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Mini Review

Regulation of Retinal Cyclic-Nucleotide Gated Channels by Calmodulin

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Abstract

Cyclic nucleotide-gated (CNG) ion channels mediate visual phototransduction in retinal photoreceptor cells. Retinal CNG channels are tetramers formed by two protein subunits (CNGA1 and CNGB1). The calcium sensor protein calmodulin (CaM) binds specifically to cytosolic regulatory domains of CNGB1. CaM binding sites within human CNGB1 (residues 565 - 587 and 1120 - 1147) bind to Ca^{2+}-bound CaM, which promote Ca^{2+}-induced inactivation of CNGA1/CNGB1 channels in retinal rods that play a role in photoreceptor light adaptation. Mutations that disable CaM binding to CNGB1 are responsible for inherited forms of blindness. In this mini review, I discuss a structural model of CaM bound to the CNG channel, which explains how CaM might decrease the channel binding affinity of cGMP and suggest how genetic mutations may lead to retinal disease.

Keywords: *CNGB1; Photoreceptor; Retina; Phototransduction; Calmodulin*

Abbreviations

CNGB1: β-Subunit of Retinal Cyclic-Nucleotide Gated Channel; CNGA1: α-Subunit of Retinal Cyclic-Nucleotide Gated Channel; CAM: Calmodulin; RETGC: Retinal Membrane Guanylyl Cyclase; CGMP: Cyclic Guanosine Monophosphate

Introduction

Phototransduction in retinal rod and cone photoreceptor cells involves light-activation of the visual pigment rhodopsin (R*) that turns on an enzymatic cascade, leading to the hydrolysis of cGMP catalyzed by phosphodiesterase PDE6 [1] (Figure 1A). The light-induced drop in cGMP level causes the closure of cGMP gated (CNG) channels in photoreceptor outer segment plasma membranes that generates a neural signal [2-4]. Photoreceptor CNG channels conduct a cation current only when the cytosolic ligand-binding domain of the channel binds to cGMP in dark-adapted photoreceptors (Figure 1B) [5,6]. Ca^{2+}-dependent regulation of photoreceptor CNG channels by CaM is important for promoting light adaptation in photoreceptor cells [7-9]. Retinal CNG channels consist of two protein subunits, CNGA1 and CNGB1 [10]. The CNGA1 subunit can form a functional homotetrameric channel when expressed alone, whereas CNGB1 does not form a functional homomeric channel [11]. Native CNG channels in retinal rods form a heteromeric tetramer comprised of a 3:1 stoichiometry of CNGA1:CNGB1 [12]. Three CNGA1 subunits form a trimer that binds tightly with a single CNGB1 subunit in a Ca^{2+}-dependent fashion. The Ca^{2+} sensor protein, calmodulin (CaM) binds to cytosolic sites in CNGB1 (residues 565 - 587 [13] and 1120 - 1147 [14]) that may regulate CNGB1 binding to CNGA1 [12] and perhaps mediate Ca^{2+}-induced CNG channel inactivation in rod cells [8,15]. Defects in the

Ca^{2+}-dependent regulation of CNG channels are genetically linked to autosomal recessive retinitis pigmentosa and other inherited forms of blindness [16]. Elucidating the CNG channel structural interaction with CaM may provide insights for the treatment of retinal diseases.

The recent atomic-resolution cryo-EM structures of the retinal CNG channel reveal features about the channel that explain the specificity of cation conduction and ligand binding [17,18]. However, the structure of the cytosolic regions of CNGB1 that interact with CaM (residues 565 - 587 and 1120 - 1147) are not well defined in the cryo-EM structures, perhaps because the bound CaM was structurally disordered or possibly not bound to the channel. The NMR structure of CaM bound to the CaM binding domain of the structurally related olfactory CNGA2 subunit reveals that both lobes of CaM bind to opposite sides of an α–helix in CNGA2, forming a collapsed structure [19]. The residues in the olfactory CNGA2 structure that contact CaM are highly conserved in CNGB1, suggesting a similar structure for the N-terminal CaM binding domain in CNGB1. In this mini review, I propose a new structural model of CaM bound to the retinal CNG channel, which explains how CaM might decrease the channel binding affinity of cGMP and suggest how genetic mutations may lead to retinal disease.

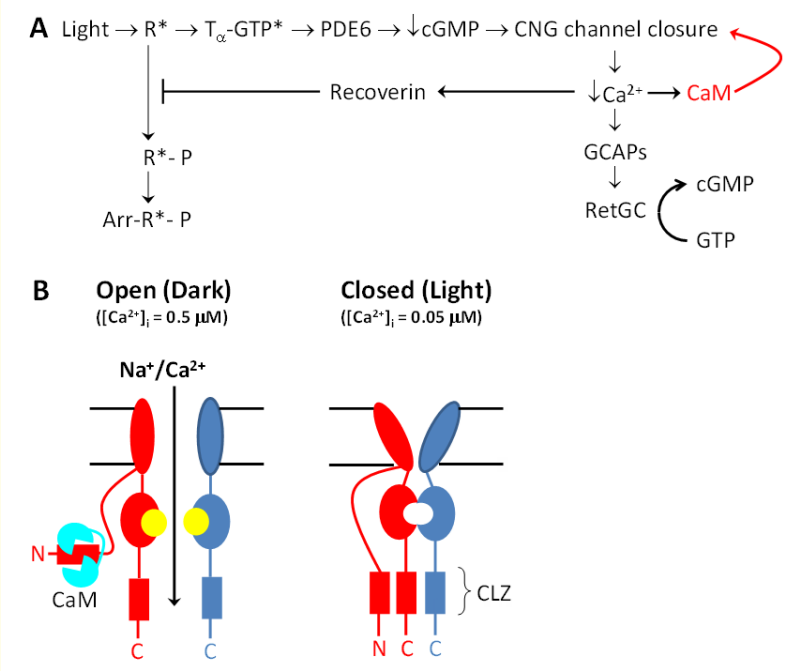

Figure 1: *Retinal CNG Channel Regulation by CaM.*

(A) Ca^{2+}-dependent regulation of visual phototransduction in retinal photoreceptor cells. Light-induced CNG channel closure promotes a drop in the cytosolic Ca^{2+} level that in turn regulates retinal membrane guanylyl cyclase (RetGC), rhodopsin kinase and CNG channel activity. Ca^{2+}-bound CaM binds to and inactivates CNG channels [13]. The Ca^{2+} sensor protein, recoverin inhibits rhodopsin kinase in a Ca^{2+}-dependent fashion [20,21], and the Ca^{2+} sensor protein GCAP1 binds to and activates RetGC [22,23]. (B) Schematic model of CNG channel regulation by CaM (CNGA1 in blue and CNGB1 in red). Bound cGMP are yellow spheres. High Ca^{2+} levels in dark-adapted rods cause Ca^{2+}-bound CaM (cyan) to bind to the N-terminal CaM binding domain (red square), which weakens cGMP binding to CNGB1 to promote light adaptation. At low Ca^{2+} levels in light-activated rods, Ca^{2+}-free CaM dissociates from CNGB1, which may allow the N-terminal CaM site to

interact with a C-terminal Leucine Zipper (CLZ) domain that in turn could enhance cGMP binding to help promote channel re-opening during visual recovery.

A structural model of the retinal cng channel bound to cam

Two CaM binding sites in CNGB1 may interact with CaM. A careful re-analysis of the recent cryo-EM electron density map of the retinal CNG channel structure identified two possible CaM binding domains of CNGB1 (residues 565 - 587, called CaM1 site and residues 1120 - 1147, called CaM2) that are structurally connected to the C-terminal Leucine zipper (CLZ) domain of CNGA1 [18,24] (Figure 2). The CLZ domain interacts closely with the cyclic-nucleotide ligand binding domain (CNBD) and is believed to modulate ligand binding. The interaction of the CaM1 site with CLZ was also suggested previously by [13,15]; however, the cryo-EM electron density map (Figure 2B) suggests that the bound CaM might also interact with the C-terminal CaM2 site in CNGB1. Two alternative models of CaM binding to the CNG channel are proposed in figure 2C. The binding of CaM to the CaM1 site (Figure 2C, left panel) may alter its interaction with the CLZ, which might explain how CaM binding could weaken channel binding to cGMP during light adaptation. Alternatively, CaM binding to the CaM2 site might interact with the CLZ instead of CaM1 (Figure 2C, right panel). Future studies are needed to distinguish and evaluate which model in Fig. 2C is physiologically most relevant.

The CaM C-lobe is constitutively anchored to CNG channels. Only one of the CaM lobes was detected in the cryoEM structure [24], which is tentatively assigned to the CaM C-lobe (Figure 2B) because the electron density matches the structure of the CaM C-lobe bound to creatine kinase (PDB: 7BF2). Also, the CaM C-lobe usually binds to target proteins with higher affinity than the N-lobe [25], which could explain why only the CaM C-lobe is bound to CNGB1 in the cryoEM structure (Figure 2B). The lower affinity binding of the CaM N-lobe might explain why it was disordered and not detected in the cryoEM structure, perhaps because the CaM N-lobe was in the Ca^{2+}-free state under the conditions of the cryoEM study ([Ca^{2+}] = 80 nM). By contrast the CaM C-lobe appears to remain calcified and bound to CNGB1 even at the low Ca^{2+} levels in light adapted rods [26], which would allow the CaM C-lobe to remain bound to CNGB1 in both dark-adapted and light-activated photoreceptors. The constitutive binding of the CaM C-lobe to CNGB1 may serve to pre-anchor CaM to the CNG channel, like what is seen for L-type Ca^{2+} channels [25]. Mutations that weaken Ca^{2+} binding to the CaM C-lobe or CaM binding to CNGB1 are predicted here to affect cGMP binding to the CNG channel, which could explain how these mutations would cause retinitis pigmentosa and other inherited forms of blindness [16]. Future atomic resolution structures of CaM bound to CNGB1 and/or the full-length CNG channel are needed to test the model in figure 2 and to determine whether the CaM C-lobe is constitutively anchored to CNGB1 as described above.

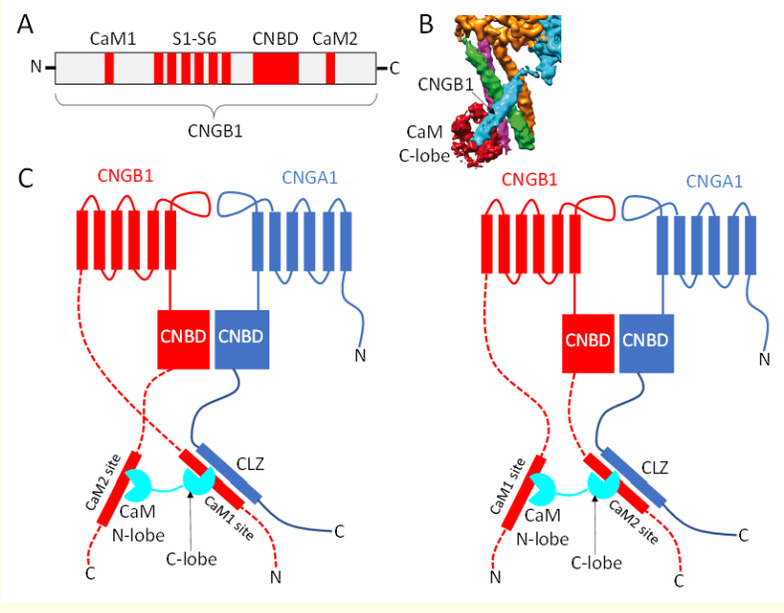

Figure 2: *Structural Models of CNG Channel Bound to CaM.*

(A) Primary structure of CNGB1. The CaM binding sites (CaM1 and CaM2), transmembrane helices (S1 - S6), and ligand binding domain (CNBD) are indicated. (B) Electron density map of CNG channel interaction with CaM (EMDB 24465); adapted from [24]. (C) Structural models of CNGB1/CNGA1 heterodimer with alternative structures of the CaM binding domains (CaM1: residues 565 - 587 and CaM2: 1120 - 1147) interacting with CaM (cyan): The model in the left panel depicts the CaM C-lobe bound to the CaM1 site and CaM N-lobe bound to CaM2 (left). The model in the right panel depicts the CaM C-lobe bound to the CaM2 site and CaM N-lobe bound to CaM1 (right). Adapted from [24]. Red dotted lines represent regions of CNGB1 that appeared structurally disordered in the original cryoEM structure [18].

Conclusion

CaM binding to the retinal CNG channel weakens cGMP binding to the channel, which helps promote light adaptation in photoreceptor cells. Recent cryo-EM structures of the CNG channel in the open state suggest that the CaM C-lobe may bind to a cytosolic helical region in CNGB1 that interacts with a cytosolic C-terminal Leucine Zipper motif (Figure 2). I propose that the CaM C-lobe is constitutively anchored to CNGB1, whereas the CaM N-lobe may serve as a Ca^{2+} sensor that could bind to CNGB1 only at high Ca^{2+} levels in dark-adapted rods to help promote light adaptation. Mutations that affect CaM binding to the CNG channel lead to inherited forms of blindness. Understanding the structural basis of CaM binding to the CNG channel may provide new insights for future drug design.

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