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,α}, G_{α}, and G_{α} are shown to activate the GTPase activity of tubulin, inhibit microtubule assembly, and accelerate microtubule dynamics. G_{α} 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,α} 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) 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-ligated 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 βγ subunits. Ga subunits bind GTP and display various levels of intrinsic GTPase activity. Certain G protein α subunits (G_{i1,α}, G_{α}, and G_{α}) 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 Ga (transactivation) (29, 31). In addition to activating Ga, the association between Ga 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 Gβγ 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...
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-32P]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-32P]GTP (31). The samples were then desalted twice on centrifugal gel filtration columns using P-6DG 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 Bio-Rad (Bio-Rad). Alternatively, when Gα concentrations were low, a buffer control was performed to avoid a reduction in protein concentration by gel filtration.

**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 stars in 80 μM PIPES, 0.8 mM Mg2+, 1 μM EGTA, pH 6.8 (PME buffer), containing 275 μM GTP in the absence or presence of Gα 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 axone seed or until the microtubule end became occluded. 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**

**Gα Activates the Intrinsic GTPase of Tubulin**—Tubulin binds to Gα and Gα with a Kd 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). Gα binding to tubulin in vitro also activates GTP hydrolysis (32). Both tubulin and Gα 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 Gα hydrolyzes the E-site-bound GTP after transfer to the Gα. The second possibility is that Gα

**TABLE I**

Comparison of the effect of Gα on microtubule assembly induced by GTP or GppNHp

| GTP        | Microtubule assembly | Samples | GppNHp | Microtubule assembly | Samples |
|------------|----------------------|---------|--------|----------------------|---------|
| Tubulin-GTP (control) | 100 | Tubulin-GppNHp (control) | 100 |
| +Gα       | 14.6 ± 3.8           | +Gα     | 15.3 ± 1.7 |
| +Gα → +5 μM GTP | 70.2 ± 5.7          | +Gα → +5 μM GppNHp | 20.4 ± 1.2 |
| +Gα → +10 μM GTP | 93.5 ± 1.5          | Gα → +10 μM GppNHp | 19 ± 1.0 |
| +Gα → +50 μM GTP | 113 ± 5.0           | Gα → +50 μM GppNHp | 22 ± 8.0 |

The tubulin-GTP or tubulin-GppNHp was preincubated with or without Gα (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 Gα was considered 100%). Values represent mean ± S.E. of at least three experiments.
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<sub>i1</sub><sub>a</sub> with a single amino acid substitution, Gln204<sup>3</sup>Lys (Q204LG<sub>i1</sub><sub>a</sub>), with incapacitated GTPase activity. However, the ability of the mutated Q204LG<sub>i1</sub><sub>a</sub> to bind GTP is unaltered (42). The mutated Q204LG<sub>i1</sub><sub>a</sub>, or wild-type G<sub>i1</sub><sub>a</sub>, was incubated with tubulin-[<sup>32</sup>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 [<sup>32</sup>P]GTP was poorly hydrolyzed in the absence of G<sub>i1</sub><sub>a</sub> (10.1 ± 1.9%, n = 10). In the presence of G<sub>i1</sub><sub>a</sub>, 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<sub>i1</sub><sub>a</sub>. When Q204LG<sub>i1</sub><sub>a</sub> was added to the tubulin, 49 ± 3% (n = 3) of the bound GTP was hydrolyzed. Because Q204LG<sub>i1</sub><sub>a</sub> cannot hydrolyze GTP, the tubulin must have been responsible for the GTP hydrolysis. Because in the presence of G<sub>i1</sub><sub>a</sub>, 71.8% of the E-site GTP was hydrolyzed, approximately 23% of the GTP must have been hydrolyzed by G<sub>i1</sub><sub>a</sub>. Myristoylated G<sub>i1</sub><sub>a</sub> was also tested for its ability to activate tubulin GTPase. The amino terminus of G<sub>i1</sub><sub>a</sub> is myristoylated in vivo, a modification that is important for association of G<sub>i1</sub><sub>a</sub> with membranes and G<sub>b</sub><sub>γ</sub> (43). We found that 80.4 ± 2.3% (n = 7) of the tubulin-bound [<sup>32</sup>P]GTP was hydrolyzed by myristoylated G<sub>i1</sub><sub>a</sub>. The slightly increased potency of myristoylated G<sub>i1</sub><sub>a</sub> to activate tubulin GTPase as compared with G<sub>i1</sub><sub>a</sub> may suggest an enhanced ability of myristoylated G<sub>i1</sub><sub>a</sub> to bind to tubulin. The results indicate that G<sub>i1</sub><sub>a</sub> may act as a GTPase activating protein for tubulin.

G<sub>i1</sub><sub>a</sub> Inhibits Microtubule Assembly in a GTP-dependent Manner—G<sub>i1</sub><sub>a</sub> has been shown previously to inhibit microtubule assembly upon association with membranes and G<sub>b</sub><sub>γ</sub> (44). We therefore attempted to test whether G<sub>i1</sub><sub>a</sub> would inhibit microtubule assembly when added to tubulin under conditions in which GTP is available. The experimental protocol was similar to that described in Table I except that GTP (at indicated concentrations) was added to the samples in which tubulin-GppNHp was preincubated with G<sub>i1</sub><sub>a</sub>, whereas GppNHp was added to G<sub>i1</sub><sub>a</sub>-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.

**TABLE II**

| Tubulin-GppNHp Samples | Microtubule assembly | % control | Tubulin-GTP Samples | Microtubule assembly | % control |
|------------------------|----------------------|-----------|---------------------|----------------------|-----------|
| Tubulin-GppNHp (control) | 100                  |           | Tubulin-GTP (control) | 100                  |           |
| + G<sub>i1</sub><sub>a</sub> | 19.2 ± 6.8           |           | + G<sub>i1</sub><sub>a</sub> | 11.1 ± 0.85          |           |
| + G<sub>i1</sub><sub>a</sub> → +10 μM GTP | 73.3 ± 9.2        |           | + G<sub>i1</sub><sub>a</sub> → +10 μM GppNHp | 17.2 ± 8.8        |           |
| G<sub>i1</sub><sub>a</sub> → +50 μM GTP | 101.6 ± 11.3         |           | G<sub>i1</sub><sub>a</sub> → +50 μM GppNHp | 17.5 ± 2.5         |           |
| G<sub>i1</sub><sub>a</sub> → +100 μM GTP | 118.5 ± 6.5         |           | G<sub>i1</sub><sub>a</sub> → +100 μM 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.](image-url)
polymerization (44). This inhibition might occur by binding of the G\textsubscript{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\textsubscript{i} resulted in \(-85\%\) inhibition of assembly, and exogenous GTP overcame the ability of G\textsubscript{i} to inhibit assembly in a GTP concentration-dependent manner. To determine whether inhibition of microtubule assembly by G\textsubscript{i} was the result of hydrolysis of the E-site GTP by G\textsubscript{i}, 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\textsubscript{i} 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\textsubscript{i} 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\textsubscript{i} in microtubules. However, some incorporation of G\textsubscript{i} into the microtubule fraction was observed by Western blotting using a G\textsubscript{i} antibody (data not shown).

**Tubulin Exchanges Nucleotide in the Go-Tubulin Complex—**Addition of exogenous GTP to the tubulin-G\textsubscript{i} complex with either GTP or GppNHp in the E-site reversed the ability of G\textsubscript{i} to inhibit polymerization (Table II). Furthermore, addition of exogenous GppNHp to the tubulin-G\textsubscript{i} 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 E-site when complexed with G\textsubscript{i}. The GTPase-deficient G\textsubscript{i} variant, Q204LGi\textsubscript{i}, also inhibited microtubule polymerization in a manner similar to G\textsubscript{i} (by 74.5 \pm 9.5\%), suggesting that GTP hydrolysis in G\textsubscript{i} does not cause the inhibition of microtubule assembly.

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

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

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

![Figure 3](image)
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). G₁α(4 μM) increased the dynamicity by 44%. Thus, G₁α increases the dynamic behavior of the microtubules primarily by increasing the catastrophe frequency.

**DISCUSSION**

In the present study, the α subunits of G proteins (G₁, G₅, and G₆) 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. 5A. 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, Ga dissociates from the tubulin-Gα complex and is now ready for another cycle of interaction with the microtubule ends.

**TABLE III**

**Effects of G₁α 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 G₁α concentration. Tubulin is 12 μM throughout. All values are ± S.E.

| Rate (μM/min)       | None  | G₁α 2.0 μM | G₁α 4.0 μM |
|---------------------|-------|------------|------------|
| Growing             | 0.45 ± 0.04 | 0.47 ± 0.045 | 0.48 ± 0.042 |
| Shortening          | 11.0 ± 2.1  | 11.6 ± 2.5  | 9.5 ± 2.1   |
| Length (μM/event)   | 1.5 ± 0.9   | 0.9 ± 0.1   | 0.9 ± 0.08  |
| Growing             | 15.0 ± 0.8  | 13.3 ± 0.4  | 2.7 ± 0.08  |
| % of total time in phase | 80.1    | 70.1       | 68.8       |
| Attenuation         | 14.1 ± 2.7  | 21.7 ± 17.6 | 19.7       |

**FIG. 4. Effect of G₁α 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 G₁α.

**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. αGα 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.
released Ga could be recycled for further interaction with newly growing microtubules, reducing the Ga concentration required to exert this effect. In fact, 4 μM G1α, 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 Ga 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 Ga and Gβγ with the microtubule cytoskeleton has been reported (21, 24–26). Furthermore, an association of Gα and -β (or -γ) 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 Gα and Gβγ, 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 Ga 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 Gα and Gα. 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 Ga and Gβγ) 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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