit assembly was disturbed in these mutants [104]. Recently, a Japanese patient with congenital achromatopsia was found to carry a point mutation within the carboxy-terminal leucine zipper (CLZ) domain (L633P) [105], which is important for proper heteromeric assembly of CNG channels [106]. Profoundly impaired channel targeting may also explain the loss of function induced by several other mutations. Alternatively, mutations may alter the biophysical properties of the channels. For example, the N471S and R563H mutants were found to have an increased cGMP affinity, which may prevent complete closure of the channels in response to light stimulation [107]. Consequently, these "gain-of-function" mutations may lead to a sustained and potentially toxic elevation of intracellular Ca^{2+} levels in cone outer segments. Another example of a mutation that leads to a channel with altered biophysical properties has been recently reported by Tränkner et al. [108]. The authors investigated a mutation in the pore region of CNGA3 (T369S) that is linked to incomplete achromatopsia. When coexpressed with the CNGB3 subunit, this mutant induced robust currents. However, the channel revealed a lower efficacy of Ca^{2+} blockage at physiological membrane potentials which tentatively would lead to a lower signal-to-noise ratio of the light response, and hence, explain the lower light sensitivity observed in the patients.

CNGA3-deficient mice display the principal hallmarks of achromatopsia described above [109]. As the only exception, deletion of CNGA3 has no obvious effect on visual acuity [110]. This finding may be explained by the fact that mice lack the cone-enriched macula that confers high-acuity vision in humans [111]. CNGA3-deficient mice reveal a complete loss of cone function. By contrast, the rod photoreceptor system remains fully intact (structurally and functionally) over the whole life span of the animals. CNGA3-deficient cones show a delayed postnatal migration

behavior, form irregular synapses, and reveal a progressive degeneration [112, 113]. In addition, the postsynaptic partners (e.g., cone bipolar cells) react to the missing cone input and form ectopic synapses with rods [114]. Interestingly, dorsal cones expressing medium-wave sensitive opsin survive significantly longer (up to 22 months) than ventral cones expressing mostly short-wave sensitive opsin (less than 3 months). The reason for this difference remains to be determined. A comparable asymmetrical pattern of cone loss was observed in the retina of mice lacking the guanylyl cyclase E (GC-E) [115]. CNGA3-deficient cones fail to transport opsins into outer segments, downregulate various proteins of the phototransduction cascade and induce apoptotic death [113]. As discussed above for CNGA1/B1-deficient rods, cones of CNGA3-deficent mice may degenerate as a result of the non-functional visual cascade. Alternatively, the CNG channel may serve as an important structural protein in outer segments. Although proteins binding to the cone channel have not yet been identified, it is reasonable to assume that the cone channel like the related rod CNG channel [116–118] is part of a highly structured protein complex. Thus, the loss of CNGA3 would lead to a structural alteration of cone outer segments. The accumulation of opsins in the inner segments and somata may result from the failure of these proteins to be targeted into these irregular outer segments. Given the high expression levels of opsins, the mislocalization and accumulation of these proteins could well induce cellular stress and apoptosis. Misrouting and accumulation of proteins has been found frequently in degenerative processes in neurons [119].

The CNGA3 subunit has been identified in a number of tissues different from retina, including a subpopulation of ORNs of the olfactory epithelium, sperm, kidney, and brain (for overview [3]). CNGA3-deficient mice reveal normal fertility, indicating that CNGA3 is not essential for sperm function [109].

CNGB3

A missense mutation in the CNGB3 gene (S435F) was identified in color-blind individuals originating from the Pingelap Atoll of Micronesia [92]. In this small island, achromatopsia is very frequent and affects nearly 10% of the native population [120]. Additional mutations of the CNGB3 gene have been identified in other patients with achromatopsia, most of which lead to truncated proteins [91, 92, 99, 102, 103, 105, 121, 122]. Overall, mutations in the CNGB3 gene account for about 50% of all cases of achromatopsia [121]. A missense mutation in exon 6 of the CNGB3 gene (D262N corresponding to human D267N) and a CNGB3 null-deletion were also identified in two canine inbred strains with cone degeneration [123].

Several disease-associated mutations have been functionally characterized in *Xenopus* oocytes [122, 124] or HEK293 cells [125]. Interestingly, some mutations resulted in an increase in apparent affinity for cAMP/cGMP, e.g., the S435F mutation. CNGA3/CNGB3$_{S435F}$ heteromeric channels exhibited a fourfold increase in cAMP affinity and a twofold increase in apparent affinity for cGMP. In addition, the mutant channel had a slightly higher open probability in the presence of saturating cyclic nucleotide concentration than wild-type channels. It is unclear whether these subtle effects can account for the severe clinical phenotype of the patients. Alternatively, it is possible that the mutation prevents correct protein folding and/or assembly with the CNGA3 subunit in vivo. The finding that null mutations of CNGB3 gene induce achromatopsia strongly suggests that the CNGA3 subunit (like the CNGA1 subunit) is not able to form homomeric channels in vivo.

Knockout Models of the Olfactory CNG Channel

CNGA2

Unlike all other CNG channel genes, the CNGA2 gene is localized on the X chromosome. So far, mutations of this gene have not been identified in humans. However, four different CNGA2-deficient mouse lines have been generated to define the physiological role of this subunit [126–129]. CNGA2-deficient mice display elevated neonatal lethality due to their inefficiency to find their mother's nipples to suckle milk. Indeed, initial electroolfactogram (EOG) recordings showed that the mice exhibit no detectable responses to odorants [126]. Moreover, in perforated patch experiments, ORNs of CNGA2-deficient mice did not respond to odors or activators of the cAMP system (IBMX/forskolin) [130]. Together, these findings indicate that olfaction is conferred by a cAMP/CNGA2-mediated signaling pathway. In addition, the results strongly argue against a major contribution of other signaling cascades which have been suggested in the past (e.g., IP3-mediated olfaction; [131]). More recent studies confirmed this principal conclusion, but also uncovered that CNGA2-deficient mice are not totally anosmic. Zhao and Reed [129] found very small but reproducible EOG responses to a variety of odorants in CNGA2-deficient mice. In behavioral tests, CNGA2-deficient mice could detect and discriminate several odorants, including 2-heptanone and 2,5-dimethylpyrazine [132]. The molecular components conferring this residual olfaction remain to be determined. Pharmacological blockers suggest that the respective signaling pathway(s) are cAMP-independent. There is evidence that a small subgroup of ORNs projecting to the so-called necklace glomeruli could be involved in

non-cAMP-mediated olfaction [132]. These neurons do not express CNGA2, but rather express the CNGA3 channel and several other components that support a cGMP-signaling pathway [36–38].

Besides its key role in olfactory transduction, CNGA2 is required for normal development of the olfactory epithelium and olfactory bulb [127]. In comparison to wild-type mice, CNGA2 knockouts display a thinner olfactory epithelium and show lower expression of an olfactory marker protein. Moreover, the olfactory bulb is smaller in size, and tyrosine hydroxylase expression is reduced in most periglomerular neurons of the main olfactory bulb, but is retained in necklace glomeruli.

In the olfactory system, sensory neurons expressing a given odorant receptor (OR) project with precision to a pair of spatially invariant glomeruli within the olfactory bulb [133, 134]. Gene-targeting experiments indicate that odorant receptors are required for precise targeting [135]. In contrast, the olfactory CNG channel, and hence, odorant-induced electrical activity, does not seem to be required for proper axon guidance [128, 136]. However, this paradigm may not hold true for all subtypes of ORNs. It was reported that CNGA2-deficient ORNs expressing the M72 OR projected to four to six instead of two glomeruli in the wild-type mouse, suggesting that at least, in M72 OR neurons, CNGA2 may be important for correct axon targeting [128]. In addition, CNGA2 knockout mice fail to mate or fight, suggesting a broad and essential role of CNGA2-mediated olfaction in regulating these behaviors [137].

As CNGA2 is expressed only in the main olfactory epithelium (MOE), but not in the vomeronasal organ, this suggests a broad and essential role for the MOE in regulating these behaviors.

The olfactory CNG channel was identified in murine hippocampus, suggesting a role of this protein in memory formation [138–140]. In support of this hypothesis, hippocampal long-term potentiation (LTP) evoked by a theta burst stimulation protocol was reduced in CNGA2-deficient mice [141]. By contrast, several measures of basal synaptic activity as well as LTP induced by high-frequency stimulation seemed to be similar in wild-type and knockout mice. Behavioral tests to determine the memory performance of CNGA2-deficient mice have not been reported so far, leaving open whether or not the observed alterations of hippocampal LTP are physiologically relevant.

CNGA4

Mice deficient for the CNGA4 subunit are fertile and show no obvious morphological differences with respect to wild-type mice [142, 143]. Moreover, the olfactory epithelium and the olfactory bulb are normally developed in these mice. Maximal EOG responses induced by IBMX, an inhibitor of the phosphodiesterase, are comparable in both genotypes. However, the dose response curves are shifted to about threefold higher IBMX concentrations in the CNGA4-deficient mice, suggesting that the mutant CNG channel is less sensitive to cAMP. Indeed, the apparent cAMP affinity of the CNG channel as determined from excised patch recordings is about tenfold lower in the knockouts than in wild-type mice. Thus, CNGA4 is clearly required to confer high cAMP affinity to the olfactory channel. CNGA4-deficient mice also show a defect in odor adaptation [142, 143]. The decay time of the EOG in response to an odorant stimulus is profoundly slower in knockouts than in wild-type mice. Moreover, in paired-pulse stimulations, both EOG responses are identical in the knockout mice, whereas the second EOG is always smaller (due to adaptation) in the wild-type animals. Electrophysiological recordings in excised patches from ORNs clarified why CNGA4 is needed for odor adaptation. The recordings revealed that in the absence of CNGA4, the kinetics of Ca^{2+}-calmodulin-dependent inhibition are profoundly slower than in its presence. Thus, CNGA4 is required for fast feedback inhibition of channel activity by Ca^{2+}-calmodulin. The result also suggested the Ca^{2+}-calmodulin binding site identified in the CNGA2 subunit [144] is probably not involved in Ca^{2+}-calmodulin-mediated inhibition in vivo. Further analysis of Ca^{2+}-calmodulin binding sites of the olfactory CNG subunits confirmed this notion by demonstrating that apocalmodulin is preassociated with an IQ motif [145] present in the C-linker of CNGA4 [146]. The authors also showed that another IQ motif present in the N terminus of the CNGB1b subunit is necessary for inhibition. It was speculated that one apocalmodulin molecule binds to both sides, thereby cross-linking the CNGA4 and the CNGB1b subunit. If Ca^{2+} flows through the channel pore, it interacts with the preassociated apocalmodulin and induces a conformational change in the channel that decreases its cAMP sensitivity. As a consequence, the CNG channel closes, and the olfactory transduction pathway is switched off. Phosphoinositides were also implicated in the inhibition of the odorant response [147]. A possible mechanism for this inhibitory effect was recently discovered [148]. According to Brady et al. [148], phosphatidylinositol-3,4,5-trisphosphate (PIP_3) inhibits the olfactory CNG channel by interacting with the previously identified "silent" Ca^{2+}-calmodulin binding site of the CNGA2 subunit [146].

CNGB1b

RP patients carrying the G993V mutation in the CNGB1 gene were reported to show no olfactory impairment [89]. In contrast, CNGB1-deficient mice that lack both the retinal CNGB1a and the olfactory CNGB1b subunit reveal a

profound olfactory phenotype [149]. As reported for CNGA2-deficient mice, CNGB1 knockouts show an increased postnatal mortality and delayed body weight gain that is most probably due to a deficit in locating their mother's nipples to suckle milk. The knockout mice have a decreased olfactory performance, and their EOG responses display a reduced maximal amplitude and decelerated onset and recovery kinetics compared to wild-type mice [149]. Moreover, fast odor adaptation is impaired, confirming that CNGB1 is absolutely necessary for Ca^{2+}-calmodulin-dependent inhibition. Interestingly, the levels of CNGA2 and CNGA4 protein are dramatically reduced in the cilia of CNGB1-deficient mice. In contrast, other ciliary proteins (e.g., adenylyl cyclase III) are normally expressed. Further analysis of these mice showed that CNGA2/CNGA4-containing channels are assembled and also inserted into the plasma membrane of the knobs, but do not localize to the cilia. Experiments with MG-132, a specific proteasome inhibitor, suggest that these channels are degraded by the action of the proteasome [149]. A recent study analyzed the ciliary targeting of CNG channels in Madin–Darby canine kidney (MDCK) epithelial cells [150]. By overexpressing wild-type and mutated olfactory CNG channel subunits in these cells, a RVXP-motif in the C terminus of CNGB1b was identified that is necessary, but not sufficient, for ciliary targeting of CNGA2 [150]. Interestingly, the same report found that CNGA4 was not needed for ciliary CNG channel targeting in MDCK cells. In contrast, analysis of $CNGA4^{-/-}$ mice revealed a ciliary targeting defect similar to that present in CNGB1 knockout mice [149]. Thus, unlike MDCK cells, native ORNs require both CNGA4 and CNGB1b for normal ciliary targeting of CNGA2.

Conclusion

The use of gene ablation combined with the analysis of mutations linked to human channelopathies allowed tremendous progress towards the identification of functional roles of CNG channel subunits in vivo (Table 1). A major insight coming from these studies was that in olfactory neurons and photoreceptors, only heteromeric CNG channels with the correct subunit composition are targeted to outer segments and cilia, respectively. By contrast, homomeric channels and, probably, also heteromeric channels, containing mutations (as found in the vast majority of CNG channelopathies) are degraded and fail to be expressed at substantial levels. Interestingly, heterologous cell systems such as *Xenopus* oocytes or HEK293 cells do not contain such a "quality control system", as they express homo- and heteromeric channels equally well. The biophysical and functional properties of CNG channels are perfectly tailored to the specific requirements of rods, cones, and ORNs. Electrophysiological measurements indicate that even small alterations in the sequence of CNG channels, let alone alterations in the subunit composition, can have severe effects on the efficiency and acuity of sensory transduction. Given the high evolutionary pressure put on the maintenance of vision and olfaction, it is tempting to speculate that the quality control system has been vital for the survival of species. Future studies need to address the molecular determinants underlying the quality control system in sensory cells. Moreover, the molecular pathways linking the loss or dysfunction of CNG channels with the induction of apoptosis are by and large unknown. Finally, it will be interesting to see whether or not the loss of a CNG channel subunit can be functionally rescued, e.g., by viral or non-viral gene transfer. Patients suffering from RP and other degenerative diseases of the retina resulting from the loss of gene function would strongly profit from the availability of such therapeutic approaches.

Acknowledgment This work was supported by the Deutsche Forschungsgemeinschaft.

References

1. Burns ME, Baylor DA (2001) Activation, deactivation, and adaptation in vertebrate photoreceptor cells. Annu Rev Neurosci 24:779–805
2. Firestein S (2001) How the olfactory system makes sense of scents. Nature 413:211–218
3. Kaupp UB, Seifert R (2002) Cyclic nucleotide-gated ion channels. Physiol Rev 82:769–824
4. Ahnelt PK, Kolb H (2000) The mammalian photoreceptor mosaic-adaptive design. Prog Retin Eye Res 19:711–777
5. Yokoyama S (2000) Molecular evolution of vertebrate visual pigments. Prog Retin Eye Res 19:385–419
6. Pugh EN Jr, Duda T, Sitaramayya A, Sharma RK (1997) Photoreceptor guanylate cyclases: a review. Biosci Rep 17:429–473
7. Yang RB, Foster DC, Garbers DL, Fulle HJ (1995) Two membrane forms of guanylyl cyclase found in the eye. Proc Natl Acad Sci USA 92:602–606
8. Yau KW, Nakatani K (1984) Cation selectivity of light-sensitive conductance in retinal rods. Nature 309:352–354
9. 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
10. Yau KW, Nakatani K (1985) Light-induced reduction of cytoplasmic free calcium in retinal rod outer segment. Nature 313:579–582
11. 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
12. Schnetkamp PP (2004) The SLC24 Na+/Ca2+-K+ exchanger family: vision and beyond. Pflugers Arch 447:683–688

13. Fain GL, Matthews HR, Cornwall MC, Koutalos Y (2001) Adaptation in vertebrate photoreceptors. Physiol Rev 81:117–151
14. Koutalos Y, Yau KW (1996) Regulation of sensitivity in vertebrate rod photoreceptors by calcium. Trends Neurosci 19:73–81
15. Buck L, Axel R (1991) A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 65:175–187
16. Bakalyar HA, Reed RR (1990) Identification of a specialized adenylyl cyclase that may mediate odorant detection. Science 250:1403–1406
17. Wong ST, Trinh K, Hacker B, Chan GC, Lowe G, Gaggar A, Xia Z, Gold GH, Storm DR (2000) Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. 27:487–497
18. Nakamura T, Gold GH (1987) A cyclic nucleotidegated conductance in olfactory receptor cilia. Nature 325:442–444
19. Frings S, Seifert R, Godde M, Kaupp UB (1995) Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. Neuron 15:169–179
20. Kurahashi T, Yau KW (1993) Co-existence of cationic and chloride components in odorant-induced current of vertebrate olfactory receptor cells. Nature 363:71–74
21. Kleene SJ, Gesteland RC (1991) Calcium-activated chloride conductance in frog olfactory cilia. J Neurosci 11:3624–3629
22. Lowe G, Gold GH (1993) Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. Nature 366:283–286
23. Pifferi S, Pascarella G, Boccaccio A, Mazzatenta A, Gustincich S, Menini A, Zucchelli S (2006) Bestrophin-2 is a candidate calcium-activated chloride channel involved in olfactory transduction. Proc Natl Acad Sci USA 103:12929–12934
24. Kaneko H, Mohrlen F, Frings S (2006) Calmodulin contributes to gating control in olfactory calcium-activated chloride channels. J Gen Physiol 127:737–748
25. Kaneko H, Putzier I, Frings S, Kaupp UB, Gensch T (2004) Chloride accumulation in mammalian olfactory sensory neurons. J Neurosci 24:7931–7938
26. Reisert J, Lai J, Yau KW, Bradley J (2005) Mechanism of the excitatory Cl- response in mouse olfactory receptor neurons. Neuron 45:553–561
27. Matthews HR, Reisert J (2003) Calcium, the two-faced messenger of olfactory transduction and adaptation. Curr Opin Neurobiol 13:469–475
28. Kurahashi T, Menini A (1997) Mechanism of odorant adaptation in the olfactory receptor cell. Nature 385:725–729
29. Wei J, Zhao AZ, Chan GC, Baker LP, Impey S, Beavo JA, Storm DR (1998) Phosphorylation and inhibition of olfactory adenylyl cyclase by CaM kinase II in Neurons: a mechanism for attenuation of olfactory signals. Neuron 21:495–504
30. Leinders-Zufall T, Ma M, Zufall F (1999) Impaired odor adaptation in olfactory receptor neurons after inhibition of Ca2+/calmodulin kinase II. J Neurosci 19:RC19
31. Borisy FF, Ronnett GV, Cunningham AM, Juilfs D, Beavo J, Snyder SH (1992) Calcium/calmodulin-activated phosphodiesterase expressed in olfactory receptor neurons. J Neurosci 12:915–923
32. Yan C, Zhao AZ, Bentley JK, Loughney K, Ferguson K, Beavo JA (1995) Molecular cloning and characterization of a calmodulin-dependent phosphodiesterase enriched in olfactory sensory neurons. Proc Natl Acad Sci USA 92:9677–9681
33. Boccaccio A, Lagostena L, Hagen V, Menini A (2006) Fast adaptation in mouse olfactory sensory neurons does not require the activity of phosphodiesterase. J Gen Physiol 128:171–184
34. Chen TY, Takeuchi H, Kurahashi T (2006) Odorant inhibition of the olfactory cyclic nucleotide-gated channel with a native molecular assembly. J Gen Physiol 128:365–371
35. Zufall F, Munger SD (2001) From odor and pheromone transduction to the organization of the sense of smell. Trends Neurosci 24:191–193
36. Meyer MR, Angele A, Kremmer E, Kaupp UB, Muller F (2000) A cGMP-signaling pathway in a subset of olfactory sensory neurons. Proc Natl Acad Sci USA 97:10595–10600
37. Fulle HJ, Vassar R, Foster DC, Yang RB, Axel R, Garbers DL (1995) A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. Proc Natl Acad Sci USA 92:3571–3575
38. Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, Beavo JA (1997) A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. Proc Natl Acad Sci USA 94:3388–3395
39. Shinoda K, Shiotani Y, Osawa Y (1989) "Necklace olfactory glomeruli" form unique components of the rat primary olfactory system. J Comp Neurol 284:362–373
40. Hofmann F, Biel M, Kaupp UB (2005) International Union of Pharmacology. XLII. Compendium of voltage-gated ion channels: cyclic nucleotide-modulated channels. Pharmacol Rev 55:587–589
41. Yu FH, Catterall WA (2004) The VGL-chanome: a protein superfamily specialized for electrical signaling and ionic homeostasis. Sci STKE 2004:re15
42. Hofmann F, Biel M, Kaupp UB (2005) International Union of Pharmacology. LI. Nomenclature and structure-function relationships of cyclic nucleotide-regulated channels. Pharmacol Rev 57:455–462
43. Körschen HG, Illing M, Seifert R, Sesti F, Williams A, Gotzes S, Colville C, Muller F, Dose A, Godde M et al (1995) A 240 kDa protein represents the complete beta subunit of the cyclic nucleotide-gated channel from rod photoreceptor. Neuron 15:627–636
44. Chen TY, Peng YW, Dhallan RS, Ahamed B, Reed RR, Yau KW (1993) A new subunit of the cyclic nucleotide-gated cation channel in retinal rods. Nature 362:764–767
45. Bönigk W, Bradley J, Muller F, Sesti F, Boekhoff I, Ronnett GV, Kaupp UB, Frings S (1999) The native rat olfactory cyclic nucleotide-gated channel is composed of three distinct subunits. J Neurosci 19:5332–5347
46. Sautter A, Zong X, Hofmann F, Biel M (1998) An isoform of the rod photoreceptor cyclic nucleotide-gated channel beta subunit expressed in olfactory neurons. Proc Natl Acad Sci USA 95:4696–4701
47. Long SB, Campbell EB, Mackinnon R (2005) Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. Science 309:897–903
48. Higgins MK, Weitz D, Warne T, Schertler GF, Kaupp UB (2002) Molecular architecture of a retinal cGMP-gated channel: the arrangement of the cytoplasmic domains. Embo J 21:2087–2094
49. Shi N, Ye S, Alam A, Chen L, Jiang Y (2006) Atomic structure of a Na+- and K+-conducting channel. Nature 440:570–574
50. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R (2002) Crystal structure and mechanism of a calcium-gated potassium channel. Nature 417:515–522
51. Giorgetti A, Nair AV, Codega P, Torre V, Carloni P (2005) Structural basis of gating of CNG channels. FEBS Lett 579:1968–1972
52. Zagotta WN, Olivier NB, Black KD, Young EC, Olson R, Gouaux E (2003) Structural basis for modulation and agonist specificity of HCN pacemaker channels. Nature 425:200–205
53. Kaupp UB, Niidome T, Tanabe T, Terada S, Bonigk W, Stuhmer W, Cook NJ, Kangawa K, Matsuo H, Hirose T, Miyata T, Numa S (1989) Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. Nature 342:762–766

54. Zong X, Zucker H, Hofmann F, Biel M (1998) Three amino acids in the C-linker are major determinants of gating in cyclic nucleotide-gated channels. Embo J 17:353–362
55. Paoletti P, Young EC, Siegelbaum SA (1999) C-Linker of cyclic nucleotide-gated channels controls coupling of ligand binding to channel gating. J Gen Physiol 113:17–34
56. Sunderman ER, Zagotta WN (1999) Mechanism of allosteric modulation of rod cyclic nucleotide-gated channels. J Gen Physiol 113:601–620
57. Kaupp UB, Seifert R (2001) Molecular diversity of pacemaker ion channels. Annu Rev Physiol 63:235–257
58. Biel M, Schneider A, Wahl C (2002) Cardiac HCN channels: structure, function, and modulation. Trends Cardiovasc Med 12:206–212
59. Robinson RB, Siegelbaum SA (2003) Hyperpolarization-activated cation currents: from molecules to physiological function. Annu Rev Physiol 65:453–480
60. Frère SG, Kuisle M, Lüthi A (2004) Regulation of recombinant and native hyperpolarization-activated cation channels. Mol Neurobiol 30:279–305
61. Warmke JW, Ganetzky B (1994) A family of potassium channel genes related to eag in Drosophila and mammals. Proc Natl Acad Sci USA 91:3438–3442
62. Schachtman DP, Schroeder JI, Lucas WJ, Anderson JA, Gaber RF (1992) Expression of an inward-rectifying potassium channel by the Arabidopsis KAT1 cDNA. Science 258:1654–1658
63. Matulef K, Zagotta WN (2003) Cyclic nucleotide-gated ion channels. Annu Rev Cell Dev Biol 19:23–44
64. Zhong H, Molday LL, Molday RS, Yau KW (2002) The heteromeric cyclic nucleotide-gated channel adopts a 3A:1B stoichiometry. Nature 420:193–198
65. Weitz D, Ficek N, Kremmer E, Bauer PJ, Kaupp UB (2002) Subunit stoichiometry of the CNG channel of rod photoreceptors. Neuron 36:881–889
66. 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
67. Peng C, Rich ED, Varnum MD (2004) Subunit configuration of heteromeric cone cyclic nucleotide-gated channels. Neuron 42:401–410
68. Zheng J, Zagotta WN (2004) Stoichiometry and assembly of olfactory cyclic nucleotide-gated channels. Neuron 42:411–421
69. Dzeja C, Hagen V, Kaupp UB, Frings S (1999) Ca2+ permeation in cyclic nucleotide-gated channels. Embo J 18:131–144
70. Bradley J, Reisert J, Frings S (2005) Regulation of cyclic nucleotide-gated channels. Curr Opin Neurobiol 15:343–349
71. Tanaka JC, Eccleston JF, Furman RE (1989) Photoreceptor channel activation by nucleotide derivatives. Biochemistry 28:2776–2784
72. Frings S, Lynch JW, Lindemann B (1992) Properties of cyclic nucleotide-gated channels mediating olfactory transduction. Activation, selectivity, and blockage. J Gen Physiol 100:45–67
73. Dryja TP, Finn JT, Peng YW, McGee TL, Berson EL, Yau KW (1995) Mutations in the gene encoding the alpha subunit of the rod cGMP-gated channel in autosomal recessive retinitis pigmentosa. Proc Natl Acad Sci USA 92:10177–10181
74. Kennan A, Aherne A, Humphries P (2005) Light in retinitis pigmentosa. Trends Genet 21:103–110
75. Kalloniatis M, Fletcher EL (2004) Retinitis pigmentosa: understanding the clinical presentation, mechanisms and treatment options. Clin Exp Optom 87:65–80
76. Mallouk N, Ildefonse M, Pages F, Ragno M, Bennett N (2002) Basis for intracellular retention of a human mutant of the retinal rod channel alpha subunit. J Membr Biol 185:129–136
77. Trudeau MC, Zagotta WN (2002) An intersubunit interaction regulates trafficking of rod cyclic nucleotide-gated channels and is disrupted in an inherited form of blindness. Neuron 34:197–207
78. Leconte L, Barnstable CJ (2000) Impairment of rod cGMP-gated channel alpha-subunit expression leads to photoreceptor and bipolar cell degeneration. Invest Ophthalmol Vis Sci 41:917–926
79. Lisman J, Fain G (1995) Support for the equivalent light hypothesis for RP. Nat Med 1:1254–1255
80. Fain GL, Lisman JE (1999) Light, Ca2+, and photoreceptor death: new evidence for the equivalent-light hyphothesis from arrestin knockout mice. Invest Ophthalmol Vis Sci 40:2770–2772
81. Pierce EA (2001) Pathways to photoreceptor cell death in inherited retinal degenerations. Bioessays 23:605–618
82. Wenzel A, Grimm C, Samardzija M, Reme CE (2005) Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration. Prog Retin Eye Res 24:275–306
83. Fain GL (2006) Why photoreceptors die (and why they don't). Bioessays 28:344–354
84. Ardell MD, Bedsole DL, Schoborg RV, Pittler SJ (2000) Genomic organization of the human rod photoreceptor cGMP-gated cation channel beta-subunit gene. Gene 245:311–318
85. Sugimoto Y, Yatsunami K, Tsujimoto M, Khorana HG, Ichikawa A (1991) The amino acid sequence of a glutamic acid-rich protein from bovine retina as deduced from the cDNA sequence. Proc Natl Acad Sci USA 88:3116–3119
86. Biel M, Zong X, Ludwig A, Sautter A, Hofmann F (1996) Molecular cloning and expression of the Modulatory subunit of the cyclic nucleotide-gated cation channel. J Biol Chem 271:6349–6355
87. Wiesner B, Weiner J, Middendorff R, Hagen V, Kaupp UB, Weyand I (1998) Cyclic nucleotide-gated channels on the flagellum control Ca2+ entry into sperm. J Cell Biol 142:473–484
88. Hüttl S, Michalakis S, Seeliger M, Luo D-G, Acar N, Geiger H, Hudl K, Mader R, Haverkamp S, Moser M, Pfeifer A, Gerstner A, Yau K-W, Biel M (2005) Impaired Channel Targeting and Retinal Degeneration in Mice Lacking the Cyclic Nucleotide-Gated Channel Subunit CNGB1. J Neurosci 25:130–138
89. Bareil C, Hamel CP, Delague V, Arnaud B, Demaille J, Claustres M (2001) Segregation of a mutation in CNGB1 encoding the beta-subunit of the rod cGMP-gated channel in a family with autosomal recessive retinitis pigmentosa. Hum Genet 108:328–334
90. Kohl S, Marx T, Giddings I, Jagle H, Jacobson SG, Apfelstedt-Sylla E, Zrenner E, Sharpe LT, Wissinger B (1998) Total colourblindness is caused by mutations in the gene encoding the alpha-subunit of the cone photoreceptor cGMP-gated cation channel. Nat Genet 19:257–259
91. Kohl S, Baumann B, Broghammer M, Jagle H, Sieving P, Kellner U, Spegal R, Anastasi M, Zrenner E, Sharpe LT, Wissinger B (2000) Mutations in the CNGB3 gene encoding the beta-subunit of the cone photoreceptor cGMP-gated channel are responsible for achromatopsia (ACHM3) linked to chromosome 8q21. Hum Mol Genet 9:2107–2116
92. Sundin OH, Yang JM, Li Y, Zhu D, Hurd JN, Mitchell TN, Silva ED, Maumenee IH (2000) Genetic basis of total colourblindness among the Pingelapese islanders. Nat Genet 25:289–293
93. Aligianis IA, Forshew T, Johnson S, Michaelides M, Johnson CA, Trembath RC, Hunt DM, Moore AT, Maher ER (2002) Mapping of a novel locus for achromatopsia (ACHM4) to 1p and identification of a germline mutation in the alpha subunit of cone transducin (GNAT2). J Med Genet 39:656–660
94. Kohl S, Baumann B, Rosenberg T, Kellner U, Lorenz B, Vadala M, Jacobson SG, Wissinger B (2002) Mutations in the cone photoreceptor G-protein alpha-subunit gene GNAT2 in patients with achromatopsia. Am J Hum Genet 71:422–425
95. Sharpe LT, Stockman A, Jägle H, Nathans J (1999) In *color vision: from genes to perception*. Cambridge University Press, Cambridge, UK, pp 3–52

96. Pokorny J, Smith VC, Pinckers AJ, Cozijnsen M(1982) Classification of complete and incomplete autosomal recessive achromatopsia. Graefes Arch Clin Exp Ophthalmol 219:121–130
97. Wissinger B, Gamer D, Jägle H, Giorda R, Marx T, Mayer S, Tippmann S, Broghammer M, Jurklies B, Rosenberg T, Jacobson SG, Sener EC, Tatlipinar S, Hoyng CB, Castellan C, Bitoun P, Andreasson S, Rudolph G, Kellner U, Lorenz B, Wolff G, Verellen-Dumoulin C, Schwartz M, Cremers FP, Apfelstedt-Sylla E, Zrenner E, Salati R, Sharpe LT, Kohl S (2001) CNGA3 mutations in hereditary cone photoreceptor disorders. Am J Hum Genet 69:722–737
98. Eksandh L, Kohl S, Wissinger B (2002) Clinical features of achromatopsia in Swedish patients with defined genotypes. Ophthalmic Genet 23:109–120
99. Johnson S, Michaelides M, Aligianis IA, Ainsworth JR, Mollon JD, Maher ER, Moore AT, Hunt DM (2004) Achromatopsia caused by novel mutations in both CNGA3 and CNGB3. J Med Genet 41:e20
100. Kellner U, Wissinger B, Kohl S, Kraus H, Foerster MH (2004) [Molecular genetic findings in patients with congenital cone dysfunction. Mutations in the CNGA3, CNGB3, or GNAT2 genes]. Ophthalmologe 101:830–835
101. Nishiguchi KM, Sandberg MA, Gorji N, Berson EL, Dryja TP (2005) Cone cGMP-gated channel mutations and clinical findings in patients with achromatopsia, macular degeneration, and other hereditary cone diseases. Hum Mutat 25:248–258
102. Varsanyi B, Wissinger B, Kohl S, Koeppen K, Farkas A (2005) Clinical and genetic features of Hungarian achromatopsia patients. Mol Vis 11:996–1001
103. Michaelides M, Hunt DM, Moore AT (2004) The cone dysfunction syndromes. Br J Ophthalmol 88:291–297
104. Patel KA, Bartoli KM, Fandino RA, Ngatchou AN, Woch G, Carey J, Tanaka JC (2005) Transmembrane S1 mutations in CNGA3 from achromatopsia 2 patients cause loss of function and impaired cellular trafficking of the cone CNG channel. Invest Ophthalmol Vis Sci 46:2282–2290
105. Goto-Omoto S, Hayashi T, Gekka T, Kubo A, Takeuchi T, Kitahara K (2006) Compound heterozygous CNGA3 mutations (R436W, L633P) in a Japanese patient with congenital achromatopsia. Vis Neurosci 23:395–402
106. Zhong H, Lai J, Yau KW (2003) Selective heteromeric assembly of cyclic nucleotide-gated channels. Proc Natl Acad Sci USA 100:5509–5513
107. 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–C198
108. Tränkner D, Jagle H, Kohl S, Apfelstedt-Sylla E, Sharpe LT, Kaupp UB, Zrenner E, Seifert R, Wissinger B (2004) Molecular basis of an inherited form of incomplete achromatopsia. J Neurosci 24:138–147
109. 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 USA 96:7553–7557
110. Schmucker C, Seeliger M, Humphries P, Biel M, Schaeffel F (2005) Grating acuity at different luminances in wild-type mice and in mice lacking rod or cone function. Invest Ophthalmol Vis Sci 46:398–407
111. Szel A, Lukats A, Fekete T, Szepessy Z, Rohlich P (2000) Photoreceptor distribution in the retinas of subprimate mammals. J Opt Soc Am A Opt Image Sci Vis 17:568–579
112. Claes E, Seeliger M, Michalakis S, Biel M, Humphries P, Haverkamp S (2004) Morphological characterization of the retina of the CNGA3(-/-)Rho(-/-) mutant mouse lacking functional cones and rods. Invest Ophthalmol Vis Sci 45:2039–2048
113. Michalakis S, Geiger H, Haverkamp S, Hofmann F, Gerstner A, Biel M (2005) Impaired opsin targeting and cone photoreceptor migration in the retina of mice lacking the cyclic nucleotide-gated channel CNGA3. Invest Ophthalmol Vis Sci 46:1516–1524
114. Haverkamp S, Michalakis S, Claes E, Seeliger MW, Humphries P, Biel M, Feigenspan A (2006) Synaptic plasticity in CNGA3 (-/-) mice: cone bipolar cells react on the missing cone input and form ectopic synapses with rods. J Neurosci 26:5248–5255
115. Coleman JE, Zhang Y, Brown GA, Semple-Rowland SL (2004) Cone cell survival and downregulation of GCAP1 protein in the retinas of GC1 knockout mice. Invest Ophthalmol Vis Sci 45:3397–3403
116. Schwarzer A, Schauf H, Bauer PJ (2000) Binding of the cGMP-gated channel to the Na/Ca-K exchanger in rod photoreceptors. J Biol Chem 275:13448–13454
117. Poetsch A, Molday LL, Molday RS (2001) The cGMP-gated channel and related glutamic acid-rich proteins interact with peripherin-2 at the rim region of rod photoreceptor disc membranes. J Biol Chem 276:48009–48016
118. Kang K, Bauer PJ, Kinjo TG, Szerencsei RT, Bonigk W, Winkfein RJ, Schnetkamp PP (2003) Assembly of retinal rod or cone Na(+)/Ca(2+)-K(+) exchanger oligomers with cGMP-gated channel subunits as probed with heterologously expressed cDNAs. Biochemistry 42:4593–4600
119. Taylor JP, Hardy J, Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. Science 296:1991–1995
120. Sacks OW (1997) *The island of the colorblind*. A.A. Knopf, New York
121. Kohl S, Varsanyi B, Antunes GA, Baumann B, Hoyng CB, Jagle H, Rosenberg T, Kellner U, Lorenz B, Salati R, Jurklies B, Farkas A, Andreasson S, Weleber RG, Jacobson SG, Rudolph G, Castellan C, Dollfus H, Legius E, Anastasi M, Bitoun P, Lev D, Sieving PA, Munier FL, Zrenner E, Sharpe LT, Cremers FP, Wissinger B (2005) CNGB3 mutations account for 50% of all cases with autosomal recessive achromatopsia. Eur J Hum Genet 13:302–308
122. Bright SR, Brown TE, Varnum MD (2005) Disease-associated mutations in CNGB3 produce gain of function alterations in cone cyclic nucleotide-gated channels. Mol Vis 11:1141–1150
123. Sidjanin DJ, Lowe JK, McElwee JL, Milne BS, Phippen TM, Sargan DR, Aguirre GD, Acland GM, Ostrander EA (2002) Canine CNGB3 mutations establish cone degeneration as orthologous to the human achromatopsia locus ACHM3. Hum Mol Genet 11:1823–1833
124. 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
125. Okada A, Ueyama H, Toyoda F, Oda S, Ding WG, Tanabe S, Yamade S, Matsuura H, Ohkubo I, Kani K (2004) Functional role of hCngb3 in regulation of human cone cng channel: effect of rod monochromacy-associated mutations in hCNGB3 on channel function. Invest Ophthalmol Vis Sci 45:2324–2332
126. Brunet LJ, Gold GH, Ngai J (1996) General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. Neuron 17:681–693
127. Baker H, Cummings DM, Munger SD, Margolis JW, Franzen L, Reed RR, Margolis FL (1999) Targeted deletion of a cyclic nucleotide-gated channel subunit (OCNC1): biochemical and morphological consequences in adult mice. J Neurosci 19:9313–9321
128. Zheng C, Feinstein P, Bozza T, Rodriguez I, Mombaerts P (2000) Peripheral olfactory projections are differentially affected in mice deficient in a cyclic nucleotide-gated channel subunit. Neuron 26:81–91

129. Zhao H, Reed RR (2001) X inactivation of the OCNC1 channel gene reveals a role for activity-dependent competition in the olfactory system. Cell 104:651–660
130. Delay R, Restrepo D (2004) Odorant responses of dual polarity are mediated by cAMP in mouse olfactory sensory neurons. J Neurophysiol 92:1312–1319
131. Boekhoff I, Tareilus E, Strotmann J, Breer H (1990) Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. Embo J 9:2453–2458
132. Lin W, Arellano J, Slotnick B, Restrepo D (2004) Odors detected by mice deficient in cyclic nucleotide-gated channel subunit A2 stimulate the main olfactory system. J Neurosci 24:3703–3710
133. Ressler KJ, Sullivan SL, Buck LB (1994) Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. Cell 79:1245–1255
134. Vassar R, Chao SK, Sitcheran R, Nunez JM, Vosshall LB, Axel R (1994) Topographic organization of sensory projections to the olfactory bulb. Cell 79:981–991
135. Wang F, Nemes A, Mendelsohn M, Axel R (1998) Odorant receptors govern the formation of a precise topographic map. Cell 93:47–60
136. Lin DM, Wang F, Lowe G, Gold GH, Axel R, Ngai J, Brunet L (2000) Formation of precise connections in the olfactory bulb occurs in the absence of odorant-evoked neuronal activity. Neuron 26:69–80
137. Mandiyan VS, Coats JK, Shah NM (2005) Deficits in sexual and aggressive behaviors in Cnga2 mutant mice. Nat Neurosci 8:1660–1662
138. Leinders-Zufall T, Rosenboom H, Barnstable CJ, Shepherd GM, Zufall F (1995) A calcium-permeable cGMP-activated cation conductance in hippocampal neurons. Neuroreport 6:1761–1765
139. Kingston PA, Zufall F, Barnstable CJ (1996) Rat hippocampal neurons express genes for both rod retinal and olfactory cyclic nucleotide-gated channels: Novel targets for cAMP/cGMP function. Proc Natl Acad Sci USA 93:10440–10445
140. Bradley J, Zhang Y, Bakin R, Lester HA, Ronnett GV, Zinn K (1997) Functional Expression of the Heteromeric "Olfactory" Cyclic Nucleotide-Gated Channel in the Hippocampus: A Potential Effector of Synaptic Plasticity in Brain Neurons. J Neurosci 17:1993–2005
141. Parent A, Schrader K, Munger SD, Reed RR, Linden DJ, Ronnett GV (1998) Synaptic transmission and hippocampal long-term potentiation in olfactory cyclic nucleotide-gated channel type 1 null mouse. J Neurophysiol 79:3295–3301
142. Munger SD, Lane AP, Zhong H, Leinders-Zufall T, Yau KW, Zufall F, Reed RR (2001) Central role of the CNGA4 channel subunit in Ca2+-calmodulin-dependent odor adaptation. Science 294:2172–2175
143. Kelliher KR, Ziesmann J, Munger SD, Reed RR, Zufall F (2003) Importance of the CNGA4 channel gene for odor discrimination and adaptation in behaving mice. Proc Natl Acad Sci USA 100:4299–4304
144. Liu M, Chen TY, Ahamed B, Li J, Yau KW (1994) Calcium-calmodulin modulation of the olfactory cyclic nucleotide-gated cation channel. Science 266:1348–1354
145. Rhoads AR, Friedberg F (1997) Sequence motifs for calmodulin recognition. Faseb J 11:331–340
146. Bradley J, Bonigk W, Yau KW, Frings S (2004) Calmodulin permanently associates with rat olfactory CNG channels under native conditions. Nat Neurosci 7:705–710
147. Spehr M, Wetzel CH, Hatt H, Ache BW (2002) 3-phosphoinositides modulate cyclic nucleotide signaling in olfactory receptor neurons. Neuron 33:731–739
148. Brady JD, Rich ED, Martens JR, Karpen JW, Varnum MD, Brown RL (2006) Interplay between PIP3 and calmodulin regulation of olfactory cyclic nucleotide-gated channels. Proc Natl Acad Sci USA 103:15635–15640
149. Michalakis S, Reisert J, Geiger H, Wetzel CH, Zong X, Bradley J, Spehr M, Hüttl S, Gerstner A, Pfeifer A, Hatt H, Yau KW, Biel M (2006) Loss of CNGB1 protein leads to olfactory dysfunction and subciliary cyclic nucleotide-gated channel trapping. J Biol Chem 281:35156–35166
150. Jenkins PM, Hurd TW, Zhang L, McEwen DP, Brown RL, Margolis B, Verhey KJ, Martens JR (2006) Ciliary Targeting of Olfactory CNG Channels Requires the CNGB1b Subunit and the Kinesin-2 Motor Protein, KIF17. Curr Biol 16:1211
151. Michaelides M, Aligianis IA, Ainsworth JR, Good P, Mollon JD, Maher ER, Moore AT, Hunt DM (2004) Progressive cone dystrophy associated with mutation in CNGB3. Invest Ophthalmol Vis Sci 45:1975–1982