This Review is the first in a thematic series on Novel Aspects of Cardiovascular G Protein-Coupled Receptor Signaling: Implications for New Therapeutics, which includes the following articles:


G-Protein–Coupled Receptor Kinases (GRKs) as Therapeutic Targets in Cardiovascular Disease [Circ Res. 2011;109:309–319]

Regulators of G-Protein Signaling in the Heart and Their Potential as Therapeutic Targets

Howard Rockman, Guest Editor

Regulators of G-Protein Signaling in the Heart and Their Potential as Therapeutic Targets

Peng Zhang, Ulrike Mende

Abstract: Signal transduction through G-protein–coupled receptors (GPCRs) is central for the regulation of virtually all cellular functions and has been widely implicated in human disease. Regulators of G-protein signaling (RGS proteins) belong to a diverse protein family that was originally discovered for their ability to accelerate signal termination in response to GPCR stimulation, thereby reducing the amplitude and duration of GPCR effects. All RGS proteins share a common RGS domain that interacts with G protein α subunits and mediates their biological regulation of GPCR signaling. However, RGS proteins differ widely in size and the organization of their sequences flanking the RGS domain, which contain several additional functional domains that facilitate protein-protein (or protein-lipid) interactions. RGS proteins are subject to posttranslational modifications, and, in addition, their expression, activity, and subcellular localization can be dynamically regulated. Thus, there exists a wide array of mechanisms that facilitate their proper function as modulators and integrators of G-protein signaling. Several RGS proteins have been implicated in the cardiac remodeling response and heart rate regulation, and changes in RGS protein expression and/or function are believed to participate in the pathophysiology of cardiac hypertrophy, failure and arrhythmias as well as hypertension. This review is based on recent advances in our understanding of the expression pattern, regulation, and functional role of canonical RGS proteins, with a special focus on the healthy heart and the diseased heart. In addition, we discuss their potential and promise as therapeutic targets as well as strategies to modulate their expression and function. (Circ Res. 2011;109:320-333.)

Key Words: RGS proteins ■ signal transduction ■ myocardium ■ cardiac myocytes ■ cardiac fibroblasts

Signal transduction through G-protein–coupled receptors (GPCRs) is essential for the regulation of cardiovascular function, including heart rate, growth, contraction, and vascular tone. Perturbations in GPCR signaling have pathophysiological consequences and are major contributors to cardiac disease.1 Ligand activation of GPCRs promotes GTP-for-GDP exchange on the α subunits of heterotrimeric G proteins (Figure 1), resulting in dissociation of GTP-bound Go from...
Gβγ. Both Gα and Gβγ subunits then activate (or inhibit) downstream signaling molecules (enzymes, kinases, and ion channels) and thereby elicit cellular responses. Their magnitude and duration depend on how long G proteins remain activated, which is determined by a GTPase activity intrinsic to Gα. On GTP hydrolysis, the resulting GDP-bound inactive Gα reassociates with Gβγ and can enter a new activation cycle. Thus, the rate of GTP hydrolysis determines the duration that Gα-GTP and Gβγ are free to interact with intracellular or membrane effectors. It long remained a conundrum that the intrinsic rate of GTP hydrolysis is insufficient to account for the rate of signal termination typically observed in vivo. Whereas some effector molecules (eg, phospholipase C β2) were found to act as GTPase-activating proteins (GAPs), the discovery of regulators of G-protein signaling (RGS proteins) introduced a new large and diverse protein family that leads to pronounced (up to 2000-fold) acceleration of GTPase activity,3,4 which decreases both Gα inactivation and thereby block Gα-mediated signal generation.147 or G-protein–independent GPCR effects.148 Furthermore, full dissociation of Gα and Gβγ subunits may not be required to trigger downstream effects.149

GTP in its transition state), provide mechanistic insight into G-protein–mediated downstream effects, and (2) competition with downstream effectors for binding to activated Gα, which inhibits only Gα-mediated signal generation. Please note that this illustration depicts the traditional view of GPCR-induced, G-protein–mediated signal transduction. It does not incorporate GPCR-independent G-protein activation167 or G-protein-independent GPCR effects.146 Furthermore, full dissociation of Gα and Gβγ subunits may not be required to trigger downstream effects.149

In the present review, we focus on the expression pattern, regulation, and functional role of canonical RGS proteins in the healthy heart and in the diseased heart, as well as their potential as therapeutic drug targets. Other reviews provide further details on the structure and function of canonical RGS proteins.8,9 Because of space constraints, only some information about the role of RGS proteins in the vasculature could be included. The reader is referred to other excellent reviews to learn more about RGS proteins in blood vessels,10,11 the nervous system,12,13 inflammation,14 and cancer15 for a broader view on the importance of RGS proteins in regulating GPCR signaling and function in health and disease.

RGS Protein Expression in the Heart

Several canonical RGS proteins are expressed in the mammalian and human myocardium16–18 as well as in cardiac myocytes19,20 (see Table 1). RGS protein expression in nonmyocytes has long been suggested19 but was only recently reported for cardiac fibroblasts.21 A comprehensive, reverse transcription–polymerase chain reaction (PCR)–based overview of canonical RGS protein expression in cardiac myocytes and fibroblasts from adult rat ventricles is shown in Figure 2 (compared to brain). Quantitative mRNA analysis revealed that RGS2, RGS3, and RGS5 are most highly expressed in the human heart.22 In contrast, RGS4, which initially garnered a lot of attention, was found only at a very low levels, consistent with Figure 2 and other studies showing lack of RGS4 in the ventricular myocardium but enrichment in the sinoatrial node.23,24

RGS protein expression profiles are most often based on Northern blots, in situ hybridization, and PCR analyses. Discrepancies between mRNA and protein levels have been reported (eg, increased mRNA but decreased protein expression for RGS4 in breast cancer tissue),25 emphasizing the importance of protein measurements. However, protein detection has been a significant challenge in the field, because

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Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AC</td>
<td>adenylate cyclase</td>
</tr>
<tr>
<td>Ang II</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>Ca2+/CaM</td>
<td>calcium calmodulin</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>GAP</td>
<td>GTPase-activating protein</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-protein–coupled receptor</td>
</tr>
<tr>
<td>PIP3</td>
<td>phosphatidylinositol 3,4,5-trisphosphate</td>
</tr>
<tr>
<td>RGS</td>
<td>regulators of G-protein signaling</td>
</tr>
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Table 1. Structure, GAP Function, Posttranslational Modifications, and Cardiac Expression of Canonical RGS Proteins

<table>
<thead>
<tr>
<th>Subfamily*</th>
<th>Gene†</th>
<th>Size Amino Acids</th>
<th>Non-RGS Domains</th>
<th>GAP for</th>
<th>Posttranslational Modifications</th>
<th>Ventricles</th>
<th>Atria</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N-Terminal</td>
<td>C-Terminal</td>
<td></td>
<td>Tissue‡</td>
<td>CM§</td>
</tr>
<tr>
<td>R4 (B)</td>
<td>RGS1†</td>
<td>209</td>
<td>A. helix</td>
<td>None</td>
<td>G₁ and G₁₁</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>RGS2</td>
<td>211</td>
<td>A. helix</td>
<td>None</td>
<td>G₁₁(&gt;,&gt;) (G₁₁)</td>
<td>S46/64/73</td>
<td>PKG</td>
</tr>
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<td></td>
<td>RGS3†</td>
<td>519</td>
<td>A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>? (PKG)</td>
<td>S264</td>
</tr>
<tr>
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<td>RGS4†</td>
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<td>A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>S52 (PKG)</td>
<td>C2,</td>
</tr>
<tr>
<td></td>
<td>RGS5†</td>
<td>181</td>
<td>A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>S166 (PKC)</td>
<td>S84?</td>
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<td>A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>T 41 (PKA)</td>
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<tr>
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<td>159</td>
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<td>G₁₁ and G₁₁</td>
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<td></td>
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<td>G₁₁ and G₁₁</td>
<td>Y168 (EGFR,</td>
<td>S258/T494</td>
</tr>
<tr>
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<td>RGS9†</td>
<td>235</td>
<td>A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>S49 (?)</td>
<td>(+)</td>
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<tr>
<td></td>
<td>RGS21</td>
<td>152</td>
<td>None</td>
<td>None</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>R7 (C)</td>
<td>RGS6†</td>
<td>472</td>
<td>DEP GGL</td>
<td>None</td>
<td>G₁₀</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>RGS7†</td>
<td>487</td>
<td>DEP GGL</td>
<td>None</td>
<td>G₁₀</td>
<td>S434 (PKCα)</td>
<td>C60,</td>
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<tr>
<td></td>
<td>RGS9†</td>
<td>484</td>
<td>DEP GGL</td>
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<td>G₁₀</td>
<td>S427/428</td>
<td>(PKA)</td>
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<tr>
<td></td>
<td>RGS11†</td>
<td>446</td>
<td>DEP GGL</td>
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<td>G₁₀</td>
<td>S168 (PKA)</td>
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<tr>
<td>R12 (D)</td>
<td>RGS10</td>
<td>446</td>
<td>DEP GGL</td>
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<tr>
<td></td>
<td>RGS12†</td>
<td>1376</td>
<td>PDZ PTB</td>
<td>RBD GoLco</td>
<td>G₁₀</td>
<td>S258/494</td>
<td>(PKA)</td>
</tr>
<tr>
<td>R12 (D)</td>
<td>RGS14†</td>
<td>566</td>
<td>None</td>
<td>RBD GoLco</td>
<td>G₁₀</td>
<td>S258/494</td>
<td>(PKA)</td>
</tr>
<tr>
<td>RZ (A)</td>
<td>RGS17</td>
<td>210</td>
<td>C string</td>
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<td>G₂ and other G₁₀</td>
<td>Likely (S151)</td>
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</tr>
<tr>
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<td>RGS19†</td>
<td>217</td>
<td>C string &amp; A. helix</td>
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<td>G₁₁ and G₁₁</td>
<td>Likely (S151)</td>
<td></td>
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<tr>
<td>RGS20†</td>
<td>241</td>
<td>C string</td>
<td>None</td>
<td>G₁₁(&gt;,&gt;) (G₁₁)</td>
<td>Likely (S151)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Nomenclatures for canonical RGS protein subfamilies are based on either a prototypical subfamily member or arbitrary alphabetical letters (in brackets). †Indicates the existence of alternatively spliced variants for the specified RGS protein genes.
‡RGS protein expression profile obtained from studies performed in the human heart (using quantitative real-time PCR) or human left ventricular myocardium (using RNase protection assays and RT-PCR).
§RT-PCR–based RGS protein expression profile in myocytes (CM) and fibroblasts (CF) from adult rat ventricles (see Figure 2).
<table>
<thead>
<tr>
<th>Additional Annotations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>For the expression profiles, + and − denote detection or lack thereof of specified isoforms and (+) indicates weak expression.</td>
</tr>
<tr>
<td>In the phosphorylation column, the phosphorylation-specific site(s) are followed by responsible kinase(s) in parentheses. Unknown sites or kinases are indicated by ?.</td>
</tr>
<tr>
<td>RGS21 has controversial tissue distribution (restricted to taste buds versus ubiquitous), and its expression was not determined (ND) in the expression studies listed.</td>
</tr>
<tr>
<td>Several canonical RGS protein isoforms are expressed in the myocardium with regional differences between atria and ventricles (Table 1). Myocytes and fibroblasts have a unique complement of RGS proteins, so that expression studies in cardiac tissue must be interpreted with caution. As will be described below, significant progress has been made in assigning signaling and functional roles for specific RGS protein isoforms in both major cell types in the heart, although much work remains.</td>
</tr>
</tbody>
</table>

Antibodies that unequivocally recognize endogenous RGS proteins are not available for many isoforms. In addition, knockout controls may be required to demonstrate specificity of protein bands of expected molecular weight. The difficulty in detecting endogenous RGS proteins with antibodies that recognize overexpressed RGS proteins very well suggests that cellular levels of endogenous RGS proteins may be quite low. Stoichiometric information on relative G protein and RGS protein levels therefore has yet to be determined.
that lack of interaction between recombinant RGS2 and mediated but not Gi/o-mediated signaling. Nevertheless, it was also shown to directly interact with and negatively regulates Gi signaling was recently demonstrated. RGS2 arose from the R4 subfamily to have specialized G protein, which has been attributed to the geometry of a Gα-binding pocket that is unfavorable to Gαi0. The structural determinants were recently pinpointed to 3 evolutionary highly conserved amino acids, leading the authors to speculate that RGS2 arose from the R4 subfamily to have specialized Gαi11 GAP activity to modulate cardiovascular function. Indeed, in adult rat cardiomyocytes, RGS2 negatively regulates Gαi11−mediated but not Gαo-mediated signaling. Nevertheless, it has been reported that RGS2 interactions with Gαo may occur, dependent on receptor-mediated Gα activation, so that lack of interaction between recombinant RGS2 and Gα may not necessarily be indicative of a lack of regulatory interaction in cells. In fact, in cultured ventricular myocytes, a novel role of RGS2 as terminator of Gi/o and Gq/11 family members. Only RGS2 generally has been considered to be selective in negatively regulating Gq/11, conserved amino acids, leading the authors to speculate that RGS2 arose from the R4 subfamily to have specialized Gαi11 GAP activity to modulate cardiovascular function. Indeed, in adult rat cardiomyocytes, RGS2 negatively regulates Gαi11−mediated but not Gαo-mediated signaling. Nevertheless, it has been reported that RGS2 interactions with Gαo may occur, dependent on receptor-mediated Gα activation, so that lack of interaction between recombinant RGS2 and Gα may not necessarily be indicative of a lack of regulatory interaction in cells. In fact, in cultured ventricular myocytes, a novel role of RGS2 as terminator of β2-receptor−mediated Gi signaling was recently demonstrated. RGS2 was also shown to directly interact with and negatively regulate select adenylate cyclase (AC) isoforms (including the major cardiac isoforms ACV and ACV1), . Gαi/o interaction albeit without GAP activity was reported as well. However, these studies were performed in HEK293 and other noncardiac cells. In adult rat ventricular myocytes, RGS2 overexpression did not affect forskolin- or isoproterenol-induced cAMP generation, suggesting that neither direct nor indirect RGS2-induced AC regulation appears to play a major role in differentiated myocytes. In neonatal rat cardiomyocytes, hypertrophy induced by β-adrenergic stimulation could be inhibited by RGS2 expression.

RGS3, which exists in several splice variants, is a unique R4 RGS protein in that a long N-terminus in some variants facilitates interactions with other proteins. For example, binding to Gβγ enables RGS3L (519 amino acids) to inhibit Gβγ-mediated signaling by acting as a scavenger and has the ability to switch Gαi/o-coupled muscarinic and adenosine receptor–induced signaling from Rac1 to Rho A activation. However, the switch is highly dependent on the expression level of endogenous RGS3L, which is markedly downregulated by fibroblast growth factor 2. This mechanism could be of pathophysiological significance in the heart but has thus far only been demonstrated in H10 cells. The N-terminus of RGS3 can also interact with Smad2, Smad3, and Smad4 through their Mad homology 2 domain and inhibit Smad-mediated gene transcription by preventing Smad3/Smad4 heteromerization. RGS3-Smad interaction has been shown to inhibit transforming growth factor-β–induced differentiation of pulmonary fibroblasts and may play a role in cardiac fibroblasts as well.

Other R4 RGS protein subfamily members can also regulate non-G-protein signaling. For example, several isoforms can interact with the regulatory p85α subunit of phosphatidylinositol-3-OH kinase. Subsequent inhibition of phosphatidylinositol-3-OH kinase activity by inhibiting p85-Gab1/2 interactions has been shown for RGS13 in mast cells and RGS16 in breast cancer cells. Investigations of potential RGS protein regulation of cardiac phosphatidylinositol-3-OH kinase are warranted in light of its importance in modulating cell survival, growth, contractility, and metabolism. Furthermore, RGS13 also acts as a nuclear repressor of cAMP response element–binding protein in B-lymphocytes that inhibits cAMP response element-binding protein–dependent transcription through disruption of promoter complexes.

R7 subfamily members are predominantly expressed in the nervous system and best known for their role in the regulation of neuronal processes, including vision, memory, motor control, reward behavior, and nociception. However, a key role of RGS6 in the heart was recently discovered (see section, Functional Role of RGS Proteins in the Heart). Through their RGS domain, R7 RGS proteins exert GAP activity primarily on Gαq/11 proteins. They also contain a G-protein γ-like domain that is structurally homologous to conventional Gγ subunits but binds only with the most distant member of the Gβ family (Gγ1), an interaction that is essential for the stability and expression of all R7 RGS proteins. Beyond protecting R7 RGS proteins from proteolysis, the role of Gγ1 is not fully understood. It is believed to participate in determining G-protein selectivity and GAP properties. The crystal structure of RGS9-Gβγ offers some insight into potential mechanisms. The N-terminus of R7 RGS proteins also contains Disheveled-EGL10-Pleckstrin homology and its helical extension domains that mediate interactions with membrane anchor proteins (ie, RGS9 anchor protein and RGS7 family–binding protein), which both play key roles in determinin-
The R12 subfamily is composed of members that are structurally very diverse in regions other than their RGS domain (Table 1). RGS10 lacks any additional domain, acts as GAP for Goαi/o and Goq/11, and is phosphorylated by protein kinase A.51 RGS12 and RGS14 are GAPs for Goαi/o only. In addition to binding to activated Goαi/o in its activated state through their C-terminal Goαi/o-Loco domain and act as GAP-dissociating inhibitors.52 Inhibition of GDP-to-GTP exchange and subsequent Goα activation provide an additional GAP-independent mechanism of regulating G-protein signaling through these RGS protein isoforms. Furthermore, RGS12 and RGS14 have recently emerged as integrators of G protein and Ras-Raf/extra-cellular signal-regulated kinase (ERK) signaling by facilitating formation of a selective Ras-Raf-MEK-ERK multiprotein complex to promote sustained ERK activation, involving their C-terminal tandem Ras-binding domains and for RGS12 its additional PSD-95 disk-large ZO-1 and phosphotyrosine-binding domains.53–55 However, existence of these mechanisms in cardiac cells remains to be investigated.

Members of the RZ subfamily56 are short in size and share an N-terminal cysteine string motif, which presumably provides substrate for palmitoylation for each isoform (reported thus far for RGS19). Similarly, phosphorylation has been demonstrated for RGS19 at two sites, one which (S151) is conserved among subfamily members. RGS19 contains an additional C-terminal PSD-95 disk-large ZO-1 binding motif that facilitates binding to a scaffolding protein (Goα-interacting protein C-terminus) that assembles receptors and signaling molecules and may promote cross-talk between G-protein and non-G-protein signaling pathways.57 The RZ subfamily was originally named because RGS20 (originally known as RGSZ1) was found to selectively accelerate GTP hydrolysis of Gαz, a more distant member of the Goαi/o family that also inhibits AC and activates potassium channels. In contrast, RGS17 and RGS19 (aka Goα-interacting protein [GAI]) have GAP activity for all Goαi/o subunits (and Goαq/11, for RGS19). Although RGS17 is not a Goαq/11,GAP in vitro, it can bind and inhibit Gq/11 mediated signaling in the cellular context through a yet-undetermined mechanism.58 In the same study, despite its GAP selectivity, RGS20 blocked Goαi/o signaling. Thus, in vitro GAP activity assays are not always good predictors of function in the cellular context; effector antagonism and non-GAP mechanism are additional determinants of RGS protein function in vivo.

Taken together, canonical RGS proteins serve as GAPs for members of the Goαi/o and Goαq/11 families. It is generally believed that they do not serve as GAPs for Goαz, evidence to the contrary regarding RGS-PX159 has yet to be confirmed. GAP activity for Goαi/o13 is displayed only by noncanonical “Gα-like” Rhoguanine nucleotide exchange factors, which are also their effectors.60 Although a wealth of information on the interactions between RGS proteins and Gα subunits has been collected over the past 15 years, it cannot account for the specificity with which RGS proteins regulate G-protein–mediated signaling in living cells. Despite tissue- and cell-specific expression for some isoforms, most cells express several RGS proteins with diverse activities, and they are rather nondiscriminatory toward G proteins. A variety of mechanisms that regulate RGS protein expression, activity, location, and interaction with other proteins are summarized below, which collectively facilitate effective and specific modulation of GPCR-induced signal transfer. Following is a brief synopsis of the current understanding of RGS protein regulation, with a special focus on mechanisms that may potentially be at play in the heart.

**Regulation of RGS Protein Expression, Activity, and Location**

**Expression of Different RGS Gene and Protein Products**
Both alternative mRNA splicing (for specific isoforms, see Table 1) and translation initiation from alternative start sites have been reported. Variations are generally not located in the core RGS domain but the additional extensions and regulatory domains, suggesting that they may play a role in fine-tune signaling responses. For example, utilization of 3 alternative translation start sites in human RGS2 was shown to yield proteins of different functionality in overexpression experiments, in that AC inhibition was compromised when the N-terminal AC binding site was missing, whereas GAP-mediated Gq/11 regulation was unaffected.61 However, the prevalence of these regulatory mechanisms in the cardiovascular system and their significance under physiological conditions are not known at this point.

**Regulation of mRNA Expression**
Numerous reports in many different cell types have shown that mRNA encoding for various RGS isoforms can be regulated by a variety of factors, including GPCR activation, second messengers, and disease states. Most recently, promotor hypermethylation–dependent silencing was reported for RGS2 in human prostate cancer, suggesting epigenetic repression as a novel mechanism for regulating RGS mRNA expression.62 In the heart, many studies have been conducted in myocardial tissue and in already-hypertrophied or failing hearts. In the diseased heart, a multitude of signaling changes occur, many of which are secondary to the remodeling process. Disparities regarding RGS protein expression changes between animal models of hypertrophy63,64 and in humans65,66 may be due to species- and model-specific differences.66

Among the various RGS proteins, RGS2 has emerged as an isoform that is highly susceptible to regulation, and it also exemplifies the dynamic nature of RGS protein regulation in the heart. In response to short-term activation of the Gq/11 signaling pathway, RGS2 mRNA is transiently upregulated in both cardiac myocytes30,67 and fibroblasts.21 This is generally viewed as a negative feedback mechanism in light of the role of RGS2 as a negative regulator of Gq/11 signaling.68,69 Interestingly, acute β-adrenergic or forskolin stimulation also cause a marked increase in RGS2 mRNA,30,39 which may point to potential cross-regulation and desensitization between Gq/11- and Gα-activated signaling pathways. Although no RGS2 regulatory effects on cAMP were detected in adult rat myocytes,30 inhibition of isoproterenol-induced hypertrophy by blunting of ERK1/2 and Akt activation was reported in...
neonatal myocytes. Importantly, in contrast to acute stimulation, marked RGS2 downregulation has been discovered in ventricles subjected to pressure overload, myocytes from mice expressing constitutively active Gaq, and myocytes and fibroblasts from rats subjected to prolonged angiotensin (Ang II) infusion in vivo and has been implicated in exacerbating cardiac remodeling in the stressed or injured hearts. Protein kinase C- dependent and Ca2+ -dependent changes are involved in Gq11 -mediated RGS2 mRNA regulation, but little is known thus far about the precise mechanisms.

Regulation of protein stability is an alternative way to modulate RGS protein expression levels. Phosphorylation-induced slowing of RGS protein degradation has been demonstrated for some isoforms (eg, RGS13, RGS16 [Y168]). N-end rule of degradation is another important mechanism to regulate cellular RGS protein levels. Whereas several RGS proteins have potentially destabilizing N-terminal residues and are predicted to be degraded by this pathway, only RGS4, RGS5, and RGS16 have been confirmed thus far in vitro and in vivo. Among them, RGS4 is best characterized and can be stabilized by mutations as well as palmitoylation of its N-terminal C2 residue. Potential clinical relevance was suggested by detection of two potentially destabilizing mutations of RGS2 in a group of hypertensive individuals from Japan, one of which (Q2L) showed much reduced protein expression in HEK293 cells that was markedly enhanced by pretreatment with a proteasome inhibitor. Furthermore, proteosomal degradation of RGS4 was recently linked to invasiveness of breast cancer.

Posttranslational Modifications

RGS isoforms from all subfamilies can be phosphorylated by a large variety of kinases (Table 1). Functional effects are diverse and include protein stabilization (see above), changes in subcellular localization (eg, membrane translocation of RGS3, RGS4; nuclear translocation of RGS10 [Z168]), and alterations in GAP activity, which can be either enhanced or reduced depending on RGS isoforms and protein kinases involved. For example, RGS2 phosphorylation by protein kinase C leads to a reduction, whereas cGMP-dependent protein kinase causes an increase. Several RGS proteins are also modified by palmitoylation near the N terminus and/or on conserved cysteine residue in the α4-helix of the RGS domain. Palmitoylation can also affect protein stabilization and membrane and lipid raft targeting (eg, RGS7, RGS16, and RGS19). It generally increases GAP activity, presumably as a result of increased membrane association, but this is not a requirement. Palmitoylation was found to be both constitutive (eg, RGS10) and dependent on GPCR activation (eg, RGS3). The extent to which RGS protein phosphorylation and/or palmitoylation occurs in myocardial cells and its functional consequences have yet to be delineated.

Subcellular Localization

RGS protein location within the cell is diverse and depends on isoform, cell type, and expression level. Although most RGS proteins were predicted to be hydrophilic, many of them can be found to varying degree in the cytosol and in the nucleus. Much information on the subcellular location of RGS proteins has been derived from overexpression studies that may lead to aberrant targeting, but a few reports suggested similar localization for some endogenous RGS proteins. The location of RGS proteins in the cell is in flux and highly regulated. Plasma membrane translocation of RGS proteins can be induced by direct recruitment by Ga-GTP or after GPCR-induced G-protein activation and is facilitated by phosphorylation and palmitoylation, as mentioned above. RGS proteins may not be able to freely interact with every available Ga protein but selectively sorted by GPCRs at the plasma membrane because GPCRs alone or in a concerted effort with their linked G proteins were shown to selectively recruit RGS proteins to the plasma membrane. Mechanisms proposed for nuclear targeting involve regions inside and outside the RGS domain and nuclear targeting/export signals. The function(s) of cytosolic and nuclear RGS proteins if/are not well understood. Sequestration of RGS proteins from G proteins localized at the plasma membrane has been proposed, but additional functions are likely and appear to include regulation of transcription factors/repres- sors (reported for RGS13 and RGS6). RGS protein-mediated regulation of G-protein signaling is also a distinct possibility in light of increasing evidence for nuclear location of functional GPCRs and G proteins as well as intracellular signaling. Many more studies are needed to fully validate novel interactions and putative regulatory roles and to delineate the subcellular localization of RGS proteins and its exact role in mediating canonical and emerging signaling processes in cardiac cells.

Interaction With GPCRs and Other Molecules

Although the R7 and R12 subfamilies of RGS proteins contain multiple well-established protein-protein interaction domains, the structurally simple R4 and RZ RGS proteins with short extensions to the RGS domain also display a remarkable ability to interact with many different binding partners. For example, RGS2 has been shown to interact with GPCRs, AC, cGMP-dependent protein kinase, TRPV channel, and tubulin through distinct regions of its N-terminus. Thus, RGS protein-binding partners are diverse and range from GPCRs, effector proteins (ion channels, enzymes), and kinases to scaffold and other auxiliary proteins, so that only a few examples can be highlighted. Interaction with GPCRs can be direct (eg, through PSD-95 disk-large ZO-1 domains in particular RGS3 or RGS12 splice variants or the N-terminus in R4 RGS proteins) or mediated by scaffolding proteins (such as GAIP-interacting protein C-terminus and spinophilin). Direct evidence for cellular interactions between full-length GPCRs and RGS proteins in living cells has yet to be demonstrated, but many functional studies have shown selective regulation of GPCR signaling, irrespective of the particular G protein coupled, demonstrating the importance of Ga- and GAP-independent mechanisms in determining selectivity of signal regulation. Interactions with several other molecules have been described, each with significant functional implications. For example, RGS3 was shown to interact with the phosphoserine-binding protein 14-3-3 through its N-terminus (S264). Because RGS3 when bound to 14-3-3 is unable to interact with G proteins, it has been proposed that 14-3-3 may act as a scavenger, regulating the amounts of
RGS proteins in the heart, overexpression strategies have been used to determine the functional capacity of cardiac RGS proteins, and, as the prototypical R4 subfamily member, RGS4 initially garnered most attention. Although nonphysiological interactions may occur on overexpression, loss-of-function studies addressing the role of endogenous RGS proteins can be hampered by the presence of different RGS isoforms with potentially overlapping functions, which can result in redundancy and/or compensatory coverage. Several strategies have been used to reduce RGS protein expression and/or function: Specific antibodies were successfully used to disrupt the RGS-Gα interface, whereas antisense oligonucleotides, ribozymes, or RNAi were used to knock down RGS protein expression in vitro. Conventional in vivo gene targeting strategies have been used to generate mouse models with global deletion of select RGS isoforms (Table 2). The Neubig laboratory introduced an elegant alternative approach (Table 2), in which endogenous Gαq/11 isoforms were replaced with a single amino acid point mutation in the Gα switch I region that blocks its interaction with RGS proteins and subsequent GTPase activation. However, it does not affect the intrinsic GTPase activity or coupling to Gβγ, receptors, and downstream effectors. This approach offered novel insight into the full extent of RGS protein–mediated regulation in modulating downstream effects of particular Gα subunits (unencumbered by functional redundancy among RGS) and into subtype-selective signaling by Gαq family members. Compared with transgenic models with Gα overexpression, knock-ins of RGS-insensitive Gα mutants maintain normal Gα expression levels and reveal both Gα- and Gβγ-mediated RGS protein–sensitive responses after GPCR-induced Gα activation. However, they cannot identify the specific RGS protein isoform(s) involved and only probe for RGS protein–mediated GAP activity regulation (and effector blockade). RGS effects that are mediated by their non-RGS domains will not be detected in these models.

RGS Proteins and Pressure Overload–Induced Cardiac Remodeling

Although several RGS proteins are expressed in the heart, the R4 subfamily has thus far been best characterized. The first cardiac mouse model (see Table 2) featured cardiomyocyte-specific transgenic RGS4 expression, which did not affect cardiac morphology or basal function but markedly compromised the heart’s ability to adapt to transverse aortic constriction and ameliorated (although only transiently) hypertrophy and heart failure in Gαq-expressing hearts, suggesting that the antihypertrophic effect of RGS4 could be beneficial or detrimental, depending on the (patho)physiological context. Mechanistic contributions of RGS4 regulation of Gαq and/or Gq/11 pathways were not examined in this model, and the physiological significance is to be viewed in light of subsequent reports on the virtual absence of RGS4 in the working myocardium. Investigation of RGS2 knockout mice first revealed that RGS2 plays a critical role in regulating contractile activity of vascular smooth muscle cells and blood pressure homeostasis, cGMP-dependent protein kinase–mediated RGS2 phosphorylation resulting in enhanced GTPase activity was identified as a key mechanism suppressing Gq-stimulated vascular contraction; an increase in sympathetic tone has been proposed to potentially contribute as well. More recently, RGS2 was shown to be required for early myocardial compensation to pressure overload and as a mediator of antihypertrophic and cardioprotective cGMP-mediated effects of sildenafil, a cGMP-selective phosphodiesterase 5 inhibitor. Similarly, counterregulatory effects of ANF on Ang II–induced hypertrophic effects were shown to be dependent on guanylyl cyclase A receptor, cGMP-dependent protein kinase, and RGS2. A role of RGS5 in protecting against cardiac hypertrophy in response to pressure overload was revealed in mice with cardiосpecific transgenic overexpression or global deletion of RGS5, presumably through regulation of MEK/ERK activation (but not JNK, p38, and Akt).

Although most studies to date have focused on myocyte regulation by RGS proteins, investigations into the role of RGS proteins in fibroblasts are emerging. This is particularly relevant because cardiac fibroblasts are also important therapeutic targets. Exacerbation of pressure overload–induced fibrosis development has been reported for mice with global deletion of RGS5 or RGS2. Both Ang II and endothelin-1 are important profibrotic factors in human cardiac fibroblasts; and their effects are mediated through Gq11-coupled AT1 receptors and ETα receptors respectively. Importantly, RGS2 was recently shown to be a functionally important and highly regulated negative regulator of Ang II–induced signaling, cell proliferation, and collagen in adult ventricular fibroblasts. These studies suggest that RGS protein targeting could become a strategy to modulate cardiac fibroblast responses. However, to establish a direct (patho)physiological role of RGS2, RGS5, and potentially other RGS isoforms in regulating fibroblast behavior and fibrosis in vivo, mouse models with fibroblast-restricted deletions are required. RGS2 and RGS5 are ubiquitously expressed, and changes that occur in fibroblasts must be discerned from those in other cell types. For example, the fact that myocyte-restricted...
RGS5 expression markedly attenuated fibrosis in pressure-overloaded hearts suggests that myocyte-fibroblast cross-talk plays a major role.112 To date, gene-targeting experiments have been hampered by the challenge of identifying fibroblast-specific promoter elements,116 but recent studies have shown promising results.117

**RGS Proteins and Heart Rate Control**

RGS proteins also play an essential role in regulating parasympathetic heart rate regulation, which involves M2-receptor activation of G\(_i/o\), release of G\(\beta\gamma\) with subsequent activation of G-protein–coupled inwardly rectifying K\(^+\) channels, resulting in acetylcholine-activated potassium current (I\(_{\text{KAch}}\)) and membrane hyperpolarization. In addition, vagal stimulation suppresses G\(_i\)–mediated AC activation, thereby reducing binding of cAMP to pacemaker current (I\(_f\)) and protein kinase A phosphorylation increase in L-type calcium channel current (I\(_{\text{Lca1}}\)). The first in vivo evidence was provided in knock-in mice expressing RGS-resistant G\(\alpha_{i2}\), which displayed markedly enhanced carbachol-induced bradycardia118 (Table 2). Direct regulation of cardiac pacemakers was subsequently suggested when isolated, perfused hearts from this model showed potentiation of muscarinic inhibition of cardiac automaticity as well as atrioventricular conduction.119 Comparison of chronotropic responses of cardiomyocytes derived from embryonic stem cells with knock-in of RGS-insensitive G\(\alpha_{i2}\) or G\(\alpha_o\) showed that endogenous RGS modules G\(\alpha_{i/o}\)–coupled receptor signaling (eg, M\(_2\), A\(_1\), and \(\beta_2\) receptors) in a G\(\alpha\)-isoform–specific manner.118

Subsequent RGS isoform–specific knockout models implicated RGS4124 and RGS6120,121 as key regulators of parasympathetic heart rate control, because their loss was associated with severely exaggerated bradycardia and atrioventricular block in response to parasympathetic stimulation in vivo. The underlying mechanisms still must be fully delineated but probably differ: Whereas RGS4 and RGS6 can both negatively regulate G\(\alpha_{i/o}\) subunits, only RGS6 has the capacity to directly interact with G\(\beta_2\) through its G-protein \(\gamma\)-like domain and to form a complex that appears to contribute to the inactivation of I\(_{\text{KAch}}\).120 Both RGS4 and RGS6 were required

**Table 2. Gain- and Loss-of-Function RGS Protein Models and RGS-Insensitive G\(\alpha\) Models and Their Cardiovascular Phenotypes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Model</th>
<th>Cardiovascular Phenotype</th>
</tr>
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<tbody>
<tr>
<td>RGS2</td>
<td>K0</td>
<td>Hypertension and enhanced vasoconstriction due to prolonged G(_s)-mediated signaling and decreased cGMP-mediated relaxation110,111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal basal cardiac phenotype and hypertrophic response to swimming; increased G(_i) signaling and hypertrophy in response to pressure overload with more rapid transition to failure and early mortality; exacerbated hypertrophy and dilution in G(\alpha_i) transgenes; lack of inhibition of G(_i/o)-coupled stimuli and suppression of maladaptive hypertrophy by cGMP-selective PDE5 inhibitor sildenafil119</td>
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<tr>
<td></td>
<td></td>
<td>Enhanced susceptibility to atrial tachycardia/fibrillation through enhanced M(_2)-receptor activity122</td>
</tr>
<tr>
<td>RGS4</td>
<td>TG</td>
<td>No basal phenotype; compromised adaption to pressure overload (rapid decompensation, increased mortality)109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In G(\alpha_i)-expressing transgenes, delay in hypertrophy onset but comparable end-stage hypertrophic phenotype109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced cardiomyopathic phenotype in PPAR(_\alpha) transgenes without change in metabolic abnormalities; resistance to streptozotocin-induced fetal gene induction142</td>
</tr>
<tr>
<td></td>
<td>K0</td>
<td>No basal phenotype; reduced hypertrophic response in mice lacking GC-A receptor143</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viable and fertile; normal neural development; subtle sensorimotor deficits23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased M(<em>2)-mediated bradycardia in conscious mice and perfused hearts; lower baseline heart rate and greater increase in response to atropine in anesthetized mice; in SA nodal cells greater sensitivity to muscarinic inhibition of spontaneous action potential firing rate and decreased level of I(</em>{\text{KAch}}) desensitization as well as slowed activation and deactivation kinetics144</td>
</tr>
<tr>
<td>RGS5</td>
<td>TG</td>
<td>Attenuated hypertrophy and fibrosis response to pressure overload112</td>
</tr>
<tr>
<td></td>
<td>K0</td>
<td>Enhanced hypertrophy and fibrosis development in response to pressure overload112</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viable and fertile; reduced blood pressure and increased heart rate; normal vasculature and remodeling response (to tumor growth and oxygen-induced retinopathy)144</td>
</tr>
<tr>
<td>RGS6</td>
<td>KO</td>
<td>Enhanced carbachol-induced bradycardia (and AV block) in conscious mice and perfused hearts; enhanced muscarinic inhibition of spontaneous action potential firing rate of SA nodal cells; reduction in time course of I(_{\text{KAch}}) activation and deactivation and extent of desensitization in atrial myocytes120,121</td>
</tr>
<tr>
<td>RGS-insensitive G(\alpha)</td>
<td>G(\alpha_{i2}), G184S</td>
<td>Reduced viability, low birth weight, growth retardation, cardiac hypertrophy, and increased baseline heart rate during daytime, enlarged spleen, elevated neutrophil and monocyte counts, behavioral hyperactivity146</td>
</tr>
<tr>
<td></td>
<td>KI</td>
<td>Enhanced muscarinic (but not adenosine-induced) bradycardic responses in intact mice and perfused hearts; delayed AV conduction118,119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smaller infarct size and enhanced contractile recovery after ischemia/reperfusion in perfused hearts; enhanced potency for carbachol’s negative inotropic effect after (\beta)-adrenergic stimulation in ventricular myocytes123</td>
</tr>
</tbody>
</table>

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for desensitization and rapid deactivation as well as normal activation of \( I_{K_{ACV}} \). Importantly, double RGS4 and RGS6 knockout mice are needed to determine whether RGS4 and RGS6 act on the same G proteins mediating G-protein–coupled inwardly rectifying K\(^+\) regulation and whether their effects will be additive. Additional RGS isoforms may be involved in heart rate regulation. For example, enhanced susceptibility to atrial fibrillation, presumably through enhanced M\(_3\) receptor activity, was reported in RGS2 knockout mice.\(^{122}\)

Taken together, gain- and loss-of-function mouse models designed to interrogate RGS protein function in vivo strongly suggest that RGS proteins play important roles in the cardiovascular system in health and disease. To date, several RGS isoforms have been implicated in the regulation of blood pressure (RGS2, RGS5), cardiac automaticity and conduction (RGS4, RGS6, and potentially RGS2), and development of both hypertrophy (RGS2, RGS4, and RGS5) and fibrosis (RGS2 and RGS5) in response to pressure overload. Most recently, RGS proteins were also implicated to suppress Go\(_{12}\)-mediated cardioprotection (Table 2).\(^{123}\) Additional models targeting other RGS isoforms and in a cell-type–specific manner will be required to obtain a comprehensive picture of the functional significance of RGS proteins in regulating GPCR signaling in the heart.

**RGS Proteins as Therapeutic Targets**

GPCRs are a cell surface receptor superfamily, with >800 genes encoding GPCRs in the human genome.\(^{124}\) They regulate virtually all known physiological processes in mammals and are estimated to be the target of approximately one-third of approved drugs.\(^{125}\) In light of the vastly greater number of GPCRs (>200 the heart,\(^{126}\)) compared with G proteins (15 Go, 5 G\(\beta\), and 12 G\(\gamma\) subunits),\(^{127}\) it has long been recognized that many different GPCRs are generally linked to the same G-protein–mediated signaling pathway, but GPCRs can also functionally couple simultaneously with distinct unrelated G proteins, leading to activation of multiple intracellular effectors by a single receptor. Targeting GPCR signaling at the receptor level has yielded substantial therapeutic benefits in the cardiovascular and many other fields, yet heart failure remains a leading cause of death morbidity and mortality in the world. A longstanding alternative strategy to target GPCR signaling at the level of the G proteins has been to mitigate G\(\beta\)\(\gamma\) signaling, initially using large peptide inhibitors and more recently small molecule inhibitors.\(^{128}\) As key regulators of G-protein signaling, RGS proteins have emerged as intriguing additional therapeutic targets, based on their physiological and pathophysiological importance in the heart, central nervous system, cancer biology, and beyond.

Therapeutic benefits can be derived from inhibition or enhancement of RGS protein function, depending on the nature of the targeted isoform, its regulatory function, and the cellular and pathophysiological context. Conceptually, RGS protein inhibitors potentiate GPCR agonist function, which would be useful for rapidly desensitizing agonists as well for minimizing GPCR agonist dosage and its side effects when given as a drug. RGS protein inhibitors could also increase the specificity of exogenous GPCR agonists, and, in addition, block effector signaling by RGS proteins. In contrast, enhancing RGS protein function could be beneficial in settings where reduction in RGS protein expression or activity is associated with pathophysiological consequences. For example, marked reduction in RGS2 in response to pressure overload and other settings with enhanced Go\(_1\) signaling is known to exacerbate myocyte and cardiac hypertrophy.\(^{26,70}\) Similarly, diminished RGS2 expression is associated with hypertension in mice\(^{129}\) and humans,\(^{129}\) whereas RGS2 levels are increased in patients with Bartter/Gitelman syndrome, which is associated with reduced Ang II signaling and vasomotor tone.\(^{130}\) Furthermore, single-nucleotide polymorphisms identified in Japanese patients with hypertension were shown to be less stable or lead to reduced plasma membrane targeting and function.\(^{10}\)

On the basis of current knowledge of RGS protein structure and function, strategies to target RGS protein function include altering GAP activity, steady-state expression, protein or lipid interactions, posttranslational modifications, and/or subcellular location. Most targeting efforts to date have focused on RGS4 as one of the best characterized isoforms. A number of peptide and small-molecule inhibitors targeting RGS-G\(\alpha\) interaction were identified through high-throughput screening,\(^{131–133}\) as summarized in an excellent review that also provides an overview of the strengths and limitations of the assay systems used in the quest for drugs targeting RGS proteins.\(^{134}\) As reviewed in detail elsewhere,\(^{73}\) the mechanism of action of one of the first RGS4 inhibitors (CCG-4986) involves covalent cysteine modifications, one of which occurs in the RGS/G\(\alpha\) interaction surface (aka “A site”), whereas the other functionally more important one is located on the opposite face of RGS (near the “B site”) and leads to allosteric inhibition of RGS-G\(\alpha\) interaction.\(^{136}\) Whereas CCG-4986 binds irreversibly and cannot function in cellular environment, another reversible small molecule inhibitor for RGS4 was recently introduced, which leads to similar allosteric inhibition.\(^{137}\) Encouraging for further drug development and a prerequisite for ultimate therapeutic utility is the fact that closely related RGS isoforms with similar sequence and structure have different responsiveness to these inhibitors. Regardless of the mechanism, disruption of RGS/G\(\alpha\) binding and subsequent inhibition of GAP function is expected to enhance both G\(\alpha\)- and G\(\beta\)\(\gamma\)-mediated effects. GAP-independent RGS protein effects that are mediated through regions outside the RGS domain could be targeted as well, particularly for isoforms with well-characterized protein–protein interaction sites (eg, RGS2 and AC\(^{26}\) and RGS3 and Smad\(^{42}\)). Stabilizing RGS protein expression is another potential strategy to enhance RGS protein function, which would affect GAP-dependent and GAP-independent RGS protein effects. This could be achieved for R7 subfamily members by disrupting the interaction between their G-protein \(\gamma\)-like domain and G\(\beta\)\(\gamma\), which is required for stable expression.\(^{48}\) Preventing proteosomal degradation could be another approach, particularly for isoforms that are subject to the N-end rule pathway. Intriguingly, progressive increase in invasiveness in human breast cancer was shown to be tightly linked to gradual reduction in RGS4 protein (but not mRNA), due to enhanced proteosomal degradation.\(^{125}\)
Taken together, RGS proteins are clearly promising targets for therapeutic development. Like many GPCRs, several RGS isoforms are ubiquitously expressed. Unlike GPCR agonists/antagonists that act on the extracellular cell surface, targeting of RGS proteins requires cell-permeable compounds. Despite the significant progress already made, much work still must be done to develop strategies that can eventually be used successfully in vivo. At this stage, computer predictions of potential drug-binding pockets indicate that the back side of the RGS domain opposing the Go interaction site may be more favorable to small-molecule inhibition. Interestingly, competitive binding of PIP3 and Cα2+/CaM with implications for GAP functions (inhibited versus no effect, respectively; see above) as well as palmitoylation leading to GAP inhibition occur in that region. In contrast to the well-characterized structure of the RGS domain, little is currently known about the structure of the other domains in the N- and C-terminal extensions of RGS proteins, which could offer additional sites of intervention. Compounds that stabilize protein expression of specific RGS protein isoforms are also believed to have significant potential.

Conclusions and Future Perspective
The importance of GPCR signaling for determining cardiac differentiation, growth, contraction, and heart rate regulation has been recognized for a very long time. After their discovery in the mid 1990s, RGS proteins were quickly appreciated as key players in the regulation of GPCR signaling. Of the 20 canonical RGS proteins, many isoforms have been detected in the heart, with a specific complement for each cell type, as shown for cardiac myocytes and fibroblasts. Many studies have been performed in various cell lines as well as primary cells from the brain and cardiovascular system, each focusing on one or a few RGS proteins. They have provided a wealth of information into the function of RGS proteins as modulators and integrators as G-protein signaling. Unfortunately, it is not possible to extrapolate from one cell type to another because of the complex expression, regulatory, and interaction patterns of RGS proteins with other molecules that is unique to each cell type. Studies investigating the role of RGS proteins (primarily a few isoforms from the R4 subfamily and more recently also RGS6) have demonstrated the central importance of cardiac RGS proteins in regulating myocyte function in vitro and in vivo. New evidence suggests that RGS proteins may also be important regulators of cardiac fibroblast function. Several important questions must be addressed. For example, what is the functional role(s) of each RGS isoform expressed in the two major cell types in the myocardium? Investigating RGS proteins individually (and in each cell type) is obviously a daunting task. The RGS-insensitive Go mutants will continue to be an essential tool to investigate global RGS protein-mediated inhibition of Go-mediated signaling in cells and animal models, and expansion to Go subunits beyond Go12 and Goi6 is eagerly anticipated. Nevertheless, identifying (the) particular RGS isoform(s) that regulate(s) specific cell signaling and functional responses will require targeted deletion of individual RGS proteins, ideally in a cell type–specific manner. The roles of RGS proteins in the other myocardial cell types that participate in maintaining normal cardiac function and determine the response to stress (eg, endothelial cells and inflammatory cells) also must be addressed. Collectively, future investigations in these areas will advance our understanding of the physiological role of RGS proteins in regulating signal transduction and cell functions in the heart as well as their contributions to the development of cardiovascular disease. Studies in larger animal models and healthy and diseased human hearts will be essential for clinical translation.

A variety of mechanisms (such as GTPase acceleration, posttranslational modifications, protein-protein/lipid interactions, and spatiotemporal-specific expression) are believed to enable RGS proteins to serve effectively as multifunctional signal regulators. This is evident by the fact that despite functional redundancy in vitro, specificity in RGS protein–mediated regulation of signal transduction and cellular function exists in cellular context and in vivo. Because many of the regulatory mechanisms were discovered in biochemical or overexpression studies, it must be determined which of them are of functional relevance under physiological conditions and what the mechanisms controlling them are. Furthermore, it is not clear at this point what regulatory mechanisms play a role in human disease and if and how they can be targeted therapeutically. To obtain insights into the regulation of endogenous RGS proteins at the protein level in primary cardiac cells and tissue, the sensitivity for RGS protein detection must be increased. Additional very useful reagents will be RGS isoform–specific inhibitors/enhancers, because they will open avenues for mechanistic studies akin to the way GPCR agonists/antagonists facilitated research into GPCR function. Although developing these reagents is a challenging task, substantial progress has already been made. With regard to the potential therapeutic use of RGS protein inhibitors or enhancers, it is hoped that further development of reversible small molecules or other compounds and validation of their properties in cells and animal models will eventually allow investigators to test the potential of targeting RGS protein expression and/or activity in vivo for the treatment of cardiac hypertrophy, heart failure, and/or heart rate irregularities.

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330


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Peng Zhang and Ulrike Mende

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