MINIREVIEW

Thinking Outside of the “RGS Box”: New Approaches to Therapeutic Targeting of Regulators of G Protein Signaling

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Received March 31, 2010; accepted July 22, 2010

ABSTRACT

Regulators of G protein signaling (RGS) proteins are emerging as potentially important drug targets. The mammalian RGS protein family has more than 20 members and they share a common ~120-residue RGS homology domain or “RGS box.” RGS proteins regulate signaling via G protein-coupled receptors by accelerating GTPase activity at active α subunits of G proteins of the G_q and $G_{i/o}$ families. Most studies searching for modulators of RGS protein function have been focused on inhibiting the GTPase accelerating protein activity. However, many RGS proteins contain additional domains that serve other

functions, such as interactions with proteins or subcellular targeting. Here, we discuss a rationale for therapeutic targeting of RGS proteins by regulation of expression or allosteric modulation to permit either increases or decreases in RGS function. Several RGS proteins have reduced expression or function in pathophysiological states, so strategies to increase RGS function would be useful. Because several RGS proteins are rapidly degraded by the N-end rule pathway, finding ways to stabilize them may prove to be an effective way to enhance RGS protein function.

Introduction

G protein-coupled receptors (GPCRs), with more than 1000 members, represent one of the largest groups of proteins. They mediate key physiological responses and their endogenous agonists include small-molecule neurotransmitters, hormones, peptides, and, for some receptors, even their own N termini. The importance of GPCRs in therapeutics is demonstrated by the fact that approximately 30% of drugs approved by the U.S. Food and Drug Administration target these receptors (Williams and Hill, 2009). GPCRs couple to heterotrimeric G proteins consisting of an α and a $\beta\gamma$ subunit. In its inactive state, the α subunit is bound to GDP. Upon agonist binding to the receptor, the G protein gets

activated and the GDP is exchanged for GTP; the α and $\beta\gamma$ subunits dissociate and both can mediate downstream signaling. The signal is turned off when the GTP is hydrolyzed back to GDP. G proteins have built-in GTPase activity; however, this process is very slow and does not correspond to the rapid turnoff of signaling events in cells. Regulators of G protein signaling (RGS) proteins have emerged in the last 15 years as important intracellular regulators of GPCR signaling (for review, see Ross and Wilkie, 2000; Zhong and Neubig, 2001; Hollinger and Hepler, 2002). They reduce the amplitude and duration of G protein signaling by binding to activated $G\alpha$ subunits and dramatically increasing their GTPase activity. This GTPase-accelerating protein (GAP) activity of RGS proteins is largely limited to α subunits of the G_q and $G_{i/o}$ families of G proteins. There are no widely accepted reports of RGS proteins acting on G_s , although it has been suggested that RGS proteins can indirectly regulate $G\alpha_s$ signaling via interactions with certain adenylate cyclase subtypes (Roy et al., 2006; Talbot et al., 2010).

The structure and function of RGS proteins (Hollinger and

This work was supported by the National Institutes of Health National Institute of General Medical Sciences [Grant R01-GM39561] and The Swedish Research council [Grant VR-524-2008-6963].

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.
doi:10.1124/mol.110.065219.

ABBREVIATIONS: GPCR, G protein-coupled receptor; RGS, regulators of G protein signaling; GAP, GTPase-accelerating protein; RH, RGS homology; AC, adenylate cyclase; DEP, disheveled-EGL10-Pleckstrin homology; R7BP, R7 binding protein; GGL, G γ -like; CCG-4986, [methyl-N-[(4-chlorophenyl)sulfonyl]-4-nitrobenzenesulfonimidoate]; NPI-0052, Salinosporamide A; HEK, human embryonic kidney; MG-132, N-benzoyloxycarbonyl (Z)-Leu-Leu-leucinal.

Hepler, 2002) and implications for them as drug targets (see, e.g., Ross and Wilkie, 2000; Zhong and Neubig, 2001; Neubig and Siderovski, 2002; Traynor and Neubig, 2005) have been extensively reviewed previously. The main focus of efforts to identify modulators of RGS protein function has thus far been targeted toward inhibiting the GAP activity and/or the interaction with $G\alpha$ subunits. However, many RGS proteins display other functions, including protein-protein interactions and regulation of cellular trafficking. This indicates that inhibiting RGS protein function might not be as simple as inhibiting GAP activity toward $G\alpha$ subunits. Recent data also suggest that RGS protein expression may be altered in certain pathophysiological states, and several RGS proteins have been shown to be rapidly degraded in cells. This review explores the possibility of pharmacologically modulating RGS protein expression as an alternative route to alter RGS protein function. Furthermore, we discuss possible ways to allosterically modulate RGS protein function through other mechanisms than competitively inhibiting GAP activity.

RGS Proteins—More Than Just GAPs on $G\alpha$

There are 20 known mammalian RGS proteins that have strong GAP activity at $G\alpha$ subunits, and they are divided into several subfamilies based on sequence homology (R4, R7, R12, and RZ). Their structure and function have been extensively reviewed elsewhere (Ross and Wilkie, 2000; Hollinger and Hepler, 2002), so those aspects will not be discussed in detail here. In addition to these four classic RGS families, there are a number of other proteins with RGS homology (RH) domains (Tesmer, 2010), which is not covered in this review. Many RGS proteins have additional domains apart from the RGS domain (Ross and Wilkie, 2000; Neubig and Siderovski, 2002; Hollinger and Hepler, 2002). This suggests that RGS proteins can have functions other than just accelerating GTP hydrolysis. Coincidentally, a recent review provided a detailed overview of non-GAP functions of RGS proteins, including regulation of receptor tyrosine kinase signaling and nuclear functions (Sethakorn et al., 2010). Given these additional functions, targeting these proteins in drug discovery may not be as simple as simply inhibiting GAP activity.

Except for RGS3, the R4 family members (RGS 1–5, 8, 13, 16, 18, and 21) are small structures with only minimal C- and N-terminal extensions flanking the RGS domain. RGS3 exists as three splice variants; the two longer forms, RGS3L and PDZ-RGS3, contain domains involved in protein-protein interactions (Kehrl et al., 2002). Despite the apparent lack of additional structured domains, several R4 family members have been shown to possess functions beyond GAP activity (for review, see Bansal et al., 2007). The N terminus of R4 family RGS proteins contains an amphipathic α -helix that serves as a membrane targeting signal. This targeting is necessary for proper function of the proteins in that it brings them close to the site of action (i.e., activated $G\alpha$ subunits) (Bernstein et al., 2000; Saitoh et al., 2001). Furthermore, several members of the R4 family have been shown to interact with components of GPCR signaling, such as receptors, adenylate cyclase (AC) subtypes and phospholipase $C\beta$ (for review, see Bansal et al., 2007). This, in addition to different affinities for $G\alpha$ protein subtypes, contributes to selectivity among R4 family RGS proteins. Most members are relatively promiscuous for $G_{i/o}$ and G_q proteins, but RGS2 has been

shown to be selective for G_q (Heximer et al., 1997). Although there are no widely accepted reports on RGS proteins with GAP activity toward $G\alpha_s$ proteins, there have been reports of RGS2 regulating $G\alpha_s$ -mediated cAMP signaling through a direct protein-protein interaction with certain AC subtypes (ACII, IV, and VI) (Roy et al., 2006).

The members of the R7 family of RGS proteins (RGS 6, 7, 9, and 11) contain a disheveled-EGL10-Pleckstrin homology (DEP) domain that is responsible for protein-protein interactions. Specifically, this domain serves as an interaction site with R7 binding protein (R7BP) in the brain and RGS9 anchoring protein in the retina to anchor the RGS protein to the plasma membrane near its site of action (Drenan et al., 2006; Jayaraman et al., 2009). The DEP domain can also interact with receptors to directly modulate GPCR signaling (Ballon et al., 2006). The R7 family members also contain a G γ -like (GGL) domain that binds $G\beta_5$, an interaction that is necessary for stable expression of both proteins. $G\beta_5$ knockout mice also lack expression of all four R7 family RGS proteins (Chen et al., 2003). Specific targeting of the DEP or GGL domain of an R7 family RGS could serve as an alternative route to target function of these proteins. Inhibition of the RGS- $G\beta_5$ interaction would down-regulate expression of that particular RGS protein. Likewise, inhibition of the interaction between the DEP domain of RGS9 and R7BP could downregulate RGS9 expression, because a couple reports have shown that RGS9 depends on this interaction for stable expression (Anderson et al., 2007a,b).

The R12 family members RGS 12 and 14 also contain additional functional domains. The C-terminal $G\alpha_{i/o}$ -Loco motif has guanine nucleotide dissociation inhibitor activity toward $G\alpha_{i1}$, $G\alpha_{i2}$ and $G\alpha_{i3}$ (Kimple et al., 2001; Siderovski and Willard, 2005). Binding of the $G\alpha_{i/o}$ -Loco motif to $G\alpha$ inhibits the activation of the G protein by preventing exchange of GDP for GTP. It also blocks association of $G\alpha$ with $G\beta\gamma$, potentially leading to prolonged $\beta\gamma$ signaling.

The members of the RZ family of RGS proteins, which are less well characterized than the other families, all have an N-terminal cysteine string motif (for review, see Nunn et al., 2006) that is a target for palmitoylation. The cysteine string can serve as a site for protein-protein interactions, and the palmitoylation could be a signal for subcellular trafficking or protein stability (for review, see Linder and Deschenes, 2007).

RGS Proteins Are Important Targets for Drug Discovery

Since their discovery approximately 15 years ago, important biological roles for RGS proteins have been identified. Given their role in regulating GPCR signaling, it is not surprising that they have been suggested to be potentially interesting targets for drug discovery. The literature on the physiological functions of RGS proteins has expanded greatly in recent years, so only selected aspects of RGS protein function are discussed here (Hepler, 1999; Ross and Wilkie, 2000; Zhong and Neubig, 2001; Hollinger and Hepler, 2002; Traynor and Neubig, 2005; Blazer and Neubig, 2009; Sjögren et al., 2010). The numerous RGS knockout mouse models have been reviewed recently (Kaur et al., 2010).

Substantial data demonstrate roles for endogenous RGS proteins in cardiovascular functions, such as regulation of blood pressure and cardiac rhythmicity. RGS2 is expressed in vascular smooth muscle cells and heart, and RGS2 knockout

mice are hypertensive, suggesting a role in blood pressure homeostasis (Heximer et al., 2003). In contrast, RGS5 knockout mice were shown in one study to be hypotensive (Cho et al., 2008), possibly suggesting a tightly regulated balance between different RGS proteins in the control of physiological responses. RGS proteins have also been demonstrated, by us and others, to regulate cardiac rhythmicity through actions in the sinoatrial and atrioventricular nodes in the heart (Fu et al., 2006, 2007; Bender et al., 2008; Cifelli et al., 2008).

There is substantial central nervous system expression of many RGS protein subtypes (Gold et al., 1997). Several, including RGS4 and RGS9, control effects of opiates and other drugs of abuse (Gold et al., 2003; Rahman et al., 2003; Zachariou et al., 2003; Traynor and Neubig, 2005). RGS4 associates with μ - and/or δ -opioid receptors in vitro and inhibits signaling via these receptors (Georgoussi et al., 2006; Wang et al., 2009b). Although RGS4 knockout mice initially showed no difference in opiate dependence (Grillet et al., 2005), targeted RGS4 knockdown in the locus ceruleus increased morphine reward, whereas in the nucleus accumbens, RGS4 acted as a negative regulator of opioid dependence (Han et al., 2010). RGS9 knockout mice have markedly enhanced opioid-induced analgesia and are more susceptible than wild-type mice to the development of morphine dependence, implicating RGS9 as an important regulator of opioid functions in the central nervous system (Zachariou et al., 2003) along with RGS4.

RGS9 is highly expressed in the striatum, and RGS9 knockout mice have impairments in motor coordination (Blundell et al., 2008). They develop more severe L-DOPA-induced dyskinesias compared with wild-type mice in models of Parkinson's disease (Gold et al., 2007). In addition, overexpression of RGS9 in the striatum in primate models of Parkinson's disease attenuates L-DOPA-induced dyskinesias (Gold et al., 2007).

Evidence is emerging of the role of RGS proteins in cancer biology. GPCR signaling in cancer progression is an area of increasing interest; several RGS proteins have been implicated in regulating GPCR-mediated signals in various cancers (for review, see Hurst and Hooks, 2009). Altered expression levels of several RGS proteins have been detected in various cancer forms, including prostate cancer (RGS2), breast cancer (RGS4), and hepatocellular carcinoma (RGS5). Reduced expression of RGS2 in androgen-independent prostate cancer leads to increased GPCR signaling linked to uninhibited cell growth (Cao et al., 2006). Overexpression of RGS2 in prostate cancer cell lines leads to decreased signaling that is also seen in part with a GAP-deficient mutant, suggesting a mechanism involving non-RGS domains of the protein. A recent study showed that RGS4 is down-regulated in breast cancer and overexpression of RGS4 in metastatic breast cancer cells reduced both invasion and migration (Xie et al., 2009 and see below).

Given these and many other biological functions attributed to RGS proteins, it is clear that they could serve as important drug targets. Several groups, including our own, have undertaken strategies for high-throughput screening for compounds that modulate RGS protein function. Young and colleagues used a yeast two-hybrid approach and identified a series of compounds (Young et al., 2004) and peptides (Wang et al., 2008) that inhibit RGS4 GAP activity by blocking the interaction with $G\alpha_{12}$. Our lab has developed biochemical

methods to screen for inhibitors of the RGS- $G\alpha$ interaction. The Flow Cytometry Protein Interaction assay is a bead-based assay that has been adapted to high-throughput screening and can be multiplexed to study the effects of compounds on several RGS proteins in the same well (Roman et al., 2009). Using this method, CCG-4986 was identified as an inhibitor of the $G\alpha$ -RGS4 interaction, with selectivity over other RGS proteins (Roman et al., 2007).

The common denominator of previous studies has been the goal of finding inhibitors of the $G\alpha$ -RGS interaction. Such compounds would be very useful pharmacological tools to increase understanding of the biological functions of RGS proteins. However, from a clinical perspective, enhancers rather than inhibitors may be more useful in drug development. This is supported by emerging data showing reductions in RGS protein expression or function in several pathophysiological states. Modulators of RGS protein expression could be an effective way to increase (or decrease) the function of these proteins. As discussed above, many RGS proteins have functions besides their GAP activity. Increased expression would also modulate those functions, whereas a RGS/ $G\alpha$ inhibitor would not. One alternative approach to increasing RGS activity that we will discuss first is allosteric modulation of RGS protein function.

Allosteric Modulation of RGS Protein Function

The presence of additional domains in many RGS proteins provides mechanisms to allosterically modulate function, both within and from outside of the RGS domain. R7 family RGS proteins form an obligatory dimer with $G\beta_5$ and this interaction is necessary for stable expression. $G\beta_5$ knockout mice lack expression of all four members of the R7 family of RGS proteins (Chen et al., 2003). Their interaction with the broadly expressed membrane anchor R7BP seems to be more complicated. In most cases, this serves to target the RGS proteins to the plasma membrane, but in the case of the brain-specific isoform of RGS9, it also stabilizes RGS9 protein expression. RGS9, but not RGS7, is rapidly degraded by cysteine proteases in the absence of R7BP (Anderson et al., 2007a,b).

Slepak and colleagues (Narayanan et al., 2007; Sandiford and Slepak, 2009) recently reported a novel receptor-specific role of the DEP domain of RGS7 that might be modulated chemically to affect RGS7 function. The RGS7 DEP domain can bind to the third intracellular loop of the M3 (but not the M1) muscarinic receptor and inhibit its coupling to G_q by a mechanism that does not involve the RGS GAP function (Narayanan et al., 2007; Sandiford and Slepak, 2009). In addition, RGS7 interacts with $G\beta_5$ via the GGL domain, as do all members of the R7 family. One study found a novel interaction between the RGS7 DEP domain and recombinant $G\beta_5$ (Narayanan et al., 2007) that was suggested to subserve an intramolecular inhibition role in the complex. In the intact RGS7/ $G\beta_5$ complex, a closed form of the DEP/ $G\beta_5$ contact was less active at inhibiting $G\alpha_q$ signaling via the muscarinic M3 receptor (Narayanan et al., 2007). Mutations in the RGS7 DEP domain disrupted the $G\beta_5$ interaction, suggesting that chemical modulation of that process might lead to increased activity of RGS7 in inhibiting G_q signaling. Perhaps this could also enhance the DEP-dependent receptor recruitment of R7 family RGS proteins to modulate their classic GAP function as well. Finally, recent crystallographic

data suggest that this DEP/G β_5 contact may also occur for RGS9 (Cheever et al., 2008).

Similar to inhibiting protein-protein interactions involving R7 family RGS proteins and their binding partners, a way to modulate RGS2 function is to inhibit interaction with AC, specifically targeting RGS2 effects on G $_s$ -mediated signaling. Other protein-protein interactions have also been identified that may have potential in drug development. As is discussed in the recent review by Sethakorn et al. (2010), RGS3 can interact with the Mad homology domain of Smad3, thereby regulating transforming growth factor- β signaling (Yau et al., 2008). This interaction was mapped to a region outside the RGS domain and may be another potentially interesting interaction to target. The full significance of the RGS3-Smad3 interaction is not yet known, but given the role for transforming growth factor- β in regulating cell growth and survival, this may prove to have clinical importance.

Phosphorylation of proteins can serve many roles, such as activation or inactivation of an enzyme. RGS16 is phosphorylated at Tyr¹⁶⁸ in the RGS domain by p60 Src kinase in vitro (Derrien et al., 2003). This phosphorylation increases GAP activity and stabilizes RGS16 protein expression in stably transfected cells. This could be an important regulatory mechanism for RGS16 activity by dual actions on GAP activity and protein expression and could serve as an alternative route in drug discovery toward RGS16 protein modulators.

Targeting the RGS-G α interaction has for several years been attempted by us and others. The R4 family of RGS proteins has been extensively studied, and it is the only family of RGS proteins for which small-molecule and peptide inhibitors have been reported (Jin et al., 2004; Roof et al., 2006; Roman et al., 2007, 2009; Wang et al., 2008). Despite the small and relatively simple structure of R4 family RGS proteins, allosteric modulation of GAP activity may be possible. The structure of the RGS domain of RGS4 bound to G α_{i1} (Tesmer et al., 1997) revealed two potential drug binding pockets on the surface of RGS4, and a recent review article described the structure of RGS domains in detail (Tesmer, 2009). The site of contact between RGS4 and the G α_{i1} subunit in the cocrystal, also known as the A site (Zhong and Neubig, 2001), is a flat surface that may be difficult to target with small molecules. The second potential drug binding pocket, on the “back” of the molecule, has been termed the B site (Zhong and Neubig, 2001). This site is more similar in size and shape to “druggable” pockets on other proteins and may serve as a possible allosteric site to modulate GAP activity. Indeed, we have shown that the potent RGS4 inhibitor CCG-4986 [methyl-*N*-[(4-chlorophenyl)sulfonyl]-4-nitrobenzenesulfonimidoate] binds to two cysteines in the RGS domain of RGS4 (Roman et al., 2010), one of which is located near the proposed B site of the molecule. A second, reversible RGS4 inhibitor also acts on those residues (Blazer et al., 2010).

This site also seems to play a role in physiological regulation of R4 family function. Wilkie and coworkers (Popov et al., 2000) showed that several members of the R4 family of RGS proteins interact with phosphatidyl-3,4,5-trisphosphate, which inhibits GAP activity. It is noteworthy that this can be reversed by Ca²⁺/calmodulin binding to a similar site on the RGS domain (Popov et al., 2000; Ishii et al., 2001, 2002, 2005). Given the role of R4 family RGS proteins in regulation of G α_q -mediated calcium signaling, this probably represents an endogenous negative-feedback mechanism whereby Ca²⁺ increases RGS activity to suppress Ca²⁺ re-

sponses. It is intriguing to consider that this natural process could be co-opted pharmacologically to inhibit or enhance R4 family RGS protein function.

RGS Proteins Can Be Rapidly Degraded

Several RGS proteins have a short in vivo half-life because of rapid degradation in cells. Furthermore, the expression of several RGS proteins is reduced in various disease states. Ubiquitin-mediated proteasomal degradation is an important regulatory system for many cellular processes, including cell-cycle control, stress responses, and breakdown of misfolded proteins. These pathways have been well described elsewhere (for example, see Hershko and Ciechanover, 1998). In brief, a protein targeted for degradation is coupled to a chain of ubiquitin molecules by a series of enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligases). The ubiquitylated protein is then recognized by the 26S proteasome and degraded. Drugs targeting protein degradation are already in use for the treatment of various cancers. The first FDA approved drug was the proteasome inhibitor bortezomib (Velcade) (for review, see Adams, 2004). Subsequently, similar drugs, such as carfilzomib and NPI-0052 (Salinoporamide A) have entered clinical trials for the treatment of various cancers (for review, see Yang et al., 2009). However, targeting general protein degradation will give rise to a number of side effects. A more focused approach could be taken by inhibiting specific enzymes in the degradation pathway. For instance, there are more than 500 known mammalian E3 ligases, and targeting one of these enzymes might be a better strategy to improve selectivity. More knowledge of specific degradation pathways of individual RGS proteins in specific tissues or under pathophysiologic situations is required for this approach to be successful.

The *N*-end rule pathway, first proposed by Alexander Varshavsky, is a collection of molecular components that tag proteins bearing an *N*-degron for proteasomal degradation. This could be considered a “front end” to the ubiquitin/proteasomal degradation pathway. *N*-degrons are created by proteolytic cleavage of the initial methionine, exposing residue 2 (from a gene-numbering perspective) at the *N* terminus of a protein. Depending on the nature of this residue, it can be stabilizing or destabilizing (Varshavsky, 1996; Meinel et al., 2006; Tasaki and Kwon, 2007; Fig. 1). *N*-degrons are, by definition, destabilizing and can be divided into primary, secondary, and tertiary destabilizing residues (Fig. 1). Destabilizing *N*-terminal residues are recognized by the *N*-end rule pathway components, ubiquitylated, and targeted to the proteasome. Primary *N*-degrons are direct targets for degradation, being recognized by E3 ligases. In mammalian cells, these are divided into type I (Arg, Lys, and His) and type II (Leu, Phe, Trp, Tyr, and Ile), denoting specificity for different classes of E3 ligases. Secondary destabilizing residues (Asp, Cys, and Glu) first need to be conjugated to Arg by R-transferase (gene name *ATE-1*) to create primary *N*-degrons. The third secondary destabilizing residue Cys requires oxidation before Arg conjugation. The two tertiary destabilizing residues Asn and Glu are deamidated into the secondary destabilizing residues Asp and Gln, respectively. In mammalian cells, this enzymatic process is carried out by *N*-terminal amidases specific for either Asn or Glu. The mammalian Asn-specific *N*-terminal amidase (Ntan1) shows high homol-

ogy to the *Saccharomyces cerevisiae* nonspecific Nta1p (Grigoryev et al., 1996), but the Glu-specific Nt-amidase (Ntaq1) has only recently been cloned and characterized (Wang et al., 2009a).

The importance of the *N*-end rule machinery is demonstrated by the fact that *ATE-1* knockout mice are embryonically lethal (Kwon et al., 2002). Moreover, several types of cancer, including breast cancer, are linked to mutations or altered expression levels of E3 ligases or other components of the ubiquitin/proteasomal pathway (reviewed in Chen et al., 2006 and Hoeller et al., 2006).

Several RGS proteins contain a potentially destabilizing *N*-terminal residue (Fig. 1), and numerous reports support *N*-end rule-mediated degradation of RGS proteins (Davydov and Varshavsky, 2000; Lee et al., 2005; Bodenstein et al., 2007). Furthermore, RGS protein expression is tightly regulated and is altered in pathophysiological states (Zmijewski et al., 2001; Derrien et al., 2003; Song and Jope, 2006; Zou et al., 2006; Anderson et al., 2007a,b; Xie et al., 2009).

The best-characterized example of *N*-end rule pathway-mediated degradation of RGS proteins is RGS4, which has an *N*-terminal cysteine (a secondary *N*-degron). The earliest report came from Davydov and Varshavsky (2000), who found RGS4 to be degraded through the *N*-end rule pathway both in vitro and in vivo. Mutants of RGS4 (C2G, C2V, and C2A) were stably expressed and not up-regulated after treatment with a proteasome inhibitor. They also found RGS16 to be regulated by the *N*-end rule pathway, and subsequent studies have identified RGS5 as another substrate (Lee et al., 2005; Bodenstein et al., 2007). These three proteins all have an *N*-terminal cysteine followed by a basic residue (lysine or arginine) that, in some systems, is a second target signal for protein degradation. In fact, the RGS4 K3S and RGS8 A2C mutants are stable despite the presence of the *N*-terminal cysteine (Davydov and Varshavsky, 2000; Bodenstein et al., 2007).

Members of our laboratory found differences in the ability of different RGS proteins of the R4 family to inhibit calcium responses elicited by G_q -coupled muscarinic receptor activation in HEK-293 cells (Bodenstein et al., 2007). This differ-

ence was due largely to low protein expression of RGS4 and RGS5 compared with RGS2 and RGS8. The proteasome inhibitor *N*-benzoyloxycarbonyl (Z)-Leu-Leu-leucinal (MG-132) increased protein expression of all RGS proteins studied (RGS 2, 4, 5, and 8), RGS4 showing the greatest increase ($>15\times$), suggesting that these proteins are degraded by the proteasome in HEK-293 cells. As in previous studies, a stabilizing mutation in RGS4 (C2S) increased basal protein expression more than 50-fold and prevented any further effect of MG-132 treatment, consistent with a role for the *N*-end rule pathway, where cysteine is a destabilizing residue and serine is a stabilizing residue.

Two potentially destabilizing mutations of RGS2, Q2L and Q2R, were found in a group of Japanese patients with hypertension (Yang et al., 2005), which is intriguing in light of the role for RGS2 expression in the regulation of blood pressure (Heximer et al., 2003). The mutations are relatively rare (5 of 1724 hypertensive subjects, or 0.3%). We showed in HEK-293 cells that one mutant (RGS2-Q2L) exhibited severely reduced protein expression and markedly increased effects of MG-132, whereas the other, Q2R, also showed reduced expression but to a lesser extent (Bodenstein et al., 2007). It is noteworthy that that same study (Yang et al., 2005) found another mutation (R44H, 6 of 1724, also 0.3%) that has been shown to reduce membrane localization and function (Gu et al., 2008). Consequently, strategies to pharmacologically enhance RGS2 expression could be quite useful in hypertension and other cardiovascular diseases.

A better understanding of the molecular mechanisms involved in the degradation of specific RGS proteins in particular tissues could provide novel pharmacological targets to enhance their activity. As shown in Fig. 1, there are numerous steps at which this could be accomplished (Ntan1, Ntaq1, ATE1, and various E3 ligases).

Stabilizing RGS Protein Expression—Implications in Pathophysiology

Compelling evidence has come from several recent studies that identified changes in RGS protein expression as a possible pathophysiological mechanism in disease states. Xie et al. (2009) identified RGS4 degradation as a possible contrib-

Fig. 1. Role of the *N*-end rule pathway in RGS protein degradation. The *N*-end rule pathway of protein degradation is based on destabilizing residues in the *N* terminus of a protein. These are represented with standard one-letter amino acid codes. The primary destabilizing residues (green) are ubiquitinated by a series of enzymes initially identified in yeast, *N*-recognin (Ubr1p, E3), Ub-conjugating enzyme (Ubc2p, E2), and Ub-activating enzyme (Uba1p, E1), and then are targeted for proteasomal degradation. The secondary residues [yellow; Asp (D) and Glu (E)] must first be arginylated by R-transferase (mammalian *ATE-1*) to create primary *N*-degrons. The third secondary destabilizing residue, Cys (C), is oxidized before arginylation. Finally, the tertiary destabilizing residues (red) Asn (N) and Gln (Q) are converted to secondary residues by the mammalian *N*-terminal amidases specific for asparagine (Ntan1) and glutamine (Ntaq1), respectively. Several RGS proteins are predicted substrates for this pathway based on their *N*-terminal residues (indicated in the figure). RGS4, RGS5, and RGS16 (purple) are so far the only RGS proteins confirmed to be degraded by this pathway (Davydov and Varshavsky, 2000; Lee et al., 2005; Bodenstein et al., 2007). Also shown are native RGS proteins predicted to be stable and mutant RGS proteins that are either stabilized or have been shown to be degraded through the proteasome (Bodenstein et al., 2007).

utor to breast cancer invasion. They found that transient expression of wild-type but not a GAP-deficient mutant of RGS4 (N128A) effectively inhibited migration of metastatic breast cancer cells (MDA-MB-231) induced by NIH-3T3 fibroblast conditioned medium. Expression of RGS4 in a xenograft tumor model in nude mice significantly reduced muscle cell invasion with no effect on tumor incidence (Xie et al., 2009). The effect of RGS4 expression was assigned to its regulation of $G\alpha_i$ -mediated signaling via the protease-activated receptor 1, which has previously been identified as an important signaling pathway in breast cancer metastasis (Boire et al., 2005). Furthermore, RGS4 protein levels are significantly reduced in human breast carcinomas, as demonstrated by immunohistochemical analysis of tissue samples (Xie et al., 2009). It is noteworthy that RGS4 mRNA expression is greatly up-regulated (>2000 times) in invasive breast cancer cell lines compared with normal epithelial cells, suggesting that the reduced protein levels of RGS4 are due to markedly increased protein degradation. By inhibiting protein degradation with MG-132, a proteasome inhibitor, RGS4 protein levels were restored and cell migration was inhibited (Xie et al., 2009). Together, these data indicate that RGS4 protein expression is an important factor in breast cancer metastasis.

RGS2 could also be an interesting target with respect to protein stabilization. RGS2 knockout mice are hypertensive (Heximer et al., 2003), and human mutations in RGS2 have been identified that are connected to hypertension. The RGS2-Q2L mutation identified in a Japanese population of patients with hypertension (Yang et al., 2005) has reduced stability compared with wild-type in transfected cells (Bodenstein et al., 2007). RGS2 is also a negative regulator of cardiac hypertrophy. α_1 Adrenergic receptor stimulation in primary ventricular myocytes leads to increases in RGS2 mRNA levels, whereas the expression of other RGS proteins (RGS 3, 4, and 5) is unaffected. Moreover, overexpression of RGS2 in these cells blocks (Zou et al., 2006), and RNA interference-mediated knockdown of RGS2 exacerbates (Zhang et al., 2006) α_1 adrenergic-induced hypertrophy. These effects have been assumed to be due to regulation of $G\alpha_q$ signaling. However, recent data suggest that additional mechanisms may be involved.

Chidiac and colleagues (Nguyen et al., 2009) recently demonstrated that RGS2 can regulate protein translation. RGS2 suppressed protein translation in vitro by binding to and inhibiting eukaryotic initiation factor 2B ϵ , the rate-controlling enzyme in protein translation. Furthermore, they found increased levels of protein translation in RGS2 knockout mice (Nguyen et al., 2009). RGS2 expression, at both the mRNA and protein levels, is known to be up-regulated by several forms of stress, such as oxidative stress, DNA damage, and mechanical stress, situations that are also exhibit reductions in de novo protein synthesis (Zmijewski et al., 2001; Song and Jope, 2006). It is possible that this up-regulation of RGS2 could be a way to restore cellular integrity by suppressing protein translation. Furthermore, this could be an alternative mechanism for RGS2 to suppress induction of cardiac hypertrophy, which is also characterized by an increase in protein synthesis.

As discussed above, several RGS proteins are involved in regulating the effects of drugs of abuse. An early study identified altered RGS protein expression in response to mor-

phine (Gold et al., 2003). RGS2 and 4 mRNA levels in the locus ceruleus increased 2- to 3-fold during opiate withdrawal, with a peak at 6 h. The levels returned to normal after 24 h. As with RGS4 in breast cancer regulation, there were discrepancies between mRNA and protein levels of RGS2 and 4. Protein levels showed a 2-fold increase but had returned to normal levels 6 h after opiate withdrawal (Gold et al., 2003). With the potential for both of these proteins to be rapidly degraded via the N-end rule pathway, these findings might not be too surprising. The authors of this study argued that increased levels of RGS proteins might be an additional cause of opiate dependence, because they can reduce signaling via opiate receptors. The increased RGS protein expression upon withdrawal could therefore explain in part the withdrawal symptoms. The exact molecular mechanism of the up-regulation of RGS proteins remains unknown.

Conclusions and Future Directions

Since their discovery 15 years ago, RGS proteins have emerged as important regulators of GPCR signaling and novel drug targets in a number of pathophysiological states. As more knowledge has been obtained about RGS protein function, it has become clear that there is more to the story than just accelerating GTPase activity on activated $G\alpha$ proteins. This review summarizes studies on alterations in RGS protein expression in several diseases and presents an alternative route in drug discovery through regulation of expression and/or allosteric mechanisms. Compounds that specifically stabilize protein expression of certain RGS proteins could potentially be useful in a clinical setting to increase the overall effects of the RGS protein. This would not be limited to the GAP activity but would be available to all aspects of RGS protein function. Furthermore, given that one RGS protein could be differentially regulated in different cell types, tissues, or pathological states, these approaches might serve to increase specificity. In the case of RGS4, which is expressed in multiple tissues and brain regions, targeting a function specific to one cell type might decrease adverse effects in other tissues. Indeed RGS4 seems to play different roles in different tissues, as described above, with various functions in the brain, cardiovascular system and in the regulation of cancer progression. Much more work is needed to elucidate regulatory mechanisms in specific cellular environments.

References

- Adams J (2004) The development of proteasome inhibitors as anticancer drugs. *Cancer Cell* 5:417–421.
- Anderson GR, Lujan R, Semenov A, Pravetoni M, Posokhova EN, Song JH, Uversky V, Chen CK, Wickman K, and Martemyanov KA (2007a) Expression and localization of RGS9–2/G 5/R7BP complex in vivo is set by dynamic control of its constitutive degradation by cellular cysteine proteases. *J Neurosci* 27:14117–14127.
- Anderson GR, Semenov A, Song JH, and Martemyanov KA (2007b) The membrane anchor R7BP controls the proteolytic stability of the striatal specific RGS protein, RGS9–2. *J Biol Chem* 282:4772–4781.
- Ballon DR, Flanary PL, Gladue DP, Konopka JB, Dohlman HG, and Thorner J (2006) DEP-domain-mediated regulation of GPCR signaling responses. *Cell* 126:1079–1093.
- Bansal G, Druey KM, and Xie Z (2007) R4 RGS proteins: regulation of G-protein signaling and beyond. *Pharmacol Ther* 116:473–495.
- Bender K, Nasrollahzadeh P, Timpert M, Liu B, Pott L, and Kienitz MC (2008) A role for RGS10 in beta-adrenergic modulation of G-protein-activated K^+ (GIRK) channel current in rat atrial myocytes. *J Physiol* 586:2049–2060.
- Bernstein LS, Grillo AA, Loranger SS, and Linder ME (2000) RGS4 binds to membranes through an amphipathic alpha-helix. *J Biol Chem* 275:18520–18526.
- Blazer LL and Neubig RR (2009) Small molecule protein-protein interaction inhibitors as CNS therapeutic agents: current progress and future hurdles. *Neuropsychopharmacology* 34:126–141.
- Blazer LL, Roman DL, Chung A, Larsen M, Greedy B, Husbands S and Neubig RR (2010) Reversible, allosteric small-molecule inhibitors of regulator of G protein signaling proteins. *Mol Pharmacol* 78:524–533.

- Blundell J, Hoang CV, Potts B, Gold SJ, and Powell CM (2008) Motor coordination deficits in mice lacking RGS9. *Brain Res* **1190**:78–85.
- Bodenstein J, Sunahara RK, and Neubig RR (2007) N-terminal residues control proteasomal degradation of RGS2, RGS4, and RGS5 in human embryonic kidney 293 cells. *Mol Pharmacol* **71**:1040–1050.
- Boire A, Covic L, Agarwal A, Jacques S, Sherif S, and Kuliopulos A (2005) PAR1 is a matrix metalloproteinase-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. *Cell* **120**:303–313.
- Cao X, Qin J, Xie Y, Khan O, Dowd F, Scofield M, Lin MF, and Tu Y (2006) Regulator of G-protein signaling 2 (RGS2) inhibits androgen-independent activation of androgen receptor in prostate cancer cells. *Oncogene* **25**:3719–3734.
- Cheever ML, Snyder JT, Gershburg S, Siderovski DP, Harden TK, and Sondek J (2008) Crystal structure of the multifunctional Gbeta5-RGS9 complex. *Nat Struct Mol Biol* **15**:155–162.
- Chen C, Seth AK, and Aplin AE (2006) Genetic and expression aberrations of E3 ubiquitin ligases in human breast cancer. *Mol Cancer Res* **4**:695–707.
- Chen CK, Eversole-Cire P, Zhang H, Mancino V, Chen YJ, He W, Wensel TG, and Simon MI (2003) Instability of GGL domain-containing RGS proteins in mice lacking the G protein beta-subunit Gbeta5. *Proc Natl Acad Sci USA* **100**:6604–6609.
- Cho H, Park C, Hwang IY, Han SB, Schimel D, Despres D, and Kehrl JH (2008) Rgs5 targeting leads to chronic low blood pressure and a lean body habitus. *Mol Cell Biol* **28**:2590–2597.
- Cifelli C, Rose RA, Zhang H, Voigtlaender-Bolz J, Bolz SS, Backx PH, and Heximer SP (2008) RGS4 regulates parasympathetic signaling and heart rate control in the sinoatrial node. *Circ Res* **103**:527–535.
- Davydov IV and Varshavsky A (2000) RGS4 is arginylation and degraded by the N-end rule pathway in vitro. *J Biol Chem* **275**:22931–22941.
- Derrien A, Zheng B, Osterhout JL, Ma YC, Milligan G, Farquhar MG, and Druey KM (2003) Src-mediated RGS16 tyrosine phosphorylation promotes RGS16 stability. *J Biol Chem* **278**:16107–16116.
- Drenan RM, Doupnik CA, Jayaraman M, Buchwalter AL, Kaltenbronn KM, Huettner JE, Linder ME, and Blumer KJ (2006) R7BP augments the function of RGS7*Gbeta5 complexes by a plasma membrane-targeting mechanism. *J Biol Chem* **281**:28222–28231.
- Fu Y, Huang X, Piao L, Lopatin AN, and Neubig RR (2007) Endogenous RGS proteins modulate SA and AV nodal functions in isolated heart: implications for sick sinus syndrome and AV block. *Am J Physiol Heart Circ Physiol* **292**:H2532–H2539.
- Fu Y, Huang X, Zhong H, Mortensen RM, D'Alecy LG, and Neubig RR (2006) Endogenous RGS proteins and Galpha subtypes differentially control muscarinic and adenosine-mediated chronotropic effects. *Circ Res* **98**:659–666.
- Georgoussi Z, Leontiadis L, Mazarakou G, Merkouris M, Hyde K, and Hamm H (2006) Selective interactions between G protein subunits and RGS4 with the C-terminal domains of the mu- and delta-opioid receptors regulate opioid receptor signaling. *Cell Signal* **18**:771–782.
- Gold SJ, Han MH, Herman AE, Ni YG, Pudiak CM, Aghajanian GK, Liu RJ, Potts BW, Mumby SM, and Nestler EJ (2003) Regulation of RGS proteins by chronic morphine in rat locus coeruleus. *Eur J Neurosci* **17**:971–980.
- Gold SJ, Hoang CV, Potts BW, Porras G, Pioli E, Kim KW, Nadjari A, Qin C, LaHoste GJ, Li Q, et al. (2007) RGS9–2 negatively modulates L-3,4-dihydroxyphenylalanine-induced dyskinesia in experimental Parkinson's disease. *J Neurosci* **27**:14338–14348.
- Gold SJ, Ni YG, Dohlman HG, and Nestler EJ (1997) Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci* **17**:8024–8037.
- Grigoryev S, Stewart AE, Kwon YT, Arfin SM, Bradshaw RA, Jenkins NA, Copeland NG, and Varshavsky A (1996) A mouse amidase specific for N-terminal asparagine. The gene, the enzyme, and their function in the N-end rule pathway. *J Biol Chem* **271**:28521–28532.
- Grillet N, Pattyn A, Contet C, Kieffer BL, Georidis C, and Brunet JF (2005) Generation and characterization of Rgs4 mutant mice. *Mol Cell Biol* **25**:4221–4228.
- Gu S, Tirgari S, and Heximer SP (2008) The RGS2 gene product from a candidate hypertension allele shows decreased plasma membrane association and inhibition of G_q. *Mol Pharmacol* **73**:1037–1043.
- Han MH, Renthal W, Ring RH, Rahman Z, Psifogeorgou K, Howland D, Birnbaum S, Young K, Neve R, Nestler EJ, et al. (2010) Brain region specific actions of regulator of G protein signaling 4 oppose morphine reward and dependence but promote analgesia. *Biol Psychiatry* **67**:761–769.
- Hepler JR (1999) Emerging roles for RGS proteins in cell signalling. *Trends Pharmacol Sci* **20**:376–382.
- Hershko A and Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* **67**:425–479.
- Heximer SP, Knutsen RH, Sun X, Kaltenbronn KM, Rhee MH, Peng N, Oliveirados-Santos A, Penninger JM, Muslin AJ, Steinberg TH, et al. (2003) Hypertension and prolonged vasoconstrictor signaling in RGS2-deficient mice. *J Clin Invest* **111**:445–452.
- Heximer SP, Watson S, Linder ME, Blumer KJ, and Hepler JR (1997) RGS2/G0S8 is a selective inhibitor of G_qalpha function. *Proc Natl Acad Sci USA* **94**:14389–14393.
- Hoeller D, Hecker CM, and Dikic I (2006) Ubiquitin and ubiquitin-like proteins in cancer pathogenesis. *Nat Rev Cancer* **6**:776–788.
- Hollinger S and Hepler JR (2002) Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. *Pharmacol Rev* **54**:527–559.
- Hurst BJ and Hooks SB (2009) Regulator of G-protein signaling (RGS) proteins in cancer biology. *Biochem Pharmacol* **78**:1289–1297.
- Ishii M, Fujita S, Yamada M, Hosaka Y, and Kurachi Y (2005) Phosphatidylinositol 3,4,5-trisphosphate and Ca²⁺/calmodulin competitively bind to the regulators of G-protein-signalling (RGS) domain of RGS4 and reciprocally regulate its action. *Biochem J* **385**:65–73.
- Ishii M, Inanobe A, Fujita S, Makino Y, Hosoya Y, and Kurachi Y (2001) Ca²⁺ elevation evoked by membrane depolarization regulates G protein cycle via RGS proteins in the heart. *Circ Res* **89**:1045–1050.
- Ishii M, Inanobe A, and Kurachi Y (2002) PIP₃ inhibition of RGS protein and its reversal by Ca²⁺/calmodulin mediate voltage-dependent control of the G protein cycle in a cardiac K⁺ channel. *Proc Natl Acad Sci USA* **99**:4325–4330.
- Jayaraman M, Zhou H, Jia L, Cain MD, and Blumer KJ (2009) R9AP and R7BP: traffic cops for the RGS7 family in phototransduction and neuronal GPCR signaling. *Trends Pharmacol Sci* **30**:17–24.
- Jin Y, Zhong H, Omnaas JR, Neubig RR, and Mosberg HI (2004) Structure-based design, synthesis, and pharmacologic evaluation of peptide RGS4 inhibitors. *J Pept Res* **63**:141–146.
- Kaur K, Kehrl JM, Charbeneau RA, and Neubig RR (2010) RGS-insensitive G_q subunits: probes of G_q subtype-selective signaling and physiological functions of RGS proteins. *Methods Mol Biol*. In press.
- Kehrl JH, Srikumar D, Harrison K, Wilson GL, and Shi CS (2002) Additional 5' exons in the RGS3 locus generate multiple mRNA transcripts, one of which accounts for the origin of human PDZ-RGS3. *Genomics* **79**:860–868.
- Kimple RJ, De Vries L, Tronchère H, Behe CI, Morris RA, Gist Farquhar M, and Siderovski DP (2001) RGS12 and RGS14 GoLoco motifs are G alpha(i) interaction sites with guanine nucleotide dissociation inhibitor Activity. *J Biol Chem* **276**:29275–29281.
- Kwon YT, Kashina AS, Davydov IV, Hu RG, An JY, Seo JW, Du F, and Varshavsky A (2002) An essential role of N-terminal arginylation in cardiovascular development. *Science* **297**:96–99.
- Lee MJ, Tasaki T, Moroi K, An JY, Kimura S, Davydov IV, and Kwon YT (2005) RGS4 and RGS5 are in vivo substrates of the N-end rule pathway. *Proc Natl Acad Sci USA* **102**:15030–15035.
- Linder ME and Deschenes RJ (2007) Palmitoylation: policing protein stability and traffic. *Nat Rev Mol Cell Biol* **8**:74–84.
- Meinert T, Serero A, and Giglione C (2006) Impact of the N-terminal amino acid on targeted protein degradation. *Biol Chem* **387**:839–851.
- Narayanan V, Sandiford SL, Wang Q, Keren-Raifman T, Levay K, and Slepak VZ (2007) Intramolecular interaction between the DEP domain of RGS7 and the Gbeta5 subunit. *Biochemistry* **46**:6859–6870.
- Neubig RR and Siderovski DP (2002) Regulators of G-protein signalling as new central nervous system drug targets. *Nat Rev Drug Discov* **1**:187–197.
- Nguyen CH, Ming H, Zhao P, Hugendubler L, Gros R, Kimball SR, and Chidiac P (2009) Translational control by RGS2. *J Cell Biol* **186**:755–765.
- Nunn C, Mao H, Chidiac P, and Albert P (2006) RGS17/RGS22 and the RZ/A family of regulators of G-protein signaling. *Semin Cell Dev Biol* **17**:390–399.
- Popov SG, Krishna UM, Falck JR, and Wilkie TM (2000) Ca²⁺/Calmodulin reverses phosphatidylinositol 3,4,5-trisphosphate-dependent inhibition of regulators of G protein-signaling GTPase-activating protein activity. *J Biol Chem* **275**:18962–18968.
- Rahman Z, Schwarz J, Gold SJ, Zachariou V, Wein MN, Choi KH, Koor A, Chen CK, DiLeone RJ, Schwarz SC, et al. (2003) RGS9 modulates dopamine signaling in the basal ganglia. *Neuron* **38**:941–952.
- Roman DL, Blazer LL, Monroy CA and Neubig RR (2010) Allosteric inhibition of the regulator of G protein signaling–G protein–protein interaction by CCG-4986. *Mol Pharmacol* **78**:360–365.
- Roman DL, Ota S, and Neubig RR (2009) Polyplexed flow cytometry protein interaction assay: a novel high-throughput screening paradigm for RGS protein inhibitors. *J Biomol Screen* **14**:610–619.
- Roman DL, Talbot JN, Roof RA, Sunahara RK, Traynor JR, and Neubig RR (2007) Identification of small-molecule inhibitors of RGS4 using a high-throughput flow cytometry protein interaction assay. *Mol Pharmacol* **71**:169–175.
- Roof RA, Jin Y, Roman DL, Sunahara RK, Ishii M, Mosberg HI, and Neubig RR (2006) Mechanism of action and structural requirements of constrained peptide inhibitors of RGS proteins. *Chem Biol Drug Des* **67**:266–274.
- Ross EM and Wilkie TM (2000) GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annu Rev Biochem* **69**:795–827.
- Roy AA, Baragli A, Bernstein LS, Hepler JR, Hébert TE, and Chidiac P (2006) RGS2 interacts with Gs and adenylyl cyclase in living cells. *Cell Signal* **18**:336–348.
- Saitoh O, Masuho I, Terakawa I, Nomoto S, Asano T, and Kubo Y (2001) Regulator of G protein signaling 8 (RGS8) requires its NH2 terminus for subcellular localization and acute desensitization of G protein-gated K⁺ channels. *J Biol Chem* **276**:5052–5058.
- Sandiford SL and Slepak VZ (2009) The Gbeta5-RGS7 complex selectively inhibits muscarinic M3 receptor signaling via the interaction between the third intracellular loop of the receptor and the DEP domain of RGS7. *Biochemistry* **48**:2282–2289.
- Sethakorn N, Yau DM, and Dulin NO (2010) Non-canonical functions of RGS proteins. *Cell Signal* **22**:1274–1281.
- Siderovski DP and Willard FS (2005) The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. *Int J Biol Sci* **1**:51–66.
- Sjögren B, Blazer LL, and Neubig RR (2010) Regulators of G protein signaling proteins as targets for drug discovery, in *Progress in Molecular Biology and Translational Science*, vol 91, pp 81–119, Academic Press, San Diego.
- Song L and Jope RS (2006) Cellular stress increases RGS2 mRNA and decreases RGS4 mRNA levels in SH-SY5Y cells. *Neurosci Lett* **402**:205–209.
- Talbot JN, Roman DL, Clark MJ, Roof RA, Tesmer JJ, Neubig RR, and Traynor JR (2010) Differential modulation of mu-opioid receptor signaling to adenylyl cyclase by regulators of G protein signaling proteins 4 or 8 and 7 in permeabilised C6 cells is Galpha subtype dependent. *J Neurochem* **112**:1026–1034.
- Tasaki T and Kwon YT (2007) The mammalian N-end rule pathway: new insights into its components and physiological roles. *Trends Biochem Sci* **32**:520–528.
- Tesmer JJ (2010) Structure and function of regulator of G protein signaling homol-

- ogy domains, in *Molecular Biology of RGS Proteins* (Fisher R ed) pp 75–113, Elsevier, Amsterdam.
- Tesmer JJ, Berman DM, Gilman AG, and Sprang SR (1997) Structure of RGS4 bound to AlF₄-activated G(i alpha1): stabilization of the transition state for GTP hydrolysis. *Cell* **89**:251–261.
- Tesmer JJ (2009) Structure and function of regulator of G protein signaling homology domains, in *Progress in Molecular Biology and Translational Science* (Rory AF ed) pp 75–113, Academic Press, San Diego.
- Traynor JR and Neubig RR (2005) Regulators of G protein signaling & drugs of abuse. *Mol Interv* **5**:30–41.
- Varshavsky A (1996) The N-end rule: functions, mysteries, uses. *Proc Natl Acad Sci USA* **93**:12142–12149.
- Wang H, Piatkov KI, Brower CS, and Varshavsky A (2009a) Glutamine-specific n-terminal amidase, a component of the N-end rule pathway. *Mol Cell* **34**:686–695.
- Wang Q, Liu-Chen LY, and Traynor JR. *J Biol Chem* **284**:18357–18367.
- Wang Y, Lee Y, Zhang J, and Young KH (2008) Identification of peptides that inhibit regulator of G protein signaling 4 function. *Pharmacology* **82**:97–104.
- Williams C and Hill SJ (2009) GPCR signaling: understanding the pathway to successful drug discovery, in *G Protein-Coupled Receptors in Drug Discovery* (Leifert WR ed), pp 39–50, Humana Press, New York.
- Xie Y, Wolff DW, Wei T, Wang B, Deng C, Kirui JK, Jiang H, Qin J, Abel PW, and Tu Y. *Cancer Res* **69**:5743–5751.
- Yang H, Zonder JA, and Dou QP (2009) Clinical development of novel proteasome inhibitors for cancer treatment. *Expert Opin Investig Drugs* **18**:957–971.
- Yang J, Kamide K, Kokubo Y, Takiuchi S, Tanaka C, Banno M, Miwa Y, Yoshii M, Horio T, Okayama A, et al. (2005) Genetic variations of regulator of G-protein signaling 2 in hypertensive patients and in the general population. *J Hypertens* **23**:1497–1505.
- Yau DM, Sethakorn N, Taurin S, Kregel S, Sandbo N, Camoretti-Mercado B, Sperling AI, and Dulin NO (2008) Regulation of Smad-mediated gene transcription by RGS3. *Mol Pharmacol* **73**:1356–1361.
- Young KH, Wang Y, Bender C, Ajit S, Ramirez F, Gilbert A, and Nieuwenhuijsen BW (2004) Yeast-based screening for inhibitors of RGS proteins. *Methods Enzymol* **389**:277–301.
- Zachariou V, Georgescu D, Sanchez N, Rahman Z, DiLeone R, Berton O, Neve RL, Sim-Selley LJ, Selley DE, Gold SJ, et al. (2003) Essential role for RGS9 in opiate action. *Proc Natl Acad Sci USA* **100**:13656–13661.
- Zhang W, Anger T, Su J, Hao J, Xu X, Zhu M, Gach A, Cui L, Liao R, and Mende U (2006) Selective loss of fine tuning of G_q/11 signaling by RGS2 protein exacerbates cardiomyocyte hypertrophy. *J Biol Chem* **281**:5811–5820.
- Zhong H and Neubig RR (2001) Regulator of G protein signaling proteins: novel multifunctional drug targets. *J Pharmacol Exp Ther* **297**:837–845.
- Zmijewski JW, Song L, Harkins L, Cobbs CS, and Jope RS (2001) Oxidative stress and heat shock stimulate RGS2 expression in 1321N1 astrocytoma cells. *Arch Biochem Biophys* **392**:192–196.
- Zou MX, Roy AA, Zhao Q, Kirshenbaum LA, Karmazyn M, and Chidiac P (2006) RGS2 is upregulated by and attenuates the hypertrophic effect of alpha1-adrenergic activation in cultured ventricular myocytes. *Cell Signal* **18**:1655–1663.

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