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Mini Review

Ca^{2+}-Dependent Regulation of Retinal Guanylyl Cyclase and Retinal Disease

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Received: June 17, 2019; **Published:** July 03, 2019

Abstract

Retinal guanylyl cyclase (RetGC), expressed exclusively in rod and cone photoreceptor cells, promotes the recovery phase of visual phototransduction. RetGC enzymatic activity is regulated by a family of Ca^{2+} sensor proteins known as guanylyl cyclase activator proteins (GCAPs), which activate RetGC in light activated photoreceptors at low cytosolic Ca^{2+} levels (50 nM) when GCAP proteins are in the Ca^{2+}-free state. By contrast, Ca^{2+}-bound GCAPs inhibit RetGC in dark adapted photoreceptors where cytosolic Ca^{2+} levels are maintained above 500 nM. Thus, the GCAPs act as both an accelerator and brake to precisely tune RetGC activity in photoreceptor cells. The Ca^{2+}-dependent regulation of RetGC by the GCAPs is important for coordinating visual recovery, and mutations in both RetGC and GCAPs are genetically linked to retinal degenerative diseases. In this mini review, I discuss a mechanism for how GCAPs control the Ca^{2+}-dependent activation of RetGC and explain why genetic mutations in GCAPs and RetGC lead to retinal disease.

Keywords: *Guanylyl Cyclase; Photoreceptor; Retina; Phototransduction; GCAP1; RetGC*

Abbreviations

GCAP: Guanylyl Cyclase Activating Protein; RetGC: Retinal Membrane Guanylyl Cyclase; cGMP: Cyclic Guanosine Monophosphate

Introduction

Visual phototransduction in retinal rod and cone cells is triggered by the light-activated hydrolysis of cGMP catalyzed by phosphodiesterase PDE6 [1,2] (Figure 1A). The light-induced decrease in cytosolic cGMP concentration promotes the closing of cyclic nucleotide gated channels (CNG channels) in photoreceptors that causes hyperpolarization of the plasma membrane [3-5]. When the light stimulus is turned off, the light-activated photoreceptor state converts back to the resting dark state by a process known as visual recovery [1,6]. Light activation of the photoreceptor causes a depletion of cGMP that can be replenished during visual recovery by the rapid re-synthesis of cGMP catalyzed by the enzyme, retinal guanylyl cyclase (RetGC). The light-dependent re-synthesis of cGMP is triggered by the light-induced drop in cytosolic Ca^{2+} concentration in light activated photoreceptor cells [4,7,8] (see figure 1A).

The Ca^{2+} sensitive RetGC enzymatic activity in photoreceptor cells (Figure 1B) is controlled by a family of Ca^{2+} sensor proteins, called guanylyl cyclase activating proteins (GCAP1-7) [7,9,10]. The Ca^{2+}-free state of GCAP proteins activate RetGC in light activated photoreceptors, when the cytosolic Ca^{2+} levels drop below 50 nM [7]. By contrast, Ca^{2+}-bound GCAPs inhibit RetGC in dark adapted photoreceptors where cytosolic Ca^{2+} levels are maintained above 500 nM [11]. This combined effect of Ca^{2+}-free and Ca^{2+}-bound GCAPs modulates the catalytic activity of RetGC more than 20-fold in rods and cones [6,12]. Electrophysiology studies on transgenic mice lacking GCAPs revealed that GCAPs increase the light sensitivity of rods in darkness but decrease the sensitivity in bright light [13]. Various mutations in GCAP1 [14-16] that prevent Ca^{2+}-sensitive activation of RetGC are genetically linked to retinal degenerative diseases [15,17,18]. Understanding

how GCAP1 structurally binds to and regulates RetGC is necessary in order to understand the molecular mechanism of human retinal dystrophies [19]. In this mini review, I discuss a structural mechanism for how GCAP1 regulates the Ca^{2+}-dependent cyclase activity of RetGC and explain why genetic mutations in GCAPs [15,16,20-22] and RetGC [17,18] each lead to retinal disease.

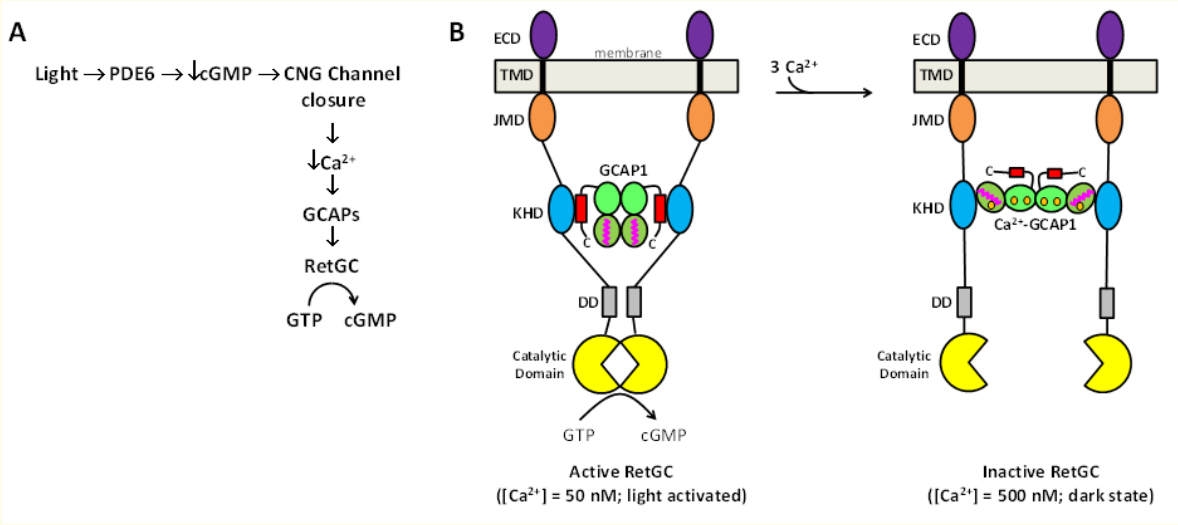

Figure 1: *Ca^{2+}-dependent RetGC activation in visual phototransduction. (A) Visual excitation pathway in retinal photoreceptor cells. Light-activated CNG channel closure promotes a drop in cytosolic Ca^{2+} level (50 nM) that causes Ca^{2+} dissociation from GCAPs that in turn switches on Ca^{2+}-free GCAPs to activate RetGC in light activated photoreceptors. Ca^{2+}-bound GCAPs inhibit RetGC at much higher Ca^{2+} levels (500 nM) in dark-adapted photoreceptors. (B) Schematic model of Ca^{2+}-dependent activation of RetGC by GCAP1. RetGC consists of six protein domains (ECD, TMD, JMD, KHD, DD and catalytic domain) that form a dimer. The dimeric GCAP1 (green) contains bound Ca^{2+} (orange circles), N-terminal myristoyl group (magenta), and Ca^{2+}-switch helix (red). Abbreviations: ECD: Extracellular Domain; TMD: Transmembrane Domain; KHD: Kinase Homology Domain; DD: Dimerization Domain.*

Discussion

The GCAP1 protein in mammalian photoreceptors helps to promote the visual recovery phase of phototransduction (Figure 1A) by switching on the catalytic activity of RetGC located on the outer segment disc membrane in light activated photoreceptors (Figure 1B, left panel). GCAP1 also switches off RetGC activity in dark-adapted photoreceptors (Figure 1B, right panel), which prevents the cGMP concentration in the photoreceptor from getting too high. Indeed, any condition or mutation that results in excessively high levels of cGMP causes retinal degeneration [23].

A mechanism of the Ca^{2+}-dependent activation of RetGC is shown in figure 1B. In the dark-adapted photoreceptor cell, the cytosolic Ca^{2+} concentration is maintained at a relatively high level (~500 nM), because CNG channels are kept open in the dark by the binding of cGMP, which allows Ca^{2+} influx. The GCAP1 protein is bound to Ca^{2+} in dark-adapted photoreceptors, because the cytosolic Ca^{2+} level (500 nM) is higher than the Ca^{2+} dissociation constant for GCAP1 (K_d = 200 nM [24]). The Ca^{2+}-bound GCAP1 crystal structure is known [25] and Ca^{2+}-bound GCAP1 forms a dimer in solution [26, 27]. The Ca^{2+}-bound GCAP1 dimer interacts with the dimeric RetGC and forms a 2:2 complex. Each Ca^{2+}-bound GCAP1 subunit (Figure 1B, green) interacts with the kinase homology domain (KHD) in RetGC [28], and this remote GCAP1 binding exerts an allosteric effect to the cyclase catalytic domain, which turns off catalysis (Figure 1B, right panel). By contrast, in light-adapted photoreceptors, the cytosolic Ca^{2+} concentration drops to below 50 nM, which causes Ca^{2+} to dissociate from

GCAP1. The structure of Ca^{2+}-free GCAP1 [29] undergoes a conformation change that involves a Ca^{2+} switch helix (Figure 1B, red), which alters its contact with RetGC and modulates the quaternary structure of the RetGC dimer. This Ca^{2+}-dependent structural change in GCAP1 and altered quaternary structure in RetGC exerts an allosteric effect to the cyclase catalytic domain, which turns on catalytic activity (Figure 1B, left panel).

Mutations in either RetGC [30,31] or GCAP1 [14-16,20,32] that abolish the Ca^{2+}-dependent regulation of RetGC are genetically linked to autosomal dominant cone dystrophy. In particular, the Y99C mutation in GCAP1 (that weakens Ca^{2+} binding to GCAP1) causes persistent activation of RetGC that in turn leads to elevated levels of cGMP, which causes retinal degeneration [16,33]. The Y99C mutation in GCAP1 creates a void in the hydrophobic core of Ca^{2+}-bound GCAP1, which destabilizes the structure of Ca^{2+}-bound GCAP1 relative to that of Ca^{2+}-free GCAP1 [25,29]. Small molecule drugs that mimic the size and shape of the Trp indole ring (e.g. a drug molecule called W7 [34,35]) might fill the void in the Y99C mutant and could possibly restore high affinity Ca^{2+}-binding affinity, which may rescue the Y99C phenotype. Indeed, structural studies on GCAP1 bound to W7 or related drugs may provide clues about how to increase the Ca^{2+}-binding affinity of Y99C and other mutants that cause autosomal dominant cone dystrophy. Future studies are needed to solve atomic-resolution structures of RetGC bound to GCAP1 that may provide molecular insights for designing new drugs to treat retinal degeneration.

Conclusion

GCAP1 binding to RetGC is essential for Ca^{2+}-dependent regulation of photoreceptor cyclase activity that dynamically controls the level of cGMP required for visual phototransduction. At high Ca^{2+} levels in dark-adapted photoreceptors, Ca^{2+}-bound GCAP1 inhibits RetGC and regulates the cytosolic cGMP levels under basal conditions. Light activation of retinal rods and cones leads to a drop in the cytosolic Ca^{2+} concentration that in turn causes Ca^{2+}-free GCAP1 to activate RetGC to replenish the cGMP concentration during visual recovery. Mutations that disable Ca^{2+}-dependent regulation of RetGC by GCAP1 are genetically linked to retinal degenerative disease. Understanding the structural basis of the GCAP1 binding interaction with RetGC may provide new insights for drug design.

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