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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 G_s, G_i, G_q, and G_{12} 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 (Bünemann 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

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ABBREVIATIONS: 7TMR, seven-transmembrane domain receptor; GPCR, G protein-coupled receptor; GEF, guanine nucleotide exchange factor; JAK, Janus kinase; STAT, signal transducer and activator of transcription; AGS, activators of G protein signaling; TPR, tetratricopeptide repeat; RTK, receptor tyrosine kinase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; PDGF, platelet-derived growth factor; IGF, insulin-like growth factor; PTX, pertussis toxin; mTORC1, mammalian target of rapamycin complex 1; Gab1, Grb2-associated binding protein 1; PI3K, phosphatidylinositol 3-kinase; EPO, erythropoietin; GM-CSF, granulocyte macrophage-colony stimulating factor; MAPK, mitogen-activated protein kinase; IL, interleukin; EPOR, erythropoietin receptor; AP-1, activator protein 1; TLR4, Toll-like receptor 4.

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). Takesono 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 $G\alpha_i$), stabilizing the GDP-bound complex as a guanine nucleotide dissociation inhibitor (AGS3 for $G\alpha_{i/o}$), 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).

$G\alpha_{i2}$ in its inactive (GDP-bound) state is found to associate with the cytosolic factor $p67^{phox}$ of the NADPH oxidase (Marty et al., 2006). A possible consequence of this association is to target $p67^{phox}$ 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 *Drosophila melanogaster* homolog Pins, which contain an N-terminal tetratricopeptide repeat (TPR) domain, such as the one in $p67^{phox}$, 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 of a GEF for Gα activation. Gα interaction with 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 $G\alpha_s$, $G\alpha_o$, and $G\alpha_{i2}$ (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). EGFR 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 $G\alpha_{i/o}$ family and uncouples these G proteins from their receptors, and cholera toxin, which alters the GTPase activity of $G\alpha_{s/olf}$ (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 EGFR interacts with $G\alpha_s$ (Nair et al., 1990; Poppleton et al., 1996) through its juxtamembrane region (Sun et al., 1997). This interaction leads to phosphorylation of $G\alpha_s$ and elevation of intracellular cAMP in cardiac myocytes (Nair et al., 1990). The activation of $G\alpha_s$ through EGFR is accompanied by augmented adenylyl cyclase activation and increased heart rate and contractility (Nair et al., 1993). It is noteworthy that EGFR also couples to $G\alpha_{i/o}$, and its choice for $G\alpha_s$ or $G\alpha_{i/o}$ 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 $G\alpha_{i/o}$ 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 $G\alpha_{i2}$. Upon receptor stimulation, $G\alpha_i$ proteins are activated with the hierarchy of $G\alpha_{i2} > G\alpha_{i1} \approx G\alpha_{i3}$. The activation of $G\alpha_i$ proteins by a simple structure of the IGF-II/man6R is reminiscent to G protein activation by the amphiphilic peptide mastoparan (Higashijima et al., 1990). Similarities also exist between $G\alpha_s$ 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 $G\alpha_{i/o}$ (Krupinski et al., 1988) and $G\alpha_{q/11}$ (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.

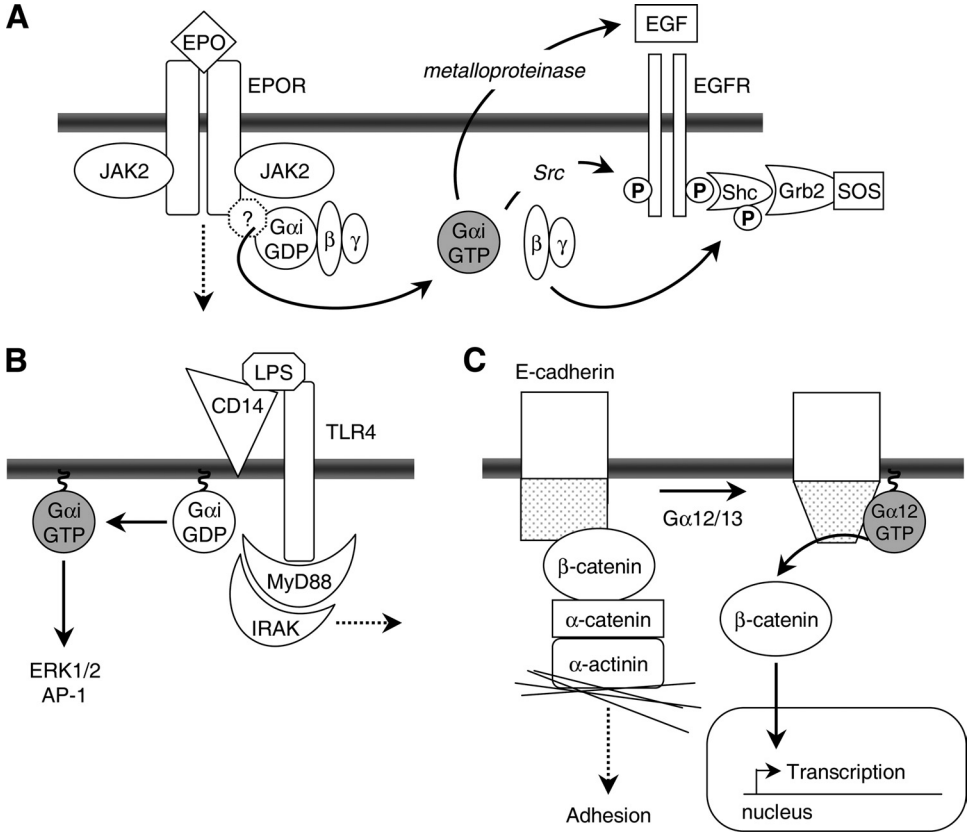

Fig. 1. Schematic representation of selected noncanonical functions of Gα proteins. A, the EPOR requires association with the soluble tyrosine kinase JAK2 to mediate signaling (dotted arrow). The direct or indirect (dotted circle) interaction between the $G\alpha_i$ protein and EPOR allows for EPO-induced activation of $G\alpha_i$ and downstream effectors of both GTP-bound $G\alpha_i$ and $G\beta\gamma$, including transactivation through EGFR. B, the Toll-like receptor TLR4 mediates LPS signaling in collaboration with the glycosylphosphatidylinositol-anchored CD14 protein. The myeloid differentiation factor 88 (MyD88) and IL-1 receptor associated kinase (IRAK) are also involved in some of the TLR4 signaling pathways. The signal is relayed downstream by adaptor proteins and results in MAPK, AP-1 and nuclear factor-κB (NF-κB) activation (dotted arrow). The interaction between CD14 and $G\alpha_{i/o}$ proteins regulates LPS-induced ERK activation and AP-1 transcriptional activity. C, the cell surface glycoprotein E-cadherin is linked to the cytoskeleton via its interaction with β-catenin, α-catenin and α-actinin. This interaction mediates cell-cell adhesion (dotted arrow). $G\alpha_{12/13}$ interaction with E-cadherin produces an allosteric effect (shaded box and trapezoid) uncoupling β-catenin from E-cadherin. Abolition of the E-cadherin/β-catenin complex disrupts cell adhesion and allows β-catenin, a known transcriptional regulator, to translocate into the nucleus. Not shown in this figure are the direct interaction of EGFR with $G\alpha_s$ and $G\alpha_s$-mediated cAMP elevation, which was depicted in Fig. 2 of the review by Patel (2004), and the proposed signaling mechanism by which $G\alpha_{i1}$ and $G\alpha_{i3}$ mediate EGF-induced Akt-mTORC1 activation, which was described in the article by Cao et al. (2009). The $G\alpha_{13}$ involvement in αIIbβ3 integrin signaling was detailed in Fig. 4G of the article by Gong et al. (2010).

TABLE 1
A nonexhaustive list of G proteins coupling to RTKs and their functions

RTK	G Proteins	Functions	References
EGFR	$G\alpha_{i1/i2}$	Inhibition of adenylyl cyclase	Stryjek-Kaminska et al., 1996
	$G\alpha_{i/o}$	Activation of PLC	Liang and Garrison, 1991
	$G\alpha_{i1/i3/(i2?)}$	Activation of Akt-mTORC1 pathway	Cao et al., 2009
	$G\alpha_s$	Activation of adenylyl cyclase	Nair et al., 1990
FGFR	$G\alpha_s$	Activation of adenylyl cyclase	
	$G\beta\gamma$	Inhibition of NADPH oxidase	Krieger-Brauer et al., 2000
Insulin receptor	$G\alpha_{i/o}$		
	$G\alpha_{q/11}$		
	$G\alpha_{i2}$	Activation of NADPH oxidase	Krieger-Brauer et al., 1997
IGF-IR	$G\alpha_i$		
	$G\beta\gamma$		Luttrell et al., 1995
IGF-IIR	$G\alpha_{i/o}$	Ca^{2+} influx and DNA synthesis	Okamoto et al., 1990
CSF-1R		PKC and Na^+ influx	Imamura et al., 1990; Corre and Hermouet, 1995

FGFR, fibroblast growth factor receptor; CSF-1R, 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 EGFR and upstream of Gab1. Exactly how the $G\alpha_i$ 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 $G\alpha_i$ protein is involved in the interaction with Gab1. The role of the $G\alpha_i$ proteins in EGFR signaling has some resemblance to that of the yeast Gα protein, which binds to the PI3K Vsp34 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 Gα 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 $G\alpha_i$-dependent, non-7TMR 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 (Durstin et al., 1993). The increase in cell surface-associated $G\alpha_{i2}$ makes them more available for 7TMR 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 $G\alpha_i$ and increased its binding of GTP. The activation of $G\alpha_i$ 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 Gα proteins (Gold et al., 1987; DeFranco and Gold, 1989; DeFranco et al., 1989). However, the identity of the Gα 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 Gα proteins came from a study showing that $G\alpha_{i/o}$ 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 Gα 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 $G\alpha_i$ 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 families may be important players in signaling through TLR4 and selected cytokine receptors.

Non-7TMRs that couple to Gα 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 Gα 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 Gα 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 (Vaiskunaite 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 $G\alpha_{12}$ for binding to the same region of E-cadherin (Kaplan et al., 2001), the $G\alpha_{12}$-dependent dissociation of β-catenin from E-cadherin may result from an allosteric effect. This finding substantiates the well characterized transforming ability of the G_{12} subfamily, because nuclear translocation of β-catenin is associated with increased transcriptional activation. $G\alpha_{12}$ and $G\alpha_{13}$ 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, $G\alpha_{12}$ and $G\alpha_{13}$ were found to play important roles in other adhesion molecule-dependent cellular functions. In platelets lacking $G\alpha_q$, coactivation of the $G\alpha_q$- and $G\alpha_{12/13}$-coupled thromboxane A2 receptor and the $G\alpha_i$-coupled ADP receptor P2Y12 resulted in irreversible integrin αIIbβ3-mediated platelet aggregation (Nieswandt et al., 2002). Because $G\alpha_q$-deficient platelets fail to respond to thromboxane A2 in αIIbβ3 activation assay, the study demonstrates converging signaling of $G\alpha_{12/13}$ and $G\alpha_i$, which is sufficient to overcome $G\alpha_q$ deficiency for αIIbβ3 activation. A recent study provided evidence for a role of the G_{12} subfamily in integrin signaling by demonstrating a direct interaction of $G\alpha_{13}$ with the cytoplasmic domain of the β3 integrin (Gong et al., 2010). Ligand binding to the integrin αIIbβ3, as well as GTP loading of $G\alpha_{13}$, promotes this interaction, supporting an "outside-in" signaling mechanism that favors cell spreading through the activation of c-Src. In platelets, inhibition of $G\alpha_{13}$ 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 $G\alpha_{13}$, which is critical to dynamic regulation of c-Src and RhoA.

The above findings are of significance in understanding the role of the G_{12} 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 $G\alpha_{12/13}$ pathway and contributes to ovarian cancer cell migration (Bian et al., 2006). Likewise, the G_{12} 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 G_{12} subfamily in cell migration may extend further than signaling downstream of 7TMRs. Shan et al. (2006) reported that $G\alpha_{13}$-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 $G\alpha_{13}$ unable to couple to 7TMRs can rescue PDGF-induced migration of cells lacking $G\alpha_{13}$, suggesting that the RTK-mediated cell migration is $G\alpha_{13}$-dependent but independent of its interaction with 7TMRs. In MEFs lacking both $G\alpha_{12}$ and $G\alpha_{13}$, rescue of cell migration was successful with $G\alpha_{13}$ but not $G\alpha_{12}$, indicating that the RTK selectively couples to $G\alpha_{13}$ for this function (Shan et al., 2006). Recent studies have also shown that the G_{12} subfamily is important for lymphocyte adhesiveness and motility (Herroeder et al., 2009). In the polarized and migrating promyelocytic leukemic HL-60 cell line, $G\alpha_{13}$ and RhoA are localized to the rear of the cell (uropod) and play a role in defining its "backness" (Xu et al., 2003). How $G\alpha_{13}$ gets there and what regulates its activation remain unknown. Studies of the integrin outside-in signaling pathways may shed light on the $G\alpha_{13}$ regulatory mechanisms, especially if $G\alpha_{13}$ can interact with other β integrins. In another study, $G\alpha_{13}$ was reported to bind to Hax-1, a cytoskeleton-associated, cortactin-interacting protein. Disruption of this interaction leads to reduced migration of NIH 3T3 cells (Radhika et al., 2004).

The G_{12} 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 $G\alpha_{12}$ or $G\alpha_{13}$ leads to an increase in the phosphatase activity (Table 2). Although $G\alpha_{12}$ and $G\alpha_{13}$ sequences are closely related, several studies have highlighted the distinction between $G\alpha_{12}$ and $G\alpha_{13}$ in their interaction with proteins other than the 7TMRs. For instance, interaction with Hsp90 and protein acylation facilitate $G\alpha_{12}$ localization to lipid rafts but have no effect on $G\alpha_{13}$ (Waheed and Jones, 2002). This observation also raises the intriguing question of whether $G\alpha_{12}$ membrane localization is dependent on $G\beta\gamma$ 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 G_{12} subfamily

Proteins	G Proteins	Functions	References
Cadherins	$G\alpha_{12/13}$	Release of β-catenin for transcriptional activation; regulation of epiboly	Kaplan et al., 2001; Meigs et al., 2001; Lin et al., 2009
Factor VIIa/TF (tissue factor)	$G\alpha_{12/13}$	Jak2 activation, Jak2/STAT5-dependent production of Bcl(XL)	Versteeg et al., 2004
Hax-1	$G\alpha_{13}$	$G\alpha_{13}$ forms complex with Hax-1, Rac and cortacin for cell movement	Radhika et al., 2004
Integrin aIIbβ3	$G\alpha_{13}$	Integrin signaling, platelet adhesion	Gong et al., 2010
Hsp90	$G\alpha_{12}$ (not $G\alpha_{13}$)	Localization of $G\alpha_{12}$ to lipid rafts	Waheed and Jones, 2002
PP2A	$G\alpha_{12}$	Increase in PP2A phosphatase activity	Zhu et al., 2004
PP5	$G\alpha_{12/13}$	$G\alpha_{12/13}$ interact with TPR domain in PP5, increasing its phosphatase activity	Yamaguchi et al., 2002
Radixin	$G\alpha_{13}$	Conformational activation of radixin and its binding to polymerized F-actin	Vaiskunaite et al., 2000
RTK	$G\alpha_{13}$ (not $G\alpha_{12}$)	Necessary for PDGF-induced cell migration	Shan et al., 2006
Socius	$G\alpha_{12/13}$	$G\alpha_{12/13}$-dependent RhoA activation	Tateiwa et al., 2005

PP2A, protein phosphatase 2A; PP5, protein phosphatase 5.

Unanswered Questions and Future Perspectives

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 $G\alpha_{13}$ is pivotal in cell migration, deletion of the $G\alpha_{13}$ gene disrupts cell migration necessary for embryonic vasculogenesis in mice, resulting in a lethal phenotype (Offermanns et al., 1997). The recent finding of $G\alpha_i$ involvement in EGF-stimulated Akt-mTORC1 pathway (Cao et al., 2009) also raises an interesting possibility for the Gα proteins to regulate important developmental processes, because deletion of $G\alpha_{i2}$ and $G\alpha_{i3}$ leads to embryonic lethality as a result 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.

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 $G\alpha_{i1}$ and $G\alpha_{i3}$ to be critical to EGFR-mediated Akt-mTORC1 activation but not that mediated by insulin receptor, IGFR, 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-β, PI3K-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 heterotrimers. It is notable that Gβγ-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 several 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 $G\alpha_{12}$ and $G\alpha_{13}$ 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 G_{12} family of Gα proteins was found to associate with cytoskeletal proteins and regulate integrin functions, activated $G\alpha_{12}$ and/or $G\alpha_{13}$ were required. It is presently unclear whether the activated $G\alpha_{12}$ and $G\alpha_{13}$ 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.

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