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Cyclic nucleotide–gated channels

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Cyclic nucleotide–gated channels

Michael D. Varnum and Gucan Dai

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24.1 INTRODUCTION
Ion channels that are activated by the direct binding of intracellular cyclic nucleotides are best known for the essential role they play in sensory transduction, including vision and olfaction. In these sensory cells, they transduce a chemical signal produced by the stimulus input—a change in the intracellular concentration of guanosine 3',5'-cyclic monophosphate (cGMP) or adenosine 3',5'-cyclic monophosphates (cAMP)—into an electrical response via a change in cation conductance through the channel pore. Cyclic nucleotide–gated (CNG) channels are tetrameric complexes of homologous subunits, with each subunit contributing a cyclic nucleotide–binding domain (CNBD), part of the ion conduction pathway, and the fundamental machinery to covert cGMP or cAMP binding into channel opening. Here, we review their functional and structural properties and their physiological and pathophysiological roles.

24.2 PHYSIOLOGICAL ROLES OF CNG CHANNELS
The most prominent known role of CNG channels is in sensory cells, where they help to convert sensory inputs that alter cyclic nucleotide concentrations into electrical responses. CNG channels were first described by Fesenko and coworkers in the outer segment membrane of retinal rod photoreceptors (Fesenko et al., 1985). In rod and cone photoreceptors, these channels conduct a cation current (the dark current) in the absence of light; photoactivation of the light receptor opsin activates the G protein transducin, which in turn activates a cGMP-specific phosphodiesterase (PDE) leading to the hydrolysis of cGMP and closure of the CNG channel (Figure 24.1) (Stryer, 1987; Burns and Arshavsky, 2005). Decreased channel conductance produces hyperpolarization of the membrane and decreased neurotransmitter release onto second-order cells. In addition, CNG channels located at cone photoreceptor synapses onto bipolar cells have been shown to modulate synaptic transmission and mediate the effects of nitric oxide (Rieke and Schwartz, 1994; Savchenko et al., 1997).

CNG channels also play a critical role in olfactory transduction (Lancet, 1986; Nakamura and Gold, 1987; Menini, 1995). Olfactory sensory neurons (OSNs) in the olfactory epithelium detect odorants via diverse G protein–coupled olfactory receptors. Olfactory receptors are coupled to G{\text{ol}} activation, which turns on adenylate cyclase (AC), thus leading to increased production of cAMP. Increased cAMP opens the olfactory CNG channels, which depolarize the olfactory neurons. Native olfactory CNG channels are highly permeable to Ca\textsuperscript{2+}, and Ca\textsuperscript{2+} amplifies olfactory signal transduction by opening Ca\textsuperscript{2+}-activated chloride channels. Chloride ions flowing out of the cell further depolarize the OSNs, which helps induce neurotransmitter release from olfactory receptor cells onto second-order neurons (Frings, 2001). A subset of olfactory receptor neurons utilize cGMP signaling components, including a specialized CNG channel, rather than the cAMP-based components found in the principal olfactory neurons (Meyer et al., 2000).
CNG channels are proposed to have several physiological functions outside of their canonical contributions to sensory signaling (vision and olfaction); they have been shown to be expressed in a wide variety of cell types (Distler et al., 1994) and to play diverse roles in multiple tissues. For example, CNG channels contribute to nerve growth cone guidance by building blocks (subunits) of CNG channels. The first CNG channel is shown within the plasma membrane of the outer segment. \( \text{Ca}^{2+} \) entering through CNG channels can regulate guanylyl cyclase (GC) via binding to GC-activating protein (GCAP). In addition, CNG channel activity can be modulated by \( \text{Ca}^{2+}-\text{CaM} \) or membrane-bound phosphoinositides (PIP\(_2\)). The potassium-dependent, \( \text{Na}^+-\text{Ca}^{2+} \) exchanger (NCKX) extrudes \( \text{Ca}^{2+} \) from the photoreceptor, which helps to balance \( \text{Ca}^{2+} \) levels within the outer segment.

### 24.3 CNG CHANNEL SUBUNIT DIVERSITY AND BASIC STRUCTURAL ORGANIZATION

Six paralogous genes in mammals encode the fundamental building blocks (subunits) of CNG channels. The first CNG channel cDNA to be cloned and heterologously expressed encoded the rod photoreceptor alpha subunit (CNGA1) (Kaupp et al., 1989). Subsequently, cDNAs for CNGA2, CNGA3, CNGA4, CNGB1, and CNGB3 were isolated (Dhallan et al., 1990; Bönigk et al., 1993; Chen et al., 1993; Bradley et al., 1994; Liman and Buck, 1994; Gerstner et al., 2000). CNG channels are homo- or heteromeric assemblies of some combination of the six possible pore-forming subunits (Figure 24.2). The CNGA1–CNGA3 subunits can form functional channels when expressed alone as homomultimers, but the other subunit types cannot. Native rod photoreceptor channels are composed of three CNGA1 and one CNGB1a subunits (Weitz et al., 2002; Zheng et al., 2002; Zhong et al., 2002); cone CNG channels are thought to consist of two CNGA3 and two CNGB3 subunits (Peng et al., 2004); but also see Zhong et al., 2003; Ding et al., 2012); and the olfactory channels have two CNGA2, one CNGB4, and one CNGB1b subunits (Zheng and Zagotta, 2004). CNG channels are part of the pore loop, cation-selective superfamily of ion channels (Jan and Jan, 1990). The basic architecture of CNG channels is similar to that of voltage-dependent K\(^+\) channels, characterized by tetrameric assembly of pore-forming subunits. Like K\(^+\) channels, each CNG channel subunit has six transmembrane domains (S1–S6), with a vestibual voltage sensor-like domain (S1–S4), and an ion conduction pathway lined by part of the pore loop linking S5 and S6 along with the distal S6 region. Each subunit also presents cytoplasmic amino- (N) and carboxy- (C) terminal domains, with a CNBD located in the C-terminal region. A C-linker domain connects the CNBD to the pore-forming domain, helping to couple ligand binding to channel opening.

The nearest relatives of CNG channels are hyperpolarization-activated and cyclic nucleotide-regulated (HCN1–4) channels and the KCNH family of voltage-gated potassium channels, which includes ether-à-go-go (EAG, Kv10), EAG-related gene (ERG, Kv11), and EAG-like (ELK, Kv12) channels. In contrast...
Ion channel families

24.3 CNG channel subunit diversity and basic structural organization

To CNG channels, HCN channels require membrane voltage changes for activation, but cyclic nucleotide binding can regulate their gating properties. KCNH channels also are voltage gated; the region homologous to the CNBD of CNG and HCN channels, however, does not bind cyclic nucleotides (Brelidze et al., 2009) but instead possesses an intrinsic ligand formed by part of the channel polypeptide that substitutes for the cyclic nucleotide within this cyclic nucleotide–binding homology domain (CNBHD) (Brelidze et al., 2012).

Diverse, related CNG channels exist in more distant parts of the phylogenetic landscape. Noted examples include the TAX2 and TAX4 subunits of the nematode Caenorhabditis elegans, forming channels that play roles in chemosensation in these organisms (Hellman and Shen, 2011) and the bacterial (Nimigean et al., 2004) and plant CNG channels (Kaplan et al., 2007). Some related channels have unique structural features that distinguish them from their mammalian relatives. For example, bacterial MloK CNG channels exhibit a dramatically shorter C-linker region proximal to the CNBD (Clayton et al., 2004). In addition, structure–function studies of MloK channels suggest that compared to mammalian CNG channels, this bacterial CNG channel exhibits less cooperativity during gating (Cukkemane et al., 2007), presumably because the CNBDs function independently (Chiu et al., 2007). More recently, a novel potassium-selective cyclic nucleotide-gated channel (CNGK) from Arbacia punctulata was discovered and characterized (Strünker et al., 2006; Bönigk et al., 2009). This channel comprises a single polypeptide chain with four pseudo-subunit domains, reflecting an architecture similar to that of voltage–dependent Na+- and Ca2+-selective channels. For CNGK channels, only the CNBD in pseudo-subunit repeat three appears to be necessary for channel activation; consistent with this observation, these channels exhibit little binding cooperativity (Bönigk et al., 2009).

In addition to the combinatorial assembly of differentially expressed subunits described earlier, CNG channel diversity also arises from alternative splicing of precursor mRNAs. The generation of CNG channel subunit variants via alternative splicing was first described for the CNGB1 gene. A long form CNGB1 subunit (CNGB1a) is expressed in rod photoreceptor cells, while olfactory receptor neurons produce a shorter isoform (CNGB1b) that lacks much of the N-terminal cytoplasmic domain present in the long variant (Körösch et al., 1995; Sautter et al., 1998). In addition, alternative splicing can generate two soluble isoforms (long and short) representing only the N-terminal glutamic acid–rich protein (GARP) domain of CNGB1, without the channel-forming region (Cukkemane et al., 2007). The GARP domain of CNGB1a and the related soluble forms have been shown to be critical for protein–protein interactions within the photoreceptor outer segment (Körösch et al., 1999; Haber-Pohlmeier et al., 2007; Zhang et al., 2009;
Figure 24.3 Alternative splicing generates CNG channel subunit diversity. (a) CNGA3 splicing patterns for constitutive and alternative cassette exons across representative species. Exon (box) but not intron (line) length is presented to scale. (b) Four protein isoforms of human CNGA3 produced by inclusion or exclusion of regions encoded by optional exons 3 and 5. (c) Protein isoforms of CNGB1 produced by alternative splicing. The long CNGB1a isoform includes a GARP domain, while the short CNGB1b isoform does not. CaM-binding sites are located within the N-terminal region. GARP domains also are expressed as soluble proteins (GARP1 and GARP2) within rod photoreceptors.

Ritter et al., 2011). Recently, examination of a disease-associated mutation in CNGB1 has demonstrated that the GARP domain can act as a gating inhibitor in the context of heteromeric channels (Michalakis et al., 2011b). Second, CNGA3 transcripts can produce subunit isoforms via the use of optional cassette exons that encode regions of the N-terminal cytoplasmic domain (Figure 24.3a and b). While alternative splicing of CNGA3 is conserved across several species, some of the incorporated optional exons and the exact splicing patterns differ among CNGA3 orthologs. One optional cassette exon found in human CNGA3 transcripts (Wissinger et al., 2001; Cassar et al., 2004) appears to be unique to humans and functionally absent even from CNGA3 of other primates. While CNGA3 alternative splicing does not influence the fundamental gating properties of the channel, it appears to confer variations in sensitivity to modulation by second messengers (Bö尼克 et al., 1996; Dai et al., 2014). Finally, sequence evidence suggests that CNGA1 can also give rise to protein variants via alternative splicing and/or alternative transcriptional start sites. Similar to CNGB1 and CNGA3 variants, these events lead to alterations within the N-terminal cytoplasmic region of the protein. To our knowledge, the functional significance of these changes has not yet been determined. Other CNG channel genes have not been extensively examined regarding possible protein variants.

While voltage sensor domains (VSDs) (S1–S6) are present in CNG channels, including positively charged residues in a repeated R/KXX pattern within S4, these do not support voltage-dependent channel activation. CNG channels instead exhibit only weak voltage dependence in their apparent affinity for cGMP, showing less than a twofold change K1/2,cGMP with a membrane voltage change from −100 to +100 mV. Tang and Papazian have shown that the S4 domain of CNGA2 can functionally replace S4 in voltage-gated EAG channels; they proposed that in CNG channels, the voltage sensor may exist in a constitutively activated position (Tang and Papazian, 1997). At the level of the transmembrane domains, CNG channels are thought to exhibit the swapped-domain configuration common to the voltage-gated channel superfamily (Long et al., 2005), with vestigial domains from each CNG channel subunit interacting with the pore-forming domains of adjacent subunits. Offset VSDs may position N-terminal cytoplasmic domains for interactions with C-terminal cytoplasmic domains. Indeed, intersubunit N-C interactions are a well-characterized feature of CNG channels, demonstrating intriguing roles in channel regulation (Gordon et al., 1997; Varnum and Zagotta, 1997; Rosenbaum and Gordon, 2002; Trudeau and Zagotta, 2002a; Zheng et al., 2003; Michalakis et al., 2011b; Dai and Varnum, 2013), although detailed structural information about these N–C interactions is currently missing.

The basic structural organization of CNG channels at the level of the C-linker and CNBD is thought to share fundamental features with the corresponding parts of homologous channels (Craven and Zagotta, 2006). Structural studies of the C-terminal region of HCN channels with bound ligand reveal a symmetrical tetramer arrangement, with contacts made between the C-linker regions of adjacent subunits (Zagotta et al., 2003; Flynn et al., 2007). Structural studies of related zELK and mEAG1 channels demonstrate dimeric assembly of C-terminal domains (Breidize et al., 2012; Haitin et al., 2013); yet, the C-linker/CNBHD structure of agERG is monomeric (Breidize et al., 2013). Another defined structural element in CNG channels is the post-CNBD region of CNGA subunits, which presents a carboxy-terminal, leucine-zipper (CLZ) domain that has been shown to play an important role in channel assembly (Trudeau and Zagotta, 2002a; Zhong et al., 2003). In addition, the CLZ domain is proposed to be a critical determinant for subunit stoichiometry and arrangement in heteromeric channels (Zhong et al., 2002, 2003; Shuart et al., 2011).
Zagotta, 2004; Zhou et al., 2004; Biskup et al., 2007), with possible state-dependent variations. There are likely to be as yet uncharacterized dynamic relationships between the C-linkers, CNBDs, post-CNBD regions, and/or N-terminal regions of CNG channels, involving changes in symmetry, orientation, and/or interactions among the domains (within and between subunits), which ultimately help govern channel gating and regulation.

### 24.4 GATING OF CNG CHANNELS

CNG channels are activated by binding of cyclic nucleotides to the CNBDs. Cyclic nucleotide binding induces allosteric conformational changes that are transduced via the C-linker, the inner helix (S6), the pore helix, and the selectivity filter, ultimately opening the CNG channel ion conduction pathway.

#### 24.4.1 LIGAND BINDING TO CNG CHANNELS

Each subunit of CNG channels contains a CNBD within the cytoplasmic C-terminal region. Apparent ligand affinity and ligand efficacy for CNG channels vary depending on subunit composition, but generally cGMP is a better agonist than cAMP (Figure 24.4). The CNBD comprises of three α-helices, termed the A-, B-, and C-helix, and a β-roll domain with eight β strands located between the A-helix and the B-helix (Figure 24.5a). The CNBDs of CNG channels are homologous to other proteins with CNBDs, including the protein kinase A (PKA) regulatory subunit, protein kinase G (PKG), the catabolite gene-activator protein (CAP), HCN channels, bacterial cyclic protein of Escherichia coli (CAP), HCN channels, bacterial cyclic nucleotide-regulated K+ (MloK) channels, and the KCNH family of K+ channels having CNBHDs. Although to date the crystal structure for a mammalian CNG channel CNBD has not been solved, the structures of many homologous CNBDs have been determined by x-ray crystallography or NMR (McKay and Steitz, 1981; Su et al., 1995; Zagotta et al., 2003; Clayton et al., 2004; Flynn et al., 2007; Das et al., 2009; Schünke et al., 2009; Brelidze et al., 2012).

There are two fundamental steps for ligand interaction with the CNBD of CNG channels. The first step is initial ligand docking to the CNBD; the second step is the conformational change in the CNBD that helps couple cyclic nucleotide binding to channel opening (Varnum et al., 1995; Flynn et al., 2007). The initial ligand-docking step is mediated mainly by interactions between the β-roll in the CNBD and the ribose and cyclic phosphate moiety of the cyclic nucleotide. There is a conserved short α-helical structure between the β-6 and β-7 strands of the CNBD for CNG channels called the phosphate-binding cassette (PBC). The PBC contains a conserved GE sequence; the glycine and glutamic acid are thought to interact with 2′-OH of the ribose for ligand docking. Furthermore, there is a conserved arginine within the β-7 strand that is mainly responsible for interacting with the cyclic phosphate of cyclic nucleotides; mutating this Arg to Glu dramatically decreases the ligand affinity but does not affect ligand efficacy (Tibbs et al., 1998). Therefore, this R-to-E mutation has been used to cripple the ligand-binding site of a CNG subunit in order to investigate subunit contributions to ligand binding and activation of CNG channels (Liu et al., 1998; Waldeck et al., 2009; Nache et al., 2012). Moreover, the threonine adjacent to this arginine within the β-7 strand also has been shown to be important for ligand docking as well as contributing to selectivity for cGMP over cAMP in CNG and HCN channels (Altenhofen et al., 1991; Varnum et al., 1995; Zagotta et al., 2003; Flynn et al., 2007; Zhou and Siegelbaum, 2007).

After the initial ligand-docking event, the C-helix of the CNBD moves to interact with the purine ring of cyclic nucleotides, and this interaction is coupled to the opening conformational change of CNG channels (Varnum et al., 1995; Sunderman and Zagotta, 1999a; Flynn et al., 2007). In addition, stabilization of the helical structure of the C-helix itself has been demonstrated to be linked to promoting channel opening after ligand binding (Taraska et al., 2009; Puljung and Zagotta, 2013). Based on the orientation of the purine ring relative to the ribose of cyclic nucleotides, cGMP and cAMP have two possible configurations: syn (with the pyrimidine ring closer to the ribose than the imidazole ring) and anti (180° rotation of the purine ring compared to the syn configuration) (Figure 24.5b). According to studies of HCN channels, cAMP binds to the CNBD in the anti configuration, while cGMP binds to the CNBD in the syn conformation (Zagotta et al., 2003; Flynn et al., 2007; Zhou and Siegelbaum, 2007); it is likely that cGMP and cAMP maintain

![Figure 24.4](image-url)
the same configuration for binding to CNG channels. The conserved threonine in the \( \beta \)-7 strand of the \( \beta \)-roll is proposed to interact with the purine ring of cGMP when cGMP is in the syn configuration (Altenhofen et al., 1991). However, this interaction between the threonine in the \( \beta \)-roll and cGMP is not sufficient to explain high selectivity for cGMP over cAMP in CNG channels. Instead, cGMP selectivity is largely dependent on a crucial ligand discrimination residue located near the C-terminal end of the C-helix (an aspartic acid in CNGA1 and CNGA3; a glutamic acid in CNGA2) (Varnum et al., 1995). For CNGA subunits, the negatively charged residue is thought to present a favorable electrostatic interaction with cGMP via two hydrogen bonds with N1 and N2 of the guanine ring; this interpretation is supported by recent structural studies of CNBDs in HCN channels (Flynn et al., 2007; Zhou and Siegelbaum, 2007). In contrast, the aspartic acid residue forms an unfavorable interaction with cAMP, possibly due to repulsion between the negative charge of the side chain and the unshared pair of electrons in the \( \text{sp}^2 \) orbital at N1 of the adenine. These differences make cGMP a nearly full agonist and cAMP a partial agonist for CNGA1 or CNGA3 channels (Varnum et al., 1995; Peng et al., 2004). Mutating this aspartic acid to methionine (D604M for CNGA1; D609M for CNGA3) reversed ligand selectivity, making cAMP a better agonist than cGMP (Varnum et al., 1995; Peng et al., 2004). Homomeric CNGA2 channels, which have a glutamic acid at this position, are still selective for cGMP over cAMP, showing an apparent affinity for cGMP about 25-fold higher than that for cAMP (Nache et al., 2012). For CNGA2 channels, cGMP and cAMP have a similar efficacy because the ligand-independent intrinsic tendency for opening of CNGA2 channels is very high. The equivalent residue within the CNBD of CNGB1 subunits is an asparagine; this difference accounts for the increase in cAMP efficacy for heteromeric rod CNG channels (Pagès et al., 2000; He and Karpen, 2001). For CNGB3 subunits, the equivalent residue is a positively charged lysine; thus, ligand discrimination for CNGB3 subunits may be mediated by a different mechanism compared to other types of CNG channel subunits. In CNGA4, the residue equivalent to D604 in CNGA1 instead is a methionine, which helps explain why CNGA4 confers high cAMP selectivity to heteromeric olfactory CNG channel (Bradley et al., 1994; Shapiro and Zagotta, 2000). However, other regions within the CNBD, including the \( \beta \)-roll, have been shown to contribute to the ligand selectivity for CNG channels (Young and Krougliak, 2004). In addition, it has been reported that an arginine in the C-helix of the CNBD helps couple ligand binding to channel activation for HCN and for bacterial cyclic nucleotide–regulated K+ channels (Zagotta et al., 2003; Clayton et al., 2004).

Several analogues of natural cyclic nucleotides have been used to characterize the CNBD of CNG channels. It was found that chemical modifications of the ribose and phosphate moiety of cyclic nucleotide are less tolerated than those of the purine ring. Modification of the ribose and cyclic phosphate group of "Cyclic nucleotide–gated channels"
cGMP significantly impaired activation of photoreceptor CNG channels (Zimmerman et al., 1985; Tanaka et al., 1989). This led to the development of a caged 4,5-dimethoxy-2-nitrobenzyl ester of cGMP that is not capable of activating CNG channels unless altered by photolysis (Nerbonne et al., 1984; Karpen et al., 1988). Caged cGMP has been used to study the kinetics of CNG channel activation by fast jumps in cGMP concentration via laser flash photolysis. In contrast, the CNBD of CNG channels can accommodate large modifications of the purine ring of cyclic nucleotides, particularly at the C8 position. Generally, cGMP analogues with substitutions at the C8 position such as 8-Br-cGMP and 8-pCPT-cGMP have higher ligand affinity, are resistant to hydrolysis by PDE, and exhibit membrane permeability (Zimmerman et al., 1985; Wei et al., 1998). The photoaffinity analogue 8-pAPT-[32P]-cGMP, which can be covalently attached to the CNBD after UV light activation, labels a region containing hydrophobic residues within the β-4 strand of the CNBD, indicating that this region is close to the C8 position of the purine ring (Brown et al., 1995). Moreover, a polymer-linked cGMP dimer has been engineered to enhance ligand affinity and to measure the distance between two ligand-binding sites in CNG channels (Kramer and Karpen, 1998).

### 24.4.2 C-LINKER OF CNG CHANNELS

The C-linker region connecting S6 and the CNBD has been demonstrated to be critical for coupling ligand binding to opening of rod, cone, and olfactory CNG channels (Gordon and Zagotta, 1995a,b; Brown et al., 1998; Zong et al., 1998; Zhou et al., 2004). Based on the crystal structure of this region in homologous HCN2 channels (Zagotta et al., 2003), the C-linker comprises six α-helices (A′–F′). The C-linker is thought to support several intersubunit interactions in the tetrameric channel, including an elbow on the shoulder contact with the A′–B′ helices of one subunit representing the elbow and C′–D′ helices of the adjacent subunit forming the shoulder (Zagotta et al., 2003; Craven and Zagotta, 2004). Ni2+ coordination sites within the A′ helix of the C-linker can potentiate or inhibit channel gating, depending on whether histidines in adjacent subunits coordinate Ni2+ better in open or closed conformations, respectively (Gordon and Zagotta, 1995a,b; Johnson and Zagotta, 2001). Disulfide-bond formation between introduced cysteines in this region of the A′ helix also can stabilize the open state (Hua and Gordon, 2005). In addition, a conserved tripeptide located in the A′ helix of the C-linker has been shown to be important for assembly and ligand-dependent gating of both CNG and HCN channels (Zhou et al., 2004). Craven and coworkers have characterized salt bridges that exist in parallel within the C-linker regions of CNG and HCN channels (Craven and Zagotta, 2004; Craven et al., 2008). In bovine CNGA1 channels, R431 in the B′ helix interacts via salt bridges with E462 in the D′ helix of the adjacent subunit and D502 in the CNBD β-roll of the same subunit (Craven and Zagotta, 2004; Craven et al., 2008). Disrupting these interactions enhanced channel opening, substantiating the view that these interactions occur in the closed state of the channel and that the C-linker region plays a critical role in CNG channel gating. The C-linker region of CNGA1 also was found to interact with the N-terminal region between and within subunits (Rosenbaum and Gordon, 2002). Considering the important roles of the C-linker and N-terminal regions for activation of CNG channels (Goulding et al., 1994; Gordon and Zagotta, 1995b; Tibbs et al., 1997; Möttig et al., 2001), N–C interactions may serve to adjust the gating properties of CNG channels.

### 24.4.3 CNG CHANNEL GATE

During CNG channel gating, the conformational change in the C-linker is transmitted to opening of the pore. For homologous voltage-gated potassium channels, the pore gate is well characterized as a bundle crossing formed by the inner helix (Hackos et al., 2002; Labro et al., 2003; Webster et al., 2004) with a hinge motif bending during channel opening (Jiang et al., 2002, 2003; Long et al., 2005). For CNG channels, the inner helix is unlikely to represent the gate, despite evidence for analogous conformational rearrangements here during channel activation (Flynn and Zagotta, 2001). Ag+, Cd2+, and some MTS reagents were able to enter the inner vestibule of CNGA1 channels in both the closed and open states (Flynn and Zagotta, 2001; Contreras and Holmgren, 2006; Nair et al., 2009). Furthermore, in contrast to intracellular quaternary ammonium (QA) block of voltage-gated K+ channels (Armstrong and Hille, 1972), the extent of blockage of CNGA1 channels by QA is inversely proportional to the open probability of the channel, indicating that QA ions can bind to the inner vestibule of CNGA1 channels more readily during the closed state (Contreras and Holmgren, 2006). Together, the results indicate that gating of CNG channels involves a movement of the S6 helix, dilating or constricting the pathway to the inner vestibule during the open or closed state, respectively. However, because of the relative lack of state-dependent accessibility in this region compared to K+ channels, the S6 helix probably does not represent the gate of CNG channels.

Other evidence favors the view that the gate of CNG channels is located at the selectivity filter (Fodor et al., 1997a; Contreras and Holmgren, 2006; Contreras et al., 2008; Martínez-François et al., 2009; Mazzolini et al., 2009). Using cysteine-scanning mutagenesis of the entire selectivity filter of CNGA1 channels, a recent study has found that the accessibility of high-affinity cysteine-binding agents, Cd2+ and Ag+, to these mutant channels is state dependent (Contreras et al., 2008). Consistent with this finding, mutation of residues in the selectivity filter not only changed the conductance of the channel but also decreased the open probability of the channel (Bucossi et al., 1996; Becchetti and Gamel, 1999; Becchetti et al., 1999). Together, these results strongly imply that there is a reorientation of the pore region of the channel during channel gating. Furthermore, subconductance states that have been observed in CNG channels at subsaturating concentrations of cyclic nucleotides (Taylor and Baylor, 1995; Ruiz and Karpen, 1997a,b) also are consistent with a conformational change in the channel pore during CNG channel gating.

If the selectivity filter acts as the gate of CNG channels, then how are the conformational changes within the C-linker and S6 helix transmitted to the pore of the channel? It has been demonstrated recently that S6 helix movement may be linked to the pore helix during the gating of CNG channels. F380 in the S6 helix has been shown to interact with L356 in the pore helix, stabilizing the open state of the channel via coupling of the S6 helix with the pore (Mazzolini et al., 2009). In addition, the pore helix has been demonstrated to present a rotational movement
during channel gating (Liu and Siegelbaum, 2000), probably induced by movement of the C-linker and S6 helix. The gate at the selectivity filter of CNG channels is reminiscent of C-type inactivation of K+ channels, where the selectivity filter acts as a secondary gate in addition to the bundle-crossing gate composed of S6 helices. Interactions between the selectivity filter and pore helix are critical for C-type inactivation of K+ channels (Cordero-Morales et al., 2006, 2011; Cuello et al., 2010). Interestingly, this type of interaction is also present in CNGA1 channels. E363 in the selectivity filter of CNGA1 has been shown to interact with Thr 355 in the pore helix; disrupting the hydrogen bond between E363 and T355 impaired gating of the channel, producing desensitization during constant cGMP application (Mazzolini et al., 2009). Furthermore, mutating residues within the selectivity filter and pore helix of CNGA1 can render the channel voltage sensitive, producing strong outward rectification at saturating concentrations of cGMP (Martínez-François et al., 2009). Together, these results suggest that gating of CNG channels and C-type inactivation of K+ channels share a similar mechanism at the selectivity filter.

24.4.4 CNG CHANNEL ACTIVATION SCHEMES AND SUBUNIT CONTRIBUTIONS

A number of different activation schemes have been employed to interpret the gating behavior of CNG channels. The Monod–Wyman–Changeux (MWC) concerted allosteric model (Monod et al., 1965) has been useful to describe ligand binding and channel activation of CNG channels (Goulding et al., 1994; Varnum and Zagotta, 1996; Tibbs et al., 1997) (Figure 24.6b). The MWC model for CNG channels assumes four equivalent binding sites; the cooperativity of ligand binding is dependent on binding energies. One advantage of the MWC model compared to sequential models is that it can account for the well-defined spontaneous channel opening events in the absence of ligand binding, which reflect the ligand-independent intrinsic gating properties of the channel (Tibbs et al., 1997). For homomeric CNG channels, the spontaneous channel open probability (P\text{O}) has been estimated to be in the following order (from low to high): CNGA1 (P\text{O} = 10^{-5}), CNGA3 (P\text{O} = 10^{-4}), and CNGA2 (P\text{O} = 10^{-3}) channels (Ruiz and Karpen, 1997; Tibbs et al., 1997; Gerstner et al., 2000). However, this concerted MWC model can be less than satisfactory in describing all properties integral to activation of CNG channels. Furthermore, multimerization of related but divergent subunit types is likely to make asymmetrical subunit contributions an essential feature of heteromeric channel gating. In order to study subunit contributions to channel gating, photoaffinity 8-pApt-cGMP was used to covalently lock rod CNGA1 channels in one, two, three, or four ligand-bound states (Ruiz and Karpen, 1997). Utilizing this approach, it was found that (1) four ligands are required to fully activate CNGA1 channels, (2) the equilibrium constant for channel opening does not increase by a constant factor for each ligand-binding event, and (3) a single ligand cannot induce appreciable channel opening (Ruiz and Karpen, 1997). Other studies differ regarding the threshold number of bound cGMP molecules necessary for appreciable channel activation, indicating that channels with a single bound ligand can have a considerable open probability (P\text{O} > 0.01) (Liu et al., 1998; Biskup et al., 2007). These features may reflect in part the specific subunit compositions of the channels. Furthermore, partially liganded channels have been found to exhibit openings to one or more subconductance states (Ruiz and Karpen, 1997); this observation is not consistent with the MWC model, where there is a single transition from closed to open conformations. However, the existence of subconductance states for CNGA1 channel activation remains controversial (Sunderman and Zagotta, 1999b).

Sequential models involving two to four ligand-binding steps followed by an allosteric opening conformational change also have been proposed to describe activation of CNG channels (Karpen et al., 1988; Gordon and Zagotta, 1995a) (Figure 24.6a). One tool used in studying ligand binding and gating of rod and olfactory CNG channels is photolysis-induced jumps of caged cGMP or cAMP (Karpen et al., 1988; Nache et al., 2005). For rod CNG channels, activation was well described by sequential cGMP-binding steps and a rapid closed-open transition with three or four ligands bound; the rate-limiting step was the third cGMP-binding event (Karpen et al., 1988). For CNGA2 channels, it was found that the activation time course generated by cGMP jumps from zero to a saturating concentration was better fit with a double-exponential trace rather than a sigmoidal trace predicted by the MWC model (Nache et al., 2005). In addition, the time

![Figure 24.6 Gating schemes proposed for CNG channels. (a) A simple sequential model for activation of CNG channels. Cyclic nucleotides bind to channel subunits independently, followed by an allosteric transition from the closed state (C) to the open state (O). (b) MWC model for activation of CNG channels. (c) A C4L-*O4L sequential model proposed for activation and deactivation of homomeric CNGA2 channels.](image-url)
24.5 Ion permeability of CNG channels

CNG channels are nonselective cation channels, conducting monovalent K⁺, Na⁺, Li⁺, Rb⁺, and Cs⁺. CNG channels also are permeable to Ca²⁺ but can be blocked by Ca²⁺ from both the intracellular and extracellular sides of the membrane (Zimmerman and Baylor, 1992; Karpen et al., 1993; Root and MacKinnon, 1993). The presence of a glutamic acid within the selectivity filter of CNGA subunits has been shown to be critical for coordination of Ca²⁺ ions, conferring Ca²⁺ permeability and Ca²⁺ block to the channel (Root and MacKinnon, 1993; Eismann et al., 1994; Derebe et al., 2011b). Ion selectivity and the fraction of current carried by Ca²⁺ differ among homomeric CNGA subunits and for heteromeric channels containing CNGB subunits (Frings et al., 1995; Dzeja et al., 1999; Seifert et al., 1999). Moreover, Ca²⁺ selectivity is much greater for native cone CNG channels compared to rod CNG channels (Picones and Korenbrot, 1995). Instead of having a glutamic acid within the selectivity filter, CNGB1 and CNGB3 subunits contain a glycine at the respective position. The difference between E and G within the selectivity filter is thought to have two opposing effects on ion permeation, considering that Ca²⁺ is both a permeant ion and a blocker for CNG channels. First, this glycine residue decreases Ca²⁺ affinity, therefore reducing the dwell time of Ca²⁺ within the selectivity filter and concomitantly increasing Ca²⁺ influx. Second, the glycine residue relieves the Ca²⁺ block of monovalent cation permeation through the pore, thus enhancing the influx of Na⁺ and K⁺ ions.

The mechanisms underlying the nonselectivity among cations in CNG channels have been studied by engineering a NaK chimera mimicking the CNG channel pore, combined with x-ray crystallography (Derebe et al., 2011a). The sequence of the selectivity filter is TIGET for CNGA1, CNGA2, and CNGA3 subunits and TIGGL for CNGB1 and CNGB3; these differ from the TVGYG signature sequence found in K⁺-selective channels. NaK channels are prokaryotic, nonselective cation channels with the TVGDG sequence in the selectivity filter. For NaK channels, the aspartic acid in the selectivity filter is equivalent to the glutamic acid of CNG channels. Ca²⁺ is also a permeable blocker for NaK channels, but the Ca²⁺ affinity is lower than that of CNG channels. In contrast to K⁺-selective channels, there are only two contiguous ion-binding sites within the selectivity filter of NaK channels, corresponding to sites 3 and 4 for K⁺-selective channels (Derebe et al., 2011a). In comparison, there are three contiguous ion-binding sites for the NaK–CNG chimera containing the CNG channel pore mimic, corresponding to sites 2, 3, and 4 for K⁺-selective channels (Derebe et al., 2011a). These results suggest that the number of ion-binding sites within the selectivity filter helps determine the selectivity properties of CNG or NaK channels. If the number of ion-binding sites is less than 4, then the channel is relatively nonselective, conducting both Na⁺ and K⁺.

The molecular mechanism underlying Ca²⁺ block of CNG channels has been determined at the structural level by engineering a NaK channel chimera mimicking CNG channels (Derebe et al., 2011b). The NaK chimera mimicking the CNG channel pore was co-crystallized with bound Ca²⁺ ions. Contrary to the opinion that Ca²⁺ is coordinated by the negatively charged side chains of the aspartic or glutamic acid residues in the selectivity filter, it was found that Ca²⁺ is chelated by backbone carbonyl oxygens at sites 2 and 3 of the selectivity filter (Derebe et al., 2011b). In addition, the crystal structure of the NaK–CNG chimera showed that the D or E side chains within the selectivity filter form hydrogen bonds with tyrosine and threonine residues within the pore helix, corroborating the view that interactions occur between the selectivity filter and pore helix for CNG channels (Derebe et al., 2011b).

The selectivity filter of CNG channels is proposed to serve a dual function as both a channel gate and an ion selectivity feature. This view is supported by studies demonstrating that gating alters...
the ion permeability of CNG channels. The selectivity of native photoreceptor CNG channels for Ca$^{2+}$ over Na$^+$ was found to increase as the open probability of the channel increased (Hackos and Korenbrot, 1999). In addition, the apparent cGMP affinity of CNGA1 channels is higher when K$^+$ is the permeant ion compared to when Na$^+$ is the permeant ion (Holmgren, 2003). K$^+$ prolonged the open time duration and increased the maximum open probability of the channel compared to Na$^+$ (Holmgren, 2003).

24.6 PHARMACOLOGY OF CNG CHANNELS

The best-known blocker for CNG channels is l-cis-diltiazem, an isomer of the clinically used drug Cardizem. l-cis-diltiazem has been shown to block CNG channels from rod and cone photoreceptors and OSNs (Koch and Kaupp, 1985; Frings et al., 1992; Haynes, 1992). The block of CNG channels by l-cis-diltiazem is voltage dependent, exhibiting an increase in current suppression with membrane depolarization (McLatchie and Matthews, 1992). Furthermore, l-cis-diltiazem block is a useful reporter to confirm the formation of heteromeric CNG channels when expressed as recombinant proteins; l-cis-diltiazem effectively blocks heteromeric CNG channels containing CNGB1 or CNGB3 subunits, while it has little effect on homomeric CNG channels (Chen et al., 1993; Gerstner et al., 2000). However, the mechanisms underlying l-cis-diltiazem block of CNG channels are not well understood; it may act by modifying channel gating instead of directly occluding the pore.

Several agents used to block other types of ion channels have been shown to block CNG channels. The local anesthetic tetracaine blocks CNG channels in a voltage-dependent and state-dependent manner (Fodor et al., 1997a,b); the profound block of CNG channels are not well understood; it may act by modifying channel gating instead of directly occluding the pore. Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993). Dequainuline, an extracellular blocker to block CNG channels (Frings et al., 1992; Nicol, 1993; Zufall and Firestein, 1993).

24.7 REGULATION OF CNG CHANNELS

Regulation of CNG channels is important for adaptation in retinal photoreceptors and in olfactory receptor neurons and for paracrine and circadian controls within photoreceptors. CNG channels can be regulated on a rapid time scale, ranging from milliseconds to seconds, for example, via Ca$^{2+}$-dependent feedback mechanisms, or on a relatively slow time scale, for example, for channel regulation by phosphorylation or by membrane phosphoinositides.

Ca$^{2+}$-dependent regulation of CNG channels, mediated by binding of calmodulin (CaM) or other calcium-sensing proteins, contributes to the rapid adaptive properties of olfactory neurons and photoreceptors. Sensory CNG channels are highly permeable to calcium, and Ca$^{2+}$–CaM serves to decrease channel apparent ligand affinity, thus providing a negative feedback signal for control of channel activity (Trudeau and Zagotta, 2003). OSNs desensitize dramatically after prolonged odorant stimulation (Reisert and Matthews, 1999). Ca$^{2+}$–mediated feedback regulation of olfactory CNG channels represents a central mechanism underlying odorant adaptation (Kurahashi and Menini, 1997). There are three CaM-binding sites located in olfactory CNG channel subunits: a CaM-binding domain within the N-terminal region of CNGA2, an IQ-type CaM-binding site within the C-linker region of CNGB4, and an IQ-type CaM-binding site within the N-terminal region of CNGB1b (Bradley et al., 2001). It was found that Ca$^{2+}$-free apocalmodulin (apoCaM) is permanently associated with the IQ-type CaM-binding sites within CNGB4 and CNGB3 subunits (Bradley et al., 2004). Homomeric CNGB2 channels also are sensitive to inhibition by Ca$^{2+}$–CaM (Liu et al., 1994); Ca$^{2+}$–CaM disrupts autoregulatory intersubunit interactions between N- and C-terminal cytoplasmic regions of CNGB2 subunits (Varnum and Zagotta, 1997; Zheng et al., 2003). However, deleting the CaM-binding site in CNGB2 does not prevent regulation of CNGB2+CNGB4+CNGB3 channels, a result that suggested that the physiologically relevant CaM-binding sites are those present in CNGB4 and CNGB3 subunits (Bradley et al., 2004). Moreover, a recent study has shown that the CNGB1b CaM-binding site is sufficient to mediate Ca$^{2+}$-dependent desensitization conferred by endogenous CaM (Waldeck et al., 2009). A mouse model where the CaM-binding domain within CNGB1b was genetically deleted has provided new insights into the physiological role of CaM regulation of olfactory CNG channels (Song et al., 2008). This study suggested that Ca$^{2+}$–CaM regulation of olfactory CNG channels is not responsible for adjusting sensitivity of olfactory neurons to repeated stimuli but instead contributes mainly to the rapid termination of the odorant response (within ~100 ms) (Song et al., 2008).

Rod CNG channels also have been shown to be directly regulated by Ca$^{2+}$–CaM, and this feature is thought to contribute to Ca$^{2+}$-dependent adjustment of phototransduction (Hsu and Molday, 1993; Gordon et al., 1995b; Haynes and Stotz, 1997). The Ca$^{2+}$ concentration within the photoreceptor outer segments is mainly determined by the opening of CNG channels and also by the activity of the K$^+$-dependent Na$^+-$Ca$^{2+}$ exchanger.
(NCKX1 in rods; NCKX2 in cones) that extrudes Ca\(^{2+}\) from the outer segment (Szerencsei et al., 2002). The N-terminal region of the rod CNGB1a subunit contains an IQ-type Ca\(^{2+}\)/CaM-binding site (Figure 24.2) (Grunwald et al., 1998; Weitz et al., 1998). Ca\(^{2+}\)–CaM binding to this site disrupts an intersubunit interaction between the N-terminal region of CNGB1a and the C-terminal region of CNGA1, which decreases cGMP affinity of CNGA1+CNGB1a channels (Trudeau and Zagotta, 2002b, 2004). However, using a caged Ca\(^{2+}\)-chelator to rapidly decrease Ca\(^{2+}\) concentration, Ca\(^{2+}\)-dependent modulation of CNG channels was detected only in cones but not in rods in intact ground squirrel photoreceptors (Rebrik and Korenbrot, 2004). In addition, a recent paper utilizing a mouse model in which the CaM-binding domain within CNGB1a was genetically ablated demonstrated that CaM-dependent modulation of rod CNG channels is not a major mechanism mediating light adaptation for rod photoreceptors (Chen et al., 2010).

The native cone CNG channel exhibits greater sensitivity to calcium feedback regulation of apparent ligand affinity than the rod channel, and this modulation is thought to be of much greater importance for adaptation in cones (Korenbrot and Rebrik, 2002; Korenbrot, 2012). The N-terminal region of CNGA3 encompasses a Baa-type CaM-binding site, but for most species, homomeric CNGA3 channels are not sensitive to Ca\(^{2+}\)–CaM regulation. However, mutations downstream of this CaM-binding site within the CNGA3 N-terminal domain can unmask CaM sensitivity (Grunwald et al., 1999). Instead, functional CaM-binding sites within the N- and C-terminal regions of CNGB3 subunits support Ca\(^{2+}\)-dependent regulation of human cone CNG channels (Peng et al., 2003a). However, the magnitude of Ca\(^{2+}\)–CaM-dependent inhibition of native and recombinant cone CNG channels appears to be too small to completely account for Ca\(^{2+}\)-dependent desensitization in intact cones (Hackos and Korenbrot, 1997; Haynes and Storz, 1997; Rebrik and Korenbrot, 1998, 2004). Furthermore, a recent study shows that CNG-modulin, a calcium-binding protein interacting with the N-terminal region of CNGB3, may be the authentic calcium sensor for Ca\(^{2+}\)-dependent control of cone CNG channels in striped bass (Rebrik et al., 2012).

Another type of CNG channel regulation operating on a relatively slow time is via phosphorylation of tyrosine and serine/threonine residues in channel subunits (Figure 24.2). Serine/threonine phosphorylation and dephosphorylation can adjust the apparent ligand affinity of native rod CNG channels (Gordon et al., 1992). In addition, tyrosine phosphorylation at positions Y498 of bovine CNGA1 and Y1097 of CNGB1a (within the respective CNBDs) inhibits the activity of CNGA1+CNGB1a channels, inducing an approximately twofold decrease in apparent cGMP affinity (Mołokanowa et al., 2003). Interestingly, there is crosstalk between regulations by tyrosine phosphorylation and CaM for rod CNG channels: CaM regulation was attenuated when the channel was phosphorylated; this effect was abolished in CNGA1-Y498F+CNGB1a channels but not in CNGA1+CNGB1a-Y1097F channels (Krajewski et al., 2003). In contrast to rod channels, the equivalent position in CNGA3 is a phenylalanine, while the equivalent tyrosine in CNGB3 (Y545) does not confer tyrosine phosphorylation-dependent regulation of cone CNG channels (Bright et al., 2007).

Signaling molecules downstream of phospholipase C (PLC) are associated with regulation of CNG channels. Diacylglycerol generated after PLC activation has been shown to inhibit rod CNG channels (Gordon et al., 1995a; Crary et al., 2000) and influence the ligand affinity of native cone CNG channels (Chen et al., 2007). Furthermore, protein kinase C (PKC) can modulate the activities of CNG channels in a subunit-specific way. It was reported that phosphorylation of olfactory CNGA2 subunits by PKC, at a serine residue within the N-terminal domain adjacent to the CaM-binding site, was able to increase apparent cGMP affinity (Müller et al., 1998). In addition, bovine CNGA3 channels were found to be regulated by PKC-mediated phosphorylation of serine residues within the CNBD, exhibiting a decrease in cGMP affinity for homomeric CNGA3 channels (Müller et al., 2001).

Membrane-bound phosphoinositides, which have been shown to be ubiquitous ion channel modulators (Gamper and Shapiro, 2007; Suh and Hille, 2008), also have been reported to regulate CNG channels. PI(4,5)P\(_2\) and PIP(3,4,5)P\(_3\) produced inhibition of rod (Womack et al., 2000) and cone CNG channels (Bright et al., 2007). Furthermore, PI(3,4,5)P\(_3\) has been reported to inhibit olfactory CNG channels in rat OSNs as well as recombinant CNGA2 channels expressed in heterologous expression systems (Spehr et al., 2002; Zhainazarov et al., 2004). Moreover, phosphoinositide regulation has been shown to interact with Ca\(^{2+}\)–CaM regulation of olfactory CNG channels (Brady et al., 2006). It was found that PIP(3,4,5)P\(_3\) inhibited the apparent ligand affinity of homomeric CNGA2 channels and heteromeric CNGA2+CNGA4+CNGB1b channels, an effect that was dependent on a putative N-terminal PIP(2,3)-binding site within CNGA2 (Figure 24.2). PIP(3) suppressed Ca\(^{2+}\)–CaM regulation of heteromeric CNGA2+CNGA4+CNGB1b channels, presumably by preventing CaM binding to CNGA4 and CNGB1b subunits (Brady et al., 2006). In addition, the molecular mechanism for PIP(3) and PIP(2,3) regulation of cone CNG channels has recently been elucidated (Dai and Varnum, 2013; Dai et al., 2013). However, the physiological role for this regulation in photoreceptors is not well established. Phosphoinositide levels within photoreceptors are controlled by light, intracellular Ca\(^{2+}\), paracrine signals, and circadian oscillators (Chen et al., 2007; Li et al., 2008; Ko et al., 2009). Thus, phosphoinositide regulation of CNG channels has the potential to contribute to several critical physiological processes necessary for normal photoreceptor function.

The ligand affinity of native cone photoreceptor CNG channels is modulated by retinal circadian oscillations, generating a low apparent cGMP affinity during the daytime and an almost twofold increase in cGMP affinity during the night (Ko et al., 2003, 2004; Chae et al., 2007; Chen et al., 2007). Tyrosine phosphorylation of chicken CNGB3 subunits has been proposed as one possible pathway responsible for circadian regulation of cone CNG channels (Chae et al., 2007), but other mechanisms such as activation of lipid kinases or lipid phosphatases, for example, PLC and/or PI3-kinase, might also contribute to changes in ligand affinity (Bright et al., 2007; Chen et al., 2007; Ko et al., 2009). Furthermore, circadian regulation of dopamine and somatostatin release may contribute to the circadian regulation of chicken cone CNG channels. Dopamine...
levels are higher during the day compared to the night; applying dopamine D2 receptor agonist decreased the cGMP affinity of CNG channels during the night but not during the daytime (Ko et al., 2003). Somatostatin release from retinal amacrine cells is high during the night and low during the day; activation of somatostatin receptors increased the ligand affinity of native cone CNG channels during the early part of the day, possibly through activation of PLC and PKC (Chen et al., 2007). Moreover, the cAMP–PKA signaling pathway, the small monomeric G protein, Ras, the mitogen-activated protein kinase (MARK) signaling pathway, and calcium–calmodulin kinase II (CaMII) all have been reported to contribute to circadian regulation of cone CNG channels (Ko et al., 2001a, 2004).

Besides regulation of photoreceptor CNG channels by the intrinsic circadian clock, light is able to indirectly regulate CNG channels. Light-induced insulin receptor activation has been shown to inhibit rod CNG channels via phosphorylation of Y498 and Y503 in CNGA1 (Gupta et al., 2012). This finding adds a novel physiological link for the tyrosine phosphorylation of rod CNG channel subunits that was previously described by Kramer and coworkers (Molokanova et al., 1999, 2003). In addition, Grb 14, an insulin receptor-binding protein, has been shown to suppress rod CNG channel activity by directly binding to the C-terminal region of CNGA1 in a light-dependent manner (Gupta et al., 2010; Rajala et al., 2012). Direct phosphorylation of CNGA1 after insulin receptor activation and Grb 14 binding to CNGA1 together have an additive effect for inhibition of rod CNG channel activity (Gupta et al., 2012). Furthermore, PI3-kinase activity was enhanced after light-dependent phosphorylation of insulin receptors, which could potentially regulate photoreceptor CNG channels by manipulating membrane PIP_3 and PIP_2 levels in the outer segment of photoreceptors (Rajala et al., 2002). Consistent with this finding, light exposure has been demonstrated to induce a robust increase in PIP_3 levels within the photoreceptor outer segment (Li et al., 2008).

24.8 CNG CHANNEL CELL BIOLOGY: BIOGENESIS, TRAFFICKING, AND TURNOVER

Compared to the detailed biophysical characterization of CNG channels described earlier, much less is known about cell biology aspects of their function, including channel biogenesis and trafficking mechanisms. CNGA (but not CNGB) subunits are modified by N-glycosylation within the extracellular pore-turret region following the fifth transmembrane domain (TMS) (Figure 24.2). The precise site for N-glycosylation varies among subunit types and shows some species-specific differences. There is no obvious functional requirement for subunit glycosylation (Rhö et al., 2000), but lack of glycan addition can report defects in channel folding or maturation (Faillace et al., 2004; Liu and Varnum, 2005; Duricka et al., 2012). In addition, CNGA subunit glycosylation at some positions can protect channels from MMP-dependent processing and subsequent potentiation of channel gating (Meighan et al., 2012, 2013). Furthermore, the N-terminal region of CNGA1 subunits (and chicken CNGA3 subunits) is subject to proteolytic processing in photoreceptors, with cleavage at amino acid 92 in bovine CNGA1 (Molday et al., 1991; Bönigk et al., 1993). The possible functional significance and tissue-type specificity of this subunit processing is not known.

There appear to be subunit-specific trafficking rules for CNG channels. In heterologous expression systems, CNGA1, CNGB2, and CNGB3 subunits exhibit efficient plasma-membrane localization in the absence of CNGB1 or CNGB3 (or CNGB4) subunits, but in the absence of CNGB1, CNGB2, or CNGB3 subunits, the CNGB or CNGB4 subunits are retained within intracellular compartments (Trudeau and Zagotta, 2002a; Peng et al., 2003b; Zheng and Zagotta, 2004; Nache et al., 2012). On the other hand, CNGB1 has been shown to be essential for ciliary trafficking of CNG channels (Hüttl et al., 2005; Michalakis et al., 2006). For the olfactory CNG channel, ciliary trafficking requires the kinesin motor protein Kif17, which interacts with the C-terminal region of CNGB1b (Jenkins et al., 2006). In addition, PACS-1 binding and serine/threonine protein kinase CK2-mediated phosphorylation of CNGB1b together regulate ciliary trafficking of the olfactory channel (Jenkins et al., 2009). The zebrafish KIF3A kinesin protein has been shown to be important for ciliary trafficking of cone CNG channels, as well as other phototransduction proteins localizing within the cone photoreceptor outer segment (Avasthi et al., 2009). Furthermore, targeting of rod CNG channels to the outer segment depends on interactions between the C-terminus of CNGB1a and the cytoskeletal protein ankyrin G (Kizhatil et al., 2009). For cone CNG channels, CNGB3 knockout in mice decreases CNGA3 protein levels and outer segment localization (Ding et al., 2009). However, the precise protein–protein interactions regulating ciliary targeting of cone CNG channels remain to be determined. There are other protein–protein interactions that are proposed to influence CNG channel localization. These include contacts made between channel subunits and protein components of the outer segment disc membranes (Kürschen et al., 1999), including peripherin-2 (Poetsch et al., 2001). Also, rod and cone CNG channels assemble with the NCKX1 and NCKX2, respectively, presumably via interactions with the CNGB subunits (Bauer and Dreichsler, 1992; Kim et al., 1998; Schnetkamp, 2004). Since NCKX1 and NCKX2 represent the primary extrusion pathway for outer segment calcium, channel–exchanger complexes are expected to create highly localized regulation of outer segment calcium levels (Prinsen et al., 2000; Schnetkamp, 1995).

Regarding channel stability, CNG channel subunits appear to be subject to degradation via the ubiquitin–proteasome system (UPS), particularly in terms of quality-control surveillance (Michalakis et al., 2006; Becirovic et al., 2010; Duricka and Varnum, unpublished data). The UPS may also exert a role in mediating normal turnover of the channels. The half-life of CNG channels in embryonic chick cone photoreceptors has been estimated to be less than 12 h (Ko et al., 2001b). This channel lifetime is much shorter than the expected turnover rate via outer segment phagocytosis mediated by the retinal pigment epithelium (RPE), which is thought to engulf about 10% of the photoreceptor outer segment per day (Kevany and Palczewski, 2010). Intriguingly, several enzymes mediating ubiquitination and deubiquitination are present in photoreceptor outer segments (Obin et al., 1996; Esteve-Rudd et al., 2010; Hajkova et al., 2010).
24.9 CNG CHANNELOPATHIES AND DISEASE MECHANISMS

The most common forms of CNG channelopathies involve disturbances of CNG channel function in the retina (Biel and Michalakis, 2007). Mutations in the genes encoding rod CNG channel subunits (CNGA1 and CNGB1) are associated with autosomal recessive retinitis pigmentosa (aRP) (Dryja et al., 1995; Paquet-Durand et al., 2011). RP is characterized by progressive degeneration of rod photoreceptors followed by loss of cones. Mutations in CNGA3 and CNGB3 have been linked to complete or incomplete achromatopsia, progressive cone dystrophy, oligocone trichromacy, inherited macular degeneration, and/or macular malfunction (Kohl et al., 1998, 2000, 2005; Sundin et al., 2000; Wissinger et al., 2001; Rojas et al., 2002; Johnson et al., 2004; Michaelides et al., 2004; Nishiguchi et al., 2005; Khan et al., 2007; Wiszniewski et al., 2007; Thadiens et al., 2010; Vincent et al., 2011). These disorders are typically inherited in an autosomal recessive manner and are characterized by absent or limited cone function (but intact rod function), compromised visual acuity, nystagmus, photophobia, and in some cases cone degeneration.

Determining the pathophysiological mechanisms, at the molecular and cellular levels, arising from disease-associated mutations in CNG channel genes is an arena of keen ongoing interest. The most commonly occurring CNGA3 mutations are missense mutations, and many have been shown to produce a loss-of-function phenotype at the molecular level—with decreased channel ligand sensitivity or absence of functional subunits, impaired folding, mislocalization, and/or increased subunit turnover (Tränkner et al., 2004; Liu and Varnum, 2005; Patel et al., 2005; Muraki-Oda et al., 2007; Reuter et al., 2008). Most CNGA1 and CNGB1 mutations also are thought to produce channel loss of function (Dryja et al., 1995; Trudeau and Zagotta, 2002a; Kizhatil et al., 2009). For some loss-of-function mutations, ER stress has been implicated in disease progression; improperly folded or misassembled channels arising from missense mutations (or null mutations in one subunit type) may accumulate in the endoplasmic reticulum and induce the unfolded protein response (Duricka et al., 2012; Thapa et al., 2012).

CNG channel gene knockouts in mice represent informative disease models that mimic pathological features arising from CNG channel null mutations in humans. Cone photoreceptors of CNGA3 −/− mice demonstrate impaired photoresponses and progressive degeneration (Biel et al., 1999; Michalakis et al., 2005, 2011a). CNGB1 knockout leads to functional and structural defects in both rod photoreceptors and olfactory receptor neurons (Hüttel et al., 2005; Michalakis et al., 2006). CNGA1 subunit knockdown using an antisense approach also leads to retinal degeneration (Lecomte and Barnstable, 2000). In contrast, the effect of CNGB3 absence appears to be less severe, with more subtle early changes, evidence of residual function, and slower progression overall (Ding et al., 2009). CNG channel subunit knockouts also have been shown to produce effects outside the retina, including impaired olfaction for CNGA2 −/− (Brunet et al., 1996) and CNGB1b −/− (Michalakis et al., 2006) and altered hippocampal LTP for CNGA2 −/− (Parent et al., 1998) and CNGA3 −/− (Michalakis et al., 2011a).

Disease-associated mutations can lead to gain-of-function changes in CNG channel activity. While the most commonly occurring CNGB3 mutations lead to subunit truncation via frameshifts, splice-site defects, and/or premature stop codons, many CNGB3 missense mutations (and some CNGA3 mutations) appear to produce gain-of-function changes in channel gating (Peng et al., 2003b; Bright et al., 2005) (Figure 24.7). For example, the achromatopsia-associated CNGB3-F525N mutation produces an approximately threefold increase in cGMP sensitivity (Figure 24.7). Hyperactive CNG channels are expected to disturb calcium homeostasis and lead to photoreceptor dysfunction and cell death (Liu et al., 2013). In addition, disease-causing mutations in other photoreceptor proteins, particularly those producing elevated cGMP levels, can result in uncontrolled CNG channel activity. Consistent with this idea, CNG channel subunit knockout or knockdown via siRNA has been shown to have a profound rescue effect, slowing progression of retinal degeneration in mice having mutations in other critical phototransduction proteins such as PDE (Paquet-Durand et al., 2011; Tosi et al., 2011).

Other functional defects arising from CNG channel mutations include altered pore properties such as ion selectivity and/or single-channel conductance (Seifert et al., 1999; Peng et al., 2003b; Tränkner et al., 2004; Koeppen et al., 2010). Finally, disease-associated mutations in CNG channels can interfere with
Cyclic nucleotide–gated channels

intersubunit interactions that are critical for channel assembly, gating, and/or regulation (Trudeau and Zagotta, 2002a; Michalakis et al., 2011b; Dai and Varnum, 2013). In one example, an achromatopsia-associated mutation in CNGA3 was shown to enhance cone CNG channel sensitivity to regulation by phosphoinositides by altering intersubunit coupling (Dai and Varnum, 2013).

24.10 CONCLUSIONS

Since the discovery of CNG channels nearly 30 years ago, detailed physiological and biophysical studies have provided many insights into fundamental CNG channel mechanisms. Information revealed by structural studies of bacterial CNG channels and of related HCN and EAG family channels also provides a rich context for understanding mammalian CNG channel structure and function. Issues that appear to require further study include several cell biology aspects of CNG channels, including a deeper characterization of the features and protein–protein interactions that control channel assembly, trafficking, and stability/turnover, as these are likely to inform our thinking about both normal physiology and pathophysiology in humans. Additional structural information, including a closer view of the interactions between N- and C-terminal cytoplasmic domains, may uncover structural mechanisms influencing ligand-dependent gating and channel regulation by second messengers.

Intriguing but incompletely characterized roles for CNG channels in nonsensory cells are another key area for additional investigation. Furthermore, to complement existing channel knockout models, the development and characterization of animal models representing CNG channel missense mutations is expected to provide important information about retinal disease mechanisms and potential treatment strategies.

REFERENCES


Cyclic nucleotide–gated channels


Ion channel families


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References

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References


