MINIREVIEW

Heterotrimeric G Protein Signaling Outside the Realm of Seven Transmembrane Domain Receptors

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ABSTRACT

Heterotrimeric G proteins, consisting of the guanine nucleotide-binding Gα subunits with GTPase activity and the closely associated Gβ and Gγ subunits, are important signaling components for receptors with seven transmembrane domains (7TMRs). These receptors, also termed G protein-coupled receptors (GPCRs), act as guanine nucleotide exchange factors upon agonist stimulation. There is now accumulating evidence for noncanonical functions of heterotrimeric G proteins independent of 7TMR coupling. Gα proteins belonging to all 4 subfamilies, including Gαs, Gαi, Gαq, and Gα12 are found to play important roles in receptor tyrosine kinase signaling, regulation of oxidant production, development, and cell migration, through physical and functional interaction with proteins other than 7TMRs. Association of Gα with non-7TMR proteins also facilitates presentation of these G proteins to specific cellular microdomains. This Minireview aims to summarize our current understanding of the noncanonical roles of Gα proteins in cell signaling and to discuss unresolved issues including regulation of Gα activation by proteins other than the 7TMRs.

Heterotrimeric G proteins are characteristically activated by seven-transmembrane domain receptors (7TMRs), also known as G protein-coupled receptors (GPCRs). The binding of an agonist to the extracellular or transmembrane domains of a 7TMR induces conformational changes in the receptor, which then functions as a guanine nucleotide exchange factor (GEF) for the associated GDP-bound Gα subunit. The switch to the active GTP-bound state triggers Gα subunit release from the receptor, leading to dissociation of Gβγ from Gα proteins and activation or inhibition of downstream effectors (Janetopoulos et al., 2001; Cabrera-Vera et al., 2003). Increasing evidence also suggests that, in some cases, upon ligand binding, the G proteins remain in the heterotrimeric form but undergo conformational rearrangement (Bünnemann et al., 2003; Digby et al., 2006). The signaling cycle is complete after hydrolysis of GTP to GDP via the GTPase activity intrinsic to the Gα subunits. It is noteworthy that 7TMRs can signal independently of heterotrimeric G proteins. Several mechanisms mediating 7TMR signaling involve β-arrestins (Luttrell and Lefkowitz, 2002; Violin and Lefkowitz, 2007), G protein-coupled receptor kinases (Penn et al., 2000), JAKs/STATs (Marrero et al., 1995), Src-family tyrosine kinases (Sun et al., 1997; Thomas and Brugge, 1997), and scaffold proteins such as postsynaptic density 95/disc-large/zona occludens (PDZ) domain-containing proteins (Hall et al., 1998; Vila-Coro et al., 1999). In recent years, heterotrimeric G proteins have also emerged as noncanonical mediators of 7TMR-independent signaling pathways (Patel, 2004). This Minireview aims to address the pivotal roles of Gα proteins in increasing evidence also suggests that, in some cases, upon ligand binding, the G proteins remain in the heterotrimeric form but undergo conformational rearrangement (Bünnemann et al., 2003; Digby et al., 2006). The signaling cycle is complete after hydrolysis of GTP to GDP via the GTPase activity intrinsic to the Gα subunits. It is noteworthy that 7TMRs can signal independently of heterotrimeric G proteins. Several mechanisms mediating 7TMR signaling involve β-arrestins (Luttrell and Lefkowitz, 2002; Violin and Lefkowitz, 2007), G protein-coupled receptor kinases (Penn et al., 2000), JAKs/STATs (Marrero et al., 1995), Src-family tyrosine kinases (Sun et al., 1997; Thomas and Brugge, 1997), and scaffold proteins such as postsynaptic density 95/disc-large/zona occludens (PDZ) domain-containing proteins (Hall et al., 1998; Vila-Coro et al., 1999). In recent years, heterotrimeric G proteins have also emerged as noncanonical mediators of 7TMR-independent signaling pathways (Patel, 2004). This Minireview aims to address the pivotal roles of Gα proteins in
regulating diverse biological responses through their interaction with proteins other than the 7TMRs.

**Regulation of Gα Activation by Accessory Proteins**

In recent years, novel accessory proteins involved in the activation of G proteins, either by directly influencing GTP binding or through their association with Gα or Gβγ subunits, have been uncovered (Sato and Ishikawa, 2010). Take-sono et al. (1999) identified the activators of G protein signaling (AGS) that stimulate G protein activity independently of 7TMRs. The AGS group now contains 10 characterized members initially isolated on the basis of yeast functional screen (for review, see Blumer et al., 2007). The different AGS proteins exhibit selectivity for G protein subunits and present diverse modes of action ranging from promoting GTP binding as a GEF (AGS1 for Gα1), stabilizing the GDP-bound complex as a guanine nucleotide dissociation inhibitor (AGS3 for Gαi/o), and interfering with subunit interaction independently of nucleotide exchange (AGS8 with Gβγ). Although the signals integrated by these accessory proteins has not been fully delineated, they are part of the intracellular changes processed through G protein activation. AGS proteins might contribute to positioning G proteins and different effectors within the cell microdomains akin to a molecular scaffold. The growing list of nonreceptor GEFs includes Gα-interacting vesicle-associated protein (Garcia-Marcos et al., 2009), resistance to inhibitors of cholinesterase 8A (Tall et al., 2003), cysteine string protein (Natochin et al., 2005), and the yeast protein Arr4 (Lee and Dohlman, 2009).

Gα2, in its inactive (GDP-bound) state is found to associate with the cytosolic factor p67phox of the NADPH oxidase (Marty et al., 2006). A possible consequence of this association is to target p67phox to a specific subcellular compartment, thereby affecting NADPH oxidase assembly. Likewise, it is possible that mammalian AGS3 and Leu-Gly-Asn repeat-enriched proteins (Marty et al., 2003) and their Drosohila melanogaster homolog Pins, which contain an N-terminal tetratricopeptide repeat (TPR) domain, such as the one in p67phox, have their activities directly regulated through association with Gα proteins. TPR domains were previously known for their interaction with small GTPases such as Rac (Das et al., 1998). Its binding to Gα proteins suggests a potentially novel regulatory mechanism for Gα activation independent of the 7TMRs. It is unclear, however, whether proteins bearing the TPR domains have the function to activate Gα proteins through their association with Gα proteins. TPR domains may also facilitate its presentation to specific intracellular microdomains. Caveolin, a major component of caveolae membranes, has been found to copurify with multiple Gα proteins, including Gαs, Gαi/o, and Gα12/13 (Li et al., 1995). In this case, caveolin functions as a scaffolding protein to recruit Gα proteins to caveolin-rich areas of the plasma membrane.

**Transactivation and Direct Activation of Gα Proteins through Receptor Tyrosine Kinases**

Noncanonical activation of Gα proteins by receptor tyrosine kinases (RTKs) may be defined as either a direct activation process involving an interaction of the Gα protein with a RTK or functional cross-talk in the form of transactivation through the RTK (Patel, 2004). Agonists for growth factor receptors, including epidermal growth factor (EGF), fibroblast growth factor, platelet-derived growth factor (PDGF), insulin, insulin-like growth factor (IGF), and neurotrophins bind to RTKs, which are single-span transmembrane receptors. These agonists stimulate intrinsic tyrosine kinase activity encompassed in the cytoplasmic domains of the RTKs, resulting in receptor auto-phosphorylation on tyrosine residues and generation of docking sites for Src homology 2 domain-containing proteins such as Shc, Grb2, and phospholipase C-γ. Some of these growth factor receptors activate G proteins through functional cross-talk termed transactivation (Daub et al., 1996). It is increasingly evident that 7TMRs and RTKs form an integrated signaling network, and transactivation of RTKs by ligand-stimulated 7TMRs is a general process allowing for pleiotropic signaling (Lowes et al., 2002; Shah and Catt, 2004; Natarajan and Berk, 2006; Delcourt et al., 2007). EGF is an extensively studied example of transactivation through RTKs (Daub et al., 1996) in which the activation of 7TMRs promotes production of EGF via metalloproteinase-catalyzed shedding of a transmembrane precursor and, in some cases, induction or tyrosine phosphorylation in the cytoplasmic tail of the RTK (Prenzel et al., 1999) (Fig. 1A). RTKs may in turn transactivate 7TMR signaling through physical association with Gα proteins, phosphorylation of the 7TMRs, and up-regulation of 7TMR ligand synthesis. Compelling evidence for the involvement of Gα proteins in RTK-mediated activation of various 7TMR effectors was provided with the use of pertussis toxin (PTX), which ADP-ribosylates the Gαi/o family and uncouples these G proteins from their receptors, and cholera toxin, which alters the GTPase activity of Gαs (Gilman, 1987). Several studies have shown that the RTK ligand-activated events are sensitive to PTX (Table 1).

In addition to transactivation through RTKs, some of these receptors directly interact with Gα proteins (Patel, 2004). Early studies have shown that EGF interacts with Gαs (Nair et al., 1990; Poppleton et al., 1996) through its juxtamembrane region (Sun et al., 1997). This interaction leads to phosphorylation of Gαs and elevation of intracellular cAMP in cardiac myocytes (Nair et al., 1990). The activation of Gαs through EGF is accompanied by augmented adenylyl cyclase activation and increased heart rate and contractility (Nair et al., 1993). It is noteworthy that EGF also couples to Gαi/o, and its choice for Gαs or Gαi/o coupling seems to be cell type-dependent. For example, EGF was found to induce hydrolysis of phosphatidylinositol 4,5-bisphosphate in rat hepatocytes, and this activity was abolished by PTX treatment (Liang and Garrison, 1991). Functional coupling of the Gα protein to EGFR was thought to be dependent on protein kinase C-induced phosphorylation of the receptor. Another single transmembrane domain receptor, IGF-II/mannose-6-phosphate receptor, was found to mediate endogenous acetylcholine release in a PTX-sensitive and PKC-dependent manner (Hawkes et al., 2006). Although no direct binding of Gαi/o proteins to the IGF-II/mannose-6-phosphate receptor was investigated in this study, Okamoto et al. (1990) found that a part of the cytoplasmic domain (residues 2410–2423 located to the C-terminal end of the receptor) is responsible for interaction with Gαi2. Upon receptor stimulation, Gαi proteins are activated with the hierarchy of Gαi2 > Gαi3 ≈ Gαi4. The activation of Gαi proteins by a simple structure of the IGF-II/mann6R is reminiscent to G protein activation by the amphiphilic peptide mastoparan (Higashijima et al., 1990). Similarities also exist between Gαi interaction with...
the EGF RTKs and its interaction with the β2-adrenergic receptor (Okamoto et al., 1991; Sun et al., 1997). Overall, however, the exact mechanism of RTK-mediated G protein activation is not fully resolved and could depend on the RTK and the pathways activated by the ligand. In other reported cases, agonist stimulation of the insulin receptor leads to phosphorylation of Go_{i/o} (Krupinski et al., 1988) and Go_{q/11} (Imamura et al., 1999). The tyrosine phosphorylation triggered through agonist binding to these receptors may promote the exchange of GDP for GTP on the Gα proteins. A partial list of RTKs that signal through Gα proteins is shown in Table 1.

**TABLE 1**

A nonexhaustive list of G proteins coupling to RTKs and their functions

<table>
<thead>
<tr>
<th>RTK</th>
<th>G Proteins</th>
<th>Functions</th>
<th>References</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>Go_{i2}</td>
<td>Inhibition of adenylyl cyclase</td>
<td>Stryjek-Kaminska et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Go_{i2}</td>
<td>Activation of PLC</td>
<td>Liang and Garrison, 1991</td>
</tr>
<tr>
<td></td>
<td>Go_{i2}</td>
<td>Activation of Akt-mTORC1 pathway</td>
<td>Cao et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Go_{i2}</td>
<td>Activation of adenylyl cyclase</td>
<td>Nair et al., 1990</td>
</tr>
<tr>
<td>FGFR</td>
<td>Go_{i2}</td>
<td>Activation of adenylyl cyclase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Go_{i2}</td>
<td>Inhibition of NADPH oxidase</td>
<td>Krieger-Brauer et al., 2000</td>
</tr>
<tr>
<td>Insulin receptor</td>
<td>Go_{i2}</td>
<td>Activation of NADPH oxidase</td>
<td>Krieger-Brauer et al., 1997</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>Go_{i2}</td>
<td></td>
<td>Luttrell et al., 1995</td>
</tr>
<tr>
<td>IGF-IR</td>
<td>Go_{i2}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSF-IR</td>
<td>Go_{i2}</td>
<td>Ca^{2+} influx and DNA synthesis</td>
<td>Okamoto et al., 1990</td>
</tr>
</tbody>
</table>

FGFR, fibroblast growth factor receptor; CSF-IR, colony stimulating factor 1 receptor.
Cao et al. (2009) reported recently that $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ are required for EGF-mediated activation of the Akt-mammalian target of rapamycin complex 1 (mTORC1) pathway. In this case, the $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ proteins form a complex with Grb2-associated binding protein 1 (Gab1), and these proteins are required for EGF-induced Gab1 phosphorylation and its subsequent interaction with the regulatory (p85) subunit of phosphatidylinositol 3-kinase (PI3K). As a result, EGF-induced cell migration and proliferation were compromised in the absence of $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$. These results place $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ downstream of EGF and upstream of Gab1. Exactly how the $G_{\alpha}$ proteins interact with Gab1 remains unclear, because PTX does not affect EGF-induced activation of the PI3K-Akt-mTORC1 pathway, indicating that a structure other than the C-terminal region of the $G_{\alpha}$ protein is involved in the interaction with Gab1. The role of the $G_{\alpha}$ proteins in EGFR signaling has some resemblance to that of the yeast $G_{\alpha}$ protein, which binds to the PI3K Vps34 for its activation (Slessareva et al., 2006). However, the yeast Gpa1 protein translocates to endosome for this activity, whereas the $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ proteins remain close to the activated EGFR. Whether $G_{\alpha_{i2}}$ has a similar function is unknown, because its absence did not affect EGF-induced Akt-mTORC1 activation (Cao et al., 2009). Deletion of the $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ genes did not affect EGF-induced activation of phospholipase C-γ and phosphorylation of STAT5, indicating that the $G_{\alpha_{i1}}$ and $G_{\alpha_{i3}}$ have a highly specialized function in EGFR signaling.

Role of $G_{\alpha}$ Proteins in Transmembrane Signaling through Other Non-7TMRs

A variety of cytokine receptors, including the receptors for erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF), and interleukin-3 require members of the cytosolic tyrosine kinase JAK family for signaling. Ligand binding brings receptor-associated JAKs into apposition and leads to autophosphorylation of the JAK molecules and consequently to kinase activity. The activated JAK then phosphorylates specific tyrosine residues on the cytoplasmic tail of the receptor, creating docking sites for Src homology 2 domain-containing proteins such as the STATs. STATs are sequentially phosphorylated by JAKs on tyrosine residues then dissociated from the receptor to form active dimer protein complexes that translocate to the nucleus, where they bind to specific DNA sequences in promoters to modulate gene transcription. Other signaling pathways that are initiated by JAK activation include the PI3K and MAPK kinase pathways (Schindler, 2002). PTX has been shown to inhibit IL-3, GM-CSF and macrophage-colony stimulating factor signaling in hematopoietic cells (He et al., 1988; Imamura and Kufe, 1988; McColl et al., 1989). In these studies, it was not clear whether PTX influences the $G_{\alpha}$-dependent, non-7TM signaling cascades in a direct or indirect way, although the experimental data indicate that the PTX-sensitive substrate is a member of the $G_{i}$ subfamily of $G$ proteins. It is noteworthy that GM-CSF stimulation of neutrophils affects the distribution of $G_{\alpha_{i2}}$ at the plasma membrane (Durst et al., 1993). The increase in cell surface-associated $G_{\alpha_{i2}}$ makes them more available for 7TM signaling and is one of the known mechanisms for GM-CSF priming of neutrophils. Whether there is a direct coupling between GM-CSFR and $G_{\alpha_{i2}}$ remains to be investigated. In the case of the EPOR, Guillard et al. (2001) discovered a novel EPO-dependent MAPK activation pathway involving the $G_{\beta\gamma}$ subunit of $G_{i}$ proteins. EPO treatment inhibited ADP-ribosylation of $G_{i}$ and increased its binding of GTP. The activation of $G_{\alpha_{i1}}$ and $G_{\beta\gamma}$ suggests that the EPOR may catalyze guanine nucleotide exchange or trigger a transactivation mechanism.

Some receptors serving immune recognition functions are found to couple to $G$ proteins. In independent studies conducted by DeFranco and colleagues, it was reported that B cell antigen receptor-mediated phosphoinositide signaling involves $G_{\alpha}$ proteins (Gold et al., 1987; DeFranco and Gold, 1989; DeFranco et al., 1989). However, the identity of the $G_{\alpha}$ proteins remains unclear, because the observed phosphoinositide signaling was not sensitive to PTX (Gold et al., 1987). An exception is that LPS signaling in the same B cell line and in macrophages was found to be sensitive to PTX inhibition (Jakway and DeFranco, 1986). Evidence for physical coupling of these receptors with the $G_{\alpha}$ proteins came from a study showing that $G_{\alpha_{i2}}$ proteins coimmunoprecipitate with the glycosylphosphatidylinositol-anchored glycoprotein CD14, which is a critical component for LPS signaling through TLR4 (Solomon et al., 1998). The involvement of the $G_{i}$ subfamily in LPS signaling was suggested in another study based on the observations that $G_{\alpha_{i2}}$ and $G_{\alpha_{i3}}$ minigenes (which block the functions of these $G_{\alpha}$ proteins) could block MAPK phosphorylation and AP-1 activation induced by a constitutively active TLR4 (Fan et al., 2004) (Fig. 1B). In some studies, the involvement of $G_{i}$ class proteins in cytokine receptor signaling was suggested based on the inhibition by PTX, such as in IL-1R1 signaling through the serine/threonine kinase IRAK (Zumbihl et al., 1995; Cao et al., 1996). In these cases, whether there is physical association between the receptors and the $G$ proteins is not entirely clear. In addition to the $G_{\alpha}$ proteins, $G_{\alpha_{q/11}}$ have been found in complex with β-arrestin-1 and Src, which couple tumor necrosis factor-α to PI3K activation and inflammatory gene expression (Kawamata et al., 2007). Together, these findings suggest that proteins from the $G_{i}$ and sometimes the $G_{q}$ family may be important players in signaling through TLR4 and selected cytokine receptors.

Non-7TMRs that couple to $G_{\alpha}$ proteins also include the C-type natriuretic peptide receptor, amyloid precursor protein, T-cell receptors, and integrin-associated protein (CD47). An excellent review (Patel, 2004) summarizes the interactions between these non-7TMRs and the $G_{\alpha}$ proteins.

Noncanonical Functions of $G_{\alpha_{12/13}}$ Proteins

Nearly all 7TM receptors that activate the $G_{12}$ subfamily ($G_{\alpha_{12}}$ and $G_{\alpha_{13}}$) also functionally couple to other subfamilies of $G_{\alpha}$ proteins, raising the intriguing questions of whether $G_{\alpha_{12}}$ and $G_{\alpha_{13}}$ are independently activated upon agonist binding to these receptors and whether they have functions different from those downstream of 7TM receptors. Research conducted thus far has produced a wealth of information supporting that idea the $G_{12}$ family of $G$ proteins have noncanonical signaling properties (Table 2). Using yeast two-hybrid screening, a study showed that $G_{\alpha_{13}}$ interacts with radixin, a member of the ERM (ezrin, radixin, and moesin) family of cytoskeleton-binding proteins (Vaikunait et al., 2000). Radixin interaction with activated $G_{\alpha_{13}}$ increases its binding to polymerized F-actin, suggesting a possible role for $G_{\alpha_{13}}$ in regulating the actin cytoskeleton. The $G_{\alpha_{12}}$ and $G_{\alpha_{13}}$
proteins also bind to the cytoplasmic domain of cadherins, causing dissociation of the transcriptional activator β-catenin from cadherins (Meigs et al., 2001). The epithelial intercellular adhesion molecule E-cadherin is linked to the cytoskeleton via its interaction with β-catenin (Fig. 1C). The association between E-cadherin and β-catenin plays a role in preventing β-catenin nuclear translocation and thus transcriptional activity. Because there is no direct competition between β-catenin and Gα12 for binding to the same region of E-cadherin (Kaplan et al., 2001), the Gα12-dependent dissociation of β-catenin from E-cadherin may result from an allosteric effect. This finding substantiates the well-characterized transforming ability of the G12 subfamily, because nuclear localization of β-catenin is associated with increased transcriptional activation. Gα12 and Gα13 also regulate zebrafish epiboly, through a mechanism involving association with the cytoplasmic terminus of E-cadherin, which leads to inhibition of the activity of E-cadherin and cell adhesion (Lin et al., 2009).

In addition to binding to cadherins, Gα12 and Gα13 were found to play important roles in other adhesion-molecule-dependent cellular functions. In platelets lacking Gαq, coactivation of the Gαq and Gα12/13-coupled thromboxane A2 receptor and the Gαi-coupled ADP receptor P2Y12 resulted in irreversible integrin αIIbβ3-mediated platelet aggregation (Nieswandt et al., 2002). Because Gαq-deficient platelets fail to respond to thromboxane A2 in αIIbβ3 activation assay, the study demonstrates converging signaling of Gα12/13 and Gαq, which is sufficient to overcome Gαq deficiency for αIIbβ3 activation. A recent study provided evidence for a role of the G12 subfamily in integrin signaling by demonstrating a direct interaction of Gα13 with the cytoplasmic domain of the β3 integrin (Gong et al., 2010). Ligand binding to the integrin αIIbβ3, as well as GTP loading of Gα13, promotes this interaction, supporting an “outside-in” signaling mechanism that favors cell spreading through the activation of c-Src. In platelets, inhibition of Gα13 activation or its expression diminishes the activity of c-Src and augments the activity of RhoA, thus promoting cell retraction (Gong et al., 2010). Therefore, the β3 integrin functions as a noncanonical receptor for Gα13, which is critical to dynamic regulation of c-Src and RhoA.

The above findings are of significance in understanding the role of the G12 subfamily in cell migration, which is relevant to development, host defense, and tumor metastasis. Recent studies have shown that lysophosphatidic acid-induced focal adhesion kinase autophosphorylation is dependent on the Gα12/13 pathway and contributes to ovarian cancer cell migration (Bian et al., 2006). Likewise, the Gα12 subfamily was found to be involved in the invasion of prostate cancer cell lines (Kelly et al., 2006). Although agonists for 7TMRs were used in these studies, the role of the Gα12 subfamily in cell migration may extend further than signaling downstream of 7TMRs. Shan et al. (2006) reported that Gα13-deficient MEFs are defective in cell migration induced by PDGF, a RTK-activating ligand. Of particular interest is the finding that C-terminal deletion mutants of Gαi3 unable to couple to 7TMRs can rescue PDGF-induced migration of cells lacking Gα13, suggesting that the RTK-mediated cell migration is Gα13-dependent but independent of its interaction with 7TMRs. In MEFs lacking both Gα12 and Gα13, rescue of cell migration was successful with Gαi3 but not Gα12, indicating that the RTK selectively couples to Gα13 for this function (Shan et al., 2006). Recent studies have also shown that the G12 subfamily is important for lymphocyte adhesiveness and motility (Herroeder et al., 2009). In the polarized and migrating promyelocytic leukemic HL-60 cell line, Gα13 and RhoA are localized to the rear of the cell (uropod) and play a role in defining its “backness” (Xu et al., 2003). How Gα13 gets there and what regulates its activation remain unknown. Studies of the integrin outside-in signaling pathways may shed light on the Gα13 regulatory mechanisms, especially if Gα13 can interact with other β integrins. In another study, Gα13 was reported to bind to Hax-1, a cytoskeleton-associated, contactin-interacting protein. Disruption of this interaction leads to reduced migration of NIH 3T3 cells (Radhika et al., 2004).

The G12 subfamily of G proteins also interacts with phosphatases, including the Ser/Thr protein phosphatase type 5 and protein phosphatase 2A (Yamaguchi et al., 2002; Zhu et al., 2004). In both cases, interaction of the phosphatases with activated Gα12 or Gα13 leads to an increase in the phosphatase activity (Table 2). Although Gα12 and Gα13 sequences are closely related, several studies have highlighted the distinction between Gα12 and Gα13 in their interaction with proteins other than the 7TMRs. For instance, interaction with Hsp90 and protein acylation facilitate Gα13 localization to lipid rafts but have no effect on Gαi3 (Waheed and Jones, 2002). This observation also raises the intriguing question of whether Gα12 membrane localization is dependent on Gβγ or other proteins and its post-translational modifications.

### TABLE 2

A partial list of non-7TMR proteins that physically and/or functionally couple to the Gα12 subfamily

<table>
<thead>
<tr>
<th>Proteins</th>
<th>G Proteins</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadherins</td>
<td>Gα12/13</td>
<td>Release of β-catenin for transcriptional activation; regulation of epithobyl Jak2 activation, Jak2/STAT5-dependent production of Bcl(XL)</td>
<td>Kaplan et al., 2001; Meigs et al., 2001; Lin et al., 2009; Versteeg et al., 2004</td>
</tr>
<tr>
<td>Factor VIIa/TF (tissue factor)</td>
<td>Gα12/13</td>
<td>Jak2 activation, Jak2/STAT5-dependent production of Bcl(XL)</td>
<td>Kaplan et al., 2001; Meigs et al., 2001; Lin et al., 2009; Versteeg et al., 2004</td>
</tr>
<tr>
<td>Hax-1</td>
<td>Gα13</td>
<td>Gα13 forms complex with Hax-1, Rac and cortacin for cell movement</td>
<td>Radhika et al., 2004; Gong et al., 2010</td>
</tr>
<tr>
<td>Integrin αIIbβ3</td>
<td>Gα13</td>
<td>Integrin signaling, platelet adhesion</td>
<td>Radhika et al., 2004; Gong et al., 2010</td>
</tr>
<tr>
<td>Hsp90</td>
<td>Gα12 (not Gα13)</td>
<td>Localization of Gα12 to lipid rafts</td>
<td>Waheed and Jones, 2002; Zhu et al., 2004</td>
</tr>
<tr>
<td>PP2A</td>
<td>Gα12</td>
<td>Increase in PP2A phosphatase activity</td>
<td>Yamaguchi et al., 2002</td>
</tr>
<tr>
<td>PP5</td>
<td>Gα12/13</td>
<td>Gα12/13 interact with TPR domain in PP5, increasing its phosphatase activity</td>
<td>Yamaguchi et al., 2002</td>
</tr>
<tr>
<td>Radixin</td>
<td>Gα13</td>
<td>Conformational activation of radixin and its binding to polymerized F-actin</td>
<td>Vaismarkaite et al., 2000</td>
</tr>
<tr>
<td>RTK</td>
<td>Gα13 (not Gα12)</td>
<td>Necessary for PDGF-induced cell migration</td>
<td>Shan et al., 2006; Tateiwa et al., 2005</td>
</tr>
<tr>
<td>Socius</td>
<td>Gα12/13</td>
<td>Gα12/13-dependent RhoA activation</td>
<td>Shan et al., 2006; Tateiwa et al., 2005</td>
</tr>
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PP2A, protein phosphatase 2A; PP5, protein phosphatase 5.
Mounting evidence for the noncanonical roles of Gα proteins in 7TMR-independent signaling suggests that these G proteins play important physiological functions in addition to coupling to the 7TMRs. For instance, consistent with the finding that Gα13 is pivotal in cell migration, deletion of the Gα13 gene disrupts cell migration necessary for embryonic vasculogenesis in mice, resulting in a lethal phenotype (Ofermanns et al., 1997). The recent finding of Gvasculogenesis in mice, resulting in a lethal phenotype (Ofermanns et al., 1997). The recent finding of Gvasculogenesis in mice, resulting in a lethal phenotype (Ofermanns et al., 1997).

Important questions have not been addressed. The recent finding of Gvasculogenesis in mice, resulting in a lethal phenotype (Ofermanns et al., 1997).

Protein interactions are very limited and far behind what we have learned of severe growth retardation. Despite the progress made thus far, our understanding of the noncanonical functions of Gα proteins is very limited and far behind what we have learned from investigating G protein coupling to 7TMRs. A number of important questions have not been addressed. The recent finding of Gvasculogenesis in mice, resulting in a lethal phenotype (Ofermanns et al., 1997).

1. It is unclear whether Gα coupling to non-7TMRs is ubiquitous or confined to a selected group of receptors. Cao et al. (2009) found Gαi4 and Gαi3 to be critical to EGF-mediated Akt-mTORC1 activation but not that mediated by insulin receptor, IGF-1R, and PDGFR, suggesting receptor-dependent selectivity.

2. At present, it is also unclear whether Gβγ proteins play a role in the noncanonical functions of Gα proteins. With the 7TMRs, Gβγ subunits serve the function of anchoring Gα proteins to the plasma membrane, where most of the 7TMRs are located. In addition, Gβγ proteins directly activate downstream effectors including phospholipase C-β1, PKC-γ, the guanine nucleotide exchange factor P-Rex1, and selected ion channels. A number of the non-7TMR Gα-binding proteins are localized intracellularly, raising the question of whether they interact with Gα protein monomers instead of heterotrimeric G proteins. It is notable that Gαi-mediated MAP kinase activation involves tyrosine kinase activation and is a pathway shared by both 7TMRs and RTKs (van Biesen et al., 1995). Therefore, Gβγ proteins should be treated separately from Gα proteins because they do not have the intrinsic GTPase activity but are direct activators of signaling effectors.

3. Based on published data, it is most likely that Gα-mediated noncanonical signaling requires these proteins to enter the activation cycle. However, because most of the published studies were conducted either with constitutively active mutants of Gα12 and Gα13 or with GTP-loading, the GEFs and GTPase-activating proteins associated with the noncanonical coupling events remain largely unidentified. In several reported studies in which the Gα12 family of Gα proteins was found to associate with cytoskeletal proteins and regulate integrin function, activated Gα12 and/or Gα13 were required. It is presently unclear whether the activated Gα12 and Gα13 proteins are derived from agonist-stimulated 7TMRs or from as-yet-unidentified GEFs. Colocalization of the relevant 7TMRs with these Gα-binding proteins and establishing functional coupling between the noncanonical activation and 7TMR-mediated activation events will shed light on the possible link between 7TMRs and non-7TMR Gα binding proteins. In conclusion, although it is exciting to observe expanded roles of Gα proteins in cell signaling beyond the traditional 7TMRs, much remains to be investigated to appreciate the physiological importance as well as pharmacological mechanisms related to the noncanonical functions of heterotrimeric G proteins.

References

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