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How regulators of G protein signaling achieve selective regulation

Guo-xi Xie and **Pamela Pierce Palmer***
Department of Anesthesia and Perioperative Care, University of California, San Francisco, California 94143, USA

Summary

The regulators of G protein signaling (RGS) are a family of cellular proteins that play an essential regulatory role in G protein-mediated signal transduction. There are multiple RGS subfamilies consisting of over twenty different RGS proteins. They are basically the guanosine triphosphatase (GTPase)-accelerating proteins that specifically interact with G protein α subunits. RGS proteins display remarkable selectivity and specificity in their regulation of receptors, ion channels, and other G protein-mediated physiological events. The molecular and cellular mechanisms underlying such selectivity are complex and cooperate at many different levels. Recent research data have provided strong evidence that the spatiotemporal-specific expression of RGS proteins and their target components, as well as the specific protein-protein recognition and interaction through their characteristic structural domains and functional motifs, are determinants for RGS selectivity and specificity. Other molecular mechanisms, such as alternative splicing and scaffold proteins, also significantly contribute to RGS selectivity. To pursue a thorough understanding of the mechanisms of RGS selective regulation will be of great significance for the advancement of our knowledge of molecular and cellular signal transduction.

Keywords

G protein-coupled receptor; GTPase; RGS; selectivity; domain

Introduction

Upon binding to their specific receptors on the cell membranes, numerous neurotransmitters, hormones, growth factors, and drug chemicals selectively activate their own specific signaling pathways, accurately and precisely transduce the signals into cells and nuclei, and accomplish their activities and functions. The G protein-coupled receptors (GPCR) and the G protein-regulated ion channels represent a major class of signal transduction systems.[1,2] It has now been well recognized that the regulators of G protein signaling (RGS) play essential regulatory roles in the G protein-mediated signal transduction.[3]

RGS proteins are a family of cellular proteins with conserved RGS domains (also called RGS box) of about 120 amino-acid residues in length. RGS proteins specifically interact with the α subunits of G proteins, greatly enhance the intrinsic GTPase activities of Gα and accelerate

*Corresponding author: Pamela Pierce Palmer, M.D., Ph.D., University of California, San Francisco, Department of Anesthesia and Perioperative Care, 513 Parnassus Avenue, Box 0464, Room S-455, San Francisco, California 94143, USA, Telephone: (415)476-6783, FAX: (415)502-5375, E-mail: palmerp@anesthesia.ucsf.edu.

the hydrolysis of GTP to GDP by Gα, thus converting G proteins from a GTP-bound active state to a GDP-bound inactive state and terminating G protein-mediated signaling.[4]

There are over 20 members in the mammalian RGS family. According to the similarity in sequence and features of structural domains, they have been classified into nine subfamilies (Table 1).[4–6] We will mainly discuss those which are directly involved in G protein-mediated signal transduction.

In animals and humans, there are hundreds of GPCRs and G protein-regulated ion channels. They are coupled to or regulated by different types of heterotrimeric G proteins that are coded by at least 20 different Gα, 6 different Gβ, and 11 different Gγ genes.[7–9] Those G proteins are themselves regulated by multiple RGS proteins. It becomes obvious from these numbers that a specific RGS protein could be responsible for regulating several different types of G proteins, receptors, and ion channels. The reverse may also be true that multiple RGS proteins could regulate a single type of G protein, receptor, or ion channel. Therefore, two fundamental questions raised here are: (1) Do RGS proteins possess specificity and selectivity in their regulation of G protein-mediated signal transduction? (2) If RGS proteins do display specificity and selectivity, what are the molecular and cellular bases underlying such properties?

Biochemical and genetic knockout analyses have convincingly demonstrated that RGS proteins are essential regulatory elements in the G protein-mediated signal transduction pathways.[10–14] Thus, it is of great significance and interest to address the two questions. In this article, we will review the recent advances in our understanding of the specificity and selectivity of RGS proteins. We will first discuss experimental evidence that supports RGS selectivity, and then focus on specific molecular mechanisms.

RGS proteins display specificity and selectivity in their interaction and regulation

Since the first recognition of RGS proteins in yeast, invertebrates and vertebrates,[10,15,16] great progress has been made in understanding the molecular structures, biochemical properties, and functions of this multigene family. Rich research data have demonstrated that RGS proteins possess specificity and selectivity in their regulation of G protein-mediated receptors, ion channels, and other signaling events. Basically the data provide two kinds of evidence indicating RGS selectivity. One class of evidence shows that some RGS proteins preferably regulate certain subtypes of signaling molecules, but have no effect on other subtypes in the same families. Another class of evidence shows that different RGS proteins display significant differences in the effectiveness in regulating a given signaling molecule.

RGS proteins selectively regulate G protein-coupled receptors

It was first discovered by Berman et al. that two RGS proteins, RGS4 and GAIP (Gα-interacting protein, also named RGS19), selectively accelerate the GTPase activities of Giα1, Giα2, Giα3, and Goα, but not that of Gsα.[17] These two RGS proteins were also found to have selectivity in their attenuation of signaling mediated by Gi or Gq-coupled M1 muscarinic acetylcholine (ACh) receptors, bradykinin receptors, and δ opioid receptors in both *in vitro* and *in vivo* cell assays.[18,19] The patterns of selectivity observed *in vivo* are similar to those seen *in vitro*. RGS4 and GAIP/RGS19 both can attenuate Gi-mediated inhibition of cyclic AMP (cAMP) synthesis and Gq-mediated activation of phospholipase Cβ (PLCβ). However, RGS4 is almost 10 times more effective than GAIP/RGS19.[18,19]

Since then more studies have shown the selectivity of RGS proteins in regulating GPCRs. A study using stable cell lines expressing 5-HT1A, 5-HT2A, or dopamine D2 receptors

demonstrates that adenovirus-delivered expressions of RGS4, RGS10, and RGS20 (also called RGSZ1) effectively attenuate Gαi-mediated 5-HT1A receptor signaling, but have no effect on that of dopamine D2 receptors. In contrast, RGS2 and RGS7 significantly decrease Gαq-mediated 5-HT2A receptor signaling, but have little effect on 5-HT1A and D2 receptors.[20]

RGS proteins have distinct selectivity for different types of muscarinic ACh receptors.[21,22] The four members of the C/R7 subfamily, RGS6, RGS7, RGS9, and RGS11, when in complex with Gβ5, are able to stimulate steady-state GTPase activity of Gαi that is coupled to muscarinic M2 receptor, but not that of Gαq/11 coupled to M1 receptor. RGS9 and RGS11 are more potent to Gαi than RGS6 and RGS7. All four exhibit similar potencies to Gαo.[22] Another study shows that RGS4 has greatest potency in regulating Gq-coupled muscarinic ACh receptors, 4 times higher than its potency in regulating Gq-coupled bombesin receptors and 33-fold more powerful than that in Gq-coupled cholecystokinin (CCK) receptors. RGS1 shows 1000-fold higher potency in inhibiting muscarinic receptors than CCK receptors. RGS16 also potently inhibits muscarinic but partially inhibits CCK receptors. However, RGS2 inhibits muscarinic and CCK receptors with equal potencies.[23]

RGS9 is an excellent example in elucidating the selectivity and specificity of an RGS protein. There are two RGS9 variants, RGS9-1 and RGS9-2, derived from an alternative splicing of the same RGS9 gene. The two variant proteins differ only in the C-terminal region. RGS9-1 is primarily expressed in the retina, while RGS9-2 is highly enriched in the striatum and certain other brain areas.[24,25] RGS9-1 is essential for acceleration of hydrolysis of GTP by Gαt, the α subunit of the G protein in phototransduction transducin, and for a prompt recovery of photoresponse of the photoreceptor rhodopsin.[12] RGS9-2, however, has been shown to specifically regulate D2 dopamine receptor and μ opioid receptor.[13,14,26] Rahman et al. have demonstrated that a viral-mediated overexpression of RGS9-2 in the nucleus accumbens significantly reduces the locomotor responses of rats to cocaine (an indirect dopamine agonist) and to D2 dopaminergic agonists, but not to D1 dopaminergic agonists. Conversely, RGS9 knockout mice show dramatically increased locomotor and rewarding responses to cocaine and dopamine agonists. Expression of RGS9-2 in Xenopus oocytes accelerates the deactivation kinetics of D2 receptor-activated potassium channels. A chronic cocaine exposure specifically increases RGS9-2 expression in nucleus accumbens.[13] These findings are supported by several independent studies.[27,28]

RGS9-2 protein also exhibits selectivity to μ opioid receptors over other types of opioid receptors.[14,26,29,30] Zachariou et al. demonstrate that RGS9-2 is expressed in CNS regions known to be involved in opioid action. Acute morphine administration increases the expression of RGS9-2 in the nucleus accumbens and other CNS regions, whereas chronic morphine treatment decreases RGS9-2 levels. More convincingly, the study demonstrates that the behavioral responses of RGS9-2-knockout mice to acute and chronic morphine are significantly enhanced, which include greatly increased morphine analgesia and reward, much delayed tolerance, and increased physical dependence and withdrawal reactions to morphine.[14] Using antisence oligonucleotide technology Garzon and colleagues show that an inhibition of RGS9-2 expression in mice greatly increases the potency and duration of morphine analgesia and prevents acute morphine tolerance.[26,31] RGS9-2 also plays an important role in μ opioid receptor desensitization.[29,30]

The selectivity of RGS proteins in regulating adrenergic receptor system has also been investigated.[32–34] RGS2 shows a selective regulation of Gq/11-mediated α1 adrenergic receptor signaling associated with hypertrophy, either in primary culture of neonatal rat cardiomyocytes or in neonatal and adult ventricular myocytes with adenoviral gene delivery.[33,34] The α1 adrenergic agonist phenylephrine (PE) significantly increases RGS2 mRNA expression, but has little or no effect on other major cardiac RGS proteins, such as RGS1,

RGS3, RGS4, and RGS5. Adenovirus-delivered overexpression of RGS2 in the cardiomyocytes dramatically blocks the effects of PE on the increase of cardiomyocyte size and on the upregulation of several protein markers of hypertrophy. Furthermore, RGS2 overexpression selectively and completely prevents the activation by PE of the Gq/11-mediated mitogen-activated protein kinase (MAPK) pathway associated with hypertrophy.[33,34] In contrast, RGS3, RGS4 and RGS5 appear to equally regulate both Gq/11- and Gi/o-mediated signaling in myocytes.[34] A selective knockdown of RGS2 by RNA interference (RNAi) increases PE- and endothelin-1-induced PLCβ stimulation, and facilitates hypertrophy development in ventricular myocytes.[35] These results indicate that RGS2 is a selective and negative regulator in cardiac hypertrophy produced by α1 adrenergic receptor activation and Gq/11-mediated signaling event in the heart.

Studies using specific ribozymes to selectively inhibit RGS expression have demonstrated RGS selectivity. RGS3 ribozyme selectively enhances cholinergic agonist carbachol-induced MAPK activity, while RGS5 ribozyme selectively enhances angiotensin II-induced MAPK activity and inositol phosphate release. RGS2 and RGS7 ribozymes have no effect on those two receptors.[36]

Our own study, using COS cells co-expressing one type each of opioid receptors and RGS proteins by transfection with specifically engineered dual-expression plasmids, demonstrated that GAIP/RGS19 is more selective to regulate the opioid-receptor-like receptor (ORL1) over the μ, δ, and κ opioid receptors, as measured by the effectiveness to increase type-selective agonist-stimulated GTPase activity and to reverse the agonist-induced inhibition of cAMP accumulation. In contrast, RGS4 is more selective to μ opioid receptors in the same assays.[37]

The G protein-coupled receptor kinases (GRKs) form another important RGS subfamily, showing significant specificity and selectivity in phosphorylation and desensitization of GPCRs.[38–41] Different GRKs also selectively interact with different Gα subunits.[42]

Although most RGS proteins do not interact with stimulatory G protein α subunits Gαs and do not show specific regulatory effect on Gs-mediated signaling, there might be exceptions. One RGS member, RGS-PX1, was reported to selectively interact with Gαs, and to regulate Gs-mediated β2 adrenergic receptor signaling and epidermal growth factor (EGF) receptor degradation.[43,44] RGS2 was also shown to interact with Gαs.[45] These exceptional findings are still controversial and have yet to be confirmed by independent laboratories.

RGS proteins selectively regulate G protein-gated ion channels

The selectivity of RGS proteins in the regulation of G protein-gated ion channels is also significant.

The G protein-gated inwardly rectifying potassium channels (GIRKs) are gated primarily by a direct interaction with Gβγ subunits via the activation of GPCRs.[46] It has been noted that cloned GIRKs, when expressed in functional expression systems such as Xenopus oocytes and mammalian cell cultures, display significantly (20 to 40 times) slower activation and deactivation kinetics than *in vivo* recordings in isolated neurons and cardiomyocytes. However, co-expression of specific RGS proteins can dramatically restore the physiological channel kinetics of GIRKs. The selectivity of a specific RGS protein to GIRK is largely dependent on which GPCR and G protein subunits control the ion channel. While several RGS proteins are shown to accelerate the activation and deactivation of muscarinic M2- and serotonin 5HT1A-activated GIRK channels,[47,48] RGS9-2 is shown to selectively accelerate the kinetics of dopamine D2-activated GIRK channels.[13] Noticeably, a very low dose of RGS9-2 alone has no effect; but co-expression of the very low doses of RGS9-2 and Gβ5 exhibits a great acceleration effect on D2-induced GIRK deactivation.[13] RGS4 is reported to selectively

accelerate the deactivation of μ or κ opioid receptor-linked GIRK channels.[49,50] Evidence shows that RGS4 is able to specifically restore the electrophysiological properties of reconstituted GIRK (Kir3.1 and Kir3.4) channels in Xenopus oocytes.[51]

Interestingly, it is reported that an alternatively spliced short RGS3 variant that is abundantly expressed in neuronal tissues differentially modulates muscarinic M2- and 5-HT1A receptor-activated neuronal GIRK (Kir3.1 and Kir3.2a) channels.[52] The short RGS3 variant and RGS4 are basically indistinguishable in modulating every aspect of the gating properties of 5-HT1A receptor-activated GIRK channels, but are found to be different in regulating muscarinic M2 receptor-activated GIRK channels. The short RGS3 causes a significant reduction (~45%) in the maximal ACh-induced GIRK current amplitude and a marked shift in the steady-state ACh dose-response curve, indicating a reduction of functional coupling between M2 receptor and GIRK channel. RGS4 has no effect on the maximal amplitude and dose-response of ACh-induced GIRK currents.[52]

Studies demonstrate that some RGS proteins selectively modulate the activation of GIRK channels, while others are more selective in deactivation. In the Xenopus oocyte expression system, RGS2, RGS5 and RGS8 appear to be more selective in accelerating the deactivation of GIRK channels than they are in accelerating the activation.[53] On the contrary, RGS7 significantly accelerates the activation of muscarinic M2 receptor-coupled GIRK channels, but has a very small effect on deactivation.[54]

RGS proteins also show selectivity in regulating the calcium ion channels. While RGS2 appears not to have significant effect on GIRK channels,[47] it shows specific regulation for Ca^{2+} channels.[55–58] Studies demonstrate that RGS2 selectively reduces or blocks the slow inhibition of L- and N-types of Ca^{2+} channels induced by muscarinic and bradykinin receptors, and accelerates their recovery from the inhibition.[55–57] In contrast, RGS2 markedly accelerates both the fast inhibition and recovery of the presynaptic P/Q-type Ca^{2+} channels controlled by the muscarinic M2 receptors.[59]

Convincing evidence shows that RGS12 selectively regulates γ-aminobutyric acid (GABA)-regulated N-type Ca^{2+} channels (Cav2.2).[60] Upon the activation of $GABA_B$ receptors, the α1 (pore-forming) subunit of the N-type Cav2.2 channels is phosphorylated by tyrosine kinases. RGS12 selectively binds to the phosphorylated α1 subunit, which dramatically alters the kinetics of termination of GABA-mediated inhibition of calcium currents.[60] A micro-injection of recombinant RGS12 into dorsal root ganglion (DRG) neurons in primary culture selectively accelerates the termination of the GABA-induced, tyrosine kinase-mediated inhibition of calcium currents in a voltage-independent manner. Other RGS proteins, such as RGS2, RGS14, and GAIP/RGS19, fail to alter the GABA-induced inhibition of calcium currents. An inhibition of endogenous RGS12 by anti-RGS12 antibody significantly slows the termination of the GABA-induced inhibition of calcium currents.[61]

A study by Cabrera-Vera et al. describes the selectivity of RGS9-2 protein for regulation of dopamine D2 receptor-mediated Ca^{2+} channels in individual rat striatal neurons. Dialysis of striatal cholinergic neurons with recombinant RGS9 proteins reduces D2 dopamine receptor-mediated inhibition of the voltage-dependent Cav2.2 channels, but does not alter M2 muscarinic receptor modulation of Cav2.2 currents in the same neurons. A mutated RGS9 that impairs its specific binding to Gα subunits fails to modulate D2 receptor-mediated Cav2.2 channel inhibition.[27]

Another interesting example demonstrating RGS selectivity for Ca^{2+} channels is a unique GAIP/RGS19 from chicken embryonic DRG neurons.[62] The chicken GAIP/RGS19 consists of a conserved RGS domain (85% identical to human counterpart) and a unique, short N-terminus (only 41% identical to known mammalian counterparts). In electrophysiological

assays using chicken DRG neurons, the chicken GAIP/RGS19 selectively reduces a voltage-independent inhibition of Ca^{2+} channels by GABA (mediated by Gαo) without affecting the voltage-dependent inhibition (mediated by Gβγo). In contrast, mammalian GAIP/RGS19 shows no selectivity between these two forms of Ca^{2+} channel inhibition in the same assays.[62]

Data also suggest that differential selectivity in regulation of N-type Ca^{2+} channels can be achieved by different combinations of Gβ/γ subunits and RGS proteins.[63]

The selective regulation of GIRKs and Ca^{2+} channels by RGS proteins, in a receptor-specific manner, has provided an important mechanism by which the ion channels are fine-tuned and the signaling from GPCRs to ion channels is modulated.

RGS proteins selectively regulate other G protein-mediated events

GAIP/RGS19 is to date the only RGS protein that is specifically involved in clathrin-coated membrane vesicles (CCVs) and selectively regulates protein trafficking, endocytosis and recycling, as well as agonist-induced receptor internalization.[64,65] Using immunogold labeling, GAIP/RGS19 is found on clathrin-coated membrane pits, buds or CCVs,[64] where it can be phosphorylated.[66] GAIP/RGS19 on CCVs possesses functional GTPase-activating protein (GAP) activity *in vitro*.[67] GAIP/RGS19 is shown to be functionally involved in the membrane- and protein-trafficking machinery in the brain, pituitary, liver, and kidney.[68,69]

GAIP/RGS19 plays a very special role in the agonist-induced receptor internalization.[65] Using immunofluorescence labeling technology with the Gαi3-coupled δ opioid receptor as a model, Elenko et al. demonstrate that in the absence of opioid agonist, the δ opioid receptor and the Gαi3 are located in uncoated regions of the cell plasma membrane, whereas GAIP/RGS19 is spatially segregated in CCVs. When δ opioid receptor is activated by a selective δ opioid agonist, the receptor and the activated (GTP-bound) Gαi3 translocate into CCVs. This translocation allows GAIP/RGS19 to interact with Gαi3, accelerate GTP hydrolysis, and terminate Gi-mediated δ opioid receptor signaling. Subsequently, the inactivated (GDP-bound) Gαi3 returns to uncoated domains of the plasma membrane, while GAIP/RGS19 remains associated with CCVs to accommodate the internalization and recycling of the δ opioid receptor.[65]

A number of RGS proteins play specific roles in a receptor-independent, G protein-mediated process of mitosis and cell division.[70–74] Recent studies have demonstrated that RGS7 and the GoLoco domain-containing RGS14, via distinct mechanisms, specifically regulate microtubule dynamics, mitotic spindle formation and movements.[70–72] RGS7 appears to promote an asymmetric G protein function by playing dual roles as both the negative regulator and the effector for Gαo.[70] RGS14 is a microtubule- and mitotic spindle-associated protein. Its GoLoco domain which can specifically binds Gαi-GDT plays a critical role in the control of mitotic spindle dynamics.[71,72] We will discuss this function in more details in the GoLoco domain section.

Through the above literature review, we can appreciate the selectivity and specificity of RGS proteins in the regulation of G protein-mediated signal transduction pathways. It is noteworthy that the apparent RGS selectivity is assay-dependent, which may explain some controversial observations *in vitro* and *in vivo*.

Molecular and cellular bases of the selectivity of RGS proteins

The important question is: what are the molecular and cellular mechanisms by which RGS proteins achieve their specific interaction and selective regulation? Although we do not yet

have a comprehensive knowledge and an integral explanation of how RGS proteins function selectively, recent advancement in understanding of the specific and selective interaction between some individual RGS proteins and their regulatory targets at the molecular and cellular levels has begun to provide answers to this essential question.

Spatiotemporal-specific expression

A prerequisite for an RGS protein to specifically regulate its preferred target is co-expression. The RGS protein must be co-expressed with its target protein(s) at the right time and the right location in order for the selective interaction to take place. This principle is best illustrated by the expression, function and selectivity of RGS9-1, RGS9-2, and RGS21.

The entirely different tissue distributions of the alternatively spliced RGS9-1 and RGS9-2 determine that they specifically interact with very different target proteins, and thus have completely different functions and selectivity. RGS9-1 mRNA and protein are specifically expressed in the rod and cone photoreceptor cell (vision sensory neurons) layers of the retina, and are co-localized with essential components in the phototransduction pathway such as rhodopsin, transducin Gαt, and cGMP phosphodiesterase (PDE).[75,76] RGS9-1 selectively regulates phototransduction through its specific interaction with Gαt and its GAP activity to accelerate the hydrolysis of GTP by Gαt. The GAP activity of RGS9-1 is greatly enhanced by the γ subunit of PDE, the effector in phototransduction.[75,76] When RGS9-1 is removed from extracts of the rod outer segments of the retina by immunodepletion using a specific RGS9 antibody, the extracts completely lose the PDEγ-enhanced GAP activities.[76] Data also show that RGS9-1, via its N-terminus, directly binds and inhibits retinal guanylyl cyclase, the enzyme that produces the second messenger cGMP for visual transduction.[77] This unique localized expression provides RGS9-1 the necessary and sufficient environment for selective regulation of photoreceptors. Unlike RGS9-1, several other RGS proteins (such as RGS3, RGS4, RGS6, RGS7, RGS11, and RGS16) that have been detected in the retina do not show any specialized expression and function related to phototransduction.[75]

Contrastingly, RGS9-2 mRNA and protein are predominantly expressed in the brain, with very high levels in nucleus accumbens, caudoputamen, dorsal striatum, and olfactory tubercle; and with lower levels in periaqueductal gray (PAG), deep layers of neocortex, medial amygdala, medial hypothalamic nuclei, and dorsal horn of the spinal cord, some of the regions known to express abundant dopamine receptors and opioid receptors.[25,78,79] It has been demonstrated that in the cell membrane preparations from mouse PAG region, RGS9-2 protein is specifically coprecipitated with μ opioid receptor, α subunits of Gi/o/z/q/11 proteins, Gβ1/2, and Gβ5. In contrast, RGS7 and RGS11 present in the same region are found not to associate with μ opioid receptors.[30] RGS9-2 is undetectable in the retina. Interestingly, the regional distribution pattern of RGS9-2 is gradually formed and strengthened with development and age.[79,80] Such spatiotemporal-specific expression allows RGS9-2 to selectively regulate dopamine D2 and opioid μ receptors.

RGS21 is so far the smallest member in the entire RGS protein family, consisting of barely a little more than an RGS domain.[81] Apparently, RGS21 does not have any particular structural and functional motif other than the lone RGS domain that could be contributing to its selectivity. It is, therefore, most likely the selective regional expression that determines the preference of RGS21 interaction and regulation. It has been shown that RGS21 mRNA is selectively expressed in the taste tissues. Specifically, RGS21 is restricted in a subpopulation of taste bud cells (taste sensory cells) of the tongue, and is coexpressed with the sweet and bitter transduction components including α-gustducin (the α subunit of G protein in taste transduction), PLCβ2, T1R2/3 sweet taste receptors and T2R bitter taste receptors. RGS21 is not detected in the surrounding epithelium, muscle, glands and connective tissues of the tongue, nor is it detected in other tissues tested.[81] *In vitro* binding assays demonstrate that RGS21

binds α-gustducin in a conformation-dependent manner, and prefers to interact with the same Gα subtypes with which sweet and bitter taste receptors interact.[81] These results imply that RGS21 is a selective regulator in sweet and bitter taste signal transduction pathways. The highly restricted distribution is the determinant for such selectivity.

The cell type-specific subcellular localizations and Gα activation-induced translocation of RGS proteins also play important roles in determining their selectivity, which will be discussed in details in the section of RGS N-terminal domain.[82]

Interestingly, studies suggest that the expression levels and ratio of different RGS proteins may also play a part in determining their selectivity. For example, expression levels of RGS4 and RGS7 proteins determine the mode of regulation of GIRKs.[83]

At the genomic structure and gene expression regulation levels, RGS genes exhibit special patterns regarding their genomic positions. A recent genomic analysis has revealed that not only are several RGS genes arranged in clusters and often adjacent to G protein-related components, such as Gα, Gγ, and GRK genes, but also some RGS genes are linked to specific receptor genes.[84] Studies by our laboratory have described the genomic coupling of GAIP/RGS19 and the ORL1 gene oprl in human chromosome 20.[85,86] The first exons of GAIP/RGS19 and oprl are separated by only 83 bp and this region functions as a bi-directional promoter for both genes.[85] Subsequently, two more RGS genes were found to be closely linked to κ and μ opioid receptor genes (oprk and oprm), respectively. RGS20 and oprk are separated by only 0.6 Mb in chromosome 8. RGS17 and oprm are separated by 1.5 Mb in chromosome 6.[84]

The tight genomic coupling between human GAIP/RGS19 and oprl genes strongly suggests a functional relationship, not only at the transcription level where the transcription of the two genes are co-regulated, but also possibly at the protein and cellular function levels.[85,86] There are a number of mammalian genes that, like the human GAIP/ RGS19 and oprl, are linked together (within a distance of about 100–200 bp) head-to-head by shared bi-directional promoters. Each of these pairs of adjacent genes studied to date has been found to have a significant functional relationship.[87] These findings suggest selectivity of certain RGS proteins to opioid receptors. Indeed, experiments confirm that GAIP/RGS19 has selectivity to ORL1, and RGS17 and RGS20 have selectivity to μ opioid receptors.[37,88–90]

Specific structural and functional domains

A large body of evidence supports the notion that specific structural domains and sites within the RGS proteins, the Gα subunits and the GPCRs, are the molecular determinants functioning individually or coordinately for the specificity and selectivity of RGS proteins.

(1) The RGS domain and the Gα subunit—The RGS domain is the characteristic structural element that defines the RGS protein family. The interaction between the RGS domain and the Gα subunit is the basis for the GAP activity of RGS proteins.[11,91] Studies show that specific RGS proteins selectively interact with particular Gα subunits, which may be determined by specific sequences within the RGS domains and the Gα subunits.[17,92] It was determined soon after the discovery of RGS proteins that GAIP/RGS19 interacts strongly with Gαi1, Gαi3 and Gαo, very weakly with Gαi2, and does not interact with Gαs and Gαq.[93] However, RGS2 and RGS4, in addition to their interactions with Gαi/o sununits, interact well with Gαq.[23] The crystal structure of RGS4 in complex with Gαi1 shows that only the amino-acid residues within the RGS domain of RGS4 specifically interact with Gαi1 and form significant contacts to residues within the three switch regions of Gαi1. Particularly, residue Asn128 in the RGS domain specifically interacts with residues Gln204, Ser206, and Glu207 of Gαi1.[94] An NMR structure of free RGS4 shows that residues Asp117, Ser118, and Arg121

form a unique binding pocket for Thr182 from Gαi1.[95] In GAIP/RGS19, there are major similarities and minor deviations. The primary sequence of the RGS domain of GAIP/RGS19 is approximately 60% identical to that of RGS4, and their three-dimensional structures are very similar. GAIP/RGS19 has Ser156 at the position corresponding to the Asn128 position in RGS4, which may contribute to the fine differences in selectivity and affinity of the two RGS proteins toward different Gαi members, and to the higher GAP activity of GAIP/RGS19 for Gαz.[96] Several other RGS proteins also have Ser instead of Asn in the corresponding position. In addition, only five residues among those that directly interact with RGS4 are different in Gαi1 and Gαs, and only two are different in Gαi1 and Gα12. These differences significantly affect RGS4 binding, which explain the selectivity of RGS4 and GAIP/RGS19 to Gαi, but not to Gαs and Gα12.[94] Interestingly, there is an Asp229 in Gαs that appears to play a crucial part in the prevention of its interaction with the majority of RGS proteins. A single mutation changing Asp229 to serine allows Gαs to readily interact with most RGS proteins.[97] However, in RGS-PX1, the only RGS protein that specifically interacts with Gαs, there are two positions, Arg457 and Thr459, that show specific interaction with the Asp229 of Gαs in both binding and functional assays, which may explain the unique selectivity of RGS-PX1 for Gαs.[43] This remains to be validated independently.

RGS9-1 and RGS9-2 share the same RGS domain. The crystal structures of the RGS domain alone as well as its complex with Gαt/i1 and PDEγ show that the binding surface on RGS9 for Gαt/i1 is predominantly positively charged and complements the negatively charged binding surface on Gαt/i1 for RGS9. The RGS9-Gαt/i1 complex appears to be similar to that of RGS4-Gαi1, but contains fewer interactions, suggesting a plasticity of RGS9 in binding of Gα subunits.[98]

It is noteworthy that certain RGS domains may have specific interaction surface for effectors, adding another layer of selectivity.[98,99] There exist unique electrostatic and hydrophobic interactions between the RGS domain of RGS9 and the effector PDEγ, which may contribute to the selectivity and specificity of RGS9 (when it is expressed as the RGS9-1 isoform in the retina) to interact with PDEγ.[98]

The importance of specific sequences within the RGS domains and the Gα subunits in determining the selectivity of RGS proteins is fully demonstrated in site-directed mutagenesis studies where single point mutations either in the RGS domains or in the Gα subunits completely block the specific interaction between respective RGS and Gα proteins, or significantly modulate the affinity and selectivity of RGS proteins to bind Gα subunits.[100,101]

Such selective recognitions between RGS domains and Gα subunits allow the development of mutated Gα subunits that are insensitive to RGS proteins as an efficient means to inhibit the function of endogenous RGS proteins.[102,103]

(2) The N-terminal region of RGS protein—Different RGS subfamilies have distinct and characteristic N-terminal regions (Table 1). Rich research data have revealed that the N-terminal regions of RGS proteins play an essential part in three functional aspects of RGS proteins, (1) plasma membrane targeting and subcellular localizations; (2) direct contact with and specific recognition of GPCRs, ion channels and effector proteins; (3) primary biological activities, such as the GAP activity. Clearly, the N-terminal regions of RGS proteins are one of the most important determinants for their selectivity.

Most members of the A/RZ and B/R4 RGS subfamilies are relatively small, consisting of a core RGS domain with short N- and C-terminal extensions. In the B/R4 subfamily, several RGS proteins have, at or near their N-termini, an amphipathic α-helix of about 30 amino-acid

residues in length and several palmitoylation sites. Studies demonstrate that the N-terminal amphipathic helices can bind vesicles containing acidic phospholipids, and are necessary and sufficient for targeting plasma membrane and subcellular localizations, as well as a translocation of the RGS proteins induced by receptor and Gα activation.[82,104,105] A precise membrane targeting and effective translocation of RGS proteins upon G protein activation are essential for their specific functions. In RGS2, the amphipathic α-helix region is located approximately from residue 28 to residue 57.[82,106] Studies using RGS2 fusion proteins with the green fluorescent protein (GFP) demonstrate that a deletion of N-terminal (1–32) segment alters the membrane targeting of RGS2, and that a further deletion of N-terminal (1–67) greatly reduces the plasma membrane and nuclear distributions, as well as the biological activities of RGS2.[82] In RGS4, the N-terminal first 33 residues form an amphipathic helix, which is responsible for the plasma membrane localization and the inhibition of the pheromone signaling pathway in yeast.[107] Point mutations that change the positively charged residues on the hydrophilic face of the helix to neutral ones, or substitute the hydrophobic residues on the hydrophobic face with polar ones, significantly impair RGS4 membrane targeting and biological activity.[107] Interestingly, the N-terminal amphipathic helices of RGS2 and RGS4 function differentially for membrane targeting.[82] One possible explanation is that RGS2 has two leucine residues in the beginning (positions 37–38) of the helix domain, while RGS4 has serine and alanine at the corresponding positions that make the hydrophobic face of the amphipathic helix less hydrophobic. Another possible reason is that RGS2 has a longer sequence N-terminal to the amphipathic helix (>30 residues in RGS2 versus < 5 in RGS4) that may confer different interaction.[82] Evidence shows that the subcellular localization of RGS proteins and their translocation upon receptor/G protein activation are RGS- and cell type-specific.[82]

Data also indicate that the N-terminal domains of RGS proteins in the B/R4 subfamily are responsible for direct and specific recognition of GPCRs. The short segment N-terminal to the amphipathic helix as well as the loop between the helix and the RGS domain may play a critical role in such selective recognition.[106] Experiments demonstrate that a full RGS2 protein, an RGS2 N-terminal (1–77) fragment, and a chimeric RGS protein with RGS2 (1–77) at the N-terminal and RGS16 at the C-terminal, all can bind the third intracellular loops of M1 muscarinic and α1A adrenergic receptors, whereas RGS16 itself and an N-terminal (1–77)-truncated RGS2 can not bind. In addition, the N-terminal (1–77)-truncation of RGS2 significantly (5–10 fold) impairs the function of RGS2 in the inhibition of cholinergic agonist-induced phosphoinositide hydrolysis.[108] Studies also show that RGS2 specifically inhibits Gq-coupled CCK-2 receptor-induced PLC stimulation and inositol phosphate accumulation, which could be completely abolished by a deletion of the N-terminal region, especially the N-terminal (54–80), of RGS2 protein.[106] Furthermore, studies demonstrate that RGS2, via its N-terminal 19 amino-acid residues, directly interacts with and inhibits type V of adenylyl cyclase, which is independent of its GAP activity and the inhibition by Gi.[109,110]

The N-terminal domain of RGS4 plays a key role in a high-affinity and selective interaction with GPCRs.[111] Full-length RGS4 protein markedly inhibits Ca^{2+} response induced by muscarinic ACh receptor activation but has less effect on CCK receptors. A deletion of N-terminal (1–58) reduces RGS4 potency more than 10^4-fold and impairs its selectivity for muscarinic receptors over CCK receptors. A synthetic RGS4 N-terminal (1–33) peptide alone can partially mimic RGS4 selectivity. Co-application of the N-terminal (1–33) peptide and the RGS4 box can partially restore the receptor selectivity and the potency of RGS4.[96] RGS10 itself is selective for Gαi-coupled receptors and has no effect on Gαq. However, a combination of RGS4 N-terminal (1–53) and RGS10 box displays a marked effect on Gq-mediated signaling.[112] It is suggested that a selective interaction of the RGS4 N-terminus with the receptor may help optimally positioning RGS4 between the Gα and the effector.[111]

Additional data have also showed that the N-terminal cysteine residues in RGS16 are required for the palmitoylation and for the modulation of Gi- and Gq-mediated signaling.[113] The N-terminal (1–68) region of RGS1 is required for its inhibition of pheromone receptor signaling.[104]

Some members in the B/R4 subfamily, such as RGS3, RGS5, and RGS8, do not have apparent amphipathic helix segments at or near the N-termini. But their N-terminal regions still contribute significantly to their membrane targeting and receptor selectivity.[52,114–118]

The N-terminal regions of most A/RZ subfamily RGS proteins do not form amphipathic helix structure, instead, they have a cysteine-rich domain called cysteine string motif or poly-Cys domain. For example, in GAIP/RGS19 there are eight cysteine residues in the N-terminal (39–49) segment and they are palmitoylated.[93] Studies suggest that the cysteine string and its palmitoylation are important for the membrane-anchoring and function of GAIP/RGS19. The sequence from the N-terminus to the cysteine string, i.e., from residue 1 to residue 38, also contributes to GAIP/RGS19 selectivity.

Studies show that GAIP/RGS19 has selectivity to Goα-mediated Ca^{2+} channels, and that selectivity is diminished by the deletion of its N-terminal (1–78) segment.[119] The selectivity of the chicken embryonic GAIP/RGS19 to GABA-induced, Goα-mediated inhibition of Ca^{2+} channels that we have mentioned above is also likely to be determined by its unique N terminus.[62]

Recently we have identified an N-terminal (1–22)-truncated GAIP/RGS19 from mouse. This N-terminal truncation does not affect the cysteine string and other known domains in GAIP/RGS19. However, the N-terminal truncated GAIP/RGS19 is much less effective than a full-length protein in enhancing GTPase activities and reversing the inhibition of cAMP production induced by opioid receptor activation, and does not discriminate among the μ, δ, κ, and ORL1 types of opioid receptors, suggesting that the major determinant of selectivity of GAIP/RGS19 for receptors lies in its N-terminal domain. Therefore, we have proposed a model in which the N-terminal domain of GAIP/RGS19 protein displays a selective interaction with a specific receptor or a specific group of receptors after it is anchored to the cell membrane.[37]

In other RGS subfamilies, there are conserved domains such as DEP and PDZ domains at or near the N-termini, which may play parts in RGS selectivity. Study shows that the N-terminal PDZ and PTB domains of RGS12 form a specific complex with platelet-derived growth factor receptor β (PDGFβ), and that overexpression of the PDZ/PTB domain significantly reduces PDGF-induced activation of p42/p44 MAPK, suggesting that RGS12 via its N-terminal PDZ/PTB domain regulates PDGFβ receptor signaling.[120]

(3) The GGL domain—There are certain conserved and well-defined domains outside the core RGS domain that also play critical roles in RGS selectivity. Members of the C/R7 RGS subfamily contain a G protein γ subunit-like (GGL) domain which has a significant similarity with Gγ subunits (~34–41% identical at the amino-acid level) and can specifically interact with Gβ5, a particular subtype of the Gβ subunit.[121,122] Thus, this subfamily of RGS proteins possesses selectivity towards receptors that are coupled to heterotrimeric G proteins consisting of Gβ5 subunit.

Co-transfection/expression studies of RGS proteins with different Gβ subunits have demonstrated that the C/R7 RGS members specifically bind Gβ5, but do not bind other Gβ subtypes. A deletion of the GGL domain completely abolishes the specific binding. The RGS/Gβ5 heterodimer complex exerts selective GAP activity to GTP-bound Gαo.[121,122] A study using fluorescence resonance energy transfer (FRET) imaging of fluorescent protein-tagged

RGS and Gβ pairs has demonstrated that the GGL domain-containing RGS proteins can directly bind to Gβ5 *in vivo*. Gβ5 interacts with RGS11 and its N-terminal region where the GGL domain resides, but not with RGS11 C-terminal region. Under the same conditions, RGS11 does not interact with Gβ1.[123]

The tissue distributions of C/R7 RGS proteins are parallel to that of Gβ5 in humans and animals. In contrast to the broad distributions of other Gβ subunits, the Gβ5 isoform is expressed in relatively restricted regions in the brain, kidney, and retina, areas associated with the functions of the C/R7 RGS proteins.[122] Co-expression with Gβ5 greatly enhances the functions of the GGL domain-containing RGS proteins.[124] More interestingly, in the Gβ5 gene-knockout mice, the C/R7 RGS proteins become unstable and their expression levels in the striatum and retina are significantly reduced.[125]

Using the crystal structures of Gβ1 and Gγ1 as a starting model, a molecular model for the GGL domain-Gβ5 complex has been deduced. It predicts that the GGL domain binds to the hydrophobic cleft of Gβ5. The amino-acid residues in both proteins at the interface fit well for specific high-affinity binding. In particular, residues unique to Gβ5 such as Val274 and Ala353, which are smaller than those at the corresponding positions in Gβ1–4, are well suited for interaction with residues in the GGL domain. Furthermore, mutagenesis studies suggest that a tryptophan residue (position 309 in RGS6, 306 in RGS7, 270 in RGS9, and 274 in RGS11) within the GGL domain plays a critical role in the selectivity for Gβ5, changing it to phenylalanine destabilizes the GGL-Gβ5 complex.[122]

The GGL-Gβ5 complex can be seen as a special Gβγ heterodimer and may play a functional role in mediating specific set of Gβγ-mediated signaling pathways.[121,122] It adds a significant mode of action and complexity to the G-protein signaling.

(4) The DEP domain—RGS proteins of the C/R7 subfamily also contain a DEP (Disheveled/EGL-10/Pleckstrin) domain which can specifically interact with a selective set of GPCRs and RGS-binding proteins. The DEP domain of RGS9-2 has been demonstrated to be a key requirement in its selective interaction with D2 dopamine receptors. A full-length RGS9-2 or its DEP domain alone is able to specifically target and colocalize with the D2 receptors in plasma membranes and intracellular compartments, but not with M1 muscarinic receptors. In contrast, a recombinant RGS9-2 without the DEP domain, or a mutated (F115S) DEP domain, or other RGS proteins that do not have a DEP domain, fail to target and colocalize with D2 receptors.[28]

The C/R7 RGS subfamily, via the N-terminal DEP domain, can bind specific RGS-binding proteins such as R7BP (R7-binding protein) and R9AP (RGS9-1-anchor protein).[126–131] R7BP and R9AP structurally resemble the syntaxin subfamily of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) involved in vesicular and protein trafficking, and membrane fusion.[126,129] R7BP is specifically expressed in the nervous system and interacts with all four C/R7 RGS members, determining their subcellular targeting and selectivity.[127,128] However, R9AP is only expressed in the retina and selectively binds to RGS9-1, anchoring it to the photo disk membrane and activating its GAP activity for photoreceptors.[129–131]

A recent study demonstrates that the DEP domain in the yeast RGS protein Sst2 specifically binds to the C-terminal tail of Ste2, the yeast pheromone response GPCR, and directs RGS activity to the pheromone signaling pathway.[132] A glutamine residue at position 304 in the DEP domain is critical for Sst2 binding and function. Furthermore, Sst2 DEP domain only binds to unphosphorylated cytosolic region of Ste2, offering a mechanism for RGS-GPCR dissociation upon GPCR phosphorylation and desensitization.[132]

(5) The GoLoco domain—RGS12 and RGS14 of the D/R12 RGS subfamily have a GoLoco domain which can specifically interact with GDP-bound Gαi. It may also act as an anchor to promote the selective binding of RGS domain to certain Gα subunits.[133] Crystal structure of the GoLoco domain of RGS14 in complex with Gαi1-GDP suggests that the C-terminal residues in the GoLoco domain and the all-helical domain in Gα contribute to the selectivity of GoLoco domains for Gαi subunits.[134] Furthermore, the RGS14 GoLoco domain shows differential selectivity for different Gαi isoforms.[135] The GoLoco domains in RGS12 and RGS14 do not show significant specific bindings to Gαo.[134]

Studies using GoLoco-domain peptides from different RGS proteins have successfully identified the selectivity of Gα subunits in coupling of different receptors to ion channels. A peptide derived from RGS12 GoLoco domain sequence is shown to selectively bind Gαi-GDP, but not Gαo-GDP, and block the association of Gαi and Gβγ subunits. This peptide dramatically causes a progressive uncoupling of D2 dopamine receptors and GIRK (Kir3.1/3.2) channels under repeated agonist application, but has no effect on the coupling of somatostatin receptors and GIRK channels, or on that of D2 receptors and Ca^{2+} channels.[136]

It is important to mention that GoLoco domain and RGS proteins also play a critical part in the mechanism of G protein-mediated centrosome/chromosome movement during cell division.[73,74] In this G protein signaling pathway, the GoLoco domain-containing protein functions as a guanine nucleotide dissociation inhibitor (GDI), specifically binding to Gαi-GDP and the nuclear mitotic apparatus protein (NuMA). In the place of GPCR, it is the guanine nucleotide exchange factor RIC-8 (Resistance to Inhibitors of Cholinesterase 8) which utilizes GoLoco/Gαi-GDP complex as specific substrate. The activation of RIC-8 stimulates the exchange of GTP for GDP and the release of Gαi-GTP and NuMA to regulate the microtubule pulling forces on centrosomes during cell division.[73,74] Thus, the GoLoco domain-containing proteins functioning as selective Gα binding partners have a broad implication in both signaling transduction and cell division.

(6) The third intracellular loop and the C-terminal region of G protein-coupled receptor—Recent studies have suggested that the third intracellular loop and the intracellular C-terminal tail of the seven-transmembrane GPCRs are specific sites for interaction with RGS proteins.

Several lines of evidence show that RGS proteins selectively bind the third intracellular loops of specific receptors. RGS2 and RGS4 specifically bind the third intracellular loops of M1 and M5 muscarinic receptors. RGS2 also weakly binds the third intracellular loop of M3 receptor. RGS16 very weakly binds those of M3 and M4. RGS1 does not bind the third intracellular loops of any muscarinic receptors.[108] Similar analyses also demonstrate that RGS2 directly and specifically binds the third intracellular loop of α1A adrenergic receptor, within which the residues Lys219, Ser220 and Arg238 are essential for RGS binding. These interactions are essential for the modulation of G protein-mediated signaling by RGS proteins. RGS2 does not bind the third intracellular loops of α1B or α1D adrenergic receptors. RGS16, a homologue of RGS2, does not bind any third intracellular loops of α1A, α1B, and α1D adrenergic receptors.[137]

The intracellular C-terminal tails of GPCRs may be as important as the third intracellular loops for specific interaction with RGS proteins. Studies demonstrate that RGS4 directly interacts with the C-terminal regions of μ and δ opioid receptors, as well as the third intracellular loop of the δ opioid receptor.[138] RGS4 is also reported to specifically interact with the C-terminal tail of the platelet-activating factor receptor (PAFR) to inhibit phosphoinositide hydrolysis and Ca^{2+} mobilization mediated by PAFR.[139] RGS10 specifically interacts with the C-terminal tail of the gonadotropin-releasing hormone receptor (GnRHR).[140] Data also show that RGS12,

via its N-terminus, selectively binds the C-terminus of the interleukin-8 receptor B.[141] As we have mentioned, the DEP domain of the yeast RGS protein Sst2 specifically binds to the C-terminal tail of the pheromone receptor Ste2.[132] These interactions are implied to be essential for RGS function and selectivity.

Specific adaptor or scaffold proteins

There are specific cellular proteins that function as adaptors or scaffolds to selectively bridge the N-terminal, C-terminal, or other special regions of RGS proteins with GPCRs, G proteins, or effectors. Thus, these adaptor/scaffold proteins can strengthen, modify, or convey additional selectivity to RGS proteins. Examples include 14-3-3 proteins, GIPN (GAIP N-terminus-interacting protein), GIPC (GAIP C-terminus-interacting protein), spinophilin, Homer 2, and α-actinin-2. In some cases, a scaffold protein is required for the selective recognition of GPCR by RGS protein.

The 14-3-3 proteins are a family of cellular proteins that specifically bind their target proteins in a phosphorylation-dependent manner to participate in signal transduction and cell-cycle processes. Studies demonstrate that 14-3-3 proteins selectively bind to specific sites in the RGS domains of RGS3, RGS7 and RGS8, inhibit their GAP activities and therefore enhance GPCR signaling.[142] The 14-3-3 proteins do not bind and inhibit RGS4.[143] Independent studies show that the 14-3-3 binding site on RGS3 is Ser264 which is outside the RGS domain.[144,145]

Mutation of this 14-3-3 binding site renders RGS3 more potent in inhibiting G protein signaling.[144] Studies also demonstrate that the phosphorylation of 14-3-3 binding sites on RGS proteins and thus the binding of 14-3-3 proteins are dynamically regulated by other extracellular signals, such as tumor necrosis factor-α (TNF-α), which provide additional regulation and selectivity for RGS proteins.[143,146]

GIPN is a unique transmembrane protein with an N-terminal leucine-rich region and a zinc-ring finger-like domain. GIPN, via its leucine-rich region, specifically binds the N-terminal cysteine strings of GAIP/RGS19 and other A/RZ RGS members. Overexpression of GIPN also down-regulates Gαi3 expression and promotes the degradation of Gαi3.[147] Therefore, GIPN provides a selective link between GAIP/RGS19 and the Gαi subunits.

GIPC, a protein with a PDZ domain in the middle, specifically binds to the C- terminus of GAIP/RGS19 where a modified PDZ-binding motif is located.[148] This interaction selectively recruits GAIP/RGS19 to modulate dopamine D2, β1-adrenergic, nerve growth factor tyrosine kinase (NGF TrkA), transforming growth factor β (TGF-β), and insulin-like growth factor-1 (IGF-1) receptors, as well as other signaling pathways.[149–153] Thus, GIPC not only strengthens GAIP/RGS19 selectivity, but also selectively links the G protein regulator to certain signaling pathways which themselves are not directly G protein-mediated.

Spinophilin is a ubiquitously expressed protein containing multidomains including the protein phosphatase 1 (PP1)-binding, F-actin-binding, PDZ, and coiled-coil domains.[154] Spinophilin specifically binds RGS1, RGS2, RGS4, RGS16, and GAIP/RGS19. In particular, the study shows that spinophilin specifically binds the N-terminal region of RGS2.[155] Studies also demonstrate that spinophilin selectively binds the third intracellular loops of α2 (including A, B and C subtypes) adrenergic and D2 dopamine receptors, and regulates their expression, functional status, and signaling.[155–160] Therefore, spinophilin functions as a scaffold protein to confer the selective receptor recognition for RGS proteins.

Homers are another group of scaffold proteins that bind GPCRs. It has been shown that Homer 2 (but not Homer 1 and Homer 3) tunes the intensity of GPCR-mediated Ca^{2+} signaling by regulating the GAP activities of RGS and PLCβ.[161] Deletion of Homer 2 significantly

increases the potency of agonist stimulation and reduces the effectiveness of RGS4 to inhibit Ca^{2+} signaling in vivo. Furthermore, Homer 2 specifically binds to PLCβ in tissue extracts and stimulates GAP activities of RGS4 and PLCβ in vitro.[161] These results suggest a novel mechanism by which Homer 2 fulfills the regulatory function by selective interaction with GPCR, RGS and PLCβ.

It is interesting to mention that RGS9-2 selectively interacts with a cytoskeleton protein, α-actinin-2, to regulate the Ca^{2+}-dependent inactivation of NMDA receptors. RGS9-2, α-actinin-2, and NMDA receptors are found to be co-expressed in the striatum, and can be coimmunoprecipitated from tissues and from transfected cells. In NMDA receptor-expressing HEK293 cells, co-expression of RGS9-2 significantly modulates NMDA receptor inactivation mediated by α-actinin-2. These findings reveal a functional interaction between RGS9-2 and α-actinin-2, and suggest a novel role for α-actinin-2 in RGS9-2 selective regulation of NMDA receptors.[162]

R7BP and R9AP are two other examples of specific RGS-binding proteins, which we have discussed in the DEP domain section.

Alternative splicing variants

One mechanism by which RGS proteins acquire selectivity and versatility is through alternative splicing. Many RGS proteins are found to have alternatively spliced variants that display distinct selectivity and functions.

We have already discussed how a single RGS9 gene produces two proteins (RGS9-1 and RGS9-2) that have completely different tissue distributions, receptor selectivity, and functions. Studies also show that the alternative splicing of the RGS8 gene, which produces two isoforms differing only in the N-terminal (1–9) region, determines the function of RGS8 in inhibiting the receptor type-specific Gq-mediated signaling.[117] Remarkably, the human RGS12 gene has at least 12 distinct alternatively spliced transcripts that exhibit different subcellular localizations, binding specificity, and signaling selectivity.[141,163]

Our own data show that human and mouse GAIP/RGS19 have several alternative splicing variants, some of which display altered potency and selectivity toward opioid receptors.[37, 85,86]

Other specific molecules and modifications

The functions of RGS proteins can be affected by ions, phospholipids, and post-translational modifications such as phosphorylation and glycosylation.[90,164] However, their contributions to RGS selectivity have yet to be addressed.

Conclusion

In vitro and *in vivo* studies have provided strong evidence supporting that RGS proteins possess specificity and selectivity in their regulation of G protein-mediated signal transduction. In an analogy to the mechanisms of the selectivity of G proteins themselves, the molecular and cellular mechanisms underlying RGS selectivity are complex and coordinated at multiple levels. Besides the specific domains and sites within RGS proteins acting as molecular determinants, every molecular component that directly or indirectly interacts with RGS proteins in the signaling pathways is probably involved and contributes to RGS specificity and selectivity. We have just begun to comprehend how RGS proteins, whose expressions, functions and targets appear to be redundant, are able to regulate hundreds of GPCR signaling with such great specificity and accuracy. Fully understanding the basis of RGS selectivity will

undoubtedly advance our knowledge of cellular signal transduction and help to develop novel therapeutic means for treating disorders involving G protein-mediated signaling.

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Abbreviations

cAMP

cyclic AMP

GAIP

G alpha interacting protein, also named RGS19

GAP
: GTPase-activating protein

GIRK
: G protein-gated inwardly rectifying K^+ channels

GPCR
: G protein-coupled receptor

GRK
: G protein-coupled receptor kinase

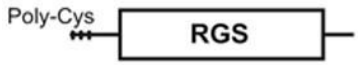
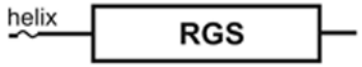
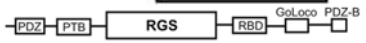
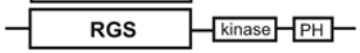
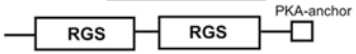
GTPase
: guanosine triphosphatase

PLC
: phospholipase C

RGS
: regulator of G protein signaling

Table 1

Classification of RGS protein subfamilies and their structural features

Subfamily	Members	Representative Protein Structure	Common Features Outside RGS Domain
RZ / A	GAIP/RGS19, RGSZ1/RGS20, RGSZ2/RGS17, Ret-RGS1		N-terminal cysteine string
R4 / B	RGS1, RGS2, RGS3, RGS4, RGS5, RGS8, RGS13, RGS16, RGS18, RGS21		N-terminal amphipathic α-helix, or without any specified domains
R7 / C	RGS6, RGS7, RGS9, RGS11		N-terminal DEP and GGL domains
R12 / D	RGS10, RGS12, RGS14		may contain PDZ, PTB, RBD, GoLoco, and PDZ-binding domains
RA / E	Axin, Conductin		GSK binding, β-catenin binding, PP2A homology, and dimerization domains
GEF / F	P115-RhoGEF, PDZ-RhoGEF, LARG		DH and PH domains
GRK / G	GRK1, GRK2, GRK3, GRK4, GRK5, GRK6, GRK7		GPCR kinase and PH domains
SNX / H	RGS-PX1 (SNX13), SNX14, SNX25		(Gsα-specific RGS domain), PXA and PX domains
D-AKAP2 / I	D-AKAP2		(two RGS domains)

Abbreviations: β-Cat, β-catenin-binding; D, dimerization domain; D-AKAP, dual-specificity A-kinase anchoring protein; DEP, Dishevelled/EGL-10/pleckstrin; DH, double homology; GEF, guanine nucleotide exchange factor; GGL, Gγ-like; GoLoco, Gαi/o-Loco; GRK, G protein-coupled receptor kinase; GSK, glycogen synthase kinase 3β- binding; PDZ, PSD95/Dlg/Z0–1/2; PH, pleckstrin homology; PP2A, protein phosphatase 2A; PTB, phosphotyrosine-binding; PX, phosphatidylinositol-binding; PXA, PX-associated; RBD, Ras-binding domain; SNX, sorting nexin