The Journal of Neuroscience, April 15, 2000, 20(8):2774–2782

Muscarinic Receptor Activation Promotes the Membrane Association of Tubulin for the Regulation of Gq-Mediated Phospholipase Cβ_1 Signaling

Juliana S. Popova[1] **and Mark M. Rasenick**[1,2]

Departments of [1]*Physiology and Biophysics, and* [2]*Psychiatry, University of Illinois at Chicago, College of Medicine, Chicago, Illinois 60612-7342*

The microtubule protein tubulin regulates adenylyl cyclase and phospholipase Cβ_1 (PLCβ_1) signaling via transactivation of the G-protein subunits Gαs, Gαi1, and Gαq. Because most tubulin is not membrane associated, this study investigates whether tubulin translocates to the membrane in response to an agonist so that it might regulate G-protein signaling. This was studied in SK-N-SH neuroblastoma cells, which possess a muscarinic receptor-regulated PLCβ_1-signaling pathway. Tubulin, at nanomolar concentrations, transactivated Gαq by the direct transfer of a GTP analog and potentiated carbachol-activated PLCβ_1. A specific and time-dependent association of tubulin with plasma membranes was observed when SK-N-SH cells were treated with carbachol. The same phenomenon was observed with membranes from Sf9 cells, expressing a recombinant PLCβ_1 cascade. The time course of this event was concordant both with transactivation of Gαq by the direct transfer of [^{32}P]P^3(4-azidoanilido)-P^1-5′-GTP from tubulin as well as with the activation of PLCβ_1. In SK-N-SH cells, carbachol induced a rapid and transient translocation of tubulin to the plasma membrane, microtubule reorganization, and a change in cell shape as demonstrated by confocal immunofluorescence microscopy. These observations presented a spatial and temporal resolution of the sequence of events underlying receptor-evoked involvement of tubulin in G-protein-mediated signaling. It is suggested that G-protein-coupled receptors might modulate cytoskeletal dynamics, intracellular traffic, and cellular architecture.

Key words: tubulin; microtubules; cytoskeleton; G-protein; phospholipase C; muscarinic receptor

Cytoskeletal proteins appear to be involved in the control of intracellular signaling. The microtubule protein tubulin regulates adenylyl cyclase and phospholipase Cβ_1 (PLCβ_1) signal transduction (Wang at al., 1990; Roychowdhury et al., 1993; Popova et al., 1994, 1997; Roychowdhury and Rasenick, 1994; Yan et al., 1996). The microtubule cytoskeleton is also suggested to be involved in the regulation of voltage-gated calcium channel activity (Unno et al., 1999). Certain isoforms of the microtubule-associated protein tau appear to regulate PLCγ (Hwang et al., 1996). Actin binds and inhibits the G-protein-coupled receptor kinase 5 (GRK5) (Freeman et al., 1998), and actin regulatory proteins, like profilin, gelsolin, and CapG, bind to the phospholipase C substrate phosphatidylinositol 4,5-bisphosphate (PIP_2) and inhibit PLC isoenzymes (Goldschmidt-Clermont et al., 1990, 1991; Banno et al., 1992; Sun et al., 1997). Although cytoskeletal elements affect growth factor-directed phospholipid metabolism (Payrastre et al., 1991; Rhee, 1991; Banno et al., 1992), the phosphorylation of PLCγ_1 by the epidermal growth factor receptor tyrosine kinase overcomes profilin-induced inhibition of this signaling pathway (Goldschmidt-Clermont et al., 1991).

Tubulin regulates adenylyl cyclase and PLCβ_1 signaling via specific interactions with the α subunits of the regulatory G-proteins, Gαs, Gαi1, and Gαq (Wang at al., 1990; Roychowdhury et al., 1993; Popova et al., 1994, 1997). The interaction of tubulin with these polypeptides involves a GTP transfer from the exchangeable GTP-binding site (E site) of tubulin to Gα, which activates the G-protein (transactivation) (Roychowdhury and Rasenick, 1994). Tubulin binds specifically to Gαi1, Gαs, and Gαq with a K_d of ~130 nM (Wang at al., 1990). Complexes of tubulin and Gαi1 or Gαs have been immunoprecipitated from brain extracts and were suggested to represent functional assemblies, responsible for the observed G-protein activation by tubulin (Yan et al., 1996).

PLCβ_1, which is regulated by Gαq (Blank et al., 1992; Boyer et al., 1992; Park et al., 1993), evokes Ca^{2+}-dependent hydrolysis of PIP_2 and generates two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Rhee and Choi, 1992). DAG is the physiological activator of protein kinase C (PKC), whereas IP_3 mobilizes stored calcium and promotes external calcium influx to activate Ca^{2+}-dependent protein kinases ((Rhee and Choi, 1992; Berridge, 1993; Noh et al., 1995). A biphasic pattern of response of PLCβ_1 to dimeric tubulin with guanosine 5′-(β,γ-imido)triphosphate (GppNHp) bound (tubulin-GppNHp) was detected in membranes from Sf9 cells expressing m_1 muscarinic receptors, Gαq, and PLCβ_1, as well as in a purified reconstituted system (Popova et al., 1997). At lower (nanomolar) concentrations, tubulin activated, whereas at higher (micromolar) concentrations, tubulin inhibited, PLCβ_1. The stimulatory effect of tubulin-GppNHp was more efficacious than that

Received Nov. 15, 1999; revised Feb. 4, 2000; accepted Feb. 4, 2000.

This study was supported by the United States National Institutes of Health Grants MH 39595 and AG 15482 and the Council for Tobacco Research Grant 4089. We thank Dr. S. G. Rhee for providing us with PLCβ_1 baculovirus and the corresponding antibody. We are also grateful to Dr. J. C. Garrison for the Gαq baculovirus. M. LoConte and H. Brown are thanked for their technical assistance. We also appreciate the advice of K. Chaney and the assistance of Dr. M. L. Chen in the performance of our confocal microscopy experiments. Dr. R. Cohen is thanked for her advice in image analysis. We thank Drs. G. Luthin, D. Manning, W. Dunn, M. Gnegy, J. Lessard, and E. Ross for their generous gifts of material.

Correspondence should be addressed to Dr. Mark M. Rasenick, Department of Physiology and Biophysics, University of Illinois at Chicago, College of Medicine, 835 South Wolcott Avenue m/c 901, Chicago, IL 60612-7342. E-mail: raz@uic.edu.

 0270-6474/00/202774-09$15.00/0

of GppNHp alone (Popova et al., 1997). The muscarinic receptor agonist carbachol significantly potentiated PLCβ_1 activation by tubulin-GppNHp and was able to counteract partially the inhibitory effect seen at higher tubulin concentrations (Popova et al., 1997). Transactivation by tubulin appeared responsible for Gq and, subsequently, PLCβ_1 activation, because nucleotide-free tubulin inhibited PLCβ_1 at any tested concentration (Popova et al., 1997). The observed interaction of tubulin with the PLCβ_1 substrate PIP_2 was thought to be responsible for enzyme inhibition at high concentrations of tubulin (Popova et al., 1997).

Most of the above-mentioned studies were performed with exogenous tubulin added to membranes or in reconstituted systems. The origin of the tubulin that might normally be involved in this process has not been investigated. It is possible that specific signals initiate a redistribution and translocation of cytosolic tubulin to the cell membrane (Popova et al., 1997; Panagiotou et al., 1999).

The present study investigates how cytoskeletal tubulin is involved in the regulation of PLCβ_1 signaling. SK-N-SH neuroblastoma cells, which naturally maintain a PLCβ_1-signaling cascade (Fisher and Snider, 1987; Fisher, 1988; Fisher and Heacock, 1988), were used to test the hypothesis that agonist stimulation evokes a redistribution of cellular tubulin, tubulin membrane association, and engagement in Gαq-PLCβ_1 regulation. Demonstration of agonist-induced reorganization of the microtubule cytoskeleton suggests that the interface between tubulin and heterotrimeric G-proteins may regulate both cellular signaling and cell shape or form.

MATERIALS AND METHODS

Cell culture. SK-N-SH neuroblastoma cells were maintained in DMEM supplemented with 10% fetal bovine serum according to standard procedures (Fisher and Snider, 1987). Sf9 cells were grown in Sf-900 II SFM media as described (Popova et al., 1997).

Baculovirus-directed protein expression in Sf9 cells. Sf9 cells were infected with baculoviruses bearing the m_1 muscarinic receptor, Gαq, or PLCβ_1 cDNAs, as described previously (Popova et al., 1997). The construction of recombinant baculoviruses was described elsewhere (Parker et al., 1991; Graber et al., 1992; Boguslavsky et al., 1994). Membranes were prepared from cells collected 60 hr after infection.

Membrane preparation and Western blotting. Cells were sonicated in ice-cold 20 mM HEPES, pH 7.4, 1 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol (DTT), and 0.3 mM PMSF (three times for 30 sec each; Branson sonifier; output control, 4). Membrane pellets were prepared as described (Popova et al., 1997). Protein concentrations were determined by the Bradford (1976) dye-binding assay with bovine serum albumin as a standard. Expression of receptors, G-proteins, and PLCβ_1 was determined by immunoblotting. Membrane proteins transferred to nitrocellulose were probed with antibodies specific for the m_1 muscarinic receptor (#71; from G. Luthin, Philadelphia, PA), Gαq/11 (#0945; from D. Manning, Philadelphia, PA), or PLCβ_1 (K-32-3, monoclonal; from S. G. Rhee, Bethesda, MD) at a dilution of 1:500 (polyclonal) or 1:5000 (monoclonal). Biotinylated goat anti-rabbit IgG or anti-mouse IgG was used as a secondary antibody, accordingly. Either streptavidin-alkaline phosphatase or -horseradish peroxidase conjugates were used for detection via colorimetric or chemiluminescent techniques, as described. Expression levels were estimated by densitometry of the corresponding protein bands (Storm 840; Molecular Dynamics, Sunnyvale, CA). They varied by no >10% for a given recombinant protein. Receptor-binding studies using [^{3}H]l-quinuclidinyl [phenyl-4(*n*)]benzilate ([^{3}H]QNB) as a ligand were also performed to monitor m_1 muscarinic receptor expression (Popova et al., 1997). When coexpressed with Gαq and PLCβ_1 in the Sf9 cells, m_1 muscarinic receptor density was estimated at 240 fmol/mg of membrane protein (Popova et al., 1997). SK-N-SH cells have a high density of m_3 muscarinic receptors (500 fmol/mg of membrane protein) that are coupled to phosphoinositide turnover (Fisher and Snider, 1987; Fisher, 1988; Fisher and Heacock, 1988).

Tubulin preparations. Microtubules were isolated as described (Shelanski et al., 1973). Microtubule-associated proteins were removed by phosphocellulose chromatography, and the remaining pure tubulin fraction was termed PC-tubulin (Wang and Rasenick, 1991). Replacement of GTP in β-tubulin was performed as described previously (Wang et al., 1990). GTP was removed from PC-tubulin by charcoal pretreatment, and tubulin was incubated with 150 μM guanine nucleotide [guanosine 5′-O-(3-thiotriphosphate) (GTPγS), GppNHp, GDP, or [^{32}P]P^3(4-azidoanilido)-P^1-5′-GTP (AAGTP)] for 30 min on ice. Before use, these samples were passed through P6-DG columns twice to remove the unbound nucleotide. This procedure yields 0.4–0.6 moles of guanine nucleotide bound per mole of tubulin dimer. Tubulin-guanine nucleotide concentrations used throughout the study were based on the protein concentration.

PLCβ_1 assays. Forty micrograms of SK-N-SH or 20 μg of Sf9 membrane protein was incubated with a [^{3}H]PIP_2 substrate mixture (30 μM final concentration, unless stated otherwise) as described (Popova et al., 1997). Ten microliters of GppNHp or tubulin-GppNHp and carbachol were added at appropriate concentrations to a final volume of 80 μl. The tubes were incubated for 10 min (unless stated otherwise) at 37°C with constant shaking, as described (Popova et al., 1997). [^{3}H]IP_3 production was quantified by liquid scintillation counting (Popova and Dubocovich, 1995; Popova et al., 1997).

Photoaffinity labeling and nucleotide transfer. Cell membranes were incubated with the indicated concentrations of dimeric tubulin with [^{32}P]AAGTP bound (tubulin-[^{32}P]AAGTP) in the presence or absence of carbachol in 100 mM 1,4-piperazinediethanesulfonic acid (PIPES) buffer, pH 6.9, 2 mM EGTA, and 1 mM $MgCl_2$ (buffer A) as described (Rasenick et al., 1994). The tubes were UV irradiated, and the reaction was quenched with ice-cold PIPES buffer, 1 mM $MgCl_2$, and 4 mM DTT. After centrifugation at 20,000 × *g* for 15 min, the membrane pellets were washed with buffer and dissolved in SDS Laemmli sample buffer with 50 mM DTT as described (Laemmli, 1970). SDS-PAGE of the samples was performed (Popova et al., 1997), and the gels were either stained (Coomassie blue) or subjected to Western blotting, followed by autoradiography (Kodak XAR-5 film) or phosphorimage analysis of the bands (Storm 840; Molecular Dynamics).

Immunoprecipitation. Membrane preparations were extracted with 1% sodium cholate in buffer A for 1 hr at 4°C with constant stirring. The tubes were centrifuged at 20,000 × *g* for 15 min at 4°C. Membrane extracts (0.5 mg/ml membrane protein) were incubated with guanine nucleotide-bound tubulin (1 μM), as described above. After UV irradiation and preclearing (Pansorbin; Calbiochem, La Jolla, CA), each membrane extract was incubated overnight with the appropriate specific antiserum or preimmune serum (1:20 dilution for polyclonal and 1:500 dilution for monoclonal antibodies) at 4°C with constant stirring. Immune complexes were precipitated with Pansorbin, and each immunoprecipitate was subjected to SDS-PAGE and autoradiography, as described (Popova et al., 1997). Where indicated, Western blotting followed by an ECL detection of the bands was performed.

Membrane association of tubulin in SK-N-SH cells. SK-N-SH cells were collected and washed three times with PBS, and aliquots of 1×10^7 cells were distributed in plastic tubes on ice. Carbachol (1 mM) was immediately added, and the samples were incubated for the indicated time periods at 37°C with constant shaking. When tested, atropine (100 μM) was added before carbachol. Samples was transferred on ice and immediately sonicated, as described. Each sample was centrifuged at 600 × *g* at 4°C (P_1 pellets). Supernatants were centrifuged at 20,000 × *g* at 4°C (P_2 pellets). SDS-PAGE of the fractions was followed by immunoblotting, using polyclonal anti-tubulin antiserum (raised against the C-terminal 422–431 amino acid region of β-tubulin) and ECL detection (Amersham, Arlington Heights, IL). The results were analyzed in a Storm 840 image system (Molecular Dynamics).

Confocal immunofluorescence microscopy. SK-N-SH cells were plated onto glass coverslips in 12-well culture plates at a density of 1×10^5. After ~24 hr, treatment with 1 mM carbachol, 10 μM atropine, or both was performed for the indicated times at 37°C. After washing with PBS buffer, the cells were fixed in −20°C methanol for 6 min. Cells were washed three times in PBS and blocked at room temperature for 30 min in 5% NGS-containing PBS. After a PBS wash, cells were incubated for 2 hr with the above-mentioned polyclonal anti-β-tubulin antiserum (1:100 dilution). After washing three times in PBS, secondary fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit antibody (EY Laboratories; 1:100 dilution) was applied for 1 hr, followed by washing and mounting. Images were acquired using a Carl Zeiss laser-scanning confocal microscope LSM 510 equipped with a 40× immersion objective. A single 488 nm beam from an argon–krypton laser was used for excitation.

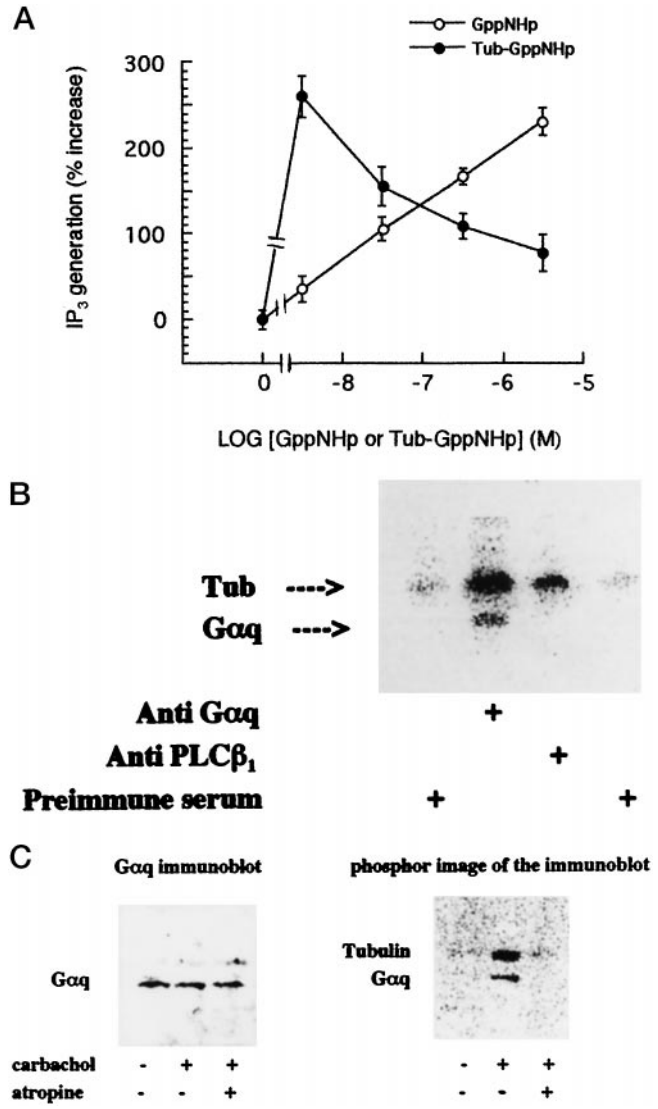

Figure 1. Tubulin regulates PLCβ signaling in SK-N-SH neuroblastoma cell membranes. *A,* Dual regulation of PLCβ_1 by tubulin-GppNHp. SK-N-SH membranes (50 μg of membrane protein) were assayed for PLCβ_1 activity in the presence of 1 mM carbachol and the indicated concentrations of GppNHp or tubulin-GppNHp. Control activity was 0.70 ± 0.08 nmol of $IP_3 \cdot min^{-1} \cdot mg$ of $protein^{-1}$. A representative of three experiments with similar results is shown. *B,* Coimmunoprecipitation of tubulin with Gαq and PLCβ_1. Extracted SK-N-SH membranes (0.5 mg/ml) and 1 μM tubulin-[^{32}P]AAGTP were tested as described. Tubulin was not precipitated when preimmune serum replaced the anti-Gαq antibody (*left lane*) or the anti-PLCβ_1 antibody (*right lane*). A phosphorimage of one of three independent experiments with similar results is shown. *C,* Carbachol-triggered membrane association of tubulin and guanine nucleotide transfer from tubulin to Gαq. SK-N-SH membranes were incubated with 100 nM tubulin-[^{32}P]AAGTP, 10 μM carbachol, and 100 nM atropine (as indicated) for 5 min at 23°C. UV irradiation, SDS-PAGE (50 μg of membrane protein in *each lane*), immunoblotting, and phosphorimage analysis of the blots were performed as described. A representative experiment of three with similar results is shown. In the absence of carbachol, no increase in tubulin membrane association or nucleotide transfer to Gαq was detected at any time point tested. *Tub*, Tubulin.

Emission from FITC was detected via an LP505 filter. Differential interference contrast (DIC) images of the cells were also acquired. Images of computer-generated cross sections of cells were collected as well (*x–z* and *y–z* planes). Coverslips were examined at random. At each time point a total of 300 randomly selected cells over four consecutive experiments were evaluated for tubulin redistribution, changes in microtubule network, and cell shape. When analyzed, cellular processes were defined as projections of >2 μm in length. Final image composites were created using Adobe Photoshop 5.0. No specific FITC labeling was observed in cells treated with preimmune serum instead of anti-β-tubulin antiserum. FITC labeling was not observed when the anti-tubulin antiserum was preincubated overnight at 4°C with PC-tubulin (1:1 ratio) and tested at the same antibody dilution (1:100) afterward. Although the anti-β-tubulin antiserum did not cross-react with actin in immunoblotting, SK-N-SH cells were tested for tubulin and actin distribution throughout the cell. Cells were double stained with the above-described polyclonal anti-β-tubulin antibody (1:100 dilution) and an anti-actin antibody (monoclonal; kindly provided by Dr. J. Lessard, University of Cincinnati, Cincinnati, OH; 1:1000 dilution). Secondary FITC-conjugated goat anti-rabbit antibody was used to detect tubulin (1:100 dilution), and Texas Red dye-conjugated AffiniPure goat anti-mouse antibody (Jackson ImmunoResearch, West Grove, PA; 1:100 dilution) was used to detect actin. Distinct protein structures were recognized by the anti-tubulin and anti-actin antibodies.

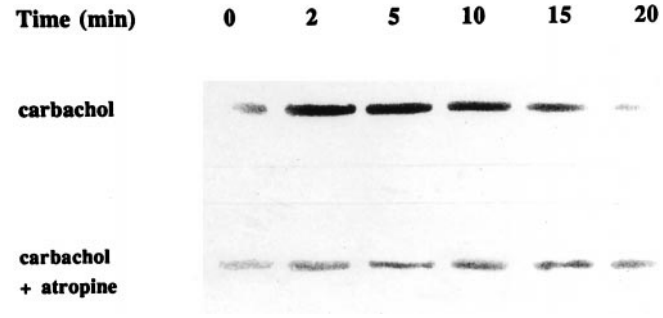

Figure 2. The activation of muscarinic receptors in SK-N-SH cells triggers the membrane association of tubulin. SK-N-SH cells were incubated for the indicated times with 1 mM carbachol with or without 100 μM atropine, as described. Membrane pellets were subjected to SDS-PAGE (50 μg of membrane protein in *each lane*) and immunoblotting with anti-β-tubulin antiserum, as described. A representative experiment of four with similar results is shown.

Materials. [α-^{32}P]GTP was from ICN Biomedicals (Cleveland, OH). [^{32}P]AAGTP and AAGTP were synthesized as described (Rasenick et al., 1994). *p*-Azidoaniline was synthesized by Dr. William Dunn (University of Illinois, Chicago, IL). [^{3}H]PIP_2 and myo-[^{3}H]inositol were from American Radiolabeled Chemicals (St. Louis, MO). [^{3}H]QNB was from Amersham. GppNHp, GTP, GDP, and GTPγS were from Boehringer Mannheim (Indianapolis, IN). Carbachol, atropine sulfate, and PIP_2 were from Sigma (St. Louis, MO). The P6-DG desalting gel was from Bio-Rad (Hercules, CA). P11 cellulose phosphate was from Whatman (Maidstone, UK). All other reagents were of analytical grade.

RESULTS

PLCβ_1 signaling is regulated by tubulin in neuroblastoma SK-N-SH cells

Both reconstitution studies with purified proteins and studies using membranes from Sf9 cells with recombinant m_1 muscarinic receptors, Gαq, and PLCβ_1 indicated that tubulin regulated PLCβ_1 signaling (Popova et al., 1997).

To test whether tubulin would regulate PLCβ_1 in a cell with endogenous expression of a PLCβ_1 cascade, membranes were prepared from SK-N-SH neuroblastoma cells, and carbachol-evoked activation of PLCβ_1 was studied. Tubulin-GppNHp evoked a biphasic regulation of PLCβ_1 in SK-N-SH cells similar to that seen in the Sf9 system. Enzyme activation at lower (nanomolar) and inhibition at higher (micromolar) concentrations of tubulin-GppNHp were observed (Fig. 1*A*). Tubulin-GppNHp potentiated carbachol-evoked phosphoinositide hydrolysis more than did GppNHp. Thus it appears that in SK-N-SH cells, tubulin might participate in the regulation of PLCβ_1 via a receptor-Gq-signaling cascade.

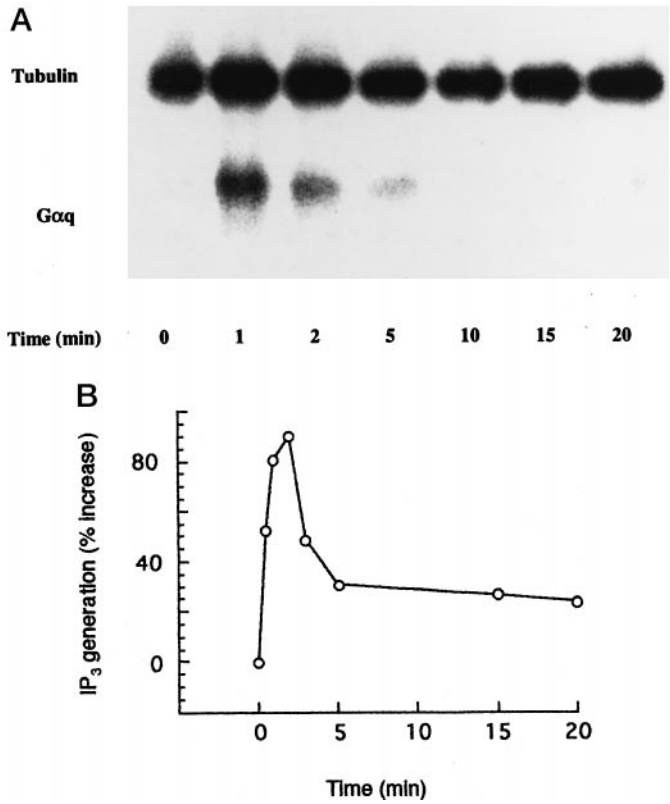

Figure 3. Tubulin membrane association, nucleotide transfer to Gαq, and PLCβ$_1$ activation follow a similar time course in membranes from Sf9 cells that contain a recombinant PLCβ$_1$ cascade. *A,* Time course of the carbachol-evoked tubulin-[^{32}P]AAGTP association with the membrane and the nucleotide transfer to Gαq. Membranes were incubated for the indicated times with 1 μM tubulin-[^{32}P]AAGTP and 10 μM carbachol at 23°C, followed by UV irradiation, SDS-PAGE (70 μg of membrane protein in *each lane*), and autoradiography. The experiment shown is representative of three with similar results. In the absence of carbachol, no increase in tubulin membrane association or nucleotide transfer to Gαq was detected at any time point tested. Both tubulin membrane association and Gαq transactivation, triggered by carbachol, were inhibited by 100 nM atropine. *B,* Carbachol-evoked activation of PLCβ$_1$ by tubulin-GppNHp. Twenty micrograms of Sf9 cell membranes containing the indicated recombinant proteins were incubated with 100 μM PIP_2 substrate as described. Carbachol (10 μM) and tubulin-GppNHp (30 nM) were added and incubated for the indicated times at 37°C as described. The experiment shown is representative of three with similar results. Control activity was 0.61 ± 0.09 nmol of $IP_3 \cdot min^{-1} \cdot mg$ of protein^{-1}. When GppNHp was tested under these experimental conditions, a linear increase in PLCβ$_1$ activation was observed. Tubulin-GppNHp activation of PLCβ$_1$ in the presence of carbachol was atropine-sensitive.

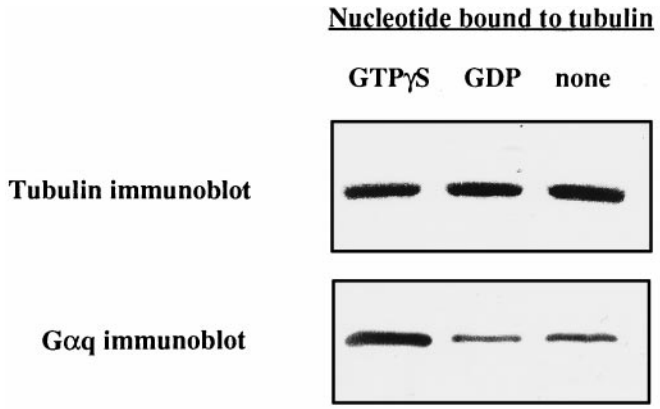

Figure 4. Preferential association of Gαq with the GTPγS-bound form of tubulin. Membrane extracts (0.5 mg/ml membrane protein) from Sf9 cells infected with baculoviruses carrying Gαq cDNA were incubated at 4°C with 1 μM tubulin-GTPγS, tubulin-GDP, or nucleotide-free tubulin. Immunoprecipitation with anti-β-tubulin antibody (polyclonal) was followed by SDS-PAGE (10% gels) and immunoblotting with anti-Gαq and anti-α-tubulin antibodies (monoclonal DM 1A). ECL detection and densitometry of the bands were used to estimate the Gαq bound to different tubulin states. No tubulin or Gαq immunoprecipitated when preimmune serum replaced the anti-β-tubulin antiserum in the immunoprecipitation. Gαq-containing Sf9 membranes were run in each experiment to ensure that the immunoprecipitated protein was Gαq. The image of one of three independent experiments with similar results is shown.

Table 1. Coimmunoprecipitation of Gαq with different tubulin-guanine nucleotide bound species

Tubulin species	Arbitrary units (% change)
Tubulin-GTPγS	100.0 ± 8.2
Tubulin-GppNHp	89.0 ± 7.4
Tubulin-GDP	31.0 ± 5.1*
Tubulin stripped of nucleotide	43.2 ± 9.3*

Membranes were extracted with sodium cholate, incubated with the indicated tubulin species, and immunoprecipitated with anti-tubulin antiserum as described in Figure 4. The results presented are the means ± SD of three independent experiments.

*Significantly less than tubulin-GTPγS ($p < 0.05$), Student's *t* test.

Tubulin transactivates Gαq in SK-N-SH cell membranes

To investigate whether tubulin interacted with Gαq in SK-N-SH membranes, these membranes were extracted with sodium cholate and incubated with tubulin-[^{32}P]AAGTP, and the extracts were immunoprecipitated with anti-Gαq or anti-PLCβ$_1$ antisera (Fig. 1*B*). Tubulin coimmunoprecipitated with endogenous Gαq and, to a lesser extent, with PLCβ$_1$.

Previous studies suggested that tubulin activated Gαq by the direct transfer of GTP (Popova et al., 1997). This was tested in SK-N-SH cells using the photoaffinity GTP analog AAGTP. Carbachol increased the association of tubulin-[^{32}P]AAGTP with SK-N-SH membranes (Fig. 1*C*). Although the amount of Gαq was similar under these experimental conditions, [^{32}P]AAGTP transfer from tubulin to Gαq (transactivation) was observed only after muscarinic receptor stimulation. Thus, an activated receptor was required to initiate the process of Gαq activation by tubulin. This effect was specific, because it was blocked by atropine.

Carbachol triggers the membrane association of tubulin in SK-N-SH cells

In the studies described above, exogenous tubulin was shown to bind to isolated membranes and regulate PLCβ$_1$ signaling. To clarify whether endogenous tubulin would gravitate toward the membrane in response to agonist stimulation, we incubated SK-N-SH cells with carbachol and quantified membrane-associated tubulin by immunoblotting (Fig. 2). Carbachol evoked a rapid and time-dependent increase in the tubulin recruited to membranes of the SK-N-SH cells. Tubulin association with membranes (postnuclear fraction) increased by ~2.5-fold [244.8 ± 31.3% (± SD)] after 2 min of carbachol treatment and gradually declined afterward. The process was atropine sensitive. A decrease in cytosolic tubulin was also detected [35.9 ± 10.5% (± SD)]. Carbachol did

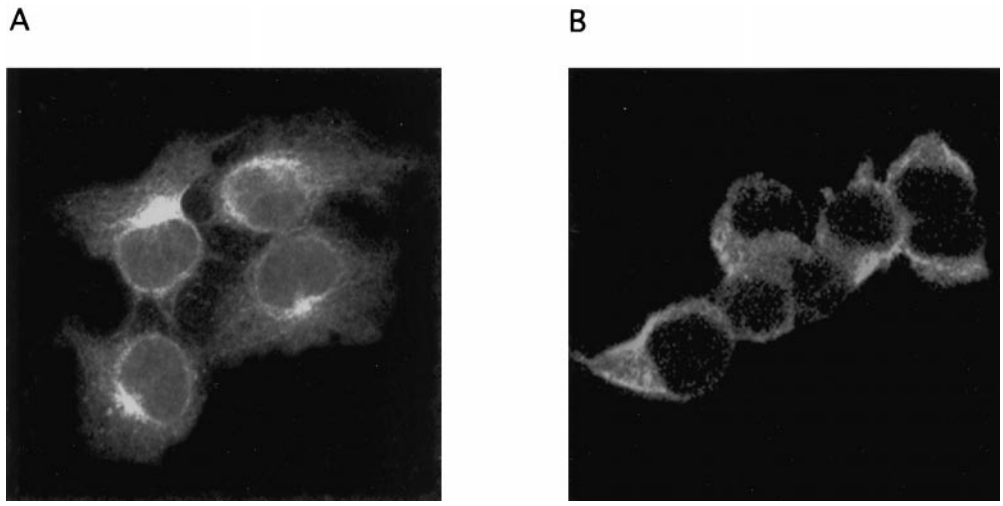

Figure 5. Carbachol stimulation causes microtubule reorganization and translocation of tubulin to the membrane in the SK-N-SH cells. Cells were untreated (*A*) or treated with 1 mM carbachol for 2 min (*B*) before fixation and immunofluorescence labeling, as described. A representative image of cells obtained in one of four independent experiments with similar results is shown.

not increase the amount of tubulin associated with the nuclear fraction.

The time courses of tubulin-[^{32}P]AAGTP membrane association, Gαq transactivation by tubulin, and PLCβ_1 activation are concordant

The activation of PLCβ_1 by tubulin shows rapid onset and decline. To understand this process, it was important to correlate the association of tubulin with the membrane and the transactivation of Gαq with the activation of PLCβ_1. This was performed in Sf9 cells in which no endogenous PLCβ_1 activity is detected unless m_1 muscarinic receptors, Gαq, and PLCβ_1 are expressed (Popova et al., 1997). Carbachol (10 μM) induced a rapid membrane association of tubulin-[^{32}P]AAGTP with Sf9 membranes containing all three elements of the PLCβ_1 cascade (Fig. 3*A*). The pattern of response was similar to that observed in the SK-N-SH membranes. The amount of membrane-associated tubulin was maximal after 1 min [an increase of 127.0 ± 15.5% (± SD)] and gradually decreased over 10 min. The transfer of [^{32}P]AAGTP from tubulin to Gαq followed the same pattern. All [^{32}P]AAGTP bound to Gαq originated from tubulin, because under this experimental condition [^{32}P]AAGTP remains bound to tubulin unless transferred directly to Gα (Roychowdhury and Rasenick, 1994). The association of tubulin with the membrane and Gαq transactivation by tubulin were dependent on m_1 receptor activation and blocked by atropine (data not shown). Carbachol did not cause the association of tubulin with native Sf9 membranes or with membranes that did not contain recombinant m_1 muscarinic receptors, Gαq, or PLCβ_1 (Popova et al., 1997).

The temporal relationship between the membrane association of tubulin and the activation of Gαq and PLCβ_1 was also tested (Fig. 3*B*). The time course of carbachol-induced activation of PLCβ_1 in the presence of tubulin-GppNHp was strikingly parallel to the membrane association of tubulin and Gαq transactivation. The enzyme activity reached a maximum in 2 min and declined afterward, although a 30% activation of the enzyme above basal was maintained for the duration of the experiment. The observed kinetics of tubulin membrane association, both *in vivo* and *in vitro*, supported the idea that tubulin was recruited to the membrane in response to receptor stimulation for the regulation of Gαq-mediated PLCβ_1 signaling.

Tubulin must have GTP (or a GTP analog) in the exchangeable binding site to interact with Gαq and activate PLCβ_1

Previous results suggested that GTP or a GTP analog had to occupy the exchangeable nucleotide-binding site of tubulin to activate PLCβ_1, whereas PLCβ_1 inhibition at high tubulin concentrations was a nucleotide-independent process. When tested, tubulin-GppNHp, but not tubulin-GDP, tubulin-GDPβS, or tubulin stripped of nucleotide, activated the enzyme (Popova et al., 1997).

To analyze further the importance of GTP in the interaction between tubulin and Gαq, tubulin with various guanine nucleotides bound was coimmunoprecipitated with Gαq. Tubulin-GTPγS, tubulin-GppNHp, tubulin-GDP, or tubulin free of nucleotide in the exchangeable GTP-binding site was incubated with detergent extracts of Sf9 cell membranes enriched in Gαq. Anti-tubulin antiserum was added, and tubulin was immunoprecipitated (Fig. 4, Table 1). Although the amounts of the tubulin species, immunoprecipitated by the anti-tubulin antiserum, were identical (this was determined by blotting with a monoclonal anti-tubulin antibody, DM 1A; Sigma), Gαq coimmunoprecipitated with the GTPγS- and GppNHp-bound forms of tubulin. Coimmunoprecipitation of Gαq with the GDP-bound form of tubulin or with tubulin devoid of nucleotide was identical to that seen with preimmune serum replacing the anti-tubulin antiserum (Fig. 4, Table 1). These results confirmed that a conformation of tubulin with GTP bound to the E site was required for tubulin interaction with Gαq.

Carbachol stimulation causes redistribution of intracellular tubulin

It is predicted that tubulin dimers translocate to the membrane in response to muscarinic receptor stimulation, transactivate Gαq, and promote activation of PLCβ_1. A possible mechanism would be that when the intracellular calcium concentration rises in defined areas of the cell, microtubules depolymerize and more dimers are available to interact with Gαq. To clarify whether muscarinic receptor stimulation causes redistribution of intracellular tubulin, SK-N-SH cells were treated with carbachol and studied with confocal laser microscopy.

During interphase, microtubules were seen in greatest density at the centrosome, radiating out to the periphery of the cell in a fine array of threads (Fig. 5). Tubulin was not seen at the plasma

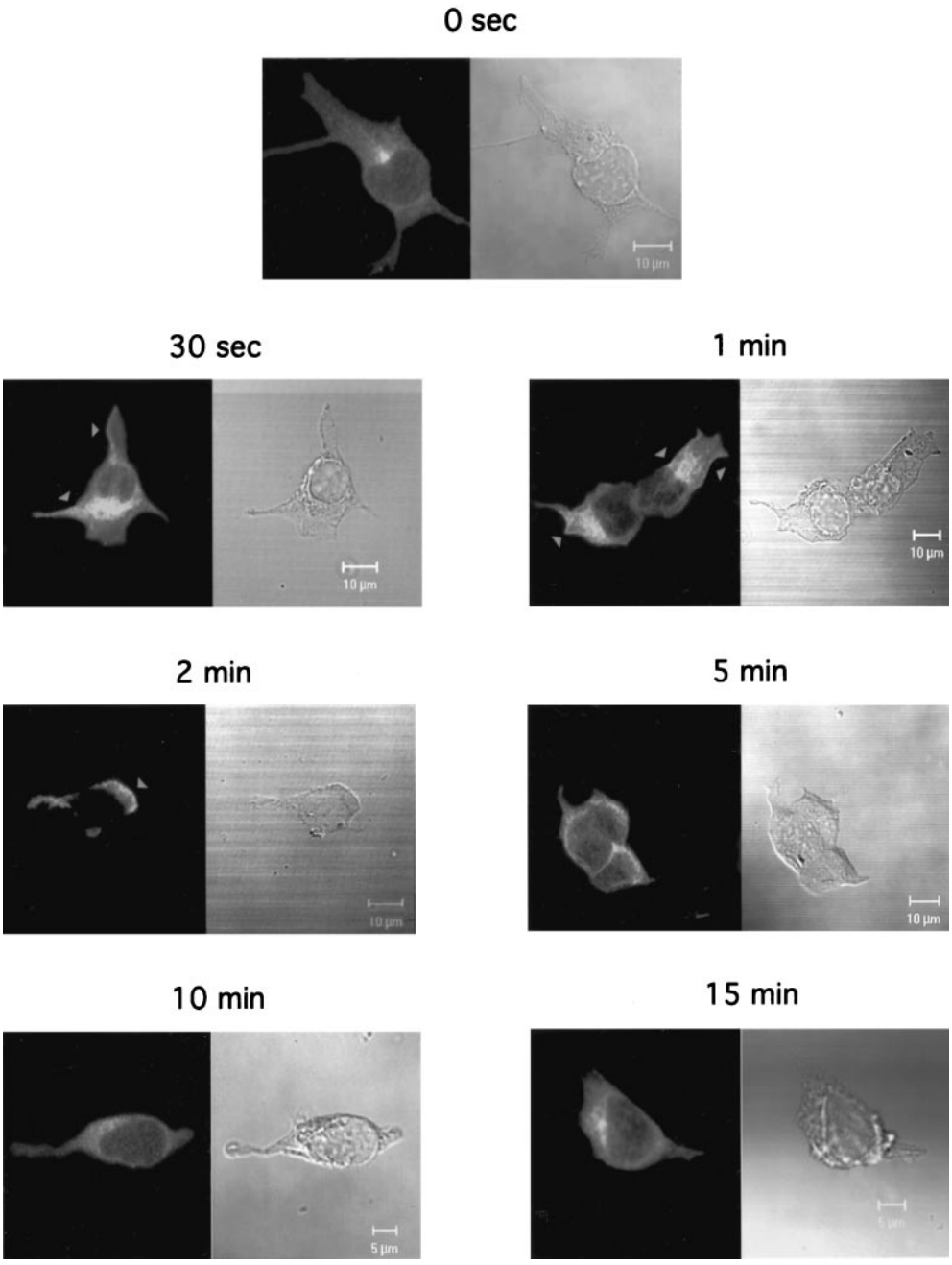

Figure 6. Time course of the redistribution and membrane translocation of tubulin during carbachol stimulation of SK-N-SH cells. Cells were treated with 1 mM carbachol for the periods indicated before fixation and immunostaining, as described. When 100 μM atropine was applied before carbachol, the images were identical to those of control cells. Confocal micrographs of the treated cells are shown to the *left*. Shown to the *right* are the differential interference contrast micrographs of the same cells. The *arrowheads* denote areas of membrane localization of tubulin. Confocal images of 1-μm-thick sections at the same level within the cell are presented. Four independent experiments with similar results were performed. The images shown are representative of ~300 cells examined at each time point.

membrane. Two minutes after carbachol exposure, microtubule depolymerization and redistribution of tubulin to areas along the plasma membrane were observed. A rapid and transient translocation of tubulin to the periphery of the cell was seen as early as 30 sec after muscarinic receptor activation (Fig. 6). The membrane relocation of tubulin was most evident at 2 min after agonist stimulation. This event was accompanied by changes in the organization of the cytoskeleton, as well as cell shape. This is seen in the corresponding DIC images. Although most of the control cells (85.5%; $n = 300$) exhibited a well developed microtubule network and cellular projections, cells treated with carbachol progressively lost and regained these features. At 2 min of stimulation, microtubules in most of the cells appeared disorganized, cell projections were either lacking or shortening, and the cells had the tendency to round up (processes appeared in only 12.6% of the cells; $n = 300$).

Five minutes after agonist stimulation, the process of tubulin redistribution back to the cytosol had commenced. Distinct membrane localization of tubulin was no longer observed after 10 min of carbachol exposure of the cells. Fifteen minutes subsequent to the initiation of carbachol exposure, the resting cell shape was restored, and the microtubule network was reorganized. Cell processes became more abundant (processes were present in 61.0% of the cells; $n = 300$). The confocal *z*-scan images, presented in Figure 7 clearly demonstrate a normal distribution of tubulin in the untreated cell and its peripheral redistribution after 2 min of carbachol exposure.

DISCUSSION

The principal finding in this study is that muscarinic receptor activation triggers a transient redistribution and membrane association of cytosolic tubulin. The intracellular reorganization of

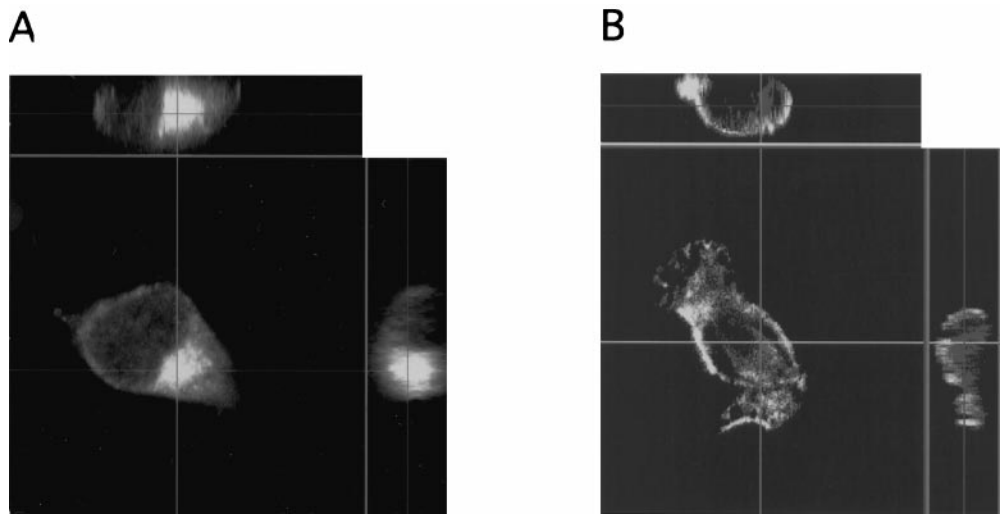

Figure 7. Carbachol exposure of the SK-N-SH cells translocates tubulin to the membrane of the cell. Computer-generated cross sections of the whole cell are displayed on the *top* (*x–z* plane) and on the *right* (*y–z* plane). *A,* Untreated SK-N-SH cell (0 time point, control). Note the random distribution of tubulin throughout the cell in the *top* and *right* computer-generated cross-section images. *B,* SK-N-SH cell treated with 1 mM carbachol (2 min exposure). Note the membrane localization of tubulin in the *top* and *right* computer-generated cross-section images of the cell. The images shown are representative of at least 30 cells subjected to a *z*-scan analysis with similar results.

this cytoskeletal protein in response to agonist stimulation was visualized in SK-N-SH neuroblastoma cells, and the carbachol-evoked association of tubulin with the plasma membrane was correlated with the regulation of Gq-mediated PLCβ_1 signaling. This translocation event carries the potential of affecting a broad spectrum of cellular functions, including other signaling pathways, intracellular trafficking, cell shape, cell movement, and cell division.

Numerous cytosolic enzymes, including phospholipases, kinases, and phosphatases, associate transiently with membrane proteins. The association of tubulin with receptors (Kirsch et al., 1991; Barsony and McKoy, 1992; Item and Sieghart, 1994), G-proteins (Wang at al., 1990; Wang and Rasenick, 1991; Roychowdhury et al., 1993; Popova et al., 1994, 1997; Rasenick et al., 1994; Roychowdhury and Rasenick, 1994; Yan et al., 1996), and regulatory enzymes (Kapeller et al., 1995; Reszka et al., 1995; Sontag et al., 1995; Popova et al., 1997; Pitcher et al., 1998) has also been reported. A direct interaction of tubulin with the α subunit of the G-proteins Gs, Gi1, and Gq is thought to mediate adenylyl cyclase and PLCβ_1 signaling (Popova et al., 1994, 1997; Yan et al., 1996). The present findings extend these observations further. They raise the possibility that at least some of the tubulin associated with the cell membrane might be transiently recruited from the cytosol in response to a signal. It is suggested that previously observed tubulin-Gα complexes (Yan et al., 1996) are transient dynamic formations, whose functional assembly is regulated and reversible.

The present data also reveal that tubulin dimers must be in the GTP conformation to interact with Gαq. A similar functional interaction of tubulin-GTP but not tubulin-GDP with the small GTPase Rac1 has been reported (Best et al., 1996). Furthermore, tubulin-GppNHp was more potent and efficacious than GppNHp for the activation of PLCβ_1. This suggested the possibility that, after receptor activation, Gαq was transactivated by tubulin-GTP more easily than activated by GTP alone. It also indicated that the effect of tubulin-GTP on PLCβ_1 could be significant in cellular compartments where the local tubulin dimer concentration is comparable with or higher than that of GTP.

Although membrane-associated tubulin has been demonstrated (Bhattacharyya and Wolff, 1976; Zisapel et al., 1980; Pfeffer et al., 1983; Stephens, 1986), there is no clear evidence that a membrane-integrated tubulin isoform exists. For tubulin to transactivate Gα, however, association with the membrane can be quite transient. In fact, the evidence in Figures 3 and 5–7 is consistent with such a transient association. It is noteworthy that a redistribution of cytoskeletal components, including actin and tubulin, has been reported to occur in T47D breast cancer cells after opioid receptor stimulation (Panagiotou et al., 1999).

In addition to elements of the cytoskeleton regulating cellular signaling, cellular-signaling molecules have been shown to regulate cytoskeletal form and function. The actin cytoskeleton is regulated by the small GTP-binding proteins Rho, Rac, Cdc42, and Ras (Zigmond et al., 1997; Hall, 1998; Nobes and Hall, 1999); the heterotrimeric G-protein subunits Gαi1, Gαq, and G$\beta_1\gamma_2$ affect microtubule polymerization dynamics (Ravindra et al., 1996; Roychowdhury and Rasenick, 1997; Roychowdhury et al., 1999).

After a rapid increase, tubulin migration to the membrane, transactivation of Gαq by tubulin, and activation of PLCβ_1 declined (Figs. 2, 3, 6). The reorganization of the microtubule cytoskeleton also appeared transient and reversible. It is possible that increases in cytosolic calcium resulting from the generation of IP_3 might evoke localized destabilization of microtubules. As a result, an increase in tubulin dimer concentrations near regions of the membrane where receptors, Gq, and PLCβ_1 are found would be expected. Because tubulin concentrations >100 nM inhibit PLCβ_1, continued activation of PLCβ_1 could effect a feedback inhibition of the enzyme via tubulin.

It is possible that an increased amount of tubulin associated with the plasma membrane would interfere with the coupling between Gαq and PLCβ_1. Tubulin coimmunoprecipitates with both Gαq and PLCβ_1, although to a lesser extend with the later (Fig. 1*B*) (Popova et al., 1997). As such, a direct tubulin-PLCβ_1 interaction (at high tubulin concentrations) might abolish Gαq-PLCβ_1 coupling. However, tubulin also binds the PLCβ_1 substrate PIP_2 (Popova et al., 1997). Rapid hydrolysis of a readily available PIP_2 pool by PLCβ_1, followed by tubulin association with the remaining substrate, might also account for the observed decrease in IP_3 generation in the course of the experiment.

It is also possible that dissociation of tubulin from the membrane after Gαq activation accounts for the observed decline in PLCβ_1 activation. Such movement of tubulin from the membrane back to its original location in the cytosol could be caused by the decrease in the local membrane concentration of PIP_2. If Gαq is the membrane anchor for tubulin, the loss of GTP from tubulin during the process of Gαq transactivation would also decrease the

affinity of tubulin for Gq and release tubulin. In addition to Gαq and PIP_2, other signaling proteins might enjoy a reversible association with tubulin at the plasma membrane. Both Gβγ (Roychowdhury and Rasenick, 1997) and the GRKs, GRK2 (Carman et al., 1998; Haga et al., 1998; Pitcher et al., 1998) and GRK5, (Carman et al., 1998) bind to tubulin. Gβγ assists the recruitment of GRK2 to the membrane (Pitcher et al., 1992). Thus, a possible scenario is that tubulin, GRK, and Gβγ are involved in a common membrane association pathway and that some interplay among these molecules is a regulatory event. Should the membrane anchor for tubulin prove to be a molecule other than those listed above, another scenario would need to be developed.

It is noteworthy that activity shapes the structure of neurons and their circuits. Synaptic activation is shown to produce rapid input-specific changes in dendritic structure (Maletic-Savatic et al., 1999). The possibility exists that the neurotransmitter-evoked recruitment of tubulin to the membrane assists with this process. In fact, it has been suggested previously that the synaptic activity-controlled balancing of monomeric, dimeric, and polymeric forms of actin and tubulin might underlie the changes in spine shape (Van Rossum and Hanisch, 1999).

In summary, the experiments described above support the notion that, in addition to its function as a structural protein that forms microtubules, tubulin serves as a signal-recruited regulator of membrane-associated signaling events. As such, tubulin is able to orchestrate the function of multiprotein signaling complexes. These results also suggest the possibility of direct cross-regulation of cellular signaling and the reorganization of the microtubule cytoskeleton. Reciprocal interactions between G-protein signaling and the cytoskeleton might channel events triggered by diverse regulatory signals to different cellular compartments. The regulation of cell division, growth, motility, and morphology, as well as the movement of intracellular components, might be coordinated along this axis.

REFERENCES

Banno Y, Nakashima T, Kumada T, Ebisawa K, Nonomura Y, Nozawa Y (1992) Effects of gelsolin on human platelet cytosolic phosphoinositide-phospholipase C isozymes. J Biol Chem 267:6488–6494.

Barsony J, McKoy W (1992) Molybdate increases intracellular 3′,5′-guanosine cyclic monophosphate and stabilizes vitamin D receptor association with tubulin-containing filaments. J Biol Chem 267:24457–24465.

Berridge MJ (1993) Inositol trisphosphate and calcium signalling. Nature 361:315–325.

Best A, Ahmed S, Kozma R, Lim L (1996) The Ras-related GTPase Rac1 binds tubulin. J Biol Chem 271:3756–3762.

Bhattacharyya B, Wolff J (1976) Polymerisation of membrane tubulin. Nature 264:576–577.

Blank JL, Brattain KA, Exton JH (1992) Activation of cytosolic phosphoinositide phospholipase C by G-protein βγ subunits. J Biol Chem 267:23062–23075.

Boguslavsky V, Rebecchi M, Morris AJ, Jhon D-Y, Rhee SG, McLaughlin S (1994) Effect of monolayer surface pressure on the activities of phosphoinositide-specific phospholipase C-β_1, -γ_1, and -δ_1. Biochemistry 33:3032–3037.

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

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254.

Carman CV, Som T, Kim CM, Benovic JL (1998) Binding and phosphorylation of tubulin by G protein-coupled receptor kinases. J Biol Chem 273:20308–20316.

Fisher SK (1988) Recognition of muscarinic cholinergic receptors in human SK-N-SH neuroblastoma cells by quaternary and tertiary ligands is dependent upon temperature, cell integrity, and the presence of agonists. Mol Pharmacol 33:414–422.

Fisher SK, Heacock AM (1988) A putative M_3 muscarinic cholinergic receptor of high molecular weight couples to phosphoinositide hydrolysis in human SK-N-SH neuroblastoma cells. J Neurochem 50:984–987.

Fisher SK, Snider RM (1987) Differential receptor occupancy requirements for muscarinic cholinergic stimulation of inositol lipid hydrolysis in brain and in neuroblastomas. Mol Pharmacol 32:81–90.

Freeman JL, De La Cruz EM, Pollard TD, Lefkowitz RJ, Pitcher JA (1998) Regulation of G protein-coupled receptor kinase 5 (GRK5) by actin. J Biol Chem 273:20653–20657.

Goldschmidt-Clermont PJ, Machesky LM, Baldassare JJ, Pollard TD (1990) The actin-binding protein profilin binds to PIP_2 and inhibits its hydrolysis by phospholipase C. Science 247:1575–1578.

Goldschmidt-Clermont PJ, Kim JW, Machesky LM, Rhee SG, Pollard TD (1991) Regulation of phospholipase Cγ_1 by profilin and tyrosine phosphorylation. Science 251:1231–1233.

Graber SG, Figler RA, Garrison JC (1992) Expression and purification of functional G protein α subunits using a baculovirus expression system. J Biol Chem 267:1271–1278.

Haga K, Ogawa H, Haga T, Murofushi H (1998) GTP-binding-protein-coupled receptor kinase 2 (GRK2) binds and phosphorylates tubulin. Eur J Biochem 255:363–368.

Hall A (1998) Rho GTPases and the actin cytoskeleton. Science 279:509–514.

Hwang SC, Jhon D-Y, Bae YS, Kim JH, Rhee SG (1996) Activation of phospholipase Cγ by the concerted action of *tau* proteins and arachidonic acid. J Biol Chem 271:18342–18349.

Item C, Sieghart W (1994) Binding of γ-aminobutyric acid A receptors to tubulin. J Neurochem 63:1119–1125.

Kapeller R, Toker A, Cantley LC, Carpenter CL (1995) Phosphoinositide 3-kinase binds constitutively to α/β-tubulin and binds to γ-tubulin in response to insulin. J Biol Chem 27:25985–25991.

Kirsch J, Langosch D, Prior P, Littauer UZ, Schmitt B, Betz H (1991) The 93-kDa glycine receptor-associated protein binds to tubulin. J Biol Chem 266:22242–22245.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Maletic-Savatic M, Malinow R, Svoboda K (1999) Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. Science 283:1923–1927.

Nobes CD, Hall A (1999) Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol 144:1235–1244.

Noh D-Y, Shin SH, Rhee SG (1995) Phosphoinositide-specific phospholipase C and mitogenic signaling. Biochim Biophys Acta 1242:99–114.

Panagiotou S, Bakogeorgou E, Papakonstanti E, Hatzoglou A, Wallet F, Dussert C, Stournaras C, Martin PM, Castanas E (1999) Opioid agonists modify breast cancer cell proliferation by blocking cells to the G2/M phase of the cycle: involvement of cytoskeletal elements. J Cell Biochem 73:204–211.

Park D, Jhon DY, Lee C-W, Lee K-H, Rhee SG (1993) Activation of phospholipase C isozymes by G protein βγ subunits. J Biol Chem 268:4573–4576.

Parker EM, Kameyama K, Higashijima T, Ross EM (1991) Constitutively active G protein-coupled receptors purified from baculovirus-infected insect cells. J Biol Chem 266:519–527.

Payrastre B, van Bergen en Henegouwen PMP, Breton M, den Hartigh JC, Plantavid M, VerkLeij AJ, Boonstra J (1991) Phosphoinositide kinase, diacylglycerol kinase, and phospholipase C activities associated to the cytoskeleton: effect of epidermal growth factor. J Cell Biol 115:121–128.

Pfeffer SR, Drubin DG, Kelly RB (1983) Identification of three coated vesicle components as α- and β-tubulin linked to a phosphorylated 50,000-dalton polypeptide. J Cell Biol 97:40–47.

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

Pitcher JA, Hall RA, Daaka Y, Zhang J, Ferguson SG, Hester S, Miller S, Caron MG, Lefkowitz RJ, Barak LS (1998) The G protein-coupled receptor kinase 2 is a microtubule-associated protein kinase that phosphorylates tubulin. J Biol Chem 273:12316–12324.

Popova JS, Dubocovich ML (1995) Melatonin receptor-mediated stimulation of phosphoinositide breakdown in chick brain slices. J Neurochem 64:130–138.

Popova J, Johnson GL, Rasenick MM (1994) Chimeric Gαs/Gαi2 pro-

teins define domains on Gαs that interact with tubulin for β-adrenergic activation of adenylyl cyclase. J Biol Chem 269:21748–21754.
Popova JS, Garrison JC, Rhee SG, Rasenick MM (1997) Tubulin, Gq, and phosphatidylinositol 4,5-bisphosphate interact to regulate phospholipase $C\beta_1$ signaling. J Biol Chem 272:6760–6765.
Rasenick MM, Talluri M, Dunn III WJ (1994) Photoaffinity guanosine 5′-triphosphate analogs as a tool for the study of GTP-binding proteins. Methods Enzymol 237:100–110.
Ravindra R, Kunapuli SP, Forman LJ, Nagele RG, Foster KA, Patel SA (1996) Effect of transient overexpression of Gqα on soluble and polymerized tubulin pools in GH3 and AtT-20 cells. J Cell Biochem 61:392–401.
Reszka AA, Seger R, Diltz CD, Krebs EG, Fisher EH (1995) Association of mitogen-activated protein kinase with the microtubule cytoskeleton. Proc Natl Acad Sci USA 92:8881–8885.
Rhee SG (1991) Inositol phospholipids-specific phospholipase C: interaction of the γ_1 isoform with tyrosine kinase. Trends Biochem Sci 16:297–301.
Rhee SG, Choi KD (1992) Regulation of inositol phospholipid-specific phospholipase C isozymes. J Biol Chem 267:12393–12396.
Roychowdhury S, Rasenick MM (1994) Tubulin-G protein association stabilizes GTP binding and activates GTPase: cytoskeletal participation in neuronal signal transduction. Biochemistry 33:9800–9805.
Roychowdhury S, Rasenick MM (1997) G protein β1γ2 subunits promote microtubule assembly. J Biol Chem 272:31576–31581.
Roychowdhury S, Wang N, Rasenick MM (1993) G protein binding and G protein activation by nucleotide transfer involve distinct domains on tubulin: regulation of signal transduction by cytoskeletal elements. Biochemistry 32:4955–4961.
Roychowdhury S, Panda D, Wilson L, Rasenick MM (1999) G protein α subunits activate tubulin GTPase and modulate microtubule polymerization dynamics. J Biol Chem 274:13485–13490.
Shelanski M, Gaskin F, Cantor C (1973) Microtubule assembly in the absence of added nucleotides. Proc Natl Acad Sci USA 70:765–768.
Sontag E, Nunbhakdi-Craig V, Bloom JS, Mumby MC (1995) A novel pool of protein phosphatase 2A is associated with microtubules and is regulated during the cell cycle. J Cell Biol 128:1131–1144.
Stephens RE (1986) Polymerisation of membrane tubulin. Biol Cell 57:95–110.
Sun H-Q, Lin K-M, Yin HL (1997) Gelsolin modulates phospholipase C activity *in vivo* through phospholipid binding. J Cell Biol 138:811–820.
Unno T, Komori S, Ohashi H (1999) Microtubule cytoskeleton involvement in muscarinic suppression of voltage-gated calcium channel current in guinea-pig ileal smooth muscle. Br J Pharmacol 127:1703–1711.
Van Rossum D, Hanisch UK (1999) Cytoskeletal dynamics in dendritic spines: direct modulation by glutamate receptors? Trends Neurosci 22:290–295.
Wang N, Rasenick MM (1991) Tubulin-G protein interactions involve microtubule polymerization domains. Biochemistry 30:10957–10965.
Wang N, Yan K, Rasenick MM (1990) Tubulin binds specifically to the signal-transducing proteins, Gsα and Giα1. J Biol Chem 265:1239–1242.
Yan K, Greene E, Belga F, Rasenick MM (1996) Synaptic membrane G proteins are complexed with tubulin *in situ*. J Neurochem 66:1489–1495.
Zigmond SH, Joyce M, Borleis J, Bokoch GM, Devreotes PN (1997) Regulation of actin polymerization in cell-free systems by GTPγS and Cdc42. J Cell Biol 138:363–374.
Zisapel N, Levi M, Gozes I (1980) Tubulin: an integral protein of mammalian synaptic vesicle membranes. J Neurochem 34:26–32.