Published in final edited form as:

Prog Mol Biol Transl Sci. 2015 ; 133: 31–45. doi:10.1016/bs.pmbts.2015.02.004.

RGS Protein Regulation of Phototransduction

Ching-Kang Jason Chen^{1,2}

Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, USA, Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas, USA, Department of Neuroscience, Baylor College of Medicine, Houston, Texas, USA

Abstract

First identified in yeast and worm and later in other species, the physiological importance of regulators of G-protein signaling (RGS) in mammals was first demonstrated at the turn of the century in mouse retinal photoreceptors, in which RGS9 is needed for timely recovery of rod phototransduction. The role of RGS in vision has been established a synapse away in retinal depolarizing bipolar cells (DBC), where RGS7 and RGS11 work redundantly and in a complex with Gβ5-S as GAPs for Goα in the metabotropic glutamate receptor 6 pathway at DBC dendritic tips. Much less is known on how RGS protein subserves vision in the rest of the visual system. The research into the roles of RGS proteins in vision holds great potential for many exciting new discoveries.

Keywords

Retina; Photoreceptor; Phototransduction; Regulators of G-protein signaling; Rate-limiting step; Heterotrimeric G-protein; Depolarizing bipolar cell; Vision

1. INTRODUCTION

Being pushed out of the brain and into the eye during development, retina lines the back of the eye and is an extension of the central nervous system. Mature retina has a beautifully layered structure with three nuclear layers (Fig. 1) and subserves vision by converting light into electrical signals in photoreceptors and by processing and encoding changes in light intensities and wavelengths in ambient environment in the rest of the retinal neurons. Because of the ease of isolation and maintenance in culture, retina has been extensively studied but as with any mature field, the more we know, the more areas we know we do not know. Since the late 1980s, knowledge on the development and functions of the retina has benefited from the identification of numerous causative mutations in patients with hereditary blinding diseases. Subsequent recapitulation of pathologic conditions of some diseases in genetically engineered model organisms enables trials to use the knowledge gained to assist the care and/or treatment of some patients. With regard to the regulators of G-protein signaling (RGS), retinal photoreceptor is where the importance of this gene family in mammalian physiology was first demonstrated. This chapter reflects on how the

²Corresponding author: ching-kang.chen@bcm.edu.

¹Ching-Kang Jason Chen is the Alice R. McPherson Retina Research Foundation Endowed Chair at Baylor College of Medicine.

phototransduction field discovered the importance of RGS proteins and describes current state of knowledge about this gene family in vision.

2. FROM PHOTON TO A NEURAL SIGNAL: THE WONDER OF PHOTOTRANSDUCTION

In the heyday of apprenticeship in classical biochemistry, the author's typical day would start with driving to a local slaughterhouse to purchase fresh bovine eyes, usually tens upward to hundreds, keeping them in the dark and on ice, driving back to the lab, dissecting retinas out in a darkroom, and spending the rest of the day running centrifugation rounds to isolate a structure of the retina high in lipid content called the outer segment.¹ With tubes of outer segment preparation in hand, the party began by adding various reagents in the dark and then exposing them to light to see what might happen. Very often we obtained outer segments from hundreds of bovine eyes and fractionated the proteins by column chromatography or other means to purify the protein(s) with the desired activity. More often than not, experimental conditions would be altered to see if and how the activity was enhanced or killed. As one would have guessed, there are many interesting light-dependent reactions in those tubes! The most popular one in the lab at that time was light-dependent activation of phosphodiesterase,² assayable by hydrolysis of exogenous radioactive cGMP.³ We did it frequently because at that time the phototransduction field has come out of the shadow of the calcium hypothesis⁴ and firmly established that cGMP is the second messenger used by photoreceptors to transduce light into an electrical signal.⁵ The enzymatic cascade found in the outer segment preparations which links photon absorption to the hydrolysis of cGMP is called phototransduction and is a canonical heterotrimeric G-protein signaling pathway (Fig. 1 and for a more detailed review, see Ref. 6). This cascade of events starts when rhodopsin absorbs a photon and the chromophore 11-*cis*-retinaldehyde (11-*cis*-retinal) is photoisomerized into the all-*trans* form. Rhodopsin is a G protein-coupled receptor and the covalently linked 11-*cis*-retinal is an inverse agonist. When 11-*cis*-retinal turns into all-*trans* retinal, rhodopsin undergoes a series of structural changes to adopt an active intermediary conformation called Meta-II (R*). R* can catalyze the exchange of GTP for GDP on the alpha subunit of the photoreceptor-specific G protein called transducin (Tα). During its short active lifetime, R* activates tens of transducins and hence provides the initial gain to the cascade. When Tα is bound to GDP, it associates tightly with the βγ subunits (Tβγ) to form a heterotrimer. The binding of GTP causes Tα to dissociate from Tβγ and subsequently binds the inhibitory subunit of the cGMP-specific phosphodiesterase (PDE6γ). The sequestration of PDE6γ by Tα relieves inhibition on the catalytic αβ subunit (PDE6αβ). Uninhibited PDE6αβ is a near-perfect enzyme, with the rate of catalysis limited only by diffusion of substrate into the catalytic site. The catalytic activity of PDE6 constitutes the major gain step in phototransduction, which results in a rapid decline of cGMP level inside the outer segment. In the dark, the cGMP-gated (CNG) nonselective cation channels on the plasma membrane stay open by binding to cGMP. The drop in cGMP level as a result of phototransduction closes many CNG channels and membrane potential decreases from -40 to about -70 mV. This light-induced hyperpolarization spreads from the outer segment to the rest of the cell, alters voltage-dependent channel activity along the way, and eventually blocks the release of glutamate at photoreceptor synaptic terminal.⁷ This

amazing chain of events takes place within a few milliseconds after photoactivation of rhodopsin and can be captured in real time in several recording configurations using pulled glass microelectrodes.^{8–10} Macroscopically, because of the layered retinal structure, phototransduction can also be picked up using a noninvasive transcorneal field potential recording technique called electroretinography (ERG), where it appears under bright stimulus conditions as the so-called A-wave. The biochemical scheme of phototransduction is simple but effective in empowering a rod cell to detect single photons, while allowing a cone cell to transduce light with faster kinetics but lesser sensitivity. Using rods and cones and connecting them to a network of not yet very well understood endogenous neuronal circuits, the retina operates with an amazing dynamic range of ten orders of magnitudes in stimulus intensity, a task unmatched in any contemporary manmade devices.

3. THE NEED FOR SPEED: DISCREPANCY ON G-PROTEIN SHUTOFF DURING PHOTOTRANSDUCTION RECOVERY

In early 1990s, the activation phase of vertebrate phototransduction was considered solved because differences in sensitivity and kinetics in rods of various vertebrate species could be nicely modeled when body temperatures and photoreceptor sizes were taken into consideration.¹¹ The field started to tackle a more difficult question: how phototransduction is turned off.¹² It was considered difficult because unlike the sequential activation of known enzymes during activation, to turn phototransduction off in a timely manner in just a few hundred milliseconds, all active intermediates such as R*, GTP-bound T α , and PDE6 $\alpha\beta$ accumulated during activation need to be deactivated and the cGMP level must be restored to reopen CNG channels. The utility of several assayable light-dependent reactions in outer segment preparations was realized in this context, such as light-dependent phosphorylation of rhodopsin¹³ and change in membrane affinity of a protein called arrestin.¹⁴ Fast forward two decades and thanks to all assiduous vision researchers, now we have a clearer picture about what is going on during phototransduction recovery. The R* got phosphorylated at its C-terminal tail Ser and Thr residues¹⁵ by an enzyme called rhodopsin kinase (aka G protein-coupled receptor kinase 1 (GRK1))¹⁶ and arrestin binds the phosphorylated R* to prevent it from further interaction with transducin.¹⁷ As a G protein, T α has an intrinsic GTPase activity that hydrolyzes bound GTP to GDP and when it occurs, GDP-bound T α dissociates from PDE6 γ and reassociates with T $\beta\gamma$ subunits. Freed PDE6 γ reinhibits PDE6 $\alpha\beta$ to its basal activity, allowing reaccumulation of cGMP inside the outer segment. To replenish cGMP in a timely manner to open CNG channels, membrane-bound guanylate cyclase (GC) activity is activated to synthesize cGMP *de novo* from GTP.¹⁸ The timing of heightened cGMP synthesis is governed by a light-dependent decline of calcium concentration inside the outer segment¹⁹ and is mediated by a small calcium-binding protein called GCAP (guanylate cyclase-activating protein)^{20,21} that in its calcium bound form binds and inhibits the membrane-bound GCs. As mentioned above, all reactions used during phototransduction recovery are assayable and hence kinetics of individual reactions can be measured under chemically defined conditions using purified enzymes. It is under the strict mandate of classical biochemistry that a glaring discrepancy concerning transducin deactivation surfaced. This is because it takes seconds for purified T α to hydrolyze bound GTP to GDP in the test tube, but recovery of phototransduction is finished within a few hundred

milliseconds²²! This suggests that GTP hydrolysis by $T\alpha$ is somehow accelerated in the outer segment. Given the availability of material and the ease of assaying GTP hydrolysis, there was a fury in the field to find the putative GAP (GTPase-accelerating protein). However, the traditional biochemical approach used so successfully in the field quickly revealed some bad news given that the GAP activity is membrane associated and became labile in the presence of detergents, making it formidable to study using conventional methods. However, these efforts did produce some leads in that PDE6 γ can enhance the GAP activity even though it is by itself not the GAP.^{23,24} Around that time, a new group of proteins coined Regulators of G-protein Signaling (RGS) were identified by forward genetic screens in yeast and worm, and later found to be abundantly present in mammals.^{25,26} These RGS proteins are simple negative regulators of heterotrimeric G-proteins in yeast and worm, but in mammals they exist with greater diversity in sizes and expression patterns in different tissues.²⁷ All RGS proteins have a conserved RGS domain of approximately 120 amino acids in length, which is necessary and sufficient for their GAP activity toward Gi/o proteins.²⁸ This new insight set off another fury in the phototransduction field to test whether the long sought-after transducin GAP might after all be an RGS protein. Several labs used degenerate oligonucleotides to screen retinal cDNA library for RGS transcripts and found a great deal of diversity in the retina. Several members of this family, such as RGSr/16, GAIP (G alpha interacting protein), RGS9, RGS4, and Ret-RGS1, were further tested for their GAP activity toward purified transducin *in vitro* and surprisingly they all possessed GAP activity, albeit to varying degrees.^{29–34} Could it be possible that multiple RGS proteins, instead of a pivotal one, are present in the outer segment to ensure timely shutoff of transducin? To gain further insight, additional criteria such as membrane affinity, photoreceptor-specific expression pattern, and whether GAP activity could be enhanced *in vitro* by exogenous PDE6 γ were considered. Among them, the telltale sign for some was whether any of these RGS proteins are similarly expressed in a photoreceptor-specific manner. Most, if not all, proteins involved in phototransduction such as rhodopsin, transducin, and rhodopsin kinase are exclusively expressed in photoreceptors. Taken all into account, RGS9 stood out as a promising candidate because of its photoreceptor-specific expression pattern (Fig. 2), while other candidates such as RGSr/16 and RGS11 were located elsewhere in the retina (Fig. 1).^{35,36} Furthermore, RGSr/16's GAP activity toward transducin was inhibited rather than enhanced by PDE6 γ ,³⁷ making it highly unlikely that it is a physiological GAP for transducin. To test whether RGS9 is indeed the GAP, rather than one of the GAPs for transducin in photoreceptors, Chen *et al.* inactivated it and found that recovery in mouse rod and cone was severely delayed in RGS9 knockout mice.^{38,39} A few years later, Nishiguchi *et al.* reported sporadic human cases of a novel ophthalmic condition called bradyopsia, where recessive mutations in RGS9 or its membrane anchoring protein (R9AP, see below) render the patients with difficulty adapting to sudden changes in luminance levels and unable to see fast-moving objects in low-contrast conditions.⁴⁰ Since then, more patients with similar conditions caused by loss-of-function mutations in RGS9 were found.^{41–43} A two-decade worth of earnest efforts to solve a long-standing controversy in phototransduction recovery brings to light the importance of RGS proteins in human biology and disease etiology. This is one of the reasons why hypothesis-driven basic research aiming to solve a mystery is always a good bet for funding agencies, because if not

supported in a timely manner, many opportunities for exciting new discoveries would have been missed.

4. CANNOT DO IT ALONE: THE TRANSDUCIN GAP IS A PROTEIN COMPLEX

Strictly speaking, RGS9 is not a photoreceptor-specific gene because it is also transcribed in other CNS regions including striatum,^{44,45} although alternative splicing there generates a larger protein, RGS9-2, with a different and bigger C-terminal domain, as opposed to the photoreceptor-specific splice form RGS9-1 with a shorter “retina-specific” C-terminal tail.⁴⁶ The gene targeting approach in laboratory mice used to settle whether transducin is “gapped” by one or many RGS proteins has around that time identified many genes critical for phototransduction.^{16,17,47–49} This reverse genetic approach sometime produces unexpected results. In the case of transducin GAP, one surprise is that RGS9-1 has two *in vivo* partners, namely Gβ5-L⁵⁰ and R9AP (RGS9 anchoring protein).⁵¹ They associate tightly with RGS9-1 in photoreceptors to form a ternary protein complex. Gβ5-L binds and guides RGS9-1 to interact with R9AP, while the transmembrane domain of R9AP ensures the entire complex is membrane associated. In mice lacking RGS9, Gβ5-L becomes unstable and likewise, in mice lacking Gβ5, RGS9-1 becomes unstable. These studies revealed a unique obligate partnership between RGS9-1 and Gβ5-L, suggesting first during biochemical characterization of then elusive transducin GAP,⁵² wherein the loss of one leads to the instability of the other despite the presence of messenger RNA. Not surprisingly, the recovery delays observed in rods without RGS9 are also apparent in rods lacking Gβ5 or R9AP.^{53,54} Equally if not more interesting is the higher expression level of this GAP complex in cone than in rod.⁵⁵ Depending on species, this difference can be as high as 10-fold.^{45,56} As mentioned earlier, rod is more sensitive to cone but responds to light in a slower fashion. The higher level of transducin GAP in cone has been suggested to be a deciding factor governing kinetic differences between these two photoreceptors. To test the idea, Chen *et al.* attempted to transgenically overexpress RGS9-1 in rod using a construct putting RGS9-1 cDNA downstream of a rhodopsin promoter. This effort failed.⁵⁷ Similar attempts by overexpressing the obligate partner Gβ5-L, by itself or in combination with overexpressing RGS9-1, were also unsuccessful in raising photoreceptor GAP level. This remained a puzzle for a few years until the anchoring protein R9AP was inactivated; it precipitously became clear that overall GAP expression level is determined in rods by R9AP expression alone as in R9AP heterozygous knockout animals transducin GAP level is halved,⁵³ while corresponding heterozygous knockouts in either RGS9 or Gβ5 have normal GAP level.^{38,57} Krispel *et al.* again put this notion to test by overexpressing R9AP using a rhodopsin promoter and found indeed that R9AP overexpression can significantly elevate transducin GAP level in rods.⁵⁸ What was even more interesting, as the focus of the investigation turned, was that photoresponses in rods with over-expressed GAP recover much faster than their wild-type controls and do so in a dose-dependent manner to GAP levels. Because raising GAP level altered rod photoresponse waveform with a faster rate of return to baseline, these findings indicate that GAP level is indeed a determining factor in kinetic differences between rod and cone.⁵⁹ This “gain-of-function” type of experiment is the first successful case in the vision field to confer a better response property to a specific

cell type and thus has a wide applicability to other retinal cell types in the quest to understand their contributions and functions in the retina. It is now possible to ask whether response duration of rod rate-limits temporal resolution in scotopic vision under normal physiological conditions. The then powerful and now routine “loss-of-function” approach like gene knockouts merely generates an artificial limiting step and hence has little or no power to shed light on what is normally limiting. In this regard, while RGS9-1/Gβ5-L/R9AP function as the transducin GAP is firmly established, what other RGS proteins may be doing in vision becomes very intriguing. Examining the roles of RGS in vision is thus a very fertile ground with the potential for many exciting new discoveries.

5. TRANSLOCATION AND REGULATION OF RGS9-1

Several proteins involved in phototransduction translocate in and out of the outer segment compartment in a light-dependent manner (for a review, see Ref. 60). Translocation of transducin and arrestin was first noted in the early 1980s and then confirmed beyond the caveat of epitope masking shortly after the turn of the century.⁶¹ The speculated roles of protein movement are in protecting photoreceptors from light-dependent degeneration and/or long-term light adaptation. A photoreceptor enriched calcium-binding protein, recoverin, which binds and inhibits rhodopsin kinase,^{62,63} also exhibits noticeable light-dependent movement out of the outer segment,⁶² although its physiological function is less clear. Given that rod photoresponse duration is controlled by transducin GAP level, it is of interest to note that RGS9-1 was recently reported to reside mainly in the inner segment under prolonged dark adaptation and move swiftly out to the outer segment upon illumination.⁶⁴ The reported translocation of RGS9-1 from inner to outer segment occurred at much dimmer illumination than those required to translocate transducin or arrestin. Interestingly, of the three components of the transducin GAP, only RGS9-1 and Gβ5-L exhibit this peculiar translocation phenomenon and the anchoring protein R9AP stays in the outer segment regardless of illumination level. While phenomenological, it was also found that under prolonged dark adaptation RGS9-1 is phosphorylated at S475⁶⁵ (presumably by PKCs⁶⁶). Light exposure leads to a rapid decrease in S475 phosphorylation, promoting the assembly of the ternary protein complex and translocation to outer segment.⁶⁴ This finding is somewhat at odd with the well-documented stereotypic nature of rod single-photon responses.⁶⁷ Should translocation of RGS9-1 and Gβ5-L in fact occur, one would expect the single-photon responses to have some degree of variability in recovery phase. However, should RGS9-1 translocation occur quickly, as shown in just a few minutes upon dim light exposure,⁶⁴ only the initial few recorded light responses will deviate from the ensemble responses typically collected hundreds of times every 5–10 s for a period of 30 + min during suction pipette recordings. Clearly, there is much work to be done here to solve this controversy. Finally, with regard to the phosphorylation of RGS9-1, there appears to be other phosphorylation site(s) catalyzed by different kinases.⁶⁸ While the stoichiometry of phosphorylation was not quantitatively measured, introducing phosphomimetic mutations into recombinant RGS9-1 reduced its GAP activity, suggesting that this may be a modulation mechanism for phototransduction recovery, akin to what was more recently shown for GRK1 by PKA.^{69,70} While reversible protein phosphorylation is a common way of modulating enzyme activity in biochemistry, the extent to which it is used to modulate

phototransduction was not understood to its full extent. This is another fertile area of discovery.

6. CONCLUSIONS: EMERGING FUNCTIONS OF RGS PROTEINS IN THE VISUAL SYSTEM

RGS9-1 belongs to the R7 subfamily of RGS proteins, which include three additional structurally similar members, namely RGS6, RGS7, and RGS11.²⁷ The four R7 RGS proteins contain the G protein gamma-like domain and use it to interact with the fifth member of the G β gene family G β 5.⁷¹ G β 5 is peculiarly spliced in photoreceptor and contains an extra N-terminal exon.⁵⁰ The resulting protein, G β 5-L, forms complex with RGS9-1 and R9AP and function as the transducin GAP. Outside of photoreceptors and in the retina, G β 5 is expressed in the so-called short form, G β 5-S,⁷² and interacts exclusively with RGS6, RGS7, and RGS11.⁷³ The redundant functions of G β 5-S/RGS7 and G β 5-S/RGS11 as the GAP for G α in the metabotropic glutamate receptor 6 pathway at dendritic tips of depolarizing bipolar cells (DBC) have been well-documented.^{36,73,74} DBCs in mice lacking RGS7 and RGS11 possess undetectable G β 5-S staining and have very poor light responses.⁷⁵ As a result, their ERG recordings are identical to those of the G β 5^{-/-} mice in that they both lack the characteristic ERG B-waves.^{73,76} In addition, DBC dendritic tips are conspicuously stunted in G β 5^{-/-} mice and in one strain of the RGS7 and RGS11 double knockout mice,^{73,76} but not in another double knockout strain with the RGS7 gene differently targeted.⁷⁵ The controversy may be worth solving for reasons mentioned previously. Perhaps more important, in the spirit of discovery, is that other than photoreceptors and DBCs, virtually nothing is known about RGS proteins in the rest of the visual system despite their abundant presence. Some RGS proteins are expressed early during development and thus may even have additional roles than merely “GAPing” heterotrimeric G-proteins. Finally, in intrinsically photosensitive retinal ganglion cells,⁷⁷ there exists another G-protein-mediated phototransduction pathway more similar to those in invertebrate ommatidia than in the one discussed above. This phototransduction pathway is initiated by melanopsin, used mainly for nonimage forming vision, and proceeds supposedly through Gq and with a much slower kinetics than the one in rod or cone.⁷⁸ Simple questions like which Gq family protein(s) or whether any RGS protein is involved in its signaling remain unanswered. While the role of RGS9-1/G β 5-L/R9AP in rod phototransduction is understood, why and how they are expressed (or maintained) in higher level in cone or whether cone phototransduction is likewise rate-limited by transducin deactivation is presently unclear. A simultaneous comparative examination of both rods and cones will provide valuable insights. Future research efforts may thus benefit from a focused approach in the retina, due to its approachability and available anatomical and neurochemical details and genetic resources.

REFERENCES

1. Papermaster DS. Preparation of retinal rod outer segments. *Methods Enzymol.* 1982; 81:48–52. [PubMed: 6212746]

2. Raport CJ, Lem J, Makino C, et al. Downregulation of cGMP phosphodiesterase induced by expression of GTPase-deficient cone transducin in mouse rod photoreceptors. *Invest Ophthalmol Vis Sci.* 1994; 35(7):2932–2947. [PubMed: 8206711]
3. Miki N, Keirns JJ, Marcus FR, Freeman J, Bitensky MW. Regulation of cyclic nucleotide concentrations in photoreceptors: an ATP-dependent stimulation of cyclic nucleotide phosphodiesterase by light. *Proc Natl Acad Sci USA.* 1973; 70(12):3820–3824. [PubMed: 4359491]
4. Hagins WA. The visual process: excitatory mechanisms in the primary receptor cells. *Annu Rev Biophys Bioeng.* 1972; 1:131–158. [PubMed: 4567751]
5. Fesenko EE, Kolesnikov SS, Lyubarsky AL. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature.* 1985; 313:310–313. [PubMed: 2578616]
6. Chen CK. The vertebrate phototransduction cascade: amplification and termination mechanisms. *Rev Physiol Biochem Pharmacol.* 2005; 154:101–121. [PubMed: 16634148]
7. Dowling JE, Ripps H. Effect of magnesium on horizontal cell activity in the skate retina. *Nature.* 1973; 242(5393):101–103. [PubMed: 4348460]
8. Fain GL, Dowling JE. Intracellular recordings from single rods and cones in the mudpuppy retina. *Science.* 1973; 180(4091):1178–1181. [PubMed: 4707063]
9. Baylor DA, Lamb TD, Yau KW. The membrane current of single rod outer segments. *J Physiol.* 1979; 288:589–611. [PubMed: 112242]
10. Sather WA, Detwiler PB. Intracellular biochemical manipulation of phototransduction in detached rod outer segments. *Proc Natl Acad Sci USA.* 1987; 84(24):9290–9294. [PubMed: 2827176]
11. Lamb TD, Pugh EN Jr. A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. *J Physiol.* 1992; 449:719–758. [PubMed: 1326052]
12. Hurley JB. Termination of photoreceptor responses. *Curr Opin Neurobiol.* 1994; 4(4):481–487. [PubMed: 7812135]
13. Kuhn H, Dreyer WJ. Light dependent phosphorylation of rhodopsin by ATP. *FEBS Lett.* 1972; 20(1):1–6. [PubMed: 11946367]
14. Wilden U, Wust E, Weyand I, Kuhn H. Rapid affinity purification of retinal arrestin (48 kDa protein) via its light-dependent binding to phosphorylated rhodopsin. *FEBS Lett.* 1986; 207(2):292–295. [PubMed: 3770202]
15. Chen J, Makino CL, Peachey NS, Baylor DA, Simon MI. Mechanisms of rhodopsin inactivation in vivo as revealed by a COOH-terminal truncation mutant. *Science.* 1995; 267(5196):374–377. [PubMed: 7824934]
16. Chen CK, Burns ME, Spencer M, et al. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc Natl Acad Sci USA.* 1999; 96(7):3718–3722. [PubMed: 10097103]
17. Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J. Prolonged photoresponses in transgenic mouse rods lacking arrestin. *Nature.* 1997; 389(6650):505–509. [PubMed: 9333241]
18. Koch KW, Stryer L. Highly cooperative feedback control of retinal rod guanylate cyclase by calcium ions. *Nature.* 1988; 334(6177):64–66. [PubMed: 2455233]
19. Yau KW, Haynes LW, Nakatani K. Study of the roles of calcium and cyclic GMP in visual transduction. *Neurosci Res Suppl.* 1987; 6:S45–S53. [PubMed: 2446221]
20. Dizhoor AM, Olshevskaya EV, Henzel WJ, et al. Cloning, sequencing, and expression of a 24-kDa Ca(2+)-binding protein activating photoreceptor guanylyl cyclase. *J Biol Chem.* 1995; 270(42):25200–25206. [PubMed: 7559656]
21. Gorczyca WA, Polans AS, Surgucheva IG, Subbaraya I, Baehr W, Palczewski K. Guanylyl cyclase activating protein. A calcium-sensitive regulator of phototransduction. *J Biol Chem.* 1995; 270(37):22029–22036. [PubMed: 7665624]
22. Arshavsky VY, Wensel TG. Timing is everything: GTPase regulation in phototransduction. *Invest Ophthalmol Vis Sci.* 2013; 54(12):7725–7733. [PubMed: 24265205]
23. Angleson JK, Wensel TG. A GTPase-accelerating factor for transducin, distinct from its effector cGMP phosphodiesterase, in rod outer segment membranes. *Neuron.* 1993; 11(5):939–949. [PubMed: 8240815]

24. Arshavsky V, Bownds MD. Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. *Nature*. 1992; 357(6377):416–417. [PubMed: 1317509]
25. Dohlman HG, Apaniesk D, Chen Y, Song J, Nusskern D. Inhibition of G-protein signaling by dominant gain-of-function mutations in Sst2p, a pheromone desensitization factor in *Saccharomyces cerevisiae*. *Mol Cell Biol*. 1995; 15(7):3635–3643. [PubMed: 7791771]
26. Koelle MR, Horvitz HR. EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell*. 1996; 84(1):115–125. [PubMed: 8548815]
27. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem*. 2000; 69:795–827. [PubMed: 10966476]
28. Wieland T, Chen CK. Regulators of G-protein signalling: a novel protein family involved in timely deactivation and desensitization of signalling via heterotrimeric G proteins. *Naunyn Schmiedebergs Arch Pharmacol*. 1999; 360(1):14–26. [PubMed: 10463329]
29. Chen CK, Wieland T, Simon MI. RGS-r, a retinal specific RGS protein, binds an intermediate conformation of transducin and enhances recycling. *Proc Natl Acad Sci USA*. 1996; 93(23):12885–12889. [PubMed: 8917514]
30. He W, Cowan CW, Wensel TG. RGS9, a GTPase accelerator for phototransduction. *Neuron*. 1998; 20(1):95–102. [PubMed: 9459445]
31. Natochin M, Granovsky AE, Artemyev NO. Regulation of transducin GTPase activity by human retinal RGS. *J Biol Chem*. 1997; 272(28):17444–17449. [PubMed: 9211888]
32. Faurobert E, Hurley JB. The core domain of a new retina specific RGS protein stimulates the GTPase activity of transducin in vitro. *Proc Natl Acad Sci USA*. 1997; 94(7):2945–2950. [PubMed: 9096326]
33. Nekrasova ER, Berman DM, Rustandi RR, Hamm HE, Gilman AG, Arshavsky VY. Activation of transducin guanosine triphosphatase by two proteins of the RGS family. *Biochemistry*. 1997; 36(25):7638–7643. [PubMed: 9201904]
34. Snow BE, Antonio L, Suggs S, Siderovski DP. Cloning of a retinally abundant regulator of G-protein signaling (RGS-r/RGS16): genomic structure and chromosomal localization of the human gene. *Gene*. 1998; 206(2):247–253. [PubMed: 9469939]
35. Dhingra A, Faurobert E, Dascal N, Sterling P, Vardi N. A retinal-specific regulator of G-protein signaling interacts with G α (o) and accelerates an expressed metabotropic glutamate receptor 6 cascade. *J Neurosci*. 2004; 24(25):5684–5693. [PubMed: 15215290]
36. Chen FS, Shim H, Morhardt D, et al. Functional redundancy of R7 RGS proteins in ON-bipolar cell dendrites. *Invest Ophthalmol Vis Sci*. 2010; 51(2):686–693. [PubMed: 19797210]
37. Wieland T, Chen CK, Simon MI. The retinal specific protein RGS-r competes with the gamma subunit of cGMP phosphodiesterase for the alpha subunit of transducin and facilitates signal termination. *J Biol Chem*. 1997; 272(14):8853–8856. [PubMed: 9083000]
38. Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI. Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature*. 2000; 403(6769):557–560. [PubMed: 10676965]
39. Lyubarsky AL, Chen C, Simon MI, Pugh EN Jr. Mice lacking G-protein receptor kinase 1 have profoundly slowed recovery of cone-driven retinal responses. *J Neurosci*. 2000; 20(6):2209–2217. [PubMed: 10704496]
40. Nishiguchi KM, Sandberg MA, Kooijman AC, et al. Defects in RGS9 or its anchor protein R9AP in patients with slow photoreceptor deactivation. *Nature*. 2004; 427(6969):75–78. [PubMed: 14702087]
41. Cheng JY, Luu CD, Yong VH, Mathur R, Aung T, Vithana EN. Bradyopsia in an Asian man. *Arch Ophthalmol*. 2007; 125(8):1138–1140. [PubMed: 17698770]
42. Hartong DT, Pott JW, Kooijman AC. Six patients with bradyopsia (slow vision): clinical features and course of the disease. *Ophthalmology*. 2007; 114(12):2323–2331. [PubMed: 17826834]
43. Michaelides M, Li Z, Rana NA, et al. Novel mutations and electrophysiologic findings in RGS9- and R9AP-associated retinal dysfunction (Bradyopsia). *Ophthalmology*. 2010; 117(1):120.e1–127.e1. [PubMed: 19818506]

44. Gold SJ, Ni YG, Dohlman HG, Nestler EJ. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci.* 1997; 17(20):8024–8037. [PubMed: 9315921]
45. Zhang K, Howes KA, He W, et al. Structure, alternative splicing, and expression of the human RGS9 gene. *Gene.* 1999; 240(1):23–34. [PubMed: 10564809]
46. Martemyanov KA, Arshavsky VY. Biology and functions of the RGS9 isoforms. *Prog Mol Biol Transl Sci.* 2009; 86:205–227. [PubMed: 20374717]
47. Rinner O, Makhankov YV, Biehlmaier O, Neuhauss SC. Knockdown of cone-specific kinase GRK7 in larval zebrafish leads to impaired cone response recovery and delayed dark adaptation. *Neuron.* 2005; 47(2):231–242. [PubMed: 16039565]
48. Lem J, Makino CL. Phototransduction in transgenic mice. *Curr Opin Neurobiol.* 1996; 6(4):453–458. [PubMed: 8794096]
49. Nikonov SS, Brown BM, Davis JA, et al. Mouse cones require an arrestin for normal inactivation of phototransduction. *Neuron.* 2008; 59(3):462–474. [PubMed: 18701071]
50. Watson AJ, Aragay AM, Slepak VZ, Simon MI. A novel form of the G protein beta subunit Gbeta5 is specifically expressed in the vertebrate retina. *J Biol Chem.* 1996; 271(45):28154–28160. [PubMed: 8910430]
51. Hu G, Wensel TG. R9AP, a membrane anchor for the photoreceptor GTPase accelerating protein, RGS9-1. *Proc Natl Acad Sci USA.* 2002; 99(15):9755–9760. [PubMed: 12119397]
52. Makino ER, Handy JW, Li T, Arshavsky VY. The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein beta subunit. *Proc Natl Acad Sci USA.* 1999; 96(5):1947–1952. [PubMed: 10051575]
53. Keresztes G, Martemyanov KA, Krispel CM, et al. Absence of the RGS9. Gbeta5 GTPase-activating complex in photoreceptors of the R9AP knockout mouse. *J Biol Chem.* 2004; 279(3):1581–1584. [PubMed: 14625292]
54. Krispel CM, Chen CK, Simon MI, Burns ME. Prolonged photoresponses and defective adaptation in rods of Gbeta5^{-/-} mice. *J Neurosci.* 2003; 23(18):6965–6971. [PubMed: 12904457]
55. Cowan CW, Fariss RN, Sokal I, Palczewski K, Wensel TG. High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. *Proc Natl Acad Sci USA.* 1998; 95(9):5351–5356. [PubMed: 9560279]
56. Zhang X, Wensel TG, Kraft TW. GTPase regulators and photoresponses in cones of the eastern chipmunk. *J Neurosci.* 2003; 23(4):1287–1297. [PubMed: 12598617]
57. Chen CK, Eversole-Cire P, Zhang H, et al. Instability of GGL domain-containing RGS proteins in mice lacking the G protein beta-subunit Gbeta5. *Proc Natl Acad Sci USA.* 2003; 100(11):6604–6609. [PubMed: 12738888]
58. Krispel CM, Chen D, Melling N, et al. RGS expression rate-limits recovery of rod photoresponses. *Neuron.* 2006; 51(4):409–416. [PubMed: 16908407]
59. Pugh EN Jr. RGS expression level precisely regulates the duration of rod photoresponses. *Neuron.* 2006; 51(4):391–393. [PubMed: 16908403]
60. Slepak VZ, Hurley JB. Mechanism of light-induced translocation of arrestin and transducin in photoreceptors: interaction-restricted diffusion. *IUBMB Life.* 2008; 60(1):2–9. [PubMed: 18379987]
61. Sokolov M, Lyubarsky AL, Strissel KJ, et al. Massive light-driven translocation of transducin between the two major compartments of rod cells: a novel mechanism of light adaptation. *Neuron.* 2002; 34(1):95–106. [PubMed: 11931744]
62. Strissel KJ, Lishko PV, Trieu LH, Kennedy MJ, Hurley JB, Arshavsky VY. Recoverin undergoes light-dependent intracellular translocation in rod photoreceptors. *J Biol Chem.* 2005; 280(32):29250–29255. [PubMed: 15961391]
63. Chen CK, Inglese J, Lefkowitz RJ, Hurley JB. Ca(2+)-dependent interaction of recoverin with rhodopsin kinase. *J Biol Chem.* 1995; 270(30):18060–18066. [PubMed: 7629115]
64. Tian M, Zallocchi M, Wang W, et al. Light-induced translocation of RGS9-1 and Gbeta5L in mouse rod photoreceptors. *PLoS One.* 2013; 8(3):e58832. [PubMed: 23555598]

65. Hu G, Jang GF, Cowan CW, Wensel TG, Palczewski K. Phosphorylation of RGS9-1 by an endogenous protein kinase in rod outer segments. *J Biol Chem.* 2001; 276(25):22287–22295. [PubMed: 11292825]
66. Sokal I, Hu G, Liang Y, Mao M, Wensel TG, Palczewski K. Identification of protein kinase C isozymes responsible for the phosphorylation of photoreceptor-specific RGS9-1 at Ser475. *J Biol Chem.* 2003; 278(10):8316–8325. [PubMed: 12499365]
67. Whitlock GG, Lamb TD. Variability in the time course of single photon responses from toad rods: termination of rhodopsin's activity. *Neuron.* 1999; 23(2):337–351. [PubMed: 10399939]
68. Balasubramanian N, Levay K, Keren-Raifman T, Faurobert E, Slepak VZ. Phosphorylation of the regulator of G protein signaling RGS9-1 by protein kinase A is a potential mechanism of light- and Ca²⁺-mediated regulation of G protein function in photoreceptors. *Biochemistry.* 2001; 40(42):12619–12627. [PubMed: 11601986]
69. Horner TJ, Osawa S, Schaller MD, Weiss ER. Phosphorylation of GRK1 and GRK7 by cAMP-dependent protein kinase attenuates their enzymatic activities. *J Biol Chem.* 2005; 280(31):28241–28250. [PubMed: 15946941]
70. Osawa S, Jo R, Xiong Y, et al. Phosphorylation of G protein-coupled receptor kinase 1 (GRK1) is regulated by light but independent of phototransduction in rod photoreceptors. *J Biol Chem.* 2011; 286(23):20923–20929. [PubMed: 21504899]
71. Snow BE, Krumins AM, Brothers GM, et al. A G protein gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to Gbeta5 subunits. *Proc Natl Acad Sci USA.* 1998; 95(22):13307–13312. [PubMed: 9789084]
72. Watson AJ, Katz A, Simon MI. A fifth member of the mammalian G-protein beta-subunit family. Expression in brain and activation of the beta 2 isotype of phospholipase C. *J Biol Chem.* 1994; 269(35):22150–22156. [PubMed: 8071339]
73. Shim H, Wang CT, Chen YL, et al. Defective retinal depolarizing bipolar cells in regulators of G protein signaling (RGS) 7 and 11 double null mice. *J Biol Chem.* 2012; 287(18):14873–14879. [PubMed: 22371490]
74. Mojumder DK, Qian Y, Wensel TG. Two R7 regulator of G-protein signaling proteins shape retinal bipolar cell signaling. *J Neurosci.* 2009; 29(24):7753–7765. [PubMed: 19535587]
75. Cao Y, Pahlberg J, Sarria I, Kamasawa N, Sampath AP, Martemyanov KA. Regulators of G protein signaling RGS7 and RGS11 determine the onset of the light response in ON bipolar neurons. *Proc Natl Acad Sci USA.* 2012; 109(20):7905–7910. [PubMed: 22547806]
76. Rao A, Dallman R, Henderson S, Chen CK. Gbeta5 is required for normal light responses and morphology of retinal ON-bipolar cells. *J Neurosci.* 2007; 27(51):14199–14204. [PubMed: 18094259]
77. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science.* 2002; 295(5557):1070–1073. [PubMed: 11834835]
78. Xue T, Do MT, Riccio A, et al. Melanopsin signalling in mammalian iris and retina. *Nature.* 2011; 479(7371):67–73. [PubMed: 22051675]

Figure 1.

Vertebrate rod phototransduction cascade. Photon absorption by rhodopsin leads to the formation of metarhodopsin II (Meta-II), which catalyzes guanine nucleotide exchange on transducin α subunit ($T\alpha$) and results in its dissociation from the $\beta\gamma$ dimer ($T\beta\gamma$). The GTP-bound $T\alpha$ ($T\alpha.GTP$) interacts with $PDE6\gamma$ to relieve its inhibition on $PDE6\alpha\beta$, freeing this near-perfect phosphodiesterase to hydrolyze cGMP into 5'-GMP. Two gain steps marked by filled ribbons endow rods with the sensitivity to detect single photons. This chapter deals with one reaction that must occur during recovery of rod phototransduction as highlighted in the dashed box. The reaction is the hydrolysis of bound GTP by $T\alpha$ to GDP, which is assisted *in vivo* by a GTPase-accelerating protein (GAP), now known to be a protein complex consists of RGS9-1, G β 5-L, and R9AP. Without the GAP complex, $T\alpha$ turns over bound GTP in seconds, a pace too slow to sustain normal vision.

Figure 2.

In situ hybridization showing localization of GRK1, RGS9, RGSr/16, and RGS11 messages in mouse retinal cross sections. The three nuclear layers: outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) are marked at left. GRK1 message is abundantly present at the inner segment (IS) layer of the retina (red (gray in the print version) asterisk) and is used as a marker for any potential photoreceptor-specific genes. Sense probes control for degree of background staining. For RGS genes tested for candidacy as a transducin GAP, only RGS9 message appears in the IS and ONL. RGSr/16 is present throughout INL and GCL but notably absent in IS or ONL. RGS11 is localized to some GCL cells and the upper-tier cells in INL. Scale bar equals 50 μ m.