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CYCLIC NUCLEOTIDE-GATED ION CHANNELS

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■ **Abstract** Cyclic nucleotide-gated (CNG) ion channels were first discovered in rod photoreceptors, where they are responsible for the primary electrical signal of the photoreceptor in response to light. CNG channels are highly specialized membrane proteins that open an ion-permeable pore across the membrane in response to the direct binding of intracellular cyclic nucleotides. CNG channels have been identified in a number of other tissues, including the brain, where their roles are only beginning to be appreciated. Recently, significant progress has been made in understanding the molecular mechanisms underlying their functional specializations. From these studies, a picture is beginning to emerge for how the binding of cyclic nucleotide is transduced into the opening of the pore and how this allosteric transition is modulated by various physiological effectors.

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INTRODUCTION

Ion channels are the transistors of the brain. They are allosteric proteins that open and close an ion-permeable pore (a process called gating), allowing ions to flow across the cell membrane in a regulated manner. Ion channels can support

currents of up to 10^8 ions/s, while at the same time, select specific ions with greater than 99.9% accuracy. The gating can be regulated by various stimuli including changes in membrane voltage, the binding of extracellular or intracellular ligands, or membrane stretch. By controlling the flow of specific ions across the lipid bilayer, ion channels play fundamental roles in electric signaling in nerve, muscle, and synapse. Mutations in ion channels cause several diseases including myotonia, paralysis, cardiac arrhythmias, diabetes, cystic fibrosis, and blindness.

Ion channels have been studied using a combination of powerful techniques, making them a good model system for understanding conformational changes in proteins. Patch-clamp recording techniques allow measurement of single molecule behavior and macroscopic function in real time (Hamill et al. 1981). Combined with molecular biology, protein chemistry, voltage-clamp fluorometry, and more recently, X-ray crystallography, ion channel physiologists are rapidly gaining an understanding of how ion channels work as allosteric proteins. This review focuses on a specific class of ion channels, the cyclic nucleotide-gated (CNG) channels, and on recent progress made in understanding the molecular mechanisms for their highly specialized functions.

Physiology of Rod CNG Channels

CNG channels were first discovered in the plasma membrane of the outer segment of vertebrate rod photoreceptors, where they play a critical role in phototransduction (Fesenko et al. 1985). CNG channels are nonselective cation channels that are opened by the direct binding of intracellular cyclic nucleotides (Yau & Baylor 1989). In the dark, the binding of guanosine 3′:5′-cyclic monophosphate (cGMP) to the CNG channels causes the channels to open, allowing Na^+ and Ca^{2+} to flow into the cell. This flow of inward current, the dark current, depolarizes the outer segments. When light hits the retina, it activates a phototransduction cascade, diagrammed in Figure 1 (for review, see Burns & Baylor 2001). This signal transduction cascade begins with the absorption of a photon of light by the chromophore of rhodopsin, 11-*cis* retinal. This absorption activates rhodopsin to bind to and activate the G protein transducin, stimulating GTP-GDP exchange. Upon binding GTP, transducin binds to and activates a phosphodiesterase that hydrolyzes cGMP into 5′-GMP. The CNG channels in the plasma membrane close in direct response to this decrease in cGMP, inhibiting the dark current and, hence, hyperpolarizing the outer segments. This hyperpolarization is transmitted to the inner segments and ultimately causes a decrease in the tonic release of the neurotransmitter glutamate from the presynaptic terminals.

The sensitivity of the rod CNG channel has been optimized to detect and signal the drop in cGMP concentration resulting from the absorption of a single photon of light (Baylor et al. 1979). The channel's relatively low affinity for cGMP leads to a fast off rate for ligand and allows the channel to close quickly in response to light (Cobbs & Pugh 1987). The fast gating kinetics of the channel reports the level of cytosolic cGMP quickly and also improves the signal-to-noise ratio for

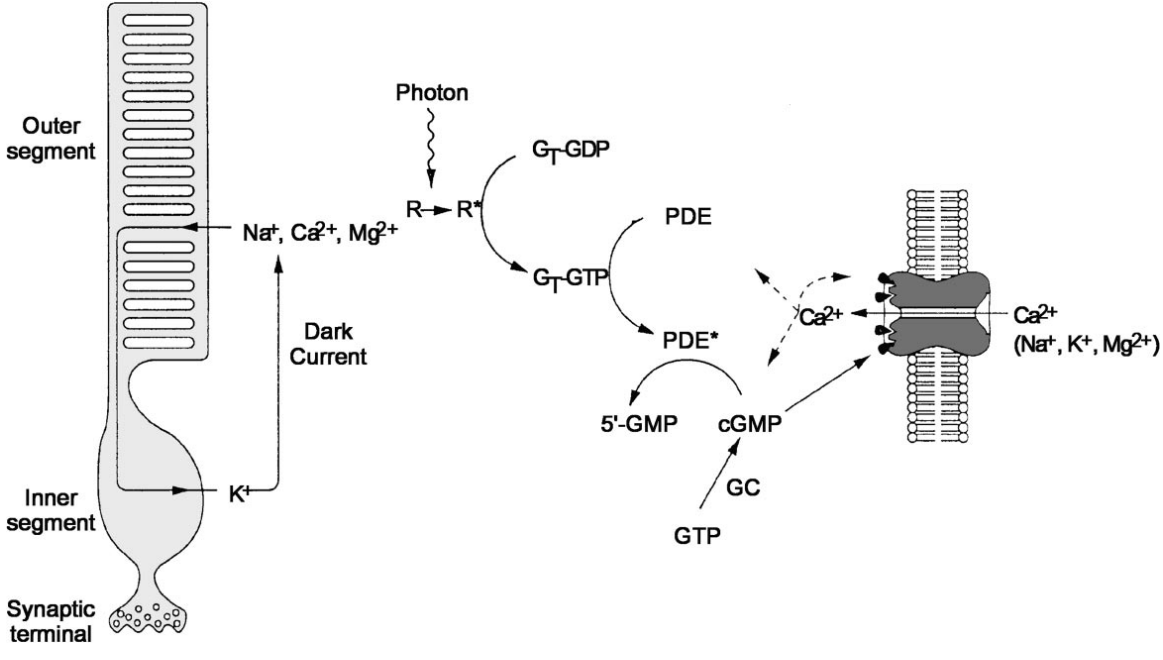

Figure 1 Phototransduction cascade. Cartoon of rod photoreceptor, showing the role of CNG channels found in the outer segment. Abbreviations: R, rhodopsin; R*, photoactivated rhodopsin; G_T-GDP, transducin bound to GDP; G_T-GTP, transducin bound to GTP; PDE, phosphodiesterase; PDE*, activated phosphodiesterase; GC, guanylyl cyclase. This figure was adapted from Zimmerman 1995.

photon detection (Haynes et al. 1986, Karpen et al. 1988, Matthews & Watanabe 1987, Zimmerman & Baylor 1986). Ca^{2+} and Mg^{2+} block CNG channels, making the single-channel conductance very small under physiological conditions and, hence, lowering the noise resulting from random gating of the channels (for review, see Yau & Baylor 1989). In addition, the closure of CNG channels reduces the cytoplasmic Ca^{2+} concentration (Yau & Nakatani 1985). This decrease in Ca^{2+} provides negative feedback in the phototransduction cascade by stimulating cGMP synthesis (Koch & Stryer 1988, Lolley & Racz 1982), increasing the channel's affinity for cGMP (Hsu & Molday 1993), reducing the catalytic rhodopsin activity produced by light (Lagnado & Baylor 1994), and accelerating rhodopsin deactivation by phosphorylation (Kawamura et al. 1993).

Family of CNG Channels

In vertebrates, six members of the CNG channel gene family have been identified. These genes are grouped according to sequence similarity into two subtypes, CNGA and CNGB (Bradley et al. 2001a). Additional genes coding for CNG channels have been cloned from *Drosophila melanogaster* and *Caenorhabditis elegans*. The phylogenetic relationship of these channels is shown in Figure 2.

The first cDNA clone for a subunit of a CNG channel (CNGA1, previously called the rod α subunit) was isolated from bovine retina (Kaupp et al. 1989). CNGA1 was expressed in rod photoreceptors and produced functional channels that were gated by cGMP when expressed exogenously either in *Xenopus* oocytes or in a human embryonic kidney cell line (HEK293). Mutations in CNGA1 in humans cause an autosomal recessive form of retinitis pigmentosa, a degenerative form of blindness (Dryja et al. 1995). Later, a second subunit of the rod channel (CNGB1, previously called the rod β subunit) was isolated and cloned (Chen et al. 1993, Korschen et al. 1995). CNGB1 subunits expressed alone do

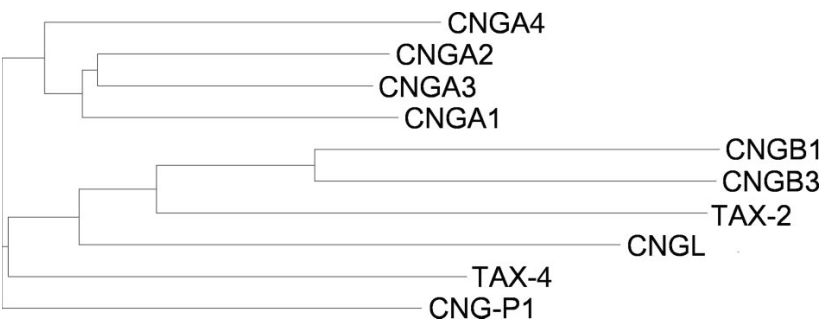

Figure 2 Phylogenetic tree of CNG channels. CNGA1, CNGA3, and CNGB1 sequences were from bovine. CNGA2 and CNGA4 sequences were from rat. The CNGB3 sequence was from human. TAX-2 and TAX-4 sequences were from *C. elegans*. CNGL and CNG-P1 sequences were from *D. melanogaster*.

not produce functional CNG channels, but coexpression of CNGA1 and CNGB1 subunits yields heteromeric channels with permeation, modulation, pharmacology, and cyclic-nucleotide specificity similar to that of native channels (Chen et al. 1993, Korschen et al. 1995). CNG channels form as tetramers (Gordon & Zagotta 1995c, Liu et al. 1996, Varnum & Zagotta 1996), and recent studies using a combination of different approaches suggest that native rod channels are composed of three CNGA1 subunits and one CNGB1 subunit (Weitz et al. 2002, Zheng et al. 2002, Zhong et al. 2002).

The CNG channels from cone photoreceptors are composed of two other cloned subunits, CNGA3 (previously called the cone α subunit) and CNGB3 (previously called the cone β subunit) (Bonigk et al. 1993, Gerstner et al. 2000). CNGA3 subunits, but not CNGB3 subunits, form functional channels when expressed exogenously. Mutations in human CNGA3 and CNGB3 have been linked to complete achromatopsia (also referred to as "rod monochromacy" or "total color blindness"), a rare, autosomal recessive inherited and congenital disorder characterized by the complete inability to discriminate between colors (Kohl et al. 1998, 2000; Sundin et al. 2000; Wissinger et al. 2001).

CNG channels have also been found in olfactory neurons, where they cause the cells to depolarize in response to increased concentrations of adenosine 3′:5′-cyclic monophosphate (cAMP) in order to transduce odorant signals (Nakamura & Gold 1987). Native olfactory channels are thought to be composed of three different subunits: CNGA2 (previously called the olfactory α subunit) (Dhallan et al. 1990), CNGA4 (previously called the olfactory β subunit) (Bradley et al. 1994, Liman & Buck 1994), and an alternately spliced form of CNGB1 (CNGB1b) (Bonigk et al. 1999, Picco et al. 2001, Sautter et al. 1998). The subunit stoichiometry and arrangement, however, are not known. CNGA2 subunits form functional channels when expressed in *Xenopus* oocytes or HEK293 cells. Mice lacking CNGA2 exhibit total anosmia (Brunet et al. 1996). CNGA4 subunits do not express functional CNG channels when expressed alone. Mice lacking CNGA4 still possess olfaction but exhibit abnormal olfactory desensitization that is associated with alterations in the Ca^{2+}/calmodulin modulation of the channels (Bradley et al. 2001b, Munger et al. 2001).

CNG channels have also been found to modulate transmitter release at the cone-bipolar cell synapse and to mediate the postsynaptic inhibitory response to glutamate of the on-bipolar cells (Nawy & Jahr 1990, Rieke & Schwartz 1994, Savchenko et al. 1997, Shiells & Falk 1990). In addition to the retina and olfactory epithelium, CNG channels are also found in nonsensory tissues such as the hippocampus, heart, testis, kidney, pancreas, adrenal gland, and colon where their functions are not currently understood (Biel et al. 1993, 1994, 1996; Bradley et al. 1997; Distler et al. 1994; Kingston et al. 1996; Weyand et al. 1994). Ca^{2+} imaging studies found that a rise in intracellular Ca^{2+} in hippocampal neurons could result from elevated intracellular cyclic nucleotide concentrations, suggesting that CNG channels play a role in the synaptic plasticity underlying learning and memory (Bradley et al. 1997, Kingston et al. 1996, Leinders-Zufall et al. 1995). Indeed,

long-term potentiation (LTP) was attenuated in mutant mice lacking CNGA2 (Parent et al. 1998).

In invertebrates, phototransduction involves a phosphoinositide enzyme cascade (Hardie et al. 1993, Minke & Selinger 1992, Ranganathan et al. 1991). However, a CNG channel subunit (CNG-P1) cloned from *D. melanogaster* is expressed in antennae and the visual system, suggesting that CNG channels may be involved in the transduction of light in invertebrates (Baumann et al. 1994). A second putative CNG-like channel subunit (CNGL) was also cloned from *D. melanogaster* and found to be expressed in the brain (Miyazu et al. 2000). In *C. elegans*, two CNG channel subunits, Tax-2 and Tax-4, have been cloned (Coburn & Bargmann 1996, Komatsu et al. 1996). These two CNG channels are required for chemosensation, thermosensation, and normal axon outgrowth of some sensory neurons in *C. elegans*.

STRUCTURE OF CNG CHANNELS

The CNG channel subunits all share the same basic architectural plan. As members of the family of voltage-dependent K^+ channels (Jan & Jan 1990), CNG channels are composed of four subunits around a centrally located pore (Gordon & Zagotta 1995c, Liu et al. 1996, Varnum & Zagotta 1996). Each subunit contains six transmembrane segments (S1-S6), a reentrant P-loop, and intracellular amino-terminal, and carboxy-terminal regions (Figure 3) (Henn et al. 1995, Kaupp et al. 1989, Liu et al. 1996, Molday et al. 1991, Wohlfart et al. 1992). The P-loop and S6 segments (Figure 3, *red*) line the ion-conducting pore, as seen in other P-loop-containing channels such as the voltage-dependent channels, the inwardly rectifying potassium channels, and the bacterial potassium channel KcsA. The carboxy-terminal region contains a cyclic nucleotide-binding domain (CNBD) and a region connecting the CNBD to the S6 segment (C-linker). These structural motifs are also seen in certain voltage-dependent potassium channels including HCN channels (Ludwig et al. 1998, Santoro et al. 1997, 1998), HERG channels (Trudeau et al. 1995), and KAT1 channels (Anderson et al. 1992). The amino-terminal region and the region following the CNBD (post-CNBD region) have specialized functions for each of the CNG channel subtypes. Each of these regions is discussed, in turn, below.

Pore

CNG channel pores are thought to be structurally similar to those of other P-loop-containing ion channels. The basic architectural plan for the pore of this family of channels was revealed by the crystal structure of KcsA, a bacterial potassium channel from *Streptomyces lividans* (Figure 4) (Doyle et al. 1998). KcsA is a tetramer of identical subunits arranged with fourfold symmetry about a centrally located pore. A single KcsA subunit has two membrane-spanning

helices, the outer and inner helices, and a reentrant P-loop. The P-loop starts from the extracellular side and enters the membrane as an α-helix (pore helix); it then exits back extracellularly as an uncoiled strand. Within this strand, permeant cations are coordinated by the backbone carbonyl oxygens of the amino acids TVGYG, recognized as the signature sequence of K^+-selective channels (Heginbotham et al. 1994). Intracellular to the selectivity filter is a large ($\sim$10 Å in diameter) water-filled vestibule. In the center of this cavity another permeant cation is stabilized through both electrostatic interactions with the pore helices and water molecules that hydrate the cation. The inner membrane-spanning helices line the vestibule of the channel and cross the membrane at an angle to form a helix bundle on the intracellular side (Doyle et al. 1998). The helix bundle defines the intracellular entrance to the pore, and KcsA may serve as a general model for the closed state for this family of ion channels.

To open the pore, a conformation change is thought to occur in the inner helix. An open conformation was revealed by the crystal structure of another ion channel, MthK (Jiang et al. 2002a,b). MthK is structurally similar to KcsA in the P-loop but exhibits a different conformation of the inner helix. The inner helix contains a gating hinge that bends this helix by 30°, creating a 12 Å opening on the intracellular side of MthK compared with the 4 Å opening of the helix bundle of KcsA. Amino acid conservation among a wide range of P-loop-containing channels suggests that the KcsA and MthK structures may serve as general models for the closed and open state conformations for this entire family of ion channels (Jiang et al. 2002b).

Experimental evidence suggests that the cytoplasmic opening of the CNG channel pore is narrow when channels are closed and widens when channels open. Substituting a cysteine (S399C) at the cytoplasmic end of the S6 (the putative inner helix) in a cysteine-free variant of CNGA1 channels promoted channel closure through the spontaneous formation of an intersubunit disulfide bond (Flynn & Zagotta 2001). Because disulfide bonds are formed between cysteine residues 5 Å apart (Careaga & Falke 1992, Falke et al. 1988), this result is consistent with a narrow cytoplasmic opening and the occurrence of a helix bundle similar to the one in KcsA. Furthermore, this disulfide bond formed faster when channels were closed than open, suggesting that a conformational change in the helix bundle of CNGA1 channels widens the intracellular entrance of the pore. A widening of the intracellular entrance is necessary to explain the voltage-dependent block by large molecules such as tetrapentylammonium ions (TPeA) that enter the inner vestibule and block the channel (Stotz & Haynes 1996). A wide intracellular entrance such as this is observed in MthK. Although the helix bundle defines a narrow cytoplasmic opening to the pore when channels are closed, its permeability to small cationic cysteine modifiers, such as Ag^+ and MTSEA, is state independent (Flynn & Zagotta 2001). These results are consistent with a model where the intracellular entrance of the pore of CNG channels widens during opening but is not itself the gate that controls permeation through the membrane.

In CNG channels, there are several pieces of evidence for conformational changes also in the selectivity filter associated with channel gating. (*a*) Mutations

in the selectivity filter have large effects on gating. This is particularly true for E363, a residue thought to create a binding site in the permeation pathway for monovalent and divalent cations (Bucossi et al. 1996, 1997; Gavazzo et al. 2000). (*b*) Tetracaine, a local anesthetic, blocks the pore of CNG channels (Fodor et al. 1997a,b). Tetracaine had a higher affinity for closed channels than for open channels, but this state dependence was abolished by mutating E363. (*c*) CNG channels that are partially activated (at subsaturating concentrations of cyclic nucleotide) have permeation properties that differ from those of fully activated (at saturating concentrations of cyclic nucleotide) channels (Hackos & Korenbrot 1999, Ruiz & Karpen 1997, Taylor & Baylor 1995). (*d*) Cysteine-scanning mutagenesis studies suggest that the pore helix, near the selectivity filter, undergoes a conformational change during channel activation (Becchetti et al. 1999, Liu & Siegelbaum 2000).

Cyclic Nucleotide-Binding Domains

The CNBDs of CNG channels share sequence similarity with other cyclic nucleotide-binding proteins including cGMP- and cAMP-dependent protein kinases (PKG and PKA, respectively) and the *Escherichia coli* catabolite gene activator protein (CAP) (Figure 5*a*). The crystal structures of CAP and PKA have been solved and are very similar (Su et al. 1995, Weber et al. 1987). The CNBD of CAP contains an eight-stranded antiparallel β roll, followed by two α-helices called the B- and C-helices (Figure 5*b*). cAMP binds to CAP in the *anti* configuration between the β roll and the C-helix. Although the overall sequence identity among the CNBDs of these cyclic nucleotide-binding proteins is only $\sim$20%, the residues that make important contacts with the bound cAMP or occur at turns between adjacent β strands are conserved (Figure 5*a*). Hence, the structure of the CNBD of CAP has been used as a model for the ligand-binding domains of CNG channels.

Channel activation can be thought of according to a model in which the initial binding of ligand is followed by a concerted allosteric opening transition (Gordon & Zagotta 1995a, Karpen et al. 1988). This model is a simplification of the more general MWC (Monod, Wyman, Changeux) model for activation of allosteric proteins, where the independent binding of ligand to each subunit stabilizes a concerted allosteric opening transition (Monod et al. 1965). Consistent with this model, the open probability of the channel is increased with increasing numbers of ligands bound (Liu et al. 1998, Ruiz & Karpen 1997). The energetics of opening with different numbers of ligands bound, however, suggests that the allosteric mechanism may be more complicated.

CNG channels exhibit a high degree of cyclic nucleotide specificity. The cyclic nucleotides cGMP, inosine 3′:5′-cyclic monophosphate (cIMP), and cAMP differ at only three positions on their purine rings (Figure 6). All three cyclic nucleotides can bind to the CNBD of the bovine CNGA1 channel subunits. However, bound cGMP promotes the allosteric opening transition approximately one order of magnitude greater than bound cIMP and three orders of magnitude greater than bound

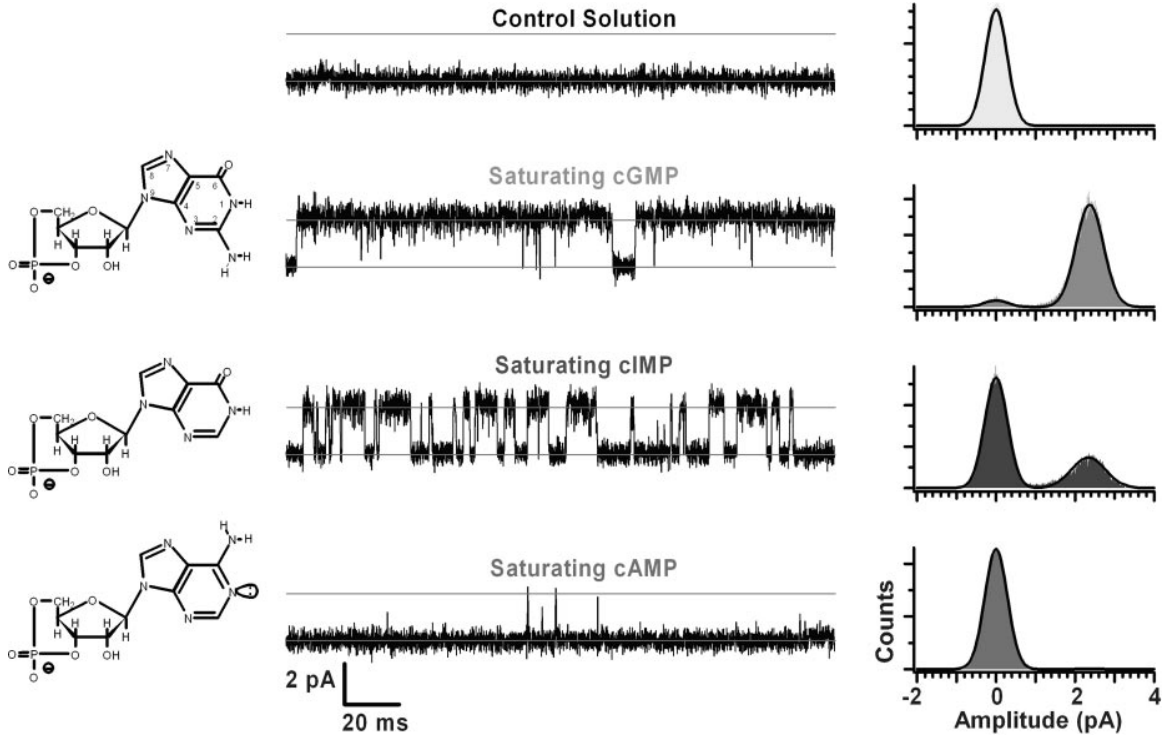

Figure 6 Cyclic nucleotide-specificity of CNGA1 channels. Structures of cGMP, cIMP, and cAMP are shown on the left. Single-channel currents recorded from inside-out patches from *Xenopus* oocytes expressing CNGA1 channels are shown in the *center* (Sunderman & Zagotta 1999a). Voltages were clamped at +80 mV. The upper and lower lines indicate the open and closed levels, respectively. Amplitude histograms are shown on the *right*.

cAMP (Figure 6) (Gordon & Zagotta 1995a, Sunderman & Zagotta 1999a, Varnum et al. 1995). Hence, the free energy of opening in CNGA1 channels is lowest with cGMP, intermediate with cIMP, and highest with cAMP. CNGA2 channels, although activated fully by saturating concentrations of both cGMP and cAMP, also have a lower free energy of opening with cGMP than with cAMP, as evidenced by the increased apparent affinity for cGMP compared with cAMP (Dhallan et al. 1990). For native olfactory channels or CNGA2/CNGA4 heteromeric channels, however, cAMP and cGMP have very similar apparent affinities (Anholt 1993, Bradley et al. 1994, Liman & Buck 1994, Nakamura & Gold 1987, Shapiro & Zagotta 2000).

What is the molecular basis for this ligand specificity? There are at least two important positions in the CNBD involved in determining the ligand specificity. The first is a threonine in the β roll corresponding to T560 in CNGA1 channels. Mutation of T560 decreases the apparent affinity of the channel for cGMP but has little effect on the cAMP apparent affinity (Altenhofen et al. 1991). Molecular modeling studies suggest that this threonine and the corresponding threonine in PKG might form an important hydrogen bond with the amino group attached to C2 on the guanine ring of cGMP (Figure 7) (Scott et al. 1996, Weber et al. 1989). This hydrogen bond formation would require cGMP to bind in the *syn* configuration. Whereas cAMP was found in the *anti* configuration in the crystal structure of CAP (Weber et al. 1987), cAMP was bound in the *syn* configuration in PKA (Su et al. 1995). T560 cannot completely explain ligand specificity, however. All CNG channel sequences identified so far have a threonine at this position, although some channels, such as the catfish olfactory channel, show nearly the same apparent affinity for cAMP as they do for cGMP (Goulding et al. 1992). In addition, while mutation of T560 decreased the apparent affinity of the channel for cGMP, cGMP still promoted opening much more than cAMP (Varnum et al. 1995).

The second residue involved in determining the ligand specificity is in the C-helix. Chimeric channels in which the C-helices of the bovine rod channel and the catfish olfactory channel were exchanged confirmed the role of the C-helix in ligand specificity (Goulding et al. 1994). Subsequently, mutations of a single residue on the C-helix of CNGA1 channels, D604, were found to dramatically alter the ligand specificity (Varnum et al. 1995). In fact, the D604M mutation caused a complete reversal of the cyclic nucleotide specificity, so that the channels were best activated by cAMP, more poorly by cIMP, and very poorly by cGMP. The presence of a methionine at this position in CNGA4 was sufficient to explain the altered ligand specificity of the native olfactory channel (Shapiro & Zagotta 2000). Single-channel recording showed that in CNGA1 channels, D604 mutations changed ligand specificity by destabilizing the free energy of opening with cGMP and stabilizing the free energy of opening with cAMP (Sunderman & Zagotta 1999b). In CAP, the residue at the homologous position on the C-helix, T127, forms a hydrogen bond with the N6 amino group in the purine ring of cAMP (Weber et al. 1987). This finding led to a model for CNG channels in which the negatively charged carboxylic acid side chain of D604 forms a pair of hydrogen bonds with

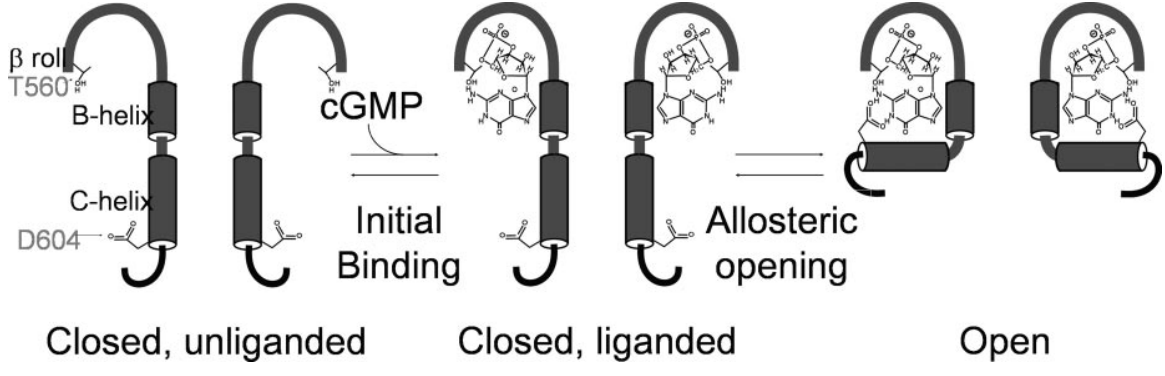

Figure 7 Proposed model of conformational changes in the CNBD during channel activation. The CNBDs of two channel subunits, with T560 and D604 side chains, are shown. The B- and C-helices are illustrated as cylinders.

the N1 and N2 hydrogen atoms on the purine ring of cGMP (Figure 7) (Varnum et al. 1995). This type of hydrogen bonding has been shown to occur in solution and in high-affinity GTP-binding proteins such as the α subunit of transducin, EF-Tu, and H-Ras. cIMP does not contain an amino group at the 2 position in its purine ring; hence, D604 could only form one hydrogen bond with cIMP, making cIMP a poorer agonist than cGMP. cAMP does not contain a hydrogen at either the 1 or the 2 position. Instead, a pair of unshared electrons at the N1 position of cAMP are proposed to cause an unfavorable interaction with D604, making cAMP a poor agonist for the rod CNGA1 channel. This unfavorable interaction is thought to generate a proton-binding site that produces a cAMP-specific potentiation of the channel at low pH (Gavazzo et al. 1997, Gordon et al. 1996).

Combined with the structure of CAP, a molecular mechanism for the conformational changes that occur in the ligand-binding domain during channel activation was proposed (Figure 7) (Varnum et al. 1995). In this mechanism, the cyclic nucleotide initially binds to the closed channel primarily by interactions between the β roll and the ribose and cyclic phosphate of the cyclic nucleotide. The purine ring of cGMP is also able to interact with T560 at this stage. Cyclic nucleotide binding is followed by a conformational change in the CNBDs that is coupled to the opening of the pore. This conformational change in the CNBDs was proposed to involve a relative movement of the C-helices toward the β rolls of each subunit, allowing D604 residues to interact with the purine rings of the bound cyclic nucleotides. This interaction could provide a significant portion of the energy required to drive an otherwise unfavorable opening conformational change.

This mechanism is supported by additional experiments. Mutation in the β roll of R559, the residue that forms the primary salt bridge with the phosphate of the cyclic nucleotides, dramatically inhibited the initial binding of ligand, decreasing the apparent affinity of the channel for cAMP and cGMP (Tibbs et al. 1998). Furthermore, cysteine modification of C505 in the β roll primarily affected the initial binding of cGMP, whereas modification of an introduced cysteine in the C-helix, G597C, primarily affected the agonist potency (Matulef et al. 1999). Recently, it was shown that cysteine residues in the C-helix produce an intersubunit disulfide bond primarily when the channel is closed (Matulef & Zagotta 2002, Mazzolini et al. 2002). This suggests that the C-helices might be nearer to each other or more flexible in the closed state of the channel and separate upon opening, as shown in Figure 7.

C-Linker

The C-linker appears to be important for the allosteric opening transition. Residues in the C-linker have been shown to be responsible for modulation of CNG channels by transition metals including Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, and Mn^{2+} (Gordon & Zagotta 1995a–c; Ildefonse & Bennett 1991; Karpen et al. 1993). Ni^{2+} modulation has been studied in much detail. In CNGA1 channels, H420, which is just below the S6 segment, coordinated Ni^{2+} between neighboring subunits and had a higher

affinity for the open state than for the closed state (Gordon & Zagotta 1995a,c). As a result, Ni^{2+} coordination at H420 decreases the free energy of the allosteric opening transition, potentiating the response of the channel to partial agonists and low concentrations of full agonists. A histidine at position 396 in CNGA2 channels, equivalent to 417 in CNGA1 channels, also coordinated Ni^{2+} but had a higher affinity for Ni^{2+} in the closed state (Gordon & Zagotta 1995b). This caused Ni^{2+} to increase the free energy of opening and inhibit CNGA2 channels. A histidine scan of the region just below the S6 in CNGA1 channels found that histidines introduced at positions 416 and 420 (Figure 8, *green*) caused Ni^{2+} to stabilize the open state (Johnson & Zagotta 2001). In contrast, histidines introduced into CNGA1 at positions 409, 413, and 417 (Figure 8, *red*) caused Ni^{2+} to stabilize the closed state, inhibiting the channels. The repetition of similar effects every four amino acids is consistent with secondary structure predictions that this region is α helical. The state dependence of Ni^{2+} coordination suggests a model in which a translation and clockwise rotation of this region relative to the central axis of the pore are involved in channel activation (Figure 8).

Other parts of the C-linker have also been found to affect the allosteric opening transition. The *C. elegans* TAX-4 channel has a much higher cyclic nucleotide efficacy and sensitivity than the bovine CNGA1 channel (Komatsu et al. 1996, Paoletti et al. 1999). These differences are largely due to three residues in the C-linker, R460, I465, and N466 (numbers correspond to CNGA1 channels) (Paoletti et al. 1999). In addition, differences between gating of CNGA3 and CNGA2 channels have been attributed to three amino acids in the C-linker (I415, D457, and D470) (Zong et al. 1998). Also, protons bind to H468 in CNGA1 channels, causing a potentiation similar to that caused by Ni^{2+} (Gordon et al. 1996). Another residue in the C-linker near the beginning of the CNBD, C481, undergoes state-dependent modification by the cysteine-modifying reagents *N*-ethylmaleimide (NEM) and methanethiosulfonate-ethyltrimethylammonium (MTSET) (Brown et al. 1998, Gordon et al. 1997). A fluorophore attached to this site has also been shown to undergo state-dependent quenching (Zheng & Zagotta 2000). These studies suggest that the entire C-linker region may be involved in the allosteric opening transition.

Amino-Terminal Domain

The amino-terminal region of some CNG channels has been found to affect the allosteric opening transition. CNGA2 channels have a lower free energy of opening compared with that of CNGA1 channels (Fodor et al. 1997b, Gordon & Zagotta 1995b, Goulding et al. 1994, Liu et al. 1994). Replacing the amino-terminal domain of CNGA1 with that of CNGA2 decreased the free energy of opening, whereas replacing the amino-terminal domain of CNGA2 with that of CNGA1 increased the free energy of opening (Gordon & Zagotta 1995b, Goulding et al. 1994). Single-channel analysis showed that the CNGA2 amino terminus stabilized the open state, causing a dramatic increase in the open probability for partial agonists

such as cAMP (Sunderman & Zagotta 1999b). Deleting part of the CNGA2 amino-terminal region decreased the open probability for cAMP and the apparent affinity for cGMP, suggesting that this region has an autoexcitatory effect on channel gating (Liu et al. 1994).

Ca^{2+}/CaM binding to the autoexcitatory region of the CNGA2 amino-terminal domain inhibits the channel's allosteric opening transition (Chen & Yau 1994, Liu et al. 1994). Olfactory channel opening allows Ca^{2+} to pass into the cell in response to odorants. This increased Ca^{2+} binds to CaM, which in turn binds to the autoexcitatory region of the CNGA2 amino-terminal domain, eliminating the autoexcitatory effect and downregulating the channel's activity (Liu et al. 1994, Varnum & Zagotta 1997). In this way, Ca^{2+}/CaM modulation of CNGA2 channels plays a significant role in olfactory adaptation (Kurahashi & Menini 1997).

How is it that the amino-terminal domain, very distant from the CNBD or pore region in primary structure, has this autoexcitatory effect on channel gating? Using polypeptides expressed in bacteria, the amino-terminal and carboxyl-terminal regions of CNGA2 were found to interact directly (Varnum & Zagotta 1997). Furthermore, it was found that the interaction of CNGA2 amino-terminal and carboxyl-terminal polypeptides was blocked by addition of Ca^{2+}/CaM, but not by the addition of either Ca^{2+} or CaM alone (Varnum & Zagotta 1997). This led to a proposed mechanism for Ca^{2+}/CaM modulation in which the CNGA2 amino-terminal domain has an autoexcitatory effect by interacting with the CNGA2 carboxyl-terminal domain, and Ca^{2+}/CaM inhibits CNGA2 channels by preventing this interaction.

Ca^{2+}/CaM inhibits rod CNG channels by binding to the amino-terminal domain of CNGB1 (Grunwald et al. 1998, Weitz et al. 1998) and disrupting amino- and carboxyl-terminal interactions (Trudeau & Zagotta 2002b). However, in rod channels, Ca^{2+}/CaM binding to an amino-terminal domain of CNGB1 prevented this domain from interacting with a carboxyl-terminal region distal to the CNBD (post-CNBD region) of CNGA1 in heteromeric channels (Figure 3). Deletion of the CNGB1 CaM-binding domain or the post-CNBD region did not affect channel gating (Trudeau & Zagotta 2002a,b). Hence, the disruption of the intersubunit interaction between these domains by CaM is proposed to directly inhibit the allosteric opening transition in CNGA1/CNGB1 channels.

Post-CNBD Region

As indicated above, the post-CNBD region of CNGA1 has been proposed to have an important role in Ca^{2+}/CaM modulation of heteromeric rod CNG channels. This region is also important for trafficking and heteromeric assembly of the rod channel. While deletion of this region has no effect on the expression or function of homomeric CNGA1 channels, it virtually eliminated functional expression of heteromeric CNGA1/CNGB1 channels (Trudeau & Zagotta 2002a). This lack of functional expression was shown to result from a trafficking defect that

prevented surface expression of the heteromeric channels, which could be rescued by deletions of the CNGB1 amino terminus. This suggests that the amino-terminal/carboxy-terminal interactions play a role in trafficking of heteromeric channels (Trudeau & Zagotta 2002a). Recently, the post-CNBD region of CNGA subunits was shown to contain a leucine zipper domain that forms trimers in solution, suggesting a further role for the region in producing the 3:1 (CNGA:CNGB) stoichiometry of the heteromeric channels (Zhong et al. 2002). Finally, the post-CNBD region of CNGA1 subunits has been shown to be truncated in a form of retinitis pigmentosa, a truncation that likely effects the trafficking of both homomeric (Dryja et al. 1995, Mallouk et al. 2002) and heteromeric (Trudeau & Zagotta 2002a) rod channels.

OTHER MODULATION OF CNG CHANNELS

CNG channels were originally thought to be static sensors of the cyclic nucleotide concentration. However, other environmental factors that can "fine tune" the sensitivity of CNG channels have now been found. As already discussed, Ca^{2+}/CaM and transition metals modulate CNG channel activity. Several other forms of modulation of CNG channels have also been described. (*a*) An endogenous Ca^{2+}-binding protein decreased cyclic nucleotide sensitivity in photoreceptors (Gordon et al. 1995b, Rebrik & Korenbrot 1998). This unknown protein competes with exogenous CaM, suggesting that they act through a similar mechanism. (*b*) Tyrosine phosphorylation of the CNGA1 ligand-binding domain at Y498 decreased the cyclic nucleotide sensitivity (Molokanova et al. 1997, 1999a). (*c*) Serine/threonine phosphorylation of native rod channels decreased the cyclic nucleotide sensitivity (Gordon et al. 1992). (*d*) In CNGA3 channels, phosphorylation of the ligand-binding domain at S577 or S579 by protein kinase C decreased the cyclic nucleotide sensitivity (Muller et al. 2001). (*e*) In CNGA2 channels, phosphorylation of the amino-terminal domain at S93 by protein kinase C increased the cyclic nucleotide sensitivity (Muller et al. 1998). (*f*) A noncatalytic interaction of a protein tyrosine kinase with regions surrounding and including the S6 domain of CNGA1 channels inhibited the saturating cGMP-elicited current (Molokanova & Kramer 2001; Molokanova et al. 1999b, 2000). (*g*) Lipid metabolites, including diacylglycerol, modulated native and expressed rod channels (Crary et al. 2000, Gordon et al. 1995a, Womack et al. 2000). (*h*) The Na/Ca-K exchanger has been found to be in close enough proximity to crosslink to CNGA1 channels in native rod photoreceptors (Schwarzer et al. 2000). (*i*) Recent studies have shown that the cGMP-sensitivity of cone photoreceptor CNG channels varies with a circadian rhythm, although the biochemical event responsible for this is not yet known (Ko et al. 2001). We are continuing to learn that CNG channels are not alone in the cell, and future studies will allow us to better understand the role of environmental factors in regulating the function of these channels.

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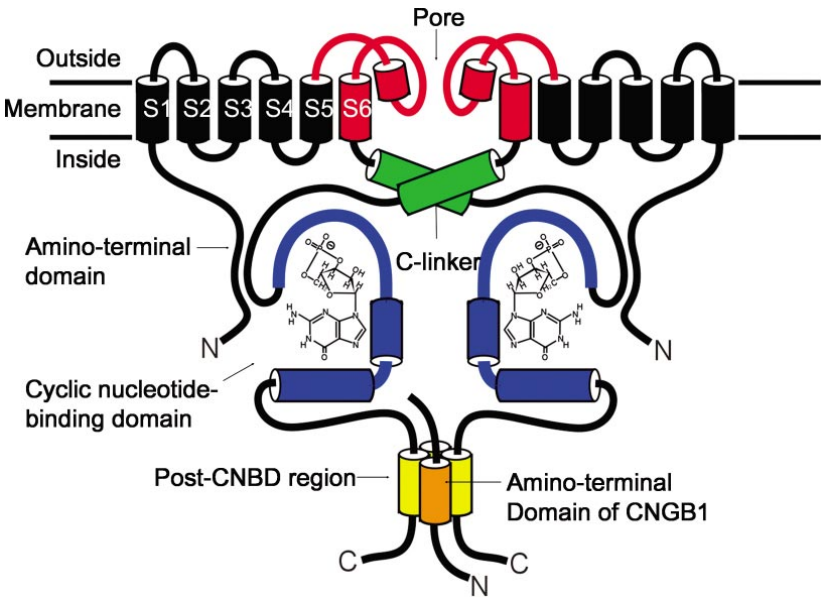

Figure 3 Structural cartoon of CNG channels. For simplicity, this figure shows two of the four subunits comprising CNG channels. The cylinders represent proposed α-helices.

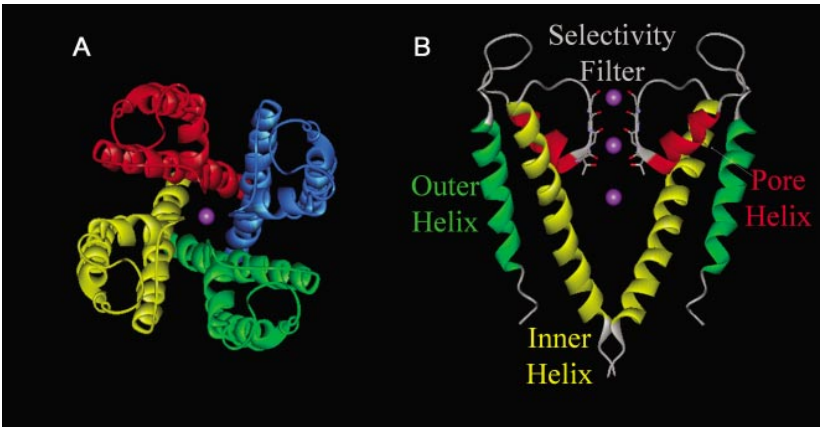

Figure 4 Structure of the KcsA channel. (*A*) Crystal structure of the KcsA channel (Doyle et al. 1998). Each subunit is shown in a different color. A K^+ ion is shown in purple. (*B*) Side view of two diagonally opposed subunits. Three K^+ ions in the permeation pathway are shown in purple.

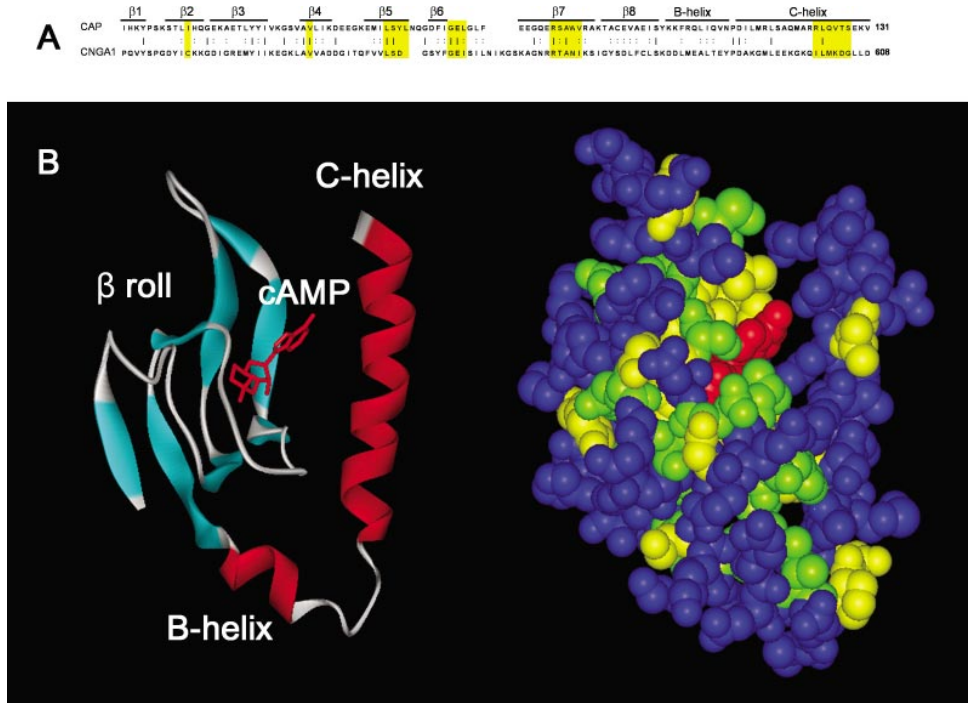

Figure 5 Structure of the CNBD. (*A*) Sequence alignment of the CNBDs of CAP and CNGA1. Lines above the sequence indicate secondary structure motifs present in the crystal structure of CAP; residues in yellow are those that line the ligand-binding pocket of CAP. (*B*) Crystal structure of the CNBD of one subunit of CAP with bound cAMP (Weber et al. 1987). Shown on the left is a ribbon diagram, and on the right is a space-filled model. Residues identical between CAP and CNGA1 are shown in yellow, conserved residues are shown in green, and other residues are shown in blue. cAMP is shown in red.

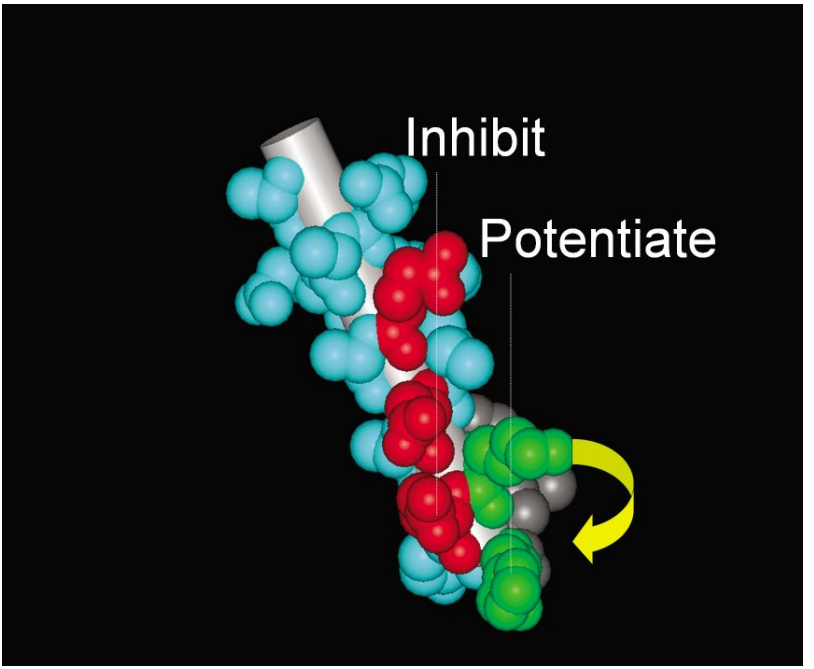

Figure 8 Structural model of the first helix of the CNGA1 C-linker. Red residues are positions where histidine substitution caused inhibition by Ni^{2+}. Green residues are positions where histidine substitution caused potentiation by Ni^{2+}. Gray residues are positions where histidine substitution had no effect of Ni^{2+}.

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ERRATA

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