G Protein Signaling in the Retina and Beyond
The Cogan Lecture

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ORGANIZATION OF G PROTEIN CASCADES: LESSONS FROM PHOTORECEPTORS

In order to survive and function properly, mammalian cells send and receive a vast number of signals that are used to adjust their behavior in response to changes in the environment. Among the multiple receptor systems that cells utilize for this purpose, the most prominent role is undoubtedly played by G protein-coupled receptors (GPCRs). These cell surface receptors respond to an incredibly wide repertoire of ligands, including ions, peptides, lipids, neurotransmitters and light. G protein-coupled receptors constitute the largest family in mammalian genomes, accounting for approximately 3% to 4% of all genes.[1] Despite their versatility, the organization of all known GPCRs is rather conserved; they share a common seven transmembrane topology and the ability to activate intracellular heterotrimeric G proteins.[2] Ligand binding causes GPCRs to undergo a conformational change, which is sensed intracellularly by G proteins, causing them to release guanosine diphosphate (GDP) in exchange for guanosine triphosphate (GTP). Nucleotide binding occurs on the Gα subunit and results in its dissociation from the Gβγ subunits. In their dissociated state, both Gα-GTP and free Gβγ are able to interact with and regulate the activity of downstream effectors, including proteins key to cellular homeostasis, such as ion channels, kinases, and second messenger-producing/degrading enzymes. This signaling is terminated upon the hydrolysis of GTP by the Gα subunit, causing its inactive Gα-GDP form to reassociate with the Gβγ subunit.

Much of what we know about the functional organization of GPCR systems is derived from the phototransduction cascade of vertebrate photoreceptors, one of the first and the best-studied G protein pathways. As a result, the lessons learned in the study of photoreceptors have had a tremendous impact on our understanding of GPCR biology and will likely continue to guide research on G protein cascades for years to come. The main sequence of the events in phototransduction is now well established and has been the subject of several excellent reviews.[3–6] In prototypic rod photoreceptors, light causes a conformational change in the photosensitive GPCR rhodopsin by inducing isomerization of the receptor-bound inverse agonist *11-cis* retinal into the full agonist *all-trans* retinal. Photoexcited rhodopsin activates G protein transducin, which in turn dissociates into Gαt1-GTP and Gβ1γ1 subunits. Activated Gαt1-GTP binds to its effector enzyme—the gamma subunit of phosphodiesterase, type 6 (PDE6γ)—and relieves the inhibitory constraint that this subunit has on the catalytic

PDE6αβ subunits, which leads to the hydrolysis of the second messenger cGMP. The declining concentrations of cGMP allow the opening of cGMP-gated ion (CNG) channels on the plasma membrane, leading to cellular hyperpolarization and the resulting inhibition of neurotransmitter release. All components of the phototransduction cascade are delegated to a special compartment of the cell called the outer segment, which is essentially an elaboration of the primary cilia. Thus, the phototransduction cascade is highly compartmentalized, revealing the first lesson from this GPCR cascade. The second lesson is provided by studies on the mechanisms that allow photoreceptors to quickly recover from excitation, a property that is essential for achieving the high temporal resolution of our vision. This process requires the deactivation of phototransduction, which involves the termination of both rhodopsin and transducin signaling.[7,8] One of the major breakthroughs in the field was the demonstration that transducin deactivation is the rate-limiting step in the termination of phototransduction reactions.[9] Transducin, as well as all other G proteins, has a very slow GTP hydrolysis rate, with kinetics that are insufficient to explain the physiologically relevant speed of photoresponse termination. The timely deactivation of transducin requires the contribution of another element of the GPCR cascade, type 9 regulator of G protein signaling (RGS9), which functions to speed up the rate of GTP hydrolysis of this G protein.[10,11] Type 9 regulator of G protein signaling belongs to a family of RGS proteins that consists of more than 30 members ubiquitously expressed in all cells and involved in the regulation of GPCR signaling.[12] Thus, the second lesson learned from the organization of the phototransduction cascade is the key involvement of RGS proteins for achieving physiologically relevant timing.

In photoreceptors, RGS9 does not act alone but requires the contribution of two proteins with which it forms a tight complex, and which are now considered to be its bona fide subunits. The first protein, an atypical member of the G protein family, type 5 beta subunit (Gβ5), is required for ensuring the correct folding and stability of the complex,[13,14] with additional contributions in guiding RGS9 to selectively recognize its correct substrate, the Gαt1-PDE6γ complex, instead of free Gαt1.[15,16] The second molecule, a SNARE-like transmembrane protein named RGS9 anchor protein (R9AP), delivers the complex to the outer segments of the photoreceptors, positioning it on the disc membranes[17,18]; R9AP also plays an essential role in determining the proteolytic stability of the complex.[19] Work on the organization and functional regulation of the RGS complex in photoreceptors by Vadim Arshavsky and

Theodore Wensel received the Proctor award in 2013[20] and highlights the third key lesson: The components are scaffolded together in tight macromolecular complexes, with intimate interplay between components that ultimately defines their function in GPCR regulation. With such deep mechanistic understanding of the vertebrate phototransduction cascade, one of the key challenges is to test how general these principles are and whether they can be used as a universal template for understanding the organization and functional regulation of other much less well-studied GPCR cascades.

THE G PROTEIN CASCADE AT THE FIRST VISUAL SYNAPSE

The initial response to light generated by photoreceptors would not be of much use unless it can be transmitted across the neural circuit in the retina and eventually to the visual cortex in the brain. To do so, photoreceptors form synaptic connections with downstream neurons, bipolar cells (BCs). Excitation of photoreceptors by light inhibits the release of the neurotransmitter glutamate by their axonal terminals, and these changes in the glutamate concentration in the synaptic cleft are differentially sensed by two classes of downstream BCs. OFF-bipolar cells signal in the dark and are inactivated upon light stimulation, whereas the ON type of BCs carry excitatory signaling to the responses produced by photoreceptors in the light.[21,22] Although cone photoreceptors responding to high-light intensities signal via both of these pathways, rods that are active under low illumination relay information exclusively to ON-BCs, making these cells essential for vision in dim light.

Remarkably, ON-BC neurons utilize a GPCR pathway that is reminiscent of the phototransduction for synaptic communication with the photoreceptors, pairing two G protein signaling systems in these synaptically connected cells. It has been established that the glutamate receptor of these cells is mGluR6[23,24] and that it is coupled via the G protein Go[25,26] to the opening of the cation channel recently identified as TRPM1.[27-29] In the dark, the pathway is constantly activated, holding the channels closed and causing the cell to hyperpolarize (Fig. 1). A decrease in glutamate release by photoreceptors during the light response reduces mGluR6 activation, triggering Go deactivation and the opening of TRPM1 channels and thus causing excitatory depolarization.[30,31] Because synaptic transmission to ON-BCs is the primary pathway for rods to communicate their signal to the retinal circuitry, the inability to transfer the signal at this point results in the loss of scotopic vision, the condition in humans known as congenital stationary night blindness (CSNB).[32] In the clinic, CSNB diagnosis is normally confirmed by performing ERG, an electrophysiological recording of light-induced changes in the field potential as an electrical signal propagates through retinal neurons. In normal subjects, a typical two-component waveform is observed: an a-wave that reflects the hyperpolarizing activity of photoreceptors and a b-wave that reflects ON-BC depolarization. In patients with CSNB, the b-wave is eliminated, indicating deficits in synaptic transmission.[33] The mapping of mutations to genes in patients with this disease manifesting in either complete or incomplete elimination of the b-wave has proven to be highly insightful for identifying the molecular players essential for this synaptic communication. In addition to known elements, such as mGluR6[34,35] and TRPM1,[36-38] as well as presynaptic calcium channels that regulate glutamate release,[39,40] these studies have revealed several new proteins, including nyctalopin,[41] orphan receptor GPR179,[42,43] and cell adhesion-like protein LRIT3,[44] the function of which in the ON-BC G protein signaling cascade
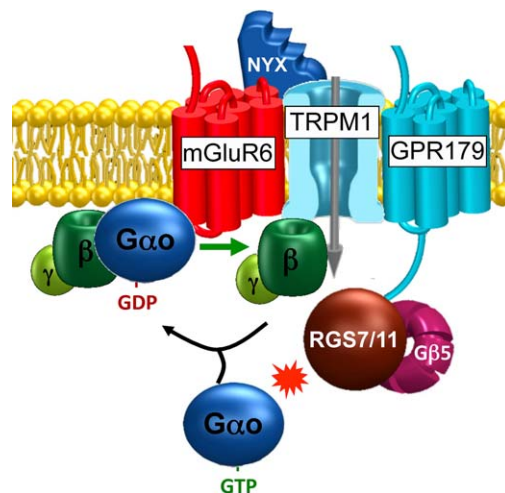

FIGURE 1. Organization of the G protein signaling cascade at the dendritic tips of retina ON-bipolar neurons.

is only beginning to be understood. A complementary and highly productive effort in the field has been provided by the phenotyping of a collection of mouse strains with random or targeted mutations for the absence of the ERG b-wave. This has led to the identification of many of the same genes, including nyctalopin,[45] TRPM1,[27-29] GPR179,[42] LRIT3,[46] and mGluR6,[47] but also several unique players, including Gαo,[25] Gβ3,[48] and Gβ5,[49] with essential roles in the synaptic transmission to ON-BCs.

ELUCIDATING THE ROLE OF RGS PROTEINS IN ON-BC SYNAPTIC TRANSMISSION

Providing that the lessons gleaned from studies on the phototransduction cascade can be used as a guide, the G protein cascade in ON-BC neurons misses a critical element: an RGS protein. Indeed, work by Sampath and Rieke[50] demonstrated that GTP hydrolysis is required for the onset of the ON-BC response; and using reconstituted system, Noga Vardi's[51] group showed that mGluR6 signaling to ion channels can, in principle, be modulated by RGS proteins. Furthermore, the discovery that the elimination of Gβ5 produces a characteristic lack of the b-wave[49] has brought attention to the R7 family of RGS proteins as possible regulators of ON-BC signaling. Similar to the case in photoreceptors, Gβ5 forms complexes with RGS proteins by irreversibly binding to a G-gamma-like domain in 3 other RGS9-like proteins, RGS6, RGS7, and RGS11, which form the R7 RGS family.[52] As with RGS9 in photoreceptors, the formation of this complex is essential for the proteolytic stability of other R7 RGS members in the rest of the nervous system, as the knockout of Gβ5 in mice drastically reduces their expression.[14] The interest in this RGS family has been further fueled by the demonstrated role of RGS9 in phototransduction, and it seems logical to delegate a related RGS protein to control the G protein cascade in the synaptically connected neuron. Our group has profiled the expression of R7 family members in the retina and found RGS7 and RGS11 to be specifically enriched in the synaptic layer, the site where photoreceptors make contacts with bipolar cells.[53] This finding was independently corroborated by several investigators including the groups of Catherine Morgans,[54] Theodore Wensel,[55] and Jason Chen.[56] To test the possible involvement of RGS7 and RGS11 in ON-BC signaling, mouse models with a

disruption in the respective genes have been used. Elimination of RGS11 or RGS7 alone did not have a substantial effect on the b-wave,[56-59] causing only minor alterations in the time required to mount a maximal response, which is inconsistent with their major role in the regulation of mGluR6 signaling, which would be predicted to produce major effects akin to what is observed in Gβ5 knockouts. Furthermore, combining RGS11 elimination with hypomorphic mutation in RGS7 did not largely exacerbate the phenotype, leading to the conclusion that neither RGS7 not RGS11 alone sufficiently impacts the mGluR6 signaling cascade of ON-BC cells. The breakthrough came when a true null allele of RGS7 was combined with the RGS11 knockout.[60,61] Mice lacking both of these RGS proteins exhibited a complete lack of the ERG b-wave and the responsiveness of ON-bipolar cells to flashes of light. However, the ON-bipolar neurons exhibited a residual response when bright steps of light were applied to the retina for a prolonged period of time.[61] The onset of the depolarizing response in retinas lacking RGS7 and RGS11 proteins was much slower than in wild type, suggesting that these proteins control the timing and sensitivity of TRPM1 channel activation. On the basis of these findings, we proposed a model in which RGS proteins in ON-bipolar neurons oppose the stimulatory action of mGluR6 to set the balance of G protein activation, such that in the dark, activated Go is produced in slight excess to effectively open TRPM1 channels. Light changes this balance, decreasing mGluR6 activity and allowing RGS proteins to deactivate Go below a certain threshold, such that TRPM1 channels begin to deactivate, generating a depolarizing response. When RGS proteins are completely eliminated, the balance is shifted to further favor Go activation, increasing the pressure to keep TRPM1 channels closed. The transient reduction in mGluR6 activity induced by light does not allow for a decrease in active Go to the action threshold that is rapid enough to open an appreciable number of channels. Hence, the opening probability of TRPM1 channels is decreased below the level needed for producing a depolarizing response. This makes the RGS proteins RGS7 and RGS11 key factors in the regulation of the mGluR6 cascade in ON-bipolar neurons, ultimately contributing to synaptic transmission. In summary, the lessons learned from photoreceptors regarding RGS involvement appear to also apply to the GPCR cascade in ON-bipolar neurons.

COMPONENTS OF THE ON-BC SIGNALING CASCADE FORM MACROMOLECULAR COMPLEXES

Recent studies on the organization of the mGluR6 signaling cascade of ON-BCs are increasingly mirroring another important lesson from phototransduction, which pertains to the intimate integration of key signal transduction components. Following identification of TRPM1 as an effector channel in the pathway, studies have focused on investigating its protein-protein interactions. Screening by both proteomics[62] and yeast two-hybrid[63] approaches have revealed that TRPM1 forms complexes with nyctalopin, a protein essential for synaptic transmission to ON-BCs. Nyctalopin was also shown to engage in complex formation with mGluR6, and the elimination of either nyctalopin or mGluR6 prevented the synaptic targeting of TRPM1.[62] These results indicate that nyctalopin is an essential component of the complex, possibly scaffolding the principal receptor mGluR6 and ion channel TRPM1 together at the dendritic tips of ON-BCs. Similarly, proteomic screening has identified orphan receptor GPR179 as a binding partner of RGS7 and RGS11.[64] Indeed, the elimination of GPR179 prevented the synaptic targeting of both RGS7 and RGS11,[64] providing a likely explanation that the synaptic deficits

observed upon GPR179 loss in mice and humans with congenital night blindness are related to a disruption in the ability of the RGS complex to regulate mGluR6 signaling to TRPM1. In addition to GPR179, one of the RGS proteins, RGS11, is additionally controlled by R9AP,[65,66] the same membrane anchor that associates with RGS9 in photoreceptors. Although the exact relationship between GPR179 and R9AP is not well understood, it is likely that RGS complexes in bipolar cells exist in several alternative configurations (e.g., RGS7/Gβ5/GPR179, RGS11/Gβ5/GPR179, and RGS11/Gβ5/R9AP).

Interestingly, there appears to be an additional layer of interactions in the scaffolding of the molecular components of the GPCR cascade in ON-bipolar neurons. Recent findings indicate that GPR179 also forms complexes with both TRPM1 and mGluR6,[67] further bringing RGS proteins into close proximity of the key components of the cascade that they regulate. It appears that mGluR6 plays a central role in the web of protein-protein interactions, as its elimination prevents the targeting of most components and also decreases their proteolytic stability.[68] Thus, multiple components are likely organized in a single macromolecular complex in which multiple signaling proteins are tightly integrated into a "signalosome." Remarkably, this organization is reminiscent of the visual cascade in fly photoreceptors, whereby GPCR also signals to the TRP channel, which likely plays a critical role in ensuring the high spatiotemporal resolution of signaling.[5]

SIMILAR PRINCIPLES GUIDE THE ORGANIZATION OF G PROTEIN CASCADES IN THE CENTRAL NERVOUS SYSTEM

Advances in the understanding of the organization and functional regulation of G protein signaling pathways in the retina raise an inevitable question: How far do the lessons learned in this system extend? Furthermore, can they be applied for understanding the organization of GPCR pathways in the nervous system in general? We hope that recent studies provide a convincingly positive answer to these questions. Taking the first case in point, the key regulator of the phototransduction RGS9 complex is also prominently expressed in the brain region called the striatum.[69-71] This structure is heavily involved in procedural learning, motivation, and reward valuation, essentially serving as the brain's reward and movement coordination center.[72-74] The striatum integrates a vast number of incoming neurotransmitter inputs, many of which signal via GPCRs. Dysfunctions in the striatum are implicated in a range of neurological disorders as well as in drug addiction. In the striatal neurons, the G protein regulator RGS9 was shown to control signaling via two specific GPCRs in this region, μ-opioid[75] and D2 dopamine receptors,[76,77] which belong to the same class as rhodopsin. The elimination of RGS9 increases the behavioral sensitivity of animals to the μ-opioid receptor agonist morphine and exacerbates the dyskinetic manifestations associated with the long-term use of antipsychotics and L-DOPA, which act on the D2 receptor. Examining the role of RGS9 in controlling intracellular signaling, we found that it regulates the homeostasis of the second messenger cAMP (Fig. 2). Type 9 regulator of G protein signaling specifically associates with the effector enzyme that produces cAMP, adenylyl cyclase type 5 (AC5), and controls the extent to which Gβγ subunits released by the μ-opioid (and possibly D2R) receptor sensitize AC5 to the stimulatory effects of Gαs/olf.[78] Chronic activation of opioid receptors has been described as resulting in the overactivation of AC5. During this process, also known as superactivation, the abrupt termination of signaling at opioid receptors, for example, through the introduction of an antagonist, leads to a surge in
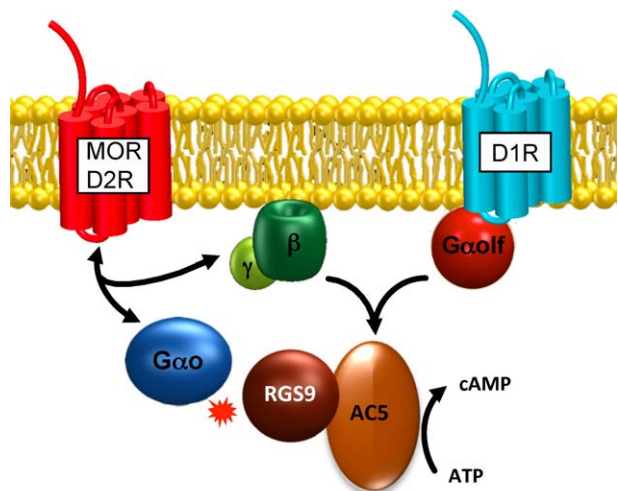
Figure 2. Organization of the G protein signaling cascade in the medium spiny neurons of the striatum.

Figure 3. Organization of the G protein signaling cascade in the hippocampal CA1 pyramidal neurons.

the cAMP concentration, which reflects homeostatic adaptation in the activity of AC5.[79] An important role in this sensitization is played by the $G\beta\gamma$ subunits that potentiate AC5 activity. We have found that by controlling the lifetime of activated $G\alpha o$, which does not directly influence AC5 activity, RGS9 selectively blunts the stimulatory effect of $G\beta\gamma$ subunits on AC5. As a result, mice lacking RGS9 show significantly higher cAMP production in their striatal regions upon withdrawal from chronic morphine administration.[78] This biochemical mechanism might help to explain the behavioral observations that are reported as more severe physical withdrawal signs as well as the higher degree of drug seeking in mice lacking RGS9.[75,76] Interestingly, as in photoreceptors, the stability and membrane localization of RGS9 require its association with protein cofactors.[80] In the striatum, RGS9 exists as a constitutive complex with the $G\beta5$ subunit, which is essential for its proteolytic stability. In addition, the expression level of RGS9 and its localization at the membrane are controlled by an R9AP-like protein called R7 family-binding protein, or R7BP.[81,82] Overall, these studies identify two significant parallels with phototransduction. Similar molecular organization of the RGS complex: RGS9/$G\beta5$/R9AP in photoreceptors and RGS9/$G\beta5$/R7BP in striatal neurons and critical roles of these complexes in controlling the timing and extent of the second messenger homeostasis: cGMP in photoreceptors and cAMP in striatal neurons.

The second illustrative example is RGS7; much like RGS9, it is also expressed in the CNS, prominently in the hippocampus, the brain region associated with spatial learning and memory.[83] Indeed, we have recently found that RGS7 plays an important role in contextual fear memories, as its knockout in mice disrupts their ability to remember the association of specific spatial cues with noxious stimulation.[84] The elimination of RGS7 results in selective disruption in the inhibitory forms of synaptic plasticity, long-term depression (LTD) and depotentiation (DP). We have further found that, just like RGS9, RGS7 forms complexes with two subunits, R7BP and $G\beta5$, in the hippocampus. Curiously, $G\beta5$ was found to be essential not only for the proteolytic stability of the RGS7 complex but also for mediating complex formation with another effector enzyme, the ion channel known as G protein-activated inwardly rectifying K^+ (GIRK) channel.[85] The GIRK channel is gated by binding to the G protein $\beta\gamma$ subunits released upon the activation of $GABA_B$ receptors, and $G\beta5$ mimics this interaction to recruit RGS7 to the complex (Fig. 3). The knockout of either
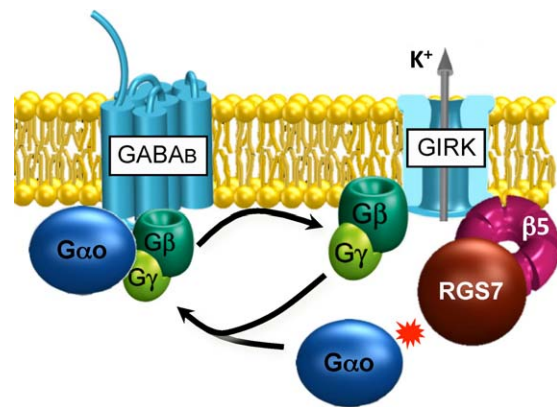
RGS7 or $G\beta5$ dramatically slows down the GIRK channel deactivation kinetics, which results in a net increase in K^+ efflux and a decrease in the resting membrane potential, thus decreasing the excitability of hippocampal neurons.[84,85] Curiously, R7BP further shapes GIRK channel regulation via the RGS7 complex by recruiting the complex to the plasma membrane and adjusting the sensitivity of its regulation by $GABA_B$ receptor stimulation.[84,86] Hence, the same parallels appear to also apply in explaining the organizational principles of the key GPCR signaling cascade in hippocampal pyramidal neurons: the essential regulation by RGS protein complexes that dictate the kinetics of effector ion channel activity, hence determining neuronal response properties.

Conclusions

The central lesson that we have learned thus far has been that despite their apparent difference, G protein signaling pathways in neurons of the retina, striatum, and hippocampus utilize similar regulatory principles based on the key involvement of RGS protein complexes, macromolecular scaffolding of the components, and cascade compartmentalization. We hope that these lessons can be further extended for a better understanding of the organization and physiological regulation of GPCR signaling systems across the nervous system.

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