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Chapter 14

Heterotrimeric G Proteins and the Regulation of Microtubule Assembly

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66929

Abstract

Microtubules (MTs), a major component of cell cytoskeleton, exhibit diverse cellular functions including cell motility, intracellular transport, cell division, and differentiation. These functions of MTs are critically dependent on their ability to polymerize and depolymerize. Although a significant progress has been made in identifying cellular factors that regulate microtubule assembly and dynamics, the role of signal transducing molecules in this process is not well understood. It has been demonstrated that heterotrimeric G proteins, which are components of G protein-coupled receptor (GPCR) signaling pathway, interact with microtubules and play important roles in regulating assembly/dynamics of this cytoskeletal filament. While α subunit of G proteins (Gα) inhibits microtubule assembly and accelerates microtubule dynamics, G$\beta\gamma$ promotes tubulin polymerization. In this chapter, we review the current status of G-protein modulation of microtubules and cellular and physiological aspects of this regulation. Molecular, biochemical, and cellular methodologies that have been used to advance this field of research are discussed. Emphasis has been given on G-protein-microtubule interaction in neuronal differentiation as significant progress has been made in this field. The outcome from this research reflects the importance of GPCRs in transducing extracellular signals to regulate a variety of microtubule-associated cellular events.

Keywords: cytoskeleton, G-proteins, microtubules, neuronal differentiation, G$\beta\gamma$, tubulin, G protein-coupled receptor, GTP-binding proteins

1. Introduction

The major component of microtubules (MTs) is the heterodimeric protein tubulin, consisting of α and β subunits, which are assembled into linear protofilaments. The protofilaments

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associate laterally to form the microtubule, a 25-nm-wide hollow cylindrical polymeric structure [1]. Due to the asymmetry of the αβ-tubulin heterodimer, MTs are polar structures with two distinct ends. These ends possess different polymerization rates: a slow-growing minus end with an exposed α-tubulin subunit, and a fast-growing plus end, at which the β-tubulin subunit is exposed [2, 3]. MT assembly occurs in two phases: nucleation, which is facilitated by a third tubulin isoform, γ-tubulin; and elongation, during which αβ-tubulin heterodimers are added to the plus end [1, 4]. Tubulin is a unique guanine nucleotide-binding protein containing one exchangeable binding site and one nonexchangeable binding site. GTP at both sites is needed for optimal assembly, and GTP at the exchangeable site is hydrolyzed after assembly [5, 6]. This hydrolysis creates an MT consisting largely of GDP-tubulin; however, a small region of GTP-bound tubulin, called a "GTP cap," remains at the end. This cap allows MTs to polymerize. The loss of the cap results in a transition from growth to shortening (called a "catastrophe"), whereas the reacquisition of the GTP cap results in a transition from shortening to growing (called a "rescue"). This behavior, known as dynamic instability, allows MTs to be remodeled rapidly in cells. An important consequence of dynamic instability is that it allows microtubules to search for specific target sites within the cell more effectively [7–9]. The MT assembly process is depicted in **Figure 1**.

MT assembly and stability can be affected by a wide variety of proteins. In this regard, microtubule-associated proteins (MAPs) play a very important role. Members of this group of proteins, such as MAP2 and tau, are known to promote MT assembly and stabilize MTs *in vivo* and *in vitro* [10–13]. The phosphorylation of MAPs is critical for their function, since

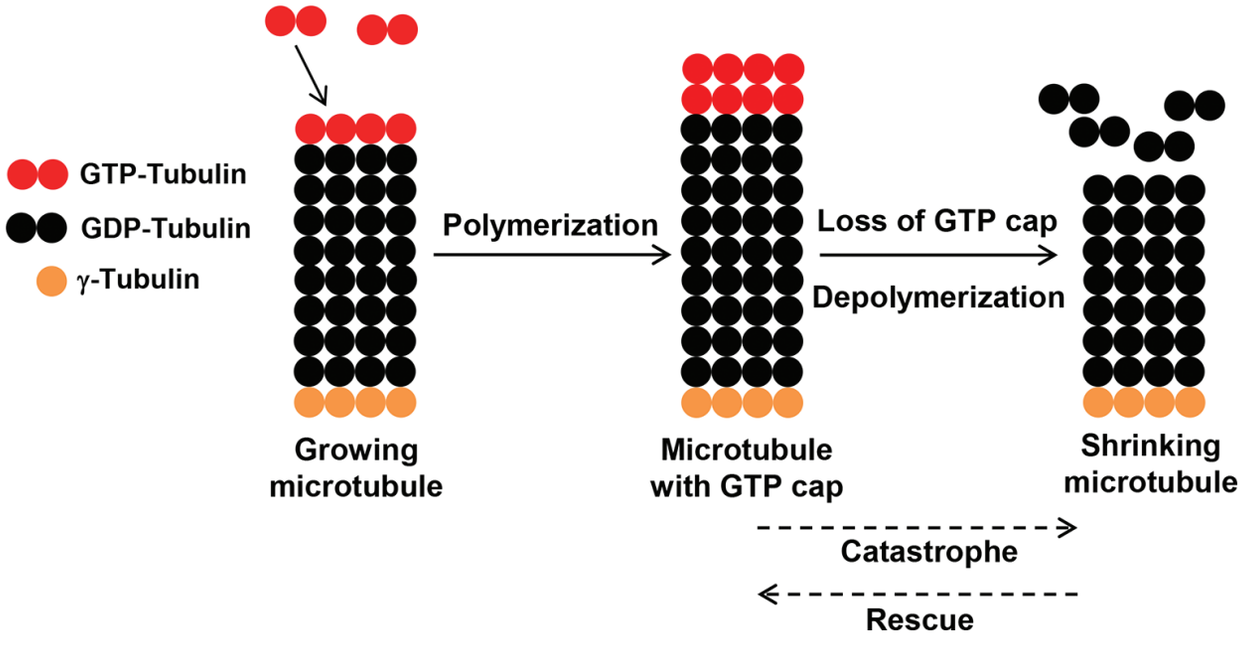


Figure 1. Polymerization/depolymerization of MTs. MTs is polymerized from tubulin heterodimer consisting of α and β subunits. A third tubulin isoform, γ-tubulin, serves as a template for nucleation, which allows proper MT assembly. As shown in the figure, MT assembly requires tubulin to be in GTP bound form. However, it is hydrolyzed to GDP when it is incorporated in MTs, except at the plus (+) end where tubulin remains at GTP bound form (a GTP cap). This cap allows MTs to polymerize. The loss of GTP cap (by hydrolysis) results in the transition to MT depolymerization (a "catastrophe"). GTP cap can be regained by binding to tubulin-GTP and MT polymerization is reestablished (a "rescue").

http://dx.doi.org/10.5772/66929

phosphorylated MAPs separate from MTs, causing MTs to become more susceptible to disassembly and destabilization [14, 15]. Destabilization of MTs can be promoted by a large number of proteins collectively termed "catastrophe promoters," as they favor the transition of MTs from elongation to shortening. Examples of these proteins include stathmin/Op18, a small heat-stable protein that is abundant in many types of cancer cells, katanin, and some kinesin-related motor proteins [16, 17]. Also, many drugs known to alter tubulin polymerization are considered valuable tools in studying the mechanisms of MT assembly. Some of these drugs, such as nocodazole, depolymerize MTs, whereas others, such as taxol, promote MT assembly [18–21]. Even though MTs are composed of α/β-tubulin heterodimers in all eukaryotic cells, MTs exhibit great functional diversity. One possible explanation is that both α- and β-tubulin undergo a series of posttranslational modifications that allow MTs to engage in a variety of cellular activities [22]. These modifications include tyrosination/detyrosination, acetylation, glutamylation, and phosphorylation [23]. Although much progress has been made in identifying and characterizing the cellular factors that regulate MT assembly and dynamics, the precise spatial and temporal control of the process is not clearly understood.

Over the past decades, an effort has been made to understand the regulation of MT assembly and dynamics by signal transducing G proteins, as reviewed in Refs. [24, 25]. G proteins are heterotrimer, consisting of guanine nucleotide-binding α plus βγ subunits. The G-protein-signaling cascade begins with the agonist-induced activation of a G protein-coupled receptor (GPCR), which allows GTP to bind to the α subunit of the heterotrimer, and subsequently, the GTP-bound-activated Gα changes its association with Gβγ in a manner that permits both subunits to participate in the regulation of intracellular effector molecules [26]. The traditional pathway for GPCR signaling is shown in **Figure 2**. The GPCR family of proteins is highly diverse; more than 1000 gene-encoding GPCRs are found in the human genome [27, 28]. GPCRs participate in the regulation of a wide variety of physiological functions, including cell growth and differentiation, neurotransmission, immune system function, and hormonal signaling. Participation in such a multitude of processes makes GPCRs a very attractive drug target, and approximately 30% of commercially available drugs are designed to target GPCRs [29]. GPCRs consist of seven transmembrane domains, connected by three extracellular loops and three intracellular loops. The extracellular region is responsible for agonist binding (neurotransmitters, hormones, and odorants, among others), and the intracellular region is responsible for interacting with heterotrimeric G proteins [30]. In humans, there are 21 isoforms of Gα subunits, 6 Gβ isoforms, and 12 isoforms of Gγ [31]. G-protein heterotrimers are typically classified into four classes depending on the Gα subunit: Gαs (for stimulation of adenylyl cyclase), Gαi (for inhibition of adenylyl cyclase), Gαq (which regulates phospholipase), and Gα12/13, which is involved in the regulation of monomeric G proteins and other molecules, such as PKC [31, 32]. Typical effectors of Gα signaling include adenylyl cyclase, phospholipase C, phospholipase A_2, ion channels, and several kinases and transcription factors. Termination of the signal occurs when GTP bound to the α subunit is hydrolyzed by its intrinsic GTPase activity that causes its functional dissociation from the effector and reassociation with βγ [26, 33–35]. Thus, G proteins act as molecular switches that can be turned "on" and "off" through the GTPase cycle. While the signal-transducing ability of heterotrimeric G proteins was once believed to depend fully on the α subunit, it has now become clear

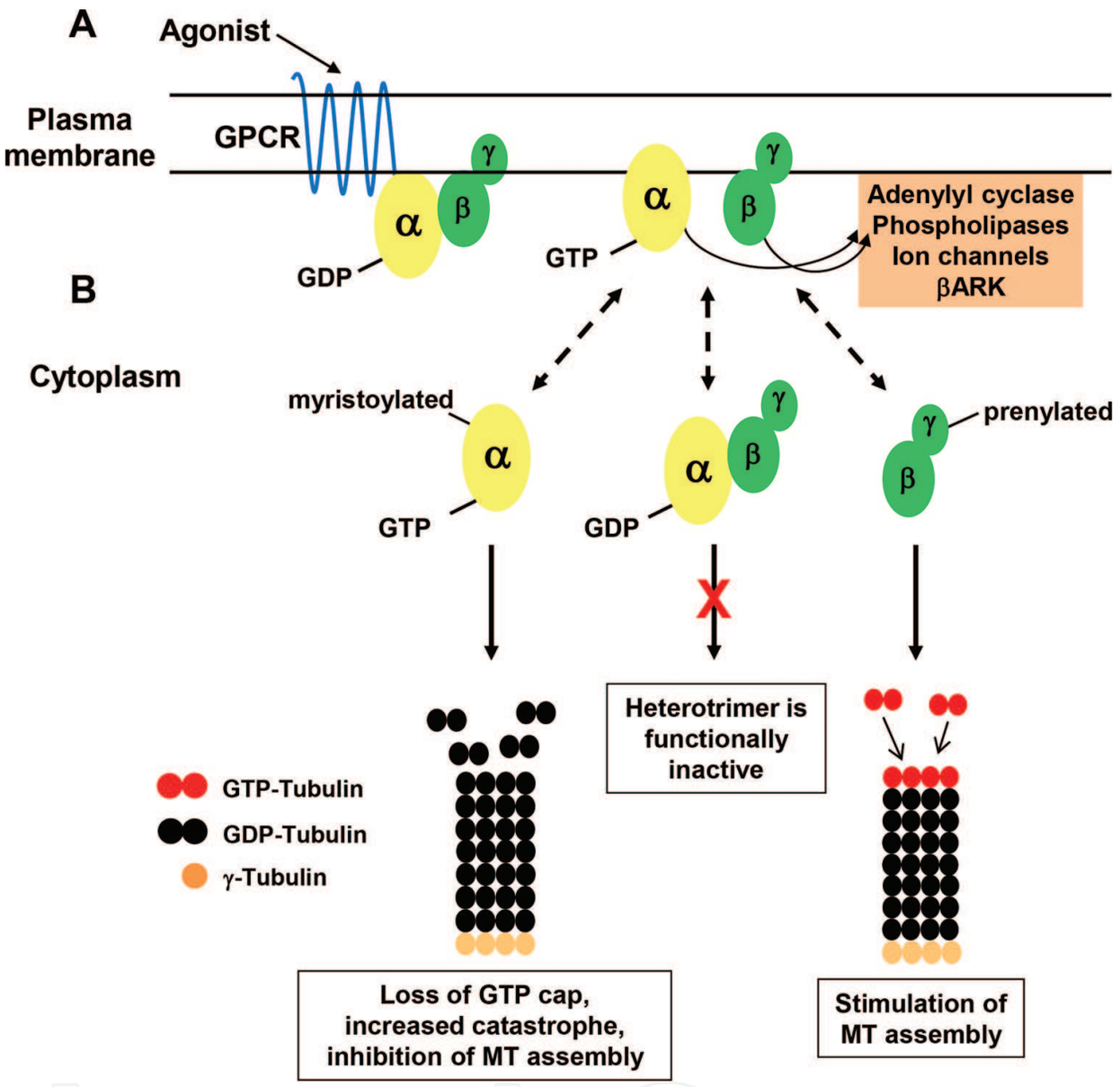


Figure 2. G-protein-mediated signaling and the regulation of MT assembly. **(A)** The traditional pathway for G-protein signaling cascade begins with the agonist-induced activation of a GPCR (G protein-coupled receptor), which allows GTP to bind to the α subunit of the heterotrimer and subsequently the GTP-bound-activated Gα changes its association with G$\beta\gamma$ in a manner that permits both subunits to participate in the regulation of intracellular effector molecules. **(B)** α and $\beta\gamma$ subunits of heterotrimeric G proteins interact with tubulin/MTs (in cytoplasm) and influence MT assembly and dynamics. Results generated from *in vitro* studies using purified proteins and cultured cells suggest that the Gα subunit inhibits MT assembly and promotes MT disassembly by interacting with tubulin-GTP and initiating GTP hydrolysis of tubulin, therefore causing MT depolymerization. The G$\beta\gamma$ subunit, on the other hand, promotes MT assembly. The G$\alpha\beta\gamma$ heterotrimer is functionally inactive (similar to that observed in traditional GPCR pathway) and does not interact functionally with Tubulin/MTs. Upon activation, Gα dissociates from G$\beta\gamma$ subunits, and both subunits then interact with tubulin/MTs and modulate assembly/dynamics. It is suggested that G-protein-MT interaction is an important step for G-protein-mediated cell activation.

that the $\beta\gamma$ subunit is capable of interacting with numerous effector molecules to influence a variety of signaling pathways [36, 37]. Among the effector molecules interacting with G$\beta\gamma$ are phospholipases, K^+ and Ca^{2+} channels, GPCR kinases, members of the MAP kinase signaling

pathway, monomeric G proteins, regulators of G protein signaling (RGS), and phosphoinositide-3 kinase (PI3K) [37–42].

Although G proteins are likely to be membrane-bound when coupled to receptors, results from several laboratories in past decades demonstrate their association with several subcellular compartments including MTs. G protein-MT interactions have been shown to modulate the assembly, dynamics and functions of MTs (**Figure 2**). This chapter focuses on our current understanding of G protein regulation of MT assembly and cellular and physiological aspects of this regulation.

2. Heterotrimeric G proteins and the tubulin/MT system

Gα and MT assembly/dynamics. Direct interactions between tubulin and α subunits of Gs, Gi1 have been demonstrated [43] and these interactions were shown to activate GTPase activity of tubulin, inhibit microtubule assembly, and accelerate microtubule dynamics [44–47]. To elucidate the role of Gα in microtubule assembly *in vitro*, purified Gα subunits as well as tubulin were used in the reconstitution assay. G protein α subunits Gi1α, Gsα, and Goα were shown to activate the GTPase activity of tubulin and inhibit microtubule assembly. The assembly of tubulin-GTP (or tubulin-GppNHp) into microtubules was inhibited by Gi1α (80–90%) in the absence of exogenous GTP. The addition of exogenous GTP—but not the addition of hydrolysis-resistant GppNHp—overcame the inhibition of microtubule assembly by Gi1α [45], thus, it appears that GTP hydrolysis resulting from the association of tubulin and Gi1α plays a critical role in modulating microtubule assembly. Gα appears to bind to tubulin and activate the intrinsic GTPase of tubulin in a manner similar to what occurs during MT formation. However, unlike the formation of microtubules from tubulin dimers, Gα dissociates from the tubulin-Gα complex subsequent to GTP hydrolysis [45]. This finding is consistent with the possibility that Gα would accelerate MT dynamic instability. Analysis of the dynamics of individual microtubules by video microscopy has demonstrated that Gi1α increases the catastrophe frequency [45]. To determine the role of Gsα in MT dynamics *in vivo*, PC12 cells were transfected with Gsα-GFP [47]. Transfected cells were treated with cholera toxin to activate Gsα-GFP or forskolin to stimulate adenylate cyclase and to increase cAMP. Cholera-toxin activation of Gsα-GFP resulted in a displacement of Gsα-GFP from the plasma membrane. It was found that activated Gsα released from the plasma membrane was directly bound to cellular microtubules and then colocalized with microtubules. As a result, activated Gsα made MTs more dynamic, decreasing the pool of insoluble MTs, without changing the total cellular tubulin content [47].

Gβγ and MT assembly. The Gβγ subunit has the opposite effect on tubulin polymerization, as it was found that Gβγ promotes MT assembly *in vitro* [48]. Assembly was monitored by negative staining electron microscopy and measuring protein in polymers collected by centrifugation. The effect of different combinations of βγ on MT assembly was tested. Tubulin that was purified free of microtubule-associated proteins was incubated at 37°C in the presence of β1γ2 or β1γ1 (transducin βγ) for 45 min to 1 h. Microtubule assembly was stimulated markedly when β1γ2 was present at ~1:20 molar ratio with tubulin; in contrast, β1γ1 had no

effect on microtubule assembly [48]. An electron microscopic analysis indicated the formation of very few microtubules either by tubulin alone or in the presence of β1γ1. In the presence of β1γ2, however, robust microtubule polymerization occurred. Protein estimation in the pellets also indicated a 71% increase in the presence of β1γ2. An SDS-PAGE of the samples further confirmed the increase in tubulin concentration in the pellet formed in the presence of β1γ2. No detectable change in pellet protein concentration (compared to controls) was observed in the presence of β1γ1. β immunoreactivity was detected exclusively in the microtubule fraction after assembly in the presence of β1γ2, suggesting a preferential association with microtubules rather than with soluble tubulin. A number of proteins, including the γ subunit of Gβγ, undergo a process of posttranslational modification termed "prenylation" and this modification is important for the biological functions of these proteins. For example, prenylation of γ subunits is required for the high-affinity interactions of Gβγ with α subunits or effector molecules [49–51]. Interestingly, it was found that a mutant β1γ2, β1γ2 (C68S), which does not undergo prenylation of γ subunit, did not stimulate the formation of MTs, suggesting that the functional interaction of Gβγ with MTs require the same specificity as other effector molecules of Gβγ [49–51].

To investigate the potential link between Gβγ and MT assembly *in vivo*, cultured PC12 and NIH3T3 cells were used. The role of Gβγ in MT assembly was demonstrated using nocodazole, a microtubule-depolymerizing drug [52]. Colchicine and the synthetic compound nocodazole are both antimitotic drugs and known to exert their effects by a similar mechanism, that is, by binding to tubulin dimers and inhibiting the subsequent addition of tubulin molecules to microtubules. However, the potential usefulness of nocodazole is due to its readily reversible and rapid activity [53, 54]. Nocodazole-induced depolymerization of microtubules drastically inhibited (~68%) the interaction between Gβγ and tubulin [52]. This result was further confirmed by the isolation of polymerized tubulin (MT) and soluble tubulin (ST) fractions from PC12 cells. Although Gβγ was found in both fractions, a tubulin-Gβγ interaction was found preferentially in MT fractions rather than ST fractions as demonstrated by coimmunoprecipitation analyses. This is consistent with *in vitro* studies, in which Gβγ was preferentially associated with MTs assembled from β1γ2 [48]. Removal of nocodazole from the cultured media allowed MTs to repolymerize to their fullest extent and tubulin-Gβγ interaction was restored completely in the MT fraction. These results clearly demonstrate that the association of Gβγ with MTs is important for MT assembly and/or stability. The interactions between Gβγ and tubulin/MTs were also assessed by immunofluorescence microscopy. Microtubules in PC12 cells are well defined and extend to the cell periphery. Gβγ was more concentrated in the perinuclear region where they were colocalized with microtubules. The network of microtubule structure collapsed and Gβγ labeling was dispersed, when cells were treated with nocodazole for 4 h. Microtubules reappeared after the removal of nocodazole, when cells were incubated in fresh media for 4 h. Gβγ labeling was also appeared in perinuclear region where they were colocalized with MTs [52]. In addition to interphase cells, Gβγ-tubulin association was also observed in mitotic spindle in PC12 cells.

Gβγ-γ-tubulin interactions. γ-Tubulin, a member of tubulin superfamily, is a centrosomal protein and its role in MT nucleation is well documented [55–58]. In addition to its binding of αβ-tubulin, Gβγ was also found to interact with γ-tubulin [33]. However, unlike αβ-tubulin,

the interaction between γ-tubulin and Gβγ is not inhibited by nocodazole, suggesting that the interaction between Gβγ and γ-tubulin is not dependent upon microtubules [33]. Both Gβγ and γ-tubulin were colocalized in the centrosomes of PC12 cells. Interestingly, γ-tubulin and Gβγ immuno-reactivity appears to be increased significantly in duplicated centrosomes at the onset of mitosis, and Gβγ was consistently found to colocalize with tubulin at mitotic spindle, particularly at the spindle pole areas [33]. Earlier studies in *Caenorhabditis elegans* and *Drosophila* have also demonstrated that Gβγ is involved in cell division by positioning the mitotic spindle and attaching microtubules to the cell cortex [21, 22]. In *C. elegans* embryos, Gβγ was shown to be important in the regulation of migration of the centrosome around the nucleus [21]. These studies collectively suggest an important role of Gβγ in centrosome functions, perhaps through its interactions with γ-tubulin. Although centrosome-associated γ-tubulin is known to be involved in MT nucleation, most γ-tubulin in cells are found in the cytoplasm as γ-tubulin ring complex (γTuRC) and it has been shown that γTuRC translocate to centrosome to mediate MT nucleation [59, 60]. Since Gβγ immunoreactivity also increases significantly in duplicated chromosomes at the onset of mitosis [52], it is possible that Gβγ may allow translocation of γ-tubulin to centrosomes. The γ-tubulin-Gβγ complex might then induce robust microtubule nucleation at the centrosome and formation of the mitotic spindle.

Gαβγ heterotrimer and MT assembly. Since G protein activation and subsequent dissociation of α and βγ subunits are necessary for G proteins to participate in signaling processes [26], it was determined if similar activation is required for modulation of microtubule assembly by G proteins. For that, Gαβγ heterotrimer was reconstituted from α and βγ subunits and its effect was tested on GTPase activation of tubulin and MT assembly. Myristoylated Gi1α and prenylated Gβ1γ2 were used to reconstitute the heterotrimer, since lipid modified G-protein subunits have been found to be more effective in interacting with tubulin and subsequent modulation of its functions [45, 48]. In addition, lipid-modified, G-protein subunits have been shown to reconstitute heterotrimers more effectively [61, 62]. Reconstituted heterotrimers have been shown to block Gi1α activation of tubulin GTPase and inhibit the ability of Gβ1γ2 to promote *in vitro* microtubule assembly [46], suggesting that G-protein activation is required for functional coupling between Gα/Gβγ and tubulin/MTs (**Figure 2**). The results also suggest that G protein-coupled receptors (GPCRs) may be involved in the regulation of MT assembly and dynamics *in vivo* by mobilizing G-protein subunits to bind to MTs. In doing so, GPCRs may control a variety of cellular activities. It appears that G-protein-MT interaction is an important step for G-protein-mediated cell activation.

3. G protein-microtubule interactions and cell division

Microtubules play a key role in cell division, participating in the exact organization and function of the spindle apparatus, a vehicle necessary for chromosomal segregation. Microtubules in the spindle are organized in such a way that the minus ends are near the spindle poles, while the plus ends extend toward the cell cortex or chromosomes [63]. Both α and βγ subunits of G proteins Gi and Go are consistently found to be associated with mitotic spindle. Genetic studies in *C. elegans*, *Drosophila*, and mammalian cells have revealed that G-protein subunits are

involved in regulating mitotic spindle for centrosome/chromosome movements in cell division [64–69]. G-protein α subunits of Gi are involved in cell division by regulating microtubule-pulling forces during chromosomal segregation through a receptor-independent pathway. Unlike the classical G-protein cycle, in which GPCR promotes the GDP/GTP exchange in Gα converting Gα in active GTP-bound form, in nonreceptor pathways, the GDP-bound form of Giα is stabilized through its interaction with guanine-nucleotide dissociation inhibitor (GDI) to regulate microtubule-pulling forces for chromosome movements [66, 70, 71]. Members of the GDI family of proteins, characterized by the presence of 20–25 amino-acid repeats termed "GPR" or "GoLoco" motifs, are known to stabilize the GDP-bound form of Giα by inhibiting the release of nucleotide. Thus, it appears that Giα participates in spindle function through a mechanism that is distinct from the receptor-mediated pathway. In addition to Giα, G$\beta\gamma$ has been shown to play a role in spindle position and orientation during cell division [64, 68]. The association of Goα and β (or G$\beta\gamma$) with spindle microtubules suggests that G-protein subunits may play an important role in the regulation of the assembly and disassembly of mitotic spindles through their ability to modulate microtubule assembly. Recently, it has been shown that reconstituted kinetochores *in vitro* bind preferentially to GTP rather than to GDP microtubules, suggesting that a protein exists in kinetochores that can distinguish between GTP conformation of the microtubules and allow the kinetochores to remain at the microtubule ends to ensure correct chromosome segregation [72]. Since Gα appears to interact preferentially to GTP (rather than the GDP-form of tubulin) and has been detected in mitotic spindles, it may be a likely candidate for segregating chromosomes through its interaction with microtubules.

4. G protein-microtubule interactions and neuronal differentiation

The process by which MT structure is remodeled in neurons is a central question in cell biology and recent research indicates an important role of G protein subunits in this process. During neuronal differentiation, two distinct domains emerge from the cell body: a long, thin axon that transmits signals, and multiple shorter dendrites, which are specialized primarily for receiving signals. The axon terminal contains synapses, specialized structures where neurotransmitters are released to communicate with target neurons. Cytoskeletal structures embodied within neurite extensions and growth cone formations are essential for establishing appropriate synaptic connections and signal transmission. MTs form dense parallel arrays in axons and dendrites that are required for the growth and maintenance of such neurites. In the axon, MTs are bundled by tau, a microtubule-associated protein (MAP), with their plus end oriented toward the nerve terminal. MAP2, a group of high molecular weight MAPs, participates in MT bundling in the dendrites (**Figure 3**). Unlike MTs, actin filaments in neurons are enriched in growth cones and organized into long bundles that form filamentous protrusions, or filopodia, veil-like sheets of branched actin that form lamellipodia [1, 7, 73]. The interaction between these two cytoskeletal filaments is important for the advancement of growth cones and axon guidance [74, 75].

It is clear that cytoskeletal components can detect biochemical signals and respond in order to change the neuronal cell morphology. However, the precise signaling pathways that lead

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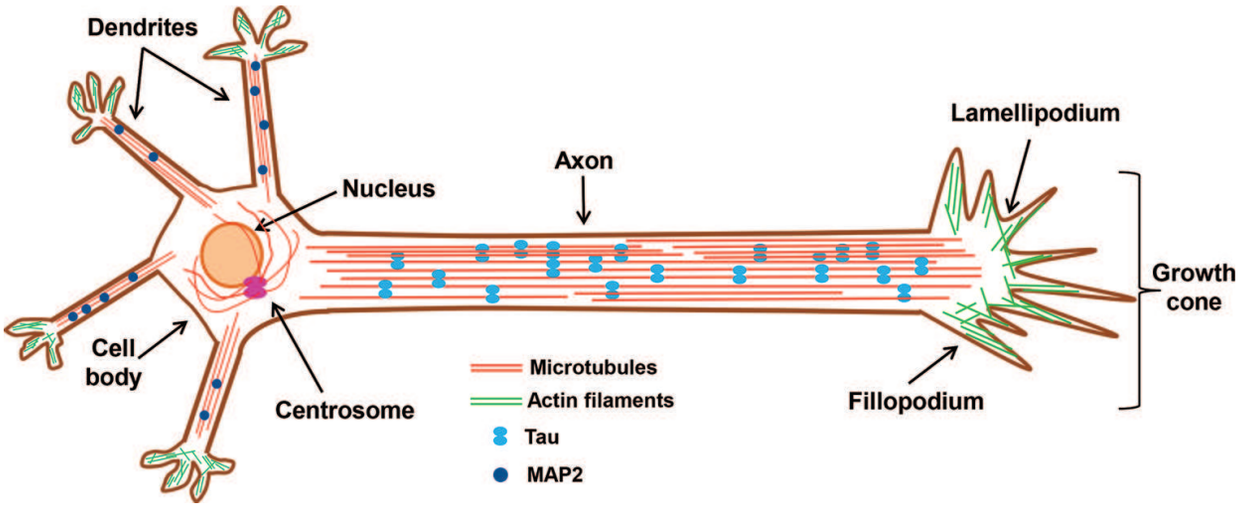


Figure 3. Neuronal cytoskeleton. The polarized and asymmetrical shape of neurons is achieved by means of a highly specialized cytoskeletal organization. In addition to cell body, MTs are found in the axon, dendrites, and the central domain of the growth cone. Tau, a microtubule-associated protein (MAP), participates in MT bundling in the axon, while MAP2 carries this function in the dendrites. Actin filaments are present in the growth cone and dendrites, where they form specialized structures such as lamellipodia and filopodia.

unique organization of MTs in neurons are not clearly understood [76]. PC12 cells have been used extensively for these studies as they respond to nerve growth factor (NGF) with growth arrest and exhibit a typical phenotype of neuronal cells that send out neurites [77]. NGF is a neurotrophic factor critical for the survival and maintenance of sensory and sympathetic neurons. The receptor commonly associated with this process is tyrosine kinase (TrkA) through which NGF exerts its effect [78]. PI3K appears to be the key molecule in this pathway and regulates localized assembly of MTs/actin filaments by downstream Akt/GSK3β pathways [79, 80]. The Rho and Ras families of small GTPases have also emerged as critical players in regulating the actin and MT cytoskeleton by modulating downstream effectors, including serine/threonine kinase, p21-activated kinase, ROCK, and mDia [81, 82]. GPCRs, as well as α and $\beta\gamma$ subunits of heterotrimeric G proteins, have also been shown to regulate neurite outgrowth [83–90]. These studies collectively suggested the role of α and $\beta\gamma$ subunits of G proteins in regulating neurite outgrowth. More recently, it has been demonstrated that both α and $\beta\gamma$ subunits of G proteins regulate neurite outgrowth and differentiation by interacting with MTs and by modulating MT assembly/dynamics [24].

Gsα and neuronal differentiation. NGF-induced neuronal differentiation of PC12 cells result in a translocation of Gsα, Gi1α, and Goα from cell bodies to cellular processes in which they appear to localize with microtubules [91]. Consistent with this, Gα in Neuro-2a cells, which spontaneously differentiate, showed a similar pattern of association of Gα with MTs [91]. The result has been further confirmed by transfecting PC12 cells with Gsα-GFP. Transfected cells were treated with cholera toxin to activate Gsα-GFP, or forskolin, to stimulate adenylyl cyclase and an increase in cAMP. Colocalization of Gsα along MTs was seen in cells treated with cholera toxin but not in those treated with forskolin, indicating that activation of Gsα induces Gsα translocation to the cytoplasm where it associates with MTs [47]. To understand the function of

Gsα/microtubule association in neuronal development and differentiation, real time trafficking of a Gsα-GFP fusion protein was used [92]. GFP-Gsα concentrated at the distal end of the neurites in NGF-differentiated living PC12 cells as well as in the cultured hippocampal neurons. Gsα appeared to translocate to the growing tip of neurites and to membrane ruffles of the newly formed extensions after NGF treatment, and it has been suggested that during neuronal differentiation, Gsα redistributes toward the areas of highly dynamic cytoskeletal activity. Neurite length as well as the number of neurites per cell was also increased in cells overexpressing Gsα-GFP in the presence of NGF. The effect was greatest in cells overexpressing constitutively active Gsα (GsQL). On the other hand, a dominant-negative Gαi-transducin chimera that interferes with Gsα binding to tubulin and activation of tubulin GTPase attenuated the neurite elongation and the neurite number both in PC12 cells and in primary hippocampal neurons. Thus, it appears that activated Gsα translocated from plasma membrane induced neurite outgrowth and development through interaction with tubulin/microtubules in the cytosol [92].

Gβγ and neuronal differentiation. The involvement of Gβγ in neuronal development and differentiation has been previously shown [68, 89]. Gβ1-deficient mice have been shown to have neural tube defects [94], and Gβ5-knockout mice have been shown to display abnormal behavior and develop multiple brain abnormalities [95]. It has also been shown that impaired Gβγ signaling promotes neurogenesis in the developing neocortex and increased neuronal differentiation of progenitor cells [68]. Although the mechanism by which Gβγ controls this process is not yet understood, the possibility that Gβγ may act on MTs has been suggested. Sachdev et al. [89] have also suggested that Gβγ-Tctex-1 complex plays a key role in regulating neurite outgrowth in primary hippocampal neurons, most likely by modulating MTs and actin filaments through activation of downstream signaling. These studies suggest a connection between Gβγ signaling and the modulation of MTs during neuronal differentiation and development.

More recently, using biochemical and immunofluorescence analysis, it has been demonstrated that Gβγ-MT interactions and modulation of MT assembly is critical for NGF-induced neuronal differentiation of PC12 cells [94]. To address this, PC12 cells were treated with NGF over the course of three days to allow for neuronal differentiation. Microtubules (MTs) and soluble tubulin (ST) fractions were extracted using a microtubule-stabilizing buffer. The interaction of Gβγ with MT and ST fractions was analyzed by coimmunoprecipitating tubulin-Gβγ complex using a Gβ-specific antibody (rabbit polyclonal anti-Gβ) or a mouse monoclonal anti-α tubulin antibody and determining tubulin and Gβγ immunoreactivity in the complex [94]. Gβγ-MT interaction was significantly increased (2–3 fold) in NGF-treated cells. We also found that MT assembly was stimulated significantly (from 45.3 ± 4.8 to 70.1 ± 3.6%) in NGF-differentiated PC12 cells. The association of Gβγ with MTs in NGF-differentiated cells was also assessed by immunofluorescence microscopy [93]. After NGF treatment, the majority of the cells displayed neurite formation. Gβγ was detected in the neurites and in cell bodies. The colocalization of Gβγ with MTs/tubulin was observed along the neuronal process and in the central portion of the growth cone, but not at the tip of the growth cones.

Overexpression of Gβγ in PC12 cells induced neurite outgrowth in the absence of NGF, further supporting the role of Gβγ in neuronal differentiation [93]. Since Gβ1γ2 promoted

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MT assembly *in vitro*—and Gβ1γ1 had no effect [48], PC12 cells were transfected by either β1γ1 or β1γ2. YFP-tagged β1, γ2, or γ1 constructs were used for transfection. Cells were cotransfected with β1 and γ2, or β1 and γ1. Within 24 h of transfection, both β1γ1- and β1γ2-transfected PC12 cells were found to overexpress the proteins. At 48 h of transfection, YFP-β1γ2 transfected cells induced neurite formation (in the absence of NGF). Overexpressed protein (YFP-Gβ1γ2) was localized in the neurite processes, growth cones, and cell bodies. Moreover, overexpressed Gβγ exhibited a pattern of association with MTs similar to that observed in NGF-differentiated cells. The average neurite length of Gβ1γ2 (42.8 ± 2.1 μm) and Gβ1γ1 (33.5 ± 1.8 μm) is significantly higher than that of control cells (18.4 ± 0.6 μm), with Gβ1γ2 having the most potent effect on neurite outgrowth. Although the average neurite length in Gβγ-overexpressing cells (42.8 ± 2.1 μm) was slightly lower than that observed in NGF-differentiated PC12 cells (53.6 ± 1.8 μm), the result clearly indicates the effectiveness of overexpressed Gβγ in inducing neurite outgrowth in the absence of NGF.

Finally, the role of Gβγ in neuronal morphology, outgrowth and differentiation was further investigated using peptides and prenylation pathway inhibitors. For example, GRK2i, a Gβγ blocking peptide known to inhibit Gβγ-dependent effector functions, induced neurite damage as well as MTs and Gβγ aggregation. In addition, cellular aggregation was also frequently observed in the presence of GRK2i. The percentage of cell-bearing neurites was reduced significantly. On the other hand, synthetic peptide mSIRK, which is known to activate Gβγ signaling in cells by promoting the dissociation of Gβγ from α subunits, stimulated neurite formation. Since, γ-subunit of Gβγ is known to be posttranslationally modified by prenyl lipid, and prenylation deficient mutant of Gβγ (C68S) was shown to be functionally inactive, inhibitors of an enzyme of prenylation pathway (PMPMEase) was tested for their effects on MT assembly and neurite outgrowth. These inhibitors were found to alter MT organization and blocked neurite outgrowth. The results further demonstrate that βγ subunit of heterotrimeric G proteins play a critical role in neurite outgrowth and differentiation by interacting with MTs and regulating MT assembly and organization.

5. Conclusion

Heterotrimeric G proteins transduce signals from cell surface receptors (G protein-coupled receptors) to intracellular effector molecules that include adenylyl cyclase, phospholipases, and ion channels. New evidence suggests that the modulation of the MTs by G proteins is an emerging field of research and therefore an in-depth understanding of G-protein-MTs interaction is important for elucidation of the function, behavior, and morphology of mammalian cells. Key results of this unique interaction may have a broader impact on health and diseases including cancer, Alzheimer's, Parkinson's, depression, and addictive behavior. We foresee that the G-protein-MT dependent pathway could be exploited for developing novel drugs to combat such diseases in the future.

Acknowledgments

Research in author's laboratory described in this report was, in part, supported by G12MD007592 (NIHMHD) to the University of Texas at El Paso. An R01 subcontract to SR from the University of Illinois at Chicago (MH39595) supported some earlier research in author's laboratory. The biochemical, molecular, and confocal microscopy experiments were carried out at the Biomolecule Analysis Core Facility, Genome Analysis Core facility, and Cytometry/screening/imaging Facility at the Border Biomedical Research Center (UTEP) supported by a grant (G12MD007592) from NIMHD (NIH). The authors like to thank Dr. Siddhartha Das for critically reading the manuscript and thoughtful suggestions.

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References

[1] Desai A, Mitchison TJ. Microtubule polymerization dynamics. Annu. Rev. Cell Dev. Biol. 1997;**13**:83–117. DOI: 10.1146/annurev.cellbio.13.1.83

[2] Jiang K, Akhmanova A. Microtubule tip-interacting proteins: a view from both ends. Curr. Opin. Cell Biol. 2011;**23**:94–101. DOI: 10.1016/j.ceb.2010.08.008

[3] Sakakibara A, Ando R, Sapir T, Tanaka T. Microtubule dynamics in neuronal morphogenesis. Open Biol. 2013;**3**:130061. DOI: 10.1098/rsob.130061

[4] Conde C, Cáceres A. Microtubule assembly, organization and dynamics in axons and dendrites. Nat. Rev. Neurosci. 2009;**10**:319–332. DOI: 10.1038/nrn2631

[5] David-Pfeuty T, Erickson HP, Pantaloni D. Guanosinetriphosphatase activity of tubulin associated with microtubule assembly. Proc. Natl. Acad. Sci. U. S. A. 1977;**74**:5372–5376. DOI: 10.1073/pnas.74.12.5372

[6] Nogales E, Wolf SG, Downing KH. Structure of the alpha beta tubulin dimer by electron crystallography. Nature. 1998;**391**:199–203. DOI: 10.1038/34465

[7] Mitchison T, Kirschner M. Dynamic instability of microtubule growth. Nature. 1984;**312**:237–242. DOI: 10.1038/312237a0

[8] Carlier MF, Didry D, Simon C, Pantaloni D. Mechanism of GTP hydrolysis in tubulin polymerization: characterization of the kinetic intermediate microtubule-GDP-Pi using phosphate analogues. Biochemistry. 1989;**28**:1783–1791. DOI: 10.1021/bi00430a054

[9] Gundersen GG, Gomes ER, Wen Y. Cortical control of microtubule stability and polarization. Curr. Opin. Cell Biol. 2004;**16**:106–112. DOI: 10.1016/j.ceb.2003.11.010

[10] Murphy DB, Borisy GG. Association of high-molecular weight proteins with microtubules and their role in microtubule assembly. Proc. Natl. Acad. Sci. U. S. A. 1975;**72**:2696–2700.

[11] Margolis RL, Rauch CT, Job D. Purification and assay of a 145-kDa protein (STOP145) with microtubule-stabilizing and motility behavior. Proc. Natl. Acad. Sci. U. S. A. 1986;**83**:639–643.

[12] Kowalski RJ, Williams RC Jr. Microtubule-associated protein 2 alters the dynamic properties of microtubule assembly and disassembly. J. Biol. Chem. 1993;**268**:9847–9855.

[13] Gamblin TC, Nachmanoff K, Halpain S, Williams Jr RC. Recombinant microtubule associated protein 2C reduces the dynamic instability of individual microtubules. Biochemistry. 1996;**35**:12575–12586. DOI: 10.1021/bi961135d

[14] Ebneth A, Drewes G, Mandelkow EM, Mandelkow E. Phosphorylation of MAP2c and MAP4 by MARK kinases leads to the destabilization of microtubules in cells. Cell Motil. Cytoskeleton. 1999;**44**:209–224. DOI:10.1002/(SICI)1097-0169(199911)44:3<209::AID-CM6>3.0.CO;2-4

[15] van der Vaart B, Akhmanova A, Straube A. Regulation of microtubule dynamic instability. Biochem. Soc. Trans. 2009;**37**:1007–1013. DOI: 10.1042/BST0371007

[16] Belmont L, Mitchison TJ. Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell. 1996;**84**:623–631. DOI: 10.1016/S0092-8674(00)81037-5

[17] Kline-Smith SL, Walczak CE. The microtubule-destabilizing kinesin XKCM1 regulates microtubule dynamic instability in cells. Mol. Biol. Cell. 2002;**13**:2718–31. DOI: 10.1091/mbc.E01-12-0143

[18] De Brabander M, Geuens G, Nuydens R, Willebrords R, Aerts F, De Mey J. Microtubule dynamics during the cell cycle: the effects of taxol and nocodazole on the microtubule system of Pt K2 cells at different stages of the mitotic cycle. Int. Rev. Cytol. 1986;**101**:215–74. DOI: 10.1016/S0074-7696(08)60250-8

[19] McGuire WP, Rowinsky EK, Rosenshein NB, Grumbine FC, Ettinger DS, Armstrong DK, Donehower RC. Taxol: a unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Intern. Med. 1988;**111**:273–279. DOI: 10.7326/0003-4819-111-4-273

[20] Wilson L, Jordan MA. Microtubule dynamics: taking aim at a moving target. Chem. Biol. 1995;**2**:569–573. DOI: 10.1016/1074-5521(95)90119-1

[21] Vasquez RJ, Howell B, Yvon AM, Wadsworth P, Cassimeris L. Nanomolar concentrations of nocodazole alter microtubule dynamic instability in vivo and in vitro. Mol. Biol. Cell. 1997;**8**:973–985. DOI: 10.1091/mbc.8.6.973

[22] Hammond JW, Cai D, Verhey KJ. Tubulin modifications and their cellular functions. Curr. Opin. Cell Biol. 2008;**20**:71–76. DOI: 10.1016/j.ceb.2007.11.010

[23] Wloga D, Gaertig J. Post-translational modifications of microtubules. J. Cell Sci. 2010;**123**:3447–3455. DOI: 10.1242/jcs.063727

[24] Roychowdhury S, Rasenick MM. Submembranous microtubule cytoskeleton: regulation of microtubule assembly by heterotrimeric G proteins. FEBS J. 2008;**275**:4654–4663. DOI: 10.1111/j.1742-4658.2008.06614.x

[25] Dave RH, Saengsawang W, Yu JZ, Donati R, Rasenick MM. Heterotrimeric G-proteins interact directly with cytoskeletal components to modify microtubule-dependent cellular processes. Neurosignals. 2009;**17**:100–108. DOI: 10.1159/000186693

[26] Gilman AG. G proteins: transducers of receptor-generated signals. Ann. Rev. Biochem. 1987;**56**:615–649. DOI: 10.1146/annurev.bi.56.070187.003151

[27] Fredriksson R, Lagerström MC, Lundin LG, Schiöth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol. Pharmacol. 2003;**63**:1256–1272. DOI: 10.1124/mol.63.6.1256

[28] Wang D, Li Y, Zhang Y, Liu Y, Shi G. High throughput screening (HTS) in identification new ligands and drugable targets of G protein-coupled receptors (GPCRs). Comb. Chem. High Throughput Screen. 2012;**15**:232–241. DOI: 10.2174/138620712799218626

[29] Salon JA, Lodowski DT, Palczewski K. The significance of G protein-coupled receptor crystallography for drug discovery. Pharmacol. Rev. 2011;**63**:901–937. DOI: 10.1124/pr.110.003350

[30] Latek D, Modzelewska A, Trzaskowski B, Palczewski K, Filipek S. G protein-coupled receptors—recent advances. Acta Biochim. Pol. 2012;**59**:515–529.

[31] Downes GB, Gautam N. The G protein subunit gene families. Genomics. 1999;**62**:544–552. DOI: 10.1006/geno.1999.5992

[32] Simon MI, Strathmann MP, Gautam N. Diversity of G proteins in signal transduction. Science. 1991;**252**:802–808. DOI: 10.1126/science.1902986

[33] Neves SR, Ram PT, Iyengar R. G protein pathways. Science. 2002;**296**:1636–1639. DOI: 10.1126/science.1071550

[34] Dohlman HG, Thorner J, Caron MJ, Lefkowitz RJ. Model systems for the study of seven transmembrane-segment receptors. Annu. Rev. Biochem. 1991;**60**:653–688. DOI: 10.1146/annurev.bi.60.070191.003253

[35] McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS. G-protein signaling: back to the future. Cell. Mol. Life Sci. 2005;**62**:551–577. DOI: 10.1007/s00018-004-4462-3

[36] Sternweis P.C. The active role of beta gamma in signal transduction. Curr. Opin. Cell Biol. 1994;**6**:198–203. DOI: 10.1016/0955-0674(94)90136-8

[37] Smrcka AV. G protein beta gamma subunits: central mediators of G protein-coupled receptor signaling. Cell. Mol. Life Sci. 2008;**65**:2191–2214. DOI: 10.1007/s00018-008-8006-5

[38] Ueda N, Iñiguez-Lluhi JA, Lee E, Smrcka AV, Robishaw JD, Gilman AG. G protein beta gamma subunits. Simplified purification and properties of novel isoforms. J. Biol. Chem. 1994;**269**:4388–4395.

[39] Wickman KD, Iñiguez-Lluhl JA, Davenport PA, Taussig R, Krapivinsky GB, Linder ME, Gilman AG, Clapham DE. Recombinant G-protein beta gamma-subunits activate the muscarinic-gated atrial potassium channel. Nature. 1994;**368**:255–257. DOI: 10.1038/368255a0

[40] Faure M, Voyno-Yasenetskaya TA, Bourne HR. cAMP and beta gamma subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. J. Biol. Chem. 1994;**269**:7851–7854.

[41] Ford CE, Skiba NP, Bae H, Daaka Y, Reuveny E, Shekter LR, Rosal R, Weng G, Yang CS, Iyengar R, Miller RJ, Jan LY, Lefkowitz RJ, Hamm HE. Molecular basis for interactions of G protein betagamma subunits with effectors. Science. 1998;**280**:1271–1274. DOI: 10.1126/science.280.5367.1271

[42] Shi CS, Lee SB, Sinnarajah S, Dessauer CW, Rhee SG, Kehrl JH. Regulator of G-protein signaling 3 (RGS3) inhibits Gbeta1gamma 2-induced inositol phosphate production, mitogen-activated protein kinase activation, and Akt activation. J. Biol. Chem. 2001;**276**:24293–24300. DOI: 10.1074/jbc.M100089200

[43] Wang N, Yan K, Rasenick MM. Tubulin binds specifically to the signal-transducing proteins, Gs alpha and Gi alpha 1. J. Biol. Chem. 1990;**265**:1239–1242.

[44] Wang N, Rasenick MM. Tubulin-G protein interactions involve microtubule polymerization domains. Biochemistry. 1991;**30**:10957–10965. DOI: 10.1021/bi00109a021

[45] Roychowdhury S, Panda D, Wilson L, Rasenick MM. G protein alpha subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. J. Biol. Chem. 1999;**274**:13485–13490. DOI: 10.1074/jbc.274.19.13485

[46] Roychowdhury S, Martinez L, Salgado L, Das S, Rasenick MM. G protein activation is prerequisite for functional coupling between Gα/G$\beta\gamma$ and tubulin/microtubules. Biochem. Biophys. Res. Commun. 2006;**340**:441–448. DOI: 10.1016/j.bbrc.2005.12.026

[47] Yu JZ, Dave RH, Allen JA, Sarma T, Rasenick MM. Cytosolic G{alpha}s acts as an intracellular messenger to increase microtubule dynamics and promote neurite outgrowth. J. Biol. Chem. 2009;**284**:10462–10472. DOI: 10.1074/jbc.M809166200

[48] Roychowdhury S, Rasenick MM. G protein beta1gamma2 subunits promote microtubule assembly. J. Biol. Chem. 1997;**272**:31476–31581. DOI: 10.1074/jbc.272.50.31576

[49] Iñiguez-Lluhi JA, Simon MI, Robinshaw JD, Gilman AG. G protein beta gamma subunits synthesized in Sf9 cells. Functional characterization and the significance of the prenylation of gamma. J. Biol. Chem. 1992;**267**:23409–23417.

[50] Higgins JB, Casey PJ. In vitro processing of G protein gamma subunits. Requirements for assembly of an active beta gamma complex. J. Biol. Chem. 1994;**269**:9067–9073.

[51] Yasuda H, Lindorfer MA, Woodfork KA, Fletcher JE, Garrison JC. Role of the prenyl group on the G protein gamma subunit in coupling trimeric G proteins to A1 adenosine receptors. J. Biol. Chem. 1996;**271**:18588–18595. DOI: 10.1074/jbc.271.31.18588

[52] Montoya V, Gutierrez C, Najera O, Leony D, Varela A, Popova J, Rasenick M, Das S, Roychowdhury S. G protein βγ subunits interact with αβ and γ tubulin and play a role in microtubule assembly in PC12 cells. Cell Motil. Cytoskeleton. 2007;**64**:936–950. DOI: 10.1002/cm.20234

[53] Hoebeke J, Van Nigen G, De Brabander MJ. Interaction of nocodazole (R 91734), a new antitumoral drug, with rat brain tubulin. Biochem. Biophys. Res. Commun. 1979;**69**:319–324. DOI: 10.1016/0006-291X(76)90524-6

[54] DeBrabander MJ, Geuens G., Nuydens R., Willebrords R., and De Mey J. Microtubule assembly in livingcells after release from nocodazole block: the effect of metabolic inhibitors, taxol and PH. Cell Biol. Int. Rep.1981;**5**:913–920

[55] Joshi HC, Palacios MJ, McNamara L, Cleveland DW. Gamma-tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. Nature. 1992;**356**:80–83. DOI: 10.1038/356080a0

[56] Oakley BR. Gamma-tubulin: the microtubule organizer? Trends Cell Biol. 1992;**2**:1–5. DOI: 10.1016/0962-8924(92)90125-7

[57] Moritz M, Braunfeld MB, Sedat JW, Alberts B, Agard DA. Microtubule nucleation by gamma-tubulin-containing rings in the centrosome. Nature. 1995;**378**:638–640. DOI: 10.1038/378638a0

[58] Moudjou M, Bordes N, Paintrand M, Bornens M. Gamma-tubulin in mammalian cells: the centrosomal and the cytosolic forms. J. Cell Sci. 1996;**109**:875–887.

[59] Moritz M, Agard DA. Gamma-tubulin complexes and microtubule nucleation. Curr. Opin. Struct. Biol. 2001;**11**:174–181. DOI: 10.1016/S0959-440X(00)00187-1

[60] Job D, Valiron O, Oakley B. Microtubule nucleation. Curr. Opin. Cell Biol. 2003;**15**:111–117. DOI: 10.1016/S0955-0674(02)00003-0

[61] Wedegaertner PB, Wilson PT, Bourne HR. Lipid modifications of trimeric G proteins. J. Biol. Chem. 1995;**270**:503–506. DOI: 10.1074/jbc.270.2.503

[62] Mumby SM, Linder ME. Myristoylation of G-protein alpha subunits. Methods Enzymol. 1994;**237**:254–268. DOI: 10.1016/S0076-6879(94)37067-2

[63] Heidemann SR, McIntosh JR. Visualization of the structural polarity of microtubules. Nature. 1989;**286**:517–519. DOI: 10.1038/286517a0

[64] Gotta M, Ahringer J. Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in Caenorhabditis elegans embryos. Nat. Cell Biol. 2002;**3**:297–301. DOI: 10.1038/35060092

[65] Schaefer M, Petronczki M, Dorner D, Forte M, Knoblich J. Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. Cell. 2001;**107**:183–194. DOI: 10.1016/S0092-8674(01)00521-9

[66] Fuse N, Hisata K, Katzen AL, Matsuzaki F. Heterotrimeric G proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions. Curr. Biol. 2003;**13**:947–954. DOI: http://dx.doi.org/10.1016/S0960-9822(03)00334-8

[67] Du Q, Macara IG. Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cells. 2004;**119**:503–516. DOI: 10.1016/j.cell.2004.10.028

[68] Sanada K, Tsai LH. G protein betagamma subunits and AGS3 control spindle orientation and asymmetric cell fate of cerebral cortical progenitors. Cell. 2005;**122**:119–131. DOI: 10.1016/j.cell.2005.05.009

[69] Siegrist SE, Doe CQ. Microtubule-induced Pins/Galphai cortical polarity in Drosophila neuroblasts. Cell. 2005;**123**:1323–1335. DOI: 10.1016/j.cell.2005.09.043

[70] De Vries L, Fischer T, Tronchère H, Brothers GM, Strockbine B, Siderovski DP, Farquhar MG. Activator of G protein signaling 3 is a guanine dissociation inhibitor for Galpha i subunits. Proc. Natl. Acad. Sci. U. S. A. 2000;**97**:14364–14369. DOI: 10.1073/pnas.97.26.14364

[71] Kimple RJ, Willard FS, Siderovski DP. The GoLoco Motif: heralding a new tango between G protein signaling and cell division. Mol. Interv. 2002;**2**:88–100. DOI: 10.1124/mi.2.2.88

[72] Severin FF, Sorger PK, Hyman AA. Kinetochores distinguish GTP from GDP forms of the microtubule lattice. Nature. 1997;**388**:888–891. DOI: 10.1038/42270

[73] Dehmelt L, Halpain S. Actin and microtubules in neurite initiation: are MAPs the missing link?. J. Neurobiol. 2004;**58**:18–33. DOI: 10.1002/neu.10284

[74] Witte H, Bradke F. The role of the cytoskeleton during neuronal polarization. Curr. Opin. Neurobiol. 2008;**18**:479–487. DOI: 10.1016/j.conb.2008.09.019

[75] Geraldo S, Gordon-Weeks PR. Cytoskeletal dynamics in growth-cone steering. J. Cell. Sci. 2009;**122**:3595–3604. DOI: 10.1242/jcs.042309

[76] Li R, Gundersen GG. Beyond polymer polarity: how the cytoskeleton builds a polarized cell. Nat. Rev. Mol. Cell Biol. 2008;**9**:860–873. DOI: 10.1038/nrm2522

[77] Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. U. S. A. 1976;**73**:2424–2428. DOI: 10.1073/pnas.73.7.2424

[78] Patapoutian A, Reichardt LF. Trk receptors: mediators of neurotrophin action. Curr. Opin. Neurobiol. 2001;**11**:272–280. DOI: 10.1016/S0959-4388(00)00208-7

[79] Cantley LC. The phosphoinositide 3-kinase pathway. Science. 2002;**296**:1655–1657. DOI: 10.1126/science.296.5573.1655

[80] Zhou FQ, Zhou J, Dedhar S, Wu YH, Snider WD. NGF-induced axon growth is mediated by localized inactivation of GSK-3beta and functions of the microtubule plus end binding protein APC. Neuron. 2004;**42**:897–912. DOI: 10.1016/j.neuron.2004.05.011

[81] Govek EE, Newey SE, Van Aelst L. The role of the Rho GTPases in neuronal development. Genes. Dev. 2005;**19**:1–49. DOI: 10.1101/gad.1256405

[82] Hall A, Lalli G. Rho and Ras GTPases in axon growth, guidance, and branching. Cold Spring Harb. Perspect. Biol. 2010;**2**:a001818. DOI: 10.1101/cshperspect.a001818

[83] Reinoso BS, Undie AS, Levitt P. Dopamine receptors mediate differential morphological effects on cerebral cortical neurons in vitro. J. Neurosci. Res. 1996;**43**:439–453. DOI: 10.1002/(SICI)1097-4547(19960215)43:4<439::AID-JNR5>3.0.CO;2-G

[84] Kwon JH, Vogt Weisenhorn DM, Downen M, Roback L, Joshi H, Wainer BH. Beta-adrenergic and fibroblast growth factor receptors induce neuronal process outgrowth through different mechanisms. Eur. J. Neurosci. 1998;**10**:2776–2789. DOI: 10.1111/j.1460-9568.1998.00315.x

[85] Lotto B, Upton L, Price DJ, Gaspar P. Serotonin receptor activation enhances neurite outgrowth of thalamic neurones in rodents. Neurosci. Lett. 1999;**269**:87–90. DOI: 10.1016/S0304-3940(99)00422-X

[86] He JC, Gomes I, Nguyen T, Jayaram G, Ram PT, Devi LA, Iyengar R. The G alpha(o/i)-coupled cannabinoid receptor-mediated neurite outgrowth involves Rap regulation of Src and Stat3. J. Biol. Chem. 2005;**280**:33426–33434. DOI: 10.1074/jbc.M502812200

[87] Igarashi M, Strittmatter S, Vartanian T, Fishman MC. Mediation by G proteins of signals that cause collapse of growth cones. Science. 1993;**259**:77–84. DOI: 10.1126/science.8418498

[88] Wolfgang WJ, Clay C, Parker J, Delgado R, Labarca P, Kidokoro Y, Forte M. Signaling through Gs alpha is required for the growth and function of neuromuscular synapses in Drosophila. Dev. Biol. 2004;**268**:295–311. DOI: 10.1016/j.ydbio.2004.01.007

[89] Sachdev P, Menon S, Kastner DB, Chuang JZ, Yeh TY, Conde C, Caceres A, Sung CH, Sakmar TP. G protein beta gamma subunit interaction with the dynein light-chain component Tctex-1 regulates neurite outgrowth. EMBO J. 2007;**26**:2621–32. DOI: 10.1038/sj.emboj.7601716

[90] Wang K, Wong YH. G protein signaling controls the differentiation of multiple cell lineages. Biofactors. 2009;**35**:232–238. DOI: 10.1002/biof.39

[91] Sarma T, Voyno-Yasenetskaya T, Hope TJ, Rasenick MM. Heterotrimeric G-proteins associate with microtubules during differentiation in PC12 pheochromocytoma cells. FASEB J. 2003;**17**:848–859. DOI: 10.1096/fj.02-0730com

[92] Sarma T, Koutsouris A, Yu JZ, Krbanjevic A, Hope TJ, Rasenick MM. Activation of microtubule dynamics increases neuronal growth via the nerve growth factor (NGF)- and Gαs-mediated signaling pathways. J. Biol. Chem. 2015;**290**:10045–10056. DOI: 10.1074/jbc.M114.630632

[93] Sierra-Fonseca JA, Najera O, Martinez-Jurado J, Walker EM, Varela-Ramirez A, Khan AM, Miranda M, Lamango NS, Roychowdhury S. Nerve growth factor induces neurite outgrowth of PC12 cells by promoting Gβγ-microtubule interaction. BMC Neurosci. 2014;**15**:32. DOI: 10.1186/s12868-014-0132-4

[94] Okae H, Iwakura Y. Neural tube defects and impaired neural progenitor cell proliferation in Gbeta1-deficient mice. Dev. Dyn. 2010;**239**:1089–1101. DOI: 10.1002/dvdy.22256

[95] Zhang JH, Pandey M, Seigneur EM, Panicker LM, Koo L, Schwartz OM, Chen W, Chen CK, Simonds WF. Knockout of G protein β5 impairs brain development and causes multiple neurologic abnormalities in mice. J. Neurochem. 2011;**119**:544–554. DOI: 10.1111/j.1471-4159.2011.07457.x

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