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G Protein α Subunits Activate Tubulin GTPase and Modulate Microtubule Polymerization Dynamics*

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G proteins serve many functions involving the transfer of signals from cell surface receptors to intracellular effector molecules. Considerable evidence suggests that there is an interaction between G proteins and the cytoskeleton. In this report, G protein α subunits $G_{i1}\alpha$, $G_s\alpha$, and $G_o\alpha$ are shown to activate the GTPase activity of tubulin, inhibit microtubule assembly, and accelerate microtubule dynamics. $G_i\alpha$ inhibited polymerization of tubulin-GTP into microtubules by 80–90% in the absence of exogenous GTP. Addition of exogenous GTP, but not guanylylimidodiphosphate, which is resistant to hydrolysis, overcame the inhibition. Analysis of the dynamics of individual microtubules by video microscopy demonstrated that $G_{i1}\alpha$ increases the catastrophe frequency, the frequency of transition from growth to shortening. Thus, Gα may play a role in modulating microtubule dynamic instability, providing a mechanism for the modification of the cytoskeleton by extracellular signals.

Microtubules, a major component of the cytoskeleton, are involved in a variety of cellular functions including chromosome movements during mitosis, intracellular transport, and the modulation of cell morphology. In general, the biological function of microtubules is based in significant part on the ability of tubulin to polymerize and depolymerize. In living cells, microtubules exist in both dynamic and stable populations, with each population called upon to carry out distinct cellular functions (1, 2). Proper control of microtubule dynamics is essential for many microtubule-dependent processes.

Microtubule ends can interconvert between slow elongation and rapid shortening, a process called dynamic instability, because of the presumed gain and loss of a small region of tubulin-liganded GTP at the microtubule end (3–5). Tubulin dimers bind 2 mol of GTP/mol of tubulin, one exchangeable (the E-site[1] in β-tubulin) and the other nonexchangeable (in α-tubulin). GTP bound to the exchangeable site becomes hydrolyzed upon incorporation of the tubulin into the microtubule. This hydrolysis creates a microtubule consisting largely of GDP-tubulin, but a small region of GTP-liganded tubulin, called a "GTP cap," remains at the end. 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) (6). The GTPase activity of tubulin is normally low, and hydrolysis of the E-site GTP requires activation. This activation normally occurs when the tubulin dimer binds to the end of a growing microtubule. It is thus suggested that one tubulin dimer might act as a GTPase activator for another during polymerization (7).

Several microtubule-associated proteins are known to regulate microtubule dynamics by stabilizing microtubules (8, 9). Stabilization of microtubules by microtubule-associated proteins is achieved, in part, by suppressing the rate and extent of microtubule shortening and by suppressing the catastrophe frequency and increasing the rescue frequency (6, 10–12). It is noteworthy that the catastrophe frequency observed in cells is much higher than that observed *in vitro* with microtubules composed of pure tubulin (13), suggesting the possible control of the process by additional cellular factors (14–17).

Studies have demonstrated that microtubule polymerization and stability are also affected by second messenger-activated protein kinases, suggesting the possibility that microtubule dynamics may be regulated by extracellular signals through G proteins (for review see Ref. 18; also Refs. 19 and 20). G proteins act as arbiters of cellular signaling, and they may associate in cells directly with microtubules (21–26). Heterotrimeric G proteins are composed of α and $\beta\gamma$ subunits. Gα subunits bind GTP and display various levels of intrinsic GTPase activity. Certain G protein α subunits ($G_{i1}\alpha$, $G_s\alpha$, and $G_q\alpha$) bind to tubulin with high affinity (27–30). This binding appears to activate the G proteins in association with a direct transfer of GTP from the E-site in tubulin to Gα (transactivation) (29, 31). In addition to activating Gα, the association between Gα and tubulin induces a GTPase activity in tubulin similar to that seen after the self-association of tubulin dimers during the formation of a microtubule (32). Recent studies have also shown that $G\beta_1\gamma_2$ binds to microtubules and promotes microtubule assembly *in vitro* (26). These studies indicate that G proteins may modulate microtubule polymerization dynamics and cytoskeletal organization or function. In the present study, the modulation of microtubule assembly and dynamics by G protein α subunits was investigated. We report here that α subunits of G proteins activate the intrinsic GTPase of tubulin (*i.e.* they act as a GTPase activating protein for tubulin), and the GTP hydrolysis modulates microtubule assembly and dynamics *in vitro*.

EXPERIMENTAL PROCEDURES

Tubulin Preparations—Tubulin for all studies except the dynamic instability analysis was purified from fresh sheep brain by cycles of assembly and disassembly (33) followed by phosphocellulose chromatography (34). The resulting tubulin preparations were more than 97% pure as determined by Coomassie Blue staining of SDS-polyacrylamide

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[1] The abbreviations used are: E-site, exchangeable site; GppNHp, guanylylimidodiphosphate; PIPES, 1,4-piperazinediethanesulfonic acid.

gels (not shown). The tubulin was stored in liquid nitrogen and used within 2 weeks. Bovine brain tubulin was used for dynamic instability analyses as described elsewhere (10). Tubulin liganded with GTP, GppNHp, or [α-^{32}P]GTP was prepared by removing exchangeable nucleotide from the tubulin by charcoal treatment followed by incubation with 0.5 mM GTP, 0.5 mM GppNHp, or 0.1 mM [α-^{32}P]GTP (31). The samples were then desalted twice on centrifugal gel filtration columns using P6-DG resin (Bio-Rad) as described previously (31). After desalting, 0.5–0.8 mol of guanine nucleotide was bound/mol of tubulin. Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard (35).

G Protein Purification—Recombinant $G_{i1}\alpha$, $G_s\alpha$, or $G_o\alpha$ were produced in *Escherichia coli* using constructs provided by Dr. Maurine Linder (Washington University, St. Louis, MO). The vector used contained $G_{i1}\alpha$, $G_s\alpha$, or $G_o\alpha$ cDNA preceded by a nucleotide sequence encoding a His_6-amino acid stretch as an affinity tag under the control of a T7 promoter. *E. coli* was grown and harvested, and G proteins were purified over a Qiagen nickel column with a subsequent MonoQ high pressure liquid chromatography step (36). The Q204L mutant of $G_{i1}\alpha$, a generous gift from Drs. J. Hepler and A. G. Gilman (University of Texas Southwestern Medical Center, Dallas, Texas), was expressed in *E. coli* and purified as described (36). Bacteria containing myristol transferase and $G_{i1}\alpha$ (a gift from Dr. M. Linder) were used to express myristoylated $G_{i1}\alpha$, which was then purified as described earlier (37).

GTP Hydrolysis—Tubulin was allowed to bind [α-^{32}P]GTP, and unbound nucleotide was removed by gel filtration using a P6-DG column (Bio-Rad). The samples were then incubated with or without Gα at 30 °C for 30 min and treated with 1% SDS at room temperature for 15 min. Nucleotide analysis was done by thin layer chromatography on polyethyleneimine cellulose plates (32, 38). Two μl of a 10 mM solution of GTP and GDP were spotted 1.5 cm apart on a polyethyleneimine cellulose thin layer plate, followed by 2–5 μl of each sample. The chromatograms were developed in 0.35 M NH_4HCO_3. The spots containing GTP or GDP were visualized with a UV lamp, and plates were exposed to film for autoradiography. Quantitative analysis was done using a Molecular Dynamics PhosphorImager system.

Microtubule Assembly—Tubulin-GTP or tubulin-GppNHp in PEM buffer (100 mM PIPES, 2 mM EGTA, 1 mM $MgCl_2$, pH 6.9) was preincubated with or without Gα at 30 °C for 30 min. Polymerization was then initiated by adding 30% glycerol and an additional 2 mM $MgCl_2$ and incubating at 37 °C for 45 min to 1 h. The extent of microtubule assembly was quantified after pelleting the microtubule polymers by centrifugation at 150,000 × *g* for 20 min at 37 °C. Pellets were resuspended in 4 °C PEM buffer, and protein concentrations in the pellet and supernatant fractions were determined (35). Before testing the effect of G proteins on microtubule assembly, free nucleotide was separated from G protein α subunits, and the buffer was changed to PEM by passage of the proteins through a rapid spin column (Bio-Gel P6DG, Bio-Rad). Alternatively, when Gα concentrations were low, a buffer control was performed to avoid a reduction in protein concentration by gel filtration.

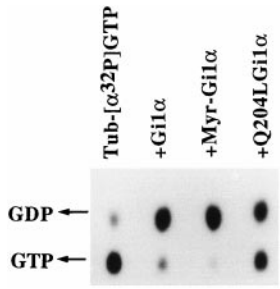

FIG. 1. **Activation of tubulin-GTPase by $G_{i1}\alpha$.** Tubulin-[α-^{32}P]GTP (1.25 μM) in PEM buffer, made by incubating phosphatidylcholine tubulin (*Tub*) with 0.1 mM [α-^{32}P]GTP followed by desalting (as described under "Experimental Procedures") was incubated with $G_{i1}\alpha$, myristoylated (*Myr*) $G_{i1}\alpha$, or a GTPase-deficient mutant of $G_{i1}\alpha$ ($G_{i1}\alpha$Q204L) (2.5 μM) at 30 °C for 30 min. The samples were then treated with 1% SDS and subjected to thin layer chromatography on polyethyleneimine cellulose plates. One of three similar experiments is shown.

Electron Microscopy—Fifteen μl of the microtubule sample was placed on a Formvar-coated nickel grid. After 10–15 s, the grids were rinsed with 10 drops of 2% uranyl acetate for negative staining, blotted dry with a filter paper, and viewed in a JEOL 100S electron microscope.

Microtubule Dynamics by Video Microscopy—Tubulin (12 μM) was mixed with *Strongylocentrotus purpuratus* flagellar seeds in 80 mM PIPES, 0.8 mM Mg^{2+}, 1 mM EGTA, pH 6.8 (PME buffer), containing 275 μM GTP in the absence or presence of $G_{i1}\alpha$ and incubated for 25 min at 37 °C for assembly to reach steady state. The seed concentration was adjusted to achieve 3–6 seeds/microscope field. 2.5 μl of the microtubule suspension was prepared for video microscopy, and the dynamics of individual microtubules were recorded at 37 °C as described previously (10). Under the experimental conditions used, microtubule growth occurred predominantly at the plus ends of the seeds as determined by the growth rates, the number of microtubules that grew, and the relative lengths of the microtubules at the opposite ends of the seeds (6, 10, 39–41). Microtubule length changes were measured in real time at 3–6 s intervals until microtubules underwent complete depolymerization to the axoneme seed or until the microtubule end became obscured. The length changes undergone by a particular microtubule as a function of time were used to create a "life history" plot. The growing and shortening rates were determined by least squares regression analysis of the data points for each growing or shortening phase. The reported mean growing and shortening rates represent the mean values for all growing and shortening events observed for a particular reaction condition. We considered a microtubule to be in a growing phase if the microtubule increased in length by >0.2 μm at a rate >0.15 μm/min and in a shortening phase if the microtubule shortened in length by >0.2 μm at a rate >0.3 μm/min. Length changes equal to or less than 0.2 μm over the duration of 6 data points were considered as attenuation phases. A total of 22–25 microtubules was analyzed for each experimental condition. The catastrophe frequency was determined by dividing the number of catastrophes by the sum of the total time spent in the growing plus attenuated states for all microtubules for a particular condition. The rescue frequency was calculated by dividing the total number of rescue events by the total time spent in the shortening states for all microtubules for a particular condition.

RESULTS

$G_{i1}\alpha$ Activates the Intrinsic GTPase of Tubulin—Tubulin binds to $G_{i1}\alpha$ and $G_s\alpha$ with a K_d of approximately 130 nM coupled with a transactivation of Gα in which 25–50% of E-site tubulin-bound GTP is transferred directly to the Gα (28, 29). $G_{i1}\alpha$ binding to tubulin *in vitro* also activates GTP hydrolysis (32). Both tubulin and $G_{i1}\alpha$ have intrinsic GTPase activities. Because the intrinsic GTPase activity of tubulin is very low, two possibilities exist to explain the higher rate of GTP hydrolysis. One possibility is that $G_i\alpha$ hydrolyzes the E-site-bound GTP after transfer to the $G_i\alpha$. The second possibility is that $G_i\alpha$

TABLE I

Comparison of the effect of $G_{i1}\alpha$ on microtubule assembly induced by GTP or GppNHp

Tubulin-GTP or tubulin-GppNHp was preincubated with or without $G_{i1}\alpha$ (as described in the Fig. 2 legend) followed by polymerization in the presence of GTP or GppNHp as indicated. Assembly was quantified by centrifuging the polymer at 150,000 × *g* and represented as % of control (assembly in the absence of $G_{i1}\alpha$ was considered 100%). Values represent mean ± S.E. of at least three experiments.

GTP		GppNHp	
Samples	Microtubule assembly	Samples	Microtubule assembly
	% control		*% control*
Tubulin-GTP (control)	100	Tubulin-GppNHp (control)	100
$+G_{i1}\alpha$	14.6 ± 3.8	$+G_{i1}\alpha$	15.3 ± 1.7
$+G_{i1}\alpha \rightarrow +5$ μM GTP	70.2 ± 5.7	$+G_{i1}\alpha \rightarrow +5$ μM GppNHp	26.4 ± 1.2
$G_{i1}\alpha \rightarrow +10$ μM GTP	93.5 ± 1.5	$G_{i1}\alpha \rightarrow +10$ μM GppNHp	19 ± 1.0
$G_{i1}\alpha \rightarrow +50$ μM GTP	113 ± 5.0	$G_{i1}\alpha \rightarrow +50$ μM GppNHp	22 ± 8.0

TABLE II

Effect of GTP or GppNHp on $G_{i1}\alpha$-mediated inhibition of microtubule assembly

Experimental protocol was similar to that as described in Table I except that GTP (at indicated concentrations) was added to the samples in which tubulin-GppNHp was preincubated with $G_{i1}\alpha$, whereas GppNHp was added to $G_{i1}\alpha$-preincubated tubulin-GTP samples. Samples were subjected to polymerization at 37 °C and quantified as in Table I. Values represent mean ± S.E. of two experiments.

Tubulin-GppNHp		Tubulin-GTP	
Samples	Microtubule assembly	Samples	Microtubule assembly
	% control		*% control*
Tubulin-GppNHp (control)	100	Tubulin-GTP	100
$+G_{i1}\alpha$	19.2 ± 6.8	$+G_{i1}\alpha$	11.1 ± 0.85
$+G_{i1}\alpha \rightarrow +10\ \mu M$ GTP	73.3 ± 9.2	$+G_{i1}\alpha \rightarrow +10\ \mu M$ GppNHp	17.2 ± 8.8
$G_{i1}\alpha \rightarrow +50\ \mu M$ GTP	101.6 ± 11.3	$G_{i1}\alpha \rightarrow +50\ \mu M$ GppNHp	17.5 ± 2.5
$G_{i1}\alpha \rightarrow +100\ \mu M$ GTP	118.5 ± 6.5	$G_{i1}\alpha \rightarrow +100\ \mu M$ GppNHp	21 ± 9

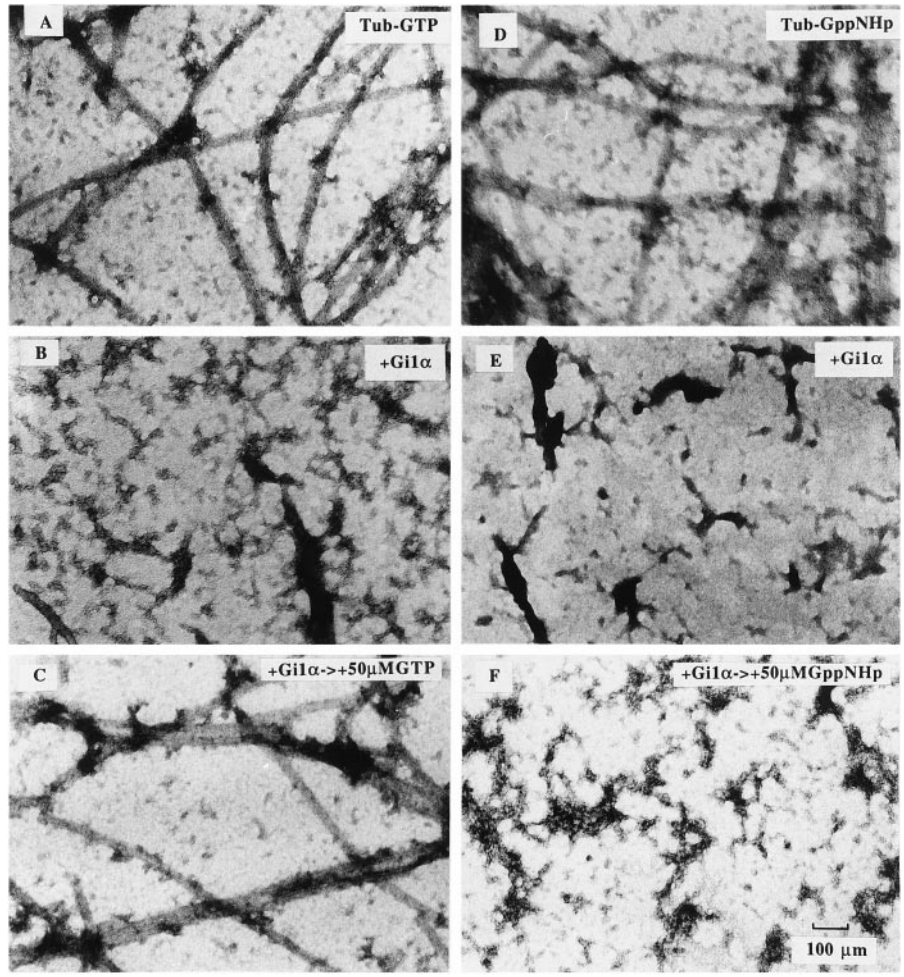

FIG. 2. **Electron microscopy of microtubules formed in the absence of Gα (*A* and *D*) or in the presence of Gα (*B, C, E,* and *F*).** *A–C*, assembly carried out in the presence of GTP. *D–F*, assembly carried out in the presence of GppNHp. Note that in *D*, some microtubule bundling occurred. *Tub*, tubulin.

activates the GTPase of tubulin by inducing a conformational change in the tubulin, similar to the way in which tubulin dimers activate neighboring GTPase activity during microtubule polymerization.

To distinguish between these possibilities, we used a mutated form of $G_{i1}\alpha$ with a single amino acid substitution, $Gln^{204} \rightarrow$ Lys ($Q204LG_{i1}\alpha$), with incapacitated GTPase activity. However, the ability of the mutated $Q204LG_{i1}\alpha$ to bind GTP is unaltered (42). The mutated $Q204LG_{i1}\alpha$, or wild-type $G_{i1}\alpha$, was incubated with tubulin-[α-^{32}P]GTP under conditions in which tubulin does not polymerize, and the extent of GTP hydrolysis was determined by thin layer chromatography. As shown in Fig. 1, the tubulin-bound [α-^{32}P]GTP was poorly hydrolyzed in the absence of $G_{i1}\alpha$ (10.1 ± 1.9%, $n = 10$). In the presence of $G_{i1}\alpha$, 71.8 ± 3.4% ($n = 10$) of the E-site-bound GTP was hydrolyzed. This hydrolysis could be a combination of that occurring in the tubulin E-site and in $G_{i1}\alpha$. When $Q204LG_{i1}\alpha$ was added to the tubulin, 49 ± 3% ($n = 3$) of the bound GTP was hydrolyzed. Because $Q204LG_{i1}\alpha$ cannot hydrolyze GTP, the tubulin must have been responsible for the GTP hydrolysis. Because in the presence of $G_{i1}\alpha$, 71.8% of the E-site GTP was hydrolyzed, approximately 23% of the GTP must have been hydrolyzed by $G_{i1}\alpha$. Myristoylated $G_{i1}\alpha$ was also tested for its ability to activate tubulin GTPase. The amino terminus of $G_{i1}\alpha$ is myristoylated *in vivo,* a modification that is important for association of $G_{i1}\alpha$ with membranes and $G\beta\gamma$ (43). We found that 80.4 ± 2.3% ($n = 7$) of the tubulin-bound [α-^{32}P]GTP was hydrolyzed by myristoylated $G_{i1}\alpha$. The slightly increased potency of myristoylated $G_{i1}\alpha$ to activate tubulin GTPase as compared with $G_{i1}\alpha$ may suggest an enhanced ability of myristoylated $G_{i1}\alpha$ to bind to tubulin. The results indicate that $G_{i1}\alpha$ may act as a GTPase activating protein for tubulin.

$G_{i1}\alpha$ Inhibits Microtubule Assembly in a GTP-dependent Manner—G_i has been shown previously to inhibit microtubule

polymerization (44). This inhibition might occur by binding of the G_i to tubulin and sequestering it, making the tubulin unavailable for polymerization. Tubulin with GTP in the E-site (1.5 mg/ml) polymerizes into microtubules in the absence of exogenous GTP as shown in Table I. Assembly of the tubulin-GTP in the presence of 0.75 mg/ml of $G_{i1}\alpha$ resulted in ~85% inhibition of assembly, and exogenous GTP overcame the ability of $G_{i1}\alpha$ to inhibit assembly in a GTP concentration-dependent manner. To determine whether inhibition of microtubule assembly by $G_{i1}\alpha$ was the result of hydrolysis of the E-site GTP by $G_{i1}\alpha$, we prepared tubulin with GppNHp (a hydrolysis-resistant GTP analog) in the E-site. As also shown in Table I, in the absence of exogenous nucleotide, $G_{i1}\alpha$ reduced the extent of microtubule polymerization by approximately 85%, and exogenous GppNHp did not restore microtubule polymerization. Thus, it appears that GTP hydrolysis resulting from the association of tubulin and $G_{i1}\alpha$ plays a critical role in modulating microtubule assembly. When the microtubule pellet was analyzed by SDS-gel electrophoresis, Coomassie Blue staining did not reveal incorporation of $G_{i1}\alpha$ in microtubules. However, some incorporation of $G\alpha$ into the microtubule fraction was observed by Western blotting using a $G_{i1}\alpha$ antibody (data not shown).

Tubulin Exchanges Nucleotide in the Gα-Tubulin Complex—Addition of exogenous GTP to the tubulin-$G_{i1}\alpha$ complex with either GTP or GppNHp in the E-site reversed the ability of $G_{i1}\alpha$ to inhibit polymerization (Table II). Furthermore, addition of exogenous GppNHp to the tubulin-$G_{i1}\alpha$ complex with GTP in the E-site, inhibited microtubule polymerization. These results indicate that exogenous GTP and GppNHp can exchange with either GppNHp or GTP in the tubulin E-site when complexed with $G_{i1}\alpha$. The GTPase-deficient $G_{i1}\alpha$ variant, $Q204LG_{i1}\alpha$, also inhibited microtubule polymerization in a manner similar to $G_{i1}\alpha$ (by 74.5 ± 9.5%), suggesting that GTP hydrolysis in $G\alpha$ does not cause the inhibition of microtubule assembly.

Microtubules Polymerized in the Presence of $G_{i1}\alpha$ Have Typical Morphology—Electron microscopic analysis of the polymers formed in the presence of $G_{i1}\alpha$ and excess GTP or GppNHp indicated that they were normal microtubules. $G_{i1}\alpha$ blocked the formation of microtubules regardless of the nucleotide bound to the tubulin (Fig. 2, *B* and *E*). The addition of 50 μM GTP reversed the $G_{i1}\alpha$-mediated inhibition of microtubule assembly, and microtubules were formed (Fig. 2*C*), whereas the addition of 50 μM GppNHp did not (Fig. 2*F*).

Specificity of G Protein α Subunits for Inhibition of Microtubule Assembly and GTPase Activity—$G_s\alpha$ binds to tubulin with an affinity similar to that of $G_{i1}\alpha$ (28). Thus, it was predicted that $G_s\alpha$ would also inhibit microtubule assembly. In the presence of $G_s\alpha$, microtubule assembly was reduced to 22% (21.8 ± 10.5%) of the control (Fig. 3*A*). Although $G_o\alpha$ does not bind to tubulin with an affinity as high as that of $G_{i1}\alpha$ or $G_s\alpha$ (28), $G_o\alpha$ inhibited microtubule polymerization similarly to $G_{i1}\alpha$ and $G_s\alpha$ (by 85%). These results are consistent with the possibility that there is a preferential interaction of G_o with oligomeric tubulin or microtubules as compared with dimeric tubulin (44). The retinal G protein transducin ($G_t\alpha$), which does not bind to tubulin or microtubules, did not inhibit microtubule assembly (Fig. 3*A*). Furthermore, the GTPase activity of tubulin was activated by $G_s\alpha$ (73.8 ± 3.8%) and $G_o\alpha$ (93 ± 2.7%) but not by $G_t\alpha$ (28.5 ± 2.5%) (Fig. 3*B*). The activation of tubulin GTPase by $G_{i1}\alpha$ was maximal at a Gα:tubulin ratio of 1:1.

$G_{i1}\alpha$ Increases Microtubule Dynamic Instability by Increasing the Catastrophe Frequency—In an effort to determine how $G\alpha$ modulates microtubule polymerization dynamics, we measured the dynamics of individual microtubules at steady state *in vitro*, in the presence or absence of $G_{i1}\alpha$, by video microscopy.

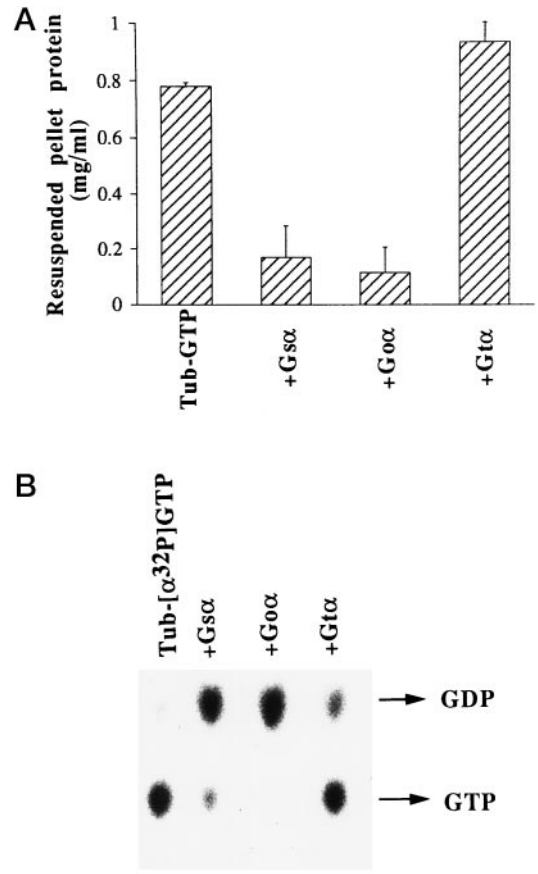

FIG. 3. **$G_s\alpha$ and $G_o\alpha$ activate tubulin GTPase and inhibit microtubule assembly**. *A*, tubulin (*Tub*)-GTP (1.25 mg/ml) was incubated with $G_s\alpha$, $G_o\alpha$, or $G_t\alpha$ (0.5 mg/ml) at 30 °C for 30 min. Samples were then polymerized as described under "Experimental Procedures." Microtubule pellets were resuspended in PEM buffer and pellets, and supernatants were analyzed for protein content. *B*, tubulin-[α-^{32}P]GTP (2 μM) was incubated with $G_s\alpha$, $G_o\alpha$, or $G_t\alpha$ (3 μM) at 30 °C for 30 min. Samples were then treated with 1% SDS and subjected to polyethyleneimine cellulose thin layer chromatography as described under "Experimental Procedures." The autoradiogram of the plate is shown. One of three similar experiments is shown.

Microtubules can alternate between phases of growing and shortening and also spend a small fraction of time in an attenuated (paused) state, neither growing nor shortening detectably, a behavior called dynamic instability (10). The transition frequencies among the growing, shortening, and attenuated states are thought to be important in the regulation of microtubule dynamics in cells (13, 14, 45). Life history traces of several microtubules in the absence (*panel A*) or presence (*panel B*) of $G_{i1}\alpha$ are shown in Fig. 4. Addition of $G_{i1}\alpha$ (4 μM) visually increased the catastrophe frequency. The dynamic instability parameters were determined quantitatively from such life history plots. As shown in Table III, $G_{i1}\alpha$ did not alter the rates of microtubule growth or shortening. However, 4 μM $G_{i1}\alpha$ significantly reduced the average length that microtubules grew per individual growth event (1.5 ± 0.2–0.9 ± 0.2 μm). $G_{i1}\alpha$ also reduced the percentage of total time that microtubules spent in the growing phase and increased the percentage of total time they spent in the shortening phase.

$G_{i1}\alpha$ significantly increased the catastrophe frequency (by 2.6-fold in the presence of 4 μM $G_{i1}\alpha$). The catastrophe frequency per micrometer of length grown was determined by dividing the total number of catastrophic events by total length increase during growing events. $G_{i1}\alpha$ also increased the catastrophe frequency per micrometer of length grown. $G_{i1}\alpha$ had no effects on the rescue frequency (transition from shortening to

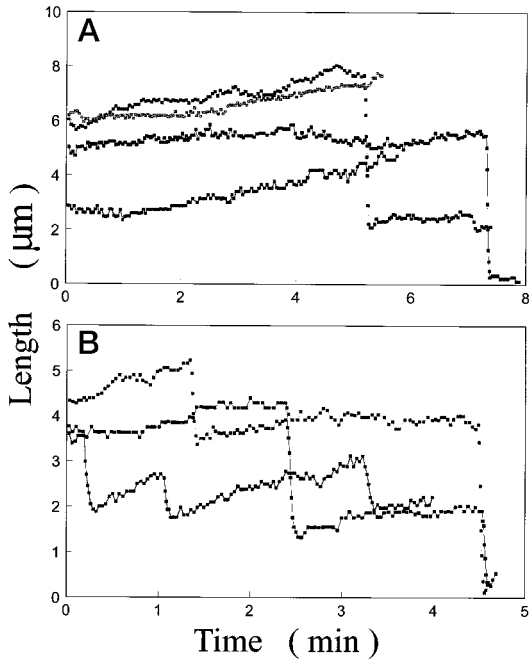

FIG. 4. **Effect of $G_{i1}\alpha$ on microtubule dynamic instability at plus ends at steady state.** Life history traces of length changes at the plus ends of individual microtubules with time are shown in the absence (*A*) or presence (*B*) of 4 μM $G_{i1}\alpha$.

TABLE III
Effects of $G_{i1}\alpha$ on the dynamics of individual microtubules

Dynamic instability parameters were determined from life history plots of individual microtubules. The reported mean growing and shortening rates represent the mean values for all growing and shortening events observed for 22–25 microtubules at each $G_{i1}\alpha$ concentration. Tubulin is 12 μM throughout. All values are ±S.E.

	None	$G_{i1}\alpha$ 2.0 μM	$G_{i1}\alpha$ 4.0 μM
Rate (μM/min)			
Growing	0.45 ± 0.04	0.47 ± 0.045	0.48 ± 0.042
Shortening	11.0 ± 3.1	11.6 ± 2.5	9.5 ± 2.1
Length (μM/event)			
Growing	1.5 ± 0.20	1.0 ± 0.1	0.90 ± 0.10
Shortening	3.5 ± 0.9	3.3 ± 0.4	2.7 ± 0.08
% of total time in phase			
Growing	80.1	70.1	68.8
Shortening	5.8	8.2	11.5
Attenuation	14.1	21.7	19.7
Transition frequency (min^{-1})			
Catastrophe	0.13 ± 0.03	0.24 ± 0.04	0.34 ± 0.05
Rescue	2.0 ± 0.5	1.6 ± 0.4	1.8 ± 0.3
Transition frequency (μM^{-1})			
Catastrophe	0.41 ± 0.09	0.88 ± 0.16	1.10 ± 0.17
Rescue	0.27 ± 0.06	0.18 ± 0.04	0.26 ± 0.05
Dynamicity (μM/min)	0.99	1.28	1.43

the growing or attenuated state) per unit of time or per unit of length shortened (Table III). Dynamicity is a parameter that reflects the overall dynamics of the microtubules (the total detectable tubulin dimer addition and loss at a microtubule end including the time spent in the attenuated state) (39). $G_{i1}\alpha$ (4 μM) increased the dynamicity by 44%. Thus, $G_{i1}\alpha$ increases the dynamic behavior of the microtubules primarily by increasing the catastrophe frequency.

DISCUSSION

In the present study, the α subunits of G proteins (G_{i1}, G_s, and G_o) were shown to activate the GTPase activity of tubulin, indicating that Gα may serve as a GTPase activating protein for tubulin. In addition, Gα inhibited microtubule assembly and increased microtubule dynamic instability *in vitro*. The assembly of tubulin into microtubules was blocked by Gα (80–90%), regardless of whether GTP or GppNHp was bound in the tubulin E-site. In addition, the addition of exogenous GTP, but not the addition of the hydrolysis-resistant GppNHp, overcame the inhibition of microtubule polymerization by Gα. A model for how Gα might interact with tubulin and how exogenous GTP might overcome the interaction is presented in Fig. 5*A*. In this model, Gα is suggested to bind to tubulin and activate the intrinsic GTPase of tubulin in a manner similar to that in which GTP hydrolysis occurs in tubulin during formation of a microtubule. However, unlike the formation of microtubules from tubulin dimers, Gα dissociates from the tubulin-Gα complex subsequent to GTP hydrolysis.

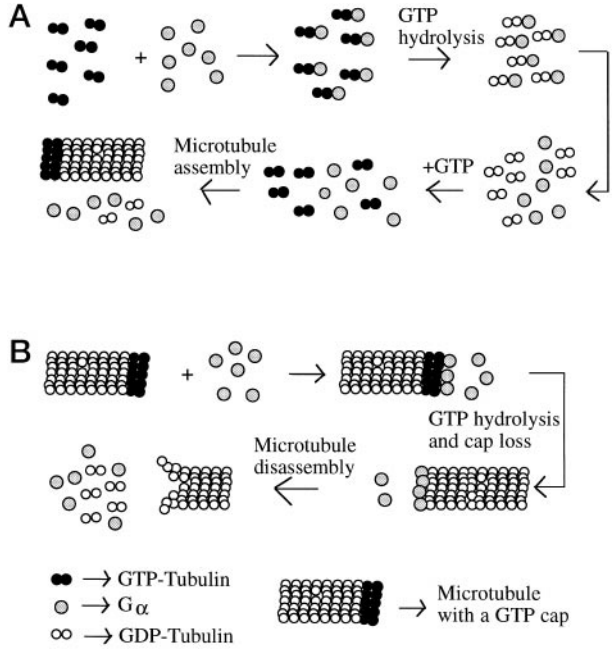

FIG. 5. **Model for the effects of Gα on microtubule assembly and dynamics.** *A*, a scheme for tubulin-Gα interaction for the regulation of microtubule assembly. The binding of Gα to tubulin-GTP inhibits microtubule polymerization and promotes GTP hydrolysis, suggesting that the binding of Gα to tubulin induces a conformation in tubulin similar to that occurring during microtubule formation. Gα dissociates from the tubulin-Gα complex after GTP hydrolysis. Addition of exogenous GTP, but not GppNHp, restores microtubule polymerization, indicating that the formation of the tubulin-Gα complex is required for the inhibition of microtubule polymerization. *B*, possible mechanism for the regulation of microtubule dynamics *in vivo* by Gα. The binding of Gα to the end of a microtubule induces hydrolysis of GTP and subsequent loss of the stabilizing cap, resulting in the transition to microtubule depolymerization (a catastrophe). GTP hydrolysis destabilizes the tubulin-Gα complex, and Gα dissociates from tubulin and is now ready for another cycle of interaction with the microtubule ends.

$G_{i1}\alpha$ altered microtubule dynamics by increasing the catastrophe frequency (the frequency of switching from growing to shortening; see Table III). Microtubules are composed of an unstable tubulin-GDP core and a stable tubulin-GTP or tubulin-GDP-P_i cap at the microtubule ends (46, 47). Microtubules grow for as long as they maintain a GTP cap, but loss of the cap exposes the labile tubulin-GDP core, and the microtubules rapidly shorten. These data are consistent with the possibility that $G_{i1}\alpha$ activates tubulin GTPase at the microtubule ends, thus resulting in loss of the GTP cap (Fig. 5*B*). Alternatively, $G_{i1}\alpha$ might increase the catastrophe frequency by reducing the effective tubulin concentration, thereby binding to and sequestering soluble tubulin. However, this sequence appears unlikely because $G_{i1}\alpha$ did not reduce the individual microtubule growth rate (Table III).

It is suggested that Gα is released from microtubules after binding and subsequent hydrolysis of the E-site GTP. The

released Gα could be recycled for further interaction with newly growing microtubules, reducing the Gα concentration required to exert this effect. In fact, 4 μM $G_{i1}\alpha$, a concentration 3-fold lower than the tubulin concentration (12 μM), increased the catastrophe frequency 2.6-fold (Table III).

Although G proteins are usually confined to the plasma membrane, translocation of activated Gα from the membrane to the cytosol has been observed (48–51). Furthermore, whereas G proteins are normally associated with second messenger-generating enzymes, or ion channels, results from several laboratories suggest that G proteins may be involved in cell growth and differentiation, perhaps through their association with cytoskeletal components (21–26). For example, an association of Gα and G$\beta\gamma$ with the microtubule cytoskeleton has been reported (21, 24–26). Furthermore, an association of $G_o\alpha$ and -β (or -$\beta\gamma$) with spindle microtubules suggests that G protein subunits may play some role in regulating the assembly and disassembly of the mitotic spindle (23, 24). The β-adrenergic receptor kinase (known as βARK or GRK2), which mediates agonist-dependent phosphorylation and desensitization of G protein coupled receptors, has been shown to associate with microtubules and to phosphorylate tubulin in an agonist-dependent manner (19, 20). Taken together, these data suggest a link between microtubules and G protein-mediated signaling that may regulate cell division and differentiation.

G proteins, particularly $G_o\alpha$ and Gβ, are abundant at the growth cone membrane of neurons (52). Growth cones at the growing tips of developing neurites are highly specialized organelles that respond to a variety of extracellular signals to achieve neuronal guidance and target recognition. Coordinated assembly of microtubules in concert with actin filaments and neurofilaments is required for growth cone motility and neurite outgrowth (53, 54). Activation of a G protein coupled receptor has been shown to collapse the growth cone cytoskeleton (55). Because some Gα appears to be released from the membrane subsequent to hormone or neurotransmitter activation (48–51), it is possible that these proteins participate in localized regulation of the cytoskeleton. Thus, microtubule dynamics at growth cones could be mediated by $G_o\alpha$ and $G_i\alpha$. Based on observations in this report as well as the emerging results from various laboratories, it is reasonable to postulate that extracellular signals orchestrate G proteins (both Gα and G$\beta\gamma$) and mobilize them to bind to microtubules. Such a process is likely to provide a venue by which extracellular signals modify cell form and growth.

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