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Lifetime Regulation of G Protein–Effector Complex: Emerging Importance of RGS Proteins

Minireview

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With their report in this issue of *Neuron* on RGS9, a rod photoreceptor GTPase activating protein (GAP), He et al. (1998) open a new chapter in the encyclopedic saga of G protein signal transduction, in the volume on the rod phototransduction cascade.

Vertebrate rod phototransduction (see Figure 1) is initiated when rhodopsin, a member of the superfamily of seven-helix G protein–coupled receptors, captures a photon; the covalently preattached ligand of rhodopsin, 11-*cis* retinal, is then isomerized to its all *trans* form, causing rhodopsin to undergo a conformational change and thus enter its enzymatically active signaling state, R*. R* catalyzes GTP/GDP exchange on the α subunit of the rod's heterotrimeric G protein, transducin. The activated moiety of transducin, $G\alpha_t$–GTP, carries the signal forward to the tertiary effector protein cGMP phosphodiesterase (PDE) by binding to and relieving an inhibitory constraint imposed by the γ subunit of the PDE; the catalytic subunits of PDE thus enter their active state, PDE*. The first three steps of the phototransduction cascade occur in or in tight association with the lamellar membranes of the rod outer segment. In the fourth step, PDE*s catalyze the hydrolysis of the cytoplasmic messenger cGMP, which in the resting state holds open cGMP-activated channels in the rod plasma membrane. The channels close as the cytoplasmic cGMP concentration declines, and they lose their bound cGMP. The extremely high amplification of rod phototransduction—universally enabling vertebrate rods to achieve single-photon signaling reliability—is achieved primarily by the cascaded action of the two enzymes R* and PDE*.

The G protein cascade theory of rod phototransduction faced a deep puzzle in the late 1980s. The molecular theory postulated that the hydrolysis of the terminal phosphate of $G\alpha_t$–GTP was a requisite step in the inactivation of the cascade, but the numbers were way, way off the mark. The responses of amphibian rods to dim flashes at room temperature were known to reach their response peak in $\sim$1 s and subsequently recover with a terminal time constant of no more than 2 s, while the dim flash responses of mammalian rods were found to be nearly ten times faster, peaking in $\sim$150 ms and recovering with a terminal time constant of $\sim$200 ms. Also in the 1980s, numerous studies of transducin–GTPase activity reported turnover numbers of 1–2 min^{-1}, nearly 100-fold too slow to account for the physiologically measured responses. An unrealistic patch of the theory was proposed—namely, that the decline in Ca^{2+} that accompanies the rod light response might accelerate guanylyl cyclase activity in such a way as to mask a long-lived activation of PDE by slowly decaying $G\alpha_t$–GTP. Physiological experiments rejected the latter hypothesis, showing that cyclase activation in situ was modest and brief at low flash intensities—certainly inadequate to bridge the chasm between the biochemically determined GTPase turnover numbers and the kinetics of the dim flash responses of rods. A gauntlet was cast.

Several laboratories engaged in photoreceptor biochemistry took up the challenge and found the rate of transducin GTPase in concentrated suspensions of rod outer segment membranes to be much higher than that observed in reconstituted systems (Arshavsky et al., 1989, and references therein). These studies provided

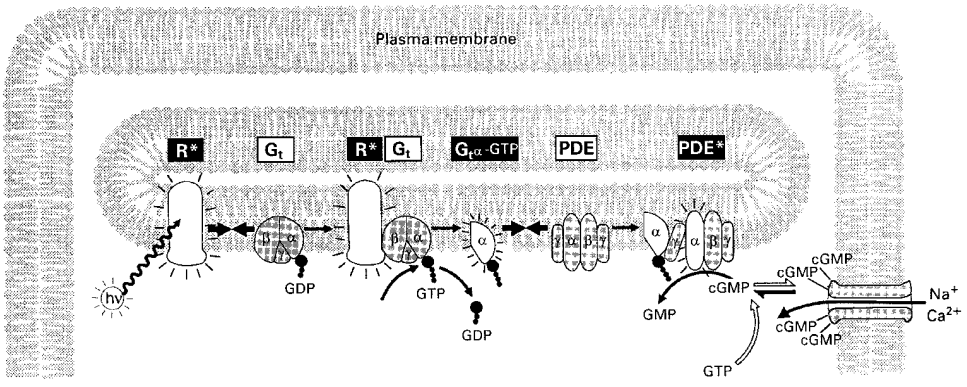

Figure 1. Schematic of the *Activation* Steps of the G Protein Phototransduction Cascade of a Vertebrate Rod Photoreceptor Outer Segment
Most of the proteins of the cascade are associated with the disk membranes of the outer segment, except for the cGMP-activated channels, which reside in the plasma membrane: cGMP serves as the internal messenger between the disk and plasma membrane. The activation steps lead to the closure of cGMP channels. *Inactivation* reactions, which lead to restoration of the resting state after light exposure, are not shown. See text for details. (Modified from Breton et al., 1994, Invest. Ophth. Vis. Sci. *35*, 295–309).

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the initial evidence for GTPase-activating proteins/factors (GAPs) in the rod outer segment. The first GAP identified was PDEγ, the γ subunit of the PDE, the tertiary effector protein of the phototransduction cascade (Arshavsky and Bownds, 1992). However, evidence accumulated rapidly that PDEγ alone is insufficient for activating transducin GTPase and that one or more factors in addition to PDEγ must also be present in the outer segment (Angleson and Wensel, 1993, 1994; Antonny et al., 1993; Arshavsky et al., 1994). The consensus conclusion of the Angleson and Wensel and the Arshavsky et al. studies was that the cooperative action of this factor and PDEγ in photoreceptors could increase the slow basal GTPase activity of transducin up to the time scale set by the photoresponse recoveries of intact rods. The identification of this factor became one of the most challenging problems in the field of phototransduction.

The strategy of He et al. (1998) in identifying the GAP for transducin follows closely on the heels of several recent reports identifying a novel family of GAPs, the regulators of G protein signaling (RGSs) (reviewed by Koelle, 1997). All characterized members of this family are strong stimulators of the GTPase activity of many G proteins, particularly those of the G_i and G_q families, including transducin. The mechanism of RGS action rests on its ability to stabilize the G protein α subunit in the conformation most favorable for hydrolyzing bound GTP (Tesmer et al., 1997). The rapidly expanding RGS family now has at least 20 members.

The hypothesis of He et al. was that the transducin GAP is a photoreceptor-specific member of the RGS family. They screened retinal libraries for cDNA representing the RGS homology domain to identify RGS proteins present in the retina and focused their efforts on those proteins that complied with three basic requirements: (1) presence in photoreceptors and specifically in rod outer segments; (2) ability to activate transducin GTPase and, most importantly, to cooperate positively with PDEγ in this activation; and (3) tight association with rod membranes. This third requirement was based on earlier data from the same laboratory showing that transducin GAP is a membrane-bound protein (Angleson and Wensel, 1993). He et al. have found that the only retinally expressed RGS protein that displays all three hallmarks of an endogenous rod outer segment GAP is RGS9. Indeed, RGS9 turned out to be a predominant RGS protein in the entire retina. The report of He et al. appears to conclude a decade-long effort to reconcile the terminal phosphate hydrolysis theory of transducin inactivation with the kinetic realities of the photoresponses of rods to dim flashes.

The conclusion that transducin GTPase is powerfully up-regulated by RGS9 and the γ subunit of its effector contributes to an interesting chapter remaining to be completed in the book of rod phototransduction—which will contain a biochemically correct and kinetically accurate theory of inactivation of the rod photoresponse. A lively page in this chapter is based on an observation that the photoreceptor recovery from response-saturating flashes is determined by a single "dominant" or rate-limiting time constant. In amphibian rods at room temperature, the dominant time constant is $\sim$2 s (Pepperberg et al., 1992), whereas in mammalian rods at body temperature, the dominant recovery time constant is 10-fold smaller: $\sim$0.2 s (Lyubarsky and Pugh, 1996). The molecular identity of the cascade intermediate whose lifetime is described by this dominant time constant is currently a subject of intense investigation. Analysis shows that there are only two possible candidates for the molecular mechanism: a first-order decay of the activity of R* or a first-order inactivation of the transducin–PDE* complex conferred through transducin GTPase. Pepperberg et al. (1992) weighed in for the R* hypothesis, but the balance seems now to be tilting toward transducin GTPase. Chief among the reasons for this tilt is the absence of calcium dependence of the dominant time constant in situ, paralleled by the absence of calcium dependence of transducin GTPase activity in rod outer segments, as well as evidence that the lifetime of R* is regulated by the calcium-binding protein called recoverin.

In the larger framework of heterotrimeric G protein theory, an entire volume may now be emerging on the topic of specificity of timing of signal inactivation. One hypothesis is that such specificity could be conferred solely by control of expression of a G protein and its GAP. A systematic study of the expression patterns of mRNA for nine different types of RGS proteins in brain indicates tissue-specific patterns of expression (Gold et al., 1997). For example, RGS9 has been localized mostly to caudoputamen, nucleus accumbens, and the olfactory tubercle. According to this hypothesis of timing specificity, the coexpression—and perhaps quantitative regulation of the amounts—of transducin and RGS9 in the rod would suffice to determine the lifetime of $G\alpha_t$ in situ. However, lifetime regulation determined solely by coexpression of a G protein and an RGS is suboptimal for cascade signaling efficacy because signaling in situ continues through a specific G protein effector. Thus, the ultimate timing problem is to regulate the lifetime of the activated G protein moiety complexed with its specific effector, since it is this complex that determines the output signal.

The simplest solution to the G protein–effector lifetime problem seems to have been found in the case of the G protein G_q, whose GTPase activity is substantially accelerated by its effector, phospholipase C-β1 (see Figure 2) (Berstein et al., 1992). Another relatively straightforward solution is offered in those pathways where the signals are conferred through G protein $\beta\gamma$ subunits. While the G$\beta\gamma$ complex is modulating the activity of its effector, the GTPase activity of the free α subunit might be stimulated by RGS. The RGS-mediated GTP hydrolysis would speed the reassociation of the G protein subunits and signal termination. It is likely that such a scenario occurs in the stimulation of G$\beta\gamma$-activated inwardly rectifying K^+ channels from the nervous system and the heart; Doupnik et al. (1997) have recently demonstrated that physiologically fast inactivation of these channels is observed when they are coexpressed in oocytes with at least three different RGS proteins, RGS1, RGS3, and RGS4. However, the more complicated solution—involving the action of RGS on lifetime of the G protein α subunit complexed with its effector—poses new problems, which appear to be largely unresolved. All data accumulated so far with recombinant

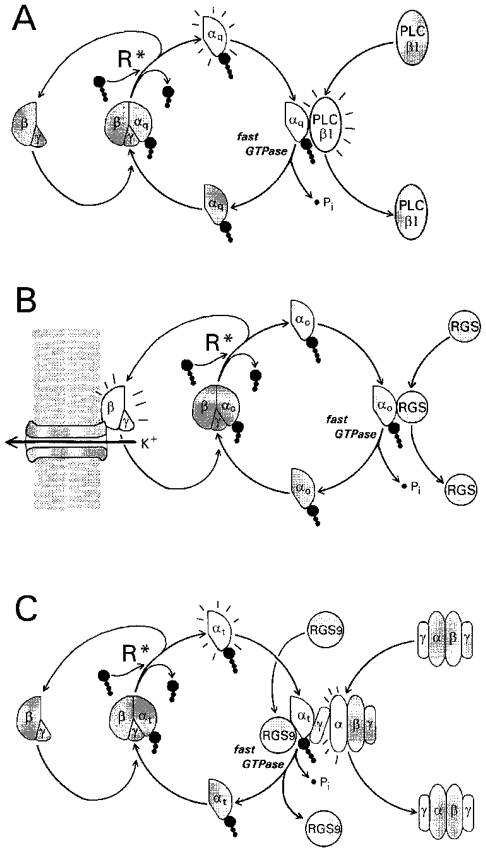

Figure 2. Three Scenarios for the Regulation of Signal Duration in G Protein–Based Signal Transduction Pathways

In all cases, the cycle of G protein activation/inactivation is initiated by the activated receptor (labeled R*).

(A) The duration of the signal is determined by the GAP ability of the effector enzyme. Phospholipase C-β1 is necessary and sufficient for activating GTP hydrolysis on the α subunit of G_q (Berstein et al., 1992).

(B) The duration of the signal is determined by the action of RGS proteins on free G protein α subunit, while the signal per se is carried by the G protein βγ subunits. Activation of G_i or G_o in the nervous system and the heart leads to stimulation of inwardly rectifying K^+ channels by corresponding βγ subunits. At the same time, various RGS proteins cause fast GTP hydrolysis on $G_{i/o}$, resulting in G protein subunit reassociation and signal termination (Doupnik et al., 1997).

(C) The duration of the signal is determined by a cooperative action of the effector enzyme and RGS protein on G protein α subunit. Transducin GTPase in the phototransduction cascade is activated by coordinated action of its effector, PDE, and RGS9 (He et al., 1998).

RGS proteins indicate them to be rather promiscuous with respect to various G protein α subunits, having no requirement for specific effectors to manifest their GAP ability. If this situation was general, instead of regulating the lifetime of the activated effectors, GAPs might short-circuit the signals, inactivating the G proteins before they have a chance to interact successfully with their specific effectors and thereby produce an output signal.

The data by He et al. (1998) help to explain how this problem of signal short-circuiting by RGS proteins has been solved in rod and in so doing suggest a general mechanism of timing specificity whereby a ternary complex of activated G protein moiety, effector, and RGS combine to determine the activated effector's lifetime. Thus, even though the recombinant RGS9 fragment used in the He et al. experiments is able to stimulate transducin GTPase on its own, PDEγ causes an additional 3-fold acceleration. This stimulatory effect of PDEγ is much more powerful in rod outer segment suspensions, where the native whole-length RGS9 is present (Angleson and Wensel, 1994; Arshavsky et al., 1994). The cooperative action of RGS9 and PDEγ allows the rod photoreceptor both to avoid premature inactivation of $G\alpha_t$–GTP and also to turn off the photoresponse in a timely manner after production of PDE*, the output signal of the membrane-associated steps of the phototransduction cascade. The exact roles of each of these components in accelerating GTP hydrolysis remain to be determined. For example, PDEγ might either contribute directly to stabilization of $G\alpha_t$–GTP transducin in a conformation favorable for GTP hydrolysis or simply enhance the affinity between $G\alpha_t$–GTP and RGS. Interestingly, there is a striking difference between inactivation of transducin by RGS9 and by other RGS proteins not present in the rod photoreceptor. Nekrasova et al. (1997) have shown that activation of transducin GTPase by two other RGS proteins, RGS4 and GAIP, is reversed rather than enhanced by PDEγ. This result indicates a precise match among all three proteins involved in the timing of the lifetime in rods of the ternary effector complex $G\alpha_t$–PDE*.

In summary, the study of transducin GTPase regulation in retinal rods provides us with the first example of a vertebrate signal transduction cascade in which the role of an RGS protein is fully defined. It is now evident that GAP activity is controlled in a sophisticated fashion and that potential problems with lack of RGS specificity have been solved by the requirement that RGS9, $G\alpha_t$–GTP, and PDE* act as an ensemble. This opens a possibility that the action of other RGS proteins will look more orderly when considered in the context of their specific signal transduction cascades. Perhaps by the end of the volume the complex of proteins regulating the activated G protein and its effector will look as multifaceted as the DNA replication fork!

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