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Review

Tubulin, actin and heterotrimeric G proteins: Coordination of signaling and structure ☆

Jeffrey M. Schappi [a], Aleksandar Krbanjevic [a,c], Mark M. Rasenick [a,b,*]

[a] Department of Physiology and Biophysics, University Of Illinois, Chicago, IL 60612, USA
[b] Department of Psychiatry, University Of Illinois, Chicago, IL 60612, USA
[c] Jesse Brown VAMC, Chicago, IL 60612, USA

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ABSTRACT

G proteins mediate signals from membrane G protein coupled receptors to the cell interior, evoking significant regulation of cell physiology. The cytoskeleton contributes to cell morphology, motility, division, and transport functions. This review will discuss the interplay between heterotrimeric G protein signaling and elements of the cytoskeleton. Also described and discussed will be the interplay between tubulin and G proteins that results in atypical modulation of signaling pathways and cytoskeletal dynamics. This will be extended to describe how tubulin and G proteins act in concert to influence various aspects of cellular behavior. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters.This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

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1. Introduction

G proteins are well-established mediators of communication from outside the cell to inside, in response to hormonal or neurotransmitter action at G-protein coupled receptors, whose effects are mediated through effectors such as adenylyl cyclases, phosphodiesterases, phospholipases, and ion channels. This review, however, will focus on the reciprocal interactions between G-proteins and the cytoskeleton.

G protein coupled receptors (GPCRs) are a large and diverse group of seven-transmembrane receptors for a variety of ligands, including hormones, odorants, and even light. Upon ligand binding, the activated receptor acts as a guanine nucleotide exchange factor for membrane-associated G protein alpha subunit, causing release of GDP and binding of GTP, resulting in an active Gα subunit. Gα subunits constitute a family of 20 proteins and include Gαs, which activates adenylyl cyclase to produce cAMP; Gαi, which decreases cAMP concentration by inhibiting adenylyl cyclase activity or activating phosphodiesterases; Gαq, which activates phopholipases to cleave membrane lipids to

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* Corresponding author at: Department of Physiology and Biophysics, University Of Illinois, Chicago, IL 60612, USA. Tel.: +312 996 6641.
E-mail address: raz@uic.edu (M.M. Rasenick).

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inositol trisphosphate (IP_3) and diacylglycerol (DAG); and Gα12/13, which regulates small GTPases affecting the actin and tubulin cytoskeleton. After activation, Gα subunits dissociate (although not necessarily physically [1]) from their bound Gβγ subunits, enabling them to interact with their effectors. Gβγ subunits may also activate effector molecules, such as ion channels. Intrinsic GTPase activity and hydrolysis to the GDP-bound form terminate Gα subunit signaling and promotes re-association with the Gβγ subunit. This intrinsic activity may also be promoted by regulators of G protein signaling (RGS) proteins, which augment Gα subunit GTPase activity to terminate signal propagation. Similarly, guanine nucleotide dissociation inhibitors (GDIs) inhibit dissociation of bound GDP, preventing exchange for GTP, and keeping the alpha subunit in its inactive state. Additional mechanisms of signal termination include receptor phosphorylation by GPCR kinases (GRKs), arrestin binding, and subsequent internalization. Gα subunits may be internalized as well, though this occurs by a separate mechanism [2]. G proteins associate with the inner layer of the cell membrane via both protein–protein and protein–lipid interactions. Gβγ subunits are prenylated, while Gα subunits require association with Gβγ subunits for proper membrane targeting and acylation [3]. Other protein interactions, such as with the cytoskeleton, will be discussed in the body of this review.

The cytoskeleton contributes to cell structure, motility, division, and intracellular transport. Microtubules are formed by polymerization of tubulin dimers, which consist of one α-tubulin and one β-tubulin subunit; both bind GTP. While β-tubulin-bound GTP is hydrolysable by the subunit's intrinsic GTPase activity, α-tubulin-bound GTP is constitutive. The intrinsic GTPase activity of β-tubulin is an important regulator of microtubule dynamics. Microtubule growth and stability are promoted by the presence at its (+) end of a "GTP cap", a series of tubulin dimers containing GTP-bound β-tubulin. GTP hydrolysis by these subunits and loss of the cap results in microtubule catastrophe, in which rapid depolymerization occurs. Various microtubule-associated proteins are known to stabilize or destabilize the microtubule structure. Cytoskeletal microfilaments are composed of polymers of globular actin. Like β-tubulin, actin subunits have intrinsic GTPase activity, which is similarly related to microfilament stability. As with microtubules, a variety of proteins are known to stabilize, destabilize, fork, and cleave microfilaments. A third class of cytoskeletal proteins, intermediate filaments, will not be considered in this review as there is no evidence for regulation by heterotrimeric G proteins.

2. Initial evidence for the involvement of the microtubule cytoskeleton in G protein signaling

Early studies identified the ability of colchicine or vinblastine, both inhibitors of microtubule polymerization and stability, to cause predictable changes in cellular behavior, particularly with respect to cAMP formation. These responses were first seen in leukocytes (lymphocytes and macrophages). Upon lymphocyte activation by lectins such as concanavalin A, cellular cAMP accumulation was increased several fold, and was further potentiated in a dose-dependent fashion by colchicine and vinblastine, but not by lumicolchicine, a congener of colchicine which does not bind tubulin [4]. Modest increases in leukocyte cAMP accumulation were also seen in response to colchicine alone, and these increases were significantly potentiated by isoproterenol and prostaglandin E1 (PGE1), which are now recognized as ligands of Gαs-linked GPCRs [5–8]. These increases in cellular cAMP could be attributed to increased production by adenylyl cyclases, decreased degradation by phosphodiesterases, or both. Notably, the potentiation of cAMP production by isoproterenol or PGE1 was observed only in the presence of the phosphodiesterase inhibitor IBMX, suggesting that the responsible phenomenon was one of increased production [5]. Further work in S49 lymphoma cells clarified the mechanisms through which microtubule disruptors potentiated adenylyl cyclase as a likely post-receptor effect involving disruption of microtubule interactions with G proteins or adenylyl cyclase; additionally, radioligand binding studies showed that the β-adrenoreceptor was unaltered by colchicine or vinblastine treatment. Finally, the potentiation was not seen in cells defective in receptor-adenylyl cyclase coupling. These data suggested a model in which microtubules regulate some factor coupling adenylyl cyclase and receptors for agonists of cAMP production [7,8].

Early studies on brain tissue similarly showed that adenylyl cyclase activity in synaptic membrane fractions enriched from rat cerebral cortex was enhanced by pretreatment with colchicine or vinblastine, as well as treatment with unsaturated fatty acids, compared to untreated controls. Membrane adenylyl cyclase activity was decreased by the addition of a membrane-washing step after treatment with colchicine or vinblastine, but not after fatty acid treatment. Furthermore, the supernatant washings collected from membranes treated with colchicine or vinblastine were able to reconstitute adenylyl cyclase activity in membranes whose endogenous Gαs activity was diminished via heat inactivation. These data were synthesized into a model wherein the ability of Gαs to activate adenylyl cyclase was constrained by membrane diffusibility and microtubule anchoring and can be increased by the described treatments (microtubule disruptors or membrane fluidization with unsaturated fatty acids) [9]. Subsequent studies demonstrated that Gαs, but not Gαi, is liberated from synaptic membranes following microtubule disruption, and that addition of tubulin in low nanomolar concentrations causes significant decreases in the adenylyl cyclase activity of synaptic membranes in vitro [10–12]. Since the time of these initial observations, a far more elaborate picture of G protein effects on the cytoskeleton, and vice versa, has developed.

3. Heterotrimeric G proteins directly interact with tubulin

Tubulin polymerized with the hydrolysis-resistant photoaffinity GTP analog, azidoanilido-GTP (^{32}P-AAGTP), and incubated with synaptic membranes, transferred directly ^{32}P-AAGTP to Gαi. These results mirrored the findings of a previous study showing a similar transfer of ^{32}P-AAGTP from Gαi to Gαs [13,14]. Complexes between the proteins were thought to occur, since large excesses of GTP in the milieu did not prevent this transfer of nucleotide (referred to as transactivation). Expanding upon previous findings, tubulin inhibition of membrane adenylyl cyclase activity was shown to require GTP- or hydrolysis resistant GTP analog (AAGTP or Gpp(NH)p)-bound tubulin. Free Gpp(NH)p was also able to similarly inhibit membrane adenylyl cyclase, but tubulin from which GTP had been extracted with charcoal was unable to inhibit adenylyl cyclase. Extracts of adenylyl cyclase lacking G proteins were neither stimulated nor inhibited with respect to cAMP production by tubulin. These data were hypothesized to suggest activation via guanine nucleotide transfer to Gαi by tubulin. In contrast, transducin-Gpp(NH)p was unable to inhibit neuronal membrane cyclase activity in the manner of tubulin-Gpp(NH)p, suggesting that transactivation requires specific protein–protein interactions and is not a general property of GTP-binding proteins [14].

Further examination of the nature of tubulin–G protein interaction occurred in the context of an expanding awareness of G proteins in general, including the recognition of varied G protein isoforms. Four alternatively spliced forms of Gαs were known, along with three forms of Gαi [15–18]; these, along with G proteins Gαo and transducin, displayed differential affinities for tubulin as demonstrated by ^{131}I-tubulin dot blot binding. Specifically, labeled tubulin bound Gαs and Gαi1 with ~100 nM affinity with insignificant binding of other Gαi isoforms, Gαo, or transducin. This binding could be abolished with excess cold tubulin as well as heat denaturation of the G protein or tubulin, suggesting a specific tubulin–G protein interaction. Furthermore, similar labeling by ^{131}I-tubulin occurred on isolated G protein α-units as well as Gαβγ heterotrimers. This suggested that the site of specific tubulin–G protein interaction differed that of Gα with Gβγ [19]. Partial digestion of α and β tubulin with subtilisin to remove MAP2 and tau binding sites at the C terminal did not affect ^{131}I-tubulin binding to these proteins. However, tubulin polymerized into microtubules showed far less affinity for G protein than

tubulin dimers. These data suggested a common site shared by tubulin polymerization sites and tubulin–G protein binding sites. These studies also suggested possible physiologic roles for tubulin beyond the formation of microtubules [20].

In addition to binding and transactivation (Fig. 1), tubulin–G protein association was also found [21] to stabilize the GTP–tubulin–G protein complex as well as activate a GTPase in this complex. ^{32}P–GTP–tubulin was retained and protected from exchange after nitrocellulose filtration in increasing amounts with the addition of Gαi1 but not other Gαi isoforms or Gαo. This interaction was apparently destabilized by the concurrent addition of Gβγ subunits, which is notable in light of previous results indicating a lack of inhibition by Gβγ subunits of tubulin binding and likely reflecting the increasingly recognized combinatorial diversity of G proteins including Gβγ subunits [22]. Also notable was the observation that hydrolysis of the tubulin-bound GTP was promoted by its association with Gαi1. This finding suggested cross-talk between pathways of G-protein signaling and microtubule formation. The rich, in situ, association of Gαs and tubulin in rat cortical membranes further suggested a role in mediation of signaling between G-protein and cytoskeleton [23].

G protein βγ subunits appear to promote assembly of microtubules, and this effect is isoform-dependent. Specifically, Gβ1γ2 promoted assembly of tubulin into microtubules in vitro, while Gβ1γ1 did not. Furthermore, when microtubules polymerized from sheep brain were embedded, thin-sectioned, and examined by electron microscopy, Gβγ was associated at regular intervals along the length of the microtubule [23].

Gβ1γ1 and Gβ1γ2 differ in part by prenylation status: Gβ1γ1 is farnesylated, while Gβ1γ2 is geranylgeranylated [24–26]. A Gβ1γ2 mutant lacking its prenylation site (C68S) also failed to promote microtubule assembly [27]. The functional basis of the specific prenylation requirement is unclear. Though this modification promotes membrane association, these findings were gathered in vitro. Prenyl-specific binding sites have been demonstrated [28], though not specifically in tubulin, to our knowledge. Lipid modification (acylation) also occurs in Gαs and Gαi subunits, which are palmitoylated and palmitoylated/myristoylated, respectively [29]. While prenylation and myristoylation are irreversible, physiologic depalmitoylation of the Gαs subunits occurs subsequent to receptor activation. Curiously, depalmitoylated, active Gαs has been observed to remain associated with the cell membrane [30].

4. Gαs and Gαi subunits can activate tubulin GTPase and destabilize microtubules

Another notable aspect of G protein/tubulin interaction is Gα subunit potentiation of tubulin GTPase activity (Fig. 2). Whereas tubulin contains intrinsic GTPase activity, this is quiescent absent the addition of other tubulin dimers to the nascent polymer, which acts as a GTPase activator protein(GAP) for the tubulin dimer [31]. Incubation of G proteins Gαs, Gαi, and Gαo with tubulin in vitro also promotes GTP hydrolysis. Use of a Q204L Gαi mutant, which is able to bind GTP but lacks GTPase activity [32], demonstrated that tubulin GTPase, rather than G protein GTPase, is activated when the complex is formed. Thus both Gαs and Gαi are GAPs for tubulin.

The G protein α subunits also promoted microtubule dynamic instability, directly visualized as an increase in microtubule catastrophe frequency and likely due to the hydrolysis of GTP–tubulin to the less polymer-stable GDP–tubulin [33]. This effect on tubulin/microtubules also required the activated, GTP-bound Gα [34], suggesting a role in the sequence of GPCR signaling. The common effects of Gαs and Gαi on microtubule instability, suggest that the microtubule's response is based upon the existence of a signal, rather than the content of the signal, and perhaps contributes to an action common to both pathways. It is noteworthy, however, that unlike Gαs, Gαi is not internalized in response to activation [2], so the ability of Gαi to modulate cytosolic microtubules is a physiologic curiosity.

Following β-adrenergic receptor activation in C6 glioma cells, Gαs is internalized on vesicles via lipid rafts/caveolae [2]. This is not seen with any other G protein. This likely facilitates its interaction with microtubules, with activation of tubulin GTPase and subsequent destabilization of microtubules and sequestration of GDP-bound tubulin by active Gαs. Upon hydrolysis of its bound GTP, Gαs affinity for tubulin decreases, tubulin is released, and can re-enter a dynamic pool for polymerization into microtubules [35]. Such a mechanism is observed in PC12 pheochromocytoma cells: active Gαs is internalized, destabilizing microtubules. This is followed by an increase in neurite outgrowth. Furthermore, this effect occurs in a cAMP-independent manner, and is an example of a unique and direct G protein regulation of cytoskeleton and cellular remodeling [36].

Furthermore, when chimeras of G1a1 and transducin (which does not bind to tubulin) were produced, these proteins bound to tubulin

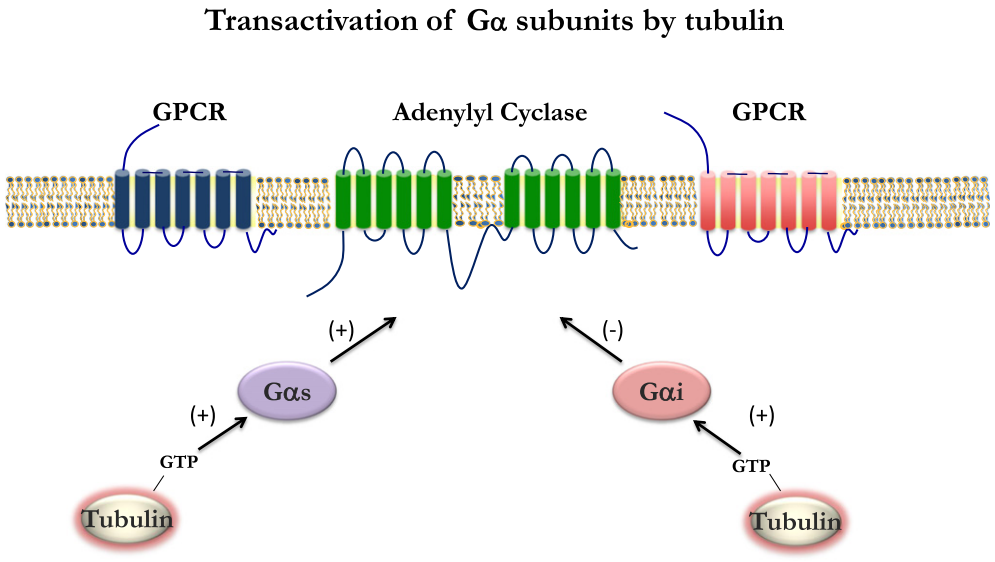

Fig. 1. Transactivation of Gαs and Gαi1 by tubulin. Tubulin, in its GTP-bound form, complexes with Gαs or Gαi1 and transfers its GTP directly to these G proteins, activating them in the absence of an activated GPCR (transactivation). The activated G proteins then interact with adenylyl cyclase, either activating (Gαs) or inhibiting (Gαi) that molecule. GDP is released from the Gα without requiring an activated GPCR.

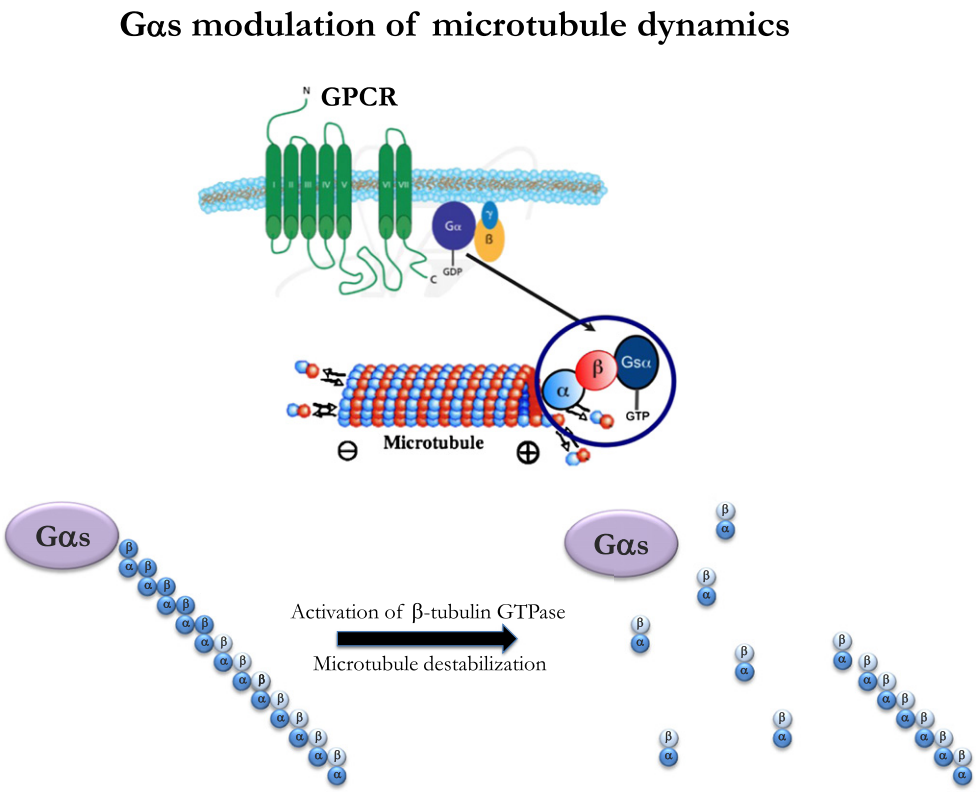

Fig. 2. Gαs promotes microtubule dynamics. Activated Gαs internalizes and associates with tubulin at the microtubule plus end. Gαs then acts as a GTPase activator for tubulin, and the resulting loss of the "GTP-cap" allows for increased microtubule dynamics. β-Tubulin–GTP is shown in blue and β-tubulin–GDP in white.

but blocked the binding of endogenous Gas. Expressing these constructs in cells resulted in a profound inhibition of microtubule-based cellular extensions [37]. Since these outgrowths require dynamic microtubules, it appeared that the inhibition of the normal association between Gsa and tubulin plays a role in the regulation of microtubule dynamics.

Recent structural studies localize the tubulin–Gαs association to the cyclase interaction region on Gαs, and on the exchangeable GTP site of β-tubulin [38]. Peptide fragments of this Gαs region are able to promote tubulin GTPase activity in the manner of full-length Gαs, supporting these structural data [39].

5. Gαq–tubulin interactions

Several of the phenomena observed in the interaction of tubulin, Gαs, and adenylyl cyclase have functional counterparts in the Gαq–phospholipase system, as well as additional behaviors not observed with the Gαs–adenylyl cyclase system. Similar to their activation of Gαs and adenylyl acyclase, the GTP analog, Gpp(NH)p, alone or complexed with tubulin, is able to activate Gαq, which stimulates phospholipase $C\beta_1$ to cleave membrane phosphatidylinositol-4,5 bisphosphate (PIP_2) into membrane diacylglycerol (DAG) and the cytosolic second messenger inositol trisphosphate (IP_3) (Fig. 3). This occurs in a receptor-independent fashion as demonstrated in membranes of Sf9 cells transfected with only Gαq and phospholipase Cβ1 (PLCβ1) and incubated with Gpp(NH)p-tubulin, suggesting transactivation of Gαq. Further increases in IP_3 production were seen in membranes of SF9 cells (optimal for Gαq production) coinfected with muscarinic M_1 receptor baculovirus and treated with the muscarinic agonist carbachol. While Gpp(NH)p promoted steady and significant increases in IP_3 production over a range of submicromolar to low micromolar concentrations, incubation of membranes with Gpp(NH)p-tubulin caused a biphasic pattern of IP_3 production, with significant increases followed by a dramatic falloff over the same concentration range as Gpp(NH)p. This suggested an inhibition of this system by tubulin at higher concentrations [40,41]. This phenomenon has not been observed in tubulin regulation of adenylyl cyclase via Gαs. The actin-associated protein profilin has been shown to associate with PIP_2 micelles, with a resulting decrease in PLC activity [42,43]. Tubulin similarly associates with PIP_2 micelles [40], suggesting a possible shared mechanism of PLC inhibition. PIP_2 inhibits microtubule polymerization in vitro [40,44], demonstrating direct and possibly regulatory interaction of these species. This phospholipid–tubulin interaction appears to be specific to PIP_2, as other prominent membrane lipids including phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine do not inhibit microtubule polymerization [45]. Activation by carbachol of M1 receptors in SK-N-SH cells evoked a transient association of tubulin with the cell membrane after muscarinic stimulation, suggesting another possible point of regulation [41]. Indeed, pretreatment of SK-N-SH cells with colchicine, which not only destabilizes microtubules but also promotes GTP hydrolysis of tubulin [46], prior to application of carbachol, results in significantly diminished IP_3 production. Taxol, which exerts a stabilizing influence on microtubules causes a similar decrease in IP3 production [47], suggesting a requirement for dynamic microtubules in the process. Thus, G protein, dynamic tubulin/microtubules, and phospholipase C operate in concert to mediate Gq signaling.

G protein βγ subunits also play a role in this scheme. Addition of purified Gβγ subunits to purified SK-N-SH membranes inhibits carbachol-stimulated association of tubulin with the membrane, and this inhibition is potentiated by PIP_2. Rapid internalization of Gβγ-tubulin occurs following carbachol stimulation as demonstrated by microscopy and coimmunoprecipitation [47]. Furthermore, Gβγ preferentially associates with GDP-bound tubulin, contrasting the slightly preferential association of Gα units with GTP–tubulin [19,21,23,45]. These observations could be explained by the flowing sequence: 1) receptor activation; 2) membrane association of GTP–tubulin; 3) transactivation of Gαq; and

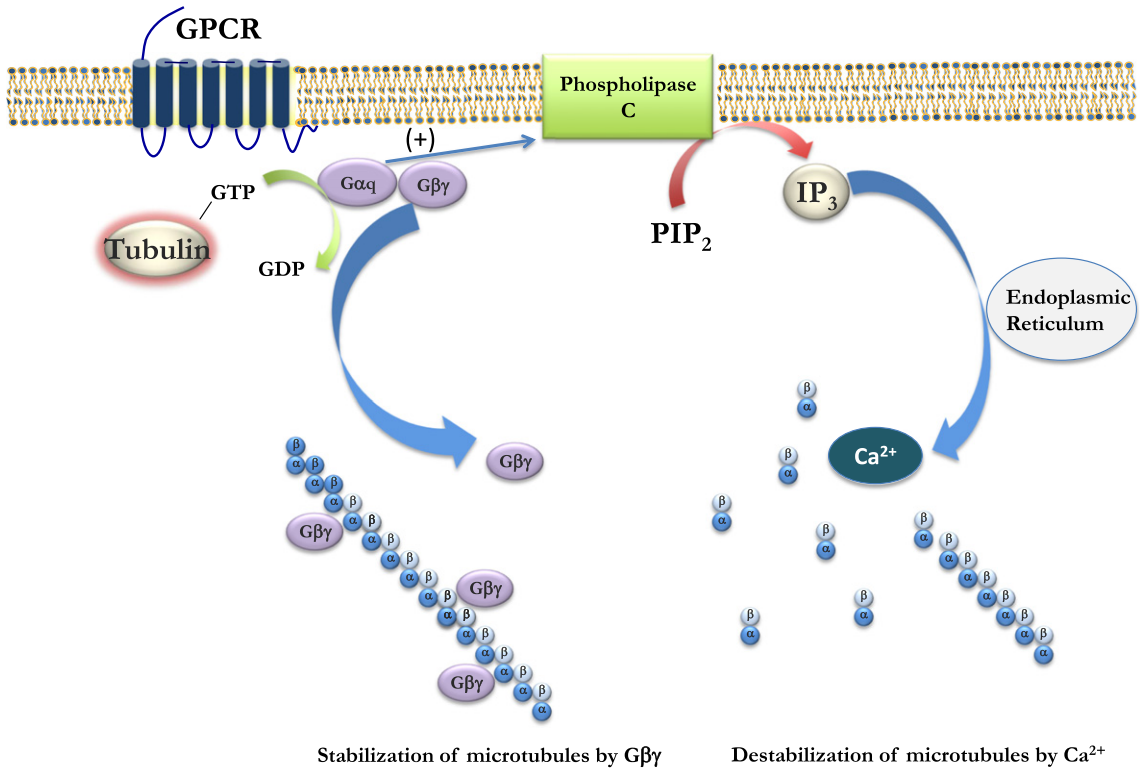

Fig. 3. Coordinated effects of Ca^{2+} and Gβγ on microtubule dynamics following Gαq activation. Activation of Gαq increases cellular Ca^{2+}, which destabilizes microtubules, particularly those subjacent to the plasma membrane. After a short time lag, Gβγ is released from the plasma membrane, where it binds to and stabilizes microtubules [47]. β-Tubulin–GTP is shown in blue and β-tubulin–GDP in white.

4) subsequent GDP–tubulin/Gβγ association and internalization [47]. While Gαs has been shown to internalize via a clathrin-independent, lipid raft/caveolar mechanism, GPCRs internalize separately via a clathrin-dependent mechanism [2]. Gβγ subunits studied in the above context of M_1 receptor activation by carbachol show co-internalization of receptor, tubulin, and Gβγ, suggesting a role for Gβγ in the association of these components [48].

6. Cytoskeletal regulation by Gα12/13 proteins

Gα12/13 proteins primarily regulate slow events like cell proliferation, transformation, shape change, locomotion and gene transcription [49]. They are activated by several receptors including serotonin 5-HT(4)R and 5-HT(7)R, angiotensin receptor AT1, endothelin receptors ETA and ETB, galanin receptor GAL2, lysophosphatidic acid receptor LPA, muscarinic M3 receptor, protease activated receptors PAR1, PAR3 and PAR4, sphingosine-1 phosphate S1P(2–5) receptors, and a few others [50–52]. Purified Gα12 demonstrates slower guanine nucleotide kinetics than other Gα subunits [53,54], which is consistent with the preferential role of these molecules in sustained reactions like modulation of cytoskeleton. The slow GTPase activity ensures that the Regulators of G Protein Signaling (RGS) proteins terminate G12/13 signaling by activating intrinsic G protein GTPases.

7. G12 proteins regulate cytoskeleton via small GTPases

Downstream effectors of G12 proteins are small (20–25 kDa) G-proteins which, like heterotrimeric G protein α subunits, exchange GDP for GTP to assume the active state, and upon hydrolysis of their bound GTP become inactive. The primary mediators of G12 activation, the Rho GTPases, RhoA, Cdc42 and Rac1, are regulated by GTPase activating proteins (GAPs), guanine nucleotide exchange factors (GEFs), as well as guanine nucleotide dissociation inhibitors (GDIs) [55]. These proteins primarily regulate dynamics of the actin cytoskeleton (Fig. 4).

The mechanism of activation of small GTPases by G12 proteins is best described for RhoA. RhoA is a downstream target of both Gα12 and Gα13. The first RGS protein discovered for Gα12 and Gα13 was p115 RhoGEF [56,57]. Soon after, two additional RhoGEFs for G12 proteins were discovered: PDZ-RhoGEF/GTRAP48 [58,59] and LARG (leukemia-associated RhoGEF [60,61]). These RhoGEFs are able to recognize activated G12 proteins (GTP-Gα12 and GTP-Gα13) in vitro via their N-terminal RH (RGS homology) domains. However, only RH domains of p115RhoGEF and LARG exhibit GAP activity for G12 proteins. These RhoGTPases also contain centrally located DH/PH (Dbl homology/pleckstrin homology) domain known to act as GEFs for small GTPases. These findings demonstrated that the RhoGEFs act as direct couplers of Gα12/13 proteins to small GTPases, and their role in this process is dual: by promoting GTP hydrolysis as inactivating GAPs on Gα12/13, and acting as activating GEFs for Rho GTPase [62,63]. RhoA is an important regulator of cell morphology, locomotion, actinomyosin contractility, and microtubule dynamics [55], with numerous RhoA downstream effectors described. RhoA activates diaphanous-related formins (DRFs; Dia) which promote the addition of actin monomers to the fast growing (barbed) end of actin filaments [64]. In addition, GTP-RhoA directly binds to and activates serine/threonine protein kinase ROCK which, cooperatively with DRF, mediates actin stress fiber formation [65]. ROCK also activates LIM kinase to phosphorylate and inhibit actin severing protein cofilin, thereby indirectly stabilizing actin filaments [66,67]. Cell locomotion additionally requires coordinated action of dynamic and stable microtubules oriented toward the leading edge of migrating cell [68]. RhoA-ROCK signaling also mediates cell locomotion by stabilizing microtubules due to phosphorylation of microtubule associated proteins [69,70].

Establishment of cell polarity is a crucial step in determining cell fate. During cell polarization, Gα12 signaling regulates Cdc42 effects on

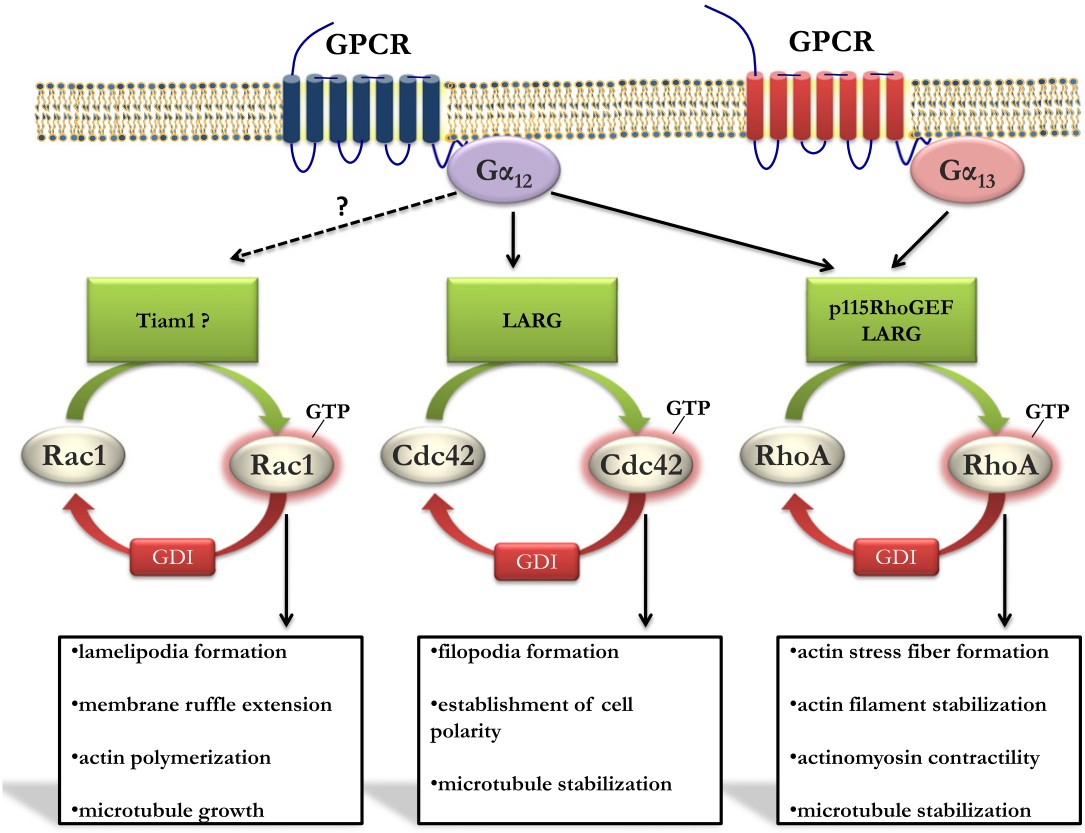

Fig. 4. G12 and G13 regulate cytoskeleton via activation of small GTPases. GPCR activation of G12 or G13 leads to activation of RhoA, cdc42, and Rac1, which then participate in cytoskeletal remodeling. These molecules cycle between an inactive (GDP-bound) form and an active (GTP-bound) form, regulated by proteins that activate GTPase (GAP), exchange GTP for GDP (GEF) or release GDP (GDI). p115RhoGEF acts as GAP for both G12 and G13 proteins and as a GEF for RhoA, whereas LARG functions as GEF for both RhoA and Cdc42. Tiam1 acts as GEF for Rac1. It is established that G12 activates directly Tiam1.

microtubule dynamics and positioning of the microtubule organizing center (MTOC), and is additionally mediated by LARG [71]. Cdc42 is thus an important intracellular effector that links extracellular cues to organelle rearrangements during cell polarization [72].

Rac1 is a downstream effector of Gα12 as its activation by Gα12 induces transformation of NIH-3T3 cells [73]. It remains unknown which GEF mediates this signaling. Rac1 is directly linked to the regulation of microtubule dynamics via activation of p21-activated kinase 1 (PAK1). It was observed that lysophosphatidic acid receptor 1 (LPA1), which couples to Gα12 [74], can induce Tiam1 activation [75]. Tiam1 acts as a GEF for Rac1 [76] and regulates neuronal morphology [77].

Thus, Gα12/13 proteins represent a unique continuum between GPCRs, heterotrimeric G proteins, small G proteins and the cytoskeleton.

8. Physiologic significance of G protein–tubulin/microtubule interactions

While the most numerous and best understood examples of G proteins influencing cellular physiology follow the canonical pathway of ligand-receptor-G protein-effector and involve target phosphorylation as well as activation of transcriptional pathways, evidence exists for the role of unique pathways as described in this review. Affected processes include cytoskeletal dynamics, cell growth and division, and morphology.

Overexpression of Gαq in GH3 cells, a rat pituitary lactosomatotrophic cell line, resulted in significant increases in TRH (Gαq-associated receptor) stimulated production of prolactin, demonstrating a functional role for the overexpressed Gαq, as well as altered tubulin dynamics. These cells also showed a 50% decrease in the ratio of soluble/polymerized tubulin compared to vector-transfected cell. Additionally, Gαq produced differential effects on polymerization of crude vs. purified extracts of tubulin (inhibition and promotion, respectively), suggesting an important role for other cellular factors in the regulation of this process [78]. G proteins of various classes including Gαs, Gαi1, and Gαo undergo cellular redistribution and directly associate with microtubules during nerve growth factor (NGF)-promoted differentiation of PC12 pheochromocytoma cells. Similar changes are seen in N2A neuroblastoma cells, which differentiate spontaneously. This may signify a G protein-mediated effect on microtubules in development occurring in response to a variety of signals [79]. Expression of Gαi1 in COS7 cells resulted in sequestration of Gβγ by Gαi1 and decreased receptor-mediated endocytosis, suggesting a role for Gβγ in endocytosis [80]. G protein βγ units have demonstrated specific ability to inhibit transfer of GTPγS, to small GTPase actin cytoskeletal regulators RhoA and Rac1, but not CDC24 [81], and have also been demonstrated to associate with actin fibers [82]. These findings display the potential for multiple, interacting levels of regulation: G protein as regulators of other G proteins, which in turn regulate factors involved in cytoskeletal structure.

Gβγ units may have a role in early embryonic spatial organization. In *Caenorhabditis elegans*, for example, Gβ is most strongly expressed at the membrane but transiently associates with asters (peri-centrisomal microtubular structures) during early mitotic cycles. In experimental embryos created deficient in Gβ, the mitotic spindle assumes random orientations, with differentiating but disorganized tissue resulting. Overexpression of Gβ likewise results in abnormalities, including slow growth and impairments in movement and egg-laying [83]. *Dictyostelium* development also illustrates the importance of Gβ action, as cells lacking these proteins do not undergo normal aggregation [84]. Multicellular

Dictyostelium development is also dependent on Gα subunit Gα5, as decreased or increased expression of this G protein results in slower or faster tip formation, respectively [85].

Adding to the likely complexity of spindle orientation regulation are the influences of guanine nucleotide dissociation inhibitors (GDIs), and guanine nucleotide exchange factors (GEFs), which promote the GDP or GTP-bound state of G protein α-subunits, respectively. Several GDIs containing GoLoco motifs make important associations in regulating spindle orientation: GPR1/2 in *C. elegans* [86], Pins in *Drosophila* [87], and mammalian AGS3 and LGN [88,89]. These proteins bind Gα and promote dissociation of Gβγ subunits, and stabilize the GDP-bound Gα subunit. The GDI-bound Gα then complexes with microtubule-associated proteins (NuMA in mammals [89], Mud in *Drosophila* [90], and Lin-5 in *C. elegans* [86]) to influence spindle orientation. Conversely, Ric-8A acts as a GEF activator of AGS3-bound Gαi1, and the effect of interplay between GEF and GDI on the activated state (GTP-bound) of G protein α-subunit may coordinate the role of Gα subunit in this process [91]. Importantly, these effects are independent of GPCR agonists, and represent poorly understood, yet potentially critical G protein functions [35].

The effect of GEF and GDI interaction on G protein may extend beyond the realm of cell division to organism-level behavior. In *C. elegans*, AGS3 and Ric-8 act upon Gαo to regulate food-seeking behavior. Animals deficient in Gαo or AGS3 fail to modify certain behaviors in response to short-term food deprivation, such as egg-laying rates and food-seeking [92]. Furthermore, AGS3 moves from a Triton X-100 insoluble to a soluble fraction in whole animal lysate within several hours of food deprivation [93]. This likely reflects intracellular information transfer involving cytoskeletal components that is mediated by G proteins and their associates.

9. Conclusion

This chapter has illustrated a complex series of interactions between cytoskeletal components and heterotrimeric G proteins. It is designed to illustrate how tubulin/microtubules participate in the process of G protein signaling as well as how G proteins, often acting as second messengers, regulate cytoskeletal dynamics and resulting cellular morphology. Much is left to learn about this process and it is hoped that the coming years will help to polymerize our knowledge.

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References

[1] M. Bünemann, M. Frank, M.J. Lohse, Gi protein activation in intact cells involves subunit rearrangement rather than dissociation, Proc. Natl. Acad. Sci. 100 (2003) 16077–16082.
[2] J.A. Allen, J.Z. Yu, R.J. Donati, M.M. Rasenick, β-Adrenergic receptor stimulation promotes Gαs internalization through lipid rafts: a study in living cells, Mol. Pharmacol. 67 (2005) 1493–1504.
[3] D.S. Evanko, M.M. Thiyagarajan, P.B. Wedegaertner, Interaction with Gβγ is required for membrane targeting and palmitoylation of Gαs and Gαq, J. Biol. Chem. 275 (2000) 1327–1336.
[4] W.C. Greene, C.M. Parker, C.W. Parker, Colchicine-sensitive structures and lymphocyte activation, J. Immunol. 117 (1976) 1015–1022.
[5] S.A. Rudolph, P. Greengard, S.E. Malawista, Effects of colchicine on cyclic AMP levels in human leukocytes, Proc. Natl. Acad. Sci. 74 (1977) 3404–3408.
[6] D. Gemsa, L. Steggemann, G. Till, K. Resch, Enhancement of the PGE1 response of macrophages by concanavalin A and colchicine, J. Immunol. 119 (1977) 524–529.
[7] P.A. Insel, M.S. Kennedy, Colchicine potentiates [beta]-adrenoreceptor-stimulated cyclic AMP in lymphoma cells by an action distal to the receptor, Nature 273 (1978) 471–473.
[8] M.S. Kennedy, P.A. Insel, Inhibitors of microtubule assembly enhance beta-adrenergic and prostaglandin E1-stimulated Cyclic AMP accumulation in S49 lymphoma cells, Mol. Pharmacol. 16 (1979) 215–223.
[9] M.M. Rasenick, P.J. Stein, M.W. Bitensky, The regulatory subunit of adenylate cyclase interacts with cytoskeletal components, Nature 294 (1981) 560–562.
[10] M.M. Rasenick, G. Wheeler, M.W. Bitensky, C. Kosack, R. Malina, P.J. Stein, Photoaffinity identification of colchicine-solubilized regulatory subunit from rat brain adenylate cyclase, J. Neurochem. 43 (1984) 1447–1454.
[11] M.M. Rasenick, M.M. Marcus, Y. Hatta, F. DeLeon-Jones, S. Hatta, Regulation of neuronal adenylate cyclase, Adv. Exp. Med. Biol. 221 (1987) 123–133.
[12] M.M. Rasenick, Regulation of neuronal adenylate cyclase by microtubule proteins, Ann. N. Y. Acad. Sci. 466 (1986) 794–797.
[13] S. Hatta, M.M. Marcus, M.M. Rasenick, Exchange of guanine nucleotide between GTP-binding proteins that regulate neuronal adenylate cyclase, Proc. Natl. Acad. Sci. USA. 83 (1986) 5439–5443.
[14] M.M. Rasenick, N. Wang, Exchange of guanine nucleotides between tubulin and GTP-binding proteins that regulate adenylate cyclase: cytoskeletal modification of neuronal signal transduction, J. Neurochem. 51 (1988) 300–311.
[15] J.D. Robishaw, M.D. Smigel, A.G. Gilman, Molecular basis for two forms of the G protein that stimulates adenylate cyclase, J. Biol. Chem. 261 (1986) 9587–9590.
[16] P. Bray, A. Carter, V. Guo, C. Puckett, J. Kamholz, A. Spiegel, M. Nirenberg, Human cDNA clones for an alpha subunit of Gi signal-transduction protein, Proc. Natl. Acad. Sci. 84 (1987) 5115–5119.
[17] H. Itoh, R. Toyama, T. Kozasa, T. Tsukamoto, M. Matsuoka, Y. Kaziro, Presence of three distinct molecular species of Gi protein alpha subunit structure of rat cDNAs and human genomic DNAs, J. Biol. Chem. 263 (1988) 6656–6664.
[18] S.Y. Kim, S.L. Ang, D.B. Bloch, K.D. Bloch, Y. Kawahara, C. Tolman, R. Lee, J.G. Seidman, E.J. Neer, Identification of cDNA encoding an additional alpha subunit of a human GTP-binding protein: expression of three alpha i subtypes in human tissues and cell lines, Proc. Natl. Acad. Sci. 85 (1988) 4153–4157.
[19] N. Wang, K. Yan, M.M. Rasenick, Tubulin binds specifically to the signal-transducing proteins, Gs alpha and Gi alpha 1, J. Biol. Chem. 265 (1990) 1239–1242.
[20] N. Wang, M.M. Rasenick, Tubulin–G protein interactions involve microtubule polymerization domains, Biochemistry 30 (1991) 10957–10965.
[21] S. Roychowdhury, M.M. Rasenick, Tubulin–G protein association stabilizes GTP binding and activates GTPase: cytoskeletal participation in neuronal signal transduction, Biochemistry 33 (1994) 9800–9805.
[22] M.I. Simon, M.P. Strathmann, N. Gautam, Diversity of G proteins in signal transduction, new series, 252 (1991) 802–808.
[23] K. Yan, E. Greene, F. Belga, M.M. Rasenick, Synaptic membrane G proteins are complexed with tubulin in situ, J. Neurochem. 66 (1996) 1489–1495.
[24] Y. Fukada, T. Takao, H. Ohguro, T. Yoshizawa, T. Akino, Y. Shimonishi, Farnesylated γ-subunit of photoreceptor G protein indispensable for GTP-binding, Nature 346 (1990) 658–660.
[25] R.K. Lai, D. Perez-Sala, F.J. Cañada, R.R. Rando, The gamma subunit of transducin is farnesylated, Proc. Natl. Acad. Sci. 87 (1990) 7673–7677.
[26] S.M. Mumby, P.J. Casey, A.G. Gilman, S. Gutowski, P.C. Sternweis, G protein gamma subunits contain a 20-carbon isoprenoid, Proc. Natl. Acad. Sci. 87 (1990) 5873–5877.
[27] S. Roychowdhury, G protein beta 1gamma 2 subunits promote microtubule assembly, J. Biol. Chem. 272 (1997) 31576–31581.
[28] Y. Kloog, A. Cox, Prenyl-binding domains: potential targets for Ras inhibitors and anti-cancer drugs, Semin. Cancer Biol. 14 (2004) 253–261.
[29] J.E. Buss, S.M. Mumby, P.J. Casey, A.G. Gilman, B.M. Sefton, Myristoylated alpha subunits of guanine nucleotide-binding regulatory proteins, Proc. Natl. Acad. Sci. 84 (1987) 7493–7497.
[30] C. Huang, J.A. Duncan, A.G. Gilman, S.M. Mumby, Persistent membrane association of activated and depalmitoylated G protein α subunits, Proc. Natl. Acad. Sci. 96 (1999) 412–417.
[31] M.F. Carlier, D. Didry, D. Pantaloni, Hydrolysis of GTP associated with the formation of tubulin oligomers is involved in microtubule nucleation, Biophys. J. 73 (1997) 418–427.
[32] D.E. Coleman, A.M. Berghuis, E. Lee, M.E. Linder, Structures of active conformations of Giai and the mechanism of GTP hydrolysis, Science 265 (1994) 1405–1412.
[33] S. Roychowdhury, D. Panda, L. Wilson, M.M. Rasenick, G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics, J. Biol. Chem. 274 (1999) 13485–13490.
[34] S. Roychowdhury, L. Martinez, L. Salgado, S. Das, M.M. Rasenick, G protein activation is prerequisite for functional coupling between Gα/Gβγ and tubulin/microtubules, Biochem. Biophys. Res. Commun. 340 (2006) 441–448.
[35] R.H. Dave, W. Saengsawang, J.-Z. Yu, R. Donati, M.M. Rasenick, Heterotrimeric G-proteins interact directly with cytoskeletal components to modify microtubule-dependent cellular processes, Neurosignals 17 (2009) 100–108.
[36] J.-Z. Yu, R.H. Dave, J.A. Allen, T. Sarma, M.M. Rasenick, Cytosolic G{alpha}s acts as an intracellular messenger to increase microtubule dynamics and promote neurite outgrowth, J. Biol. Chem. 284 (2009) 10462–10472.
[37] N.F. Chen, A Specific Domain of Gialpha Required for the Transactivation of Gialpha by Tubulin Is Implicated in the Organization of Cellular Microtubules, J. Biol. Chem. 278 (2003) 15285–15290.
[38] B.T. Layden, W. Saengsawang, R.J. Donati, S. Yang, D.C. Mulhearn, M.E. Johnson, M.M. Rasenick, Structural model of a complex between the heterotrimeric G protein, Gsα, and tubulin, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1783 (2008) 964–973.
[39] R.H. Dave, W. Saengsawang, M. Lopus, S. Davé, L. Wilson, M.M. Rasenick, A molecular and structural mechanism for G protein-mediated microtubule destabilization, J. Biol. Chem. 286 (2011) 4319–4328.
[40] J.S. Popova, J.C. Garrison, S.G. Rhee, M.M. Rasenick, Tubulin, Gq, and phosphatidylinositol 4,5-bisphosphate interact to regulate phospholipase Cbeta1 signaling, J. Biol. Chem. 272 (1997) 6760–6765.
[41] J.S. Popova, M.M. Rasenick, Muscarinic receptor activation promotes the membrane association of tubulin for the regulation of Gq-mediated phospholipase Cbeta(1) signaling, J. Neurosci. 20 (2000) 2774–2782.

[42] P.J. Goldschmidt-Clermont, L.M. Machesky, J.J. Baldassare, T.D. Pollard, The actin-binding protein profilin binds to PIP$_2$ and inhibits its hydrolysis by phospholipase C, New Series 247 (1990) 1575–1578.
[43] P.J. Goldschmidt-Clermont, J.W. Kim, L.M. Machesky, S.G. Rhee, T.D. Pollard, Regulation of phospholipase C-γl by profilin and tyrosine phosphorylation, New Series 251 (1991) 1231–1233.
[44] S.-H. Lee, G. Flynn, P.S. Yamauchi, D.L. Purich, Several metabolic factors governing the dynamics of microtubule assembly and disassembly, Ann. N. Y. Acad. Sci. 466 (1986) 519–528.
[45] J.S. Popova, A.K. Greene, J. Wang, M.M. Rasenick, Phosphatidylinositol 4,5-bisphosphate modifies tubulin participation in phospholipase Cbeta1 signaling, J. Neurosci. 22 (2002) 1668–1678.
[46] T. David-Pfeuty, C. Simon, D. Pantaloni, Effect of antimitotic drugs on tubulin GTPase activity and self-assembly, J. Biol. Chem. 254 (1979) 11696–11702.
[47] J.S. Popova, Gbetagamma mediates the interplay between tubulin dimers and microtubules in the modulation of Gq signaling, J. Biol. Chem. 278 (2003) 34299–34308.
[48] J.S. Popova, M.M. Rasenick, Clathrin-mediated endocytosis of m3 muscarinic receptors roles for Gbetagamma and tubulin, J. Biol. Chem. 279 (2004) 30410–30418.
[49] S. Siehler, Regulation of RhoGEF proteins by G 12/13-coupled receptors, Br. J. Pharmacol. 158 (2009) 41–49.
[50] E.G. Ponimaskin, J. Profirovic, R. Vaiskunaite, D.W. Richter, T.A. Voyno-Yasenetskaya, 5-Hydroxytryptamine 4(a) receptor is coupled to the Galpha subunit of heterotrimeric G13 protein, J. Biol. Chem. 277 (2002) 20812–20819.
[51] N.A. Riobo, D.R. Manning, Receptors coupled to heterotrimeric G proteins of the G12 family, Trends Pharmacol. Sci. 26 (2002) 146–154.
[52] E. Kvachnina, G. Liu, A. Dityatev, U. Renner, A. Dumuis, D.W. Richter, G. Dityateva, M. Schachner, T.A. Voyno-Yasenetskaya, E.G. Ponimaskin, 5-HT7 receptor is coupled to G alpha subunits of heterotrimeric G12-protein to regulate gene transcription and neuronal morphology, J. Neurosci. 25 (2005) 7821–7830.
[53] W.D. Singer, R.T. Miller, P.C. Sternweis, Purification and characterization of the alpha subunit of G13, J. Biol. Chem. 269 (1994) 19796–19802.
[54] T. Kozasa, A.G. Gilman, Purification of recombinant G proteins from Sf9 cells by hexahistidine tagging of associated subunits: characterization of alpha 12 and inhibition of adenylyl cyclase by alpha z, J. Biol. Chem. 270 (1995) 1734–1741.
[55] Y. Takai, T. Sasaki, T. Matozaki, Small GTP-binding proteins, Physiol. Rev. 81 (2001) 153–208.
[56] J.A. Glaven, I.P. Whitehead, T. Nomanbhoy, R. Kay, R.A. Cerione, Lfc and Lsc oncoproteins represent two new guanine nucleotide exchange factors for the Rho GTP-binding protein, J. Biol. Chem. 271 (1996) 27374–27381.
[57] T. Kozasa, X. Jiang, M.J. Hart, P.M. Sternweis, W.D. Singer, A.G. Gilman, G. Bollag, P.C. Sternweis, p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13, Science 280 (1998) 2109–2111.
[58] T. Nagase, K. Ishikawa, D. Nakajima, M. Ohira, N. Seki, N. Miyajima, A. Tanaka, H. Kotani, N. Nomura, O. Ohara, Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro, DNA Res. 4 (1997) 141–150.
[59] S. Fukuhara, C. Murga, M. Zohar, T. Igishi, J.S. Gutkind, A novel PDZ domain containing guanine nucleotide exchange factor links heterotrimeric G proteins to Rho, J. Biol. Chem. 274 (1999) 5868–5879.
[60] P.J. Kourlas, M.P. Strout, B. Becknell, M.L. Veronese, C.M. Croce, K.S. Theil, R. Krahe, T. Ruutu, S. Knuutila, C.D. Bloomfield, M.A. Caligiuri, Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 2145–2150.
[61] N. Suzuki, S. Nakamura, H. Mano, T. Kozasa, Galpha 12 activates Rho GTPase through tyrosine-phosphorylated leukemia-associated RhoGEF, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 733–738.
[62] N. Suzuki, N. Hajicek, T. Kozasa, Regulation and physiological functions of G12/13-mediated signaling pathways, Neurosignals 17 (2009) 55–70.
[63] T. Kozasa, N. Hajicek, C.R. Chow, N. Suzuki, Signalling mechanisms of RhoGTPase regulation by the heterotrimeric G proteins G12 and G13, J. Biochem. 150 (2011) 357–369.
[64] K. Nakano, K. Takaishi, A. Kodama, A. Mammoto, H. Shiozaki, M. Monden, Y. Takai, Distinct actions and cooperative roles of ROCK and mDia in Rho small G protein-induced reorganization of the actin cytoskeleton in Madin–Darby canine kidney cells, Mol. Biol. Cell 10 (1999) 2481–2491.
[65] T. Ishizaki, M. Maekawa, K. Fujisawa, K. Okawa, A. Iwamatsu, A. Fujita, N. Watanabe, Y. Saito, A. Kakizuka, N. Morii, S. Narumiya, The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase, EMBO J. 15 (1996) 1885–1893.
[66] K. Ohashi, K. Nagata, M. Maekawa, T. Ishizaki, S. Narumiya, K. Mizuno, Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop, J. Biol. Chem. 275 (2000) 3577–3582.
[67] K. Riento, A.J. Ridley, Rocks: multifunctional kinases in cell behaviour, Nat. Rev. Mol. Cell Biol. 4 (2003) 446–456.
[68] Y. Zheng, G protein control of microtubule assembly, Annu. Rev. Cell Dev. Biol. 20 (2004) 867–894.
[69] M. Amano, M. Ito, K. Kimura, Y. Fukata, K. Chihara, T. Nakano, Y. Matsuura, K. Kaibuchi, Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase), J. Biol. Chem. 271 (1996) 20246–20249.
[70] A.J. Ridley, M.A. Schwartz, K. Burridge, R.A. Firtel, M.H. Ginsberg, G. Borisy, J.T. Parsons, A.R. Horwitz, Cell migration: integrating signals from front to back, Science 302 (2003) 1704–1709.
[71] P. Goulimari, H. Knieling, U. Engel, R. Grosse, LARG and mDia1 link Galpha12/13 to cell polarity and microtubule dynamics, Mol. Biol. Cell 19 (2008) 30–40.
[72] S. Etienne-Manneville, Cdc42—the centre of polarity, J. Cell Sci. 117 (2004) 1291–1300.
[73] T. Tolkacheva, B. Feuer, M.V. Lorenzi, R. Saez, A.M. Chan, Cooperative transformation of NIH3T3 cells by G alpha12 and Rac1, Oncogene 15 (1997) 727–735.
[74] J.W. Choi, D.R. Herr, K. Noguchi, Y.C. Yung, C.W. Lee, T. Mutoh, M.E. Lin, S.T. Teo, K.E. Park, A.N. Mosley, J. Chun, LPA receptors: subtypes and biological actions, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 157–186.
[75] F.N. Van Leeuwen, C. Olivo, S. Grivell, B.N. Giepmans, J.G. Collard, W.H. Moolenaar, Rac activation by lysophosphatidic acid LPA1 receptors through the guanine nucleotide exchange factor Tiam1, J. Biol. Chem. 278 (2003) 400–406.
[76] F. Michiels, G.G. Habets, J.C. Stam, R.A. van der Kammen, J.G. Collard, A role for Rac in Tiam1-induced membrane ruffling and invasion, Nature 375 (1995) 338–340.
[77] K.F. Tolias, J.B. Bikoff, A. Burette, S. Paradis, D. Harrar, S. Tavazoie, R.J. Weinberg, M.E. Greenberg, The Rac1-GEF Tiam1 couples the NMDA receptor to the activity-dependent development of dendritic arbors and spines, Neuron 45 (2005) 525–538.
[78] R. Ravindra, S.P. Kunapuli, L.J. Forman, R.G. Nagele, K.A. Foster, S.A. Patel, Effect of transient overexpression of Gqα on soluble and polymerized tubulin pools in GH3 and AtT-20 cells, J. Cell. Biochem. 61 (1996) 392–401.
[79] T. Sarma, T. Voyno-Yasenetskaya, T.J. Hope, M.M. Rasenick, Heterotrimeric G-proteins associate with microtubules during differentiation in PC12 pheochromocytoma cells, FASEB J. 17 (2003) 848–859.
[80] H.C. Lin, J.A. Duncan, T. Kozasa, A.G. Gilman, Sequestration of the G protein βγ subunit complex inhibits receptor-mediated endocytosis, Proc. Natl. Acad. Sci. 95 (1998) 5057–5060.
[81] R. Harhammer, A. Gohla, G. Schultz, Interaction of G protein Gβγ dimers with small GTP-binding proteins of the Rho family, FEBS Lett. 399 (1996) 211–214.
[82] K.E. Carlson, M.J. Woolkalis, M.G. Newhouse, D.R. Manning, Fractionation of the beta subunit common to guanine nucleotide-binding regulatory proteins with the cytoskeleton, Mol. Pharmacol. 30 (1986) 463–468.
[83] R.R. Zwaal, J. Ahringer, H.G.A.M. van Luenen, A. Rushforth, P. Anderson, R.H.A. Plasterk, G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos, Cell 86 (1996) 619–629.
[84] P. Lilly, L. Wu, D.L. Welker, P.N. Devreotes, A G-protein beta-subunit is essential for *Dictyostelium* development, Genes Dev. 7 (1993) 986–995.
[85] J.A. Hadwiger, K. Natarajan, R.A. Firtel, Mutations in the *Dictyostelium* heterotrimeric G protein alpha subunit G alpha5 alter the kinetics of tip morphogenesis, Development 122 (1996) 1215–1224.
[86] D.G. Srinivasan, R.M. Fisk, H. Xu, S. van den Heuvel, A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C. elegans*, Genes Dev. 17 (2003) 1225–1239.
[87] M. Schaefer, M. Petronczki, D. Dorner, M. Forte, J.A. Knoblich, Heterotrimeric G proteins direct two modes of asymmetric cell division in the *Drosophila* nervous system, Cell 107 (2001) 183–194.
[88] J.B. Blumer, M.L. Bernard, Y.K. Peterson, J.-I. Nezu, P. Chung, D.J. Dunican, J.A. Knoblich, S.M. Lanier, Interaction of activator of G-protein signaling 3 (AGS3) with LKB1, a Serine/threonine kinase involved in cell polarity and cell cycle progression: phosphorylation of the g-protein regulatory (gpr) motif as a regulatory mechanism for the interaction of gpr motifs with Giα, J. Biol. Chem. 278 (2003) 23217–23220.
[89] Q. Du, I.G. Macara, Mammalian pins is a conformational switch that links NuMA to heterotrimeric G proteins, Cell 119 (2004) 503–516.
[90] K.H. Siller, C. Cabernard, C.Q. Doe, The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts, Nat Cell Biol 8 (2006) 594–600.
[91] C.J. Thomas, G.G. Tall, A. Adhikari, S.R. Sprang, Ric-8A catalyzes guanine nucleotide exchange on Gαi1 bound to the GPR/GoLoco exchange inhibitor AGS3, J. Biol. Chem. 283 (2008) 23150–23160.
[92] C. Hofler, M.R. Koelle, AGS-3 alters *Caenorhabditis elegans* behavior after food deprivation via RIC-8 activation of the neural G protein Gαo, J. Neurosci. 31 (2011) 11553–11562.
[93] C. Hofler, M.R. Koelle, The G protein regulator AGS-3 allows *C. elegans* to alter behaviors in response to food deprivation, Worm 1 (2012) 56–60.