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Regulators of G Protein Signaling in the Heart and their Potential as Therapeutic Targets:

Zhang and Mende RGS Proteins in the Heart

Peng Zhang, MD[1] and **Ulrike Mende, MD**[1]

[1]Cardiovascular Research Center, Cardiology Division, Rhode Island Hospital and Alpert Medical School of Brown University, Providence, RI

Abstract

Signal transduction via 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 biologic 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 exist 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 and diseased heart. In addition, we discuss their potential and promise as therapeutic targets as well as strategies to modulate their expression and function.

Keywords

RGS proteins; signal transduction; myocardium; cardiac myocytes; cardiac fibroblasts

1. Introduction

Signal transduction via G protein-coupled receptors (GPCRs) is essential for the regulation of cardiovascular function, including heart rate, growth, contraction, and vascular tone. Pertubations in GPCR signaling have pathophysiological consequences and are major

Corresponding Author: Ulrike Mende, MD Cardiovascular Research Center Rhode Island Hospital & Alpert Medical School of Brown University Coro West Building (5th floor, room 5105) 1 Hoppin Street, Providence, RI 02903 Phone: (401) 444-9854 Fax: (401) 444-9203 ulrike_mende@brown.edu.

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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 Gα 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. Upon 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*. While some effector molecules (e.g., phospholipase C β_1 [2]) were found to act as GTPase-activating proteins (GAPs), the discovery of RGS proteins introduced a new large and diverse protein family that leads to pronounced (up to 2000-fold) acceleration of Gα GTPase activity [3, 4], which decreases the amplitude and duration of both Gα- and Gβγ-mediated downstream signaling. Structures derived from NMR and x-ray crystallography of the RGS domain, both alone or bound to Gα subunits (in the presence of GDP and AlF_4^- to mimic the γ-phosphate of GTP in its transition state) provide mechanistic insight into RGS protein/Gα subunit interactions (for details see [5, 6]). Binding of RGS proteins to activated Gα can also antagonize effector activation and thereby block Gα-mediated signal generation. It is generally not possible to distinguish whether RGS protein-mediated signal inhibition is due to GAP activity and/or effector antagonism, unless constitutively active GTPase-deficient Gα subunits are used for signal activation [7].

The RGS protein superfamily is divided into subfamilies based on sequence homology within the RGS domain and the nature and identity of non-RGS domains that facilitate protein-protein interactions, target specificity, protein stability and subcellular location (Table 1). Twenty canonical RGS proteins in 4 subfamilies share the prototypical RGS domain (app. 130 amino acids) that binds to GTP-bound Gα subunits. Nineteen other “RGS-like” proteins (i.e., GRKs, RhoGEFs, axins, D-AKAP2, nexins, RGSL) contain a RGS protein homology domain. Only some of them have been shown to interact with Gα subunits, and their GAP activity is much weaker than that of canonical RGS. Their structure and function was recently reviewed [8].

In the present review, we focus on the expression pattern, regulation and functional role of canonical RGS proteins in the healthy and 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]. Due to 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 cancer [15] for a broader view on the importance of RGS proteins in regulating GPCR signaling and function in health and disease.

2. RGS Protein Expression in the Heart

Several canonical RGS proteins are expressed in the mammalian and human myocardium [16-18] as well as in cardiac myocytes [19, 20] (see Table 1). RGS protein expression in non-myocytes has long been suggested [19], but was only recently reported for cardiac fibroblasts [21]. A comprehensive, reverse transcription (RT)-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 hybridizations and PCR analyses. Discrepancies between mRNA and protein levels have been reported (e.g., 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 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 [26, 27]. 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.

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 need to 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.

3. RGS Protein Subfamilies: Structural and Functional Properties

Most ***R4 subfamily members*** (for RGS3 see below) are “small” RGS proteins with short N- and C-terminal extensions to the conservative RGS core domain. They are mostly non-discriminatory in their binding to and GAP activity for all $G_{i/o}$ and $G_{q/11}$ family members. Only RGS2 generally has been considered to be selective in negatively regulating $G_{q/11}$, which has been attributed to the geometry of a Gα binding pocket that is unfavorable to $G\alpha_{i/o}$ [28]. The structural determinants were recently pinpointed to three evolutionary highly conserved amino acids [29], leading the authors to speculate that RGS2 arose from the R4 subfamily to have specialized $G\alpha_{q/11}$ GAP activity to modulate cardiovascular function. Indeed, in adult rat cardiomyocytes, RGS2 negatively regulates $G_{q/11}$ but not $G_{i/o}$-mediated signaling [30]. Nevertheless, it has been reported that RGS2 interactions with $G\alpha_{i/o}$ may occur dependent on receptor-mediated Gα activation [31, 32], so that lack of interaction between recombinant RGS2 and $G_{i/o}$ [33, 34] 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 β_2-receptor mediated G_i signaling was recently demonstrated [35]. RGS2 was also shown to directly interact with and negatively regulate select adenylate cyclase (AC) isoforms (including the major cardiac isoforms ACV and ACVI) [36, 37]. $G\alpha_s$ interaction albeit without GAP activity was reported as well [38]. However, these studies were performed in HEK293 and other non-cardiac cells. In adult rat ventricular myocytes, RGS2 overexpression did not affect forskolin- or isoproterenol-induced cyclic AMP (cAMP) generation [30], 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 [39].

RGS3, which exists in several splice variants (reviewed in [17]), 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 [40] and has the ability to switch $G_{i/o}$-coupled muscarinic and adenosine receptor-induced signaling from Rac1 to RhoA activation [41]. However, the switch is highly dependent on the expression level of endogenous RGS3L, which is

markedly down-regulated by fibroblast growth factor 2. This mechanism could be of pathophysiological significance in the heart, but has so far only been demonstrated in H10 cells. The N-terminus of RGS3 can also interact with Smad2, Smad3 and Smad4 via their Mad homology 2 domain and inhibit Smad-mediated gene transcription by preventing Smad3/Smad4 heteromerization [42]. RGS3-Smad interaction has been shown to inhibit TGFβ induced differentiation of pulmonary fibroblasts [42], and may potentially 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 (PI3K). Subsequent inhibition of PI3K activity by inhibiting p85-Gab1/2 interactions has been shown for RGS13 in mast cells [43] and RGS16 in breast cancer cells [44]. Investigations of potential RGS protein regulation of cardiac PI3K are warranted in light of its importance in modulating cell survival, growth, contractility, and metabolism [45]. Furthermore, RGS13 also acts as a nuclear repressor of cAMP response element binding protein (CREB) in B lymphocytes that inhibits CREB-dependent transcription through disruption of promoter complexes [46].

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 (reviewed in [12]). However, a key role of RGS6 in the heart was recently discovered (see section 5 below). Through their RGS domain, R7 RGS proteins exert GAP activity primarily on $G\alpha_{i/o}$ proteins [47]. They also contain a G protein gamma-like (GGL) domain that is structurally homologous to conventional Gγ subunits but binds only with the most distant member of the Gβ family ($G\beta_5$), an interaction that is essential for the stability and expression of all R7 RGS proteins [48]. Beyond protecting R7 RGS proteins from proteolysis, the role of $G\beta_5$ is not fully understood. It is believed to participate in determining G protein selectivity and GAP properties. The crystal structure of RGS9-$G\beta_5$ offers some insight into potential mechanisms [49]. The N-terminus of R7 RGS proteins also contains Disheveled-EGL10-Pleckstrin homology (DEP) and DEP helical extension (DHEX) domains that mediate interactions with membrane anchor proteins (i.e., RGS9 anchor protein [R9AP] and RGS7 family binding protein [R7BP]), which both play key roles in determining the catalytic activity, subcellular localization and R7 RGS protein expression levels (reviewed in [12, 50]).

The ***R12 subfamily*** is comprised 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 $G\alpha_{i/o}$, and $G\alpha_{q/11}$ and is phosphorylated by protein kinase A (PKA) [51]. RGS12 and RGS14 are GAPs for $G\alpha_{i/o}$ only. In addition to binding to activated $G\alpha_{i/o}$ in its activated state through their RGS domain, they can bind GDP-bound $G\alpha_{i1-3}$ via their C-terminal GoLoco domain and act as GDP-dissociating inhibitors (GDI) [52]. Inhibition of GDP-to-GTP exchange and subsequent Gα 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/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 (RBD) and for RGS12 its additional PSD-95 disk-large ZO-1 (PDZ) and phosphotyrosine binding (PTB) domains [53-55]. However existence of these mechanisms in cardiac cells remains to be investigated.

Members of the ***RZ subfamily*** (reviewed in [56]) are short in size and share a N-terminal cysteine string motif, which presumably provides substrate for palmitoylation for each isoform (reported so 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 PDZ-binding motif (PM) that facilitates binding to a scaffolding protein (GIPC, GAIP-interacting protein C-terminus) that assembles receptors and signaling molecules and may promote crosstalk between G protein and non-G protein signaling pathways (reviewed [57]). The RZ subfamily was originally named because RGS20 (originally known as RGSZ1) was found to selectively accelerate GTP hydrolysis of $G\alpha_z$, a more distant member of the $G\alpha_{i/o}$ family that also inhibits AC and activates potassium channels. In contrast, RGS17 and RGS19 (aka GAIP) have GAP activity for all $G_{i/o}$ α subunits (and $G\alpha_{q/11}$ for RGS19). Although RGS17 is not a $G\alpha_{q/11}$GAP *in vitro*, it can bind and inhibit $G_{q/11}$-mediated signaling in the cellular context through a yet undetermined mechanism [58]. In the same study, despite its GAP selectivity, RGS20 blocked $G\alpha_{i/o}$ 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 $G_{i/o}$ and $G_{q/11}$ families. It is generally believed that they do not serve as GAPs for $G\alpha_s$; evidence to the contrary regarding RGS-PX1 [59] has yet to be confirmed. GAP activity for $G\alpha_{12/13}$ is displayed only by non-canonical “RGS-like” RhoGEFs, which are also their effectors (reviewed in [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 non-discriminatory towards 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.

4. 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 three 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 $G_{q/11}$ 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, promoter 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 hypertrophy (e.g., [63, 64]) and in humans (e.g., [16, 65]) may be due to species- and model-specific differences (reviewed in [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 $G_{q/11}$ signaling pathway, RGS2 mRNA is transiently up-regulated in both cardiac myocytes [30, 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 $G_{q/11}$ 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 $G_{q/11}$- and G_s-mediated signaling pathways. While 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 [39]. Importantly, in contrast to acute stimulation, marked RGS2 down-regulation has been discovered in ventricles subjected to pressure overload, myocytes from mice expressing constitutively active $G\alpha_q$* [26] as well as myocytes and fibroblasts from rats subjected to prolonged angiotensin (Ang II) infusion *in vivo* [21] and has been implicated in exacerbating cardiac remodeling in the stressed or injured hearts [21, 26, 70]. Protein kinase C (PKC)- and Ca^{2+}-dependent changes are involved in $G_{q/11}$-mediated RGS2 mRNA regulation, but little is known so far about the precise mechanisms [68].

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 (e.g., RGS13 [71], RGS16 [Y168] [72]). N-end rule of degradation is another important mechanism to regulate cellular RGS protein levels (reviewed in [73]). While 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 so far *in vitro* [74] and *in vivo* [75]. Among them, RGS4 is best characterized and can be stabilized by mutations [74] as well as palmitoylation [76] 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 [77], one of which (Q2L) showed much reduced protein expression in HEK293 cells that was markedly enhanced by pre-treatment with a proteasome inhibitor [78]. Furthermore, proteosomal degradation of RGS4 was recently linked to invasiveness of breast cancer [25].

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 (e.g., membrane translocation of RGS3, RGS4, [79]; nuclear translocation of RGS10 [51]) 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 PKC leads to a reduction [80], whereas cGMP-dependent protein kinase (PKG) causes an increase [81]. 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 [82]. Palmitoylation can also affect protein stabilization and membrane and lipid raft targeting (e.g., RGS7 [83], RGS16 [84], RGS19 [85]). It generally increases GAP activity, presumably as a result of increased membrane association, but this is not a requirement [86]. Palmitoylation was found to be both constitutive (e.g. RGS10) and dependent on GPCR activation (e.g., RGS3) [87]. 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 (reviewed in [88, 89]). 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 (for specific examples, see [88, 89]). Plasma membrane translocation of RGS proteins can be induced by direct recruitment by Gα-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 Gα protein, but selectively sorted by GPCRs at the plasma membrane, since GPCRs alone or in a concerted effort with their linked G proteins were shown to selectively recruit RGS proteins to the plasma membrane [90]. 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 is/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/repressors (reported for RGS13 [46] and RGS6 [91]). 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 (e.g., [92, 93]) as well as intracrine signaling (reviewed in [94, 95]). 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, PKG, TRPV channel, and tubulin via distinct regions of its N-terminus (reviewed in [96]). Thus, RGS protein binding partners are diverse and range from GPCRs, effector proteins (ion channels, enzymes) and kinases to scaffold and other auxilliary proteins (reviewed in [57]), so that only a few examples can be highlighted. Interaction with GPCRs (reviewed in [97]) can be direct (e.g., via PDZ domains in particular RGS3 or RGS12 splice variants or the N-terminus in R4 RGS proteins) or mediated by scaffolding proteins (such as GIPC 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 (e.g., [98]), demonstrating the importance of Gα- 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 via its N-terminus (S264). Since 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 RGS3 available for binding G proteins [99]. Another important binding partner for several RGS isoforms (best characterized for RGS4) is the calcium sensor calmodulin (Ca^{2+}/CaM), which binds to the well conserved α4 and α5 helices in the RGS domain without affecting GAP activity; however, Ca^{2+}/CaM competes with phosphatidylinositol 3,4,5-trisphosphate (PIP_3) binding to the same region, and PIP_3 inhibits GAP function [100]. Therefore, by relieving PIP_3-mediated inhibition of RGS proteins, Ca^{2+}/CaM promotes RGS-mediated inhibition of effector function. Ca^{2+}/CaM-dependent facilitation of RGS protein action has so far been

demonstrated for the modulation of intracellular Ca^{2+} oscillations in polarized cells [101] and voltage-dependent relaxation of I_{KAch} (reviewed [102]). Furthermore, direct binding of RGS2 to eIF2ε (eukaryotic initiation factor 2B ε subunit) via a 37 amino stretch within its RGS domain has been linked to inhibition of protein translation, implicating RGS2 as a novel regulator of protein translation [103].

5. Functional Role of RGS Proteins in the Heart

Experimental Strategies

Since the discovery 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. While non-physiological interactions may occur upon 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 utilized to reduce RGS protein expression and/or function: specific antibodies [37] or inhibitory RGS peptides [104] were successfully used to disrupt the RGS-Gα interface, while anti-sense oligonucleotides [105], ribozymes [98], or RNAi [26] were used to knock-down RGS protein expression *in vitro*. Conventional *in vivo* gene targeting strategies have been employed 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 $G\alpha_{i/o}$ 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 [106]. However, it does not affect the intrinsic GTPase activity or coupling to Gβγ, receptors, and downstream effectors [107]. 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 $G_{i/o}$ family members. Compared to 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 upon GPCR-induced Gα activation. However, they cannot identify the specific RGS protein isoform(s) involved and only probes for RGS protein-mediated GAP activity regulation (and effector blockade). RGS2 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 so 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 [108] and ameliorated (although only transiently) hypertrophy and heart failure in $G\alpha_q$-expressing hearts [109], suggesting that the anti-hypertrophic effect of RGS4 could be beneficial or detrimental depending on the (patho)physiological context. Mechanistic contributions of RGS4 regulation of $G_{i/o}$ and/or $G_{q/11}$ 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 [23, 24]. 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 [81, 110]. PKG-mediated RGS2 phosphorylation resulting in enhanced GTPase activity was identified as a key mechanism suppressing G_q-stimulated vascular contraction [81]; an increase in sympathetic tone has been proposed to potentially contribute as well [111]. More recently, RGS2 was shown to be required for early myocardial compensation to pressure overload and as a

mediator of anti-hypertrophic and cardioprotective cGMP-mediated effects of sildenafil, a cGMP-selective phosphodiesterase (PDE) 5 inhibitor [70]. Similarly, counter-regulatory effects of ANF on Ang II-induced hypertrophic effects were shown to be dependent on guanylyl cyclase A (GC-A) receptor, PKG and RGS2 [27]. A role of RGS5 in protecting against cardiac hypertrophy in response to pressure overload was revealed in mice with cardiac-specific transgenic overexpression or global deletion of RGS5, presumably via regulation of MEK/ERK activation (but not JNK, p38 and Akt) [112].

While 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, since cardiac fibroblasts are also important therapeutic targets [113]. Exacerbation of pressure overload-induced fibrosis development has been reported for mice with global deletion of RGS5 [112] or RGS2 [70]. Both Ang II and endothelin-1 are important profibrotic factors in human cardiac fibroblasts; and their effects are mediated via $G_{q/11}$-coupled AT_1 receptors [114] and ET_A receptors [115], 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 [21]. These studies suggest that RGS protein targeting could become a strategy to modulate cardiac fibroblast responses. However, in order 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 myocyte-fibroblast crosstalk to play 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 (e.g., [117]).

RGS Proteins and Heart Rate Control

RGS proteins also play an essential role in regulating parasympathetic heart rate regulation, which involves M_2-receptor activation of $G_{i/o}$, release of Gβγ with subsequent activation of G protein-coupled inwardly rectifying K^+ (GIRK) channels, resulting in acetylcholine-activated potassium current (I_{KAch}), and membrane hyperpolarization. In addition, vagal stimulation suppresses G_s-mediated AC activation, thereby reducing binding of cAMP to pacemaker current (I_f) and PKA-phosphorylation increase in L-type calcium channel current ($I_{Ca\text{-}L}$). The first *in vivo* evidence was provided in knock-in mice expressing RGS-resistant $G\alpha_{i2}$, which displayed markedly enhanced carbachol-induced bradycardia [118] (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 modulate $G_{i/o}$-coupled receptor signaling (e.g., M_2, A_1 and β_2 receptors) in a Gα isoform-specific manner [118].

Subsequent RGS isoform-specific knockout models implicated RGS4 [24] and RGS6 [120, 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 need to be fully delineated but likely differ: while RGS4 and RGS6 can both negatively regulate $G\alpha_{i/o}$ subunits, only RGS6 has the capacity to directly interact with $G\beta_5$ via its GGL domain and to form a complex that appears to contribute to the inactivation of I_{KAch} [120]. Both RGS4 and RGS6 were required for desensitization and rapid deactivation as well as normal activation of I_{KACh}. Importantly, double RGS4 and RGS6 knockout mice are needed to determine

whether RGS4 and RGS6 act on the same G proteins mediating GIRK 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 via 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, RGS5) and fibrosis (RGS2, RGS5) in response to pressure overload. Most recently, RGS proteins were also implicated to suppress $G\alpha_{i2}$-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.

6. RGS Proteins as Therapeutic Targets

GPCRs are a cell surface receptor superfamily with more than 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 to G proteins (15 Gα, 5 Gβ and 12 Gγ 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 long-standing alternative strategy to target GPCR signaling at the level of the G proteins has been to mitigate Gβγ signaling, initially using large peptide inhibitors and more recently small molecule inhibitors (reviewed in [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 G_q signaling is known to exacerbate myocyte and cardiac hypertrophy [26, 70]. Similarly, diminished RGS2 expression is associated with hypertension in mice [110] and humans [129], whereas RGS2 levels are increased in patients with Bartter's/Gitelman's 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 (reviewed in [10]).

Based on 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-Gα interaction were identified via high-throughput screening [131-133], as summarized by [134], 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. 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/Gα interaction surface (aka "A site", [135]), 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-Gα interaction [136]. While 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/Gα binding and subsequent inhibition of GAP function is expected to enhance both Gα and Gβγ-mediated effects. GAP-independent RGS protein effects that are mediated via regions outside the RGS domain could be targeted as well, particularly for isoforms with well characterized protein-protein interaction sites (e.g., RGS2 and AC [36], RGS3 and Smad [42]). Stabilizing RGS protein expression is another potential strategy to enhance RGS protein function, which would affect GAP-dependent and -independent RGS protein effects. This could be achieved for R7 subfamily members by disrupting the interaction between their GGL domain and $G\beta_5$, 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 be tightly linked to gradual reduction in RGS4 protein (but not mRNA) due to enhanced proteosomal degradation [25].

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 needs to be done to develop strategies that can eventually be used successfully *in vivo*. At this stage, computer predictions of potential drug binding pockets indicate the back side of the RGS domain opposing Gα interaction site may be more favorable to small molecule inhibition [134]. Interestingly, competitive binding of PIP_3 and Ca^{2+}/CaM [138] with implications for GAP functions (inhibited vs. 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.

7. 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 due to 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 need to be addressed. For example, what is/are 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 Gα mutants will continue to be an essential tool to investigate global RGS protein-mediated inhibition of Gα-mediated signaling in cells and animal models, and expansion to Gα subunits beyond $G\alpha_{i2}$ and $G\alpha_o$ 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 (e.g., endothelial cells and inflammatory cells) also need to 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*. Since many of the regulatory mechanisms were discovered in biochemical or overexpression studies, it must be determine 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. In order 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, failure and/or heart rate irregularities.

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Non-standard Abbreviations and Acronyms

AC	Adenylate cyclase
Ang II	Angiotensin II
Ca^{2+}/CaM	Calcium Calmodulin
CREB	cAMP response element binding protein
DEP	Disheveled-EGL10-Pleckstrin homology domain
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
GAIP	Gα interacting protein
GAP	GTPase-activating protein
GEF	Guanine nucleotide exchange factor
GIPC	GAIP-interacting protein C-terminus
GIRK	G protein-coupled inwardly rectifying K^+
GGL	Gγ-like domain
GoLoco	$G\alpha_{i/o}$-Loco
GPCR	G protein-coupled receptor
MEK	MAPK/ERK kinase
PDE	Phosphodiesterase
PDZ	PSD-95 disk-large ZO-1 domain
PI3K	Phosphatidylinositol-3-OH kinase
PIP_3	Phosphatidylinositol 3,4,5-trisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
PM	PDZ docking motif
PTB	Phosphotyrosine binding domain
RGS	Regulators of G protein signaling

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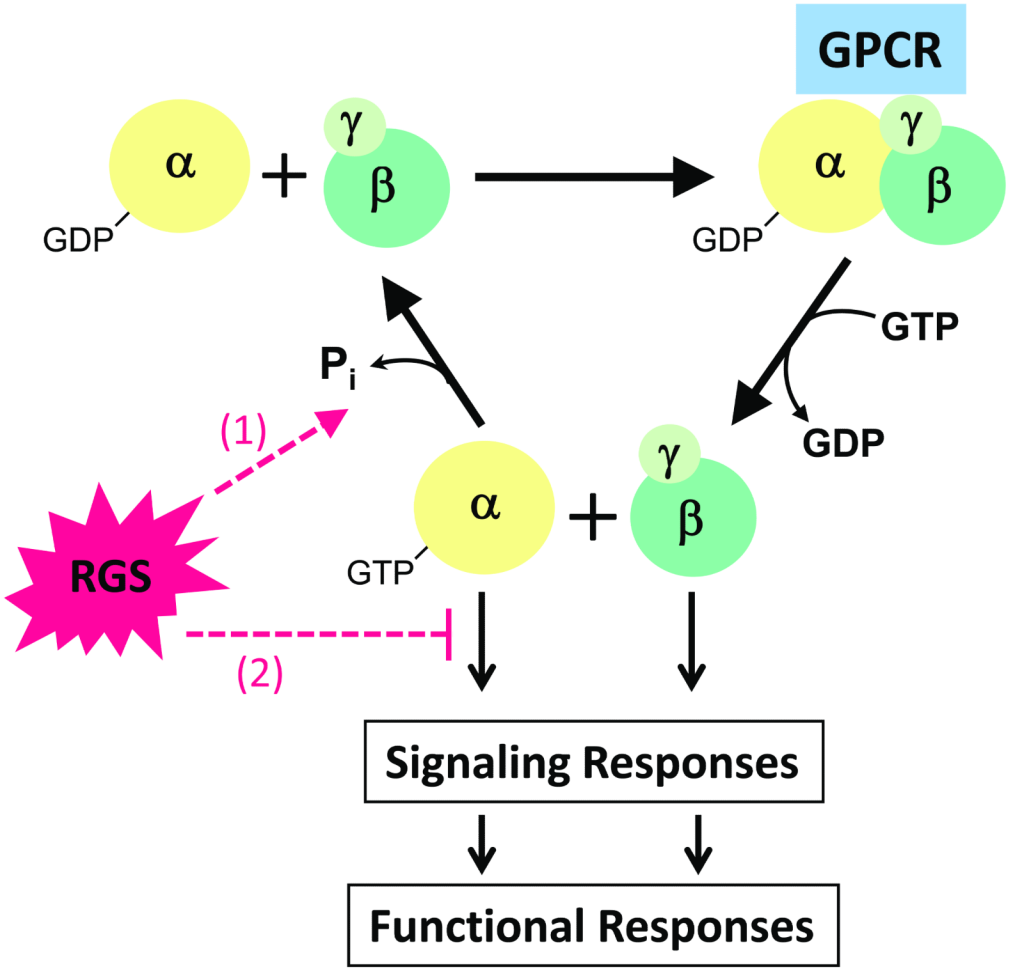

Figure 1. Regulation of G Protein-mediated Signaling by RGS proteins

See text for detail on the G protein activation/inactivation cycle. RGS proteins regulate G protein-mediated signaling via (1) marked acceleration of Gα GTPase activity, which decreases both Gα- and Gβγ -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 cartoon depicts the traditional view of GPCR-induced, G protein-mediated signal transduction. It does not incorporate GPCR-independent G protein activation (reviewed in [147]) or G protein-independent GPCR effects (reviewed in [148]). Furthermore, full dissociation of Gα and Gβγ subunits may not be required to trigger downstream effects (e.g., [149]).

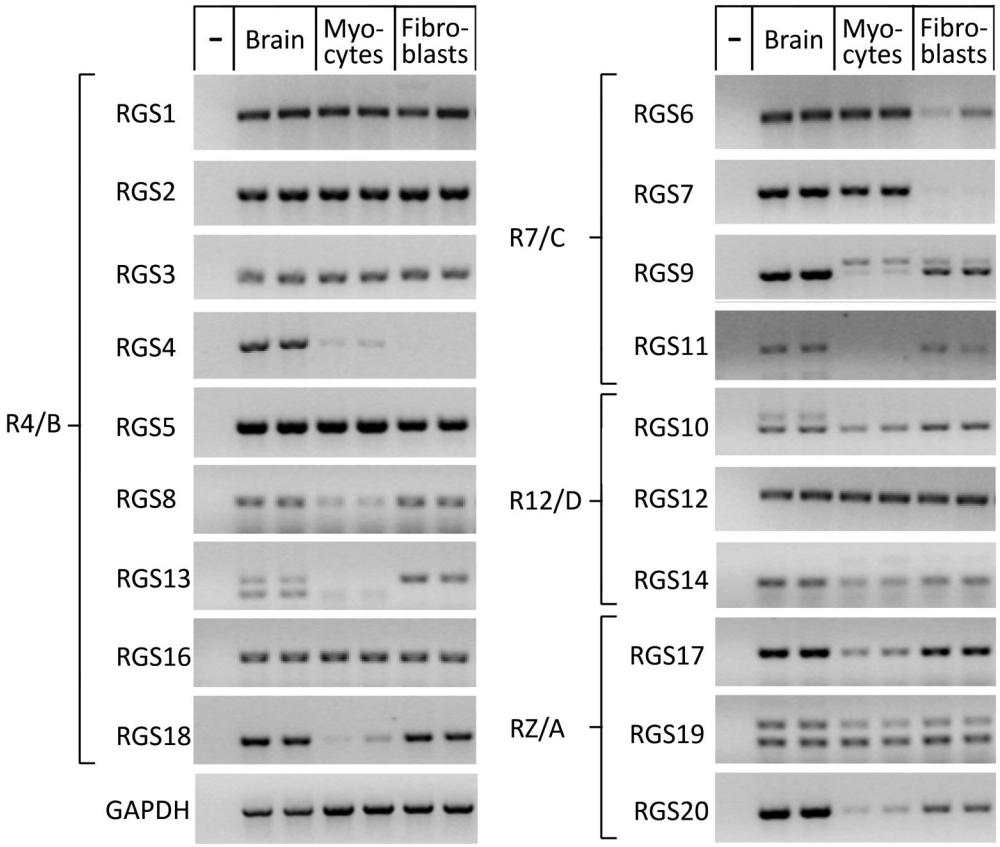

Figure 2. RGS mRNA Expression Profile in Adult Rat Ventricular Myocytes and Fibroblasts

Reverse transcription (RT) PCR analysis of freshly isolated ventricular myocytes and fibroblasts from male Sprague-Dawley rats (5 weeks old). Rat brain was used for comparison. Total RNA (1 μg) was reverse-transcribed and amplified using SuperScript One-Step RT-PCR Kit (Invitrogen, Carlsbad, CA) with RGS isoform-specific primers (primer sequences and PCR conditions available upon request). (−) denotes absence of template. GAPDH was used as internal control. RT-PCR products were visualized on ethidium bromide stained agarose gels. RGS are organized according to subfamily affiliation.

Table 1

Structure, GAP Function, Posttranslational Modifications and Cardiac Expression of Canonical RGS Proteins

* Sub-family	Gene†	Size	Non-RGS Domains		GAP for	Post-Transl. Modifications		Ventricles			Atria	
		a. acids	N-term.	C-term.		Phosphorylation	Palmitoyl.	Tissue ‡	CM §	CF §	Tissue !!	CM !!
R4 (B)	RGS1 †	209	A. helix	None	$G_{i/o}$ and $G_{q/11}$			(+)	+	+	–	–
	RGS2	211			$Gq/11 \ll (G_{i/o})$	S46/64/73 (PKG) ? (PKC)	C106, C116, C199	+	+	+	+	+
	RGS3 †	519			$G_{i/o}$ and $G_{q/11}$	? (PKG) S264 (PKA)	+	+	+	+	+	+
	RGS4 †	205			$G_{i/o}$ and $G_{q/11}$	S52 (PKA, PKG)	C2, C12, C95	(+)	(+)	–	+	+
	RGS5 †	181			$G_{i/o}$ and $G_{q/11}$	S166 (PKC) (S84?)		+	+	+	+	–
	RGS8 †	180			$G_{i/o}$ and $G_{q/11}$			–	(+)	+	–	–
	RGS13	159			$G_{i/o}$ and $G_{q/11}$	T 41 (PKA)		–	–	+	–	–
	RGS16	202			$G_{i/o}$ and $G_{q/11}$	Y168 (EGFR, src, Lyn kinase) S194/S53 (?)	C2, C12, C98	+	+	+	+	–
	RGS18	235			$G_{i/o}$ and $G_{q/11}$	S49 (?)		(+)	(+)	+	+	–
	RGS21	152	None	None	ND			ND	ND	ND	ND	ND
R7 (C)	RGS6 †	472	DEP GGL	None	$G_{i/o}$			+	+	+	+	+
	RGS7 †	487			$G_{i/o}$	S434 (PKCα)	C60, C133	–	+	–	–	–
	RGS9 †	484			$G_{i/o}$	S427/428 (PKA) S475 (PKC)		(+)	(+)	+	–	–
	RGS11 †	446			$G_{i/o}$			+	–	+	–	–
R12 (D)	RGS10	181	None	None	$G_{i/o}$ and $G_{q/11}$	S168 (PKA)	C60, C66	+	+	+	+	+
	RGS12 †	1376	PDZ PTB	RBD GoLoco PM	$G_{i/o}$			+	+	+	+	–
	RGS14 †	566	None	RBD GoLoco	$G_{i/o}$	S258/T494 (PKA)		–/(+)	+	+	–	–

* Sub-family	Gene†	Size	Non-RGS Domains		GAP for	Post-Transl. Modifications		Ventricles			Atria	
		a. acids	N-term.	C-term.		Phosphorylation	Palmitoyl.	Tissue ‡	CM §	CF §	Tissue !!	CM !!
RZ (A)	RGS17	210	C string (plus A. Helix RGS19)		G_z & other $G_{i/o}$	Likely (S151)	Likely	–	+	+	+	+
	RGS19 †	217		PM	G_z & other $G_{i/o}$ and $G_{q/11}$	S24 (CK2/PKC) S151 (ERK)	+	–	+	+	+	+
	RGS20 †	241			$G_z \ll (G_{i/o})$	Likely (S151)	Likely	(+)	(+)	+	–	–

*Nomenclatures for canonical RGS protein subfamilies are based on either a prototypical subfamily member[3] or arbitrary alphabetical letters [139] (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) [22] or human left ventricular myocardium (using RNase protection assays and RT-PCR) [16]

§RT-PCR-based RGS protein expression profile in myocytes (CM) and fibroblasts (CF) from adult rat ventricles (see Figure 2)

!!Single cell RT-PCR-based RGS protein expression profile established in spontaneously beating rat atrial CM [19]

Domain Abbreviations: A. Helix - amphiphatic helix; CM – cardiac myocyte; CF – cardiac fibroblast; GAP – GTPase activating protein; GGL - Gγ-like domain; DEP - Disheveled-EGL10-Pleckstrin homology domain; RBD - Ras-binding domain; GoLoco - $G\alpha_{i/o}$-Loco domain; PDZ - PSD-95 disk-large ZO-1 domain; PM - PDZ docking motif; PTB - phosphotyrosine binding domain

Additional Annotations:

- For the expression profiles, + and − denote detection or lack thereof of specified isoforms and (+) indicates weak expression.
- In the phosphorylation column, the phosphorylation-specific site(s) are followed by responsible kinase(s) in brackets. Unknown sites or kinases are indicated by ?.
- RGS21 has controversial tissue distribution (restricted to taste buds [140] vs. ubiquitous [141]) and its expression was not determined (ND) in the expression studies listed.

Table 2

Gain- and Loss-of-Function RGS Protein Models and RGS-insensitive Gα Models and their Cardiovascular Phenotypes

Gene	Model	Cardiovascular Phenotype
RGS Protein Transgenic (TG) & Knockout (KO) Models:		
RGS2	**KO**	Hypertension and enhanced vasoconstriction due to prolonged G_q-mediated signaling and decreased cGMP-mediated relaxation [81, 110]
		Normal basal cardiac phenotype and hypertrophic response to swimming; increased G_q signaling and hypertrophy in response to pressure overload with more rapid transition to failure and early mortality; exacerbated hypertrophy and dilation in $G\alpha_q$ transgenes; lack of inhibition of G_q-coupled stimuli and suppression of maladaptive hypertrophy by cGMP-selective PDE5 inhibitor sildenafil [70]
		Enhanced susceptibility to atrial tachycardia/fibrillation via enhanced M_3-receptor activity. [122]
RGS4	**TG**	No basal phenotype; compromised adaption to pressure overload (rapid decompensation, increased mortality) [108]
		In $G\alpha_q$-expressing transgenes, delay in hypertrophy onset, but comparable end-stage hypertrophic phenotype [109]
		Reduced cardiomyopathic phenotype in PPARα transgenes without change in metabolic abnormalities; resistance to streptozotocin-induced fetal gene induction [142]
		No basal phenotype; reduced hypertrophic response in mice lacking GC-A receptor [143]
	KO	Viable and fertile; normal neural development; subtle sensorimotor deficits. [23]
		Increased M_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_{KAch} desensitization as well as slowed activation and deactivation kinetics [24]
RGS5	**TG**	Attenuated hypertrophy and fibrosis response to pressure overload [112]
	KO	Enhanced hypertrophy and fibrosis development in response to pressure overload [112]
		Viable and fertile; reduced blood pressure and increased heart rate; normal vasculature and remodeling response (to tumor growth and oxygen-induced retinopathy) [144]
		No gross abnormalities; low blood pressure without change in heart rate; decreased body weight [145]
RGS6	**KO**	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_{KAch} activation and deactivation and extent of desensitization in atrial myocytes [120, 121]
RGS-insensitive Gα Knock-in (KI) Model:		
$G\alpha_{i2}$ G184S	**KI**	Reduced viability, low birth weight, growth retardation, cardiac hypertrophy and increased baseline heart rate during day time, enlarged spleen, elevated neutrophil and monocyte counts, behavioral hyperactivity [146]
		Enhanced muscarinic (but not adenosine-induced) bradycardic responses in intact mice and perfused hearts; delayed AV conduction [118, 119]
		Smaller infarct size and enhanced contractile recovery after ischemia/reperfusion in perfused hearts; enhanced potency for carbachol's negative inotropic effect after β-adrenergic stimulation in ventricular myocytes [123].