ELSEVIER

Seminars in Cell & Developmental Biology 17 (2006) 390–399

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

www.elsevier.com/locate/semcdb

Review

RGS17/RGSZ2 and the RZ/A family of regulators of G-protein signaling

Caroline Nunn [a], Helen Mao [b], Peter Chidiac [a], Paul R. Albert [b,*]

[a] *Department of Physiology and Pharmacology, University of Western Ontario, London, Ont., Canada, N6A 5C1*
[b] *Ottawa Health Research Institute (Neuroscience), University of Ottawa, 451 Smyth Road, Ottawa, Ont., Canada, K1H-8M5*

Available online 7 April 2006

Abstract

Regulators of G-protein signaling (RGS proteins) comprise over 20 different proteins that have been classified into subfamilies on the basis of structural homology. The RZ/A family includes RGSZ2/RGS17 (the most recently discovered member of this family), GAIP/RGS19, RGSZ1/RGS20, and the RGSZ1 variant Ret-RGS. The RGS proteins are GTPase activating proteins (GAPs) that turn off G-proteins and thus negatively regulate the signaling of G-protein coupled receptors (GPCRs). In addition, some RZ/A family RGS proteins are able to modify signaling through interactions with adapter proteins (such as GIPC and GIPN). The RZ/A proteins have a simple structure that includes a conserved amino-terminal cysteine string motif, RGS box and short carboxyl-terminal, which confer GAP activity (RGS box) and the ability to undergo covalent modification and interact with other proteins (amino-terminal). This review focuses on RGS17 and its RZ/A sibling proteins and discusses the similarities and differences among these proteins in terms of their palmitoylation, phosphorylation, intracellular localization and interactions with GPCRs and adapter proteins. The specificity of these RGS protein for different Gα proteins and receptors, and the consequences for signaling are discussed. The tissue and brain distribution, and the evolving understanding of the roles of this family of RGS proteins in receptor signaling and brain function are highlighted.

Keywords: G-protein; Palmitoylation; Ubiquinylation; Cysteine string; GIPC; GAP; MAPK; cAMP; Calcium; Receptor; Dopamine; GPCR; Clathrin; Golgi; Vesicles

Contents

* Corresponding author. Tel.: +1 613 562 5800 8307; fax: +1 613 562 5403.
E-mail address: palbert@uottawa.ca (P.R. Albert).

1084-9521/$ – see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2006.04.001

1. Regulators of G-protein signaling (RGS proteins)

1.1. RGS proteins and G-protein signaling

G-protein coupled receptors (GPCRs) comprise a superfamily of cell surface, seven transmembrane-spanning proteins that mediate the physiological actions of a diverse group of stimuli including odorants, light, Ca^{2+}, hormones, neurotransmitters, small peptides and proteins [1,2]. GPCRs are functionally coupled to intracellular heterotrimeric guanine nucleotide binding proteins (G-proteins), which consist of Gα, Gβ and Gγ subunits. Activation of the receptor causes a conformational change that allows it to act as a guanine nucleotide exchange factor (GEF) to catalyse the exchange of GTP for GDP on the Gα subunit, and this ultimately leads to the propagation of downstream signaling pathways via regulation of specific effector proteins. G-protein signaling is terminated through hydrolysis of the bound GTP to GDP by the intrinsic GTPase activity of the Gα subunit. More recently it has become evident that, in addition to coupling to heterotrimeric G-proteins and effectors, GPCRs can interact with multiple intracellular regulatory proteins that affect the localization, conformation and activity of the receptor, as well as serving as scaffolds to form multimeric complexes with other signaling proteins [3,4].

RGS proteins were first discovered in the 1990s as a group of proteins that negatively modulate GPCR signaling [5,6]. These proteins act as GTPase activating proteins (GAPs) to increase the hydrolytic rate of the GTP-bound Gα subunit to inactivate it [5]. Thus, RGS proteins are integral components of GPCR signaling that accelerate the turn-off rate of G-protein signaling by 100-fold or greater in in vitro studies [5,6].

There are at least 20 RGS proteins identified in mammals to date, and these have been grouped into 4 subfamilies according to sequence homology (R4/B, RZ/A, R7/C and R12/D) [6,7]. While all RGS proteins possess a central highly conserved ∼130 amino acid RGS box which is both necessary and sufficient for GAP activity, the flanking regions differ greatly between subfamilies and contribute to the diversity of functions observed between them. For example, members of the R7/C subfamily (RGS6, RGS7, RGS9 and RGS11) contain a Disheveled/EGL-10/Pleckstrin homology (DEP) domain that serves as a protein binding interface, as well as a G-protein γ subunit-like (GGL) domain, that is required to form a stable complex with the Gβ5 subunit [8,9]. RGS12 and RGS14, which are members of the R12/D family, both have two additional G-protein binding domains—the Rap binding domain (RBD) and GoLoco homology domain at their carboxyl terminus [7,10]. Members of the other two RGS subfamilies, RZ/A (GAIP, Ret-RGS, RGSZ1 and RGS17) and R4/B (RGS1-5, RGS8, RGS13, RGS16, RGS18 and RGS21) have simpler structures wherein the conserved RGS domain is flanked by relatively short amino- and carboxyl-termini.

1.2. RZ/A family of RGS proteins

Members of the RZ/A family of RGS proteins are small proteins (∼20–30 kDa, except for Ret-RGS that is 45 kDa due to an extended amino terminus) that are characterized by a very short (10–11 amino acid) carboxyl terminus [11–13]. One distinguishing feature of this RGS subfamily is the presence of a conserved cysteine string in the amino-terminal region, which mediates intracellular localization and protein interactions (Fig. 1). Ret-RGS also contains a putative transmembrane-spanning domain in its amino terminus, which is not found in other RZ/A family members [14]. The RZ/A subfamily displays a selectivity profile for Gα subunits that is unique among the RGS proteins, and the aim of this review is to discuss in detail the RZ/A family members, with an emphasis on the most recently characterized member, RGS17.

The first RZ/A member discovered, RGS19 (GAIP), was found through a yeast two-hybrid screen for Gαi3-interacting proteins [11]. Ret-RGS was originally identified as a GAP for transducin (Gαt) in retina [14]. RGSZ1 (originally named Gz GAP) was discovered in a search for Gαz-specific GAP activity in crude bovine brain membrane fractions [15]. RGSZ1 cDNA was cloned and shown to be homologous to GAIP (RGS19) and Ret-RGS1, and these were categorized together as the RZ/A subfamily of RGS proteins [16,17]. RGS17, the newest member of the RZ/A group, is a 210-amino acid protein which was first isolated from a yeast two-hybrid screen of a chick dorsal root ganglion library using constitutively active Gαo as bait [18]. A subsequent yeast two-hybrid screen of a human brain cDNA library using a different constitutively active Gαo mutant led to the isolation of the first mammalian RGS17 clone [13].

The four RZ/A proteins are encoded by three genes in mammals—*RGS*17, *RGS*19 (encodes GAIP) and *RGS*20 (encodes both RGSZ1 and Ret-RGS), which are located on human chromosomes 6, 20 and 8, respectively [19]. RZ/A genes are the most highly conserved RGS subfamily in metazoans and are most closely related to the R4/B subfamily which itself may have evolved from an RZ/A progenitor [19]. All three genes contain multiple introns and are alternatively spliced to yield multiple mRNA molecules [12,13,19,20]. The alternative RNA transcripts of human GAIP and RGS17 differ only in their non-coding regions and therefore splice variants of the proteins do not exist [13,20]. However, in rodents, GAIP is alternatively spliced to generate a variant that is amino-terminally truncated by 22 amino acids [20]. In contrast, alternative splicing of *RGS*20 produces at least six mRNAs, which encode protein variants. These proteins include RGSZ1 and Ret-RGS (which is identical to residues 24–241 of RGSZ1 but contains an additional 156 amino-terminal amino acids, Fig. 1) and 4 additional proteins that have yet to be characterized but possess amino termini that vary in size, hydrophobicity and the presence of a cysteine string [12]. Furthermore, RGSZ1 mRNA contains at least three translational start codons that may account for the heterogeneous molecular weight of RGSZ1 that is observed following purification from bovine brain [12].

RGS17 is highly conserved between species, with the human proteins having greater than 90% homology to rat, chicken and mouse [13]. Furthermore, there is high homology between RGS17 and other members of the RZ/A family (62% with Ret-RGS and RGSZ1 and 52% with GAIP) and the similarity between members increases in both the 13-amino acid cysteine

Fig. 1. Alignment of RZ/A proteins. The amino acid sequences of four human RZ/A RGS proteins were aligned using Vector NTI software (Invitrogen) with the blosum62mt2 matrix. The NCBI consensus RGS box is underlined (Ret-RGS, 258–278; RGSZ1, 111–231; RGS17, 80–200 and GAIP, 86–206) and the cysteine rich string is outlined in black. Bold region (RGS17, 111–120; GAIP, 115–126) is putative consensus leucine-rich nuclear export sequence.

string region (100% homology with Ret-RGS and RGSZ1, and 85% with GAIP) and the RGS box (91% homology with Ret-RGS and RGSZ1 and 70% with GAIP) (Fig. 1).

2. RZ/A protein domains-modifications and localization

Although RZ/A proteins consist primarily of an RGS box, a number of post-translational modifications and interactions that may contribute to their function have been identified or postulated.

2.1. Cysteine string motif

The highly conserved cysteine string in the amino-terminus of RGS17 is a potential site for palmitoylation. Palmitoylation is a reversible post-translational lipid modification that involves the addition of palmitate to a cysteine residue through a thioester linkage [21]. GAIP incorporates [^{3}H] palmitate to a much greater extent than Gα [22], which contains only a single palmitoylation site [23]. This suggests that GAIP is palmitoylated at more than one site, most likely on its cysteine string motif [22]. To date, palmitoylation of other RZ/A members including RGS17 has yet to be demonstrated.

The function of palmitoylation is not well understood, but addition of this lipid moiety appears to be involved in membrane association and/or targeting of the modified protein to specific membrane subdomains. For example, palmitoylation of p21ras and heterotrimeric G-protein Gα subunits enhances their membrane localization [24,25], while palmitoylation of non-receptor tyrosine kinases is required for their inclusion into lipid rafts [21,24]. Furthermore, palmitoylation of Gα subunits can enhance their binding to Gβγ and limit their sensitivity to the GAP effects of RGS proteins [25,26].

Because RZ/A family RGS proteins may be extensively palmitoylated on their cysteine string region, palmitoylation may be very important for membrane localization and activity of these proteins (Fig. 2). For example, palmitoylated GAIP is enriched in the membrane fraction, while GAIP is present in both membrane-bound and cytosolic pools [22]. Furthermore, it has been shown that GAIP is associated with endocytic clathrin-coated buds and vesicles, as well as with vesicles located within the trans-Golgi network [27–30]. RGSZ1 displays a similar pattern of localization within the *trans*-Golgi network and this was abolished in a truncated mutant lacking the first 49 amino acids (including the cysteine string motif) [31]. This localization suggests a role for GAIP and RGSZ1 (and possibly other RZ/A

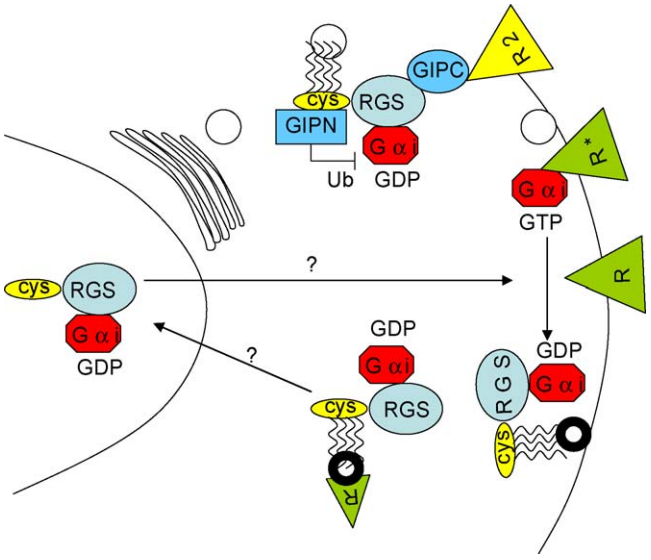

Fig. 2. A model for RZ/A protein signaling. A schematic diagram synthesizes various aspects of RZ/A protein signaling, interactions, and subcellular localization. The RZ/A protein is shown with RGS domain (RGS) and cysteine string motif (cys); the latter is either palmitoylated (wavy lines) or not. The RZ/A protein is either localized in nucleus (RGS17) or Golgi and secretory vesicles (GAIP, RGSZ1). GAIP associates with GIPC, which recruits it to GPCR or tyrosine kinase receptors (R2); GAIP also interacts with GIPN, which ubiquiylates (Ub) Gαi protein. Upon activation of GPCR (R*), the RGS protein translocates to the receptor-Gαi-GTP complex and its GAP activity at the G-protein yields the GDP-bound inactive G-protein. In addition, by association with clathrin-coated endocytotic vesicles (bold circles) the RGS protein helps to maintain the G-protein in the inactive GDP-bound state. See text for details.

family members) in maintaining G-proteins in an inactivated state during vesicular trafficking and internalization, and possibly in vesicle trafficking, which is known to be regulated by heterotrimeric G-proteins [32–34].

To examine the subcellular distribution of RGS17 protein, an antibody to mouse RGS17 (RGSZ2) was generated that specifically recognized RGS17, but did not bind to RGS-GAIP, RGSZ1, RGS2 and RGS4 [13]. A single endogenous RGS17 species that migrated at ∼27 kDa was detected by Western blot in neuronal (raphe RN46A, septum SN48) and neuroendocrine cells (pituitary tumor cell GH4C1), but not in HEK293 cells [13] (Mao et al., unpublished data). By subcellular fractionation we found that unlike other RZ/A members, RGS17 is predominantly localized to the nucleus of cells (Mao et al., unpublished data). Deletion of the N-terminal domain of RGSZ1 enhanced its nuclear localization, suggesting that differences in the N-terminal domains of these RZ-RGS proteins may lead to specific localization [31]. While RGS17 is predicted to be nuclear, no consensus nuclear localization signal was identified. However, there is a putative nuclear export sequence or leucine-rich motif [31] embedded in the RGS domain of RGS17 (Fig. 1), which suggests that RGS17 export to the cytoplasm may be regulated. Furthermore, RGS17 is present in the synaptosomal fraction of mouse brain [35], hence at least a portion of RGS17 is localized to cytoplasmic and perhaps membrane structures.

Nuclear localization has been shown for several other RGS proteins including RGS2 [36], RGS3T [37], RGS10 [31,38], RGS12TS-S [39], and RGS9-2 [40]. Two general functions of nuclear localization have been described: to regulate nuclear functions (e.g., gene transcription), or to sequester RGS proteins. In the case of RGS12TS-S, a unique N-terminal domain distinct from the RGS domain localizes the protein to the nucleus and mediates repression of transcription at the nuclear matrix. Similarly, nuclear localization signals at the N-terminal domain of RGS3T target it to the nucleus where it augments apoptosis [37]. For RGS9-2, a proline-rich carboxyl-terminal domain and interaction with Gβ5 are necessary for its partial nuclear localization, and transfected RGS9-2 can weakly regulate preprotachykinin A gene transcription [40]. In other cases (especially for small RGS proteins), nuclear localization appears to sequester RGS proteins. For example, PKA-induced phosphorylation of RGS10 inactivates its ability to inhibit coupling to potassium channels at the plasma membrane by promoting its translocation to the nucleus [38]. Similarly, RGS8 is targeted to the nucleus by its N-terminal domain, but translocates to the cytoplasm upon Gαo activation to regulate ion channel coupling [41], while RGS2 is recruited to the plasma membrane upon the co-expression of Gαq, Gαs, or receptors that couple to these G-proteins [42]. In the case of RGS17 the function of nuclear localization is unclear, but our preliminary data suggest it may translocate to the membrane upon receptor/G-protein activation. However, RGS17 may also have roles in regulating nuclear function or signaling.

2.2. Phosphorylation

RGS17 contains a number of serine and threonine residues that meet the criteria as potential phosphorylation sites for protein kinase C (PKC, six sites) and casein kinase 2 (CK2, three sites) [13], suggesting that phosphorylation of RGS17 may be involved in its function and/or regulation. A number of these sites are conserved among the other RZ/A family members and phosphorylation of GAIP is detected in rat liver extracts, in part due to clathrin-coated vesicle-associated CK2 [43]. GAIP is also phosphorylated by PKC and ERK1/2 (but not PKA or p38 MAPK) in human colon cancer HT-29 cells [44]. Functionally, ERK2-mediated phosphorylation of Ser^{151} of GAIP (which lies within the RGS box) leads to an increased rate of GTP hydrolysis for Gαi3 [44]. Ser^{151} is conserved among all four RZ/A family members suggesting this may be a putative ERK phosphorylation site in all RZ/A proteins.

3. RZ/A protein interactions and signaling

There is increasing evidence that the cellular functions of RGS proteins (including RZ/A proteins) may extend beyond their GAP activities, and can involve interactions with receptors or intracellular signaling proteins (for review see Abramow-Newerly et al. [7]).

3.1. GIPC

GAIP contains a PDZ (PSD-95/DLG/ZO-1) binding motif at its extreme carboxyl terminus which was found to interact with the novel 36 kDa PDZ domain-containing protein, GIPC (GAIP-

interacting protein, C terminus), in a yeast two-hybrid screen using a rat pituitary cell cDNA library [45]. The PDZ binding motif is unique to GAIP among the RZ/A family members suggesting that this interaction may be specific for GAIP. GIPC is a highly promiscuous protein with multiple membrane-associated protein binding partners including GPCRs (D_2 and D_3 dopaminergic, β_1-adrenergic and lutropin receptors), receptor tyrosine kinases (IGF-1, Trk-A and TGF-βIII receptors), the Glut-1 glucose transporter, semaphorin F and α6 integrin [29,46–55]. Proteins with PDZ domains have multiple roles including the formation of protein complexes at the plasma membrane, and spatially clustering and anchoring transmembrane proteins within specific subcellular domains [56,57]. GIPC in particular has been shown to play important roles in organising signaling cascades and anchoring proteins into subcellular compartments [51].

The role of the GIPC-GAIP interaction was initially thought to facilitate palmitoylation of the RGS protein, since GIPC contains an acyl carrier protein domain that is an acceptor motif for acyl moieties (including palmitate) [45]. However, it is now known that GIPC functions as a scaffolding protein that links GAIP to form complexes with other signaling molecules including G-proteins, GPCRs, the NGF receptor TrkA and the IGF-1 receptor [29,49–53]. The formation of these complexes mediates the GAP activity of GAIP, regulation of endocytic trafficking, and cross-talk with other signaling pathways [7] (Fig. 2). The TrkA receptor forms a complex with GIPC/GAIP, and overexpression of GIPC reduces coupling of TrkA to MAPK activation [29]. Interaction of GIPC with the C-terminal of the β_1-adrenergic receptor also decreases coupling to Gi-mediated MAPK activation [51]. Conversely, the IGF receptor/GIPC interaction with GAIP is required for coupling of the IGF receptor to pertussis toxin-sensitive, Gi-mediated stimulation of MAPK, presumably by recruiting Gi to the IGF receptor [53]. Interactions of GIPC with TGF-βIII, lutropin receptor [52], HPV E6 protein, neuropilin, and other proteins affect their intracellular targeting and degradation, but these appear to be GAIP-independent actions [47,48,54,58]. Thus, GIPC appears to mediate both GAIP-and G-protein dependent actions, as well as actions that seem to be RGS- and G-protein independent (Fig. 2).

3.2. GIPN

Recently the cysteine string region of RGS17, RGSZ1 and GAIP (but not Ret-RGS) was found to be involved in a novel interaction with a 38-kDa integral membrane protein named GIPN (GAIP-interacting protein N terminus) [59]. GIPN is a RING-finger like protein with E3 ubiquitin ligase activity. Ubiquitylation is a post-translational modification in which ubiquitin is conjugated to specific lysine residues in protein targets, ultimately leading to their degradation or internalization. In this case the substrate for ubiquitylation by GIPN appears not to be the RGS proteins themselves, since overexpression of GIPN in cells did not influence GAIP degradation, but Gαi3 whose half-life was significantly decreased by overexpression of GIPN [59] (Fig. 2). Therefore, it is possible that the RGS protein acts as a bifunctional adaptor to bring GIPN in close proximity with its Gα substrate. This putative mechanism would represent a unique function for RGS17, RGSZ1 and GAIP in the degradation of Gα subunits, thereby providing an additional mechanism by which this RGS subfamily is able to attenuate G-protein signaling.

3.3. Superior cerival ganglia, neural specific 10 (SCG10)

RGSZ1 was found to interact with a neuronal growth-associated protein SCG10 in a yeast two-hybrid screen [60]. In vitro pull-down experiments confirmed the interaction with the amino-terminal domain of RGSZ1 (including the cysteine string region) and immunofluorescence revealed co-localization of RGSZ1 and SCG10 within the Golgi apparatus in PC12 cells [60]. SCG10 is a member of the stathmin protein family, which destabilize microtubule formation. Overexpression of SCG10 in PC12 cells enhances neurite out-growth, indicating its involvement in neuronal differentiation [61,62]. The functional effect of the RGSZ1-SCG10 interaction seems to be independent of GAP activity but appears to inhibit SCG10-mediated disassembly of microtubules, thus providing a possible link between G-protein signaling and neuronal development [60].

4. RZ/A-G-protein selectivity

As previously discussed, RGSZ1 was first isolated from bovine brain as a GAP for Gαz, and the RZ/A family was subsequently named after this activity. In spite of its relatively low sequence similarity (∼60% amino acid identity [63]), Gαz is considered to be a member of the Gαi/o family of G-proteins based upon this homology and its ability to inhibit adenylyl cyclase and stimulate K^+ channels [64,65]. However, it is not a typical Gαi/o protein, since it is insensitive to pertussis toxin and its expression is limited to retina, brain adrenal medulla and platelets (compared to the ubiquitous distribution of other Gαi/o proteins) [63]. Gαz is also unusual in that it hydrolyses GTP very poorly compared to other heterotrimeric G-proteins—its half-life of hydrolysis is about 7 min compared to 10–20 s for other Gαi/o members [66]. This slow intrinsic rate of hydrolysis suggests that activated Gαz does not turn off immediately after receptor activation and implies that an efficient Gαz GAP is a required component of the Gαz signaling system. Besides the RZ/A family members, a number of RGS proteins including RGS4 and RGS10 have been shown to act as GAPs for Gαz [67–69].

4.1. RZ/A proteins as Gα GAPs

Despite the implication that RZ/A family members are Gαz-selective proteins, the RZ/A family members can bind to multiple G-proteins and their individual G-protein binding profiles vary between reports. One generally agreed upon fact is that RZ/A family members bind to Gαz and, with varying degrees, to other members of the Gαi/o family based on studies using yeast two-hybrid, immunoprecipitation and pull-down experiments [11,13,16,22,70]. Binding to Gαq has only been observed

for RGS17 by co-immunoprecipitation [13]. In contrast, no interaction has been observed with other G-protein subfamilies including Gαs and Gα13 [11,13,16,22,71].

RGSZ1 is generally considered to be a Gαz selective GAP. In single-turnover GTPase assays (in which the rate of a single catalytic turnover of GTP hydrolysis is examined in G-proteins which are pre-bound to [γ-^{32}P]GTP), RGSZ1 was able to increase the rate of GTP hydrolysis of Gαz up to 400-fold with nanomolar K_m [15–17,70]. In contrast, RGSZ1 displayed low apparent affinity for isolated Gαi1 [17] and high concentrations (21 μM) of RGSZ1 had a 5-fold greater effect on Gαz than on Gαi2 or Gαo [16]. Using the non-hydrolysable Gα-GTPγS combination to quantify RGSZ1 binding confirmed a 100-fold greater affinity for Gαz (2 nM) over Gαi1 [17]. Similarly, in an M_2 muscarinic receptor driven steady-state GAP assay, RGSZ1 increased Gz-GTP hydrolysis with an EC_{50} of 12 nM [17]. However, recent studies suggest that RGSZ1 does interact with Gαi1 to enhance its single-turnover GTPase activity, with 20 nM RGSZ1 producing a 3-fold (i.e. 200%) increase in the rate of GTP hydrolysis [70]. Our own studies [13] show that 30 nM RGSZ1 increased GTPase activity by about 40% and 110% on free Gαi1 and Gαo, respectively, with a higher concentration producing effects similar to those described by Wang et al. [70]. In membrane-based steady-state GTPase assays, RGSZ1 produced comparable GAP effects on receptor-coupled Gi1, Gi2, Gi3, and Go albeit with a more pronounced effect on Gz [13]. Together, these data suggest that RGSZ1 may function as a Gαz selective GAP and a general inhibitor of signals mediated via members of the Gαi subfamily.

In contrast to RGSZ1, RGS17 appears to be a relatively non-selective GAP for Gαz and other Gαi/o proteins in vitro. For example, RGS17 efficiently increased the rate of GTP hydrolysis of both Gαi and Gαo in single-turnover assays [13]. In steady-state M_2 muscarinic receptor driven assays, RGS17 had an appreciable GAP effect on Gi1, Gi2, Gi3, Go and Gz [13]. The size of the effect was lower than that of RGS4 for all subunits, however relative to RGS4, RGS17 displayed a slight preference for Gαo and Gαz [13]. No RGS17 GAP effect could be determined on M_1 muscarinic receptor stimulated G11 (a Gq homologue), possibly due to low assay sensitivity, since RGS17 interacted with Gq and reduced Gq-mediated signaling [13]. GAIP has been shown to increase the rate of GTP hydrolysis of Gαi1, Gαi2, Gαi3, Gαo, Gαz and Gαq subunits but does not have GAP activity towards Gαs subunits [67,71]. Furthermore, GAIP was found to interact more strongly with Gαi3 and Gαi1 than with Gαi2 and this was shown to be the result of a single amino acid substitution (D229 in Gαi1 and Gαi3, A230 in Gαi2) [22,71]. Finally, Ret-RGS, which was discovered in a screen for a GAP for transducin in the retina has been shown to be an efficient GAP for both Gαz and transducin [14,17].

4.2. Actions on downstream signaling

RGS protein GAP effects are typically invoked to explain their inhibitory effects on downstream signaling; however, this usually cannot be verified due to technical limitations in measuring G-protein GTPase activity in cells or tissues. Moreover, RGS proteins have been found to limit G-protein signals through mechanisms that do not involve changes in the rate of GTP hydrolysis, but are otherwise poorly understood [6,7]. Additional factors, such as GPCRs, effectors, and scaffolding proteins can have profound effects on RGS protein-G-protein interactions and thus the final effect of RGS proteins on cellular signaling can be very different from the suggested selectivity and function from GTP hydrolysis assays [7]. For the RZ/A subfamily RGS proteins, effects on G-protein GTPase activities and downstream signaling are largely similar, although a number of discrepancies remain apparent.

The downstream actions of RGS17 were examined in both cAMP accumulation (Gαi/Gαs) and Ca^{2+} mobilization (Gαq) assays [13]. These studies revealed that RGS17 blocked dopamine D_2 receptor-mediated inhibition of cAMP accumulation (presumably via Gi/o) but did not have any effect on Gαs-coupled, dopamine D_1 receptor-stimulated cAMP accumulation. These data further demonstrate the functional specificity of RGS17 for Gi/o proteins. Pertussis toxin insensitive G-protein point mutants were used to elucidate the individual Gi/o proteins involved and it was found that while GAIP and RGSZ1 blocked the inhibition of adenylyl cyclase activity by Gαi2, Gαi3 and Gαz, RGS17 did not significantly interfere with Gαz-mediated inhibition of cAMP accumulation. These results suggest that in vivo, RGS17 effects on Gαi may be more important than its effects on Gαz [13]. In Ca^{2+} mobilization assays, RGS17 (as well as GAIP and RGSZ1) blocked thyrotropin releasing hormone-stimulated Ca^{2+} mobilization, demonstrating that in a cellular context RGS17 is able to act on Gαq, either as a GAP or as an effector antagonist (in which the RGS protein interacts with the G-protein in a manner that inhibits its interaction with its effector) [13].

GAIP has been shown to have inhibitory effects on Gαq signaling in HEK293 and NG-108 cells [72,73], and also interferes with the negative regulation of adenylyl cyclase by Gαi/o proteins [13,72–74]. Furthermore, in chick dorsal root ganglion neurons, GAIP is selective for Gαo over Gαi in blocking norepinephrine-induced inhibition of N-type Ca^{2+} channel activity [75]. The amino terminus of GAIP seems to confer this molecular selectivity since a truncated mutant of GAIP (consisting only of the RGS box) had a similar effect on both Gαi and Gαo pathways [75].

In addition to being a Gαz GAP in bovine brain extracts, RGSZ1 also inhibits Gαz in pancreatic β cells where it blocks Gz-mediated inhibition of glucose-stimulated insulin secretion [76]. The functional activity of RGSZ1 has also been extended to Gαi/o proteins in a study of three different functional assays that act via pertussis toxin sensitive Gαi/o proteins: yeast pheromone response, $\alpha 2_A$ adrenoceptor-induced MAP kinase activation, and dopamine D_2 receptor-stimulated activation of the serum response element were inhibited by RGSZ1 [70]. RGSZ1 also blocked D_2 receptor-mediated inhibition of cAMP accumulation mediated by Gαi2 and Gαi3 [13]. Thus, despite being relatively selective for Gz in vitro, RGSZ1 can also block Gαi/o signaling either via its GAP activity for Gαi/o proteins or possibly via alternative mechanisms, such as effector antagonism.

4.3. Receptor selectivity

It has been established that RGS proteins can display selectivity for GPCRs such that their effects on an individual G-protein can differ depending on the receptor to which that G-protein is coupled. For example, RGS4, RGS1 and RGS16 block Gαq-mediated M_3 muscarinic receptor-stimulated Ca^{2+} mobilization roughly 30-, 1000- and 100-fold, respectively, more potently than they block the same response mediated via cholecystokinin receptors [77].

There is some evidence that RZ/A family members may display receptor selectivity in their actions on G-proteins. Despite the fact that GAIP has GAP activity on purified Gαi1, Gαi2, Gαi3 and Gαo, GAIP was able to increase the rate of hydrolysis by Gαo but not the others when these proteins were expressed as fusions with the α2A-adrenoceptor [78]. These data suggest that this receptor may limit at least some GAIP-G-protein interactions. Another factor may be that accessory proteins are involved in mediating receptor selectivity. For example, the inhibitory actions of GAIP on dopamine D_2 receptor signaling required the scaffolding protein GIPC that acts to recruit GAIP into a plasma membrane complex that includes GIPC and the receptor [49]. Since GIPC is able to bind to dopamine D_2 and D_3 receptors, but not D_4 receptors, this may result in the selective targeting of GAIP to certain receptor subtypes and pathways in vivo [50].

Members of the RZ/A family of RGS proteins modulate opioid receptor function in the CNS and display receptor selectively towards μ-opioid receptors over δ-opioid receptors [35,79–81]. Studies using antisense oligonucleotides directed against RGSZ1 and RGS17 demonstrated that impairment of the expression of these proteins augmented the antinociceptive response to morphine (a μ-opioid receptor agonist) but not to [D-Pen^{2,5}]-enkephalin or [D-Ala^2] deltorphin II (δ-opioid receptor agonists) [35,81]. This potentiation led to increased tolerance to subsequent doses of morphine [35,79,81], implying that the association of RGS17 with Gαz may contribute to morphine tolerance by preventing μ-opioid receptor signaling upon subsequent morphine challenge [35]. A recent study using recombinant mouse GAIP and opioid receptors in COS-7 cells suggested that GAIP blocks the inhibitory effect on adenylyl cyclase activity of the ORL1 (opioid receptor-like 1) receptor but not μ-, δ- or κ-opioid receptors [74]. Taken together these studies suggest different receptor specificities of RZ/A proteins, but the precise mechanisms for this specificity vary, and can involve RGS interactions with adapters like GIPC, or unknown complexes that are receptor selective. To date there is no evidence for direct interactions of RZ/A proteins with receptors.

5. Tissue and subcellular distribution of RZ/A proteins

In order to provide insight into RGS17 function in vivo, we examined the distribution of RGS17 mRNA in human tissues by Northern blot [13]. Our study showed that RGS17 is expressed in both human CNS and peripheral tissues, which is consistent with its GAP activity towards multiple Gα subtypes that are widely distributed in these tissues. Thus, RGS17 mRNA was expressed as a single ∼2 kb transcript at a low level in a variety of human peripheral tissues with higher levels in spleen, lung and blood leukocytes (Fig. 3, lanes 6, 11, 12). The size of this transcript is similar to the 1.8-kb RGSZ1 [16,17] and the 1.6-kb RGS-GAIP [11]. By contrast, at least five RGS17 transcripts were found in the brain. A similar pattern of RGS17 transcripts was observed in various brain regions with three dominant bands at 8 kb, 3 kb and 2 kb [13]. Throughout the CNS the 8-kb RGS17 transcript was expressed most abundantly in the cerebellum. Consistent with this, quantitative RT-PCR studies also show that RGS17 is most strongly expressed in human cerebellum, nucleus accumbens and parahypocampal gyrus [82]. The presence of multiple RGS17 RNA transcripts in brain, as anticipated, yielded only a single RGS17 protein species as detected by Western blotting (Mao et al., unpublished). The exact structure of the 8-kb brain-specific transcript remains unclear. However, as noted above, brain cDNA had only a single 5′-untranslated and coding region [13] suggesting that the additional sequence in the 8-kb RGS17 RNA species may be

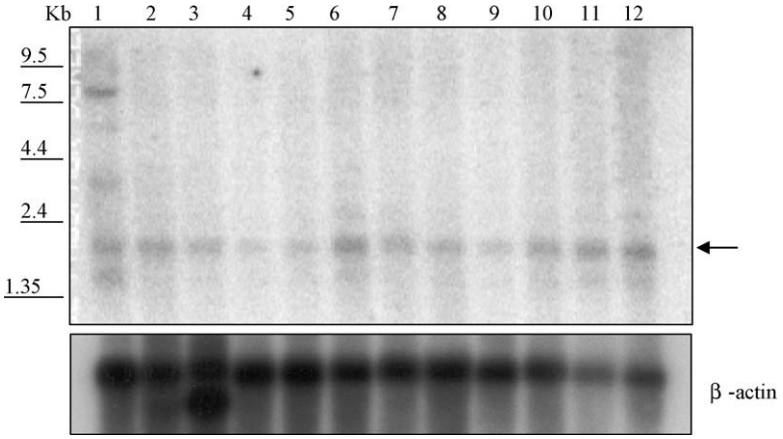

Fig. 3. Northern blot of RGS17 RNA in human tissues. Northern blotting was performed using human cDNA of RGS17 open reading frame labeled with ^{32}P as a probe; see Mao et al. [13] for details. Clontech blot (#7780-1) of human 12 tissues. (1) brain; (2) heart; (3) skeletal muscle; (4) colon (no mucosa); (5) thymus; (6) spleen; (7) kidney; (8) liver; (9) small intestine; (10) placenta; (11) lung; (12) peripheral blood leukocyte. Shown is the major 2-kb RGS17 RNA species (arrow) present in most tissues; in brain, additional RGS17 species were observed, including a predominant 8-kb band.

located at the 3′-untranslated region. We did not detect evidence of an amino-terminal extension as observed for Ret-RGS/RGS-Z1. Alterations in the 3′-untranslated sequence of RGS17 RNA may regulate the subcellular localization or stability of the longer RNA species, but this remains to be tested.

In contrast to RGS17, RGSZ1 is expressed exclusively throughout the brain [16,17,82] and in female sexual organs [12], while RGS-GAIP is highly expressed in lung, heart, placenta, and liver and to a lesser degree in brain, skeletal muscle, pancreas, and kidney [11]. Our study showed that RGS17 is expressed in both human CNS and peripheral tissues [13] (Fig. 3), which is consistent with its GAP activity towards multiple Gα subtypes that are widely distributed in these tissues. Immunohistochemistry revealed that RGS17 is expressed throughout the rat CNS, but is most abundant in the granule and Purkinje cell layers of the cerebellum and weakly expressed in the molecular layer (Mao et al., unpublished). In addition, RGS17 is enriched in mouse periaqueductal gray area, where it negatively regulates μ-opioid receptor mediated analgesia [35,79]. In cerebellar Purkinje cells, RGS17 was localized primarily to the nucleus, as observed in cultured cells (Mao et al., unpublished). Localization of RGS17 mRNA and protein in the cerebellum suggests a role in regulating Gi-mediated signaling particularly in cerebellar granule and Purkinje neurons in which RGS17 is most strongly expressed. Recently PDZ-RGS3 was found to interact with Ephrin-B C-terminal, and shown to attenuate Gi-mediated chemo-attractant signaling via CXCR1 receptor activation in cerebellar granule cells via its RGS function [83]. The PDZ-containing RGS-GAIP interacting protein GIPC has also been shown to interact with and regulate axonal guidance molecules such as semaphorins or their receptors, neuropilins [47,48], as well as mediating G-protein dependent actions on TrkA or IGF receptor signaling [29,53]. As an RGS-GAIP homologue, RGS17 may also regulate axonal outgrowth or adhesion signaling in the cerebellum.

6. Conclusion

Although they have a conserved simple structure, RZ/A family proteins display an unexpected diversity in G-protein specificity, post-translational modification, subcellular localization, receptor selectivity, and tissue localization. Further studies are required to define their distinct roles and importance in vivo, and the mechanisms by which RZ/A proteins regulate G-protein-dependent and -independent signaling.

Acknowledgements

We thank Aya Goto for assistance with immunohistochemistry and Dr Chau Nguyen for his comments on the manuscript, and we acknowledge funding for studies of RGS proteins in our laboratories by Canadian Institutes of Health Research (CIHR) (to P.R.A. and P.C.) and Ontario Mental Health Foundation (P.R.A.). P.R.A. is the CIHR/Novartis Michael Smith Chair in Neurosciences and C.N. holds a Postdoctoral Fellowship from the CIHR.

References

[1] Bockaert J, Dumuis A, Fagni L, Marin P. GPCR-GIP networks: a first step in the discovery of new therapeutic drugs? Curr Opin Drug Discov Devel 2004;7:649–57.
[2] Kostenis E, Waelbroeck M, Milligan G. Techniques: promiscuous Galpha proteins in basic research and drug discovery. Trends Pharmacol Sci 2005;26:595–602.
[3] Presland J. Identifying novel modulators of G protein-coupled receptors via interaction at allosteric sites. Curr Opin Drug Discov Devel 2005;8:567–76.
[4] Rebois RV, Hebert TE. Protein complexes involved in heptahelical receptor-mediated signal transduction. Receptors Channels 2003;9:169–94.
[5] Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem 2000;69:795–827.
[6] Hollinger S, Hepler JR. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol Rev 2002;54:527–59.
[7] Abramow-Newerly M, Roy AA, Nunn C, Chidiac P. RGS proteins have a signalling complex: interactions between RGS proteins and GPCRs, effectors, and auxiliary proteins. Cell Signal 2006;18:579–91.
[8] Snow BE, Krumins AM, Brothers GM, Lee SF, Wall MA, Chung S, et al. A G protein gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to Gbeta5 subunits. Proc Natl Acad Sci USA 1998;95:13307–12.
[9] Witherow DS, Slepak VZ. A novel kind of G protein heterodimer: the G beta5-RGS complex. Receptors Channels 2003;9:205–12.
[10] Kimple RJ, Kimple ME, Betts L, Sondek J, Siderovski DP. Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. Nature 2002;416:878–81.
[11] De Vries L, Mousli M, Wurmser A, Farquhar MG. GAIP, a protein that specifically interacts with the trimeric G protein G alpha i3, is a member of a protein family with a highly conserved core domain. Proc Natl Acad Sci USA 1995;92:11916–20.
[12] Barker SA, Wang J, Sierra DA, Ross EM. RGSZ1 and Ret RGS: two of several splice variants from the gene RGS20. Genomics 2001;78:223–9.
[13] Mao H, Zhao Q, Daigle M, Ghahremani MH, Chidiac P, Albert PR. RGS17/RGSZ2, a novel regulator of Gi/o, Gz, and Gq signaling. J Biol Chem 2004;279:26314–22.
[14] Faurobert E, Hurley JB. The core domain of a new retina specific RGS protein stimulates the GTPase activity of transducin in vitro. Proc Natl Acad Sci USA 1997;94:2945–50.
[15] Wang J, Tu Y, Woodson J, Song X, Ross EM. A GTPase-activating protein for the G protein Galphaz. Identification, purification, and mechanism of action. J Biol Chem 1997;272:5732–40.
[16] Glick JL, Meigs TE, Miron A, Casey PJ. RGSZ1, a Gz-selective regulator of G protein signaling whose action is sensitive to the phosphorylation state of Gzalpha. J Biol Chem 1998;273:26008–13.
[17] Wang J, Ducret A, Tu Y, Kozasa T, Aebersold R, Ross EM. RGSZ1, a Gz-selective RGS protein in brain. Structure, membrane association, regulation by Galphaz phosphorylation, and relationship to a Gz gtpase-activating protein subfamily. J Biol Chem 1998;273:26014–25.
[18] Jordan JD, Carey KD, Stork PJ, Iyengar R. Modulation of rap activity by direct interaction of Galpha(o) with Rap1 GTPase-activating protein. J Biol Chem 1999;274:21507–10.
[19] Sierra DA, Gilbert DJ, Householder D, Grishin NV, Yu K, Ukidwe P, et al. Evolution of the regulators of G-protein signaling multigene family in mouse and human. Genomics 2002;79:177–85.
[20] Xie GX, Han X, Ito E, Yanagisawa Y, Maruyama K, Sugano S, et al. Gene structure, dual-promoters and mRNA alternative splicing of the human and mouse regulator of G protein signaling GAIP/RGS19. J Mol Biol 2003;325:721–32.
[21] Dunphy JT, Linder ME. Signalling functions of protein palmitoylation. Biochim Biophys Acta 1998;1436:245–61.
[22] De Vries L, Elenko E, Hubler L, Jones TL, Farquhar MG. GAIP is membrane-anchored by palmitoylation and interacts with the activated

(GTP-bound) form of G alpha i subunits. Proc Natl Acad Sci USA 1996;93:15203–8.
[23] Degtyarev MY, Spiegel AM, Jones TL. The G protein alpha s subunit incorporates [^{3}H]palmitic acid and mutation of cysteine-3 prevents this modification. Biochemistry 1993;32:8057–61.
[24] Mumby SM. Reversible palmitoylation of signaling proteins. Curr Opin Cell Biol 1997;9:148–54.
[25] Iiri T, Backlund Jr PS, Jones TL, Wedegaertner PB, Bourne HR. Reciprocal regulation of Gs alpha by palmitate and the beta gamma subunit. Proc Natl Acad Sci USA 1996;93:14592–7.
[26] Tu Y, Wang J, Ross EM. Inhibition of brain Gz GAP and other RGS proteins by palmitoylation of G protein alpha subunits. Science 1997;278:1132–5.
[27] De Vries L, Elenko E, McCaffery JM, Fischer T, Hubler L, McQuistan T, et al. RGS-GAIP, a GTPase-activating protein for Galphai heterotrimeric G proteins, is located on clathrin-coated vesicles. Mol Biol Cell 1998;9:1123–34.
[28] Wylie F, Heimann K, Le TL, Brown D, Rabnott G, Stow JL. GAIP a Galphai-3-binding protein, is associated with Golgi-derived vesicles and protein trafficking. Am J Physiol 1999;276:C497–506.
[29] Lou X, Yano H, Lee F, Chao MV, Farquhar MG. GIPC and GAIP form a complex with TrkA: a putative link between G protein and receptor tyrosine kinase pathways. Mol Biol Cell 2001;12:615–27.
[30] Wylie FG, Lock JG, Jamriska L, Khromykh T, Brown DL, Stow JL. GAIP participates in budding of membrane carriers at the trans-Golgi network. Traffic 2003;4:175–89.
[31] Chatterjee TK, Fisher RA. Cytoplasmic, nuclear, and golgi localization of RGS proteins. Evidence for N-terminal and RGS domain sequences as intracellular targeting motifs. J Biol Chem 2000;275:24013–21.
[32] Stow JL, Heimann K. Vesicle budding on Golgi membranes: regulation by G proteins and myosin motors. Biochim Biophys Acta 1998;1404:161–71.
[33] Stow JL. Regulation of vesicular transport by GTP-binding proteins. Curr Opin Nephrol Hypertens 1995;4:421–5.
[34] Watson EL. GTP-binding proteins and regulated exocytosis. Crit Rev Oral Biol Med 1999;10:284–306.
[35] Garzon J, Rodriguez-Munoz M, Lopez-Fando A, Sanchez-Blazquez P. The RGSZ2 protein exists in a complex with mu-opioid receptors and regulates the desensitizing capacity of Gz proteins. Neuropsychopharmacology 2005;30:1632–48.
[36] Heximer SP, Lim H, Bernard JL, Blumer KJ. Mechanisms governing subcellular localization and function of human RGS2. J Biol Chem 2001;276:14195–203.
[37] Dulin NO, Pratt P, Tiruppathi C, Niu J, Voyno-Yasenetskaya T, Dunn MJ. Regulator of G protein signaling RGS3T is localized to the nucleus and induces apoptosis. J Biol Chem 2000;275:21317–23.
[38] Burgon PG, Lee WL, Nixon AB, Peralta EG, Casey PJ. Phosphorylation and nuclear translocation of a regulator of G protein signaling (RGS10). J Biol Chem 2001;276:32828–34.
[39] Chatterjee TK, Fisher RA. RGS12TS-S localizes at nuclear matrix-associated subnuclear structures and represses transcription: structural requirements for subnuclear targeting and transcriptional repression. Mol Cell Biol 2002;22:4334–45.
[40] Bouhamdan M, Michelhaugh SK, Calin-Jageman I, Ahern-Djamali S, Bannon MJ. Brain-specific RGS9-2 is localized to the nucleus via its unique proline-rich domain. Biochim Biophys Acta 2004;1691:141–50.
[41] Saitoh O, Masuho I, Terakawa I, Nomoto S, Asano T, Kubo Y. Regulator of G protein signaling 8 (RGS8) requires its NH2 terminus for subcellular localization and acute desensitization of G protein-gated K^+ channels. J Biol Chem 2001;276:5052–8.
[42] Roy AA, Lemberg KE, Chidiac P. Recruitment of RGS2 and RGS4 to the plasma membrane by G proteins and receptors reflects functional interactions. Mol Pharmacol 2003;64:587–93.
[43] Fischer T, Elenko E, Wan L, Thomas G, Farquhar MG. Membrane-associated GAIP is a phosphoprotein and can be phosphorylated by clathrin-coated vesicles. Proc Natl Acad Sci USA 2000;97:4040–5.
[44] Ogier-Denis E, Pattingre S, El Benna J, Codogno P. Erk1/2-dependent phosphorylation of Galpha-interacting protein stimulates its GTPase accelerating activity and autophagy in human colon cancer cells. J Biol Chem 2000;275:39090–5.
[45] De Vries L, Lou X, Zhao G, Zheng B, Farquhar MG. GIPC, a PDZ domain containing protein, interacts specifically with the C terminus of RGS-GAIP. Proc Natl Acad Sci USA 1998;95:12340–5.
[46] Bunn RC, Jensen MA, Reed BC. Protein interactions with the glucose transporter binding protein GLUT1CBP that provide a link between GLUT1 and the cytoskeleton. Mol Biol Cell 1999;10:819–32.
[47] Cai H, Reed RR. Cloning and characterization of neuropilin-1-interacting protein: a PSD-95/Dlg/ZO-1 domain-containing protein that interacts with the cytoplasmic domain of neuropilin-1. J Neurosci 1999;19:6519–27.
[48] Wang LH, Kalb RG, Strittmatter SM. A PDZ protein regulates the distribution of the transmembrane semaphorin M-SemF. J Biol Chem 1999;274:14137–46.
[49] Jeanneteau F, Guillin O, Diaz J, Griffon N, Sokoloff P. GIPC recruits GAIP (RGS19) to attenuate dopamine D2 receptor signaling. Mol Biol Cell 2004;15:4926–37.
[50] Jeanneteau F, Diaz J, Sokoloff P, Griffon N. Interactions of GIPC with dopamine D2 D3 but not D4 receptors define a novel mode of regulation of G protein-coupled receptors. Mol Biol Cell 2004;15:696–705.
[51] Hu LA, Chen W, Martin NP, Whalen EJ, Premont RT, Lefkowitz RJ. GIPC interacts with the beta1-adrenergic receptor and regulates beta1-adrenergic receptor-mediated ERK activation. J Biol Chem 2003;278:26295–301.
[52] Hirakawa T, Galet C, Kishi M, Ascoli M. GIPC binds to the human lutropin receptor (hLHR) through an unusual PDZ domain binding motif, and it regulates the sorting of the internalized human choriogonadotropin and the density of cell surface hLHR. J Biol Chem 2003;278:49348–57.
[53] Booth RA, Cummings C, Tiberi M, Liu XJ. GIPC participates in G protein signaling downstream of insulin-like growth factor 1 receptor. J Biol Chem 2002;277:6719–25.
[54] Blobe GC, Liu X, Fang SJ, How T, Lodish HF. A novel mechanism for regulating transforming growth factor beta (TGF- beta) signaling. Functional modulation of type III TGF-beta receptor expression through interaction with the PDZ domain protein, GIPC. J Biol Chem 2001;276:39608–17.
[55] Tani TT, Mercurio AM. PDZ interaction sites in integrin alpha subunits. T14853, TIP/GIPC binds to a type I recognition sequence in alpha 6A/alpha 5 and a novel sequence in alpha 6B. J Biol Chem 2001;276:36535–42.
[56] Kim E, Sheng M. PDZ domain proteins of synapses. Nat Rev Neurosci 2004;5:771–81.
[57] Harris BZ, Lim WA. Mechanism and role of PDZ domains in signaling complex assembly. J Cell Sci 2001;114:3219–31.
[58] Bohlson SS, Zhang M, Ortiz CE, Tenner AJ. CD93 interacts with the PDZ domain-containing adaptor protein GIPC: implications in the modulation of phagocytosis. J Leukoc Biol 2005;77:80–9.
[59] Fischer T, De Vries L, Meerloo T, Farquhar MG. Promotion of G alpha i3 subunit down-regulation by GIPN, a putative E3 ubiquitin ligase that interacts with RGS-GAIP. Proc Natl Acad Sci USA 2003;100:8270–5.
[60] Nixon AB, Grenningloh G, Casey PJ. The interaction of RGSZ1 with SCG10 attenuates the ability of SCG10 to promote microtubule disassembly. J Biol Chem 2002;277:18127–33.
[61] Charbaut E, Curmi PA, Ozon S, Lachkar S, Redeker V, Sobel A. Stathmin family proteins display specific molecular and tubulin binding properties. J Biol Chem 2001;276:16146–54.
[62] Riederer BM, Pellier V, Antonsson B, Di Paolo G, Stimpson SA, Lutjens R, et al. Regulation of microtubule dynamics by the neuronal growth-associated protein SCG10. Proc Natl Acad Sci USA 1997;94:741–5.
[63] Ho MK, Wong YH. G(z) signaling: emerging divergence from G(i) signaling. Oncogene 2001;20:1615–25.
[64] Wong YH, Conklin BR, Bourne HR. Gz-mediated hormonal inhibition of cyclic AMP accumulation. Science 1992;255:339–42.
[65] Jeong SW, Ikeda SR. G protein alpha subunit G alpha z couples neurotransmitter receptors to ion channels in sympathetic neurons. Neuron 1998;21:1201–12.

[66] Casey PJ, Fong HK, Simon MI, Gilman AG. Gz a guanine nucleotide-binding protein with unique biochemical properties. J Biol Chem 1990;265:2383–90.
[67] Popov S, Yu K, Kozasa T, Wilkie TM. The regulators of G protein signaling (RGS) domains of RGS4, RGS10, and GAIP retain GTPase activating protein activity in vitro. Proc Natl Acad Sci USA 1997;94:7216–20.
[68] Hunt TW, Fields TA, Casey PJ, Peralta EG. RGS10 is a selective activator of G alpha i GTPase activity. Nature 1996;383:175–7.
[69] Tu Y, Nayak SK, Woodson J, Ross EM. Phosphorylation-regulated inhibition of the Gz GTPase-activating protein activity of RGS proteins by synapsin I. J Biol Chem 2003;278:52273–81.
[70] Wang Y, Ho G, Zhang JJ, Nieuwenhuijsen B, Edris W, Chanda PK, et al. Regulator of G protein signaling Z1 (RGSZ1) interacts with Galpha i subunits and regulates Galpha i-mediated cell signaling. J Biol Chem 2002;277:48325–32.
[71] Woulfe DS, Stadel JM. Structural basis for the selectivity of the RGS protein, GAIP, for Galphai family members. Identification of a single amino acid determinant for selective interaction of Galphai subunits with GAIP. J Biol Chem 1999;274:17718–24.
[72] Hepler JR, Berman DM, Gilman AG, Kozasa T. RGS4 and GAIP are GTPase-activating proteins for Gq alpha and block activation of phospholipase C beta by gamma-thio-GTP-Gq alpha. Proc Natl Acad Sci USA 1997;94:428–32.
[73] Huang C, Hepler JR, Gilman AG, Mumby SM. Attenuation of Gi- and Gq-mediated signaling by expression of RGS4 or GAIP in mammalian cells. Proc Natl Acad Sci USA 1997;94:6159–63.
[74] Xie GX, Yanagisawa Y, Ito E, Maruyama K, Han X, Kim KJ, et al. N-terminally truncated variant of the mouse GAIP/RGS19 lacks selectivity of full-length GAIP/RGS19 protein in regulating ORL1 receptor signaling. J Mol Biol 2005;353:1081–92.
[75] Diverse-Pierluissi MA, Fischer T, Jordan JD, Schiff M, Ortiz DF, Farquhar MG, et al. Regulators of G protein signaling proteins as determinants of the rate of desensitization of presynaptic calcium channels. J Biol Chem 1999;274:14490–4.
[76] Kimple ME, Nixon AB, Kelly P, Bailey CL, Young KH, Fields TA, et al. A role for G(z) in pancreatic islet beta-cell biology. J Biol Chem 2005;280:31708–13.
[77] Xu X, Zeng W, Popov S, Berman DM, Davignon I, Yu K, et al. RGS proteins determine signaling specificity of Gq-coupled receptors. J Biol Chem 1999;274:3549–56.
[78] Hoffmann M, Ward RJ, Cavalli A, Carr IC, Milligan G. Differential capacities of the RGS1 RGS16 and RGS-GAIP regulators of G protein signaling to enhance alpha2A-adrenoreceptor agonist-stimulated GTPase activity of G(o1)alpha. J Neurochem 2001;78:797–806.
[79] Garzon J, Rodriguez-Munoz M, Sanchez-Blazquez P. Morphine alters the selective association between mu-opioid receptors and specific RGS proteins in mouse periaqueductal gray matter. Neuropharmacology 2005;48:853–68.
[80] Sanchez-Blazquez P, Rodriguez-Munoz M, Montero C, Garzon J. RGS-Rz and RGS9-2 proteins control mu-opioid receptor desensitisation in CNS: the role of activated Galphaz subunits. Neuropharmacology 2005;48:134–50.
[81] Garzon J, Rodriguez-Munoz M, Lopez-Fando A, Garcia-Espana A, Sanchez-Blazquez P. RGSZ1 and GAIP regulate mu- but not delta-opioid receptors in mouse CNS: role in tachyphylaxis and acute tolerance. Neuropsychopharmacology 2004;29:1091–104.
[82] Larminie C, Murdock P, Walhin JP, Duckworth M, Blumer KJ, Scheideler MA, et al. Selective expression of regulators of G-protein signaling (RGS) in the human central nervous system. Brain Res Mol Brain Res 2004;122:24–34.
[83] Lu Q, Sun EE, Klein RS, Flanagan JG. Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein-coupled chemoattraction. Cell 2001;105:69–79.