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Mini Review

Retinal Photoreceptor Degeneration Caused by Mutations in Guanylate Cyclase Activating Proteins (GCAPs)

James B Ames*

Department of Chemistry, University of California, Davis, CA, USA

***Corresponding Author:** James B Ames, Department of Chemistry, University of California, Davis, CA, USA.

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Abstract

Retinal photoreceptor degenerative diseases such as rod-cone dystrophies are caused by mutations in retinal Ca^{2+}-binding proteins (called GCAPs) that weaken their binding to Ca^{2+} and cause dysregulation of retinal guanylyl cyclase (RetGC). The enzymatic activity of RetGC is activated by its binding to Ca^{2+}-free GCAPs in light-adapted photoreceptors, whereas RetGC is inhibited by its binding to Ca^{2+}-bound GCAPs in dark-adapted photoreceptors. This Ca^{2+}-dependent regulation of cyclase activity is important for coordinating the recovery phase of visual phototransduction. Mutations in GCAP1 (G86R, Y99C, I143N) that weaken its Ca^{2+} binding also cause constitutive activation of RetGC that in turn leads to rod-cone dystrophies. In this mini review, I propose a structural mechanism to explain how GCAPs control the Ca^{2+}-dependent activation of RetGC.

Keywords: *Guanylyl Cyclase; Cone-Rod Dystrophy; Retina; Phototransduction; GCAP1; RetGC*

Abbreviations

GCAP: Guanylyl Cyclase Activating Protein; RetGC: Retinal Membrane Guanylyl Cyclase; cGMP: Cyclic Guanosine Monophosphate; KHD: Kinase Homology Domain

Introduction

Visual phototransduction in photoreceptor rod and cone cells is promoted by light-activated hydrolysis of cGMP catalyzed by phosphodiesterase [1,2] (Figure 1A). The cytosolic cGMP concentration decreases following illumination and promotes the closure of retinal cyclic nucleotide gated channels (CNG channels), which hyperpolarizes the plasma membrane and generates a neural signal [3-5]. When the light stimulus is removed, the light-activated photoreceptor returns to the resting dark state in a process called visual recovery [1,6]. The light-induced depletion of cGMP can be replenished during visual recovery by the rapid re-synthesis of cGMP catalyzed by the enzyme, retinal guanylyl cyclase (RetGC). Light-dependent cyclase activation is triggered by a light-induced drop in cytosolic Ca^{2+} concentration in light activated photoreceptor cells [4,7,8] (See figure 1A).

Ca^{2+}-dependent cyclase activity in photoreceptor cells (Figure 1B) requires a family of Ca^{2+} sensor proteins, called guanylyl cyclase activating proteins (GCAP1-7) [7,9,10]. The Ca^{2+}-free GCAPs activate RetGC in light activated photoreceptors at low cytosolic Ca^{2+} levels below 50 nM [7]. The Ca^{2+}-bound GCAPs inhibit RetGC in dark adapted photoreceptors at high cytosolic Ca^{2+} levels above 500 nM [11].

Thus, the GCAPs serve as both an accelerator and brake that modulates the RetGC catalytic activity more than 20-fold inside photoreceptor cells [6,12]. Transgenic mice that lack GCAPs revealed decreased light sensitivity of rods in darkness in contrast to increased sensitivity in bright light [13]. Mutations in GCAP1 (G86R, Y99C, I143N) [14-16] that weaken Ca^{2+} binding also prevent Ca^{2+}-sensitive activation of RetGC and cause rod-cone dystrophies [15,17,18]. An important unresolved question is to understand how GCAP1 structurally binds to RetGC to promote Ca^{2+}-dependent conformational changes that switch on cyclase activation [19]. In this mini review, I propose a structural mechanism for how GCAP1 regulates the Ca^{2+}-dependent cyclase activity of RetGC, which explains why mutations in GCAP1 (G86R, Y99C, I143N) that weaken or disable Ca^{2+} binding [15,16,20-22] also lead to retinal disease.

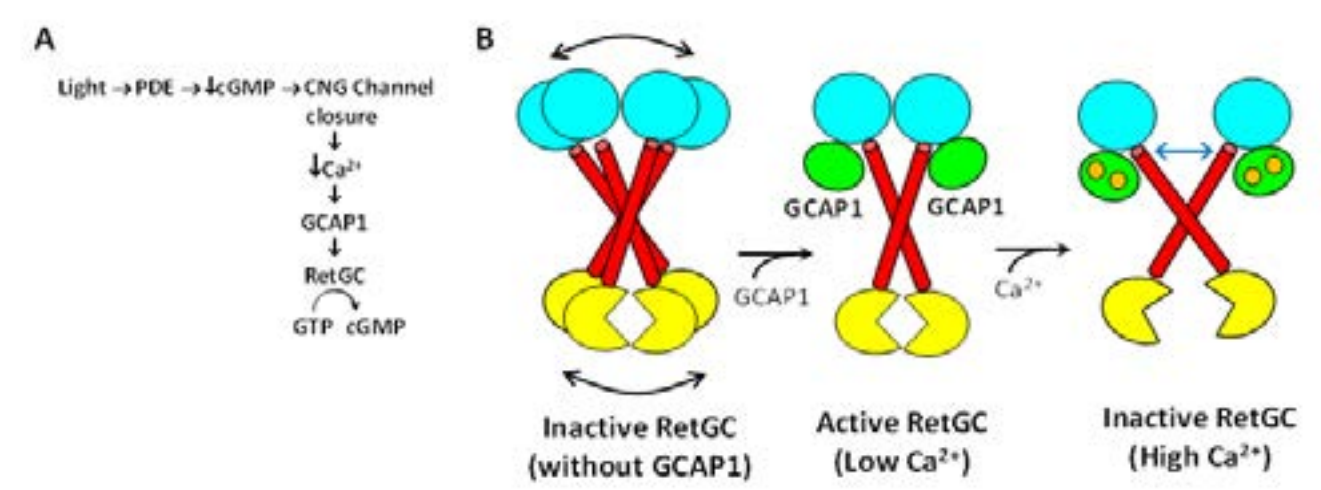

Figure 1: *$Ca2^{+}$-dependent RetGC activation mediated by GCAP1. (A) Visual excitation pathway in retinal photoreceptor cells. Light-activated CNG channel closure promotes a drop in cytosolic Ca^{2+} level (50 nM) that causes Ca^{2+} dissociation from GCAP1 that in turn switches on Ca^{2+}-free GCAPs 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 three cytosolic domains: KHD (cyan), DD (red) and catalytic domain (yellow) that forms a dimer. Inactive RetGC in the absence of GCAP1 (left panel). Ca^{2+}-free GCAP1 (green) bound to RetGC (middle panel) and Ca^{2+}-bound GCAP1 (bound Ca^{2+} as orange circles) bound to RetGC (right panel).*

Discussion

A structural mechanism of the Ca^{2+}-dependent activation of RetGC is shown in figure 1B. In dark-adapted photoreceptors, the cytosolic Ca^{2+} concentration is maintained at a high level (~500 nM), because CNG channels are kept open 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 [23]). The Ca^{2+}-bound GCAP1 crystal structure is known [24] and Ca^{2+}-bound GCAP1 forms a dimer in solution [25,26]. However, the dimeric GCAP1 structure in solution may be an artifact of the high protein concentrations used for NMR and EPR [25], because GCAP1 is monomeric under physiological conditions [27]. Also, a mutation in GCAP5 (R22A) that abolishes GCAP5 dimerization also causes a 3-fold higher activation of RetGC [28], implying the GCAP5 dimerization is not essential for cyclase activation. In our model (Figure 1B), GCAP1 is proposed to bind to RetGC at the junction between the kinase homology domain

(KHD) and the dimerization domain (DD). The model suggests that a Ca^{2+}-induced change in RetGC tertiary structure near the KHD/DD junction (caused by Ca^{2+} binding to GCAP1) could alter the quaternary structure of DD and perturb the dimer interface of the RetGC catalytic domain, causing a modulation in catalytic activity (Figure 1B). In essence, GCAP1 binding to RetGC1 is proposed to cause a scissoring-like motion of the helices in the DD (red coiled-coil in figure 1B) that modulates the quaternary structure of the catalytic subunits (akin to the T → R transition for hemoglobin [29]). I propose that Ca^{2+}-free GCAP1 binding to RetGC1 may rigidify the coiled-coil and cause it to adopt a conformation that connects the two catalytic subunits to achieve maximal catalytic activity at low Ca^{2+} levels (Figure 1B, middle). By contrast, the binding of Ca^{2+}-bound GCAP1 to RetGC1 is predicted to increase the interhelical angle in the DD (red coiled coil), which may disrupt the connection between the catalytic subunits, causing the catalytic activity to turn off at high Ca^{2+} levels (Figure 1B, right panel). This model also accounts for the low catalytic activity in the absence of GCAP1, because the RetGC dimerization domain is less restrained (scissoring-like motion, double-headed arrows in figure 1B, left) and only transiently adopts the optimal conformation required for cyclase activation. Therefore, the absence of GCAP1 gives rise to conformational heterogeneity that causes lower catalytic activity (see double-headed arrows in figure 1B, left panel).

Mutations in GCAP1 (G86R, Y99C, I143N) [14-16,20,30] that weaken Ca^{2+} binding also abolish the Ca^{2+}-dependent activation of RetGC that causes autosomal dominant cone dystrophy. The weaker Ca^{2+} binding affinity of the GCAP1 disease mutants (G86R, Y99C, I143N) allows the Ca^{2+}-free GCAP1 to persist even at high Ca^{2+} levels in the dark, which causes constitutive activation of RetGC. In particular, the Y99C mutation in GCAP1 (that weakens Ca^{2+} binding to the third EF-hand in GCAP1) causes the Ca^{2+}-free mutant to constitutively activate RetGC that in turn leads to elevated levels of cGMP, which causes retinal degeneration [16,31]. Future studies are needed to solve atomic-resolution structures of RetGC bound to GCAP1 to test the model in figure 1B.

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. Ca^{2+}-bound GCAP1 inhibits RetGC at high Ca^{2+} levels in dark-adapted photoreceptors. Light activation of retinal photoreceptors 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. GCAP1 Mutations (G86R, Y99C, I143N) that weaken Ca^{2+} binding also cause constitutive activation of RetGC, which causes retinal degenerative diseases. A structural model was presented to understand the structural basis of the GCAP1 binding interaction with RetGC, which suggests the central dimerization domain allosterically couples the GCAP1 binding site (in KHD) with the catalytic domain.

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