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ACCESSORY PROTEINS FOR G PROTEINS: Partners in Signaling

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■ **Abstract** Accessory proteins involved in signal processing through heterotrimeric G proteins are generally defined as proteins distinct from G protein–coupled receptor (GPCR), G protein, or classical effectors that regulate the strength/efficiency/specificity of signal transfer upon receptor activation or position these entities in the right microenvironment, contributing to the formation of a functional signal transduction complex. A flurry of recent studies have implicated an additional class of accessory proteins for this system that provide signal input to heterotrimeric G proteins in the absence of a cell surface receptor, serve as alternative binding partners for G protein subunits, provide unexpected modes of G protein regulation, and have introduced additional functional roles for G proteins. This group of accessory proteins includes the recently discovered Activators of G protein Signaling (AGS) proteins identified in a functional screen for receptor-independent activators of G protein signaling as well as several proteins identified in protein interaction screens and genetic screens in model organisms. These accessory proteins may influence GDP dissociation and nucleotide exchange at the G_α subunit, alter subunit interactions within heterotrimeric $G_{\alpha\beta\gamma}$ independent of nucleotide exchange, or form complexes with G_α or $G_{\beta\gamma}$ independent of the typical $G_{\alpha\beta\gamma}$ heterotrimer. AGS and related accessory proteins reveal unexpected diversity in G protein subunits as signal transducers within the cell.

THE CONCEPT OF ACCESSORY PROTEINS

Nature has evolved several clever mechanisms for cells to process external stimuli. One such system incorporates a seven-membrane-span receptor at the cell surface that is activated by a stimulus, and transfers this signal to membrane-associated heterotrimeric G proteins, initiating signal propagation to the cell interior. Via coupling of such receptors to heterotrimeric G proteins, these receptors regulate a variety of effectors, including adenylyl cyclases, phospholipases, ion channels, and protein kinases. Many of the groups of proteins involved in this signal propagation

exist as isoforms or closely related subtypes with different regulatory properties. Indeed, in mammalian systems there are 20 G_α, 5 G_β, and 12 G_γ isoforms assembled in various combinations that generate a diverse population of heterotrimeric $G_{\alpha\beta\gamma}$ complexes (1, 2). This diversity allows complex signal integration and presents opportunities for cells to engineer highly specific responses to an external stimulus.

In many cases, the large number of stimuli processed by G protein–coupled receptors (GPCRs)* utilizes a similar cadre of downstream signaling molecules, yet the final cellular response to a particular stimulus is highly specific. Thus, one of the major questions in terms of cell signaling is how individual cells are able to integrate the myriad of external stimuli that they receive in such a manner as to allow specificity of cell response. This question is of particular interest, as the great majority of diseases either involve a defect in signal propagation that accounts for or contributes to the disease process or the therapeutic management of the disease targets one of the molecules involved in signal propagation. Determinants of signaling specificity include: (*a*) cell-specific and developmentally regulated expression and processing of receptor, G protein, and effectors; (*b*) the stoichiometry of receptors, G proteins, and effectors; (*c*) cell architecture and segregation of specific signaling cassettes; and (*d*) accessory proteins.

Accessory proteins may regulate the strength/efficiency/specificity of signal transfer from receptor to G protein or G protein to effector, help position these three core signaling components in the right microenvironment, and/or contribute to the formation of a functional signal transduction complex. Such a complex may exist in the absence of the stimuli or its formation may be initiated by receptor activation. The signal transduction network for this system may parallel that used by receptors with a single-membrane-span motif, where binding of agonist initiates a series of protein interactions that depend on protein phosphorylation (218). This hypothesis is consistent with data suggesting the existence of receptor dimers and multimeric G protein subunit complexes, the isolation of receptor or G protein

*Abbreviations: AGS, activator of G protein signaling; APP, amyloid precursor protein; bFGF, basic fibroblast growth factor; Dbl, diffuse B-cell lymphoma; DIC, dynein intermediate chain; ERK, extracellular signal-regulated kinase; GAP, GTPase-activating protein; GAP-43, growth-associated protein with *Mr* of 43,000; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; GDP, guanosine-5′-diphosphate; GIV, G_α-interacting vesicle-associated protein; GPCR, G protein–coupled receptor; GPR, G protein regulatory motif; GRK, G protein–coupled receptor kinase; GRIN, G protein–regulated inducer of neurite outgrowth; GST, glutathione S-transferase; GTP, guanosine-5′-triphosphate; GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; HSP90, heat shock protein—90 kDa; KSR, kinase suppressor of Ras; MAP, mitogen-activated protein; MEK-1, mitogen-activated protein kinase kinase-1; NG-GPA, NG108-15 G protein activator; NMDA, N-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NuMA, nuclear mitotic apparatus protein; Pcp2/L7, purkinje cell protein-2; PBP, phosphatidylethanolamine-binding protein; RACK1, receptor for activated C kinase 1; RGS, regulator of G protein signaling; Ric, resistant to inhibitors of cholinesterase; RKIP, Raf kinase inhibitor protein; RNAi, RNA interference; SPR, surface plasmon resonance; TPR, tetratricopeptide repeat motif.

subunits together with some effectors, the existence of additional proteins that influence the activation state of G protein, and the identification of proteins interacting with receptor subdomains or intact receptor (3, 4, 8 and references therein, 28, 29, 219–223).

As an outgrowth from this line of thought, a flurry of studies have identified a group of accessory proteins for G protein signaling pathways that provide signal input to heterotrimeric G proteins in the absence of a cell surface receptor, serve as alternative binding partners for G protein subunits, provide unexpected modes of G protein regulation, and have introduced additional functional roles for G proteins beyond their role as GPCR signal transducers (Figure 1). One of the major classes of such accessory proteins are defined by the RGS (regulator of G protein signaling) family, which function to accelerate the GTPase activity of specific G_α subunits and may also function as scaffolding proteins or effectors (3, 4). The initial studies that suggested the existence of such regulatory proteins were based on analysis of signal termination for the pheromone response pathway in *Saccharomyces cerevisiae*, the differences in the turnover rate for GTP hydrolysis of purified $G_{\alpha q}$ versus the rapid signal processing by this G protein in the cell, and genetic studies focused on G protein-mediated control of egg laying in *Caenorhabditis elegans* (28, 29, 224–226).

Parallel studies involving copurification of entities with G proteins and cell type–specific differences in the transfer of signal from receptors to G proteins and the basal activity of G protein signaling led to the hypothesis that there were additional accessory proteins that influenced the nucleotide binding properties of G proteins (5–8). Three lines of investigation led to the discovery of proteins that directly activated G protein signaling independent of a cell surface GPCR. One strategy was based on the purification of proteins that influenced the binding of GTPγS to G protein (7, 9, 10). Several different laboratories reported on peptides, toxins, and small molecules that directly activated G proteins (11–19), the wasp venom mastoporan being perhaps the most studied (18, 20–24). This general idea was extended with the development of an expression cloning strategy in *S. cerevisiae* that allowed the screening of mammalian cDNAs for their ability to activate the $G_{\beta\gamma}$-regulated pheromone response pathway in the absence of a GPCR. The latter approach resulted in the identification of activators of G protein signaling, AGS proteins, with AGS1–8 referring specifically to the proteins functionally defined in the yeast-based screen (25, 26). The reader is referred to other reviews for a more detailed discussion of the discovery and function of AGS proteins (27–29). AGS proteins were numbered in the order in which they were identified in the functional screen and can be divided into three subgroups based on the mechanism by which they influence G protein activity, as discussed below. A third strategy involved the use of G protein subunits in yeast two-hybrid (Y2H) screens to identify novel binding partners. Proteins identified via these various strategies revealed unexpected diversity for G protein subunits as signal transducers and resulted in the appreciation of additional mechanisms for impacting the traditional G protein activation-deactivation cycle.

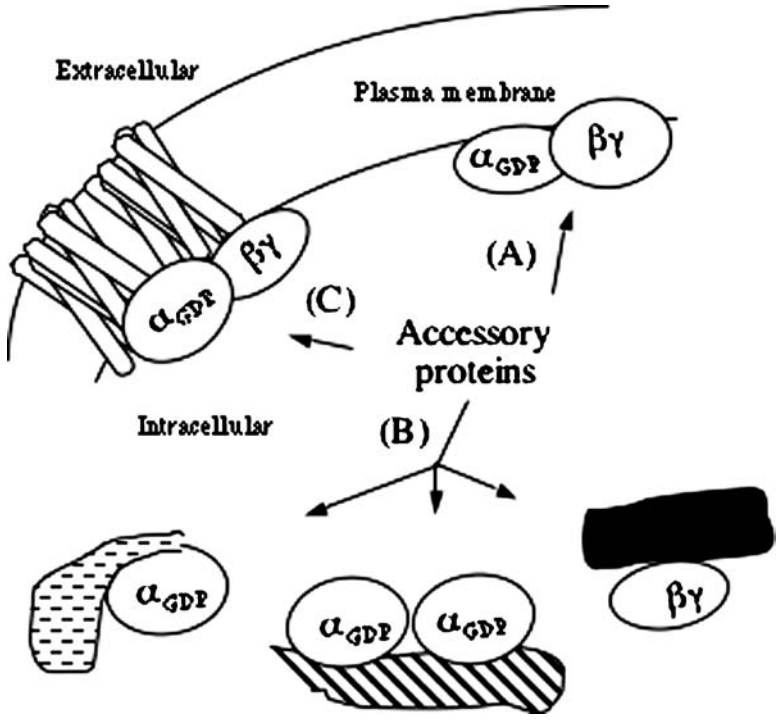

Figure 1 Roles for accessory proteins in G protein signaling systems. Accessory proteins are defined as proteins distinct from G protein–coupled receptor (GPCR), G protein, or classical effectors that regulate the strength/efficiency/specificity of signal transfer via $G_{\alpha\beta\gamma}$. This schematic illustrates the role of accessory proteins for this system providing signal input to heterotrimeric G proteins in the absence of a cell surface receptor and serving as alternative binding partners for G protein subunits. (*A*) Accessory proteins may directly regulate the activation state of G proteins in the membranes independent of a cell-surface GPCR. (*B*) G protein subunits may also be complexed with alternative binding partners (*textured or filled objects*) independent of the typical $G_{\alpha\beta\gamma}$ heterotrimer. Such complexes may exist in intracellular organelles such as the Golgi or at the plasma membrane. G_{α}-GDP complexed with such a binding partner may be regulated by nonreceptor guanine nucleotide exchange factors. (*C*) Accessory proteins may regulate signal transfer from receptor to G protein.

A critical aspect in the development of ideas regarding receptor-independent signaling roles for G proteins was the realization from studies involving subcellular fractionation and immunocytochemistry that specific G proteins (G_{α} and $G_{\beta\gamma}$), although clearly predominant at the plasma membrane, were also distributed in intracellular organelles, and that G_{α} was not always localized with $G_{\beta\gamma}$ within

the cell (30–39). Parallel studies that suggested unexpected functional roles of G protein subunits in secretion (40–47), Golgi stability (48, 49), and cell polarity in *Drosophila melanogaster* and *C. elegans* (50–53) provided further impetus for concepts related to diversification of G proteins as signal transducers. This review focuses on accessory proteins for G protein signaling (Figure 1), covering the topics of (*a*) guanine nucleotide exchange factors (GEFs) (Table 1), (*b*) guanine nucleotide dissociation inhibitors (GDIs) (Table 1), (*c*) protein binding partners for G protein subunits (Tables 1 and 2), and (*d*) regulators of signal transfer from receptor to G protein (Table 3). With one exception, only mammalian proteins are listed in the Tables.

NONRECEPTOR GEFs FOR G PROTEINS

GPCRs are widely recognized to serve as GEFs for heterotrimeric G proteins. In addition, numerous nonreceptor accessory proteins have been identified that also influence nucleotide binding by G_α subunits. These include GAP-43, NG-GPA, β-APP, presenilin I, AGS1, PBP/RKIP, and Ric-8 (Table 1). Each of these proteins directly increases GTPγS binding to purified G proteins. GAP-43, also known as F1, B-50, pp46, or neuromodulin, is a synaptic phosphoprotein enriched in neuronal growth cones where it is postulated to play a role in neurite outgrowth and axonal pathfinding. GAP-43, which interacts with $G_{\alpha o}$, the major heterotrimeric G protein in brain tissue, increases the rate of GDP dissociation and GTPγS association to purified brain heterotrimeric G protein and purified $G_{\alpha o}$ free of $G_{\beta\gamma}$ (5, 6). Pertussis toxin, which ADP-ribosylates a cysteine residue near the carboxyl terminus of $G_{\alpha i}$ and $G_{\alpha o}$, disrupts receptor-G protein coupling but does not alter the activation of brain G protein by GAP-43.

Two proteins implicated in Alzheimer's disease, β-APP and presenilin 1, interact with $G_{\alpha o}$ and exhibit preference for the GDP-bound conformation (54, 55). Presenilin 1 is primarily in the Golgi and endoplasmic reticulum, and it directly activates $G_{\alpha o}$ (54). The role of the interaction of these proteins with $G_{\alpha o}$ in the neurodegenerative disease process is not well understood.

NG-GPA, whose exact identity has not been determined, was partially purified in an assay involving the analysis of detergent-solubilized extracts of NG108-15 cells on GTPγS binding to purified heterotrimeric brain G protein (7, 9, 10). This bioactivity increases GTPγS binding to purified brain heterotrimeric G protein and G proteins in cell membranes, as well as purified $G_{\alpha o}$ and $G_{\alpha i1-3}$ (7, 9). ADP-ribosylation of purified brain G protein by pertussis toxin did not alter the ability of the NG-GPA to increase GTPγS binding to G protein, as also observed for GAP-43. As with GAP-43, NG-GPA acts on both heterotrimeric G protein and purified $G_\alpha$and its action is not altered by pertussis toxin pretreatment of cells, which effectively blocks receptor-mediated activation of G proteins in membrane preparations (10). This insensitivity to pertussis toxin suggests that these proteins promote nucleotide exchange by a mechanism differing from that of a GPCR.

TABLE 1 Accessory proteins interacting with G_α

Protein	Identification of interaction	Analysis of interaction	Functional role of interaction	References
A. Non-receptor guanine nucleotide exchange factors				
AGS1 (DexRas1; RASD1)	Functional screen for receptor-independent activators of G protein signaling	GST pull down: purified $G_{\alpha i1}$; activates $G_{\alpha i2}$ and $G_{\alpha i3}$ but not $G_{\alpha s}$ or $G_{\alpha 16}$ in functional screen	Increases GTPγS binding to $G_{\alpha i/o}$; activates pertussis toxin–sensitive activation of ERK1/2 in transfected cells; antagonizes GPCR signaling (see Table 3); involved in NMDA receptor signaling events	(25, 26, 56–62, 64)
β-APP	Amino acid similarity to IGF-II receptor	Coimmunoprecipitation with $G_{\alpha o}$	β-APP peptide (His^{657}-Lys^{676}) stimulates GTPγS binding to $G_{\alpha o}$; pertussis toxin sensitive	(55)
GAP-43	Biochemical	Direct binding to G protein not determined; GAP-43 may be the $M_r \sim 40{,}000$ protein associating with $G_{\alpha o}$ during purification of brain G proteins	Increases GTPγS binding to purified brain G-protein; pertussis toxin insensitive; promotes GDP dissociation	(5, 6, 154–158)
NG-GPA	Biochemical	Direct binding to G protein not determined	Increases GTPγS binding to $G_{\alpha i1-3}$ and $G_{\alpha o}$; pertussis toxin insensitive; promotes GDP dissociation	(7, 9, 10, 159)
PBP/RKIP (Raf kinase inhibitor protein)	Biochemical	Direct binding to G protein not determined	Increases GTPγS binding to cellular membranes and soluble $G_{\alpha i1}$; augments receptor coupling to G protein (see Table 3); pertussis toxin sensitive	(66–70)

Presenilin 1	Biochemical	GST pull down and coimmunoprecipitation; binds GDP-$G_{\alpha o}$	Increases GTPγS binding to $G_{\alpha o}$; pertussis toxin sensitive	(54)
Ric-8	8A isoform-Y2H with constitutive active $G_{\alpha o}$ Q205L as bait; 8B isoform – Y2H with $G_{\alpha s}$-long Q227L G_{α}olf as bait Genetic screens in *C. elegans*	8A isoform: GST pull down and coimmunoprecipitation; prefers GDP-bound conformation of $G_{\alpha i1}$; binds $G_{\alpha i}$, $G_{\alpha q}$, and $G_{\alpha o}$ in Y2H; 8B isoform: binds $G_{\alpha s}$ and $G_{\alpha q}$ in Y2H	8A isoform: increases GTPγS binding to $G_{\alpha i1}$, $G_{\alpha o}$, $G_{\alpha q}$, and $G_{\alpha 13}$ but not heterotrimeric G-protein; enhanced GPCR-mediated ERK1/2 activation (see Table 3) 8B isoform: role as GEF not determined	(71, 72, 76, 77)
Tubulin	Biochemical	^{125}I-tubulin blot overlay with purified G_{α} subunits; coimmunoprecipitation	Transactivates $G_{\alpha s}$, $G_{\alpha i}$, and $G_{\alpha q}$ by direct transfer of GTP to G_{α} subunits; also interacts with GPCR (see Table 3)	(80, 81, 160)
B. Inhibitors of guanine nucleotide exchange				
AGS3 (GPSM1)	Functional screen for receptor-independent activators of G protein signaling	GST pull downs: purified G_{α} and tissue extracts, coimmunoprecipitation; 4 GPR motifs*; activates $G_{\alpha i2}$ and $G_{\alpha i3}$ but not $G_{\alpha s}$ or $G_{\alpha 16}$ in functional screen	GPR motifs inhibit GTPγS binding to $G_{\alpha i/o}$, stabilize G_{α} in GDP-bound conformation, and compete with $G_{\beta\gamma}$ for $G_{\alpha i}$ binding; may be a substrate for LKB1 phosphorylation Scaffolding function, G-protein stability, neuronal adaptation	(25, 26, 90, 97–103, 120, 123–125)
AGS4 (GPSM3)	Functional screen for receptor-independent activators of G protein signaling	GST pull down and coimmunoprecipitation; contains 3 GPR motifs*; activates $G_{\alpha i2}$ and $G_{\alpha i3}$ but not $G_{\alpha s}$ in functional screen	Inhibits GTPγS binding to $G_{\alpha i/o}$	(91, 161)

(*Continued*)

TABLE 1 (*Continued*)

Protein	Identification of interaction	Analysis of interaction	Functional role of interaction	References
Caveolin	Biochemical	GST pull downs; binds $G_{\alpha i2}$ and $G_{\alpha o}$ Coimmunoprecipitation of Cav-1 and $G_{\alpha t}$	Scaffolding function; interaction with $G_{\alpha t}$ regulated by light; Cav-2 peptides inhibit GTPase activity and GTPγS binding to $G_{\alpha o}$; also interacts with GPCR (see Table 3)	(162–164)
LGN (GPSM2, AGS5)	Y2H: $G_{\alpha i2}$ as bait Functional screen for receptor-independent activators of G protein signaling	GST pull down and coimmunoprecipitation; $G_{\alpha i}$, $G_{\alpha o}$, $G_{\alpha t}$ Contains 4 GPR motifs*	Interaction with G protein influenced by binding partner NuMA Scaffolding function, G protein stability, cell division	(92, 100, 102, 123, 129–132, 165, 166)
Neuroglobin	Amino acid sequence similarity to RGS motifs	Binds $G_{\alpha i1-3}$ by SPR; prefers GDP-bound $G_{\alpha i1-3}$; interaction appears to be selective for oxidized ferric neuroglobin	Inhibits GTPγS binding to $G_{\alpha i1-3}$	(167, 168)
Pcp2/L7	Y2H: $G_{\alpha o}$	GST pull down and coimmunoprecipitation; $G_{\alpha o}$, $G_{\alpha i1-3}$ Contains 1 or 2 GPR motifs*	Influences receptor-mediated inhibition of $Ca_V2.1$ Ca^{2+} channels	(93, 100, 108, 169, 231)
Phosducin	Biochemical	Coimmunoprecipitation $G_{\alpha o}$, $G_{\alpha t}$	Inhibits GDP dissociation for $G_{\alpha q}$; also binds $G_{\beta\gamma}$	(170)
RapIGAPII (there is nonclarity on Rap1GapII versus RapIGap1 in database)	Y2H: $G_{\alpha i1}$, constitutive active $G_{\alpha o}$ Q205L and $G_{\alpha z}$ Q205L as bait	GST pull downs and coimmunoprecipitation; $G_{\alpha o}$, $G_{\alpha z}$, $G_{\alpha i1-3}$; for $G_{\alpha o}$: wild type > Q205L; $G_{\alpha z}$: Q205L > wild type; $G_{\alpha i}$ Q205L Contains 1 GPR motif*	Attenuates the ability of $G_{\alpha z}$ to inhibit adenylyl cyclase V; can enhance GPCR-mediated ERK1/2 activation; blocks $G_{\alpha o/i}$-induced neurite outgrowth; $G_{\alpha o}$ induces proteasomal degradation of RapIGAPII	(94–96, 118, 119)

RGS12 (AGS6)	In silico Functional screen for receptor-independent activators of G-protein signaling	GPR motif binds $G_{\alpha i1}$ and $G_{\alpha i3}$ by SPR Y2H: binds $G_{\alpha i1-3}$ Contains one GPR motif*	RGS motif for GAP activity	(171)
RGS14	In silico	GPR motif binds $G_{\alpha i1}$ in vitro by SPR Contains one GPR motif * Y2H: GPR motif binds $G_{\alpha i1-3}$; inhibition of nucleotide dissociation activity observed with $G_{\alpha i1}$ and $G_{\alpha i3}$, but not $G_{\alpha i2}$	RGS motif for GAP activity; PKA phosphorylation proximal to the RGS14-GPR motif may influence G_{α}-GPR interaction	(101, 104, 110, 171–174)
C. GTPase-activating proteins				
RGS proteins	Y2H: G_{α} subunits as bait Genetic screens in *C. elegans* and *S. cerevisae*	$G_{\alpha i/o}$, $G_{\alpha q}$, $G_{\alpha s}$, and $G_{\alpha 12/13}$	GTPase-activating proteins for $G_{\alpha i/o}$, $G_{\alpha q}$, $G_{\alpha s}$, and $G_{\alpha 12/13}$; can serve as scaffolding proteins for the formation of signaling complexes	(3, 4)
GRK2	Amino acid similarity with RGS motif; GST-GRK2 affinity matrix binds $G_{\alpha q}$	Weak GAP activity on $G_{\alpha q}$	Functional role of RGS motif and $G_{\alpha q}$ binding not fully defined	(175)
D. Other Gα interacting proteins				
GIV	Y2H: $G_{\alpha i3}$ as bait	GST pull down; $G_{\alpha i1-3}$, $G_{\alpha o}$, $G_{\alpha t}$, $G_{\alpha s}$	Unknown; colocalizes with $G_{\alpha i3}$ in Golgi transport vesicles	(143)

(*Continued*)

TABLE 1 (*Continued*)

Protein	Identification of interaction	Analysis of interaction	Functional role of interaction	References
GRIN1 GRIN2	^{32}P-$G_{\alpha z}$ probed cDNA λ expression library; GRIN2 also identified in Y2H with $G_{\alpha o}$Q205L as bait	His-tag and GST pull downs and coimmunoprecipitation; $G_{\alpha o}$, $G_{\alpha z}$	Selective for GTP-bound/activated $G_{\alpha i}$ and may be an effector; induces neurite formation in Neuro2a cells	(176, 177)
Gpb1, Gpb2 (*S. cerevisiae*)	Y2H: Gpa2 as bait	GST pull down and coimmunoprecipitation Selective for GDP-bound Gpa2	Contain seven kelch repeats; modulates cAMP production in response to glucose; inhibits filamentous differentiation; mammalian homolog not defined	(178)
Hax-1 (HS-1-associated protein X-1)	Y2H: $G_{\alpha 13}$ "effector interacting domain" (amino acids 221–347)	Coimmunoprecipitation Interaction selective for activated $G_{\alpha 13}$ mutants	Enhances $G_{\alpha 13}$-mediated Rac activity	(179)
HSP90	Y2H: constitutive active $G_{\alpha 12}$ Q226L as bait	Coimmunoprecipitation with $G_{\alpha 12}$ independent of nucleotide status but not $G_{\alpha 13}$, $G_{\alpha s}$	Geldanamycin, an Hsp90 inhibitor, alters $G_{\alpha 12}$ signaling	(180)
TPR1	Y2H: constitutive active $G_{\alpha 16}$ Q212L as bait	GST pull down and coimmunoprecipitations independent of nucleotide status ($G_{\alpha 16}$, $G_{\alpha q}$, $G_{\alpha s}$, $G_{\alpha i2}$)	Not defined; TPR1 interacts with Ha-Ras and promotes active Ras	(181)
UNC5H2	Y2H: constitutive active $G_{\alpha i2}$-Q205L as bait	GST pull down and coimmunoprecipitation Prefers $G_{\alpha i2}$-GTP	Inhibited receptor mediated inhibition of adenylyl cyclase	(182)

*GPR motifs inhibit GTPγS binding to $G_{\alpha i/o}$, stabilize G_α in GDP-bound conformation, and compete with $G_{\beta\gamma}$ for $G_{\alpha i}$ binding.

TABLE 2 Accessory proteins interacting with G_{β}, G_{γ} or $G_{\beta\gamma}$

Protein	Identification of interaction	Analysis of interaction	Functional role of interaction	References
AGS2 (tctex1) AGS7* AGS8**	Functional screen for receptor-independent activators of G protein signaling	GST pull down: purified $G_{\beta\gamma}$	Not defined; AGS2/tctex1 is light chain of cytoplasmic motor protein dynein; AGS2 also interacts with rhodopsin (see Table 3)	(26)
CSP (cysteine string protein)	Functional	GST pull down: purified $G_{\beta\gamma}$ and $G_{\beta\gamma}/G_{\alpha}$ from tissue lysate; coimmunoprecipitation: $G_{\beta\gamma}$calcium channel-β1 subunit, syntaxin from tissue lysate; $G_{\beta\gamma}$interaction regulated by ATP	Tonic inhibition of calcium channel activity by $G_{\beta\gamma}$	(183)
Dbl and Dbl family members kalirin and OST	Functional	GST pull down: purified $G_{\beta1\gamma2}$; coimmunoprecipitation: cotransfected $G_{\beta1\gamma2}$. Conserved amino-terminal region of kalirin and OST, coimmunoprecipitation; cotransfected $G_{\beta1}$	Interaction involved in JNK1 activation	(184)

(*Continued*)

TABLE 2 (*Continued*)

Protein	Identification of interaction	Analysis of interaction	Functional role of interaction	References
DIC (dynein intermediate chain)	Y2H: bovine $G_{\beta 1}$ as bait	Coimmunoprecipitation with purified $G_{\beta 1\gamma 1}$	Not defined	(145)
GRK2	Biochemical and functional	GST pull down: purified $G_{\beta\gamma}$, $G_{\beta 1}$, $G_{\beta 2}$; coimmunoprecipitation: cotransfected $G_{\beta 1\gamma 2}$	$G_{\beta\gamma}$ binding to GRK2 required for receptor phosphorylation	(185, 186)
KSR-1	Y2H: KSR-1 as bait binds $G_{\gamma 10}$,$_{\gamma 2}$, and $_{\gamma 3}$	GST pull down: G_{β} from cell lysate; coimmunoprecipitation: cell lysate and cotransfected $G_{\beta 1\gamma 3}$	Inhibition of $G_{\beta 1\gamma 3}$-mediated activation of ERK1/2	(146)
Phosducin	Biochemical	Co-purified with $G_{\beta\gamma}$; GST pull down: purified $G_{\beta\gamma}$; coimmunoprecipitation: purified $G_{\beta\gamma}$	Interaction influences visual signal duration; phosducin competes with $G_{\alpha t}$ for binding $G_{\beta\gamma}$; inhibits GTPγS binding to $G_{\alpha q}$ (see Table 1)	(187–192)
Phosducin-like protein (PhLP)	Cloned as an ethanol-responsive brain cDNA with homology to phosducin	GST pull down: purified $G_{\beta\gamma}$; coimmunoprecipitation: cotransfected $G_{\beta 1\gamma 2}$	PhLP antagonized enhancement of $G_{\beta\gamma}$-mediated rhodopsin phosphorylation; inhibits ADP-ribosylation of brain $G_{\alpha o}$	(193–195)

RACK1(receptor for activated C kinase 1)	Y2H: bovine $G_{\beta 1}$ as bait	GST pull down: purified $G_{\beta 1\gamma 1}$; coimmunoprecipitation: purified $G_{\beta 1\gamma 1}$ and cotransfected $G_{\beta 1\gamma 2}$	Inhibition of $G_{\beta 1\gamma 2}$-mediated activation of PLC-β2 and AC II	(145, 196)
RGS6, 7, 9, 11	Biochemical	Coimmunoprecipitation with $G_{\beta 5}$; copurified with $G_{\beta 5}$	RGS/$G_{\beta 5}$ inhibits $G_{\beta 1\gamma 2}$-mediated activation of PLC-β2	(197–200)
Syntaxin1A	Functional	GST pull down: purified $G_{\beta\gamma}$and G_{β} from tissue lysate; coimmunoprecipitation	Inhibition of N-type calcium channel activity by $G_{\beta\gamma}$	(201, 202)
Tubulin	Functional	Interaction with purified tubulin: $G_{\beta 1\gamma 2}$; coimmunoprecipitation: purified $G_{\beta\gamma}$; selective for $G_{\beta 1\gamma 2}$ versus $G_{\beta 1\gamma 1}$	Interaction involved in microtuble dynamics; tubulin-$G_{\beta\gamma}$ complex translocated to cytosol following muscarinic receptor activation; endocytosis of muscarinic receptors	(84, 203, 204)

*M.J. Cismowski, M. Sato, S.M. Lanier, unpublished observation.

**M. Sato, W.M. Chilian, S.M. Lanier, unpublished observation.

TABLE 3 Accessory proteins interacting with a GPCR and G protein and/or influencing signal transfer from receptor to G protein

Protein	Identification of interaction*	Functional role of the interaction	References
AGS1 (DexRas1; RASD1)	Functional	AGS1 blocks GPCR regulation of GIRK channels and ERK1/2	(59, 62)
AGS2 (tctex1)	Y2H: rhodopsin carboxyl terminus as bait	AGS2 regulates rhodopsin trafficking within the rod segments: $G_{\beta\gamma}$ interacts with both receptor and AGS2 $G_{\beta\gamma}$ may serve as an adaptor protein or perhaps regulate dynein function through interaction with AGS2	(147, 205, 206)
Caveolin	ET1 receptor coimmunoprecipitated with caveolin-1 following reconstitution in phospholipids vesicles	Caveolin binding to G_{α}-GDP protein and inhibition of GTP binding (see Table 1) may inhibit activation of G protein by the receptor; scaffolding function	(207)
Calmodulin**	GST-affinity matrix: mGluR4, 7a,b, 8a,b carboxyl terminus as bait	$G_{\beta\gamma}$ and calmodulin bind to mGluRs in a mutually exclusive manner; calmodulin may displace $G_{\beta\gamma}$ from the receptor to allow agonist-induced regulation of P/Q type calcium channel	(208, 209)
Coupling cofactor	Functional	Required for high-affinity binding of agonist to A1 adenosine receptors; stabilizes ternary complex with $G_{\alpha i}$ but not $G_{\alpha o}$	(149, 210)
GAP-43	Functional	GAP-43 increases M2 muscarinic receptor mediated GTP hydrolysis of $G_{\alpha o}$ and receptor-induced chloride channel opening in *Xenopus* oocytes	(211)
GPR motif	Functional	GPR peptide inhibits high-affinity agonist binding for 5-HT_{1A} receptors expressed in Sf9 cells reconstituted with $G_{\alpha i}$ or $G_{\alpha o}$ proteins; The GPR motif of RGS14 inhibits GPCR-mediated activation of ERK1/2; GPR peptide alters GPCR regulation of GIRK channels	(109–111)
GRK2	GST-affinity matrix: α_{2A}-adrenergic receptor i2 and i3 loop as bait; GRK2 coimmunoprecipitates with metabotropic glutamate receptor (mGluR1a) and $G_{\alpha q/11}$	$G_{\beta\gamma}$ binds directly to the i3 loop of some GPCRs where it may position GRK2 on its substrate; GRK2 inhibits signal transfer from mGluR1a to $G_{\alpha q/11}$ by dissociating mGluR1a from $G_{\alpha q/11}$; GAP activity of GRK2 for $G_{\alpha q/11}$ (see Table 1)	(206, 212, 213)
PBP/RKIP	Functional Copurification with the μ opioid receptor	Coexpression of PBP with μ opioid receptor, δ opioid receptor, and somatostatin receptor 2 augments agonist-induced signaling PBP may influence β-adrenergic receptor signaling	(68–70)

(*Continued*)

TABLE 3 (*Continued*)

Protein	Identification of interaction*	Functional role of the interaction	References
Periplakin**	Y2H:melanin-concentrating hormone receptor-1 and μ-opioid receptor carboxyl terminus as bait Melanin-concentrating hormone receptor-1 coimmunoprecipitated with periplakin	Periplakin interaction inhibits $G_{\alpha o}$ and $G_{\alpha i1}$ protein activation in response to melanin-concentrating hormone and DAMGO, respectively	(214, 215)
RGS2	GST-affinity matrix: i3 loop of M1, M3, and M5 muscarinic receptors	RGS2 inhibits agonist-induced production of inositol phosphates	(216)
Ric-8	Functional	Ric-8A enhances ERK1/2 activation by GPCR agonist LPA; Ric-8B augments β2-adrenergic and D1-dopaminergic receptor activation of adenylyl cyclase through $G_{\alpha olf}$	(77, 217)
Spinophilin	GST-affinity matrix: i3 loop of α2A-, α2B-, α2C-, and α1B-adrenergic receptors Y2H: D2 dopamine receptor i3 loop as bait	Spinophilin impedes α2-adrenergic receptor phosphorylation and desensitization by diminished interaction of receptor with GRK2 and arrestin; spinophilin binds RGS2, which act together to diminish α1B-adrenergic calcium signaling	(150–153)
Tubulin	GST-affinity matrix: carboxyl terminus of glutamate receptors (mGluR1, 7a, 7b); mGluR1a coimmunoprecipitates with tubulin	Tubulin interaction with mGluR may influence G-protein activation by receptor; scaffolding function	(80–84)

*See Tables 1 and 2 for G protein interaction.

**Compete with G protein binding sites on GPCR.

Alternatively, a similar mechanism may be used to promote nucleotide exchange, but the ADP-ribose group impedes the interaction of receptor but not the NG-GPA with G protein. It is not known if the protein conferring the NG-GPA bioactivity actually represents the activity of a known nonreceptor guanine nucleotide exchange factor (Table 1) or whether it represents an additional, yet to be defined, member of this group of accessory proteins.

Studies with NG-GPA provided an impetus for the development of a yeast-based functional screen for receptor-independent activators (25, 26). AGS1, which was isolated in this screen, increases GTPγS binding to brain heterotrimeric G proteins as well as purified $G_{\alpha o}$, $G_{\alpha i1}$, and $G_{\alpha i2}$. AGS1, initially identified as a dexamethasone-inducible cDNA (56), has several interesting biological properties (25, 26, 57–64). It is a member of the Ras subgroup of small G proteins, thus providing a connection for cross communication across different signaling "cassettes." In transfected cell systems, the activation of a $G_{\beta\gamma}$-regulated effector by AGS1 was blocked by pertussis toxin pretreatment of the cells, suggesting that AGS1 acts in a manner similar to a GPCR (57). However, in both *Xenopus* oocytes and transfected mammalian cells, AGS1 inhibited GPCR-mediated activation of G protein signaling pathways (59, 62) (Table 3). AGS1 was subsequently implicated

as a key player in NMDA receptor signaling and the processing of signals in the suprachiasmatic nuclei during the circadian cycle (61, 64, 65).

PBP (phosphatidylethanolamine-binding protein) or raf kinase interacting protein (RKIP) is a 23-kDA soluble basic protein that has surfaced in a variety of discovery platforms. It directly interacts with Raf-1 and the GPCR kinase GRK2, and it was also copurified with the μ opioid receptor (66–69). PBP/RKIP increases GTPγS binding to purified $G_{\alpha i1}$ and cell membrane G proteins (70). The activation of G protein signaling by PBP/RKIP was blocked by pertussis toxin treatment of cells (70). PBP/RKIP inhibits Raf-1 phosphorylation of MEK (mitogen-activated protein kinase kinase) and the activity of the receptor kinase GRK-2, providing another mechanism for signal crosstalk (66–68). The relative importance of these different actions of PBP/RKIP within the cell is not known.

The most recently identified member of this group is the protein Ric-8, which was implicated in multiple aspects of G protein signaling in *C. elegans*, where it was initially isolated in a genetic screen for survival of the neurotoxic effects of cholinesterase inhibitors reflected by altered neurotransmitter release (71). Ric-8 mutations or knockdown also result in defects in asymmetric cell division in *C. elegans* (72–75). Mammalian Ric-8 was isolated in Y2H screens with a constitutively active mutant of $G_{\alpha o}$ (Ric-8A) or $G_{\alpha s}$-long (Ric-8B) (76). In biochemical assays, Ric-8A increased GTPγS binding to purified $G_{\alpha o}$ alone but not when $G_{\alpha o}$ was complexed with $G_{\beta\gamma}$ (76). Ric-8A also increased GTPγS binding to $G_{\alpha q}$ and $G_{\alpha i}$ (76). Ric-8A overexpression was recently reported to augment the activation of ERK1/2 by either a GPCR or the $G_{\beta\gamma}$-binding peptide SIRK (77).

Tubulin, also included in this group, differs in its mechanism of G protein regulation: it increases GTP binding to G_α subunits by exchange of GTP bound to tubulin for the GDP bound to G_α (78–81). Tubulin also interacts with the carboxyl terminus of the metabotropic glutamate receptor Types 1α (82), 7a and 7b (83), as well as $G_{\beta\gamma}$ (84). Although not fully delineated, the tubulin interaction with receptor and G protein subunits may play multiple roles in trafficking, G protein activation, and the control of microtubule dynamics (84).

Members of this class of accessory proteins generally prefer the $G_{\alpha i/\alpha o}$ subgroup of G proteins. However, this may be due in part to the ease of access to these G proteins in their purified form for biochemical studies. A more extensive analysis of the interaction of some members of this group with other members of the G protein family would be of value. If this group of GEFs is indeed selective for $G_{\alpha i}/G_{\alpha o}$ subgroup of G proteins, then one must ask if this reflects particular properties of nucleotide binding for this subgroup versus other G proteins. Another question of interest is, What special aspect of $G_{\alpha i}/G_{\alpha o}$ signaling has Nature found useful to regulate in such a manner? The mechanistic aspects as to how proteins listed in Table 1 integrate into receptor-dependent or -independent regulation of G protein signaling merits further investigation. Subsequent sections of this review discuss alternative binding partners for G_α subunits independent of $G_{\beta\gamma}$, and one question is whether the GEFs listed in Table 1 can function as GEFs for G_α when it is complexed with a protein(s) other than $G_{\beta\gamma}$.

Signal Input for Nonreceptor GEFs

While accessory proteins in Table 1 provide alternative modes of signal input to G protein signaling systems, two major questions are: What upstream signal drives an individual GEF and how is this signal regulated? The expression of both GAP-43 and AGS1 is developmentally regulated and AGS1 is markedly upregulated by glucocorticoids (56, 85, 86). This information, along with the cycling of AGS1 mRNA during the circadian rhythm (65), suggests that transcriptional control may be an important factor. As a guanine nucleotide binding protein, AGS1 may be either constitutively active or activated by specific GEFs. AGS1 is activated by NMDA receptor agonists, providing an unexpected entre to G protein signaling cascades via a postulated nNOS-mediated nitrosylation of AGS1 (61, 87). Phosphorylation of GAP-43 and PBP/RKIP binding proteins also provides a focal point for signal control or protein positioning within the cell. GAP-43 is in the cytosol and is membrane-associated, and its subcellular location is altered by bFGF, perhaps in a manner that relates to GAP-43 phosphorylation (88). Palmitoylation of GAP-43 blocks its ability to increase GTPγS binding to purified G protein (89). Efforts to understand the mechanistic aspects of signal input to this class of proteins will be a fruitful area of investigation. Signal termination would presumably involve the hydrolysis of bound GTP.

GUANINE NUCLEOTIDE DISSOCIATION INHIBITORS FOR G_α

The second major class of accessory proteins listed in Table 1 actually appears to inhibit the dissociation of GDP from G_α subunits, in direct contrast to the guanine nucleotide exchange factors discussed in the preceding section. The majority of this subgroup of accessory proteins share a common structural feature termed the G protein regulatory (GPR) (26), or GoLoco motif (90). Various GPR proteins were identified in Y2H screens with G_α subunits and in a functional screen for receptor-independent activators of G protein signaling (25, 26, 91–96). Protein interaction assays indicate the binding of GPR proteins to G_α and not $G_{\beta\gamma}$. Biochemical and structural studies indicate that the GPR motif stabilizes $G_{\alpha i}/G_{\alpha o}$ subunits in the GDP-bound conformation and competes with $G_{\beta\gamma}$ for G_α binding (97–103). Identification of key amino acids within this core motif as well as regions outside of the core GPR motif, some of which anchor to the helical domain of $G_{\alpha i}$, provide a basis for GPR selectivity among different G_α subunits and a platform for the development of peptides or small molecules that might act as GPR agonists or antagonists (98, 101, 104–106, 227).

Such proteins would be predicted to impede signaling through $G_{\alpha i}$ by virtue of stabilizing the GDP-bound conformation, but several members of this group containing GPR motifs were identified as AGS proteins in the yeast-based functional screen. This likely reflects the increased availability of $G_{\beta\gamma}$ free of G_α for

activation of the MAP kinase cascade (26, 91). It is not clear if these proteins function by promoting subunit dissociation independent of nucleotide exchange or whether they "grab" G_α during basal cycling of the heterotrimer between its GDP, nucleotide-free and GTP-bound state and thus prevent rebinding of $G_{\beta\gamma}$. A GPR peptide promotes subunit dissociation of purified heterotrimer (107, 108), competes with $G_{\beta\gamma}$ binding to G_α, and influences receptor coupling to G protein (109–111), providing multiple opportunities for integration into cell signaling events. $G_{\alpha i}$ GDP complexed with a GPR protein is itself postulated to act as an effector (73, 112, 113).

The majority of functional studies with GPR proteins have dealt with the surprising role of selected GPR proteins in asymmetric cell division during development (51–53, 73, 75, 112–117). The process of asymmetric cell division in both *D. melanogaster* neuroblasts and *C. elegans* early embryos involves an intrinsic cue that does not involve a cell surface receptor, observations consistent with the postulated role of these and other accessory proteins as receptor-independent activators of G protein signaling. Most GPR proteins are also expressed in adult tissues and likely subserve functions beyond a role in cell division (94–96, 118–124). Whereas LGN is widely expressed in multiple tissues, the expression of other members of this group is more restricted (91, 92, 123). Rap1GAP, which contains one GPR motif, is implicated in signaling events involving $G_{\alpha i}$, $G_{\alpha o}$, and $G_{\alpha z}$ (94–96, 118, 119). Full-length AGS3 is enriched in brain with a short version lacking the TPR motifs enriched in heart (123, 124). A short version of AGS3 containing only GPR motifs blocks the sensitization of adenylyl cyclase observed with prolonged stimulation of a GPCR coupled to $G_{\alpha i}/G_{\alpha o}$ (121). Within the prefrontal cortex, AGS3 is implicated in the neural adaptation to cocaine withdrawal, and this action likely involves G protein signaling via the GPR motifs in AGS3 (120). Whether AGS3 and related proteins serve a more general role in neural adaptation or synaptic plasticity is a focus of several current research efforts. To fully define the functional role of each GPR-containing protein, one should also move beyond traditional questions or experimental paradigms that are often posed in the context of GPCRs.

Signal Input for GPR-Containing Proteins

A key question in the field is what regulates the interaction of GPR proteins with G proteins and whether they are complexed with G_αGDP free of $G_{\beta\gamma}$ in the cell awaiting a signal from a GEF. Many of the GPR proteins contain additional protein interaction domains for binding of regulatory partners (28, 29). Phosphorylation of GPR proteins may provide another mode of regulation (125, 126). Both phosphorylation and specific binding partners may be crucial for controlling the subcellular location of GPR proteins and the interaction of the GPR motifs with G proteins.

The importance of subcellular targeting for GPR proteins is dramatically illustrated for PINS, the AGS3 and LGN ortholog in *D. melanogaster* (51–53, 112, 116, 127). The mammalian proteins AGS3 and LGN exhibit ~65% amino acid homology with a similar domain structure. In *D. melanogaster* and *C. elegans*,

there is only one version of this mammalian gene. During asymmetric division of the neuroblast in *D. melanogaster*, a specific binding partner(s) translocates PINS from the cytosol to the apical cortex of the dividing cell where it complexes with G_α (51–53, 112, 127, 128). AGS3, LGN, and PINS each contain three to four GPR motifs in the carboxyl region of the protein and seven tetratricopeptide repeats in the amino terminal half of the protein (102). The subcellular location of these proteins is regulated by TPR and GPR motifs to varying extents, and the relative importance of one domain versus the other in this regulation likely depends on the functional and developmental status of the cell (51–53, 112, 116, 123, 124, 129–135).

Asymmetric division of the one cell embryo in *C. elegans* also requires spatial enrichment of a GPR protein (GPR1/2) (113, 115, 117), although this GPR protein is different from the AGS3, LGN ortholog in *C. elegans* (AGS3.1). Knockdown of GPR1/2 or GOA-1/GPA-16 in *C. elegans* results in symmetric cell division and subsequent embryonic death. GPR1/2, G proteins, Ric-8, and RGS7 all play crucial roles in the generation of spindle-pulling forces of different strengths at the anterior and posterior poles (72–75, 113, 115, 117, 136).

The role of the mammalian proteins LGN and AGS3 in asymmetric cell division is not yet defined. The TPR domains of LGN bind the nuclear mitotic apparatus protein NuMA, and this interaction localizes LGN to spindle poles during cell division and influences its interaction with G proteins (129, 130). LGN is also found in the nucleus of some cells during interphase, and is enriched in the midbody during late telophase and cytokinesis (123). It is not known what regulates the targeting of the protein to this region or if there is a G protein-mediated signal involved in mitotic spindle dynamics or cytokinesis during cell division. The midbody is a crucial site for control of cell abscission and it is enriched in a number of signaling proteins (137). Knockdown of LGN by RNAi results in multinucleated cells, although a role for G_α in this process is not defined (130). The overall process of symmetric cell division is not dramatically altered by pertussis toxin treatment, but it is not clear if the population of G_α involved (i.e., a population complexed with a GPR protein rather than $G_{\beta\gamma}$) is a substrate for the toxin.

Both AGS3 and LGN are phosphoproteins and the GPR domains of both proteins are phosphorylated, but the control of phosphorylation and the kinases involved are not known. AGS3 and LGN are phosphorylated in immunoprecipitates of the serine/threonine kinase LKB1 from cell homogenates (125). LKB1, which is implicated in Peutz-Jeghers Syndrome (an inherited intestinal polyposis disorder) and more recently characterized as an upstream regulator of AMP-regulated kinase, is the mammalian counterpart of the *C. elegans* gene *par-4* (138). Disruption of members of the *par* group of genes results in partitioning defects in early embryogenesis, which may relate to a role for AGS3/LGN orthologs and G proteins in cell polarity. LKB1 orthologs are also involved in epithelial cell polarity in *D. melanogaster* and in division of *Xenopus* oocytes (139, 140). LKB1 immunoprecipitates phosphorylate AGS3 in its GPR domain, and phosphorylation of a consensus GPR peptide inhibits its ability to regulate the activation state

of G proteins. Phosphorylation of serines/threonines within the GPR motif may serve as a regulatory mechanism that controls the interaction with and/or regulation of heterotrimeric G proteins by AGS3, LGN, and other proteins containing GPR motifs.

The subcellular distribution of LGN, but not AGS3, in primary cortical neurons is rapidly altered in response to activation of NMDA receptors or increases in intracellular calcium (123). With increasing understanding of the biochemistry and cell biology of GPR proteins and the availability of specific antibodies to GPR-containing proteins, it is likely that additional external stimuli will be identified that control the subcellular location of GPR proteins with interesting functional consequences.

ADDITIONAL G_α-AND $G_{\beta\gamma}$-BINDING PROTEINS

In addition to the two classes of accessory proteins for G proteins discussed in the preceding sections of this review and the well-known effectors for G_α and $G_{\beta\gamma}$, there are several binding partners identified for G_α and $G_{\beta\gamma}$ subunits that do not readily fit into these categories and deserve further discussion (Tables 1 and 2). These binding partners were identified in Y2H screens by copurification with G protein subunits or connected through functional observations. Protein phosphatase 2a and 5 were also identified in Y2H screens with selected G_α subunits, but are not included in Table 1 as this interaction was selective for the activated form of G_α and hence these proteins may be considered as effectors (141, 142). Several of these binding partners are found in intracellular compartments, providing further support for the concept of G protein signaling distinct from that operating in the context of signal transfer for GPCRs. Members of this group of proteins may also be G protein effectors or influence the subcellular location of G protein subunits. Although these proteins are divided into those binding G_α or $G_{\beta\gamma}$ subunits, some may have more complex interactions with the $G_{\alpha\beta\gamma}$ heterotrimer.

Many of the interactions of G_α subunits with various accessory proteins may occur in microdomains within the cell, such as the Golgi apparatus or caveolae. Calnuc and G_α-interacting vesicle-associated protein (GIV) were identified as $G_{\alpha i3}$-binding partners in Y2H screens (143, 144). $G_{\alpha i3}$ in the Golgi membrane is suggested to interact with cytoplasmic Calnuc, which may regulate the function of $G_{\alpha i3}$ by virtue of its calcium-binding capacity (144). GIV, which is localized to vesicles transporting proteins from the endoplasmic reticulum to the Golgi, interacts with $G_{\alpha i1-3}$, $G_{\alpha o}$, and $G_{\alpha t}$, as well as $G_{\alpha s}$, but has no effect on the nucleotide-binding or hydrolysis properties of G_α subunits (143).

While it is somewhat more direct to define a functional consequence of protein interaction with the G_α subunit in terms of nucleotide binding or hydrolysis, a similar insight as to the functional role of proteins that bind to the $G_{\beta\gamma}$ subunit is not as straightforward. $G_{\beta\gamma}$ binding partners such as RACK1, DIC, or Ksr-1 were identified in Y2H screens with a G_β or G_γ subunit as bait (145, 146). However,

with the exception of the interaction of $G_{\beta 5}$ with proteins other than the typical G_{γ} subunits, the $G_{\beta\gamma}$ complex generally functions as a single entity in the cell. Three $G_{\beta\gamma}$ binding partners (AGS2, AGS7, AGS8) were identified in the yeast-based functional screen for receptor-independent activators of G protein signaling. AGS2, which is identical to a light chain of cytoplasmic motor protein dynein, also binds to the carboxyl terminus of rhodopsin (25, 26, 147). Phosducin, which was copurified with $G_{\beta\gamma}$ from retina, interferes with reassociation of $G_{\alpha t}$ and $G_{\beta\gamma}$ following light-mediated G protein activation. The functional role of the interaction of these various proteins with $G_{\beta\gamma}$ and G protein heteotrimer in the cell, both in the context of GPCR signaling and the role of $G_{\beta\gamma}$ as a transducer of signals unrelated to a GPCR, is an active area of study in the field.

ALTERED INTERACTIONS OF G PROTEIN SUBUNITS INDEPENDENT OF NUCLEOTIDE EXCHANGE

One of the most interesting features of the group of AGS proteins identified in the yeast functional screen was their use of different mechanisms for G protein signaling activation (25, 26). One group of AGS proteins (AGS2–8) was active in the presence of a G204A-G_{α} mutant that does not stably bind GTP, whereas a second group (AGS1) was not. The second group, but not the first group, was also antagonized by coexpression of RGS4 or RGS5, which would accelerate the GTPase activity of the G_{α} subunit to promote signal termination. These observations suggest that the second group (AGS1) functions as a guanine nucleotide exchange factor, although it cannot be ruled out that RGS4 or RGS5 simply competed for AGS1 interaction with G protein. The first group of proteins (AGS2–8) is thus activating the system in the absence of guanine nucleotide exchange. Although the nucleotide-binding and hydrolysis properties of the G204A $G_{\alpha i}$ mutant are not fully characterized, if the G_{α} subunit is indeed "locked" in its GDP-bound conformation complexed with $G_{\beta\gamma}$, then such proteins may act by one of three mechanisms: (*a*) AGS2–8 may promote subunit dissociation in the absence of nucleotide exchange via an interaction with heterotrimeric $G_{\alpha\beta\gamma}$; (*b*) the on-off rate of G204A-$G_{\alpha i}$ association with $G_{\beta\gamma}$ may be such that when the two are apart, interaction of either subunit with an AGS protein may prevent subunit reassociation, and (*c*) AGS2–8 may bind to G_{α} or $G_{\beta\gamma}$ before they have a chance to associate at the membrane. If the same system operates in mammalian systems, then one could achieve selective activation of $G_{\beta\gamma}$-sensitive effectors or selective increase in the duration of $G_{\beta\gamma}$ signaling once heterotrimeric G proteins are activated by a GPCR.

The AGS2-8 group of proteins could be further distinguished by their selectivity for different G_{α} in the yeast system and their ability to interact with mammalian G_{α} or $G_{\beta\gamma}$. AGS3 functioned in yeast strains expressing $G_{\alpha i2}$ and $G_{\alpha i3}$ but not $G_{\alpha 16}$, $G_{s\alpha}$, or Gpa1 (25, 26). In contrast, AGS2, 7, and 8 functioned in each of the yeast strains expressing mammalian G_{α} ($G_{\alpha i2}$, $G_{\alpha i3}$, $G_{\alpha s}$, $G_{\alpha 16}$), suggesting

that their bioactivity was independent of a specific mammalian G_α subunit. The GPR-containing proteins in this group bind $G_{\alpha i}$GDP, whereas AGS2, 7, and 8 bind mammalian $G_{\beta\gamma}$. Thus, in the latter case, AGS bound to $G_{\beta\gamma}$ would either dissociate prior to $G_{\beta\gamma}$ interaction with its effector or allow simultaneous binding to an effector.

Although the yeast-based screen has proven extremely powerful for such studies, it is important to realize that the $G_{\alpha i2}$, $G_{\alpha i3}$, and $G_{\alpha 16}$ substituted for the yeast Gpa1 contains the amino terminal 41 amino acids of Gpa1, whereas the substituted $G_{\alpha s}$ is modified with an E10K mutation. These modifications allow effective coupling to yeast $G_{\beta\gamma}$. In addition, protein interaction assays with tagged AGS proteins involve mammalian $G_{\beta\gamma}$ and not yeast $G_{\beta\gamma}$, which contains additional structural features that distinguish it from mammalian $G_{\beta\gamma}$.

ACCESSORY PROTEINS AND SIGNAL TRANSFER FROM RECEPTOR TO G PROTEIN

Although there is clearly a developing story for the impact of AGS and related proteins on G proteins independent of a receptor, the extent to which such proteins influence signals initiated by activation of a cell-surface GPCR is not clear. The increasing realization that these receptors function in the context of a larger signal transduction complex with receptor dimers presents an attractive environment for proteins that regulate G protein activation and deactivation (219, 221, 228, 229). Several accessory proteins interact with both a GPCR and a G protein subunit and/or influence the efficiency of signal transfer from receptor to G protein and alter signaling kinetics (Table 3). AGS3 and LGN can both bind up to four G_α free of $G_{\beta\gamma}$(102, 105), which may provide a signaling scaffold, perhaps akin to other oligomeric complexes in G protein signaling (148). Dynamic signaling complexes may also be assembled and disassembled within the context of caveolae, lipid rafts, or clathrin-coated vesicles (228–230). Three of the receptor-independent GEFs (GAP-43, PBP/RKIP, Ric-8) augment such activation, whereas AGS1 and GPR proteins may impede GPCR-mediated activation of G proteins. An as-yet unidentified coupling cofactor influences agonist affinity at adenosine A1 receptors possibly by regulating the interaction of receptor and G protein (149). RGS proteins may be complexed with the intracellular domains of a receptor directly or via interaction with other proteins such as spinophilin (150–153, 216). Many GPR proteins have multiple protein interaction domains. RGS12 and RGS14 both have a single GPR motif paired with the RGS motif that accelerates GTPase activity of G_α. Depending on the context, AGS and related accessory proteins may impede signal transfer from receptor to G protein by competing with receptor for G protein binding. Alternatively, such proteins may augment signal transfer or signal duration by influencing interactions between G_α and $G_{\beta\gamma}$ or participating in the organization of a larger signaling complex.

PERSPECTIVE

Accessory proteins may (*a*) provide a cell-specific mechanism for signal amplification by acting in concert with GPCRs, (*b*) influence the population of activated G protein/effectors within the cell independent of receptor activation, (*c*) be "effectors" subject to receptor regulation providing attractive targets for cross talk among diverse signaling systems, and/or (*d*) provide alternative modes of input to G protein-regulated signaling pathways independent of classical GPCRs. Such accessory proteins thus have potentially broad physiological and pharmacological significance relative to the cell biology and functional properties of G proteins themselves. By contributing to the amplification of biological stimuli commonly observed with signaling events involving heterotrimeric G proteins, these proteins may be of particular importance in tissues requiring rapid signal processing or under conditions of aberrant cell growth. The modulation of key signaling pathways should present some interesting opportunities for drug development. Agents that influence the activity of these accessory proteins may impact GPCR signaling by altering signal duration or intensity and perhaps modulate receptor regulatory mechanisms such as desensitization.

One of the biggest challenges facing the field is determining how these accessory proteins integrate into various signaling systems and their potential contribution to signaling dysfunction in disease. Another focus of current efforts is defining the stimulus that drives the signal input of these accessory proteins, and what lies downstream subsequent to signal input, in terms of a particular pool of G proteins. For asymmetric cell division, this stimulus is postulated to be intrinsic to the cell, whereas in other situations external stimuli may act through cell surface, receptor-mediated mechanisms. Transcriptional control of accessory proteins or proteins involved in controlling their subcellular distribution also likely plays an important role. While G proteins activated by accessory proteins may influence known G protein effectors, it is likely that unexpected modes of G protein regulation also interface to distinct, as yet undefined, groups of effector proteins. For example, the role of GPR proteins and G_α in spindle-pulling forces during asymmetric cell division may involve the motor protein dynein as an effector.

The identification of alternative binding partners for G protein subunits and the action of AGS and related proteins on the G protein activation-deactivation cycle suggest an expanded functional repertoire for $G_{\alpha\beta\gamma}$ or the individual subunits G_α and $G_{\beta\gamma}$ independent of each other. One of Nature's signature features is to find something that works and adapt it time and again for different functions. One of the best examples of this phenomenon is the diverse nature of signals mediated by the seven-membrane span receptor motif and the "G-switch" involving nucleotide exchange and hydrolysis. It is thus likely that the unexpected functional roles of G protein subunits (i.e., asymmetric cell division, Golgi structure, vesicular trafficking) and their regulation, as discussed in this review, are just the first examples of such functional diversification for G_α and $G_{\beta\gamma}$.

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Annual Review of Pharmacology and Toxicology
Volume 46, 2006

CONTENTS

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS: HOW THEIR EFFECTS ON MACROPHAGES CAN LEAD TO THE DEVELOPMENT OF A NEW DRUG THERAPY AGAINST ATHEROSCLEROSIS, *Andrew C. Li and Wulf Palinski* 1

CYTOCHROME P450 AND XENOBIOTIC RECEPTOR HUMANIZED MICE, *Frank J. Gonzalez and Ai-Ming Yu* 41

HUMAN FLAVIN-CONTAINING MONOOXYGENASES, *John R. Cashman and Jun Zhang* 65

CANNABINOID RECEPTORS AS THERAPEUTIC TARGETS, *Ken Mackie* 101

REGULATION OF DRUG-METABOLIZING ENZYMES AND TRANSPORTERS IN INFLAMMATION, *Alison E. Aitken, Terrilyn A. Richardson, and Edward T. Morgan* 123

ACCESSORY PROTEINS FOR G PROTEINS: PARTNERS IN SIGNALING, *Motohiko Sato, Joe B. Blumer, Violaine Simon, and Stephen M. Lanier* 151

THE PROTEASOME AND PROTEASOME INHIBITORS IN CANCER THERAPY, *Peter M. Voorhees and Robert Z. Orlowski* 189

NUCLEAR AND MITOCHONDRIAL COMPARTMENTATION OF OXIDATIVE STRESS AND REDOX SIGNALING, *Jason M. Hansen, Young-Mi Go, and Dean P. Jones* 215

THE REGULATION AND PHARMACOLOGY OF ENDOTHELIAL NITRIC OXIDE SYNTHASE, *David M. Dudzinski, Junsuke Igarashi, Daniel Greif, and Thomas Michel* 235

REGULATION OF PLATELET FUNCTIONS BY P2 RECEPTORS, *Christian Gachet* 277

FUNCTIONAL IMAGING OF TUMOR PROTEOLYSIS, *Bonnie F. Sloane, Mansoureh Sameni, Izabela Podgorski, Dora Cavallo-Medved, and Kamiar Moin* 301

PHARMACOGENOMICS OF ACUTE LEUKEMIA, *Meyling H. Cheok, Sanne Lugthart, and William E. Evans* 317

REGULATION OF PHOSPHOLIPASE C ISOZYMES BY RAS SUPERFAMILY GTPASES, *T. Kendall Harden and John Sondek* 355

ROLE OF ABCG2/BCRP IN BIOLOGY AND MEDICINE, *P. Krishnamurthy and J.D. Schuetz* 381

CO AS A CELLULAR SIGNALING MOLECULE, *Hong Pyo Kim, Stefan W. Ryter, and Augustine M.K. Choi* 411

FUNCTION OF RETINOID NUCLEAR RECEPTORS: LESSONS FROM GENETIC AND PHARMACOLOGICAL DISSECTIONS OF THE RETINOIC ACID SIGNALING PATHWAY DURING MOUSE EMBRYOGENESIS, *Manuel Mark, Norbert B. Ghyselinck, and Pierre Chambon* 451

MOLECULAR MECHANISM OF 7TM RECEPTOR ACTIVATION—A GLOBAL TOGGLE SWITCH MODEL, *Thue W. Schwartz, Thomas M. Frimurer, Birgitte Holst, Mette M. Rosenkilde, and Christian E. Elling* 481

INDEXES

Subject Index 521
Cumulative Index of Contributing Authors, Volumes 42–46 535
Cumulative Index of Chapter Titles, Volumes 42–46 538

ERRATA

An online log of corrections to *Annual Review of Pharmacology and Toxicology* chapters may be found at http://pharmtox.annualreviews.org/errata.shtml