RGS proteins: more than just GAPs for heterotrimeric G proteins

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Members of the newly described RGS family of proteins have a common RGS domain that contains GTPase-activating activity for many Gα subunits of heterotrimeric G proteins. Their ability to dampen signalling via $G\alpha_i$-, $G\alpha_q$- and $G\alpha_{12/13}$-coupled pathways makes them crucial players in mediating the multitude of cellular processes controlled by heterotrimeric G proteins. Some RGS proteins also contain additional motifs that link them to other signalling networks, where they constitute effector-type molecules. This review summarizes recent findings on RGS proteins, especially those that implicate RGS proteins in more than just enhancing the GTPase activity of their Gα subunit targets.

Regulator of G-protein signalling (RGS) proteins are now recognized as key elements that reduce the signal generated by binding of hormones, peptides or other ligands to G-protein-coupled receptors (GPCRs). Signal reduction, or 'desensitization' as it is generally called, can take place at the level of the receptors themselves or at the level of the heterotrimeric G proteins, which comprise α, β and γ subunits. Both the receptor-activated α subunit, a GTPase, and the βγ subunit can transmit signals to downstream effector molecules such as adenylyl cyclase, phospholipase C or ion channels. RGS proteins are GTPase-activating proteins (GAPs) that reduce the signal transmitted by the receptor-activated (GTP bound) Gα subunit by rapidly returning it to the inactive state (GDP bound; see Fig. 1).

GAPs for small (or monomeric) G proteins were described more than 10 years ago, and, although the $G\alpha_q$ effector phospholipase C-β1 showed modest GAP activity, members of the recently discovered RGS protein family are now considered to be the major GAPs for α subunits of heterotrimeric G proteins. The RGS family now contains more than 20 members (Fig. 2) and is defined by the diagnostic RGS domain that binds to Gα subunits and is responsible for GAP function. But, in many RGS proteins, there are additional motifs and domains that define or predict interactions with proteins other than Gα subunits. This implies that some RGS proteins are not just GAPs but can also act as linker molecules to a multitude of other signalling pathways.

For the detailed mechanistic aspects (including mutational analysis) of their GAP activity as well as other aspects of RGS proteins, the reader should consult Refs 1–3. This review focuses on the diversity of cellular processes that are regulated by RGS proteins and on how RGS proteins might be regulated themselves.

Discovery of the RGS family and its GAP function for Gα subunits

The mammalian RGS family, whose signature is the homologous (45–80%) 120-residue RGS domain, was recognized as a distinct family simultaneously by three groups just three years ago[4–6]. They demonstrated that RGS proteins are negative regulators of several G-protein-mediated signalling pathways[5–8]. Insight into the mechanism came quickly when two family members, GAIP and RGS4, were shown to serve as GAPs for both $G\alpha_i$ and $G\alpha_q$ subunits by stabilizing the GTP-to-GDP transition state of Gα subunits[9,10]. Many RGS proteins subsequently tested *in vitro* for GAP activity interacted specifically with a subset of G proteins – that is, the $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12/13}$ subfamilies – but some have not been tested. Subtle differences in sequence between RGS domains among different RGS proteins probably dictate specific Gα interaction preferences[11,12]. None of the RGS proteins tested so far seems to be a GAP for $G\alpha_s$.

Thus, in principle, RGS proteins could modulate most if not all α_i-, α_o- and $\alpha_{12/13}$-mediated signalling pathways. In fact, there are already indications that RGS proteins are involved in modulating a variety of cell functions, such as proliferation, differentiation, response to neurotransmitters, membrane trafficking and embryonic development[1,3,10,13].

Indications that RGS proteins are more than just GAPs came from electrophysiological studies of G-protein-coupled inward-rectifying K^+ (GIRK) channels, which are directly opened by Gβγ subunits that have dissociated from activated $G\alpha_i$ subunits. RGS proteins speed up the turning off of the channel (deactivation). The accelerated turning off of the K^+ current can be explained by the GAP activity of the RGS protein (faster formation of inactive heterotrimers). However, RGS4 and RGS8 also accelerated the turning on of the current (activation) without a reduction in the peak current by increasing the number of G proteins that can activate the channels. Thus, there seems to be an additional function besides GAP activity[13–16]. This function, however, might still be explained by the GAP activity of RGS proteins on the Gα subunit itself: enhanced kinetics of the GTPase cycle could lead to a more sustained signal. In addition, certain RGS proteins function as effector antagonists because they can bind to the switch regions in the Gα subunit where the effectors interact[17]. Clearly, many fine characteristics of RGS function have not yet been explored.

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The main question that arose from the initial findings was: in which cellular processes are RGS proteins involved? Clues might come from examining their tissue distribution, subcellular compartmentalization and transcriptional regulation. RGS protein partners interacting with non-RGS domains could also direct specificity.

RGS proteins are expressed widely

At least one RGS protein is expressed in every organ, and many tissues express multiple RGS proteins (see Table 1). A survey of different regions of the brain by *in situ* hybridization revealed at least nine highly expressed RGS proteins, with some restricted to specific regions and cell types[18]. There are at least nine different RGS mRNAs and/or proteins in pituitary[19–21] and three – RET-RGS1, RGS16, RGS9 – in the retina. There is now compelling evidence that RGS9 is the physiological GAP for transducin in phototransduction in retinal cones[22], which have the best-defined G-protein-signalling cascade. The GAP function of RGS9 for transducin explains the long-standing discrepancy between the observed time for the eye to shut off signalling after the light stimulus is gone (milliseconds) and the GTPase activity of transducin that regulates this shut off (seconds).

RGS3, RGS5, GAIP, RGS12, RGS14 and RGSr mRNAs have the broadest expression patterns, but some show significant differences in expression levels from one tissue or organ to another[4,6,23–25]. Thus, it will be very difficult to deduce a specific physiological role for a given RGS protein from our present knowledge of their tissue expression. The use of *in vivo* conditional knockout systems (cre–lox mice, tissue-specific) might well be informative.

Two pools of RGS proteins: membrane and cytosolic

All the RGS proteins whose intracellular localization is known have both membrane-associated and cytosolic pools. Most GAPs for monomeric G proteins are cytosolic, except for RasGAP, which can translocate to the plasma membrane via a Ca^{2+}-dependent lipid-binding motif[26]. Gα subunits are also known to be both membrane associated and cytosolic; $G\alpha_s$ and $G\alpha_{i1}$ translocate to membranes from a cytosolic pool, a process that is regulated by palmitoylation and myristoylation[27,28]. The same might hold true for some RGS proteins[29–31].

With which membranes do RGS proteins associate? This is an important question because Gα subunits, usually associated with signalling events at the plasma membrane, are also localized on intracellular membranes, where they play a role in the regulation of vesicular trafficking[32]. $G\alpha_s$ regulates endosome fusion[33]. $G\alpha_{i3}$ occurs on Golgi membranes[34–36] and retards exocytic transport through the Golgi[34] as well as regulating the lysosomal–autophagic pathway[37]. There are few data on the localization of RGS proteins. GAIP, RGS3, RGS4, RGS9, RGSZ1 and Sst2p are all membrane bound[20,29–31,38,39], but only GAIP, RGS4 and Sst2p have been localized. Sst2p was localized by immunofluorescence to both the Golgi region and the plasma membrane of yeast[7],

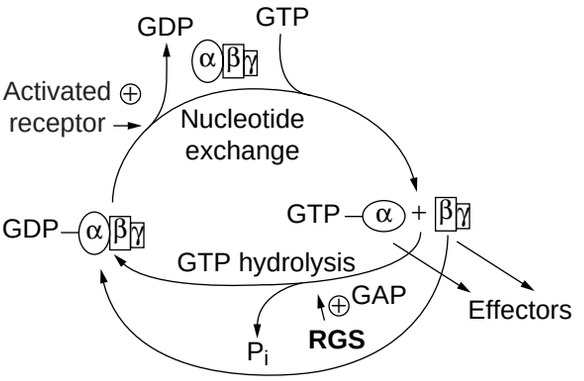

FIGURE 1

Effect of regulator of G-protein signalling (RGS) proteins on the heterotrimeric G protein activation–inactivation cycle. The cycle starts with an activated receptor enhancing displacement of GDP from an inactive heterotrimer. GTP then replaces GDP (nucleotide exchange), aided by Mg^{2+} and a GDP–GTP exchange factor (GEF). The activated (GTP-bound) Gα subunit dissociates from βγ subunits, and both entities activate their respective effectors. Inactivation of the Gα subunit takes place by GTP hydrolysis, a reaction greatly enhanced by RGS proteins, the GTPase-activating proteins (GAPs) for Gα. The inactivated (GDP-bound) Gα subunit has a greater affinity for βγ subunits, allowing the heterotrimer to reassociate, thus completing the cycle.

and there is a membrane-associated pool of RGS4 at the plasma membrane[30,31]. Interestingly, GAIP was found by immunoelectron microscopy on clathrin-coated vesicles (CCVs) located near the plasma membrane (Fig. 3) and the *trans* Golgi (Fig. 4) but not on Golgi or plasma membranes themselves[21]. This led to a model in which GAIP, the GAP, comes into contact with its target Gα located on the plasma membrane or on Golgi membranes only at the time of vesicle fusion[21].

How are RGS proteins anchored to membranes? For RGS3, RGS4 and RGS9, no precise mechanism has been proposed as to how these proteins bind to membranes[20,30,31,38]. Membrane binding of GAIP is probably mediated by palmitoylation of its cysteine-string motif[29]. RGSZ1, the $G\alpha_z$-specific GAP closely related to RET-RGS1, also possesses a cysteine-string motif and is tightly membrane bound[39]. RET-RGS1 is assumed to be membrane bound because it has a cysteine-string motif and a putative transmembrane domain[40]. Many RGS proteins contain putative N-terminal myristoylation sites, although nothing is known about the role of myristoylation in membrane association of RGS proteins.

In summary, the subcellular distribution of most RGS proteins is still poorly documented, but the study of their localization and functions will surely serve to help pinpoint the nature of the G-protein signalling pathways that they regulate. Although it is still too early to generalize, it seems likely that RGS proteins, like Gα subunits, fluctuate between membrane-bound and cytoplasmic pools. This could facilitate their access to the membrane-bound Gα subunits if the localization of the RGS and the Gα is on the same type of membrane. Alternatively, as in the case of GAIP, the presence of Gα subunits and RGS proteins on different membranes, which

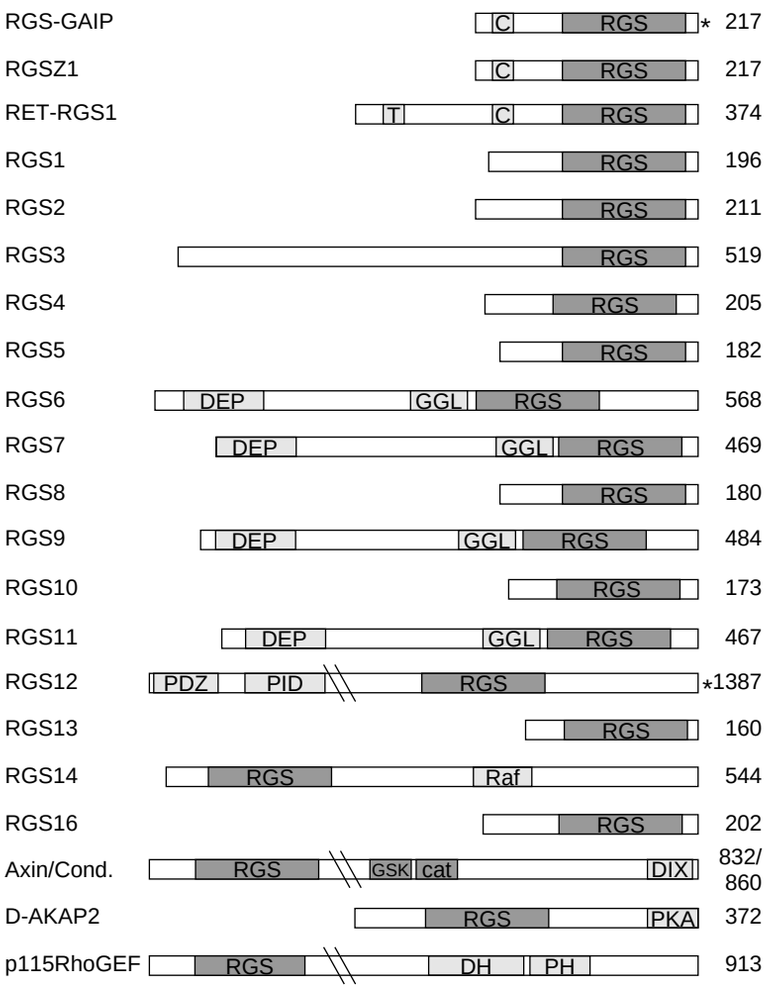

FIGURE 2

Schematic representation of mammalian regulator of G-protein signalling (RGS) proteins. The total number of amino acids for each family member is indicated to the right. Abbreviations: C, cysteine-string domain; cat, β-catenin-binding domain; DEP, DEP domain (Dishevelled/EGL-10/pleckstrin homology); DH, dbl homology domain; DIX, dishevelled-homology domain; GGL, GGL domain (homology to Gγ); GSK, glycogen synthase kinase 3b binding domain; (*), PDZ-binding motif; PDZ, PDZ domain (PSD95/Dlg/ZO1 homology); PH, pleckstrin-homology domain; PID, PID domain (phosphotyrosine-interacting domain); PKA, PKA-anchoring domain; Raf, B-raf-homology domain; RGS, RGS domain; T, transmembrane domain.

come into contact during vesicular trafficking, might add yet another level of regulation.

RGS proteins can be activated at the transcriptional level

As RGS proteins are negative regulators, their transcriptional upregulation should create a classical negative-feedback mechanism on GPCR pathways. Transcriptional upregulation of mammalian RGS proteins was first suggested by the positive effect of pheromone on the induction of the yeast *SST2* gene[41,42]. More recent examples of upregulation of RGS proteins at the transcriptional level have come from the demonstration that RGS1 mRNA is induced by B-cell mitogens[43] and that RGS2 mRNA increases rapidly after cycloheximide treatment of mononuclear blood cells[44]. This suggests the involvement – via transcriptional upregulation – of RGS1 and RGS2 in the negative regulation of incoming (GPCR) signals in haematopoietic cells.

Particularly intriguing is the demonstration that RGS16 mRNA (formerly A28-RGS14 or RGS-r)[45] is induced by the p53 tumour suppressor and that the gene encoding RGS16 has p53-binding sites[46]. Furthermore, RGS16 mRNA is induced by serum, suggesting the presence of serum-response elements (SREs) in its promoter. Overexpression of the corresponding protein inhibits activation of the mitogen-activated protein (MAP) kinase signalling pathway. These findings suggest that RGS16 acts as a transcriptionally inducible component of a p53-controlled negative-feedback mechanism involved in cell proliferation and/or apoptosis.

Interestingly, RGS proteins also appear to play a role in desensitization to psychostimulatory agents. RGS2 mRNA was induced rapidly and transiently in the rat striatum after treatment with the neurostimulator amphetamine[47], and expression of RGS3 mRNA was enhanced after induction of neuronal plasticity[48]. These findings, and the fact that many RGS proteins are expressed in different regions of the brain, point to the potential importance of RGS proteins in the regulation of neuronal signalling pathways in neuro- and psycho-pharmacology.

These few examples suggest the need for a more widespread survey to find out how RGS proteins are regulated at the transcriptional level. No data are available on the stability of the mRNAs for RGS proteins. So far, only the promoter regions of genes encoding RGS2, RGS3 and RGS16 have been studied[44,46,49]. Analysis of the promoters of RGS proteins will certainly provide clues as to which signalling pathways can induce or silence their transcriptional expression.

Non-RGS domains in RGS proteins provide links to small GTPases, protein kinase A and Wnt signalling pathways

There are two main classes of RGS proteins: small (160–217 residues) and large (372–1387 residues; see Fig. 2). In small RGS proteins, the RGS domain predominates, and the proteins have short (~50 residue) N-termini and even shorter C-termini. By contrast, the larger RGS proteins are composite molecules containing additional signalling motifs, which establish links between heterotrimeric G proteins and other signalling pathways.

One of the most exciting links is that established recently between p115RhoGEF, the GPCRs and the small GTPase RhoA. p115RhoGEF has an RGS domain that acts as a GAP for $G\alpha_{13}$ and a DH (dbl homology) domain that acts as a GDP–GTP exchange factor (GEF) for the small GTPase RhoA. GEF activity is stimulated when activated $G\alpha_{13}$ interacts with the RGS domain of p115RhoGEF[12,50]. Thus, the mechanism by which thrombin can induce morphological changes in endothelial cells[51] has become clear: thrombin receptors couple to $G\alpha_{13}$, which links to p115RhoGEF. This RGS protein serves as an effector of $G\alpha_{13}$ and activates RhoA, inducing downstream cytoskeletal changes[52]. Here, we have a completely defined pathway, whereby an extracellular ligand induces intracellular

TABLE 1 – RGS PROTEINS: THEIR Gα TARGETS AND TISSUE DISTRIBUTION[a]

RGS	Target Gα[b]	Tissue expression	Refs
RGSGAIP	$G\alpha_i$, $G\alpha_t$, $G\alpha_z > G\alpha_q$	Ubiquitous, low in brain	4, 9, 39
RGSZ1	$G\alpha_z > G\alpha_i$	Brain	39
RET-RGS1	$G\alpha_z$, $G\alpha_i$, $G\alpha_t$	Retina	39, 40
RGS1	$G\alpha_i$	B-lymphocytes, lung	6, 43
RGS2	$G\alpha_q$	Ubiquitous	6, 44, –[c]
RGS3	$G\alpha_i > G\alpha_q$	Ubiquitous	6, 18
RGS4	$G\alpha_i > G\alpha_q$	Brain, heart	6, 9, 18
RGS5	ND	Ubiquitous	18, 24
RGS6	ND	Brain	18
RGS7	$G\alpha_q$, $G\alpha_i$	Brain	18, 62, –[d]
RGS8	$G\alpha_i$, $G\alpha_0$	Brain	15
RGS9	$G\alpha_t$	Retina (cones > rods), neurons	22
RGS10	$G\alpha_i$	Brain	–[e]
RGS11	$G\alpha_o$	Brain	18, 61
RGS12	$G\alpha_i$ ($G\alpha_{12/13}$?)	Lung, brain, spleen, testis	23, 64, 65
RGS13	ND	Lung	GB
RGS14	ND	Brain, spleen, lung	23
RGS16*	$G\alpha_i$	Retina, pituitary, liver, ubiquitous?	19, 27, 46
Axin/Conductin	ND	Thymus, testis > ubiquitous	55, 56
D-AKAP2	ND	Testis > ubiquitous	54
p115RhoGEF	$G\alpha_{13} > G\alpha_{12}$	Ubiquitous, high in leukocytes	12, 50

[a]Compiled from data published or submitted to GenBank; for some RGS proteins, very little information is available. ND not determined, GB data from GenBank, (*) data combined from RGS-r, RGS16 and A28-RGS14, which have the same protein sequence[45] but show differences in distribution that still remain to be clarified.
[b]Note that these assignations of target Gα are mostly based on *in vitro* analyses that are in many cases not comprehensive. There is also no guarantee that an interaction identified *in vitro* is physiologically relevant. These assignations of target Gα should therefore be considered tentative pending further experimental confirmation, including intracellular localization and tissue-specific expression.
[c]Heximer, S. P. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 14389–14393
[d]Shuey, D. J. *et al.* (1998) *J. Neurochem.* 70, 1964–1972
[e]Hunt, T. W. *et al.* (1996) *Nature* 383, 175–177

morphological changes, and the pivotal effector molecule of the pathway belongs to the RGS family.

Another family member, RGS14, which is identical to a recently reported Rap1/Rap2 interacting protein (GenBank# U85055), provides a putative link to small GTPases involved in cell proliferation. Rap1 and Rap2 are small GTPases with highest homology to p21Ras, and Rap1, like Ras, has been implicated in the transmission of mitogenic effects. RGS14 contains an N-terminal RGS domain for which GAP activity has not yet been demonstrated[23]. The Rap1/Rap2-interacting domain in RGS14 is not known, but a possible candidate is a 70-residue region homologous to protein kinase B-raf, one of the effectors of Ras. In Raf, this region is included in the Ras-binding site, and the Raf-binding domain of Ras (residues 32–40, the effector domain) is highly conserved in Rap1A[53]. Thus, RGS14 might represent another linker molecule between heterotrimeric and small GTPases that plays a role in cell proliferation.

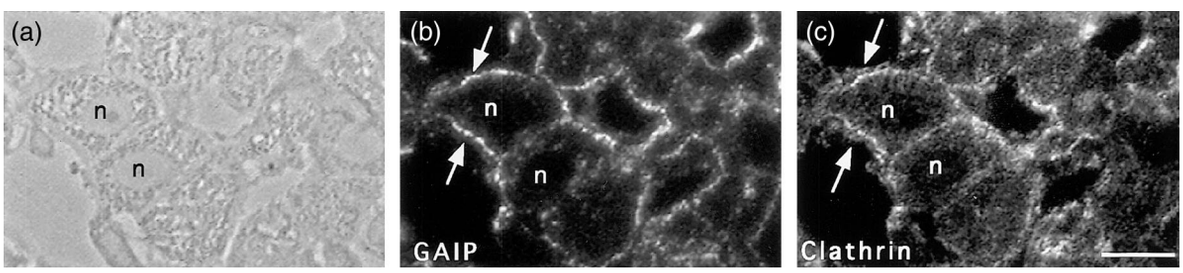

FIGURE 3

GAIP colocalizes with clathrin near the cell membrane in rat liver. Immunofluorescence micrograph showing staining for both GAIP (b) and clathrin (c) at the periphery of hepatocytes concentrated along the sinusoidal domain of the cell membrane (arrows). The corresponding phase-contrast image is shown in (a). Abbreviation: n, nucleus. Bar, 10 μm. (Reproduced, with permission, from Ref. 21.)

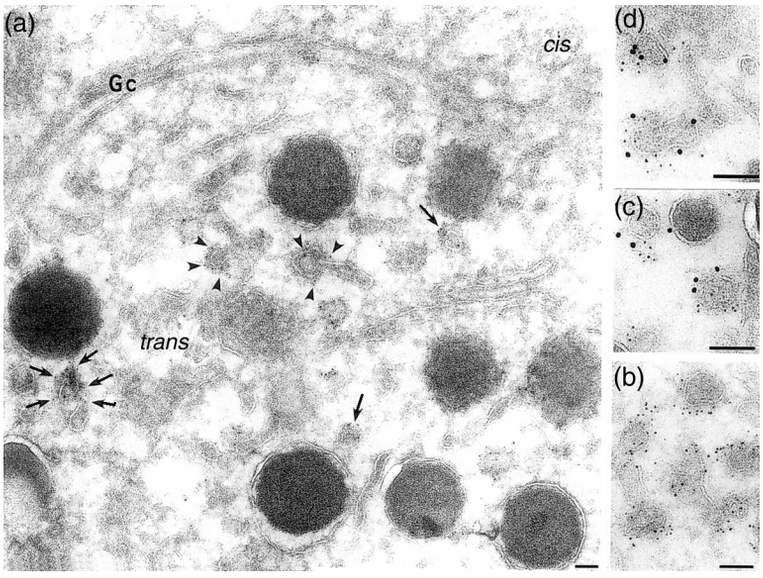

FIGURE 4

GAIP is found on clathrin-coated vesicles located in the Golgi region of rat pituitary cells by immunogold labelling. (a,b) Immunoelectron micrographs of pituitary sections labelled with affinity-purified antibodies against GAIP. IgG detected with 5 nm gold. GAIP is localized predominantly on the outer (cytoplasmic) surface of coated buds (arrowheads) or vesicles (arrows) found on the *trans* side of the Golgi cisternae (Gc) in a somatotrope (growth-hormone-producing cell). (c,d) Similar sections double-labelled for GAIP (5 nm gold) and clathrin (10 nm gold) showing their presence on the same vesicles. Bars, 0.1 μm. (Reproduced, with permission, from Ref. 21.)

Another novel link between cAMP and G-protein signalling pathways was provided by the discovery of the protein kinase A (PKA) anchoring protein D-AKAP2[54]; AKAPs are adaptor proteins that can assemble signalling complexes, including PKA, calcineurin and protein kinase C, at the plasma membrane. D-AKAP2 can bind to the two subtypes of regulatory subunits of PKA (PKA anchor) and contains an N-terminal RGS domain for which a Gα target has not yet been identified. Although G-protein signalling and cAMP pathways are linked directly via the activation or inhibition of adenylyl cyclase by $G\alpha_s$ and $G\alpha_i$, respectively, D-AKAP2 provides an additional link, via PKA anchoring and Gα binding, between the two pathways. Future research should establish which G-protein signalling pathway is involved here and what the implications of this link are for downstream signalling via both PKA and G proteins.

Recently, RGS proteins have been detected in pathways involved in cell differentiation, formation of adhering junctions and tissue development through the Wnt signalling pathway. Wnt signals are detected by the frizzled family of seven-transmembrane receptors, for which the transducing G proteins have remained elusive. Two components of this pathway, axin and its homologue conductin, contain an N-terminal RGS domain whose Gα target has not yet been defined[55,56]. Interestingly, however, the RGS domain of axin/conductin interacts directly with the tumour-suppressor adenomatous polyposis coli (APC), a non-Gα-like molecule[56,57], raising the possibility that APC might be able to compete with a Gα subunit for this RGS domain. Deletion of the RGS domain enhances the down-regulation of β-catenin, a protein linking cadherins to the actin cytoskeleton and also activating specific transcription factors that induce changes in cell adhesion, motility and morphology in mammals[58]. Here again, the RGS proteins axin and conductin are located at a crucial point in the Wnt pathway and possibly behave like p115RhoGEF (i.e. GAP and effector domains in one molecule). Both proteins could play a role in switching the cell from a proliferative to a more differentiated state.

Functional links through DEP, GGL and PDZ domains

RGS6, RGS7, RGS9 and RGS11 have N-terminal DEP (Dishevelled homology/EGL-10/pleckstrin homology) domains. The DEP domain of the *Drosophila* protein Dishevelled is responsible for its localization to membranes[59]. Thus, by analogy, the DEP domain of RGS9 might be responsible for its tight membranes association[38]. It is still not clear whether DEP domains can regulate membrane association directly or through interaction with other proteins. This domain might regulate heterotrimeric G proteins and small G proteins indirectly[60], perhaps by translocation of the DEP-domain-containing protein towards the membrane-bound G proteins.

RGS6, RGS7, RGS9 and RGS11 also have GGL domains with homology to the G protein γ subunit[61] and form dimers with the Gβ5 subunit, the least homologous of the five Gβ subunits. Gβ5 binds to both RGS7[62] and the GGL domain of RGS11[61]. In heterotrimeric G proteins, γ subunits form very tight associations with β subunits, so the presence of a Gγ-like domain suggests a link between these RGS proteins and Gβ subunits. Although the significance of this association is not yet clear, this connection between RGS proteins and the Gβ5 subunit greatly expands the possibilities for mediation of G-protein signalling. The fact that the DEP and GGL domains exist together in the same RGS proteins is intriguing. Perhaps both are needed for a common function?

PDZ domains are involved in the clustering of signalling molecules and play an important role in organizing protein networks on membranes. This is accomplished by binding to consensus C-terminal motifs in target proteins[63]. RGS12 is a GAP for $G\alpha_i$ and probably $G\alpha_{12/13}$ subunits; it is the largest RGS protein described so far, with an N-terminal PDZ domain, a C-terminal serine/proline-rich coiled-coil structure that could interact with cytoskeletal proteins, a PID/PTB domain that suggests interaction with a tyrosine-phosphorylated protein, and a PDZ binding motif[64,65]. Although the *in vivo* targets for the PDZ domain and the PDZ binding site on RGS12 are still unknown, results from yeast two-hybrid and *in vitro* interactions revealed that the chemokine interleukin-8 receptor B (CXCR-2) can specifically interact with the PDZ domain of RGS12[64]. Thus, RGS12 might be an important scaffold molecule for components of G-protein-linked chemokine signalling pathways.

Interactions of the C-terminus of GAIP with a PDZ-domain protein

So far, the RGS domain and the N-terminal regions of some (but not all) of the small RGS proteins (see Fig. 2) have been studied in some detail, but little is known about the role of their short C-termini. The only information available comes from studies on RGS-GAIP, which has an interaction partner that binds to its extreme C-terminus. The newly isolated protein GIPC (for 'GAIP-interacting protein C-terminus') binds through its central PDZ domain to the C-terminus of GAIP, which has a putative PDZ binding motif (SSEA)[66]. GIPC binds specifically to GAIP and not to other RGS proteins tested (RGS2, RGS4, RGS16 and RET-RGS1), which lack the PDZ binding motif at their C-termini. The physiological relevance of the GAIP–GIPC interaction is not known, but GIPC, like GAIP, is also found on vesicles close to the plasma membrane. The identification of GIPC suggests that there might be more as-yet-undiscovered molecules associated with G-protein-mediated signalling networks that bind to the C-termini of other RGS proteins. The N-terminus of GAIP, other than its cysteine-string motif (probably involved in membrane attachment), might also have additional functions. For example, the N-terminus of RGS4 is involved in its specificity for $G\alpha_q$-coupled receptors[67]. That GAIP has the potential to associate with other molecules suggests that even the small RGS proteins are more than just GAPs – just as has been argued for other, larger, RGS proteins in this review.

Towards closing the gaps in our knowledge

The discovery of the RGS proteins has given a new impetus and new directions to investigators working on heterotrimeric G-protein signalling pathways and has provided novel tools for dissecting the physiological relevance of these pathways. This has already led and will lead to the discovery of novel heterotrimeric G-protein-mediated signalling pathways involved in cell proliferation, motility, differentiation and vesicular trafficking. We hope it will also shed further light on the involvement of these pathways in membrane trafficking and cell motility and provide insights as to how heterotrimeric G-protein signalling complexes – including RGS proteins – transmit information that controls cellular functions.

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The subapical compartment: a novel sorting centre?

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Establishment of plasma membrane polarity involves numerous intracellular sorting events. In the past few years, it has become apparent that there is a subapical, non-Golgi compartment located in the hub of the sorting pathways involved. This 'subapical compartment', which probably consists of a heterogeneous subset of functionally distinct domains related to endosomes, contains some well-characterized components involved in polarity-dependent sorting and targeting of proteins and lipids. This article discusses the evidence supporting the existence of such a compartment, its biogenesis and its role in cell polarity.

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Polarized cells have distinct plasma membrane (PM) domains – a basolateral and an apical domain – each characterized by a specific composition of proteins and lipids[1]. The underlying mechanisms by which polarized cells generate and maintain such specific compositions are still largely unknown. An important step towards understanding this requires a detailed characterization of the intracellular 'roadmap'. Such studies will include identification of 'traffic stations' along the different transport routes where molecules are sorted and targeted to their preferred PM domains. Highly relevant issues are whether novel sorting mechanisms are induced upon polarization, involving biosynthesis of new regulatory components, and whether these events occur in unique compartments typical of polarized cells. It is also possible that polarized sorting is achieved by taking advantage of existing cognate apical and basolateral membrane-directing machineries, known to be present in nonpolarized cells[2], operating in a polarized fashion when required.

In recent years, one particular endosomal compartment in polarized cells has gained increasing attention. This compartment appears to connect the apical- and basolateral-derived transport pathways and is involved in transcytosis and recycling of both proteins[3–9] and, as revealed very recently, lipids[10,11]. Gradually, a picture has emerged of it being a 'sorting station' in polarized cells, which differs from and operates next to the *trans*-Golgi network (TGN). It appears that, in the presence of the continuous transcellular transport typical of polarized cells, this non-Golgi compartment serves as an important intermediate in sorting and, subsequently, targeting molecules derived from and destined for either the apical or basolateral PM domain. These features have made this compartment, which we refer to as the subapical compartment (SAC), the focus of intense investigations into morphological and molecular aspects of the complex sorting machinery it contains. Moreover, its similarity to endosomal compartments in nonpolarized cells has implications for its biogenesis and the establishment of polarized sorting.

The first evidence for the existence of a specialized compartment at the intersection of the apical and basolateral endocytic pathways came from the transient colocalization of basolaterally derived proteins with proteins that had been internalized from the apical surface[3–5,9] (Fig. 1). One such basolaterally derived protein found in the interconnecting compartments is the polymeric immunoglobulin receptor (pIgR). This well-characterized transcytotic marker mediates basolateral-to-apical transcytosis of both IgA and IgM[3]. Following endocytosis from the basolateral surface, pIgR-bound IgA (pIgR–IgA) first reaches peripheral early 'sorting' endosomes. Here, the pIgR–IgA complex is sorted from molecules that recycle or have destinations downstream in the late endosomal/lysosomal route and is subsequently targeted to the SAC, prior to delivery to the apical PM domain[3,4,10]. Sphingolipids[10], as well as transcytosing apical membrane-resident proteins[12,13], appear to follow the same SAC-mediated route. In the reverse apical-to-basolateral transcytotic pathway, the SAC also appears to serve as an intermediate compartment for transport of both proteins and lipids[6,10], although Ihrke *et al.*[14] failed to detect such a reverse pathway in WIF-B cells.

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