A Specific Domain of G\(_i\alpha\) Required for the Transactivation of G\(_i\alpha\) by Tubulin Is Implicated in the Organization of Cellular Microtubules*

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G\(_i\alpha\), G\(_i\alpha_1\), and G\(_i\alpha_2\) subunits bind tubulin with high affinity, whereas transducin (G\(_t\alpha\)) does not. The interaction between tubulin and Ga, which also involves the direct transfer of GTP from tubulin to Ga (transactivation), is not yet fully understood. This study, using chimeras of G\(_i\alpha\) and G\(_t\alpha\), showed that the G\(_i\alpha\) (215–295) segment converted G\(_t\alpha\) to bind to tubulin and this chimera (chimera 1) could be transactivated by tubulin. Insertion of G\(_i\alpha\) (237–270) into chimera 1 to form chimera 2 resulted in a protein that, like G\(_i\alpha_1\), did not bind tubulin. Thus, it was thought that the G\(_i\alpha\) (237–270) domain was essential to modulate the binding of G\(_i\alpha_1\) to tubulin. Surprisingly, when domain (237–270) of G\(_i\alpha\) was replaced by G\(_i\alpha\) (237–270) to form chimera 3, the chimera bound to tubulin with a similar affinity (K\(_D\) \(\approx\) 120 nM) as wild-type G\(_i\alpha_1\). However, even though chimera 3 displayed normal GTP binding, it was not transactivated by GTP-tubulin. Furthermore, when these chimeras were expressed in COS-1 cells, cellular processes in cells overexpressing G\(_i\alpha_1\) or chimera 1 were more abundant and longer than those in native cells. Ga was seen throughout the length of the process. Morphology of cells expressing chimera 2 was identical to controls. Consistent with the role of Chimera 3 as a “dominant negative” Ga, cells transfected with chimera 3 had only few truncated processes. This study demonstrates that although G\(_i\alpha\) (237–270) is not obligatory for the binding of G\(_i\alpha\) to tubulin, it is crucial for the transactivation of Ga by tubulin. These results also suggest that the transactivation of Ga by tubulin may play an important role in modulating microtubule organization and cell morphology.

G proteins act as intracellular transducers to propagate a variety of signals across the plasma membrane. Due to their interaction with transmembrane receptors and lipid modification, G proteins are usually associated with the plasma membrane. Recently, increasing evidence has emerged that G proteins are usually associated with the plasma membrane. Therefore, increasing evidence has emerged that G proteins are usually associated with the plasma membrane. Furthermore, when these chimeras were expressed in COS-1 cells, cellular processes in cells overexpressing G\(_i\alpha_1\) or chimera 1 were more abundant and longer than those in native cells. Ga was seen throughout the length of the process. Morphology of cells expressing chimera 2 was identical to controls. Consistent with the role of Chimera 3 as a “dominant negative” Ga, cells transfected with chimera 3 had only few truncated processes. This study demonstrates that although G\(_i\alpha\) (237–270) is not obligatory for the binding of G\(_i\alpha\) to tubulin, it is crucial for the transactivation of Ga by tubulin. These results also suggest that the transactivation of Ga by tubulin may play an important role in modulating microtubule organization and cell morphology.

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1 The abbreviations used are: G\(_i\alpha_1\), a subunit of the heterotrimeric G protein; G\(_i\alpha_2\), transducin; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; TRITC, tetramethylrhodamine isothiocyanate.

MATERIALS AND METHODS

Construction of Chimeras—G\(_i\alpha_1\)G\(_t\alpha_1\) chimera A, (G\(_i\alpha_1\) 1–237/G\(_t\alpha_1\) 237–270/G\(_i\alpha_1\) 270–295/G\(_t\alpha_1\) 295–314/G\(_i\alpha_1\) 314–354), and G\(_i\alpha_1\)G\(_t\alpha_1\) chimeras.
mera B, (Gα1–236/Gαα3, 236–350) were first constructed. These were used to construct chimeras 3 by replacing the chimera A C-terminal domain (270–350) with the chimera B Gαα3 (270–350) domain. To assemble DNA fragments encoding chimera 3, both plasmids were digested with HindIII and AsuII. A short AsuII-HindIII DNA fragment from the expression vector encoding chimera B was ligated with a large DNA fragment derived from the expression vector encoding chimera A. This new chimera Gαα3/Gαα3, construct, chimera 3, was confirmed by restriction analysis and DNA sequencing. Chimeras 1 and 2 had been previously described (21). In addition, all chimeras and His tag Gαα3 were subcloned into PcdNA3 vector (Invitrogen) for expression in the mammalian cells.

Expression and Purification of Chimeras 3 and Gαα3—The Escherichia coli BL21 cells transformed with the vectors harboring chimera 3 and Gαα3 were grown in 2×YT medium with 100 μg/ml of ampicillin at room temperature up to A600 of 0.5 and then induced with 30 μM isopropyl-1-thio-β-D-galactopyranoside at room temperature for 16–20 h. The cell pellet was resuspended in 1:20 of a cell culture volume of buffer containing 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, 5 mM MgCl2, 50 μM GDP, 0.1 mM phenylmethylsulfonyl fluoride, and 5 mM β-mercaptoethanol (buffer A) and disrupted by ultra-sonication using eight 30 s pulses with 1 min breaks in between pulses. The crude cell lysate was cleared by centrifugation at 100,000 × g for 60 min. The supernatant was collected and adjusted to 20 mM Tris-HCl, 500 mM NaCl, and 20 mM imidazole (binding buffer). The cell lysate was loaded onto 5 ml of nickel-nitrilotriacetic acid-agarose equilibrated with the binding buffer. After washing the column with 10 bed volumes of binding buffer, the bound material was eluted with 20 mM Tris-HCl, 500 mM NaCl, and 100 mM imidazole and subjected to overnight dialysis against buffer A with 20% glycerol. The protein samples were directly applied to 1 ml Mono Q column equilibrated with buffer A without GDP and β-mercaptoethanol. Proteins were eluted with 0–1 ν NaCl gradients in buffer A. An increase in tryptophan fluorescence was measured with excitation at 280 nm and emission at 340 nm to monitor AlF4 binding. The eluted proteins were supplemented with GDP, β-mercaptoethanol, and phenylmethylsulfonyl fluoride, and a concentration of 60 μM, 2 mM, and 0.1 mM, respectively, aliquoted and stored at –80 °C for several months with no loss of functional activity (21).

AlF4−-dependent Conformational Change of Ga—Binding of AlF4 to Ga-GDP mimics the GFP conformation of the molecule and changes in Trp fluorescence can be used to monitor the “visability” of a Ga construct. To measure this, 200 μM Ga, or the chimeras were incubated at room temperature in 50 mM Tris-HCl, pH 8.0, 50 mM NaCl, and 2 mM MgCl2. Fluorescence was measured in an Aminco-Bowman Series 2 Spectrometer (SLM-Aminco) using excitation of 280 nm and emission at 340 nm. Measurements were taken before and 5 min after the addition of 10 mM NaF and 30 μM AlCl3. Fluorescence increases were expressed as a percent change from the initial fluorescence: (F − F0)/F0 × 100, where F is the initial fluorescence and F0 is the fluorescence after addition of fluoride. This method is described in Ref. 21.

Tubulin Iodination—Ovine brain tubulin was made by two assembly-disassembly cycles (22), in the presence of microtubule-associated proteins, which were subsequently removed by phosphocellulose chromatography. An aliquot of 100 μg of PC-tubulin in 100 μl of PIPES buffer was applied to a 12 × 75 mm glass tube precoated with 100 μg IODO-GEN (Pierce), which was dissolved in 100 μl of triethanolamine and dried in a ventilated hood. 2 μCi of Na125I (Amer sham Biosciences) was then added, and the reaction was allowed to proceed with gentle agitation for 15 min. The reaction was terminated by addition of 100 μl of cold 10% TCA containing 4 mM dithiothreitol. The free iodide was removed by ultrafiltration by loading the tubulin suspension on a 3-ml P-6DG (Bio-Rad) desalting column twice. The desalted 125I-tubulin was centrifuged at 11,500 rpm for 10 min to remove denatured protein, and the supernatant containing iodinated tubulin was used in protein binding experiments (21).

Binding of Tubulin to Ga—Purified Ga, chimera 3, bovine transducin, and ovalbumin (Sigma) were applied to nitrocellulose membrane (Midwest Scientific) in the amounts of 150, 100, 50, 25 ng, respectively. Nitrocellulose was incubated with 10% bovine serum albumin in 100 mM PIPES buffer at room temperature for 2 h to block nonspecific binding and then was incubated with 100 μM iodinated tubulin in 100 mM PIPES buffer at room temperature for 4 h. Nitrocelluloses 3 times washed three times with PIPES buffer. Binding of tubulin to G protein α subunits was detected by radiodensitometry. The affinity of tubulin for chimera 3 was estimated by immobilizing chimera 3 upon nitrocellulose and hybridizing with varying concentrations of 125I-tubulin. This was quantified in a gamma counter (Beckman Gamma 9000). The binding was dose-dependent and saturable (14). Immobilized chimeras (other than chimera 2) bound 0.3–0.5 μl of tubulin/1 μl of Ga.

Photoaffinity Labeling and Nucleotide Transfer—Tubulin was incubated with [32P]PP3–1, 4-azidoanilido-p1–5′-GTP (AAGTP) on ice for 30 min in buffer (10 mM HEPES, pH 7.4, 5 mM MgCl2, 150 mM NaCl, 1 mM β-mercaptoethanol). Free AAGTP was removed with two passes through a P-50 DG column. The labeled tubulin was incubated with equimolar concentrations of Gαα3, chimera 3, and Gα at 30 °C for 30 min. UV irradiation, SDS gel electrophoresis, and analysis of results were performed as described in Ref. 16. AAGTP transferred from tubulin to G protein was quantified in a phosphorimaging device (Storm 840; Molecular Dynamics).

Transfection—Plasmids were purified using the Qiagen Maxi purification kit. COS-1 cells were split and plated in a 1:15 dilution to 10 cm plates the day before transfection. Cells were transfected either with calcium phosphate or Lipofectin (Invitrogen). For calcium phosphate transfection, 20 μg DNA was dissolved in 0.5 ml of 0.2 M CaCl2, then added drop by drop to the 0.5 ml bubbling 2 × HBS (280 mM NaCl, 10 mM KCl, 1.5 mM Na2HPO4, 12 mM dextrose, 50 mM HEPES). The precipitates were kept at room temperature for 30 min and were then applied to the plates. After 6 h of transfection, the cells were washed with PBS twice and changed to complete medium. Transfections with Lipofectin were done according to the manufacturer’s instruction.

Immunocytochemistry—COS-1 cells were grown on cover slips in 24-well plates containing Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum and 50 μg penicillin and streptomycin. Before staining, the medium was removed, the cells were washed twice with PBS, fixed with freezing cold 100% methanol in –20 °C for 10 min, and washed again with PBS twice. The cells were then incubated with 5% normal goat serum in PBS for 1 h and incubated in 1:100 dilution of primary antibody in blocking buffer for 1 h. Subsequently, the cells were washed with PBS four times and incubated with 1:100 dilution secondary antibodies labeled with fluorescein isothiocyanate or TRITC (rhodamine) (EY Labs) in blocking buffer for 45 min. Finally, the cells were washed with PBS four times and mounted on the slide with polyvinyl alcohol mounting medium. The slides were air dried and examined with a fluorescence microscope with a 100 watt mercury arc lamp (Nikon, TE300). Images were collected with an interline charge-coupled device camera (Model 1300, Roper Scientific, Trenton, NJ) driven by IP lab software (Scanalytics Inc., Suitland, VA), and assembled in Adobe Photoshop.

The quantification of images was done by assembling fluorescence images from cells transfected with the indicated constructs as well as non-transfected cells (examined by differential-interference contrast microscopy). Images were counted by two individuals blind to experimental condition, and the number and extent of processes for His positive cells (and control cells) was determined.

RESULTS

Construction and Expression of Gαα3-Transducin Chimeras—To map tubulin-binding sites on Gαα3, we constructed Gαα3- Gα chimera where we exchanged several corresponding regions of these two structurally related proteins. The structures of chimeras are schematically illustrated in Fig. 1. The recombinant α subunits were expressed in E. coli and purified using a combination of affinity chromatography on nickel-nitrilotriacetic acid agarose and Mono Q high pressure liquid chromatography. Both the GTP binding capability and the AlF4– induced conformational change as measured by tryptophan fluorescence at 340 nm were comparable for Gαα3 and each of the 3 chimeric proteins. For Ga, Gαα3 chimera 1, and chimera 2, the AlF4– ΔF (% increase) was 55, 70, 50–55, and 60–70, respectively. For chimera 3, the AlF4– ΔF (% increase) was 50. The values given represent the increase in fluorescence upon Ga and Go chimera activation. Ranges are given for multiple preparations (between two and six, depending upon the protein). Where a single value is given, two preparations gave the same results. Detected changes in intrinsic Trp fluorescence indicated that Gαα3 and chimeras were fully functionally active. Those values, which are dependent upon the number of tryptophan residues, are similar to the values reported previously for Gαα3, Gα and Gαα3-Gαα3 chimeras (21).
Binding of Tubulin by Gα-chimeric Proteins—Previous results had suggested that Gα1 binds tubulin with a KD of 120 nM, whereas the affinity of Gα for tubulin was too low to be measured reliably (14). A site on Gα thought to interact with effectors was mapped to the residues 237–270 (21). Therefore, two reciprocal chimeras containing the region 237–270 from Gα1 or Gα (chimeras 1 and 2, respectively) were constructed. Fig. 2 shows that chimera 1 was virtually indistinguishable from Gα1 in its ability to bind 125I-tubulin, whereas chimera 2, similar to transducin, did not bind tubulin in any measurable manner. The third chimera, chimera 3, was constructed to measure the contributions of regions 237–270 directly. The replacement of this region of Gα1 with the analogous region of transducin resulted in a protein that bound tubulin to roughly the same extent as Gα1 (Fig. 2A). Scatchard analysis of tubulin binding to Gα1 and chimera 3 reveals saturable binding and a KD of 120 and 123 nM, respectively.

Chimera 3 Is Not Transactivated by AAGTP-Tubulin—The above data show that both chimera 1 and chimera 3 bind to tubulin with about the same affinity as Gα1. Chimera 1 can be transactivated via transfer of GTP from tubulin to a similar extent as Gα1. Approximately 50% of the AAGTP is distributed in each protein during this process, and GTP in the milieu has no access to either Ga or tubulin once a complex has formed (19). Fig. 3 demonstrates that chimera 3 failed to serve as a substrate for transactivation despite the fact that it bound to tubulin. When tubulin and Gα1 were incubated along with chimera 3, transactivation of Gα1 by tubulin was substantially diminished. Equimolar chimera 3 inhibits transactivation of Gα by 49.9 ± 13%. When the concentration of chimera 3 was increased to twice that of Gα1, transfer of AAGTP to Ga was blocked, and the AAGTP bound to tubulin was also decreased. These data suggest that chimera 3 cannot be transactivated by tubulin, and it inhibits the transfer of GTP from tubulin to Gα1. Thus, chimera 3 may act as a dominant negative to inhibit the transactivation of Gα1 by tubulin.

Chimera 3 Blocks the Formation of Cellular Outgrowths—Previous studies, as well as data obtained in Figs. 2 and 3, suggested that Gα might alter cellular projections containing microtubules (11, 17, 19). To test this, Gα1 and chimeras 1–3 were expressed in COS-1 cells. The expression of each of these chimeras, which was about 3-fold greater than the endogenous Gα, was identified with an antibody against the His6 tag on the protein, and the expression level for each one of the constructs appears to be comparable. This was determined by both the immunostaining seen in the Fig. 4 and by Western blot (not shown). Fig. 4 also demonstrates the effect that the expression of the various constructs has on the formation of cellular outgrowths (indicated by arrows).

Native or vector-transfected COS-1 cells extend moderate length cellular processes that tend to be slightly shorter than the body of the cell (Fig. 4 and Table I). Transfection with Gα1 or chimera 1 increased both the number of cells displaying processes and the mean length of those processes (Table I). Thus, a moderate increase in the expression of Gα increases the length and extent of microtubule-bearing cellular outgrowths. By contrast, chimera 2, which did not bind to tubulin, did not significantly affect cellular outgrowths on COS-1 cells. Both the number and size of processes in chimera 2-transfected cells were similar to control. When cells were transfected with chimera 3, cellular processes were sparse and extremely short (Fig. 4 and Table I). The expression of chimera 3 prevented the transactivation of endogenous Ga by tubulin and prevented cells from sending out these microtubule-rich processes (Fig. 4 and Table I).

DISCUSSION

Data in this study suggest that distinct regions on Ga mediate the binding and the transactivation of Ga protein and tubulin. Gα1 (237–270) plays a crucial role for the transactivation of Gα by tubulin, even though it is not a region required for the binding of those two molecules. Based on the crystal structure of Ga protein, this region includes the helix of α3, a loop, and the β5 strand. The (237–270) domain in Gα1 is one of the binding sites for effectors such as adenylyl cyclase (23). Similarly, this domain in transducin binds the phosphodiesterase γ subunit allowing activation of retinal phosphodiesterase (31). The importance of the domain implies that the interaction of Gα1 and tubulin, which evokes transactivation of the later molecule, may play a role in modulating the physiology of signaling pathway and may orchestrate organization of cytoskeleton.

The α subunits of heterotrimeric G proteins are a family of proteins of 39–52 kDa that display a similarity of about 45–80% at the amino acid level. Previous studies (14) suggested that Gα1 bound tubulin with high affinity (KD = 120 nM) and Gα was transactivated by directly transferring GTP. Transducin neither bound tubulin nor was its substrate for transactivation (14). In this study, when the Gα1 (215–295) segment replaced the Gα sequence, chimera 1 could bind to tubulin and was transactivated by transfer of GTP from tubulin in vitro. This suggests that the region 215–295 might be important for mediating Gα1 interaction with tubulin. However, in a manner similar to transducin, chimera 2 did not bind tubulin in vitro. Thus, we initially thought that the Gα1 (237–270) domain might play a key role in rendering Gα1 able to bind tubulin in vitro. Strangely, chimera 3 bound to tubulin with the same high affinity as wild-type Gα1 did. Nonetheless, it appears that chimera 3 inhibited the “productive” association between Gα1 and tubulin, which allows for the transactivation of Gα. Previous studies (14, 17, 24) have shown that there may be multiple binding sites on the tubulin dimer for Gα1, and part of the receptor interaction domain of Ga may mediate this binding. Based on data in this study, it is likely that the Gα1 (237–270) domain is involved in the binding of Gα and tubulin; however, there is an additional site (or sites) for Gα1 interaction with tubulin, and this is located within 1–215 and 295–354 regions of Gα1. As shown in a solid phase binding assay, replacement of Gα1 237–270 with Gα1 237–270 in Gα sequence did not significantly affect the binding affinity of chimera 3 and tubulin (KD = 123 nM for either molecule), implying that this second site(s) is crucial for the high affinity binding of Gα1 to tubulin.

Tubulin is able to transfer nucleotide (GTP or AAGTP) directly to various G protein-α subunits (Gα1, Gα1, and Gα) (13, 16, 19). This phenomenon has been referred to as transactivation, and during the process nucleotide is not released into the milieu but rather is transferred directly from the tubulin to the
The interaction of G proteins and tubulin is shown. Proteins by directly transferring GTP to Gts.

The activation of wild-type Gi requires the participation of tubulin. Chimera 3 also inhibited GTP transactivation by tubulin, but chimera 3 did not serve as a substrate for transactivation, and samples were prepared for SDS-PAGE. ³²P[AAGTP bound to tubulin or Gαi was made covalent by UV irradiation, and samples were prepared for SDS-PAGE. ³²P[AAGTP bound to tubulin or Gαi (as a result of transactivation) was quantified by phosphoimaging analysis. Images shown are from one of three similar experiments.

**FIG. 2.** Specificity and affinity of ¹²⁵I-tubulin binding to Gα. A, ¹²⁵I-tubulin binding to G protein α subunits. Gαi, chimera 1, chimera 2, chimera 3, and Gα were applied to a nitrocellulose sheet in the amounts indicated and air-dried at room temperature. Following this, tubulin binding was assessed by overlay with ¹²⁵I-tubulin and autoradiography as described under “Materials and Methods.” One of three similar experiments is shown. B, saturation isotherm and Scatchard plot for tubulin binding to Gαi and chimera 3. Data were derived from dot blotting performed with a method similar to that for A. 100 ng of Gαi or chimera 3 were applied to each spot. Triplicate nitrocellulose spots corresponding to the total binding and nonspecific binding (determined in the presence of 100-fold excess unlabeled tubulin) were cut out and counted in an LKB Rack gamma counter. The graph on the left shows the saturation isotherm for specific binding of ¹²⁵I-tubulin to chimera 3 (○) or Gαi(○). On the right are the Scatchard plots derived from these data. The Kd and Bmax for Gαi were 121 nM and 386 fmol/ng, respectively. For chimera 3, these data were 123 nM and 473 fmol/ng, respectively. Data were calculated from two similar experiments.

**FIG. 3.** Transactivation of Gα constructs by tubulin. Freshly prepared tubulin-[³²P[AAGTP was incubated with equimolar Gαi, chimera 3, Gα1, Gαi plus chimera 3, or twice molar Gα1, or chimera 3 as indicated. AAGTP binding to tubulin or Gα was made covalent by UV irradiation, and samples were prepared for SDS-PAGE. ³²P[AAGTP bound to tubulin or Gα (as a result of transactivation) was quantified by phosphoimaging analysis. Images shown are from one of three similar experiments.
mine cell shape and in a variety of cell movements, including some forms of cell locomotion, the intracellular transport of organelles, and the separation of chromosomes during mitosis. Several studies (11, 19) have suggested G proteins might participate in modulation of the cytoskeleton. Association between tubulin or microtubules and Go has been established for some time (14, 27, 28). In this study, overexpression of His6-Giα1 and chimera 1 in COS-1 cells increases the length and number of cellular processes, whereas chimera 2, which does not bind tubulin, had no effect. This is consistent with the notion that the binding and transactivation of Gαi by tubulin might have a role in the regulation of microtubules. Previous studies also show that Gβγ stabilizes microtubules (4), whereas Gα1 increases microtubule dynamics by increasing the frequency of microtubule catastrophe (11). Although these data are from in vitro studies, they are consistent with the possibility that Go may modulate the dynamics of microtubules in cells. Note that an increase in microtubule dynamics is not inconsistent with increased length and number of cellular processes induced by increased expression of Gα1 or chimera 1. In fact, microtubules in areas of dynamic cellular extension, such as growth cones, display more dynamic behavior (32). A recent study (33) has demonstrated an increase in the association of Gαi (and Gαo) with microtubules in cellular processes induced by nerve growth factor and other “differentiating” agents in PC12 pheochromocytoma cells.

Curiously, expression of chimera 3 blocks the extension of cellular processes. Although it would be premature to suggest that Gα normally accelerates the extension of cellular process thorough its interaction with tubulin, these results are consistent with such a possibility. To draw more specific conclusions, more studies in vitro and in vivo are required. The role of Go transactivation by tubulin in this process is also unclear. Although transactivation seems pertinent to the regulation of adenyl cyclase or phospholipase C by tubulin (16, 20), its role in regulation of microtubule dynamics is unknown. Nevertheless, it is noteworthy that activation of Gαi and Gαo has been linked to a rapid increase in microtubule depolymerization (29, 30).

In summary, this study reveals that distinct regions on Gαi mediate the tubulin binding and the transactivation process. Gαi 219–295 is important to the binding of Gαi1 and tubulin, and Gαo 237–270 contributes to the transactivation by tubulin of Gαi1. The data in this study implicate Gαi transactivation by tubulin in the regulation of microtubule organization. Such a regulation might provide new insight into the relationship between hormone or neurotransmitter action and cell morphology or other aspects of the dynamic cytoskeleton.

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FIG. 4. G protein and tubulin dual staining of transiently transfected COS-1 cells. COS-1 cells were transfected with 10 μg of pCNA3 coding for Gα1, chimera 1, chimera 2, and chimera 3, respectively, and grown in normal Dulbecco’s modified Eagle’s medium for 48 h. The cells were fixed with −20 °C methanol and stained with rabbit anti-His, tag antibody and fluorescein isothiocyanate goat anti-rabbit IgG and with α-tubulin antibody and rhodamine-coupled goat anti-mouse IgM. Go constructs appear green and microtubules are seen in red. The figure shows the overlay of the tubulin and His tag antibodies. Areas of tubulin/Gα overlap appear yellow. The arrows indicate cellular processes. Images shown are typical of five experiments in which at least 60 cells of each type were examined.

Table I
Number and extent of cell process formation in COS 1 cells expressing various chimeric G protein constructs

| Construct | Processes/cell | Avg. process length (% cell body length) |
|-----------|----------------|----------------------------------------|
| Vector    | 2.22 ± 0.40    | 81 ± 25                                |
| Gα1       | 3.74 ± 0.60    | 138 ± 19                               |
| Chimera 1 | 3.48 ± 0.37    | 118 ± 3                                |
| Chimera 2 | 2.41 ± 0.35    | 92 ± 24                                |
| Chimera 3 | 0.47 ± 0.17    | 37 ± 11                                |
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