Journal of Cellular Biochemistry 70:553–562 (1998)

G Protein β Subunit Is Closely Associated With Microtubules

Han-Chung Wu,[2] **Pei-Hsin Huang,**[1] **and Chin-Tarng Lin**[1,2]*

[1]Institute of Pathology, College of Medicine, National Taiwan University, Taipei, Taiwan
[2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

Abstract Previously, we have identified the association of G protein β subunit (Gβ) with mitotic spindles in various mammalian cells. Since microtubules are the main component of mitotic spindles, here we have isolated bovine brain microtubules and purified Gβ subunit to identify the close association of Gβ subunit with purified brain microtubules and have shown the direct incorporation of Gβ subunit into the microtubules both *in vitro* and *in vivo.* It was found that: (1) microtubular fraction isolated from bovine brain contained Gβ subunit, (2) coimmunoprecipitation demonstrated that Gβ subunit could be coprecipitated with tubulin, (3) addition of purified Gβ subunit into cytosolic extract for microtubule assembly caused direct incorporation of Gβ subunit into assembled microtubules and increased the association of microtubule-associated proteins with microtubules, and (4) incubation of exogenous Gβ subunit with detergent-permeabilized cells resulted in direct incorporation of Gβ subunit into microtubule fibers and depolymerized tubulin molecules. We conclude that G protein β subunit is closely associated with microtubules and may play an important role in the regulation of microtubule formation in addition to its regulatory role in cellular signal transduction. J. Cell. Biochem. 70:553–562, 1998. © 1998 Wiley-Liss, Inc.

Key words: coimmunoprecipitation of Gβ subunit and tubulin; in situ incorporation of Gβ protein into microtubules; microtubule assembly

Heterotrimeric GTP-binding regulatory proteins (G proteins) are a family of signal coupling proteins that function in the transduction of signals from a variety of transmembrane receptors to appropriate intracellular effector proteins, such as adenylyl cyclase, phosphatidylinositol-specific phospholipase C, cGMP phosphodiesterase, and ion channels [Gilman, 1987; Neer and Clapham, 1988; Bourne et al., 1990; Simon et al., 1991, Chen et al., 1997]. The G proteins consist of two functional units: an α-subunit that binds GTP and a βγ-subunit that functions as a single entity. Activation of the G proteins by receptors is achieved by exchange of bound GDP for GTP. The binding of GTP promotes the dissociation of α-subunit from βγ-subunits, except that GTP binding to Gs does not promote subunit dissociation *in vitro.* in certain condition [Basi et al., 1996]. The free GTP bound-α subunit and βγ subunits each activate target effectors. The functional state of G α-GTP is hydrolyzed to GDP by the intrinsic GTPase activity of α-subunits. All isoforms of α subunits are GTPases, although the intrinsic rate of GTP hydrolysis varies greatly from one type of α-subunit to another [Carty et al., 1990; Linder et al., 1990]. Once GTP is cleaved to GDP, α and βγ subunits reassociate, become inactive, and return to the receptor. Although the βγ subunit does not bind GTP, its active lifetime depends on the rate of GTP hydrolysis by an α subunit.

Mammalian cells have > 21 different G protein α subunits (17 gene products, some with alternatively spliced isoforms) [Simon et al., 1991; Kaziro et al., 1991]. Most α subunits are widely expressed. Individual cells usually contain at least four or five types of α subunit [Neer et al., 1994; Neer, 1995]. The β subunit consists of five subtypes and the β 5 subunit has been identified in the vertebrate retina [Watson et al., 1996]. The amino acid sequence of each has between 53% and 90% identity [Watson et al., 1994; Simon et al., 1991].

Contract grant sponsor: National Science Council; Contract grant number: NSC-87-2314-B-002-316; Contract grant sponsor: Academia Sinica, Taipei (CTL).

*Correspondence to: Dr. Chin-Tarng Lin, Department of Pathology, National Taiwan University Hospital, Taipei 100, Taiwan.

Received 24 March 1998; Accepted 26 March 1998

© 1998 Wiley-Liss, Inc.

In the past, $G\beta\gamma$ were thought to be only a negative regulator to stabilize, localize, and reverse the action of $G\alpha$ by reforming the inactive heterotrimer and guiding $G\alpha$ back to the receptor for reactivation. Recent experiments have shown that $\beta\gamma$ subunits can also direct downstream regulation [Clapham et al., 1993]. For example, $G\beta\gamma$ and $G\alpha$ both activate the cardiac $K+$ channel [Codina et al., 1987; Logothetis et al., 1987; Logothetis et al., 1988] and muscarinic potassium channels [Reureny et al., 1994]. $G\beta\gamma$ also regulate the activity of adenylyl cyclase by activating type II [Chen et al., 1997] and IV, inhibiting type I, and have no effect on type III, V, and VI [Tang and Gilman, 1991; Federman et al., 1992; Taussig, 1993] and activate the β-subfamily of phospholipase C ($\beta 3 > \beta 2 > \beta 1$) [Boyer et al., 1992; Camps et al., 1992; Katz et al., 1992; Park et al., 1993; Smrcka and Sternweis, 1993]. $G\beta\gamma$ modulate receptor function by controlling receptor phosphorylation and subsequent desensitization [Haga and Haga, 1992; Inglese et al., 1992; Pitcher et al., 1992] and activate phospholipase A2 [Jelsema and Axelrod, 1987; Kim et al., 1989] and phosphoinositide 3 kinase [Stephens, 1994]. $G\beta\gamma$ is also the major mediator of the yeast mating pheromone pathway [Whiteway et al., 1989].

G proteins are not only present in the plasma membrane of each mammalian cell type, but also in the intracellular organelles, e.g., Gαi-3 on Golgi membrane [Stow et al., 1991; Wilson et al., 1994], Giα in RER [Audigrer et al., 1988], and within lysosome [Lewis et al., 1991] and in the nucleus [Takei et al., 1992], and Gn in neutrophil cytoplasm [Bakoch et al., 1988].

We have shown the association of Gβ protein [Lin et al., 1992] and Goα protein [Wu and Lin, 1994] with mitotic spindles. Since microtubules are the major component of mitotic spindles, in this report we describe the observed association between Gβ protein and microtubules. Our results show direct association of the Gβ subunit with microtubules both in culture cells and in a cell-free system.

MATERIALS AND METHODS

Preparation of Antibodies Against Gβ Subunit

Polyclonal rabbit antibodies against the N-terminus of β2 subunit of G protein was previously described [Lin et al., 1992]. Other antibodies, such as anti-α- and -β-tubulins, were purchased from Sigma Chemical Co. (St. Louis, Mo). The peroxidase and fluorescence-labeled antirabbit antibodies were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA).

Procedures for Purification of Microtubule Proteins

Two methods were used for the purification of microtubule proteins. The first method used temperature-dependent reversible self-assembly of microtubules [Vallee and Borisy, 1978; Vallee, 1986a] and the other used taxol to promote assembly [Vallee, 1982; Vallee, 1986b]. Briefly, bovine brains were obtained from a local slaughterhouse. The brain tissue was homogenized with a Teflon-in-glass homogenizer in 1.5 volumes of PEM buffer (0.1 M PIPES, pH 6.6, 1 mM EGTA, 1 mM $MgCl_2$). The homogenate was centrifuged at 30,000 xg for 90 min. The supernatant was recovered and centrifuged at 180,000 xg for 90 min. The recovered supernatant from this step is referred to as the cytosolic extract. GTP and ATP were then added to the cytosolic extract until a final concentration of 0.1 and 2.5 mM, respectively, was reached. The cytosolic extract was warmed to 37°C for 15 min with gentle swirling to induce microtubule polymerization. Prewarmed sucrose underlayer solution (PEM buffer containing 10% sucrose and 1 mM GTP) was carefully introduced to the bottom of the tube with a Pasteur pipette. The centrifuge tubes were carefully placed in a prewarmed rotor and centrifuged at 30,000 xg at 37°C for 30 min. The tubes containing the microtubular pellets were rinsed with PEM buffer and resuspended by addition of ice-cold PEM buffer containing 1 mM GTP. The resuspended microtubules were incubated on ice for 30 min to depolymerize the microtubules and then centrifuged at 30,000 xg at 4°C for 30 min. The supernatant containing depolymerized microtubule proteins was warmed to 37°C for 15 min again to induce microtubule polymerization. The supernatant was centrifuged further at 30,000 xg at 37°C for 30 min. The tubes containing the microtubular pellets were rinsed with PEM buffer, immersed in liquid nitrogen, and stored at −80°C. Microtubules were also purified by using taxol to promote assembly. The above cytosolic extract preparation was mixed with 20 μM taxol and 1 mM GTP and then incubated at 37°C for 15

min for microtubule assembly. The sample was centrifuged at 37°C at 30,000 xg for 30 min and the pellets were rinsed with PEM buffer. The pellets were resuspended in PEM buffer containing 20 μM taxol and 1 mM GTP. The microtubule fraction was then analyzed by Western blotting.

Western Blot Analysis

The cytosolic extract, the partially purified G protein from bovine brains [Lin et al., 1992] and microtubular pellets were subjected to Western blot analysis using 12% SDS-PAGE [Lin et al., 1992]. Proteins were transferred electrophoretically to polyvinylidene difluoride (PVDF) microporous membranes using a Hoefer Transblot apparatus (Hoefer Scientific Instruments, San Francisco, CA) in a transfer buffer (25 mM Tris base, 190 mM glycine, 0.01% SDS, and 20% methanol). The PVDF membranes were incubated with 5% skim milk in TBS solution (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) to block nonspecific binding sites on the blot and then incubated with primary antibodies at 37°C for 1 hr. For the negative control, the antibodies were pretreated with excess peptide antigen. Peroxidase-conjugated goat antirabbit IgG was used as the second antibody. The blot was immersed in the substrate solution (0.05% 3,3′ diaminobenzidine-4HCl plus 0.01% H_2O_2 in 50 mM Tris-HCl, pH 7.4) at room temperature with agitation until the bands appeared.

Immunoprecipitation Experiment

For immunoprecipitation experiments [Lin et al., 1992], the cytosolic extract solution that contained protease inhibitors (1 mM PMSF, 50 μg/ml aprotinin, and 50 μM leupeptin), 150 mM NaCl, 0.1% SDS, and 1 mM EDTA in 0.1 M PEM buffer (pH 6.6) was mixed with affinity-purified anti-GβN antibodies at 4°C for 1 hr. Antibodies were precipitated by incubation with protein A Sepharose (Sigma) for 1 hr at 4°C. The protein A-Sepharose pellet was then washed three times with 25 mM Tris-HCl (pH 8.0) buffer, containing 50 mM NaCl, 0.5% sodium deoxycholate and 0.2% NP-40. Finally, it was dissolved in the sample buffer for Western blot analysis by 12% SDS-PAGE.

Isolation of βγ Subunits of G Protein

Pertussis-toxin substrate G proteins were partially purified from bovine brain according to a previously published method [Sternweis et al., 1981] with some modifications [Lin et al., 1992]. These G proteins were dialyzed against buffer A (20 mM Tris, pH 8.0, 0.1 M NaCl, 0.3% cholate, 6 mM $MgCl_2$, 1 mM EDTA, 10 mM NaF, 10 μM $AlCl_3$, and 1 mM DTT), then applied to Octyl Sepharose column equilibrated with buffer A. After the column was washed with a twofold gel volume of buffer A, βγ subunits were eluted with linear gradients of NaCl (100–50 mM) and cholate (0.3–1.2%). The purity of the βγ subunits was determined by 12% SDS-PAGE, and the gel was stained with silver reagent.

In Situ Incorporation of Purified Gβγ Proteins Into Microtubules in Permeabilized Cells

Permeabilized cells were produced by a procedure described by Shiina et al. [1994] with some modifications. Briefly, human fibroblasts (HFY) were treated with 80 PME [80 mM Pipes (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA, 0.2 mM PMSF, leupeptin (2 μg/ml), pepstatin (2 μg/ml), 1% aprotinin, and 1 mM DTT] containing 0.5% Triton X-100 for 2 min at 37°C. The cells were then washed with 80 PME buffer and incubated with 80 PME buffer or 80 PME buffer containing different concentrations of purified Gβγ proteins at 37°C for 30 min. The cells were washed with 80 PME buffer and fixed with 3% paraformaldehyde for immunofluorescence staining.

Immunofluorescence Staining and Double Localizations of Gβ and Tubulin

The localization of Gβ and tubulin by immunoflurorescence staining was performed according to our previously published method [Wu and Lin, 1994]. Briefly, fibroblasts were fixed in 3% paraformaldehyde for 10 min, then incubated overnight with affinity purified rabbit antibodies against Gβ protein [Lin et al., 1992] or monoclonal antibody against tubulin, respectively, followed by incubation with fluorescein or rhodamine-labeled second antibodies, and then mounted for fluorescent microscopic observation. For double localization of Gβ and tubulin, a similar immunofluorescence method to that described above was used, except that two mixed primary and two mixed secondary antibodies were applied. The antibodies anti-Gβ protein and anti-β tubulin were used at a concentration of 2 μg/ml and 1 μg/ml, respectively.

RESULTS

Identification of Gβ Protein in Purified Microtubular Fraction

To elucidate the actual relationship between the microtubules and Gβ protein, the temperature-dependent reversible assembly method [Vallee and Borisy, 1978; Valle, 1986a] and the taxol stabilization method [Vallee, 1982; Vallee, 1986b] were used to isolate microtubules from bovine brains. When the *in vitro* assembled microtubule preparations from bovine brain were analyzed by Western blotting, a Gβ protein band of Mr 35 ± 1 kD was identified in the purified microtubular fraction by a specific anti-Gβ antibody (Fig. 1, lane 2). An α-tubulin band was also found by an anti-α tubulin antibody (Fig. 1, lane 3). The specific Gβ protein band was not seen if the antibodies were preincubated with excess Gβ peptide antigen (Fig. 1, lane 1). When microtubules were prepared by the taxol stabilization method, Gβ protein was detectable in the cytosolic extract, postmicrotubular supernatant, and microtubular fraction by Western blotting. A clear Gβ band was shown in the cytosolic extract (Fig. 1, lane 2) but reduced in the postmicrotubule supernatant (Fig. 1, lane 4); Gβ protein was markedly enriched in the microtubular fraction (Fig. 1, lane 6). When anti-Gβ antibodies were incubated with cytosolic extracts of bovine brain and precipitated by protein A-conjugated beads and immunoprecipitates then analyzed by Western blotting using anti-Gβ, anti-α-tubulin, and anti-β-tubulin antibodies, a clear Gβ protein band (Mr 35 ± 1 kD) and an IgG heavy chain band were detected (Fig. 2A, lane 1). Similarly, an α-tubulin (Fig. 2A, lane 2) and a β-tubulin band (Fig. 2A, lane 3) were also identified in the immunoprecipitate. If a preimmune serum was used to precipitate the protein, no specific protein band (Fig. 2B, lane 4, 5, 6) could be seen except for the IgG heavy chain band (Fig. 2B, lane 4).

Gβ protein Is Incorporated Into Microtubular Fraction in Cell and Cell-free Systems

For further confirmation that Gβ protein can be incorporated into the microtubular fraction, Gβγ proteins were purified from bovine brain and tested for their ability to be co-purified during microtubule assembly in a cell-free system. When Gβγ proteins obtained from partially purified bovine brain G proteins were analyzed with 12% SDS-PAGE and stained by the silver reagent, a single band of Gβ protein (Mr 35 ± 1 kD) was seen (Fig. 3). (The γ subunit has a molecular mass of 8 kD and did not show in the gel.) If these purified Gβγ proteins were added in increasing amounts to the brain cytosolic extracts and the microtubules were purified by temperature-dependent assembly-disassembly, the SDS-PAGE analysis of these microtubular fractions showed one enriched Gβ band in a concentration-dependent manner.

Several possible microtubule-associated protein bands also appeared in the lanes that had 25 and 50 μg/ml of Gβγ protein added (Fig. 4A, lanes 3 and 4). The nature of the prominent band at ~40kD was not clear. However, in the postmicrotubular supernatant (Fig. 4B), no Gβ band, but a prominent BSA band in lane 5 could be seen. The presence of Gβ protein in the microtubular fraction and the gradual increase in its quantity were confirmed by Western blotting using anti-Gβ antibodies (Fig. 5). When human fibroblasts were permeabilized with a detergent (0.5% Triton X-100) and then incubated with different concentrations of purified Gβγ protein, a direct incorporation of Gβγ protein into the microtubular region was shown, especially when 5 and 50 μg/ml of Gβγ protein were added (Fig. 6D,F). In the 5 μg/ml of Gβγ case, the association of Gβγ protein to the microtubules may be limited to the peripheral part and certain central region of the cytoplasm only due to the low dose of Gβγ protein (Fig. 6D). But in the high dose of Gβγ protein (50 μg/ml) case, not only the peripheral microtubular areas, but the central cytoplasmic region also revealed fibrillar and granular staining of Gβγ protein (Fig. 6F). The immunofluorescent staining of antitubulin in those cells (Fig. 6ACE) revealed partial depolymerization (granular appearance) of microtubules, mainly because in the present experiment we have permeabilized the fibroblasts with 0.5% Triton X-100 for 2 min. The effect of this detergent on the individual fibroblast may cause leakage of some important soluble cytosolic proteins that are important for microtubule polymerization. Since some microtubules showed different degree of focal depolymerization in these damaged cells, the distribution of Gβγ protein could also reveal partial overlap with microtubules and partial overlap with disorganized, rearranged tubulin molecules (Fig. 6D,F).

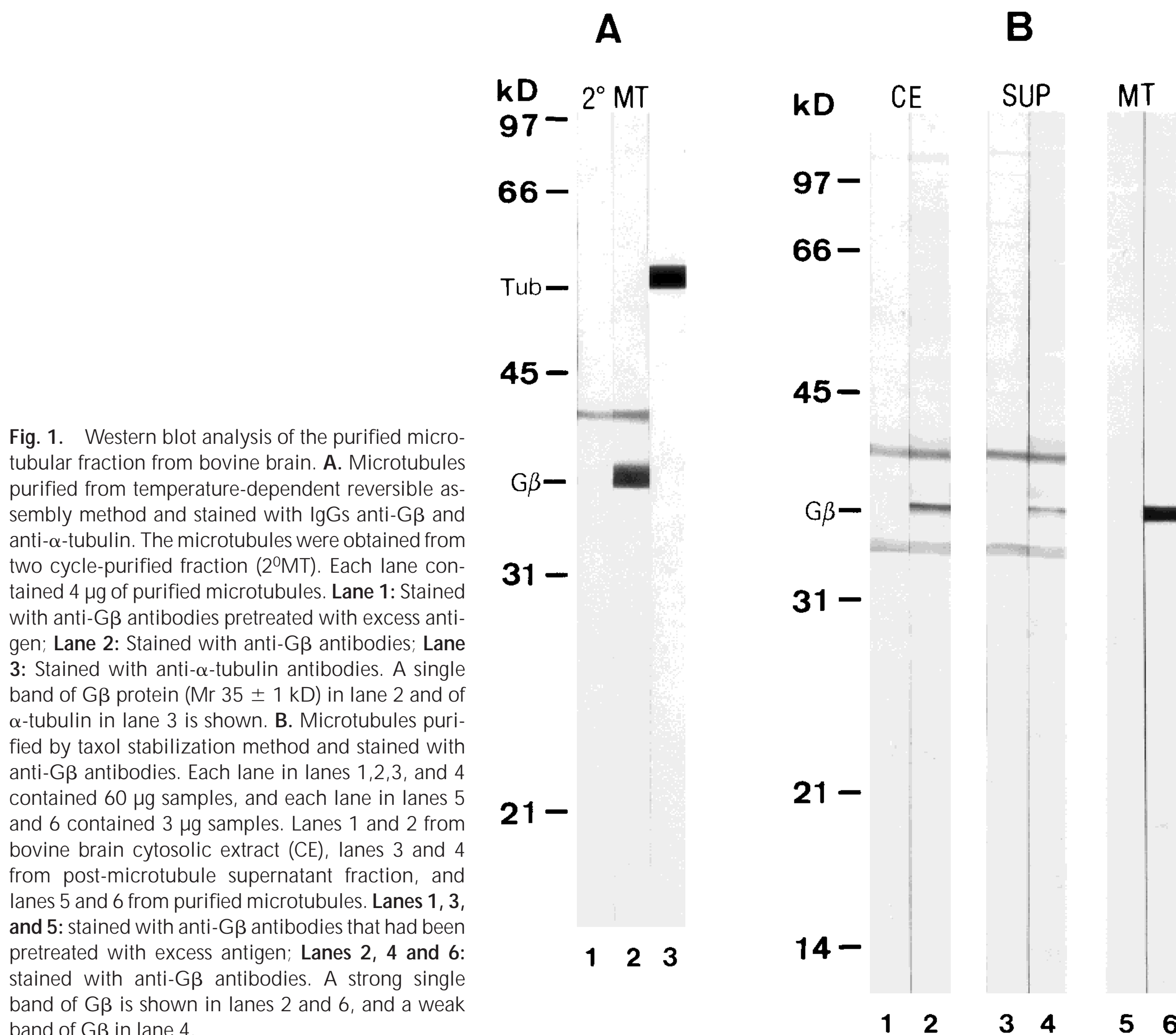


Fig. 1. Western blot analysis of the purified microtubular fraction from bovine brain. **A.** Microtubules purified from temperature-dependent reversible assembly method and stained with IgGs anti-Gβ and anti-α-tubulin. The microtubules were obtained from two cycle-purified fraction (2^0MT). Each lane contained 4 μg of purified microtubules. **Lane 1:** Stained with anti-Gβ antibodies pretreated with excess antigen; **Lane 2:** Stained with anti-Gβ antibodies; **Lane 3:** Stained with anti-α-tubulin antibodies. A single band of Gβ protein (Mr 35 ± 1 kD) in lane 2 and of α-tubulin in lane 3 is shown. **B.** Microtubules purified by taxol stabilization method and stained with anti-Gβ antibodies. Each lane in lanes 1,2,3, and 4 contained 60 μg samples, and each lane in lanes 5 and 6 contained 3 μg samples. Lanes 1 and 2 from bovine brain cytosolic extract (CE), lanes 3 and 4 from post-microtubule supernatant fraction, and lanes 5 and 6 from purified microtubules. **Lanes 1, 3, and 5:** stained with anti-Gβ antibodies that had been pretreated with excess antigen; **Lanes 2, 4 and 6:** stained with anti-Gβ antibodies. A strong single band of Gβ is shown in lanes 2 and 6, and a weak band of Gβ in lane 4.

DISCUSSION

To investigate the relationship between Gβ protein and microtubules, we have isolated microtubules from bovine brains by two methods: one used temperature-dependent reversible self-assembly of microtubules and the other used taxol to promote microtubule assembly. The microtubules isolated by both methods all showed a prominent tubulin band and several weak bands, including the ~36kD band, in the Coomassie staining of SDS-PAGE gel (data not shown), and a clear Gβ band in Western blotting (Fig. 1), indicating that Gβ protein is associated with microtubules. In addition, in the immunoprecipitation experiment, the α and β tubulins could be coprecipitated with Gβ protein by antibody against Gβ protein (Fig. 2), suggesting that Gβ protein may interact with tubulin directly or indirectly. Furthermore, with the addition of purified Gβγ protein into the brain cytosolic extract, the exogenous Gβ protein was clearly incorporated into the microtubular fraction, and the quantity of incorporated Gβ protein was dose-dependent (Figs. 4, 5). These results strongly indicate that Gβγ protein can be incorporated into the polymerized microtubular fraction. To further support this finding, an *in situ* visualization of incorporation of exogenous Gβγ proteins into permeabilized cellular microtubules was performed. Results showed a fraction of exogenous Gβγ subunits incorporating onto the microtubule fibers and depolymerized tubulin molecules in the detergent treated cells, and the degree of incorporation was variable in each cell and also dose-dependent (Fig. 6). The above experiments

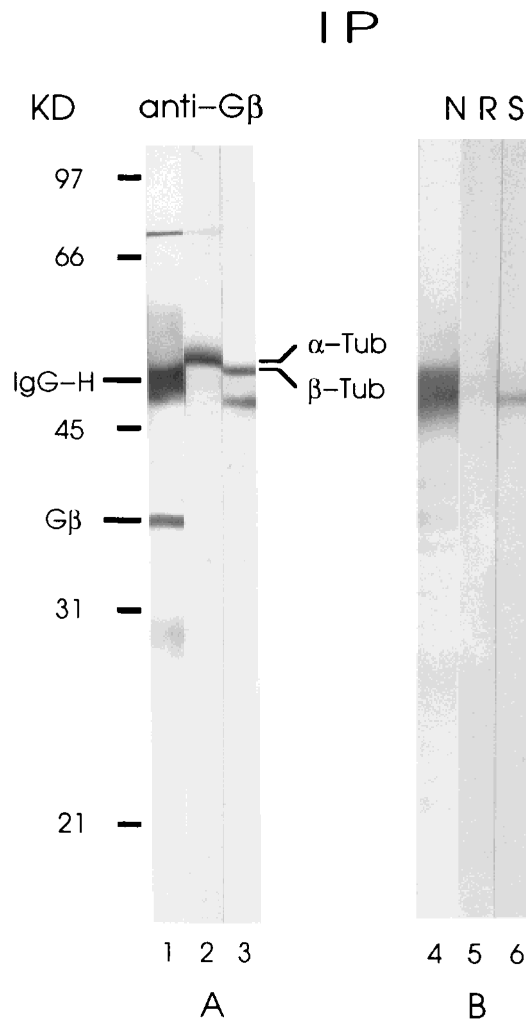


Fig. 2. Coimmunoprecipitation and Western blotting of Gβ protein and tubulin from bovine brain cytosolic extracts. The cytosolic extracts were immunoprecipitated by affinity purified IgG anti-Gβ and second antibodies **(A, lanes 1, 2, 3)** and by a preimmune serum **(B, lanes 4, 5, 6)**. The precipitates were analyzed by 12% SDS-PAGE and immunoblotting, and stained with antibodies anti-Gβ (lanes 1 and 4), anti-α-tubulin (lanes 2 and 5), and anti-β-tubulin (lanes 3 and 6). Lane 1 shows a Gβ protein band, one IgG heavy chain band and a nonspecific band; lane 2 reveals a single band of α-tubulin, and in lane 3 a β-tubulin band and a nonspecific band appears. No specific band in lanes 4 (except for the IgG heavy chain band), 5, and 6 is seen. NRS: normal rabbit serum.

suggest that the Gβ subunit is closely associated with microtubules and may play some role in regulation of microtubule formation.

It has been shown that the structure of the β subunit contains seven repeating units (WD repeats) as described by Neer [1995] and Garcia-Higuera et al. [1996]. This family of proteins is engaged not only in signal transduction, but also in control of cell division, cytoskeletal assembly, vesicle fusion, etc. In addition, the WD-repeat protein IC78 has also been shown to bind to tubulin [King et al., 1991] and to be regulated for assembly of dynein onto flagellar microtubules [Wilkerson et al., 1995]. The present experiments provide evidence to support the notion that Gβ protein can bind to microtubules and may engage in microtubule assembly.

It is well known that the extent of microtubule stabilization by the microtubule-associated proteins (MAPs) depends on the affinity of these proteins for the microtubules and that this affinity is under cellular control. Phosphorylation of MAPs often suppresses microtubule assembly [Gelfand, 1991; Lindwall and Cole, 1994]. Several MAPs have been reported to be phosphorylated by protein kinases, such as tau protein [Correas et al., 1992; Baudier and Cole, 1987] and MAP2 [Hoshi et al., 1992]. In our experiments, the quantity of microtubule-associated proteins (MAPs) in the microtubular fraction was also increased when the amount of incorporated Gβ proteins in polymerized microtubules was increased (Fig. 5A, lane 4). This may be due to a modification of MAPs by the Gβ subunits and, subsequently, increased affinity

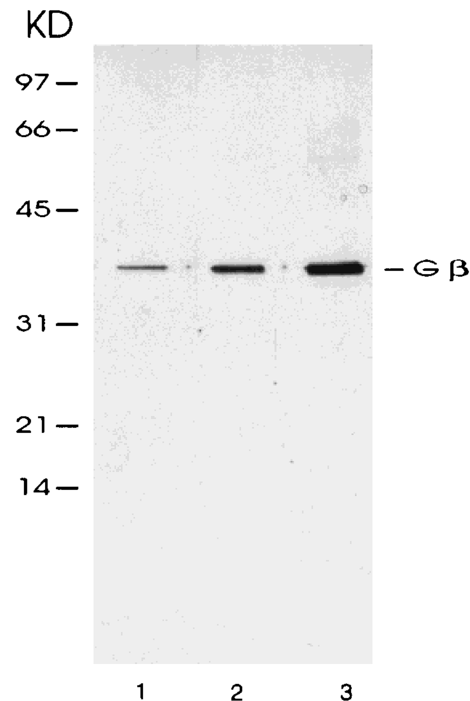
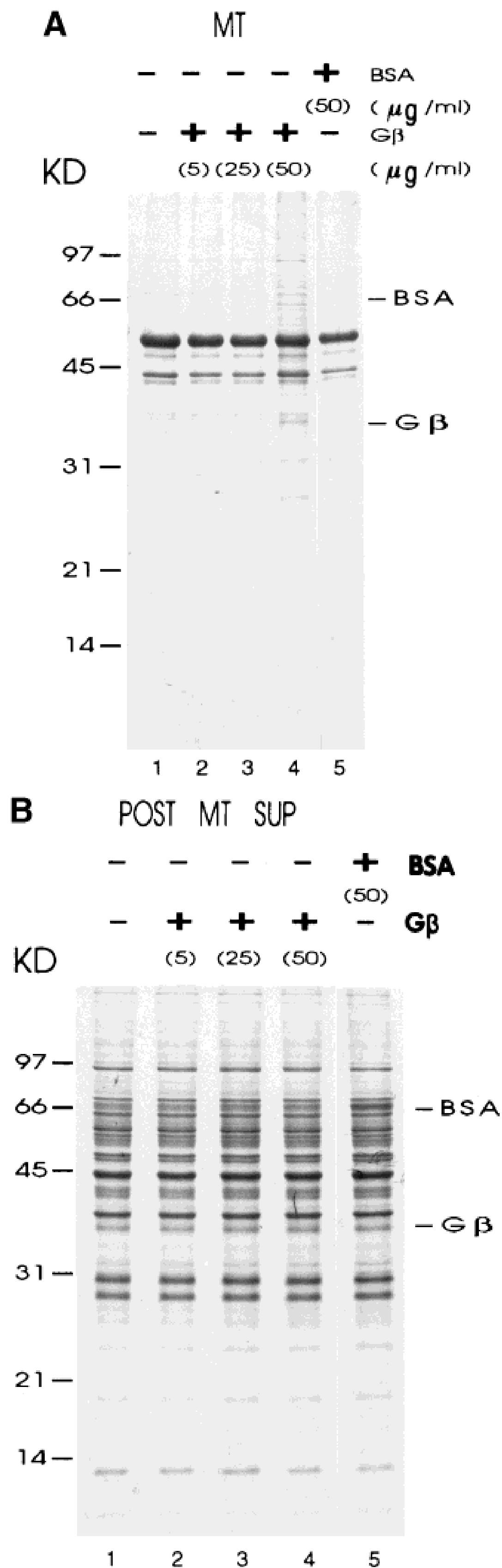


Fig. 3. SDS-PAGE analysis of purified Gβγ subunit proteins. The gel was stained with silver reagent. **Lane 1** contained 0.1 μg; **lane 2**, 0.2 μg; and **lane 3**, 0.5 μg of Gβγ subunit. A single band of Gβ protein (Mr 35 ± 1 kD) in each lane is shown. The Gγ band is invisible in this gel due to its small molecular weight.



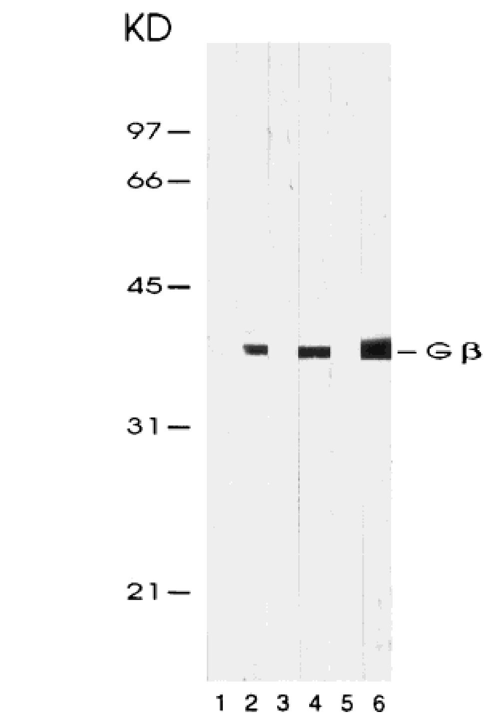


Fig. 5. Western blot analysis of Gβ protein in the microtubular fractions obtained from (Fig. 4A). **Lanes 1** and **2** were added with 0 μg/ml, **Lanes 3** and **4** with 5 μg/ml, and **Lanes 5** and **6** with 50 μg/ml of Gβγ subunit proteins. Lanes 2, 4, and 6 were stained with anti-Gβ IgG, and Lanes 1, 3, and 5 were stained with the same antibodies pretreated with excess antigen. A strong band of Gβ protein is seen in Lane 6, a weaker band in Lane 4 and the weakest in Lane 2; no band is shown in Lanes 1, 3, 5.

of the MAPs to microtubule assembly. However, the detailed mechanism of Gβ protein in regulation of microtubule assembly needs further investigation. Currently an intensive study using transfection of Gβ antisense oligonucleotide into NPC cells and fibroblasts and observation of the change of microtubules and cell behavior in these target cells is underway.

Fig. 4. Demonstration of incorporation of purified exogenous Gβγ subunits into microtubular fraction. SDS-PAGE analysis of Gβγ subunits in the microtubule-assembled (MT) and postmicrotubule supernatant (post MT sup) fractions. The cytosolic extracts from bovine brain were added to 0 μg/ml (**A** and **B**, **Lane 1**), 5 μ g/ml (**A** and **B**, **Lane 2**), 25 μg/ml (**A** and **B**, **Lane 3**), 50 μg/ml of Gβγ proteins (**A** and **B**, **Lane 4**), and 50 μg/ml of bovine serum albumin (BSA) (**A** and **B**, **Lane 5**), respectively, then the microtubules were assembled at 37°C for 30 min. The microtubular fraction **(A)** and postmicrotubular supernatant **(B)** were analyzed with 12% SDS-PAGE and stained with Coomassie blue dye. In A, Lanes 3 and 4, one Gβ band and several possible microtubule-associated protein bands are seen; no other specific band is revealed in Lane 2 when compared with Lanes 1 and 5. In B, Lane 5 shows a prominent BSA band, but no clear Gβ band is seen in Lanes 1–5.

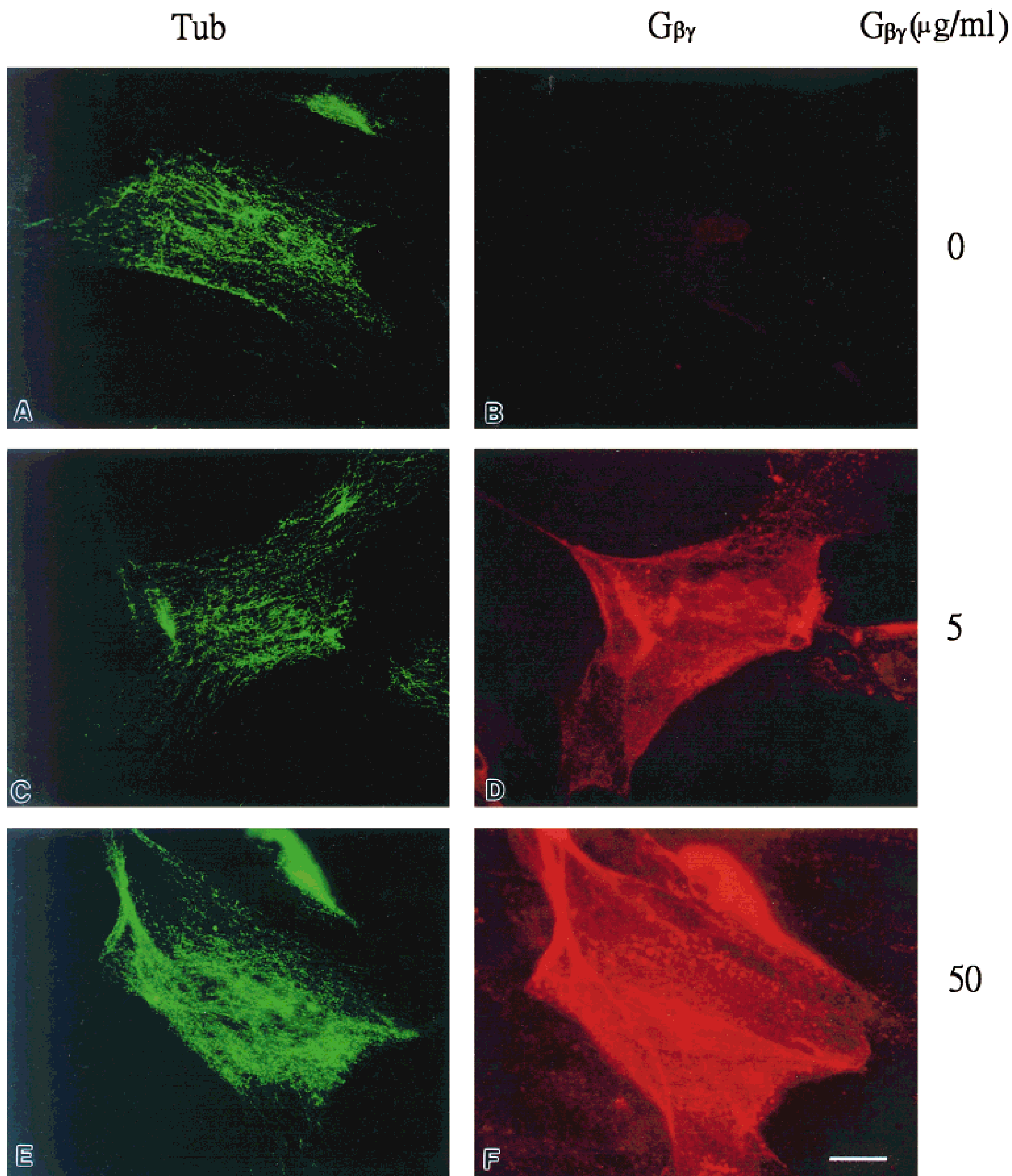


Fig. 6. *In situ* visualization of exogenous Gβ protein-incorporated microtubules in permeabilized cells by double localization of tubulin (tub) and Gβ protein. The permeabilized fibroblasts were incubated with 0 **(A, B)**, 5 **(C, D)**, and 50 **(E, F)** μg/ml of purified Gβγ proteins for 30 min, washed and fixed for double immunofluorescence staining. A, C, E show microtubule distribution. The detergent-treated cells reveal marked depolymerization of microtubules with individual or aggregated granular appearance of tubulin distributed in the cytoplasm. B, D, F reveal anti-Gβ staining. More Gβ staining was shown in D and F when the cells were incubated with exogenous Gβ proteins. However, some fibrillar and aggregated granular staining also can be seen, roughly corresponding to the tubulin distribution in C and E (Scale bar = 10 μm).

ACKNOWLEDGMENTS

We thank Drs. K-J. Chang, Y.J. Chern, and T. Tan for their critical reading of this manuscript.

REFERENCES

Audigrer Y, Nigam SK, Blobel G (1988): Identification of a G protein in rough endoplasmic reticulum of canine pancreas. J Biol Chem 263:16352–16357.

Bakoch GM, Bickford K, Bohl BP (1988): Subcellular localization and quantitation of the major neutrophil pertussis toxin substrate, Gn. J. Cell Biol 106:1927–1936.

Basi NS, Okuya S, Rebois RV (1996): GTP binding to Gs does not promote subunit dissociation. Cellular Signalling 8:209–215.

Baudier J, Cole RD (1987): Phosphorylation of tau proteins to state like that in Alzheimer's brain is catalyzed by a calcium/calmodulin dependent kinase and modulated by phospholipids. J Biol Chem 262:17577–17583.

Bourne HR, Sanders DA, McCormick F (1990): The GTPase superfamily: A conserved switch for diverse cell function. Nature (Lond) 348:125–132.

Boyer JL, Waldo GL, Karden TK (1992): βγ-subunit activation of G-protein-regulated phospholipase C. J Biol Chem 267:25451–25456.

Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P (1992): Isozyme-selective stimulation of phospholipase C-β2 by G protein βγ-subunits. Nature (Lond) 360:684–686.

Carty DJ, Padrell E, Codina J, Brinbumer L, Hildebrant JD, Lyehgar RJ (1990): Distinct guanine nucleotide binding and release properties of the three Gi proteins. J Biol Chem 265:6268–6273.

Chen Y, Weng G, Li J, Harry A, Pieroni J, Dingus J, Hildebrandt JD, Guarnieri F, Weinstein H, Iyengar R (1997): A surface on the G protein beta-subunit involved in interactions with adenylyl cyclases. Proc Natl Acad Sci USA 94:2711–2714.

Clapham DE, Neer E (1993): New roles for G-protein βγ-dimers in transmembrane signalling. Nature (Lond) 365: 403–406.

Codina J, Yatani A, Grenet D, Brown AM, Birnbaumer L (1987): The alpha subunit of Gk opens atrial potassium channels. Science (Wash. D.C.) 236:442–445.

Correas I, Diaz NJ, Avila J (1992): Microtubule-associated protein tau is phosphorylated by protein kinase C on its tubulin binding domain. J Biol Chem 267:15721–15728.

Federman AD, Conklin BR, Schrader KA, Reed RR, Bourne HR (1992): Hormonal stimulation of adenylyl cyclase through Gi-protein βγ subunits. Nature (Lond) 356:159–161.

Garcia-Higuera I, Fenoglio J, Li Y, Lewis C, Panchenko MP, Reiner O, Smith TF, Neer EJ (1996): Folding of proteins with WD-repeats: Comparison of six members of the WD-repeat superfamily to the G protein beta subunit. Biochemistry 35:13985–13994.

Gelfand VI (1991): Microtubule dynamic: mechanism, regulation, and function. Annu Rev Cell Biol 7:93–116.

Gilman AC (1987): G Proteins: Tranducers of receptor-generated signals. Annu Rev Biochem 56:615–649.

Haga K, Haga T (1992): Activation by G protein subunits of agonist-or light-dependent phosphorylation by muscarinic acetylcholine receptors and rhodopsin. J Biol Chem 267:2222–2227.

Hoshi M, Ohta K, Mori A, Murofushi H, Shkaj H, Hishida E (1992): Mitogen-activated-protein kinase catalyzed phosphorylation of microtubule-associated proteins, microtubule-associated protein 2 and microtubule-associated protein 4, induces an alteration in their function. Eur J Biochem 20:43–52.

Inglese J, Koch WJ, Caron MG, Lefkowitz RJ (1992): Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinase. Nature (Lond) 359:147–150.

Jelsema CL, Axelrod J (1987): Stimulation of phospholipase A2 activity in bovine rod outer segments by the subunits of transducin and its inhibition by the alpha subunit. Proc Natl Acad Sci USA 84:3623–3627.

Katz A, Wu D, Simon MI (1992): Subunits βγ of heterotrimeric G protein activate β2 isoform of phospholipase C. Nature (Lond) 360:686–689.

Kaziro Y, Itoh H, Kazasa T, Hakafuku M, Satoh T (1991): Structure and function of signal-transducing GTP-binding proteins. Annu Rev Biochem 60:349–400.

Kim D, Lewis DL, Graziade L, Neer EJ, Bar-Sagi D, Clapham DE (1989): G-protein beta/gamma subunits activate the cardiac muscarinic K^+-channel via phospholipase A2. Nature (Lond) 337:557–560.

King SM, Wilkerson CG, Witman GB (1991): The Mr. 78,000 intermediate chain of Chlamydomonas outer arm dynein interacts with α-tubulin in situ. J Biol Chem 266:8401–8407.

Lewis JM, Woolkalis MJ, Gerton GL, Smith RM, Farett L, Manning DR (1991): Subcellular distribution of the a subunit(s) of Gi: Visualization by immunofluorescent and immuogold labeling. Cell Regulation 2:1097–1113.

Lin CT, Wu HC, Cheng HF, Chang JT, Chang KJ (1992): Identification of β-subunit of GTP-binding regulatory protein in mitotic spindle. Lab Invest 67:770–778.

Linder ME, Ewald DA, Miller RJ, Gilman AG (1990): Purification and characterization of Go and three types of Gi after expression in Escherichia coli. J Biol Chem 265; 8243–8251.

Lindwall G, Cole RD (1994): Phosphorylation affects the ability of tau protein to promote microtubule assembly. J Biol Chem 259:5301–5305.

Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE (1987): The βγ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. Nature (Lond) 325: 321–326.

Logothetis DE, Kim D, Northup JK, Neer EJ, Clapham DE (1988): Specificity of action of G protein subunits on the cardiac muscarinic K^+ channel. Proc Natl Acad Sci USA 85:5814–5818.

Neer EJ, Clapham DE (1988): Roles of G protein subunits in transmembrane signalling. Nature (Lond) 33:129–134.

Neer EJ, Schmidt CJ, Nambudripad R, Smith TF (1994): The ancient regulatory-protein family of WD-repeat proteins. Nature 371:297–300.

Neer EJ (1995): Heterotrimeric G protein: organizers of transmembrane signals. Cell 80: 249–257.

Park D, John DY, Lee KH, Goo S (1993): Activation of phospholipase C isozymes by G protein-βγ subunits. J Biol Chem 268:4573–4576.

Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kin C, Benovic JL, Kwatra MM, Caron MG, Lefkowitz RJ (1992): Role of βγ subunits of G protein in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science (Wash DC) 257:1264–1267.

Reureny E, Slesinger PA, Inglese J, Morales JM, Iniguez-Lluhi JA, Lefkowitz RJ, Bourne HR, Jan YN, Jan LY (1994): Activation of the cloned muscarinic potassium channel by G protein beta gamma subunits. Nature (Lond) 370:143–146.

Shiina N, Gotoh Y, Kubomura N, Iwamatsu A, Nishida E (1994): Microtubule severing by elongation factor I. Science 266:282–285.

Simon MI, Strathmann MP, Gautam N (1991): Diversity of G protein in signal transduction. Science (Wash DC) 252:802–808.

Smrcka AV, Sternweis PC (1993): Regulation of purified subtypes of phosphatidylinositol specific phospholipase Cβ by G protein α and β/γ-subunits. J Biol Chem 268: 9667–9674.

Stephens L, Smrcka A, Cooke FT, Jackson TR, Sternweis PC, Hawkins PT (1994): A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by Gβγ protein subunits. Cell 77:83–93.

Sternweis PC, Northup JK, Smioge MD, Gilman AG (1981): The regulatory component of adenylate cyclase, purification and properties. J Biol Chem 256:11517–11526.

Stow JL, de Almeida JB, Narula N, Holtzman EJ, Ercolani L, Ausiello DA (1991): A heterotrimeric G protein, Gαi-3, on Golgi Membranes regulates the secretion of a heparan sulfate proteoglycan in LLC-PK1 epithelial cells. J Cell Biol 114:1113–1124.

Takei Y, Kurosu H, Takahashi K, Katada T (1992): A GTP-binding protein in rat liver nuclei serving as the specific substrate of pertussis toxin-catalyzed ADP-ribosylation. J Biol Chem 267:5085–5089.

Tang WJ, Gilman AG (1991): Type -specific regulation of adenylyl cyclase by G protein βγ subunits. Science (Wash DC) 254:1500–1503.

Taussig RL, Quarmby M, Gilman AG (1993): Regulation of purified type I and type II adenylyl cyclase by G protein subunits. J Bio Chem 268:9–12.

Vallee RB, Borisy GG (1978): The non-tubulin component of microtubule protein oligomers. J Biol Chem 253:2834–2845.

Vallee RB (1982): A toxol-dependent procedure for the isolation of microtubules and microtubule-associated proteins (MAPs). J Cell Biol 92:435–442.

Vallee RB (1986A): Reversible assembly purification of microtubule without assembly-promoting agents and further purification of tubulin, microtubule-associated proteins, and MAP fragments. Methods Enzymol 134:89–104.

Vallee RB. (1986B): Purification of brain microtubules and microtubule-associated protein 1 using taxol. Methods Enzymol 134:104–115.

Watson AJ, Katz A, Simon MI (1994): A fifth member of the mammalian G-protein β-subunit family. J Biol Chem 269:22150–22156.

Watson AJ, Aragay AM, Slepak VZ, Simon MI (1996): A novel form of the G protein beta subunit Gβ5 is specifically expressed in the vertebrate retina. J Biol Chem 271:28154–28160.

Whiteway M, Hougan L, Dignard D, Thomas DY, Bell L, Saari GC, Grant FJ, O'Hara, Mackay VL (1989): The STE4 and STE18 genes of yeast encode potential beta and gamma subunits of the mating factor receptor-coupled G protein. Cell 56:467–477.

Wilkerson CG, King SM, Koutoulis A, Pazour GJ, Witman GB (1995): The 78,000 Mr. intermediate chain of Chlamydomonas outer arm dynein is a WD-repeat protein required for arm assembly. J Cell Biol 129:169–178.

Wilson BS, Kamuro M, Farquhar MG (1994): Cellular variations in heterotrimeric G protein localization and expression in rat pituitary. Endocrinology 134:233–244.

Wu HC, Lin CT (1994): Association of heterotrimeric GTP binding regulatory protein (Go) with mitosis. Lab Invest 71:175–181.