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Non-canonical signaling and localizations of heterotrimeric G proteins

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Abstract

Heterotrimeric G proteins typically transduce signals from G protein-coupled receptors (GPCRs) to effector proteins. In the conventional G protein signaling paradigm, the G protein is located at the cytoplasmic surface of the plasma membrane, where, after activation by an agonist-bound GPCR, the GTP-bound $G\alpha$ and free $G\beta\gamma$ bind to and regulate a number of well-studied effectors, including adenylyl cyclase, phospholipase $C\beta$, RhoGEFs and ion channels. However, research over the past decade or more has established that G proteins serve non-canonical roles in the cell, whereby they regulate novel effectors, undergo activation independently of a GPCR, and/or function at subcellular locations other than the plasma membrane. This review will highlight some of these non-canonical aspects of G protein signaling, focusing on direct interactions of G protein subunits with cytoskeletal and cell adhesion proteins, the role of G proteins in cell division, and G protein signaling at diverse organelles.

Keywords

G proteins; G protein-coupled receptors; cytoskeleton; cell division; Golgi

Introduction

The heterotrimeric G proteins, composed of α , β and γ subunits are ubiquitous and crucial signaling molecules which have been well established as central mediators of a vast number of signaling pathways and functions in the cell. G proteins transduce signals from extracellular ligands, such as hormones, neurotransmitters and chemokines, via G protein coupled receptors (GPCRs), and mutations or dysregulation in G protein signaling have been shown to be the cause of major diseases including cancer, heart disease and neurodegenerative disorders [1, 2]. While GPCRs are the most widely targeted receptors in pharmaceutical therapeutics, G proteins are also becoming increasingly important targets in disease control.

G proteins transduce signals from GPCRs to downstream effector molecules, thereby initiating signaling pathways. Activation of GPCRs by their respective ligands catalyzes the

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release of GDP from $G\alpha$ and subsequent binding of GTP. Therefore the ligand occupied GPCRs function as guanine exchange factors (GEFs) for $G\alpha$ subunits. This process requires all three G protein subunits, and the binding of GTP to $G\alpha$ results in the activation of the G protein and the dissociation of $G\alpha$ from $G\beta\gamma$. β and γ subunits stay associated and function as a dimer while $G\alpha$ functions alone. $G\alpha$ -GTP and free $G\beta\gamma$ bind and activate downstream targets [3]. In addition, there is evidence that some heterotrimers may not fully dissociate, but only undergo a rearrangement, upon GPCR-mediated activation [4]. The intrinsic GTP hydrolysis activity of $G\alpha$ returns $G\alpha$ to the GDP-bound state, thus allowing formation of the inactive $\alpha\beta\gamma$ heterotrimer and completion of the G protein cycle. Other than GPCRs, additional regulators of the G protein cycle include regulator of G protein signaling (RGS) proteins, which bind to $G\alpha$ and accelerate GTP hydrolysis, and several families of proteins which can activate G proteins in the absence of a GPCR.

G proteins are peripheral membrane proteins, typically associating tightly with membranes by virtue of lipid modifications. Most $G\alpha$ undergo N-terminal modification of one or more cysteines by palmitoylation, a reversible and regulated modification, and some $G\alpha$ also are stably modified at an extreme N-terminal glycine by myristate. Moreover, all γ subunits of the $G\beta\gamma$ dimer are stably modified by a farnesyl or gerangeranyl group at a cysteine in a well-characterized C-terminal Caax box motif. Upon synthesis, G proteins are trafficked along the cytoplasmic surface of organelles, which act as locations for lipid modifications and heterotrimer assembly. The $G\beta\gamma$ subunits are synthesized in the cytoplasm, a process which is facilitated by chaperone proteins. The CCTI chaperonin complex and the phosducin-like protein (PhLP) promote the proper folding of the $G\beta$ subunit into its seven-bladed propeller structure. The dopamine receptor interacting protein 78 (DRiP78) is a chaperone protein for $G\gamma$, and PhLP may assist in the assembly of $G\beta\gamma$. $G\gamma$ undergoes lipid modification, the $G\beta\gamma$ dimer is formed and then targeted to the cytoplasmic surface of the endoplasmic reticulum (ER). Similarly, proper folding of $G\alpha$ may also be facilitated by such proteins as Ric-8 [5, 6]. It is proposed that the ER or the Golgi is the site where the $G\alpha$ undergoes palmitoylation and the heterotrimer is assembled. The heterotrimeric G protein complex is then translocated to the plasma membrane via a pathway that has not been fully characterized [7–9].

Although G proteins regulate their conventional signaling pathways while bound to the cytoplasmic surface of the plasma membrane (PM), G protein localization is dynamic. It is now clear that G protein subunits can translocate reversibly from the PM to endomembranes, such as endosomes and Golgi [7, 10]. Evidence exists for constitutive G protein trafficking and for trafficking in response to GPCR activation of a G protein heterotrimer. In the case of activation-dependent G protein translocation, it appears that the GPCR and the G protein subunits do not necessarily share the same trafficking itinerary. On the other hand, it is less clear whether $G\alpha$ and $G\beta\gamma$ traffic together or whether they follow distinct routes. There is evidence to support both models. Although much of the mechanistic details of G protein trafficking remains to be worked out, such trafficking has important implications for novel G protein signaling at diverse subcellular locations, as will be discussed in this review.

In humans, there are genes encoding 16 $G\alpha$, 5 $G\beta$ and 12 $G\gamma$ subunits; several splice variants, particularly for $G\alpha$, further increase the diversity of potential heterotrimers [11]. On the basis of sequence similarity, the $G\alpha$ have been divided into four families, $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12/13}$. Typically, $G\alpha_s$ stimulates adenylyl cyclase and increases cellular levels of cyclic AMP (cAMP), while $G\alpha_i$ inhibits adenylyl cyclase and lowers cAMP levels. $G\alpha_q$ binds to and activates members of the phospholipase C isoform β family (PLC β) which cleaves phosphatidylinositol biphosphate (PIP2) to generate inositol trisphosphate (IP3) and diacylglycerol (DAG). $G\alpha_{12/13}$ directly binds to and activates three different RGS

domain-containing Rho guanine exchange factors (RGS-RhoGEFs), p115-RhoGEF, LARG, and PDZ-RhoGEF. $G_{\alpha_{12/13}}$ also regulates a number of other proteins (discussed below and reviewed in [12]), but the RGS-RhoGEFs are the best studied. $G\beta\gamma$ regulates a number of effectors, many of which are also regulated by G_{α} , including PLC β and adenylyl cyclase. Ion channels are another class of important proteins that are directly regulated by $G\beta\gamma$. Another role for $G\beta\gamma$ is the activation of phosphatidylinositol 3 kinase (PI3K), which results in the activation of the Akt survival pathway. Activation of PI3K also leads to the activation of small GTPases Ras and Rac, where Ras activates the Raf/MEK/ERK (extra cellular signal regulated kinase) pathway leading to cell proliferation and Rac activates p21 activating kinases (PAKs) leading to cell migration. Collectively, the heterotrimeric G proteins regulate a plethora of signaling pathways in the cell [1, 2].

Conventional G protein signaling, in which GPCR-activated G proteins regulate well-studied effector proteins at the PM, represents one of the most widespread signal transduction paradigms; however, recent discoveries indicate novel effectors and signaling pathways for G proteins which involve direct interactions with cytoskeletal elements, unique roles in cell division, and signaling at subcellular locations such as the golgi, mitochondria, ER, endosomes and the nucleus. This review will focus on discussing some of these new and emerging effectors, signaling pathways and functions of G proteins. Studies in this exciting area of non-canonical G protein functions and subcellular localizations are shedding light on diverse areas of cell biology with implications for novel targets in the treatment of diseases.

G Proteins and Interactions with Cytoskeletal and Adhesion Proteins

G proteins have been shown to play critical roles in regulating cellular functions through direct interactions with cytoskeletal and cell adhesion elements leading to the activation of important regulatory events related to cellular motility, migration and development (Table 1 and Figure 1). These novel and non-canonical interactions of G proteins bypass classical signaling pathways, which occur via interactions with signaling proteins such as PLC β and adenylyl cyclase.

A number of studies have demonstrated direct interactions of G proteins with tubulin and microtubules (reviewed in [13]). It has been shown that G_{α_i} and G_{α_s} destabilize microtubules by stimulating the tubulin-GTP hydrolysis rate, apparently by binding to the microtubule plus ends. This increases the catastrophic frequency of microtubules which results in the conversion of long microtubules to a greater number of shorter ones, increasing microtubule dynamics and decreasing microtubule stability. This results in an increase in process formation, changes in cell shape, and cellular differentiation. A recent report used mutational or cholera toxin activation of expressed G_{α_s} to show that the activated, GTP-bound form of G_{α_s} preferentially binds to microtubules in neuronal PC12 cells, suggesting a model in which activated G_{α_s} translocates from the PM to intracellular microtubules to directly effect microtubule dynamics [14]. Moreover, using a PC12 cell line deficient in cAMP-dependent protein kinase (PKA), it was shown that activation of G_{α_s} promotes neurite outgrowth, consistent with the idea that G_{α_s} directly regulates microtubules independent of the classical cAMP-PKA pathway. Another recent report shed some light on the mechanism of the interaction between G_{α_s} and tubulin [15]. A molecular modeling study proposed several surfaces that mediate the interaction of the two proteins [16], and using this model as guide it was shown that a peptide corresponding to the $\alpha 3$ - $\beta 5$ region of G_{α_s} could bind tubulin, promote tubulin GTPase activity and enhance the dynamic instability of microtubules. However, a chimera in which the $\alpha 3$ - $\beta 5$ region of transducin G_{α_t} was substituted into G_{α_s} retained the ability to increase microtubule dynamics. This was surprising since neither G_{α_t} nor peptides corresponding to its $\alpha 3$ - $\beta 5$ region interact with

tubulin [15]. Thus, other surfaces of $G\alpha_s$ likely play major roles in tubulin interaction, and the $G\alpha_s$ N-terminus is one such region also identified in this study. In contrast to $G\alpha$, $G\beta\gamma$ binds along the length of microtubules, promotes microtubule assembly and increases microtubule stability. Therefore, $G\alpha$ and $G\beta\gamma$ have opposing effects on microtubule stability. The consequences of the dynamics between active $G\alpha$ and $G\beta\gamma$ are not understood, but it has been proposed that the two subunits may act on different subsets of microtubules. Additionally, it has also been shown that inactive $G\alpha_s$ interferes with functional $G\beta\gamma$ -tubulin interactions resulting in process outgrowth. $G\alpha_q$ also binds tubulin, but it appears to not have the same consequence as $G\alpha_i$ and $G\alpha_s$ binding to tubulin. Activation of $G\alpha_q$ results in recruitment of GTP-tubulin to the membrane [17]. This may effect PLC β activation, but more studies are needed. In summary, it is proposed that G proteins can modulate the microtubule cytoskeleton, thus affecting many aspects of cell morphology, by direct binding rather than or in addition to impacting the cytoskeleton via regulation of signaling pathways. These direct interactions of G proteins with the fundamental building blocks of the cytoskeleton could prove to be beneficial in providing a more efficient and potent mechanism for restructuring activities of the cytoskeleton [13].

G proteins can also interact with the actin cytoskeleton. $G\alpha_{q/11}$ has been shown to co-localize with F-actin in cells, and disruption of cellular F-actin with cytochalasin inhibited $G\alpha_{q/11}$ -mediated generation of IP $_3$ in cells [18, 19]. Likewise, select $G\beta\gamma$ subunits were found to localize to the actin cytoskeleton in cells. One study showed that specifically $G\gamma_{12}$ -containing $G\beta\gamma$ were localized to F-actin, and expression of $G\beta_{1\gamma_{12}}$ in NIH3T3 cells resulted in cell rounding, disruption of stress fibers and increased cell migration [20, 21]. Another study showed that $G\gamma_5$ -containing $G\beta\gamma$ were localized to F-actin and most prominently to focal adhesions [22]; the focal adhesion localization of $G\gamma_5$ was also observed by others [20]. Although these studies describing localization of $G\alpha_{q/11}$ and $G\beta\gamma$ were compelling, a lack of follow up studies leaves the physiological significance and biochemical mechanisms unknown.

Several studies have also suggested that G proteins act via a number of scaffolding proteins to regulate the actin cytoskeleton. One study demonstrated that $G\alpha_{13}$ interacts directly with and activates radixin, a member of the ERM (ezrin, radixin, moesin) family of proteins that anchor actin filaments to the cell membrane [23]. Evidence was also provided that radixin mediates, at least partly, neoplastic transformation caused by $G\alpha_{13}$ independently of the small GTPase RhoA. This is in contrast to a more conventional view in which changes in the organization of the actin cytoskeleton initiated by $G\alpha_{13}$ are typically thought to be dependent on RhoA. -* Another study found that $G\alpha_{13}$ interacts with Hax-1, a cytoskeleton associated, cortactin-interacting, intracellular protein and that this interaction induces $G\alpha_{13}$ -stimulated cell migration [24]. It was demonstrated that interaction with Hax-1 inhibits $G\alpha_{13}$ stimulation of Rho while potentiating $G\alpha_{13}$ stimulated activity of Rac. This activation of Rac also recruits cortactin, also an actin-associated protein, which can initiate actin polymerization, cell protrusion and cell migration. Hax-1 has been found to be overexpressed in metastatic tumors and tumor cell lines; thus the $G\alpha_{13}$ -Hax-1 interaction could prove to be a significant target in the treatment of these tumors. Although the physiological role of the interaction of $G\alpha_{12/13}$ with actin cytoskeleton-associated proteins such as radixin and Hax-1 remains to be established, such interactions provide a potential direct connection for regulation of actin by $G\alpha_{12/13}$ that bypasses the more conventional regulation mediated by Rho and downstream Rho effectors.

G proteins, specifically $G\alpha_{12}$ and $G\alpha_{13}$, also bind to and regulate cell adhesion proteins, which are plasma membrane glycoproteins that connect the intracellular actin cytoskeleton to adhesion to other cells or to the extracellular matrix. $G\alpha_{12/13}$ interacts directly with the cytoplasmic domain of several members of the cadherin family of cell surface adhesion

molecules. This interaction was shown to cause the release and translocation of the transcriptional activator β -catenin to the cytoplasm and the nucleus [25]. Cadherins depend on the binding of their cytoplasmic, C-terminal domain to the actin cytoskeleton via key cytoplasmic proteins, such as β -catenin, and β -catenin has been shown to be required for the effects of cadherin on cellular functions. A mutationally activated $G\alpha_{12}$ inhibited cadherin-mediated adhesion and was able to reverse inhibition of cell migration mediated by ectopically expressed E-cadherins, independently of Rho activation [26]. Therefore, the $G\alpha_{12}$ -cadherin interaction negatively regulates E-cadherin mediated cell-cell adhesions and interferes with E-cadherin's activities in preventing cell detachment and cell migration, reflecting an important role for $G\alpha_{12}$ -cadherin interactions in cancer metastasis and development events. The physiological importance of the $G\alpha_{12/13}$ -cadherin connection was established in a study using zebrafish [27]. The authors found that depletion of $G\alpha_{12/13}$ in zebrafish embryos resulted in defects in epiboly, a coordinated cell movement involved in the physical restructuring of the early embryo. Importantly, genetic experiments provided compelling evidence that $G\alpha_{12/13}$ regulates epiboly through cadherins. A cadherin mutant of zebrafish results in epiboly defects; however, reduced expression of $G\alpha_{12/13}$ suppressed the epiboly defects, but overexpression of $G\alpha_{12/13}$ enhanced the defects. This result is entirely consistent with $G\alpha_{12/13}$ inhibiting cadherin function.

Additional ways in which $G\alpha_{12/13}$ can regulate cadherin have been revealed by studies showing that $G\alpha_{12/13}$ can interact with certain cadherin binding proteins. One study showed that the N terminal region of $G\alpha_{12}$ also directly interacts with α -SNAP, a protein of the NSF-SNAP-SNARE complex of the membrane fusion machinery, and that this interaction is involved in VE-cadherin trafficking to the plasma membrane of endothelial cells, where it contributes to the formation of adherens junctions and the control of cell confluence and endothelial barrier function [28]. Other studies showed that $G\alpha_{12/13}$ binds to p120 catenin, one of the catenins that bind cadherins [29, 30]. p120 catenin has several functions, including promoting the stability and cell surface localization of cadherin. Increased expression of $G\alpha_{12}$ promoted the binding of p120 catenin to E-cadherin, suggesting that $G\alpha_{12}$ could thus increase cadherin function [29]. In contrast, as described above, direct binding of $G\alpha_{12/13}$ to cadherins decreased cadherin function. Thus, it is possible that $G\alpha_{12/13}$ could exert opposing regulation of cadherin signaling depending upon context, and further experiments are necessary to determine the physiological relevance of the $G\alpha_{12/13}$ -p120 catenin interaction and the role of $G\alpha_{12/13}$ in regulating the function of cadherins.

Our knowledge of G protein regulation of cell adhesion molecules was further expanded by a recent study showing that $G\alpha_{13}$ interacted directly with integrins and mediated integrin "outside-in" signaling leading to the regulation of RhoA and cell spreading [31]. Integrins are cell adhesion molecules that bind specific extracellular matrix proteins and promote cell spreading, retraction, migration and proliferation. Integrins have bidirectional signaling functions where signals from within the cell activate the binding to extracellular matrix ligands ("inside-out" signaling) which in turn triggers a signaling cascade within the cell ("outside-in" signaling). In this study, it was shown that $G\alpha_{13}$ activates the platelet integrin $\alpha_{IIb}\beta_3$ signaling pathway and initiates cell spreading by binding directly to the cytoplasmic domain of $\alpha_{IIb}\beta_3$ which in turn activates c-Src leading to the inhibition of RhoA. This is in contrast to the conventional role of $G\alpha_{13}$ where it functions to activate RhoA. Thus, this study shows that $G\alpha_{13}$ is not only binding to a novel effector, integrin $\alpha_{IIb}\beta_3$, but also promoting a surprising signaling response, inhibition of RhoA. The authors propose a model to resolve how in the context of platelet signaling activation of $G\alpha_{13}$ could both inhibit RhoA yet retain its more conventional role of activation RhoA: $G\alpha_{13}$ mediates initial GPCR-induced RhoA activation which causes changes in cell shape, followed by integrin-induced RhoA inhibition leading to cell spreading. Therefore, the $G\alpha_{13}$ -integrin connection is a

novel interaction which mediates integrin signaling to c-Src and RhoA, thus regulating cell spreading important in wound healing, cell migration and proliferation.

As it has been suggested before [32], an intriguing idea is that localization to and the direct interactions of G proteins with cytoskeletal elements provides a system by which the cytoskeleton can be modulated rapidly and more efficiently by a readily available pool of signaling molecules, while it can also be important in generating a high potency response. Indeed it has been shown previously that other signaling molecules such as PLC β can also be translocated to cytoskeletal elements and that G protein interactions with tubulin can regulate second messenger signaling cascades [17]. Therefore, the cytoskeleton could act to synergize signaling pathways by facilitating close proximity of reactants, creating a readily available pool of reactants or removing interacting molecules from their substrates.

G Proteins and Cell Division

Over the last decade, a large body of work has demonstrated a role for heterotrimeric G proteins in cell division. Early work indicated that G protein subunits functioned in asymmetric cell division in the model organisms *Caenorhabditis elegans* and *Drosophila melanogaster*; however, more recently, this non-canonical signaling function for G proteins has been extended to mammalian cells and to symmetrical cell division.

Normally, a one-cell *C. elegans* embryo undergoes asymmetric cell division in which unequal forces on the two spindle poles yield two daughter cells of differing sizes and contents. Such asymmetric cell division is essential for determining ultimate cell fate and is thus required for proper embryogenesis and development of the organism. However, RNAi-mediated depletion of select G α and G $\beta\gamma$ causes defects in asymmetric cell division in *C. elegans* embryos. Loss of GPB-1, the single *C. elegans* G β subunit, or GPC-2, one of two *C. elegans* G γ subunits, results in incorrect positioning of the mitotic spindle axes, and this improper orientation of cell cleavage results in developmental defects in the embryo [33, 34]. In addition, other studies have shown that depletion of G $\beta\gamma$ causes increased pulling forces on the anterior side of the embryo and hyperactive spindle movements characterized by increased “rocking” of the one-cell embryo [35, 36]. On the other hand, depletion of two *C. elegans* G α_i family members, GOA-1 and GPA-16, produces a loss of asymmetry characterized by equal but greatly decreased pulling forces on both anterior and posterior spindle poles and a loss of rocking motion [33, 35, 36]. These seminal results and others have led to a model in which G α functions as a force generator, whereas G $\beta\gamma$ serves as a negative regulator of force generation on the spindle poles [37]. Deciphering the exact role of G α versus G $\beta\gamma$ based on knockdown studies is complicated due to the fact that depletion of one subunit not only would result in activation of the other subunit by virtue of loss of its binding partner and thus an inability to form the inactive heterotrimer, but would also result in decreased amount and decreased membrane localization of the other subunit [7].

In *Drosophila* embryos, asymmetric cell division occurs in neuroblasts and sensory organ precursor (SOP) cells, and these cells have been used as model systems to study the process. As in *C. elegans* embryos, depletion of G α_i and G $\beta\gamma$ causes defects in proper asymmetric cell division in these model *Drosophila* cells [38, 39]. Importantly, a number of additional studies have made it clear that a function for G proteins in cell division is not restricted to asymmetric cell division in invertebrates. G protein signaling has been implicated in asymmetric cell division in mouse neuronal progenitor cells [40], as well as in symmetric cell division in cultured mammalian cells [41]. Taken together, a number of studies confirm that G proteins, and particularly G α_i , regulate pulling forces on mitotic spindle poles during cell division [42, 43].

How are G proteins regulated and what proteins do they regulate during this process? A multitude of studies have provided a unique model for how G proteins function to regulate microtubule pulling forces in cell division. The discussion below will focus on asymmetric cell division in *C. elegans*, but it is clear that G proteins function similarly in cell division in *Drosophila* and invertebrates; some of these similarities will be highlighted. Two particularly non-canonical elements of this pathway are that 1) $G\alpha$ cycles between nucleotide bound states without the involvement of a GPCR, and 2) the GDP bound form of $G\alpha$, rather than the GTP bound form, is the important form in force generation. Although a requirement for a GPCR has not been absolutely ruled out, not only has a GPCR not been implicated in this pathway, but *C. elegans* and *Drosophila* embryos are encased in impermeable membranes arguing against involvement of a GPCR activated by an extracellular agonist. The key proteins that interact with $G\alpha$ in this non-canonical cycle in *C. elegans* include the two GDIs GPR-1 and GPR-2, the GEF Ric-8 and RGS-7 (Figure 2). Depletion of GPR1/2 in *C. elegans* embryos leads to the same loss of asymmetry and decreased pulling forces as observed for $G\alpha$ depletion [44–46]. GPR1/2 contains a GoLoco motif which functions to bind $G\alpha$, and the GoLoco motif-containing proteins Pins and LGN have also been demonstrated to play a key role in asymmetric and symmetric cell division in *Drosophila* and mammalian cells, respectively [41, 47, 48]. These proteins can function as GDIs to inhibit GDP release from $G\alpha$. However, the major role of GPR1/2 appears to be to connect plasma membrane localized $G\alpha$ -GDP to regulation of microtubules, and thus we can think of GPR1/2 as an effector of $G\alpha$ -GDP in this non-canonical pathway. GPR1/2 binds the coiled-coil protein LIN-5, and GPR1/2, $G\alpha$ and LIN-5 exist in a complex [33, 44–46, 49, 50]. LIN-5 is also required for asymmetric division in *C. elegans* [50], and the related proteins NuMA and Mud appear to play similar roles in mammals and *Drosophila*, respectively. The connection of GPR1/2, $G\alpha$ and LIN-5 to force generation was illuminated recently in two elegant studies demonstrating that loss or decreased function of the microtubule motor protein dynein resulted in decreased pulling forces during asymmetric cell division, and importantly that dynein formed a complex with LIN-5 and GPR1/2 [51, 52]. Thus, the current model for *C. elegans* states that plasma membrane localized $G\alpha$ -GDP localizes GPR1/2 and LIN-5 at the cell cortex where they bind dynein, and dynein generates the force necessary to control spindle pole positioning. Although many of the key proteins appear to be conserved in different organisms, the detail of how the $G\alpha_i$ and associated proteins regulate microtubules may differ [42, 43]. Additional studies will be necessary to fully understand unique components in different organisms.

Although a GPCR is not involved in this process, it is still necessary to regulate the nucleotide bound state of $G\alpha$, and that is where Ric-8 and RGS-7 come into play (Figure 2). Interestingly, loss of RGS-7 resulted in increased force at the anterior spindle pole [53]. This increased force and at only one pole has been difficult to understand in terms of exactly how RGS-7 fits into the pathway, but nonetheless the RGS-7 study clearly implicates RGS-7 in the G protein-dependent regulation of cell division, providing evidence that $G\alpha$ needs to progress through the G protein cycle. Loss of Ric-8, a non-GPCR GEF that can promote dissociation of GDP from $G\alpha$ [54], shows a similar phenotype as loss of $G\alpha$ or GPR1/2 – loss of asymmetry and reduced pulling forces [36, 55]. At first glance, this result seems counterintuitive in that Ric-8 should promote the formation of $G\alpha$ -GTP; thus loss of Ric-8 might be predicted to lead to more $G\alpha$ -GDP and increased, rather than decreased, pulling forces. However, this can be resolved by a model in which Ric-8 function is part of the activation process [36], and thus necessary for $G\alpha$ to cycle from inactive to active states (Figure 2). Importantly, Ric-8 has another function that plays a role in promoting G protein regulation of cell division. As clearly shown in studies of asymmetric cell division in *Drosophila*, Ric-8 is necessary for membrane targeting of $G\alpha_i$ [56–59], and more recent studies have confirmed that Ric-8 plays a role in $G\alpha$ stability, and by extension, proper

localization of $G\alpha$ [5, 6, 60]. Thus, a major role for Ric-8 in cell division may be simply allowing for proper levels of $G\alpha$.

In canonical G protein signaling, $G\beta\gamma$ is an essential part of the G protein cycle, but the role of $G\beta\gamma$ in regulation of microtubule force generation in cell division is not clear. Although depletion or inhibition of $G\beta\gamma$ results in cell division and pulling force defects in various systems, proteins that would mediate the function of $G\beta\gamma$ in cell division have not been identified. Instead a major role of $G\beta\gamma$ may be to restrict the function of $G\alpha$ by preventing the interaction with GPR1/2/Pins/LGN. In this regard, a recent report showed that differential trafficking of $G\beta\gamma$ occurs at the anterior and posterior side of *C. elegans* one-cell embryos during [37]. The authors demonstrated through live cell imaging of fluorescent $G\beta$ that $G\beta\gamma$ displays a dynamic localization whereby it trafficks between the cell cortex and endosomes. During mitosis, trafficking of $G\beta\gamma$ was increased as determined by an increased presence on endosomes. Moreover, the use of dynamin and rab-5 mutants revealed that trafficking was asymmetric, showing greater trafficking on the anterior side. These results prompted the speculation that less $G\beta\gamma$ at the posterior side would result in more $G\alpha$ available to interact with GPR1/2 and promote force generation compared to the anterior side. Thus, $G\beta\gamma$ may function here in its traditional role of promoting the formation of the inactive heterotrimer (Figure 2).

Although most models suggest that G proteins reside at the plasma membrane to regulate microtubule forces in cell division, there is also evidence the G protein subunits can regulate mitosis in additional ways via localization at non-canonical subcellular sites. $G\alpha_i$ as well as $G\beta\gamma$ have been localized to centrosomes/spindle poles [33, 61–63]. Also, an earlier report indicated localization of $G\alpha_i$ at kinetochores during mitosis [64]. Additional G protein regulators, such as LGN, Ric-8A and RGS-14, have been reported to co-localize with $G\alpha_i$ at centrosomes in mammalian cells [61, 62]. Such G protein complexes may regulate microtubule forces directly at spindle poles, in addition to the cell cortex; alternatively, localization at centrosomes may indicate a site of transport to the cell cortex. All three $G\alpha_i$ subunits have also been detected at the midbody of mammalian cells during late mitosis [61, 62], and, interestingly, $G\alpha_{i1}$ localization shifts from the centrosome to the midbody as NIH3T3 cells proceed through mitosis. Midbody localization is suggestive of having a role in cytokinesis, and indeed siRNA-mediated depletion of all three $G\alpha_i$ subunits in HeLa cells resulted in cytokinesis defects, including greatly increased time spent in cytokinesis, abnormally extended intercellular bridges, microtubule misalignment and increased multinucleation [62]. Future studies will be needed to reveal the mechanisms of how G proteins regulate cytokinesis.

G Proteins at Organelles

Though classically G protein signaling occurs at the plasma membrane, there is mounting evidence that G proteins function at subcellular locations such as mitochondria, Golgi, ER, endosomes and the nucleus. Recent reports have provided unique insights regarding the activation and function of G proteins at locations in the cell distinct from the plasma membrane where they play crucial roles in the formation of signaling complexes, controlling the architecture of organelles and regulating physiological functions of the cell (Table 2 and Figure 3).

G proteins at the mitochondria

The mitochondrion has often been described as the “power plant” of the cell as it generates most of the cell’s supply of adenosine triphosphate (ATP) which is used as chemical energy. Mitochondria are also involved in a variety of other processes ranging from signaling to cellular differentiation and regulation of intracellular calcium dynamics to apoptosis.

Mitochondria are motile, dynamic organelles which undergo constant fission, fusion and branching leading to remodeling of the mitochondrial network, and defects in this mitochondrial fission/fusion machinery have been shown to cause respiratory and neurodegenerative disorders, and embryonic lethality. It has been recently discovered that G protein subunits are specifically targeted to the mitochondria where they play a critical role in controlling mitochondrial morphology and dynamics. Approximately 40% of $G\alpha_{12}$ in human umbilical vein endothelial cells was localized at the outer surface of mitochondria, and the N-terminal domain of $G\alpha_{12}$ but not other $G\alpha$ subunits, was shown to contain a mitochondrial targeting sequence [65]. Interestingly, mutations in the targeting sequence did not prevent targeting of $G\alpha_{12}$ to the mitochondria indicating the importance of factors in addition to the N-terminus; the authors speculate that Hsp90 could play such a role since it has been shown to bind $G\alpha_{12}$ and can localize and target other proteins to the mitochondria [66–68]. Depletion of $G\alpha_{12}$ increased the percentage of motile mitochondria and inhibited LPA-induced decreases in mitochondrial motility. Therefore, mitochondrial motility may be regulated by $G\alpha_{12}$ through a Rho-JNK-kinesin pathway, although this remains to be confirmed. Also, $G\alpha_{12}$ mutants unable to bind and stimulate Rho transformed the mitochondrial network into punctae and caused loss of mitochondrial membrane potential. This mutant also decreased cellular levels of Bcl2 and since Bcl-2 is an antiapoptotic protein and is important in inhibiting mitochondrial fragmentation and membrane permeabilization, it is inferred that $G\alpha_{12}$ may play a key role in regulating the functions of Bcl-2 at the mitochondrial membrane. In another study it was shown that depletion of $G\beta_2$ also resulted in mitochondrial fragmentation [69]. $G\beta_2$ was found to be enriched on the mitochondrial surface and was shown to play an important role in mitochondrial fusion by interacting specifically with mitofusin (Mfn1), a mitochondrial GTPase in the dynamin family which mediates the fusion of mitochondrial membranes. In this study, it was shown that $G\beta_2$ physically interacts with Mfn1, limits the membrane motility of Mfn1 at the mitochondrial surface and regulates mitochondrial fusion. Interestingly, expression of $G\gamma$ had no effect in these studies, and expression of a $G\beta_2$ that could not bind $G\gamma$ retained the ability to promote mitochondrial fusion; thus, this is potentially a case where $G\beta_2$ functions without being bound to $G\gamma$. Taken together, these studies show the localization and the importance of G proteins at the mitochondria and specifically the action of $G\alpha_{12}$ and $G\beta_2$ in regulating the structure, morphology and dynamics of the mitochondria. Additional studies have localized $G\alpha_i$ and $G\alpha_s$ to mitochondria [70, 71], further suggesting that the mitochondria is an important site of heterotrimeric G protein action.

G proteins at the Golgi

The Golgi complex is composed of stacks of membrane bound structures known as cisternae and is an important site for modifying, sorting and packaging macromolecules for secretion or delivery to other membranes and organelles. It has been shown that protein kinase D (PKD) is a central mediator of a signaling pathway at the trans Golgi network (TGN) involved in the fission of transport carriers that are destined for the plasma membrane. Seminal studies in the '90s demonstrated that $G\beta\gamma$ subunits could stimulate the vesiculation of the Golgi through activation of PKD, suggesting the involvement of $G\beta\gamma$ in TGN-to-PM traffic [72, 73]; such vesiculation was interpreted as overactivation of a signaling pathway that stimulated the production of TGN-derived transport carriers. A recent study built upon and confirmed predictions from earlier studies by demonstrating that $G\beta\gamma$ subunits could function at the Golgi, rather than the classical PM localization, to regulate TGN-to-PM transport of proteins [74]. Constitutive or inducible targeting of $G\beta\gamma$ subunits to the Golgi caused vesiculation of the Golgi in a PKD-dependent manner, whereas PM-targeted $G\beta\gamma$ had no effect on the Golgi. Importantly, it was further shown that inhibition of endogenous $G\beta\gamma$ by the inhibitor gallein [75] and by the $G\beta\gamma$ sequestering protein GRK2ct inhibited constitutive TGN-to-PM transport of two model cargo proteins. Moreover, Golgi-targeted

GRK2ct, but not PM-targeted GRK2ct, inhibited protein transport, providing evidence that endogenous G $\beta\gamma$ is functioning at the Golgi [74].

The mechanism by which G $\beta\gamma$ is recruited to the Golgi is yet unknown; however, there is evidence that certain G $\beta\gamma$ subunits can translocate reversibly from the PM to Golgi [76–78]. Consistent with this, a recent study provided evidence that GPCR-dependent translocation of G $\beta\gamma$ can induce Golgi fragmentation and can regulate the secretion of insulin [79]. In this study, it was shown that agonist activation of M3 muscarinic acetylcholine receptors promoted Golgi fragmentation. The fragmentation depended upon overexpression of G γ_{11} in HeLa cells or the presence of endogenous G γ_{11} in A569 cells. G γ_{11} was previously identified by the authors as one of a select group of G γ subunits that are competent for PM to Golgi translocation [76, 78]. Moreover, expression of a non-translocating G γ_3 in both cell lines prevented M3 receptor induced fragmentation. Similarly, insulin secretion was examined in a pancreatic β cell line NIT-1. Activation of the M3 receptor in NIT-1 cells promotes insulin secretion, and, interestingly, NIT-1 cells contain the translocation competent subunits G γ_5 , G γ_{10} and G γ_{13} . Overexpression of the non-translocating G γ_3 , which appears to function in a dominant negative manner to prevent translocation of endogenous G $\beta\gamma$, inhibited M3 receptor promoted insulin release. Thus, a model emerges from these studies in which extracellular activation of a cell-surface GPCR promotes G $\beta\gamma$ translocation to the Golgi, which in turn regulates the fission of PM-destined or secreted cargo [79]. In addition, other models for G $\beta\gamma$ activation need to be considered, such as regulation by an internal GPCR or G protein activation by a non-GPCR activator, since it is unlikely that regulation of transport of all G $\beta\gamma$ -regulated cargo would require a signal emanating from the extracellular environment.

In a related study, it was shown that in cells undergoing senescence the expression of translocation competent G γ_{11} is upregulated [80]. Cells that have undergone genotoxic stress or replicatively senescent cells have upregulated secretions such as cytokines and have been shown to exhibit dispersed Golgi due to the traffic of secretory proteins. In this study, depletion of G γ_{11} or expression of non-translocating/dominant negative G γ_3 inhibited the dispersed structure of the TGN. Again, these results are consistent with a model in which translocating G $\beta\gamma$ subunits initiate vesiculation of the Golgi. Cellular senescence is thought to be cancer protective, and it has been shown that G γ_{11} is downregulated in several types of cancer cells implicating an important role for G γ_{11} -containing G $\beta\gamma$ complexes at the Golgi in the regulation of cellular senescence and in the control of cell proliferation.

A great number of studies have identified proteins involved in the G $\beta\gamma$ -regulated signaling pathway at the Golgi [81]. It has been shown that PLC β_3 is required for G $\beta\gamma$ -promoted Golgi vesiculation, as inhibition of PLC β_3 inhibits vesiculation, and therefore it is proposed that Golgi-localized G $\beta\gamma$ activates PLC β_3 [82]. In this model, the activation of PLC β_3 leads to the formation of diacylglycerol (DAG) which in turn activates Golgi-localized PKC η and also recruits PKD to the Golgi [83]. PKC η then phosphorylates the activation loop of PKD, a central mediator of the generation of post Golgi transport vesicles. A early study showed that G $\beta\gamma$ could directly bind to and activate PKD [73]; thus, the direct effector(s) of G $\beta\gamma$ at the Golgi -- PLC β_3 , PKD and/or other signaling proteins -- remains to be more firmly established. PKD activation then leads to the phosphorylation and activation of membrane fission machinery which includes activation of phosphatidylinositol 4 kinase III β (P4IKIII β) and the recruitment of proteins such as oxysterol binding protein 1 (OSBP1) and the ceramide transfer protein (CERT) which is necessary for the production of modified lipids such as DAG, phosphatidic acid (PA) and lyso-PA (LPA); these lipid constituents are involved in the generation of plasma membrane specific vesicles [81]. Further mechanistic details of the fission process are still being uncovered, but it is clear that a G $\beta\gamma$ -regulated

Golgi-localized signaling pathway regulates the activation of numerous proteins and the production of several membrane lipids that collaborate to generate transport carriers.

In addition to the clear evidence for a role of $G\beta\gamma$ in regulating the fission of Golgi-to-PM carriers, there has been long-standing evidence that $G\alpha$, particularly $G\alpha_{i3}$, localizes at the Golgi and regulates the secretory pathway [84–88]. However, the mechanistic details of how $G\alpha_{i3}$ regulates transport of cargo from the Golgi to the PM have remained elusive. It is possible that some of the observed effects of overexpression or inhibition of $G\alpha_{i3}$ are due to interactions with $G\beta\gamma$ and thus regulating $G\beta\gamma$'s role in the secretory pathway. More recently, a calcium-binding protein, calnuc, has been identified as a protein that interacts with $G\alpha_{i3}$ at the Golgi [89–91]. A recent report showed that calnuc can regulate secretion and regulate the localization of $G\alpha_i$ subunits [90]. Therefore, accumulating evidence suggests that calnuc can regulate $G\alpha_{i3}$ localization and possibly $G\alpha_{i3}$'s interactions with other Golgi-localized proteins, and thus function to integrate calcium signaling with G protein regulation of secretion.

G proteins at the Endoplasmic Reticulum (ER)

Several reports also reveal G protein signaling and interactions of G proteins with proteins of the ER. Calcium signaling is typically initiated by the activation of GPCRs and G proteins which then activate PM-localized $PLC\beta$, which in turn generates the diffusible second messenger inositol trisphosphate (IP₃); IP₃ binds to IP₃ receptors on the ER membrane stimulating the release of calcium from ER internal stores. However, in contrast to this conventional G protein signaling paradigm, $G\beta\gamma$ can interact directly with and activate the IP₃ receptors located on the ER membrane [92]. Introduction of $G\beta\gamma$ into cells evoked calcium release via IP₃ receptors that was independent of $PLC\beta$. $G\beta\gamma$ also interacted directly with the IP₃ receptors while it also inhibited binding of IP₃ to the IP₃ receptors, and importantly, activated single IP₃ receptors in native ER membranes as potently as IP₃ molecules. The authors proposed that since it has been observed that activation of Gi-coupled receptors do not generate detectable amounts of IP₃, direct activation of IP₃ receptors by $G\beta\gamma$ is particularly significant for Gi-coupled receptor signaling pathways [92]. Additional insights about the function of G proteins at the ER have also emerged from studies performed in plants [93, 94]. $G\beta\gamma$ signaling is involved in cell death associated with the unfolded protein response (UPR) in *Arabidopsis thaliana* [93]. The UPR response, which occurs as a protective response when protein folding and modification is disrupted, causes ER stress and triggers a cascade of events which either promote proper protein folding or, if that fails, cell death. Dysregulation of this pathway has been shown to contribute to human diseases such as diabetes, alzheimer's, atherosclerosis and cancer. In this study, it was observed that plants lacking $G\beta$ were more resistant to growth inhibition by the protein glycosylation inhibitor tunicamycin, while they also had less accumulation of BiP chaperone proteins which promote proper protein folding and secretion. $G\beta\gamma$ was shown to localize at the ER in wild type plant cells, and in fact a majority of $G\beta$ was found in an ER fraction rather than a PM fraction in cell fractionation experiments. $G\beta$ was also degraded during UPR. Effectors of $G\beta\gamma$ in plants and the exact mechanism by which $G\beta\gamma$ triggers UPR-associated cell death have not yet been identified; however, as in mammalian cells, $G\beta\gamma$ could be activating $PLC\beta$ and/or ER-localized IP₃ receptors leading to the generation of calcium and the initiation of calcium signaling pathways. However, the involvement of $G\beta\gamma$ in UPR in mammalian cells has yet to be demonstrated. Lastly, a recent report linked $G\alpha_{i2}$ to the small GTPase Sar1, a protein that regulates the generation of COPII vesicles from the ER membrane which are destined to the Golgi [95]. It was shown that the Gi protein activator, mastoparan 7, suppressed the translocation of Sar1 to the ER in a cell free, microsomal system, while a negative regulator of the Gi protein, pertussis toxin, recovered this suppression. Moreover, $G\alpha_{i2}$ was localized to the ER by cell fractionation,

suggesting that $G\alpha_{i2}$ is a resident ER protein. The exact mechanism(s) by which this G protein regulates Sar1 translocation to the ER requires further investigation.

G Proteins at Endosomes

Several recent studies have highlighted novel rules for G proteins at endosomes. In the yeast *Saccharomyces cerevisiae*, it was shown that the $G\alpha$ subunit, Gpa1, is present at endosomes, and regulates pheromone signaling. The yeast phosphatidylinositol 3-kinase (PI3K), which phosphorylates phosphatidylinositol at the 3' position, is composed of a catalytic (Vps34) and regulatory (Vps15) subunit and it was demonstrated in this study that GDP bound Gpa1 interacts with the regulatory Vps15 subunit which is similar to the $G\beta$ subunit in that it has a seven WD repeat structure. Further experiments in this study showed that upon exchange of GDP for GTP Gpa1 dissociated from the Vps15 subunit and formed interactions with the catalytic Vps34 subunit, promoting the production of phosphatidyl inositol 3 phosphate, and the recruitment of Bem1, a protein involved in pheromone signaling in yeast. This is a novel example of a $G\alpha$ protein being recruited to the endosome by a $G\beta$ -like subunit (Vps15) where it initiates G protein signaling [96].

Although it is not clear that a mammalian $G\alpha$ is similarly activating PI3K at endosomes, there is evidence that certain mammalian $G\alpha$ subunits can localize to endosomes [97–100]. One report showed that $G\alpha_s$ localizes to early endosomes together with the endocytic protein Hrs, interacts with Hrs and regulates the degradation of the EGF receptor [100]. It will be interesting to see if G proteins have a wider role in regulating endosomal complexes involved in sorting and degradation of diverse receptors.

Another recent report, demonstrated a role for $G\beta\gamma$ in signaling at endosomes [101]. It was shown that LPA activation of cells, which occurs via a GPCR, resulted in increased interaction of $G\beta\gamma$ and Rab11a and in their colocalization at endosomes. In turn, LPA promoted a $G\beta\gamma$ -dependent endosomal recruitment of PI-4,5P-specific PI3K γ , along with endosomal recruitment and activation of Akt. PI3K γ is known to be a direct effector of $G\beta\gamma$, although it was previously assumed that $G\beta\gamma$ -mediated activation of PI3K γ and Akt occurred at the PM. Moreover, this report suggests that Rab11a is required for the trafficking of $G\beta\gamma$ to endosomes upon activation by a cell-surface GPCR. It will be important to define in the future how the mechanisms that control $G\alpha$ and $G\beta\gamma$ trafficking to endosomes differ from the mechanisms involved in trafficking of G proteins to other organelles, such as Golgi. Taken together several results provide evidence for endosomes not only serving as a point on the G protein trafficking itinerary but also providing a non-canonical site for G protein signaling.

G Proteins in the Nucleus

In one of the earliest reports on the activity of G proteins in the nucleus, it was shown that $G\gamma_5$ interacted with the novel transcriptional repressor adipocyte enhancer-binding protein 1 (AEBP1) and regulated its activity. AEBP1 complexes with $G\gamma_5$, but not $G\gamma_7$, in nuclei of 3T3-L1 cells and attenuates the transcriptional repression activity of AEBP1 [102]. The $G\gamma_5$ interaction with AEBP1 was detected initially in a two-hybrid screen; thus, it is possible that $G\gamma_5$ in the absence of $G\beta$ binds AEBP1 in cells, but $G\beta$ subunits were also detected in a co-immunoprecipitation with AEBP1, suggesting that AEBP1 binds to $G\beta\gamma$ heterodimers containing $G\gamma_5$. This study used antibodies directed against $G\beta_{1-4}$ and $G\gamma_5$ to demonstrate by cell fractionation and immunofluorescence microscopy that $G\beta\gamma$ localized to the nucleus. Subsequently, several other studies have shown a nuclear localization of $G\beta\gamma$ and its interaction with and regulation of diverse transcription factors.

In another study, It has been shown that the actions of the glucocorticoid receptor (GR) are modulated by the interaction with G $\beta\gamma$ [103, 104]. G $\beta\gamma$ interacts with the GR, co-migrates with it into the nucleus and suppresses GR-induced transactivation of the GR and its responsive genes. Glucocorticoids play a critical role in maintaining the homeostasis of the immune, cardiovascular and the central nervous systems. Upon ligand binding, GR in the cytoplasm dissociates from heat shock proteins, homodimerizes and translocates to the nucleus where it interacts with glucocorticoid response elements (GRE) and transcriptional factors, thus activating target genes. The GRE-bound GR stimulates transcriptional activation by forming a transcription initiation complex via its activation function domains AF-1 and AF-2. It was shown that G $\beta\gamma$ acts on GR by suppressing AF-2 but not AF-1 by interacting directly or indirectly with the AF-2 domain. Interestingly, it appears that G $\beta\gamma$ in which the G γ is not prenylated is the form responsible for the antiglucocorticoid action [103]. Treatment of cells with the statin drug lovastatin, which blocks prenylation, induced the antiglucocorticoid action of G $\beta\gamma$ by increasing its nuclear translocation. Because prenylated G $\beta\gamma$ is expected to associate with membranes, it makes sense that prenylation-deficient G $\beta\gamma$ would be the form that binds to soluble transcription factors. However, prenylation is considered to be an irreversible modification; there may be a fraction of G $\beta\gamma$ that escapes normal prenylation, or alternatively certain proteins may bind to G $\beta\gamma$ and sequester the hydrophobic lipid. Future studies will determine whether transcription factor regulation by non-prenylated G $\beta\gamma$ is a common theme. Regardless, this antiglucocorticoid action of G $\beta\gamma$ may have significant implications for the treatment of diseases associated with increased glucocorticoid activity. In another more recent study, it was shown that G $\beta\gamma$ interacts with the transcription factors cFos and FosB, which, along with members of the Jun family, are members of the dimeric activator protein-1 (AP-1) transcription factor family and control cell proliferation and differentiation [105]. It was shown that G $\beta\gamma$ interacts directly with cFos or FosB without blocking Fos/Jun dimerization or their interaction with DNA. However, the interaction of G $\beta\gamma$ with the Fos/Jun dimer results in the recruitment of histone deacetylase HDAC5 to the nucleus and the repression of AP-1 mediated transcriptional activity. This study also demonstrated that G $\beta\gamma$ could be found in nucleus. It should be noted that cFos transcription is also activated by G protein coupled receptors through a G $\beta\gamma$ -regulated signaling pathway and that the repression of the AP-1 complex by G $\beta\gamma$ could be part of a negative feedback loop. However, the source of G $\beta\gamma$ which mediates the repression activity may be different in that it may not be generated by GPCRs. The factors that regulate the interaction of G $\beta\gamma$ with AP-1 remain to be clarified.

In the AP-1 studies, it was shown that the interaction of G $\beta\gamma$ with AP-1 results in HDAC5 nuclear recruitment, and thus HDAC function may contribute to the observed repression of AP-1 mediated transcription. Indeed, previous work showed that G $\beta\gamma$ interacts with HDAC5 [106]. HDACs act as transcriptional co-repressors by regulating the acetylation state of histones. The C-terminal domain of HDAC5 binds directly to the G $\beta\gamma$, and this binding blocks HDAC5's inhibitory action on the myocyte enhancer factor 2 (MEF2) transcription factor. HDAC4 is also able to form complexes with G $\beta_1\gamma_2$ through its C-terminus which shares significant similarities with HDAC5, suggesting that the C-terminus of class II HDACs harbor a G $\beta\gamma$ binding motif. The study further showed that when G $\beta\gamma$ was sequestered using a GRK2 C-terminal peptide, HDAC resumed its inhibitory action on MEF2. HDAC is most prevalent in skeletal and cardiac tissue and the brain while MEF2 is involved in regulating cardiac function. Dysregulation of HDAC function has been linked with hypertrophy, and therefore, G $\beta\gamma$ appears to play a major role in fine-tuning the activity of HDAC5 and MEF2C.

Although the studies described above are all consistent with the idea that G $\beta\gamma$, in the absence of G α subunits, localizes to the nucleus and regulates important transcription factors, G α itself can also localized to nuclei [32] and interact with nuclear proteins. One

interesting recent report identified an interaction of $G\alpha_{16}$ with three related transcription factors: transcription factor E3 (TFE3), microphthalmia-associated transcription factor, and transcription factor EB [107]. These three proteins were found in a yeast-based screen designed to identify $G\alpha_{16}$ -selective AGS proteins. The interaction of $G\alpha_{16}$ and TFE3 was further analyzed in this study. Expression of TFE3 in COS cells induced nuclear localization of co-expressed $G\alpha_{16}$; however, TFE3 did not promote nuclear localization of $G\alpha_{13}$ or $G\alpha_s$. One particular gene, claudin 14, an important protein involved in membrane structure in cardiomyocytes, was highly upregulated by the co-expression of $G\alpha_{16}$ and TFE3 in HEK293 cells, and siRNA knockdown of both $G\alpha_{16}$ and TFE3 in cardiomyocytes decreased claudin 14 expression. The mechanism of action of the $G\alpha_{16}$ -TFE3 complex in the nucleus remains to be determined; the authors speculate that such an interaction may help to assemble a transcription complex or that TFE3 may activate $G\alpha_{16}$ so that it can subsequently activate nuclear PLC β . It will be interesting to see if other AGS proteins function to promote nuclear localization of $G\alpha$ subunits.

Lastly, it is worth noting that GPCRs have been detected in the nucleus or in nuclear membranes, and there are clear examples in which nuclear-localized GPCRs can induce G protein-dependent signaling [108]. Moreover, effector proteins such as PLC β and the related phosphoinositide cycles exist in the nucleus which could possibly lead to the generation and regulation of second messenger systems such as calcium signaling within the nucleus [109, 110]. This adds to the complexity of how and where G proteins that function in nucleus are activated. $G\alpha$ or $G\beta\gamma$ subunits may be activated in the traditional manner by PM-localized GPCRs, followed by translocation into the nucleus; G proteins existing at the PM or other locations may be activated in a GPCR-independent manner; or nuclear-localized GPCRs may activate G proteins *in situ*.

Conclusions

This review focused on three broad areas of novel G protein function -- 1) G protein interactions with cytoskeleton and cell adhesion proteins; 2) G protein function in cell division; and 3) G proteins at subcellular organelles – and has highlighted some of the recent progress in these areas. Of course, many important questions remain as these areas of research move forward. Some of these future questions are:

- Which novel functions are regulated by GPCRs and which are GPCR-independent?
- What determines the selectivity of a G protein subunit's participation in a particular process? For example, why have only $G\alpha_{12/13}$ been linked to cell adhesion molecules? Will we find that other G protein subunits also regulate cell adhesion?
- G proteins appear to follow an unusual cycle during regulation of cell division? What are the additional regulators that will allow us to better understand this novel G protein cycle?
- Localization of G proteins is dynamic. What are the mechanisms and interacting proteins that regulate G protein trafficking? Understanding how G proteins translocate to different subcellular environments will provide insight into G protein function at varied locations.
- GPCR localization is also dynamic. Are G protein signaling functions at subcellular organelles regulated by co-localized GPCRs, possibly responding to intracellular agonists?

These questions and more will surely keep many investigators busy. It is now clear that G proteins have many underappreciated functions at diverse subcellular locations. The G

protein signaling field looks forward to much exciting research in the future, as we better understand novel functions for heterotrimeric G proteins.

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Highlights

- G proteins directly interact with cytoskeletal and cell adhesion proteins.
- G proteins regulate cell division.
- G proteins function at diverse subcellular organelles.

Figure 1. Novel G protein interactions with cytoskeletal and cell adhesion proteins

Heterotrimeric G protein subunits directly interact with and regulate microtubules, actin-binding proteins, integrins, cadherins and cadherin-binding proteins. See text and Table 1 for details.

Figure 2. A non-canonical heterotrimeric G protein cycle in cell division

A, In conventional G protein signaling, a GPCR acts as a guanine-nucleotide exchange factor to transition the G protein from the inactive heterotrimer to the active, GTP-bound Gα and free Gβγ. **B**, During G protein function in cell division, the “active” element is GDP-bound Gα. The cycle appears to function in the absence of GPCR regulation. According to one model the guanine-nucleotide exchange factor Ric-8 and a GTPase activating RGS protein work in concert to transition the G protein from an inactive heterotrimer to an active Gα-GDP. The details of this transition await further clarification as do other points of regulation (denoted by ?) of this novel G protein cycle (adapted from [36, 111]).

Figure 3. Novel subcellular localizations of G proteins

Heterotrimeric G proteins have been identified to regulate effectors and their respective signaling pathways at diverse subcellular organelles. Indicated are select G protein subunits which have been identified at these subcellular locations, along with their interacting effector proteins. See text and Table 2 for details.

TABLE 1

Novel G protein interactions with cytoskeletal and cell adhesion proteins.

Cytoskeletal or cell adhesion element	G protein subunit	Function(s) of interaction	References
Microtubules	G α_i , G α_s	Destabilize microtubules, promote tubulin GTPase activity, increase process formation	[13]
	G α_q	Promotes membrane association of tubulin	[13]
	G $\beta\gamma$	Promotes microtubule assembly, increases microtubule stability	[13]
Actin	G $\alpha_{q/11}$	Regulates G $\alpha_{q/11}$ activation of PLC β	[18,19]
	G $\beta\gamma$	Promotes cell rounding and migration	[20–22]
Radixin	G α_{13}	Activates radixin, promotes its binding to polymerized F-actin and neoplastic transformation	[23]
Hax-1	G α_{13}	Potentiates activation of Rac, inhibits activation of Rho, recruits cortactin to complex	[24]
Cadherins	G $\alpha_{12/13}$	Releases beta-catenin and negatively regulates cadherin mediated adhesion regulates epiboly	[25–27]
α -SNAP	G α_{12}	Traffics VE-cadherins to the PM, forms adherens junctions	[28]
p120 catenin	G $\alpha_{12/13}$	Increase binding of p120 catenin to E-cadherin	[29,30]
Integrin $\alpha_{IIb}\beta_3$	G α_{13}	Mediates integrin “outside-in” signaling, inhibits RhoA, increases cell spreading	[31]

TABLE 2

Novel subcellular localizations of G proteins.

Organelle	G protein subunit	Function at organelle	References
Mitochondria	G α_{12}	Regulates mitochondrial motility and antiapoptotic functions of Bcl-2 at the mitochondrial membrane	[65]
	G β_2	Regulates activity of mitofu1n (Mfn1) and mitochondrial fusion	[69]
Golgi	G $\beta\gamma$	Regulates golgi to PM transport of proteins including insulin, regulates cellular senescence	[72–74, 79–83]
	G α_{13}	Interacts with calnuc	[84–91]
Endoplasmic Reticulum	G $\beta\gamma$	Directly activates IP3 receptors and stimulates calcium release, regulates unfolded protein response in <i>Arabidopsis</i>	[92–94]
	G α_{12}	Inhibits translocation of Sar1	[95]
Endosomes	Gpa1 (yeast)	Regulates pheromone signaling via PI3K in yeast	[96]
	G α_8	Interacts with endocytic protein Hrs and regulates the degradation of the EGF receptor	[100]
	G $\beta\gamma$	Interacts with Rab11 and recruits and activates PI3K γ and Akt	[101]
Nucleus	G $\beta\gamma_5$	Regulates the transcriptional repressor, adipocyte enhancer-binding protein	[102]
	G $\beta\gamma$	Suppresses glucocorticoid receptor (GR) activity; recruits HDAC5 to the nucleus, represses AP-1 transcriptional activity; blocks HDAC5's inhibitory action on MEF2, regulates cardiac function	[103–106]
	G α_{16}	Regulates activity of Transcription Factor E3 (TFE3) and the cardiomyocyte membrane protein, claudin 14	[107]