Endocrine, vol. 7, no. 2, 127–143, October 1997 0969-711X/97/7:127-143/$12.25

Review

Is Signal Transduction Modulated by an Interaction Between Heterotrimeric G-Proteins and Tubulin?

Rudravajhala Ravindra

Endocrine-Metabolic Division, Veterans Affairs Medical Center and Finch University of Health Sciences/The Chicago Medical School, North Chicago, IL

Although it is generally accepted that tubulin plays an important role in G-protein-mediated signal transduction in a variety of systems, the mechanism of this phenomenon is not completely understood. G-protein-tubulin interaction at the cell membrane and the cytosol, and the influence of such an interaction on cellular signaling are discussed in this review article.

Because the diameter of a microtubule is 25 nm and the plasma membrane is 9–11 nm thick, it is not possible for membrane-associated tubulin to assemble into a complete microtubule in the membrane environment. However, tubulin heterodimers may be able to function in the membrane environment as individual heterodimers or as polymers arranged into short protofilaments. At the cell membrane, membrane-associated tubulin may influence hormone–receptor interaction, receptor-G-protein coupling, and G-protein-effector coupling. Structural proteins, such as tubulin, can participate in cellular signaling by communicating through physical forces. By virtue of its interaction with the submembranous network of cytoskeletal proteins, tubulin, when perturbed in one locus, can transmit large changes in conformations to other points. Thus, GTP binding to membrane-associated tubulin might lead to a conformational change in either receptors or G proteins. This may, in turn, influence the binding of an agonist to its receptor.

On the other hand, in the cell cytosol, subsequent to agonist-induced translocation of G-proteins from the membrane compartment to the cytosol, G-proteins may affect microtubule formation. In GH_3 and AtT-20 cells (stably expressing TRH receptor), transiently transfected with Gqα cDNA, soluble tubulin levels decreased in Gqα-transfected GH_3 and AtT-20 cells, by 33% and 52%, respectively. These results suggest that G-proteins may have a direct effect on the microtubule function in vivo.

Because tubulin and G-protein families are ubiquitous and highly conserved, an interaction between these two protein families may occur in vivo, and this, in turn, can have an impact on signal transduction. However, the physiological significance of this interaction remains to be demonstrated.

Key Words: Tubulin; G-proteins; colchicine; paclitaxel; GTP.

Introduction

The discovery of cAMP had a great impact on the field of signal transduction *(1)*, finally leading to the discovery of heterotrimeric guanine nucleotide binding proteins (G-proteins). G-proteins are a family of closely related proteins involved in the transfer of information from surface receptors to biochemical effector mechanisms in a variety of systems *(2)*. At about the same time that cAMP was discovered, Peters *(3)* proposed that hormones act on cells by modifying their cytoskeleton. This interesting theory was not received with much enthusiasm at that time, probably owing to the general perception of cytoskeleton as a rigid structure, merely involved in cellular movements and the maintenance of cell shape. We now know that some of the components of the cytoskeleton, such as actin and tubulin, are dynamic proteins, with a number of different functions, including the ability to bind ATP or GTP, and hydrolyze them *(4)*. Although these proteins can also polymerize into large filamentous structures known as microtubules or microfilaments, they also appear to have a number of important functions while in the unpolymerized

Received December 20, 1996; Accepted January 8, 1997.

Author to whom all correspondence and reprint requests should be addressed: R. Ravindra, PhD, Endocrine-Metabolic Division, Veterans Affairs Medical Center, Building 1, 151, North Chicago, IL 60064. E-mail: ravindra@n-chicago.va.gov

state. There is a general consensus that actin and tubulin play an important role in signal transduction in a variety of systems *(5–7)*.

A comprehensive review of the role of cytoskeletal proteins in signal transduction was published *(5)*. The role of actin in signal transduction was described in several recent reviews *(6–9)*.

This article will critically examine the following:

- G-protein–tubulin interaction at the cell membrane and the cytosol.
- The influence of such an interaction on cellular signaling.
- The importance of this interaction for signal transduction.

G-Proteins

G-proteins are a family of closely related proteins involved in the transfer of information from surface receptors to biochemical effector mechanisms in a variety of systems *(2,10–22)*. G-protein-linked responses regulate many cellular processes in vivo, such as the hormonal regulation of metabolic pathways (e.g., lipolysis, glycogenolysis, and gluconeogenesis). In addition to defining the specificity by which a particular receptor modulates a certain intracellular signaling system, G-proteins regulate the duration of the signal. G-proteins consist of a variable α-subunit (39–46 kDa) and β- and γ-subunits (35–36 and 8 kDa, respectively). Several species of G_{α}-subunits have been identified: $G_{s\alpha}$ mediates stimulation of adenylyl cyclase by β-adrenergic (and other) receptors, $G_{i\alpha}$ mediates inhibition of adenylyl cyclase by muscarinic (and other) receptors *(23–26)*; $G_{q\alpha}$ regulates phospholipase C in liver and brain *(27–30)*. $G_{o\alpha}$ mediates signaling pathways that regulate the locomotion, egg-laying, and male mating behavior in the roundworm *Caenorhabditis elegans (31,32)*. Other G-proteins transduce signals from special sense receptors. Gustducin is implicated in the transduction of taste in the rat taste buds *(33)*. The expression of transducin in some taste receptor cells suggests that transducin, in addition to its function in vision *(34–36)*, is involved in taste transduction *(37,38)*.

The regulation of a particular effector is determined by the type of G-protein that is coupled to a specific hormone receptor. For example, in the pituitary, corticotropin-releasing hormone receptor coupling to $G_{s\alpha}$ results in the stimulation of adenylyl cyclase, somatostatin receptor coupling to $G_{i\alpha}$ causes the inhibition of adenylyl cyclase, and thyrotropin-releasing hormone (TRH) receptor coupling to $G_{q\alpha}$ results in the stimulation of phospholipase C *(2)*.

Until recently, it was thought that the α-subunit interacts with effector molecules, such as adenylyl cyclase and phospholipase C, and β- and γ-subunits merely regulate the levels of the free α-subunits. However, recent evidence emerging from various laboratories suggests that βγ-subunits directly interact with receptor as well as effector molecules *(39,40)*. For example, purified βγ-subunits associate with β_1-adrenoceptor purified from turkey erythrocyte membranes *(41)*, and somatostatin receptors from rat brain and AtT-20 pituitary cells *(42)*. βγ-subunits enhance the agonist-stimulated receptor phosphorylation and desensitization in the β-adrenergic and rhodopsin systems *(43)*. βγ-subunits stimulate type II adenylyl cyclase *(44–46)*, and different isoforms of phospholipase C *(47–49)*. These results suggest that an agonist binding to its receptor can stimulate one effector pathway through the α-subunit and an entirely different pathway through the βγ-subunits.

Tubulin

Tubulin has a mol wt of 100 kDa and is composed of two nonidentical subunits, α and β *(50,51)*. A new member of the tubulin family, the γ-tubulin (50 kDa), is associated with microtubule nucleating structures, such as spindle-pole bodies in fungal cells and centrosomes in vertebrate cells *(52,53)*. γ-Tubulin was first discovered in the fungus *Aspergillus nidulans (54)*. Subsequently, using homology-based approaches, γ-tubulin genes were cloned from animals *(55,56)*, plants *(57)*, and other fungi *(55)*. Recently, a γ-tubulin-like protein was identified in the yeast *Saccharomyces cerevisiae (58,59)*. γ-Tubulin binds with high affinity to the minus end of microtubules and is present mainly at the ends of microtubules occurring near the microtubule-organizing centers, suggesting that large amounts of γ-tubulin are unlikely to be present in microtubules *(60)*. γ-Tubulin nucleates tubulin assembly by interacting with β-tubulin, and is essential for centrosome function in vivo *(61)* and in vitro *(62,63)*. Microinjection of anti-γ-tubulin antibodies into mammalian cells inhibits the assembly of mitotic spindles *(61)*.

Tubulins have remained very stable in evolution, histones apparently being the only class of proteins that have undergone less change since the origin of eukaryotes; for example, common antigenic determinants in microtubules from mammals, birds, reptiles, teleosts, and diptera have been reported *(64,65)*.

Tubulin has two guanosine nucleotide binding sites per dimer. One is exchangeable (E-site on β-tubulin), the other nonexchangeable (N-site on α-tubulin). Although at both sites, GTP is bound noncovalently, the N-site GTP can only be removed by denaturing the protein, whereas the E-site GTP is exchangeable with free GTP, and is hydrolyzed during tubulin polymerization *(66–72)*. Tubulin migrates on electrophoresis columns with several associated proteins of higher molecular weight. The presence of these microtubule-associated proteins (MAPs), often in stoichiometric relation to tubulin, suggests a regulatory role of MAPs in the structure of microtubules *(73)*. Tubulin from the rat brain can be assembled in vitro to form microtubules with an exterior diameter of 25 nm. Microtubules generally grow in definite directions from initiation centers, such as

basal bodies or centrioles, and studies conducted in vitro on the directionality of microtubule growth from such initiation sites reveal that microtubules increase their length mainly in one direction. Tubulin assembly appears to be a sequential process, consisting of nucleation, elongation, monomer–polymer equilibrium, and length redistribution. Tubulin polymerization appears to be regulated by phospholipids. For example, in brain extracts having the capacity to form microtubules, polymerization is inhibited by phospholipase A *(74–78)*.

In addition to serving as a component of cytoarchitecture, microtubules, because of their ability to depolymerize and repolymerize, are an integral part of mitotic spindle, cilia, and flagella *(79,80)*. Moreover, tubulin is involved in the intracellular transport and secretion of proteins *(81)*, chromosome movement *(82)*, protein mobility in cell membranes *(83,84)*, axoplasmic transport in nerve tissue *(85)*, and signal transduction *(86–89)*. Microtubule function is generally investigated by the use of colchicine, vinblastine, paclitaxel, and deuterium oxide (D_2O). Colchicine and vinblastine inhibit the assembly of tubulin monomers into microtubule polymers. Paclitaxel alters the equilibrium between the soluble and polymerized forms of tubulin by overstabilizing them in their polymerized form *(90–98)*. D_2O increases the initial rate of tubulin polymerization as well as the final extent of the polymers; the total number and the length of microtubules is increased by D_2O *(99)*. Antitubulin antibodies, which inhibit the formation of microtubules *(100)*, are also used as probes to investigate the role of tubulin in cellular processes.

Most of the information regarding the biochemical properties of tubulin is derived from tubulin purified from cytosol of mammalian brain, since this is an abundant source of this protein. Data obtained in various species suggest that a small amount of tubulin is associated with the cell membranes. The identification and physicochemical properties of membrane-associated tubulin from various sources, such as nerve synaptosomes and myelin, platelets, thyroid, brain, liver, cilia, and cell cultures, were extensively reviewed *(101)*. When identifying membrane-associated tubulin, it is important to rule out the contamination of plasma membranes with soluble tubulin (of cytosolic origin) as a consequence of homogenization and isolation procedures. Tubulin comprises of 13–15% of the soluble protein of mouse brain *(102,103)*; in the synaptosomal fraction tubulin content is 28% of the total protein *(103)*. In cell cultures, the amount of tubulin is about 3.5% of total cell protein in 3T3 *(102)* and HeLa cells *(104)*. Therefore, it is imperative that the enrichment of plasma membranes is monitored using plasma membrane marker enzymes. In addition, membrane-associated tubulin should be unambiguously identified using two-dimensional gel electrophoresis. One-dimensional gel electrophoresis and immunoblotting techniques are not sensitive enough to detect the small amounts of membrane-associated tubulin.

The molecular weight of membrane-associated tubulin, isolated from various sources, is similar to that isolated from brain cytosol. It appears that α-tubulin is more tightly associated with plasma membranes. In purified synaptosomal membranes *(105,106)* and scallop ciliary membranes *(101)*, α-tubulin is more abundant than β-tubulin. Using immunofluorescence and two-dimensional gel electrophoresis, the presence of α- and β-tubulin subunits in association with purified plasma membranes in the rat anterior pituitary lobe and GH_3 cells was demonstrated *(107)*. The total amount of membrane-associated tubulin (α + β tubulin subunits) in GH_3 cells is approx 40% of that present in the rat pituitary. Twenty-seven percent of the membrane-associated tubulin in the rat pituitary is β-tubulin and 73% is α-tubulin; 17% of the GH_3 cell tubulin is β-tubulin, and 83% is α-tubulin. That this disproportionate distribution of the two subunits may have some functional significance is suggested by the observation that in GH_3 cell membranes, $G_{q\alpha}$-GTPase activity is stimulated by α-tubulin antibody and inhibited by β-tubulin antibody *(86)*.

Membrane-associated tubulin appears to differ from cytosolic tubulin in the ability to associate with lipids, the lack of carboxy-terminal tyrosine, and in isoelectric point *(101)*. One important difference between these two tubulins is that tubulin in the cytosol can assemble into microtubules. On the other hand, membrane-associated tubulin cannot form into microtubules in the membrane environment. Tubulin heterodimers can polymerize into linear protofilaments, which are roughly 2 nm in diameter and assemble into a complete microtubule, which is a hollow cylinder made up of 13 protofilaments *(75)*. Since the diameter of a microtubule is 25 nm and the plasma membrane is 9–11 nm thick, it is not possible for membrane-associated tubulin to assemble into a complete microtubule in the membrane environment. However, tubulin heterodimers may be able to function in the membrane environment as individual heterodimers or as polymers arranged into short protofilaments. Tubulin obtained from the membranes of brain, thyroid, algae, and avian erythrocytes can copolymerize with pure tubulin (obtained from the rat brain cytosol) in vitro *(101)*.

G-protein–Tubulin Interaction at Cell Membrane

Heterotrimeric G-proteins and tubulin are both "G-proteins" in the sense that their activity is regulated by guanine nucleotides *(108)*. Similarities between G-proteins and tubulin include:

1. Considerable homology and a highly conserved nature *(108,109)*.
2. ADP ribosylation sites for both cholera and pertussis toxins: ADP ribosylation inactivates both $G_{i\alpha}$ and $G_{s\alpha}$ *(14)*, and inhibits tubulin assembly into microtubules *(110)*.
3. Mg^{2+}-dependent binding and hydrolysis of GTP; GTP causes the dissociation of G_α-subunit from βγ-subunits

and GDP promotes the association of G_α-subunit with βγ-subunits; GTP-bound G_α is active, whereas GDP-bound G_α is inactive *(12)*. On the other hand, the polymerization of tubulin into microtubules is accompanied by tubulin-induced hydrolysis of GTP *(68,69)*. The K_m of G-protein GTPase activity (EC 3.6.1.-) is quite low, for example, 0.4–0.7 μ*M* in the rat striatum *(111,112)*, 0.1 μ*M* in the turkey erythrocytes *(113)*, and 0.6 μ*M* in the rat anterior pituitary lobe (Ravindra and Aronstam, unpublished results). Tubulin-GTPase activity is not a typical enzyme activity and hence a K_m value for this reaction has not been determined *(66,67)*. K_d values of GTP binding to tubulin, reported by various laboratories, range from 0.01–1 μ*M*; this large variation may be owing to the use of different experimental conditions and methods. It appears that pH of the reaction mixture has a profound influence on nucleotide affinity for tubulin. K_d for GDP was 0.02 and 1.0 μ*M* at pH 7.0 and 6.0, respectively; K_d values for GTP are also in a similar range (for references, *see 90*). K_d values for GTP[γS], GTP, and GDP binding to $G_{i\alpha}$ are 12, 25, and 25 n*M*, respectively *(114)*. K_d for GDP binding to $G_{o\alpha}$ is approx 40 n*M*, and in the presence of βγ-subunits, it is 0.1 n*M* *(12)*. Thus, GDP/GTP binding to G_α-subunits is highly dependent on βγ-subunits.

Hormone–Receptor Interaction

It is generally believed that the magnitude of the response of a particular receptor system is directly proportional to the number of receptors activated by an agonist. In addition, according to the prevailing dogma, G-protein-coupled receptors exist in an inactive form, and become active on binding of an agonist to its receptor. Recent evidence using transgenic mice suggests that a reappraisal of this dogma is in order. In one transgenic model, a class of excitatory cardiac receptor is overexpressed by 200-fold. Even in the absence of the physiological agonist, the animals' hearts behave as if they are maximally stimulated. These results suggest that in a pool of unoccupied receptors, some can always exist in an active form. According to the new model of ligand–receptor interaction, on exposure to an agonist, the inactive receptors become active and join the already active ones. The net result would be an increase in the responsiveness of a particular system in response to the newly added agonist *(115)*.

The present understanding of the mechanism of the G-protein signal transduction pathway is mainly obtained by studies with reconstituting purified components. Although the information gained with such elegant biochemical approaches is very valuable, the physiological significance of the data remains uncertain, since the cell membranes of eukaryotic cells are more complex than the reconstituted systems. The membrane is made up of heterogeneous components, and its dynamic properties are probably governed by localized interactions.

Therefore, it is conceivable that a particular region of the membrane may behave in a different manner compared to another region. Moreover, compared to intact cells, the specificity of receptor–G-protein interaction appears to be less in the reconstituted systems. Furthermore, in view of the observations that G-proteins and receptors interact with cytoskeletal proteins in the membrane environment, it seems prudent to reconsider the topology of the components of the signal transduction system.

The organization of receptors, G-proteins, and cytoskeletal proteins in the native membrane environment is not clear. It is generally believed that receptors, G-proteins, and effectors are freely floating around in the plasma membrane, and the specificity of their interaction is governed by the three-dimensional structure of the sites of protein–protein interaction. However, several studies suggest that receptors exist in the native membrane environment in a more orderly manner. In the frog erythrocyte membranes, using steady-state fluorescence depolarization, it was observed that propranolol– (a high-affinity β-adrenergic receptor antagonist) receptor complex is dynamically constrained. When the membranes were exposed to colchicine, a release in the constraint was observed, suggesting that receptors are complexed with tubulin *in situ (116)*. Using target size analysis, it was determined that the functional size of the transduction system (prior to activation by glucagon) in the rat liver membranes is approx 1500 kDa; after activation with the hormone, the functional size is reduced by a factor of 4. In addition, the combined molecular weight of glucagon receptor, $G_{s\alpha}$, and adenylyl cyclase is not more than about 210 kDa, suggesting that in order to account for 1500 kDa, the transduction system should comprise of other structures than these three components *(17,18,117,118)*. In the rat brain synaptoneurosomes, crosslinking of proteins causes the formation of crosslinked G-protein subunits similar to that of crosslinked actin or tubulin *(119)*. Since detergents, such as Lubrol and sodium cholate, are generally used to extract G-protein subunits, the possibility that these detergents disrupt the multimeric structure of G-proteins was considered. The size of G-protein structures, extracted with various detergents, was determined by hydrodynamic properties on sucrose gradients, and it appears that octyl glucoside preserves the multimeric structure. Extraction of main classes of G-proteins with octyl glucoside results in large, polydisperse structures sensitive to disaggregation by GTP[γS] and agonists *(120,121)*. In addition, G-proteins extracted with digitonin and subjected to crosslinking do not enter the stacking gel, suggesting that multimeric structures are obtained with this detergent; in contrast, Lubrol-extracted G-proteins are much smaller in size *(122)*. Taken together, these observations suggest that G-proteins occur in the native membrane environment as part of larger complexes, and that agonist activation promotes the disaggregation of a putative multimeric structure containing receptors, G-proteins, and effector molecules *(17,18)*.

GTP[γS] binding to purified brain G-proteins is potentiated by the addition of PC-12 cell membrane prepara-

tions, supporting the theory that G-proteins associate with other protein factors in the native membrane environment *(123)*. Association of the erythrocyte cytoskeleton with G_s *(124)* and the β-subunit of a G-protein with the cytoskeleton of S49 mouse lymphoma cells *(125)* have been observed. In WRK1 cells, a rat mammary tumor cell line, using immunofluorescence and immunoblotting techniques, it was observed that actin and $G_{q\alpha}$ are closely associated *(126)*. Actin cytoskeleton may play a role in the association of P_{2y}-purinergic receptor, $G_{q\alpha}$, and phospholipase C in the turkey erythrocytes *(127)*. CD2, a 50-kDa glycoprotein located on the surface of human T-lymphocytes, interacts with tubulin; this interaction appears to take place at the membrane-proximal part of the cytoplasmic domain of the CD2 molecule. Activation of CD2 with anti-CD2 antibodies disrupts the CD2–tubulin complex, suggesting that T-cell activation involves the dynamic interaction of CD2 with tubulin *(128)*. Thus, these results suggest the interaction of G-proteins and receptors with cytoskeletal proteins.

Influence of Cytoskeletal Proteins on Agonist–Receptor Interaction

It was proposed that certain cell-surface receptors interact reversibly with membrane-associated tubulin, and both the components influence each other. The state (conformation or nucleotide binding) of tubulin may be altered by one set of receptors leading to perturbation in another set of receptors *(129)*. Alteration of cytoskeletal proteins may influence the mobility of integral membrane proteins, leading to the expression of more ligand binding sites on cell surfaces. Recent results suggest that cell-surface receptors are linked by membrane proteins to structures (possibly cytoskeletal proteins) within the cytoplasmic domain. Glycophorin A, an integral protein in the red cell membrane, was observed to be one such protein *(130)*. Gephyrin, a 93-kDa microtubule binding protein, appears to link glycine receptor to tubulin in synaptic membranes *(131)*. In the rat brain membranes, γ-aminobutyric $acid_A$ ($GABA_A$) receptors copurify with tubulin, and coassemble with exogenous tubulin through three cycles of polymerization and depolymerization, suggesting that $GABA_A$ receptors are associated with tubulin *(132)*. Muscimol- (a GABA agonist) stimulated chloride uptake by mouse cerebral cortical microsacs is inhibited by drugs known to influence microtubule function *(133)*. Guanine nucleotides mimic gonadotropin-releasing hormone (GnRH) in that they stimulate luteinizing hormone (LH) secretion and inositol phosphate accumulation in the rat anterior pituitary cell cultures *(134)*. GTP and GTP[γS] decrease the affinity of pituitary membranes for GnRH and stimulate LH secretion in the rat anterior pituitary cell cultures *(135)*. Guanine nucleotides inhibit the binding of [^{125}I]GnRH agonist to bovine anterior pituitary membranes without affecting the binding of [^{125}I]GnRH antagonist *(136)*. TRH binding to plasma membrane receptors leads to the phospholipase C-mediated hydrolysis of phosphatidylinositol, which gives rise to inositol phosphate and diacylglycerol, as weld as an increase in cytosolic free calcium ion concentration *(137)*; these effects of TRH are potentiated in the presence of GTP and its analogs *(138–140)*. GTP and GTP analogs also inhibit [^{3}H]TRH binding to pituitary cell membranes by decreasing receptor affinity *(141)*. These observations raise the possibility that the effects of guanine nucleotides might be owing to their interaction with membrane-associated tubulin and/or G-proteins.

Structural proteins, such as tubulin, can participate in cellular signaling by communicating through physical forces. By virtue of its interaction with the submembranous network of cytoskeletal proteins, tubulin, when perturbed in one locus, can transmit large changes in conformations to other points *(142)*. Thus, GTP binding to membrane-associated tubulin might lead to a conformational change in either receptors or G-proteins. This may in turn influence the binding of the agonist to its receptor.

Microtubules in a cell are in a state of dynamic equilibrium, undergoing polymerization and depolymerization, and any alteration in the monomer/polymer status can lead to a change in the number of receptors and/or the affinity of a hormone to its receptor. Ward and Hammer *(143)* postulated that polymerization of ligand receptor–cytoskeleton complexes leads to receptor clustering and that the formation of this complex is sensitive to changes in the affinity of cytoskeletal proteins. Data from various systems suggest that the integrity of the cytoskeletal proteins that are associated with the plasma membrane plays an important role in receptor mobility and surface distribution. Vinblastine blocks GnRH-induced patching and capping on cultured rat pituitary cells *(144)*. Cytochalasin B (which disrupts microfilaments) treatment of *Xenopus* oocytes inhibits muscarinic (m3-like) receptor expression. In *Xenopus* oocytes transfected with TRH receptor, colchicine and vinblastine do not affect the expression of TRH receptor, whereas cytochalasin B inhibits the expression of TRH receptor by 70% *(145)*. Cytochalasin B causes a significant increase in the binding of fMet-Leu-Phe (formyl peptide receptor agonist) to the human leukemia cells without altering the affinity of the ligand to the receptors *(146)*. fMet-Leu-Phe or GTPγS (which directly activates G-proteins) promotes the association between formyl peptide receptor and actin-cytoskeleton in the human neutrophils; it appears that subsequent to this association, $G_{i\alpha 2}$-subunits are released from actin-cytoskeleton. Interestingly, GDPβS, a GDP analog that inhibits the activation of G-proteins, prevents the association of the receptor with the cytoskeleton as well as the release of $G_{i\alpha 2}$-subunits, leading to the proposal that the interaction of cytoskeleton with formyl peptide receptor is governed by G-proteins *(147)*. In AtT-20 cells (stably expressing TRH receptors), colchicine, vinblastine, and paclitaxel stimulate [^{3}H]mTRH binding to cell-surface receptors, without altering the K_a of the ligand

to the receptor. Thus, perturbation of cytosolic microtubules leads to a reorganization of the spatial location of hormone receptors *(148)*. Conceivably, either depolymerization or polymerization of the cytosolic microtubules appears to unmask new receptor binding sites. These results suggest that under normal conditions, a significant portion of receptors are cryptic and not available for ligand binding. It is plausible that microtubules provide one of the mechanisms to prevent desensitization. In view of the evidence that colchicine interacts with and modifies the behavior of membrane proteins in the ciliate eukaryote Tetrahymena pyriformis *(149)*, the increase in cellular TRH receptor concentration by colchicine suggests that in addition to polymerization and depolymerization cycles of tubulin, perturbation of membrane proteins may be responsible for the action of the drug. Since this work *(148)* was carried out with intact cells, it is not clear whether the effect on TRH receptors is mediated via the drug action on cytosolic microtubules or a direct action of the drugs on membrane proteins, independent of the drug effect on microtubules.

Receptor–G-Protein Coupling

According to the generally accepted model of G-protein function, the binding of an agonist to its receptor facilitates an exchange of GTP for GDP on the α-subunit. The activated αGTP-subunit dissociates from the βγ-subunits, and interacts with effector molecules, such as adenylyl cyclase; this dissociation of G-protein subunits was shown to take place in solution as well as *in situ* (i.e., for membrane-bound G-proteins; *13*). An intrinsic GTPase activity of the α-subunit hydrolyzes GTP to GDP, releasing inorganic phosphate (Pi); αGDP then recombines with βγ, ending the activation cycle. However, the observation that in vertebrate rod photoreceptors, deactivation of signal transduction occurs without concomitant GTP hydrolysis suggests an alternative mechanism for ending the G-protein activation cycle *(150)*. Moreover, phospholipase C β1 stimulates the GTPase activity of $G_{q\alpha}$, suggesting that the ability to generate second messengers as well as G-protein GTPase activity can reside in the effector molecule itself *(151)*.

To examine the role of tubulin in receptor–G-protein coupling, the effect of various compounds that influence tubulin function was studied on G-protein GTPase activity (EC 3.6.1.-) associated with cell membranes. The GTPase activity determined was identified as being associated with G-proteins on the basis of:

1. Its low K_m.
2. Its stimulation by G-protein-coupled receptor agonists.
3. Its inhibition by specific anti-G_α protein antibodies.
4. Its copurification with G-proteins during chromatographic procedures *(86,152)*.

Colchicine inhibits acetylcholine- (ACh) stimulated G-protein GTPase activity in the rat striatal membranes; lumicolchicine, an inactive isomer of colchicine, does not influence ACh action. Interestingly, colchicine inhibition of ACh-stimulated GTPase activity was observed only at a lower concentration of the neurotransmitter and not at 100 μ*M* ACh, suggesting that higher concentrations of the neurotransmitter can override the inhibitory effects of the drug *(152)*. Colchicine and paclitaxel inhibit TRH- and GnRH-stimulated G-protein GTPase activity in membranes from the rat anterior pituitary lobe *(153)*. In GH_3 cell membranes colchicine, but not paclitaxel, inhibits TRH-stimulated G protein GTPase activity *(86)*. Independent of its effects on tubulin polymerization, and depending on the buffer conditions employed, colchicine either stimulates or inhibits tubulin-dependent GTP hydrolysis. For example, in the presence of glutamate, colchicine stimulates tubulin-GTP hydrolysis *(154)*; in the presence of glycerol, this drug inhibits tubulin-GTP hydrolysis *(155)*. It was determined that under the buffer conditions used to assay G-protein GTPase activity, purified tubulin does not hydrolyze GTP *(86)*. Moreover, in view of the similarity between tubulin and G-proteins, the possibility that colchicine acts directly on G-proteins was investigated. The drug does not affect the GTPase activity of a mixture of partially purified bovine brain G-proteins comprising $G_{i\alpha}$, $G_{s\alpha}$, and $G_{q\alpha}$ *(86)*.

Anti-α-tubulin and β-tubulin antibodies stimulate basal G-protein GTPase activity in the rat striatal membranes and potentiate ACh-stimulated activity *(112)*. In contrast, in GH_3 cell membranes, anti-α-tubulin antibody stimulates the GTPase activity, whereas anti-β-tubulin antibody inhibits the enzymatic activity. However, in the presence of either of these antibodies, TRH-stimulated GTPase activity is inhibited *(86)*. Tubulin–G-protein interaction might be perturbed in the presence of tubulin antibodies, leading to a destabilization of hormone receptor–G-protein interaction. In some tissues, such as the rat striatum, this destabilization can cause excessive activation of G-protein(s) by an agonist *(112)*, and in other systems, such as the GH_3 cells, it can depress the activation of G-protein(s) by the hormone *(86)*. The possibility that tubulin antibodies used in this study may crossreact with G-proteins present in GH_3 cell membranes *(156,157)* was investigated by immunoblotting purified bovine brain Gα-subunits with tubulin antibodies. The tubulin antibodies used in this study do not crossreact with $G_{i\alpha}$, $G_{s\alpha}$, and $G_{q\alpha}$ *(86)*.

Phosphocellulose-purified tubulin, devoid of MAPs, was used at concentrations (0.4–1.4 n*M*) that probably reflect those in the native membrane environment; tubulin was not subjected to charcoal treatment, and therefore, the nucleotide binding site should be occupied by GTP. Tubulin (1.4 n*M*) inhibits basal and TRH-stimulated GTPase activity of GH_3 membranes. Tubulin is capable of hydrolyzing GTP under specific buffer and pH conditions, different from those used to assay G-protein GTPase activity *(90)*. Under the buffer conditions employed to assay G-protein GTPase activity, purified tubulin (1–100 n*M*) does not hydrolyze GTP *(86)*.

Since tubulin antibodies inhibit TRH receptor–G-protein coupling, one might have expected purified tubulin to have an opposite effect. In fact, purified tubulin also inhibits hormone receptor–G-protein interaction. Addition of purified tubulin to GH_3 cell membranes may alter the dynamics of an interaction between G_q and endogenous membrane-associated tubulin. Whether tubulin-induced inhibition of TRH-stimulated GTPase activity results from an indirect effect of tubulin interaction with other protein(s) in the membrane remains to be investigated. It appears that in GH_3 cells, an excess of tubulin in the membrane environment may cause the system to be refractory to hormone stimulation *(86)*.

Colchicine, purified tubulin, and tubulin antibodies only modestly inhibit [^{3}H]TRH binding to GH_3 cell membranes; the inhibition ranged from 7–26% *(86)*. These results are similar to previous observations with S49 mouse lymphoma cells, wherein the binding of β-adrenergic antagonist, [^{3}H]dihydroalprenolol, is not significantly influenced by colchicine *(158)*. The partial inhibition of [^{3}H]TRH binding does not explain the complete suppression of TRH-stimulated GTPase activity by these compounds. These results suggest that these compounds act at a site beyond hormone–receptor interaction to bring about their complete inhibition of TRH-stimulated GTPase activity *(86)*. However, an inhibitory effect of the compounds on receptor activation after TRH binding cannot be ruled out.

G-Protein–Effector Coupling

To study the role of tubulin in G-protein-mediated signal transduction, many investigators used the cAMP-adenylyl cyclase system. Association of adenylyl cyclase with microtubules has been reported *(159,160)*. Microtubule-disrupting drugs increase cAMP accumulation in S49 mouse lymphoma cells *(158,161)*, mixed human leukocytes *(162)*, leukemia cells *(163)*, human lymphocytes *(164)*, rat synaptosomal membrane-enriched fractions *(165)*, and myometrium *(166)*. In contrast, colchicine inhibits adrenocorticotropic hormone- (ACTH) stimulated cAMP production in primary cultures of the rat adrenal glomerulosa cells *(167)*. In S49 cells, colchicine and vinblastine potentiate isoproterenol- and cholera toxin-stimulated cAMP production in S49 lymphoma cells, suggesting that the drugs influence the interaction of G-protein with adenylyl cyclase *(89)*. This conclusion is supported by the potentiation of cholera toxin-stimulated cAMP production by colchicine in UNC S49 cells with impaired receptor–G-protein coupling (owing to a mutation in $G_{s\alpha}$)

Tubulin, purified from rat brain, was stripped of bound GTP by charcoal treatment, and then polymerized at 37°C in the presence of 5'guanylylimidodiphosphate (Gpp[NH]p); this process yields tubulin bound to Gpp(NH)p at the exchangeable site on β-tubulin subunit of the heterodimer. Incubation of 400 μg of the rat synaptic membrane-enriched fractions with 1–10 μ*M* tubulin-Gpp(NH)p causes a concentration- dependent inhibition of adenylyl cyclase activity; this inhibition is not reversible after washing the membranes. Charcoal-extracted tubulin (i.e., without GTP at β-tubulin) is unable to inhibit adenylyl cyclase activity, whereas Gpp(NH)p alone inhibits the enzymatic activity *(168)*. The use of very high concentrations of tubulin (1–10 μ*M*) raises the question of whether the native membrane environment is ever exposed to such high levels of tubulin. Tubulin-Gpp(NH)p stimulates adenylyl cyclase activity in C6 glioma cell membranes and inhibits the enzymatic activity in the rat cerebral cortex cell membranes *(88,169)*. Using immunoblotting, it was observed that $G_{s\alpha}$ and $G_{i\alpha1}$ are present in the cortex membranes, whereas only $G_{s\alpha}$ is detectable in C6 cell membranes. Therefore, it was concluded that the lack of $G_{i\alpha1}$ in C6 membranes allows tubulin-Gpp(NH)p to interact with $G_{s\alpha}$, leading to the stimulation of adenylyl cyclase activity *(88)*. In spite of the presence of both $G_{i\alpha1}$ and $G_{s\alpha}$ in the rat cortex membranes, it is not clear why $G_{i\alpha1}$ interacts specifically with tubulin-Gpp(NH)p; specific binding of [^{125}I]tubulin was reported to both $G_{i\alpha1}$ and $G_{s\alpha}$ *(170)*. Based on detergent extraction and photoaffinity labeling studies, it was postulated that most of the $G_{s\alpha}$ in the rat cortex membranes is already bound to tubulin; therefore, exogenous tubulin-Gpp(NH)p would have to bind $G_{i\alpha1}$ *(88)*. Tubulin polymerized in the presence of GTP was also shown to inhibit adenylyl cyclase activity in the rat synaptic membrane-enriched fractions; this is reversible after washing the membranes. In the rat synaptic membrane-enriched preparations, monoclonal anti-α-tubulin and β-tubulin antibodies block the inhibition of adenylyl cyclase activity by tubulin-Gpp(NH)p. These antibodies do not influence the basal enzymatic activity *(171)*. It would be interesting to know the effect of the antibodies on agonist-stimulated adenylyl cyclase activity, since this would indicate whether endogenous tubulin participates in the regulation of the cAMP-adenylyl cyclase system.

COS 1 cells were transiently transfected with $G_{s\alpha}$ and then permeabilized with saponin. Cells were removed from the culture dish and challenged with isoproterenol (a β-adrenergic receptor agonist), Gpp(NH)p, or tubulin-Gpp(NH)p; tubulin-Gpp(NH)p potentiates isoproterenol-stimulated adenylyl cyclase activity. These observations led to the hypothesis that tubulin-Gpp(NH)p binds to heterotrimeric G_s, and facilitates the release of GDP from $G_{s\alpha}$ and the dissociation of $G_{s\alpha}$ from the βγ-subunits. Subsequently, tubulin-Gpp(NH)p–$G_{s\alpha}$ complex activates adenylyl cyclase *(172)*. In this scheme, the effect of βγ-subunits on adenylyl cyclase activity was not taken into consideration. βγ-subunits stimulate type II adenylyl cyclase *(44–46)* and different isoforms of phospholipase C *(47–49)*. Moreover, some agonists acting via G-protein-coupled receptors induce the reorganization of the cytoskeleton (*see* Hormone-Induced Reorganization of Cytoskeleton); it is not known whether isoproterenol is one of these agonists. In addition, cytoskeletal reorganization might influ-

ence receptor–G-protein and/or G-protein–effector coupling (*see* Influence of Cytoskeletal Proteins on Agonist–Receptor Interaction). Therefore, the effect of tubulin-Gpp(NH)p on adenylyl cyclase activity using intact cells may not solely reflect the association of membrane-associated tubulin with $G_{s\alpha}$. The potentiation of adenylyl cyclase activity by tubulin-Gpp(NH)p could be owing to the cumulative effect of both cytosolic and membrane-associated tubulin.

G-Protein–Tubulin Interaction in Cell Cytosol

In addition to an interaction at the plasma membrane, recent studies suggest an interaction between these two protein families in the cytosol.

Agonist-Induced Translocation of G-Proteins into Cytosol

That G-proteins act as shuttles between the plasma membrane and cytoplasm was proposed by Chabre *(173)*. It was proposed that although G-proteins interact transiently with receptors and effectors, the important interactions are not in the membrane, but in the cytosol. This theory is supported by the demonstration of G-proteins in the cytosol of neutrophils *(174–176)*, and in primary cultures of the rat anterior pituitary lobe *(157,177)*. Furthermore, agonist-induced translocation of G_{α}-proteins from the plasma membrane to nonplasma membrane compartments of cells has been demonstrated in various systems *(178)*.

In human embryonal kidney cells using immunofluorescence and immunoblotting methods, it was observed that isoproterenol activation of β-adrenergic receptor causes a shift of $G_{s\alpha}$ from the plasma membrane to the cytosol. A threefold increase in $G_{s\alpha}$ is observed in the soluble fraction on receptor activation. As a fraction of the total amount of $G_{s\alpha}$ in the cell, the quantity of $G_{s\alpha}$ in the soluble fraction is quite small. Approximately 90% of $G_{s\alpha}$ is found to be associated with the particulate fraction after activation of the cells with isoproterenol *(179)*. In mouse mastocytoma cells, addition of cytosol to [^{32}P]ADP-ribosylated cell membranes potentiates GTP[γS]-induced release of $G_{i2\alpha}$ from the membranes; the cytosolic factor, which promotes the release, is heat-labile. By gel filtration, it is found that the molecular weight of [^{32}P]AD]P-ribosylated $G_{i2\alpha}$ released by cytosol and GTP[γS] is about 100 kDa, whereas the molecular weight of [^{32}P]ADP-ribosylated $G_{i2\alpha}$ released by GTP[γS] alone is about 40 kDa, suggesting that $G_{i2\alpha}$ associates with a protein in the cytosol. Moreover, in cells exposed to thrombin, the amount of $G_{i2\alpha}$ in the membranes is dramatically reduced with a concomitant increase in the cytosol; the agonist-stimulated translocation is blocked in cells pretreated with pertussis toxin *(180)*. In mouse mastocytoma cells, iloprost, a stable prostacyclin analog, promotes the translocation of both 42- and 45-kDa $G_{s\alpha}$ from the membrane to the cytosol. The higher-mol wt $G_{s\alpha}$ remains in the cytosol for a longer period of time and appears to form a complex with unidentified components in the cytosol; using Superose-12 gel filtration, the molecular weight of this complex is estimated to be about 120 kDa *(181)*. In S49 lymphoma cells, β-adrenergic receptor activation by isoproterenol causes the translocation of $G_{s\alpha}$ from the cell membrane into the cytosol. Cytosolic $G_{s\alpha}$ levels increased from 11% (of total detectable cellular $G_{s\alpha}$) under basal conditions to 50% after activation of the cells by isoproterenol *(182)*. Activation of platelets by thrombin involves an alteration in cell shape, reorganization of the cytoskeleton, and the translocation of G_{α}-subunits to actin-cytoskeleton. In addition, immunoprecipitation studies suggest that $G_{i\alpha 2}$, $G_{s\alpha}$, and $G_{q\alpha}$ are associated with actin *(183)*. In contrast, in the human leukemia cells, the amount of $G_{i\alpha}$-subunits associated width microfilaments is decreased by cytochalasin B and is accompanied by an increase in the amount of $G_{i\alpha}$ in the membrane fraction, suggesting the translocation of G-proteins from the cytosol to the cell membrane *(146)*.

Hormone-Induced Reorganization of Cytoskeleton

It is established that biological response to hormones and growth factors is associated with an alteration of cellular cytoskeleton (reviewed in *5,184,185*). Preincubation of cultured rat renal proximal tubule cells *(186)*, the frog adrenals *(187)*, and the rat adrenal glomerulosa cells (Gallo-Payet, personal communication) with colchicine or cytochalasin D prevents angiotensin II-stimulated production of inositol phosphates, indicating that microtubules and microfilaments play a role in angiotensin II-mediated signal transduction. In addition, angiotensin II causes a rapid reorganization of microfilamentous as well as microtubular network in the rat adrenal glomerulosa cells (Gallo-Payet, personal communication). The alteration in the morphological appearance of osteoblastic cells induced by parathyroid hormone (PTH) is accompanied by a rapid decrease in the amount of polymerized actin *(188)*. PTH causes a 40–64% decrease in the levels of polymerized actin and tubulin in the human osteoblastic cells *(189)*. In the mouse osteoblastic cells, PTH inhibits the synthesis of actin and tubulin *(190)*. In the mouse fibroblast cell lines expressing human cholecystokinin (CCK) receptors, physiological concentrations of CCK induce the formation of microfilaments *(191)*. In the prothoracic glands of tobacco hornworm *Manduca sexta*, prothoracicotropic hormone (PTTH) stimulates the specific synthesis of β-tubulin; this newly synthesized β-tubulin is capable of assembling into microtubules *(192)*. In the rat ovarian granulosa cells, concomitant follicle-stimulating hormone-induced changes in cell shape, cAMP production, and steroidogenesis may be mediated by alterations in the microfilament system *(193,194)*. The reorganization of microfilamentous and microtubular network as well as a marked alteration of cell morphology is observed in duck, rat, bovine, and human adrenocortical cells exposed to ACTH (reviewed in *195*). For example, incubation of the rat adrenal glomerulosa cells

with ACTH for 1 and 15 min causes a dramatic increase and decrease, respectively, in the amount of actin in the membrane fraction; a concomitant increase in the amount of polymerized actin in the cytosol is observed at 15 min *(167)*. In contrast, ACTH induces a rapid increase in the amount of tubulin associated with the membrane fraction; this is accompanied by a commensurate decrease in polymerized tubulin concentration in the cytosol *(167)*. GH_4C_1 cells exposed to TRH exhibit a decrease in actin-rich staining, suggesting that TRH causes depolymerization of microfilaments *(196)*. In GH_3 cells exposed to TRH, the appearance of fluorescent tubulin-rich blebs at the periphery of the cells is accompanied by an attenuation of the cortical microtubular network. Five minutes after incubation with TRH, the blebs disappear, and the microtubular network is similar to that of control cells *(197)*. In another study, exposure of GH_3 cells to physiological concentrations of TRH for 1 min causes a 50% increase in polymerized tubulin levels and a 50% increase in soluble tubulin levels at 5 min. At 10 min, TRH does not cause any appreciable change in the tubulin pools, suggesting that the tubulin–microtubule system may have reached an equilibrium in preparation for the next cycle of depolymerization and polymerization *(198)*. Thus, hormones acting via G-proteins modulate the function of cytoskeletal proteins in concert with their eliciting other biological responses.

Effect of G-Proteins on Microtubules

$G_{o\alpha}$, but not $G_{i\alpha}$ and $G_{s\alpha}$, is colocalized with the mitotic spindle apparatus in normal and malignant cell lines *(199)*, suggesting that G-proteins may participate in cellular signaling in the cytosol, away from their generally accepted role at the cell membrane. In the rat adrenal glomerulosa cells, immunoprecipitation analyses indicate that $G_{q\alpha}$ or $G_{s\alpha}$ associates with tubulin and actin; in addition, when these cells are challenged with angiotensin II or ACTH, a marked increase in the amount of $G_{q\alpha}$ or $G_{s\alpha}$ association with both microtubules and microfilaments is observed (Gallo-Payet, personal communication; *167*).

To study G_q-tubulin interaction in the cytosol, GH_3 and AtT-20 cells (stably expressing TRH receptor) were transiently transfected with $G_{q\alpha}$ cDNA. Forty-eight hours after transfection, TRH-stimulated prolactin (PRL) secretion by $G_{q\alpha}$-transfected GH_3 cells increased by 90% compared to mock-transfected cells. In addition, using immunocytochemistry, it was observed that $G_{q\alpha}$-specific staining was much more prominent in $G_{q\alpha}$-transfected GH_3 and AtT-20 cells (also transfected with $G_{q\alpha}$) compared to mock-transfected cells. Thus, transfection results in successful overexpression of functional $G_{q\alpha}$. Forty-eight hours after transfection, cells were challenged with TRH and processed to obtain soluble and polymerized tubulin fractions, and tubulin levels were determined in these fractions by immunoblotting. Compared to mock-transfected cells, soluble tubulin levels decreased in $G_{q\alpha}$-transfected GH_3 and AtT-20 cells by 33% and 52%, respectively. Moreover, compared to mock-transfected cells, a 50% reduction in the ratio (an index of the flux between tubulin pools) of soluble and polymerized tubulin levels is observed in $G_{q\alpha}$-transfected GH_3 and AtT-20 cells. These results suggest that $G_{q\alpha}$ has a direct effect on the polymerization/depolymerization cycles of microtubules in vivo *(87)*. It would be important to ascertain the specificity of $G_{q\alpha}$ effect; can TRH influence tubulin levels in cells overexpressing $G_{i\alpha}$ or $G_{s\alpha}$? In addition, it is important to recognize that overexpression of proteins might relocate signaling components from their normal environment into an abnormal compartment.

To determine whether the changes in soluble and polymerized tubulin levels observed subsequent to overexpression of $G_{q\alpha}$ were mediated by G_q directly, the influence of purified heterotrimeric G_q on tubulin polymerization in GH_3 cell cytosol was investigated. The method to estimate the formation of microtubule polymers in a crude cell lysate was developed by Sandoval and Cuatrecasas *(200)*. Crude cytosol (100,000*g* supernatant) preparation was polymerized in the presence of GTP. The reaction mixture consisted of 7.4 mg/mL of cytosol fraction (assuming that tubulin comprises of 2% of the total protein in GH_3 cell cytosol preparation, tubulin concentration is estimated to be 1.5 μM), purified G_q (0.5 μM), and 1 mM GTP. Samples were incubated for 30 min at 37°C, and then spun at 100,000*g* at 37°C to sediment the polymers formed during incubation with GTP. An aliquot of the supernatant was removed, and the unpolymerized tubulin present therein was detected by immunoblotting and quantified by densitometric scanning of the immunoblots. The amount of polymers formed was determined by subtracting the amount of monomers remaining in the supernatant after the incubation at 37°C from those present before the reaction. G_q inhibited tubulin polymerization, suggesting an interaction between G_q and the tubulin–microtubule system in the cell cytosol *(87)*.

Examination of G-Protein–Tubulin Interaction Using Purified Proteins

Binding of G-Proteins to Tubulin

Evidence suggests a physical interaction between G-proteins and tubulin. Tubulin binds to a G_i-affinity column. However, colchicine-bound tubulin does not bind to G_i-Sepharose column, suggesting that tubulin–G-protein interaction may reside at the colchicine binding site on the tubulin molecule *(201)*. [^{125}I]tubulin specifically binds to $G_{i\alpha 1}$ or $G_{s\alpha}$. In these experiments, purified G-proteins were first dotted on to a nitrocellulose sheet, and [^{125}I]tubulin was added to the G-protein spots. After autoradiography, the nitrocellulose spots were cut out and the radioactivity counted. K_d values for tubulin binding to $G_{i\alpha 1}$ ranged from 110–140 nM, immobilized $G_{i\alpha 1}$ bound 0.3–0.6 mol of tubulin/mol of $G_{i\alpha 1}$ *(170)*.

Transfer of Guanine Nucleotide from Tubulin to G-Proteins

The observations that guanine nucleotides are transferred from tubulin to Gα subunits suggest that tubulin facilitates G-protein function. Charcoal-extracted tubulin (i.e., without GTP at β-tubulin) was polymerized in the presence of [^{32}P]AAGTP (azidoanilido GTP; a nonhydrolyzable analog of GTP). Tubulin-AAGTP was incubated with the rat synaptic membranes, and the membranes were washed and exposed to UV irradiation. [^{32}P]AAGTP incorporation into $G_{i\alpha}$ is observed, suggesting that on a physical association between tubulin and $G_{i\alpha}$, nucleotide is transferred from tubulin to $G_{i\alpha}$ *(168)*. Tubulin–nucleotide complex has a different conformation in the presence of GTP than when GDP is bound *(202)*. In addition, enhanced microtubule stability following Gpp(NH)p-induced assembly has been reported *(90)*, suggesting that tubulin bound to Gpp(NH)p has a different conformation than when bound to GTP. In another study *(203)*, at a 1:4 molar ratio of tubulin and $G_{i\alpha 1}$, a fourfold increase in GTP binding is observed. Incubation of tubulin with $G_{i\alpha 1}$ appears to form a complex, leading to the stabilization of GTP binding. Without $G_{i\alpha 1}$, very little GIP binds to tubulin, suggesting that only after an interaction between tubulin and $G_{i\alpha 1}$, an increase in GTP binding to the complex is observed. The presence of either $G_{i\alpha 1}$ or tubulin (bound to GTP) does not cause the hydrolysis of GTP; however, when both the proteins are added together, about 70% of the GTP is hydrolyzed; in this experiment, 1 μ*M* tubulin and 2.5 μ*M* $G_{i\alpha 1}$ were added together. These results suggest that on a physical interaction between these two proteins, GTP is transferred from tubulin to $G_{i\alpha 1}$ *(203)*. Since extremely low amounts of tubulin associated with plasma membranes were observed *(101,107)*, the stoichiometry of tubulin and G-proteins in this study does not allow us to conclude that these elegant biochemical studies *(203)* are of physiological significance.

Influence of G-Proteins on Tubulin Polymerization

Conversely, recent reports indicate that G-proteins influence tubulin polymerization. Fifteen micromolars of tubulin (free of MAPs) were preincubated with 20 μ*M* $G_{i\alpha}$ or $G_{o\alpha}$ for 2 h, the samples were placed in a recording spectrophotometer at 37°C, and microtubule formation was monitored by changes in turbidity. Polymerization was inhibited by 57 and 40% by $G_{i\alpha}$ and $G_{o\alpha}$, respectively *(204)*. The stoichiometry of tubulin and G-proteins used in this study may not reflect that found in vivo. Extremely low amounts of G-proteins were observed in the cytosol of various systems *(157,179)*.

In another study *(87)*, the effect of low concentrations of purified heterotrimeric G_q on phosphocellulose-purified tubulin (free of MAPs) polymerization was investigated *(87)*. It was determined that 0.12 μ*M* G_q stimulated by 160% the polymerization of 28.2 μ*M* tubulin.

The observation that G_q inhibits tubulin polymerization in GH_3 cell cytosol preparation (*see* Effect of G-Proteins on Microtubules) appears to contradict the finding that G_q promotes the polymerization of purified tubulin. This may be explained in two ways. First, the dynamic properties of tubulin in the cytosol fraction may differ from those of highly purified tubulin. The MAPs present in the cytosol preparation may influence tubulin-G_q interaction. Purified tubulin used in this study *(87)* is devoid of MAPs. Second, the relative concentrations of G_q and tubulin present in the reaction mixture might determine whether tubulin polymerization is promoted or inhibited. It was reported that 20 μ*M* of $G_{i\alpha}$ or $G_{o\alpha}$ inhibits the polymerization of 15 μ*M* purified tubulin *(204)*. In contrast, it was observed that 0.12 μ*M* G_q promotes the polymerization of 28.2 μ*M* purified tubulin *(87)*. On the other hand, using GH_3 cell cytosol preparation it was determined that 0.5 μ*M* G_q inhibits the polymerization of 1.5 μ*M* tubulin *(87)*. Taken together, it appears that local compartmental domains of G-proteins, GTP/GDP, and tubulin as well as their concentrations may influence the nature of this regulation, i.e., stimulation or inhibition.

In the future, with the availability of sufficient quantities of purified G-proteins, the assembly reaction should be conducted using fixed amount of G-proteins and variable amounts of tubulin, and fixed amount of tubulin and variable amounts of G-proteins. Once the stoichiometry of G-proteins and tubulin is determined, experiments should be conducted to confirm the hypothesis that excess GDP in the reaction mixture inhibits the stimulation of tubulin polymerization by G_q; the effect of G-proteins on tubulin polymerization should be investigated in the presence of various concentrations of GTP. In order to determining if G-proteins decorate the microtubule polymer, samples should be processed for pre-embedding electron microscopic immunocytochemistry using colloidal gold markers.

Is G-Protein–Tubulin Interaction Essential for Signal Transduction?

Many hormones and growth factors acting via enzyme-linked receptors are also known to induce cytoskeletal reorganization in a variety of systems. In the murine embryonic epithelial cells, epidermal growth factor (EGF) may directly affect the organization of the intermediate filament system *(205)*. In the chick brain, nerve growth factor (NGF) appears to modulate actin polymerization *(184,206)*. Colchicine causes the total disappearance of intact microtubules in rat hepatocytes. However, when cells were exposed to colchicine in the presence of insulin, a higher microtubular stability was observed, suggesting that insulin can override the inhibitory effect of colchicine *(207)*. In addition, insulin causes the depolymerization of microfilaments in fibroblasts *(208)*. Therefore, a direct coupling of receptors to G-proteins may not be required to

alter cellular cytoskeleton. On the other hand, perturbation of cytoskeletal proteins alters the nature of many cell-surface receptors; these receptors do not directly interact with G-proteins. Colchicine and vinblastine treatment of the human erythroleukemia cells cause a significant, time-dependent increase in cell-surface binding of [^{125}I]transferrin, suggesting that disruption of cytoplasmic microtubules may be the cause of increased expression of transferrin receptors *(209)*. High-affinity transferrin receptors on the surfaces of retinal pigment epithelial cells are associated with the cytoskeleton *(210)*. Colcemid and cytochalasin D, which disrupt microtubules and microfilaments, respectively, stimulate the number of NGF receptors in PC12 neuronal cells; paclitaxel also has a similar effect in these cells *(211)*. The centripetal movement of fibrinogen receptor–ligand complexes requires an intact microfilament system as indicated by the inhibition of this movement by the treatment of platelets with cytochalasins D and E *(212)*. Hyaluronic acid-induced receptor capping in platelets is blocked by cytochalasin D, but not by colchicine *(213)*. In the human myogenic cells, disruption of microfilaments decreases the stiffness and stiffening response measured by magnetic twisting cytometry, suggesting that urokinase receptor is coupled to the cytoskeleton *(214)*. High-affinity EGF receptors in epidermoid carcinoma cells are associated with the cytoskeleton *(215)*. Thus, in many enzyme-linked receptor systems, signal transduction across membranes appears to be influenced by an interaction of receptor proteins with cytoskeletal proteins. The proponents of the view that tubulin-G-protein interaction is essential for the downstream events in signal transduction might argue that the effects of ligands acting via enzyme-linked receptors is owing to the transactivation of a G-protein-mediated pathway. In fact, recent evidence from various laboratories suggests that integration of information between tyrosine kinase and G-protein-mediated pathways takes place: 1) In a variety of systems, insulin binding to its receptor was followed by an activation of G-protein(s). Insulin stimulates GDP release by G-proteins in the rat and human membranes *(216)*, G-protein GTPase activity in membranes from human platelets *(217)* and BC_3H-1 murine myocytes *(218)*, and [^{35}S]GTPγS binding to membranes from BC_3H-1 murine myocytes *(218)*. In addition, insulin receptor directly phosphorylates β_2-adrenergic receptors *(219)*. Moreover, using antisense RNA to block the production of $G_{i\alpha 2}$ in mice, it was recently demonstrated that $G_{i\alpha 2}$ is critical for insulin action in that $G_{i\alpha 2}$ deficiency in adipose tissue and liver produces hyperinsulinemia, impaired glucose tolerance, and resistance to insulin in vivo *(220)*. 2) Ligands known to activate the G-protein signaling system led to the stimulation of enzyme-linked receptors. For example, EGF receptor becomes rapidly tyrosine-phosphorylated on stimulation of Rat-1 cells with G-protein-coupled receptor agonists endothelin-1 and thrombin *(221)*. In addition, insulin receptor substrates 1 and 2 in the heart are rapidly tyrosine-phosphorylated in angiotensin II-injected rats *(222)*.

The association between tubulin and G-protein(s) in the plasma membranes should be demonstrated by both indirect immunofluorescence and immunoelectron microscopy using colloidal gold markers. Colocalization studies using both antitubulin and anti-G-protein antibodies on the same cells with contrasting fluorochromes (using rhodamine and fluorescein isothiocyanate) should confirm the coincident spatial localization of these two proteins. In addition, immunoelectron microscopy should be used to confirm a structural association and determine how a G-protein(s) is spatially deployed along the length of the microtubule in the cytosol. The demonstration of this association *in situ* will strengthen the hypothesis that an interaction between these two proteins takes place. However, to establish unequivocally that tubulin-G-protein interaction is crucial for signal transduction, physiological significance of this interaction should be determined.

Molecular approaches might also prove futile. Since tubulin is an essential gene, blocking the production of tubulin using antisense RNA will not be successful. Overexpression of tubulin in cells is complicated by the fact that the protein comprises α- and β-subunits, and both these subunits appear to be balanced in some systems. Excess α-tubulin is degraded in a *Drosophila* mutant that produces an unstable β-tubulin *(223)*. A similar degradation of α-tubulin was reported in mammalian cells that express unstable β-tubulin *(224)*. Excess β-tubulin is rapidly degraded after overexpression of normal β-tubulin in mammalian cells *(225)*. A cell line that constitutively overexpresses α-tubulin has not been identified, suggesting that α-tubulin overexpression is lethal to mammalian cells *(226)*. An avenue that might be fruitful is to use antisense technology to inhibit the production of G-proteins and then investigate whether a particular hormone that acts via a certain G-protein retains the ability to reorganize the cytoskeletal proteins.

Summary

Role of Membrane-Associated Tubulin in Signal Transduction

Evidence presented in this article suggests that compounds that interact with tubulin influence agonist-stimulated G-protein GTPase as well as adenylyl cyclase activities. Since both GTPase and adenylyl cyclase activities are membrane-associated, we propose that these compounds (e.g., colchicine, antitubulin antibodies, or purified tubulin) interact with membrane-associated tubulin, leading to alteration in either receptor–G-protein coupling or G-protein–effector interaction. Colchicine and antitubulin antibodies inhibit microtubule polymerization; given the right conditions, addition of purified tubulin is expected to increase the length of microtubules. These assumptions are

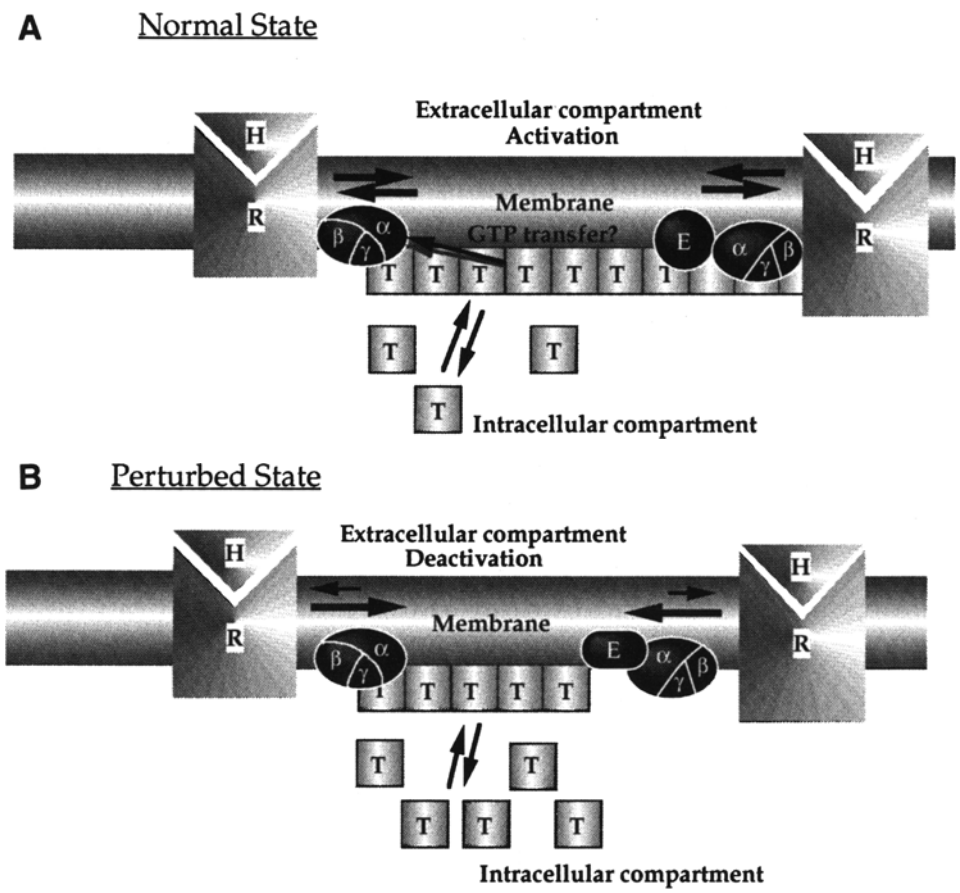

Fig. 1. Model depicting the role of membrane-associated tubulin in hormone receptor–G-protein–effector coupling. **(A)** Under normal conditions, tubulin protofilaments (T) are associated with the cytoplasmic face of the cell membrane. The binding of tubulin protofilaments to a heterotrimeric G-protein (comprising α-, β-, and γ-subunits) modulates hormone receptor–G-protein and/or G-protein–effector interaction. **(B)** Any alteration in the length of the protofilaments might influence the equilibrium between tubulin protofilaments and G-protein(s). Thus, the length of tubulin protofilaments can be reduced by colchicine or tubulin antibody, and increased by tubulin heterodimers. This might, in turn, impair the ability of G-protein(s) to interact with the hormone receptor. It should be pointed out that an effect of tubulin on hormone-induced receptor activation *per se* cannot be ruled out. H, hormone; R, receptor; T, tubulin heterodimer; α-, β-, γ-subunits of G-proteins; E, effector (adenylyl cyclase or phospholipase C).

based on our knowledge of the effect of these compounds on cytosolic microtubules, in vitro or in vivo. However, we do not know how these compounds influence membrane-associated tubulin. Taking these limitations into consideration, and based on the data from various laboratories, we propose the following theory regarding the role of membrane-associated tubulin in signal transduction. As discussed in the Tubulin section, membrane-associated tubulin cannot form into microtubules in the membrane environment. Tubulin heterodimers may be able to function in the membrane environment as individual heterodimers or as polymers arranged into short protofilaments. Under normal conditions, tubulin protofilaments are associated with the cytoplasmic face of the cell membrane. An interaction of tubulin protofilaments with a G-protein modulates hormone receptor-G-protein interaction. Any alteration in the length of the protofilaments might influence the equilibrium between tubulin protofilaments and G-protein(s). Thus, the length of tubulin protofilaments can be reduced by colchicine or tubulin antibody, and increased by tubulin. This might, in turn, influence the ability of G-protein(s) to interact with receptors and/or effectors (Fig. 1).

In general, beyond the fact that there is an increased GTP-GDP exchange by G-proteins in response to hormone binding, very little is known about receptor coupling to G-proteins. What are the mechanisms operating in the binding of GTP to the site vacated by GDP? Is there a mechanism for sequestering GTP so that an indiscriminate end to G-protein cycles can be prevented? In other words, are the cellular levels of GTP regulated? If so, other GTP binding proteins might have to participate in the GDP-GTP exchange of the G-proteins. Can tubulin regulate the local GTP concentrations in the native membrane environment? How does the transfer of guanine nucleotides from tubulin to G_α-subunits fit into this scheme? In the absence of an agonist, tubulin might inhibit GDP-GTP exchange of G-proteins; this inhibition can be overcome on agonist stimulation of the receptor. Potentiation of isoproterenol-

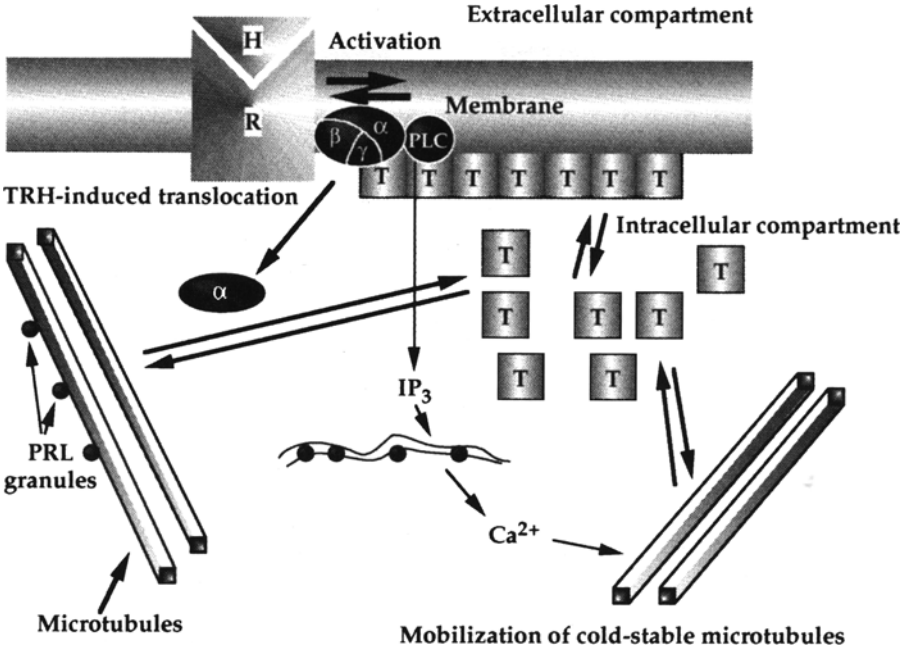

Fig. 2. Model depicting the role of $G_{q\alpha}$ in modulating tubulin function in the cell cytosol. In response to TRH stimulation, $G_{q\alpha}$ is translocated into the cytosol, and once in the cytosol, it interacts with tubulin pools to influence the monomer–polymer equilibrium. $G_{q\alpha}$ stimulates phospholipase C, leading to the production of IP_3; IP_3-induced release of Ca^{2+} might mobilize cold-stable microtubules. H, hormone; R, receptor; T, tubulin heterodimer; α-, β-, γ-subunits of G-proteins; PRL, prolactin; PLC, phospholipase C; IP_3, inositol triphosphate; Ca^{2+}, calcium.

stimulated adenylyl cyclase activity in COS 1 cells by tubulin-Gpp(NH)p, and stimulation of basal G-protein GTPase activity and potentiation of ACh-stimulated G-protein GTPase activity by antitubulin antibodies in the rat striatum support this idea. In contrast, the inhibitory effect of tubulin antibodies as well as purified tubulin on TRH-stimulated G-protein GTPase activity in GH_3 cells does not support this hypothesis. Moreover, the amount of GTP in cells does not appear to be in short supply *(227)*. Thus, with plenty of GTP around, what can prevent its binding to G-proteins or tubulin? Since G-proteins may exist in the native membrane environment as multimeric structures complexed with other G-proteins as well as tubulin and/or actin, it is difficult to envisage GTP binding to these multimeric structures; high-affinity GTP binding to G_α-subunit occurs after the dissociation of βγ-subunits. Since the intracellular concentration of GTP appears to be abundant, is there a need for the transfer of GTP from tubulin to G-proteins?

G-Protein Modulation of Cytosolic Tubulin

To propose a function for G-proteins in the cytosol, I will use TRH-stimulated PRL secretion by the pituitary as a model (Fig. 2). According to this hypothesis, immediately after TRH receptor couples to G_q, the α-subunit of G_q is translocated into the cytosol. The observation that $G_{q\alpha}$ is present in GH_3 cell cytosol supports this idea *(157)*. Once in the cytosol, $G_{q\alpha}$ influences tubulin monomer–polymer equilibrium (Fig. 2). Although the actual mechanism is not clear, it is generally accepted that in the pituitary, microtubules are involved in the transport of PRL secretory granules from the Golgi to the cell membrane *(81)*. Apart from facilitating the secretion of polypeptide hormones and other proteins, G-proteins in the cytosol may play a role in a variety of other functions *(228)*. The recent demonstration of $G_{o\alpha}$ in association with the mitotic spindle apparatus in proliferating malignant and normal cells suggests a role for G-proteins in cell division *(199)*. Future work in various other systems should be conducted to elucidate the involvement of G-proteins in other cellular functions in the cytosol.

Exposure to Ca^{2+} causes the depolymerization of those microtubules not depolymerized by cold alone *(229)*. This "Ca^{2+} labile" pool is generally referred to as "cold-stable" microtubules. Although the presence of cold-stable microtubules has been reported in the mammalian brain *(229, 230)*, the physiological role of this particular population of microtubules is not clear. G_q mediates IP_3 production, which in turn mobilizes intracellular stores of Ca^{2+} *(27)*. In view of the reorganization of the tubulin–microtubule system by TRH (*see* Hormone-Induced Reorganization of Cytoskeleton), it is tempting to speculate a role for cold-stable microtubules in signal transduction (Fig. 2).

Given the ubiquitous and highly conserved nature of both G-proteins and tubulin, it is conceivable that these two protein families interact with each other, leading to

the modulation of signal transduction in a variety of receptor systems. Until the physiological significance of this interaction can be established, this hypothesis will remain just that.

Acknowledgments

The author would like to express sincere gratitude to Ernest Hamel and Robert Nagele for helpful discussions. Gallo-Payet is also thanked for providing her preprints prior to publication. The author is especially indebted to Rocco Carsia, who provided the impetus for some of our work in this area, and for critically reading this manuscript. The author is grateful to Yan Gao, Paula Abbey, and Becky Lucas for their help with the preparation of this manuscript. To limit the number of references, reviews were cited, and therefore, the work of some colleagues could not be recognized.

References

1. Rall, T. W., Sutherland, E. W., and Wasilait, W. O. (1956). *J. Biol. Chem.* **218,** 483–488.
2. Pennington, S. R. (1994). *Protein Profile* **1,** 169–342.
3. Peters, R. A. (1956). *Nature* **177,** 426.
4. Mitchison, T. J. (1992). *Mol. Biol. Cell.* **3,** 1309–1315.
5. Zor, U. (1983). *Endocr. Rev.* **4,** 1–21.
6. Jesaitis, A. J. and Klotz, K. N. (1993). *Eur. J. Haematol.* **51,** 288–293.
7. Omann, G. M., Allen, R. A., Bokoch, G. M., Painter, R. G., Traynor, A. E., and Sklar, L. A. (1987). *Physiol. Rev.* **67,** 285–322.
8. Aderem, A. (1992). *Trends Biochem. Sci.* **17,** 438–443.
9. Ridley, A. J. (1994). *BioEssays* **16,** 312–327.
10. Burrin, J. M. (1994). *Ann. Clin. Biochem.* **31,** 205–214.
11. Birnbaumer L., Abramowitz, J., and Brown, A. M. (1990). *Biochim. Biophys. Acta* **1031,** 163–224.
12. Gilman, A. G. (1987). *Annu. Rev. Biochem.* **56,** 615–649.
13. Gilman, A. G. (1995). *Biosci. Rep.* **15,** 65–97.
14. Spiegel, A. M., Shenker, A., and Weinstein, L. S. (1992). *Endocr. Rev.* **13,** 536–565.
15. Conklin, B. R. and Bourne, H. R. (1993). *Cell* **73,** 631–641.
16. Rens-Domiano, S. and Hamm, H. E. (1995). *FASEB J.* **9,** 1059–1066.
17. Rodbell, M. (1992). *Curr. Topics Cell. Reg.* **32,** 1–47.
18. Rodbell, M. (1995). *Biosci. Rep.* **15,** 117–133.
19. Neer, E. J. (1995). *Cell* **80,** 249–257.
20. Simon, M. I., Strathmann, M. P., and Gautam, N. (1991). *Science* **252,** 802–808.
21. Neubig, R. R. (1994). *FASEB J.* **8,** 939–946.
22. Gudermann, T., Kalkbrenner, F., and Schultz, G. (1996). *Annu. Rev. Pharmacol. Toxicol.* **36,** 429–459.
23. Felder, C. C. (1995). *FASEB J.* **9,** 619–625.
24. Levitzki, A. and Bar-Sinai, A. (1991). *Pharmacol. Ther.* **50,** 271–283.
25. Boege, F., Neumann, E., and Helmreich, J. M. (1991). *Eur. J. Biochem.* **199,** 1–15.
26. Strosberg, A. D., Camoin, L., Thin, N., and Maigret, B. (1993). *Drug Design Discovery* **9,** 199–211.
27. Berridge, M. J. (1993). *Nature* **361,** 315–325.
28. Exton, J. H. (1994). *Annu. Rev. Physiol.* **56,** 349–369.
29. Exton, J. H. (1996). *Annu. Rev. Pharmacol. Toxicol.* **36,** 481–509.
30. Sternweis, P. C. and Smrcka, A. V. (1993). *Ciba Foundation Symp.* **176,** 96–111.
31. Segalat, L., Elkes, D. A., and Kaplan, J. M. (1995). *Science* **267,** 1648–1651.
32. Mendel, J. E., Korswagen, H. C., Liu, K. S., Hajdu-Cronin, Y. M., Simon, M. I., Plasterk, R. H., et al. (1995). *Science* **267,** 1652–1655.
33. McLaughlin, S., McKinnon, P., and Margolskee, R. F. (1992). *Nature* **357,** 563–569.
34. Chabre, M. and Deterre, P. (1989). *Eur. J. Biochem.* **179,** 255–266.
35. Stryer, L. (1991). *J. Biol. Chem.* **266,** 10,711–10,714.
36. Hargrave, P. A. and McDowell, J. H. (1992). *FASEB J.* **6,** 2323–2331.
37. Ruiz-Avila, L., McLaughlin, S. K., Wildman, D., McKinnon, P. J., Robichon, A., Spickofsky, N., et al. (1995). *Nature* **376,** 80–85.
38. Kolesnikov, S. S. and Margolskee, R. F. (1995). *Nature* **376,** 85–88.
39. Clapham, D. E. and Neer, E. J. (1993). *Nature* **365,** 403–406.
40. Sternweis, P. C. (1994). *Curr. Opinion Cell Biol.* **6,** 198–203.
41. Heithier, H., Frohlich, M., Dees, C., Baumann, M., Haring, M., Gierschik, P., et al. (1992). *Eur. J. Biochem.* **204,** 1169–1181.
42. Law, S. F., Manning, D., and Reisine, T. (1991). *J. Biol. Chem.* **266,** 17,885–17,897.
43. Pitcher, J. A., Inglese, J., Higgins, J. E3., Arriza, J. L., Casey, P. J., Kim, C., et al. (1992). *Science* **257,** 1264–1267.
44. Taussig, R., Quarmby, L. M., and Gilman, A. G. (1993). *J. Biol. Chem.* **268,** 9–12.
45. Federman, A. D., Conklin, B. R., Schrader, K. A., Reed, R. R., and Bourne, H. R. (1992). *Nature* **356,** 159–161.
46. Sunahara, R. K., Dessauer, C. W., and Gilman, A. G., (1996). *Annu. Rev. Pharmacol. Toxicol.* **36,** 461–480.
47. Camps, M., Carozzi, A., Schnabel, P., Scheer, A., Parker, P. J., and Gierschik, P. (1992). *Nature* **360,** 684–686.
48. Katz, A., Wu, D., and Simon, M. I. (1992). *Nature* **360,** 686–689.
49. Blank, J. L., Brattain, K. A., and Exton, J. H. (1992). *J. Biol. Chem.* **267,** 23,069–23,075.
50. Dustin, P. (1984). *Microtubules*. Springer-Verlag, Berlin, pp. 1–482.
51. Fosket, D. E. and Morejohn, L. C. (1992). *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43,** 201–240.
52. Oakley, B. R. (1992). *Trends Cell Biol.* **2,** 1–5.
53. Joshi, H. C. and Cleveland, D. W. (1990). *Cell Motil. Cytoskeleton* **16,** 159–163.
54. Oakley, C. E. and Oakley, B. R. (1989). *Nature* **338,** 662–664.
55. Stearns, T., Evans, L., and Kirschner, M. (1991). *Cell* **65,** 825–836.
56. Zheng, Y., Jung, M. K., and Oakley, B. R. (1991). *Cell* **65,** 817–823.
57. Fuchs, U., Moepps, B., Maucher, H. P., and Schraudolf, H. (1993). *Plant Mol. Biol.* **23,** 595–603.
58. Marschall, L. G., Jeng, R. L., Mulholland. J., and Stearns, T. (1996). *J. Cell Biol.* **134,** 443–454.
59. Spang, A., Geissler, S., Grein, K., and Schiebel, E. (1996). *J. Cell Biol.* **134,** 429–441.
60. Li, Q. and Joshi, H. C. (1995). *J. Cell Biol.* **131,** 207–214.
61. Joshi, H. C., Monica, L., McNamara, L., and Cleveland, D. W. (1992). *Nature* **356,** 80–83.
62. Felix, M.-A., Antony, C., Wright, M., and Maro, B. (1994). *J. Cell Biol.* **124,** 19–31.
63. Stearns, T. and Kirschner, M. (1994). *Cell* **76,** 623–638.
64. Luduena, R. F., Banerjee, A., and Khan, I. A. (1992). *Curr. Opinion Cell Biol.* **4,** 53–57.
65. Burns, R. G. and Surridge, C. (1990). *FEBS Lett.* **271,** 1–8.
66. Carlier, M.-F. (1982). *Mol. Cell Biochem.* **47,** 97–113.
67. Carlier, M.-F. (1989). *Int. Rev. Cytol.* **115,** 139–170.
68. Caplow, M. and Shanks, J. (1996). *Mol. Biol. Cell* **7,** 663–675.
69. Erickson, H. P. and O'Brien, E. T. (1992). *Annu. Rev. Biophys. Biomol. Struct.* **21,** 145–166.

70. Purich, D. L. and Angelastro, J. M. (1994). *Adv. Enzymol. Related Areas Mol. Biol.* **69,** 121–154.
71. Caplow, M. (1992). *Curr. Opinion Cell Biol.* **4,** 58–65.
72. Drechel, D. N. and Kirschner, M. W. (1994). *Curr. Biol.* **4,** 1053–1061.
73. MacRae, T. H. (1992). *Biochem. Cell Biol.* **70,** 835–841.
74. Cassimeris, L. (1993). *Cell Motil. Cytoskeleton* **26,** 275–281.
75. Engelborghs, Y. (1990). In: *Microtubule Proteins.* Avila, J. (ed.) CRC, Boca Raton, FL, pp. 1–35.
76. Bayley, P. M. (1990). *J. Cell Sci.* **95,** 329–334.
77. Mandelkow, E. and Mandelkow, E.-M. (1990). *Curr. Opinion Cell Biol.* **2,** 3–9.
78. Avila, J. (1990). *FASEB J.* **4,** 3284–3290.
79. Murphy, D. B. (1991). *Curr. Opinion Cell Biol.* **3,** 43–51.
80. Joshi, H. C. (1993). *BioEssays* **15,** 637–643.
81. Ravindra, R. and Grosvenor, C. E. (1990). *Mol. Cell. Endocrinol.* **71,** 165–176.
82. McIntosh, J. R. (1994). In: *Microtubules.* Hyams, J. S. and Lloyd, C. W. (eds.) Wiley-Liss, New York, pp. 413–434.
83. Jacobson, K., Sheets, E. D., and Simson, R. (1995). *Science* **268,** 1441,1442.
84. Travis, J. L. and Bowser, S. S. (1990). *Protoplasma* **154,** 184–189.
85. Dillman, J. F., III, Dabney, L. P., and Pfister, K. K. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 141–144.
86. Ravindra, R., Forman, L. J., and Patel, S. A. (1996). *Endocrine* **4,** 43–52.
87. Ravindra, R., Kunapuli, S. P., Forman, L. J., Nagele, R. G., Foster, K. A., and Patel, S. A. (1996). *J. Cell. Biochem.* **61,** 392–401.
88. Yan, K., Greene, E., Belga, F., and Rasenick, M. M. (1996). *J. Neurochem.* **66,** 1489–1495.
89. Leiber, D., Jasper, J. R., Alousi, A. A., Martin, J., Bernstein, D., and Insel, P. A. (1993). *J. Biol. Chem.* **268,** 3833–3837.
90. Hamel, E. (1990). In: *Microtubule Proteins.* Avila, J. (ed.) CRC, Boca Raton, FL, pp. 89–191.
91. Hamel, E. (1996). *Med. Res. Rev.* **16,** 207–231.
92. Rowinsky, E. K. and Donehower, R. C. (1991). *Pharmacol. Ther.* **52,** 35–84.
93. Himes, R. H. (1991). *Pharmacol. Ther.* **51,** 257–267.
94. Rowinsky, E. K., Onetto, N., Canetta, R. M., and Arbuck, S. G. (1992). *Sem. Oncol.* **19,** 646–662.
95. Foa, R., Norton, L., and Seidman, A. D. (1994). *Int. J. Clin. Lab. Res.* **24,** 6–14.
96. Arnal, I. and Wade, R. H. (1995). *Curr. Biol.* **5,** 900–908.
97. Vyas, D. M. and Kadow, J. F. (1995) *Prog. Med. Chem.* **32,** 289–337.
98. Wilson, L. and Jordan, M. A. (1994). In: *Microtubules.* Hyams, J. S. and Lloyd, C. W. (eds.) Wiley-Liss, New York, pp. 59–83.
99. Sato, H., Kato, T., Choku Takahashi, T., and Ito, T. (1982). In: *Biological Functions of Microtubules and Related Structures.* Sakai, H., Mohri, H., and Borisy, G. G. (eds.) Academic, New York, pp. 211–226.
100. Pepper, D. A. and Brinkley, B. R. (1979). *J. Cell Biol.* **82,** 585–591.
101. Stephens, R. E. (1986). *Biol. Cell* **57,** 95–110.
102. Anderson, P. J. (1979). *J. Biol. Chem.* **254,** 2168–2171.
103. Feit, H., Duton, G. R., Barondes, S. H., and Shelanski, M. L. (1971). *J. Cell Biol.* **51,** 138–147.
104. Thrower, D., Jordan, M. A., and Wilson, L. (1993). *Methods Cell Biol.* **37,** 129–145.
105. Gozes, I. and Littauer, U. Z. (1979). *FEBS Lett.* **99,** 86–90.
106. Zisapel, N., Levi, M., and Gozes, L. (1980). *J. Neurochem.* **34,** 26–32.
107. Ravindra, R., Nagele, R. G., Patel, S. A., and Freeman, T. A. (1994). *NeuroReport* **5,** 1653–1656.
108. Burns, R. G., Farrell, K. W., and Surridge, C. D. (1993). *Ciba Foundation Symp.* **176,** 248–267.
109. Sternlicht, H., Yaffe, M. B., and Farr, G. W. (1987). *FEBS Lett.* **214,** 226–235.
110. Scaife, R. M., Wilson, L., and Purich, D. L. (1992). *Biochemistry* **31,** 310–316.
111. Onali, P., Olianas, M. C., Schwartz, J. P., and Costa, E. (1983). *Mol. Pharmacol.* **24,** 380–386.
112. Ravindra, R. and Aronstam, R. S. (1990). *Biochem. Pharmacol.* **40,** 457–463.
113. Cassel, D. and Selinger, Z. (1976). *Biochim. Biophys. Acta* **452,** 538–551.
114. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M., and Gilman, A. G. (1984). *J. Biol. Chem.* **259,** 3560–3567.
115. Bond, R. A., Leff, P., Johnson, T. D., Milano, C. A., Rockman, H. A., McMinn, T. R., et al. (1995). *Nature* **374,** 272–276.
116. Cherksey, B. D., Zadunaisky, J. A., and Murphy, R. B. (1980). *Proc. Natl. Acad. Sci. USA* **77,** 6401–6405.
117. Schlegel, W., Kempner, E. S., and Rodbell, M. (1979). *J. Biol. Chem.* **254,** 5168–5176.
118. Rodbell, M. (1980). *Nature* **284,** 17–22.
119. Coulter, S. and Rodbell, M. (1992). *Proc. Natl. Acad. Sci. USA* **89,** 5842–5846.
120. Nakamura, S.-I. and Rodbell, M. (1990). *Proc. Natl. Acad. Sci. USA* **87,** 6413–6417.
121. Nakamura, S.-I. and Rodbell, M. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 7150–7154.
122. Jahangeer, S. and Rodbell, M. (1993). *Proc. Natl. Acad. Sci. USA* **90,** 8782–8786.
123. Sato, M., Kataoka, R., Dingus, J., Wilcox, M., Hildebrandt, J. D., and Lanier, S. M. (1995). *J. Biol. Chem.* **270,** 15,269–15,276.
124. Sahyoon, N., LeVine, H., III, Davis, J., Hebdon, G. M., and Cuatrecasas, P. (1981). *Proc. Natl. Acad. Sci. USA* **78,** 6158–6162.
125. Carlson, K. E., Woolkalis, M. J, Newhouse, M. G., and Manning, D. R. (1986). *Mol. Pharmacol.* **30,** 463–468.
126. Ibarrondo, J., Joubert, D., Dufour, M. N., Cohen-Solal, A., Homburger, V., Jard, S., et al. (1995). *Proc. Natl. Acad. Sci. USA* **92,** 8413–8417.
127. Vaziri, C. and Downes, C. P. (1992). *J. Biol. Chem.* **267,** 22,973–22,981.
128. Offringa, R. and Bierer, B. E. (1993). *J. Biol. Chem.* **268,** 4979–4988.
129. Edelman, G. M., Yahara. I., and Wang, J. L. (1973). *Proc. Natl. Acad. Sci. USA* **70,** 1442–1446.
130. Knowles, D. W., Chasis, J. A., Evans, E. A., and Mohandas, N. (1994). *Biophys. J.* **66,** 1726–1732.
131. Kirsch, J., Wolters, I., Triller, A., and Betz, H. (1993). *Nature* **366,** 745–748.
132. Item, C. and Sieghart, W. (1994). *J. Neurochem.* **63,** 1119–1125.
133. Whatley, V. J., Mihic, S. J., Allan, A. M., McQuilkin, S. J., and Harris, R. A. (1994). *J. Biol. Chem.* **269,** 19,546–19,552.
134. Andrews, W. V., Staley, D. D., Huckle, W. R., and Conn, P. M. (1986). *Endocrinology* **119,** 2537–2546.
135. Limor, R., Schvartz, I., Hazum, E., Ayalon, D., and Naor, Z. (1989). *Biochem. Biophys. Res. Commun.* **159,** 209–215.
136. Perrin, M. H., Haas, Y., Porter, J., Rivier, J., and Vale, W. (1989). *Endocrinology* **124,** 798–804.
137. Gershengorn, M. C. and Osman, R (1996). *Physiol. Rev.* **76,** 175–191.
138. Straub, R. E. and Gershengorn, M. C. (1986). *J. Biol. Chem.* **261,** 2712–2717.
139. Lucas, D. O., Bajjalieh, S. M., Kowalchyk, J. A., and Martin, T. F. J. (1985). *Biochem. Biophys. Res. Commun.* **132,** 721–728.
140. Martin, T. F. J., Lucas, D. O., Bajjalieh, S. M., and Kowalchyk, J. A. (1986). *J. Biol. Chem.* **261,** 2918–2927.
141. Taylor, R. L. and Burt, D. R. (1981). *Mol. Cell. Endocrinol.* **21,** 85–91.
142. Cuatrecasas, P. (1986). *The Harvey Lectures* Series **80,** 89–128.

143. Ward, M. D. and Hammer, D. A. (1994). *J. Math. Biol.* **32,** 677–704.
144. Conn, P. M. and Hazum, E. (1981). *Endocrinology* **109,** 2040–2045.
145. Matus-Leibovitch, N., Gershengorn, M. C., and Oron, Y. (1993). *Cell. Mol. Neurobiol.* **13,** 625–637.
146. Meyer zu Heringdorf, D., Liedel, K., Kaldenberg-Stasch, S., Michel, M. C., Jakobs, K. H., and Wieland, T. (1996). *Eur. J. Biochem.* **235,** 670–676.
147. Sarndahl, E., Bokoch, G. M., Boulay, F., Stendahl, O., and Andersson, T. (1996). *J. Biol. Chem.* **271,** 15,267–15,271.
148. Ravindra, R., McIlroy, P. J., and Patel, S. A. (1997). *Pharmacol. Toxicol.* **80,** 24–29.
149. Wunderlich, F., Muller, R., and Speth, V. (1973). *Science* **182,** 1136–1138.
150. Erickson, M. A., Robinson, P., and Lisman, J. (1992). *Science* **257,** 1255–1258.
151. Berstein, G., Blank, J. L., Jhon, D.-Y., Exton, J. H., Rhee, S. G., and Ross, E. M. (1992). *Cell* **70,** 411–418.
152. Ravindra, R. and Aronstam, R. S. (1991). *Pharmacol. Toxicol.* **69,** 259–262.
153. Ravindra, R. and Aronstam, R. S. (1992). *J. Reprod. Fertil.* **95,** 669–677.
154. Lin, C. M. and Hamel, E. (1981). *J. Biol. Chem.* **256,** 9242–9245.
155. Arai, T. and Kaziro, Y. (1977). *J. Biochem.* **82,** 1063–1071.
156. Hsieh, K. P. and Martin, T. F. J. (1992). *Mol. Endocrinol.* **6,** 1673–1681.
157. Wilson, B. S., Komuro, M., and Farquhar, M. G. (1994). *Endocrinology* **134,** 233–244.
158. Insel, P. A. and Kennedy, M. S. (1978). *Nature* **273,** 471–473.
159. Margolis, R. and Wilson, L. (1979). *Cell* **18,** 673–679.
160. Wolff, J. and Cook, G. H. (1985). *Biochim. Biophys. Acta* **844,** 34–41.
161. Kennedy, M. S. and Insel, P. A. (1979). *Mol. Pharmacol.* **16,** 215–223.
162. Rudolph, S. A., Greengard, P., and Malawista, S. E. (1977). *Proc. Natl. Acad. Sci. USA* **74,** 3404–3408.
163. Simantov, R. and Sachs, L. (1978). *FEBS Lett.* **90,** 69–73.
164. Greene, W. C., Parker, C. M., and Parker, C. W. (1976). *J. Immunol.* **117,** 1015–1022.
165. Rasenick, M. M., Stein, P. J., and Bitensky, M. W. (1981). *Nature* **294,** 560–562.
166. DoKhac, L., Tanfin, Z., and Harbon, S. (1983). *Biochem. Pharmacol.* **32,** 2535–2541.
167. Cote, M., Payet, M.-D., and Gallo-Payet, N. (1997) *Endocrinology* **138,** 69–78.
168. Rasenick, M. M. and Wang, N. (1988). *J. Neurochem.* **51,** 300–311.
169. Hatta, S., Ozawa, H., Saito, T., and Ohshika, H. (1995). *J. Neurochem.* **64,** 1343–1350.
170. Wang, N., Yan, K., and Rasenick, M. M. (1990). *J. Biol. Chem.* **265,** 1239–1242.
171. Roychowdhury, S. and Rasenick, M. M. (1994). *Biochemistry* **33,** 9800–9805.
172. Popova, J. S., Johnson, G. L., and Rasenick, M. M. (1994). *J. Biol. Chem.* **269,** 21,748–21,754.
173. Chabre, M. (1987). *Trends Biochem. Sci.* **12,** 213–215.
174. Bokoch, G. M., Bickford, K., and Elohl, B. P. (1988). *J. Cell Biol.* **106,** 1927–1936.
175. Rotrosen, D., Gallin, J. I., Spiegel, A. M., and Malech, H. L. (1988). *J. Biol. Chem.* **263,** 10,958–10,964.
176. Rudolph, U., Koesling, D., Hinsch, K.-D., Seifert, R., Bigalke, M., Schultz, G., et al. (1989). *Mol. Cell. Endocrinol.* **63,** 143–153.
177. Muller, L., Picart, R., Barret, A., Bockaert, J., Homburger, V., and Tougard, C. (1994). *Mol. Cell. Neurosci.* **5,** 556–566.
178. Milligan, G. (1993). *Trends Pharm. Sci.* **14,** 413–418.
179. Wedegaertner, P. B., Bourne, H. R., and von Zastrow, M. (1996). *Mol. Biol. Cell* **7,** 1225–1233.
180. Takahashi, S., Negishi, M., and Ichikawa, A. (1991). *J. Biol. Chem.* **266,** 5367–5370.
181. Negishi, M., Hashimoto, H., and Ichikawa, A. (1992). *J. Biol. Chem.* **267,** 2367–2369.
182. Ransnas, L., Svoboda, P., Jasper, J., and Insel, P. A. (1989). *Proc. Natl. Acad. Sci. USA* **86,** 7900–7903.
183. Ozawa, K., Takahashi, M., and Sobue, K. (1996). *FEBS Lett.* **382,** 159–163.
184. Szego, C. M. and Pietras, R. J. (1984). *Int. Rev. Cytol.* **88,** 1–302.
185. Szego, C. M. (1994). *Endocrine* **2,** 1079–1093.
186. Schelling, J. R., Hanson, A. S., Marzec, R., and Linas, S. L. (1992). *J. Clin. Invest.* **90,** 2472–2480.
187. Feuilloley, M., Desrues, L., and Vaudry, H. (1993). *Endocrinology* **133,** 2319–2326.
188. Egan, J. J., Gronowicz, G., and Rodan, G. A. (1991). *J. Cell Biol.* **45,** 101–111.
189. Lomri, A. and Marie, P. J. (1990). *Bone Miner.* **10,** 1–12.
190. Lomri, A. and Marie, P. J. (1990) *Biochim. Biophys. Acta* **1052,** 179–186.
191. Taniguchi, T., Takaishi, K., Murayama, T., Ito, M., Iwata, N., Chihara, K., et al. (1996). *Oncogene* **12,** 1357–1360.
192. Rybczynski, R. and Gilbert, L. I. (1995). *Dev. Biol.* **169,** 15–28.
193. Lawrence, T. S., Ginzberg, R. D., Gilula, N. B., and Beers, W. H. (1979). *J. Cell Biol.* **80,** 21–36.
194. Aharoni, A., Dantes A., and Amsterdam, A. (1993). *Endocrinology* **133,** 1426–1436.
195. Feuilloley, M. and Vaudry, H. (1996). *Endocrine Rev.* **17,** 269–288.
196. Kiley, S. C., Parker, P. J., Fabbro, D., and Jaken, S. (1992). *Mol. Endocrinol.* **6,** 120–131.
197. De Moortele, S. V., Rosenbaum, E., Tixier-Vidal, A., and Tougard, C. (1991). *J. Cell Sci.* **99,** 79–89.
198. Ravindra, R., Forman, L. J., Foster,. K. A., and Patel, S. A. (1995). *NeuroReport* **6,** 1405–1408.
199. Wu, H. C. and Lin, C. T. (1994). *Lab. Invest.* **71,** 175–181.
200. Sandoval, I. V. and Cuatrecasas, P. (1978). *Eur. J. Biochem.* **91,** 151–161.
201. Higashi, K. and Ishibashi, S. (1985). *Biochem. Biophys. Res. Commun.* **132,** 193–197.
202. Geahlen, R. L. and Haley, B. E. (1979). *J. Biol. Chem.* **254,** 11,982–11,987.
203. Roychowdhury, S., Wang, N., and Rasenick, M. M. (1993). *Biochemistry* **32,** 4955–4961.
204. Wang, N. and Rasenick, M. M. (1991). *Biochemistry* **30,** 10,957–10,965.
205. Keski-Oja, J., Lehto, V.-P., and Virtanen, I. (1981). *J. Cell Biol.* **90,** 537–541.
206. Calissano, P., Monaco, G., Castellani, L., Mercanti, D., and Levi, A. (1978). *Proc. Natl. Acad. Sci. USA* **75,** 2210–2214.
207. Haussinger, D., Stoll, B., vom Dahl, S., Theodoropoulos, P. A., Markogiannakis, E., Gravanis, A., et al. (1994). *Biochem. Cell Biol.* **72,** 12–19.
208. Martin, S. S., Rose, D. W., Salteil, A. R., Klippel, A., Williams, L. T., and Olefsky, J. M. (1996). *Endocrinology* **137,** 5045–5054.
209. Thatte, H. S., Bridges, K. R., and Golan, D. E. (1994). *J. Cell. Physiol.* **160,** 345–357.
210. Hunt, R. C., Dewey, A., and Davis, A. A. (1989). *J. Cell Sci.* **92,** 655–666.
211. Spoerri, P. E. and Roisen, F. J. (1992). *J. Neurosci. Res.* **31,** 494–501.
212. Olorundare, O. E., Simmons, S. R., and Albrecht, R. M. (1993). *Eur. J. Cell Biol.* **60,** 131–145.
213. Bourguignon, L. Y., Lokeshwar, V. B., Chen, X., and Kerrick, W. G. (1993). *J. Immunology* **151,** 6634–6644.
214. Wang, N., Planus, E., Pouchelet, M., Fredberg, J. J., and Barlovatz-Meimon, G. (1995). *Am. J. Phys.* **268,** C1062–1066.

215. van Bergen en Henegouwen, P. M. P., Defize, L. H. K., de Kroon, J., van Damme, H., Verkleij, A. J., and Boonstra, J. (1989). *J. Cell. Biochem.* **39,** 455–465.
216. Ravindra, R. and Caro, J. F. (1993) *J. Cell. Biochem.* **53,** 181–189.
217. Gawler, D. and Houslay, M. D. (1987). *FEBS Lett.* **216,** 94–98.
218. Luttrell, L., Kilgour, E., Lamer, J., and Romero, G. (1990) *J. Biol. Chem.* **265,** 16,873–16,879.
219. Baltensperger, K., Karoor, V., Paul, H., Ruoho, A., Czech, M. P., and Malbon, C. C. (1996). *J. Biol. Chem.* **271,** 1061–1064.
220. Moxham, C. M. and Malbon, C. C. (1996). *Nature* **379,** 840–844.
221. Daub, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996) *Nature* **379,** 557–560.
222. Velloso, L. A., Folli, F., Sun, X. J., White, M. F., Saad, M. J. A., and Kahn, C. R. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 12,490–12,495.
223. Kemphues, K. J., Kaufman, T. C., Raff, R. A., and Raff, E. C. (1982). *Cell* **31,** 655–670.
224. Boggs, B. and Cabral, F. (1987). *Mol. Cell. Biol.* **7,** 2700–2702.
225. Sisodia, S. S., Gay, D. A., and Cleveland, D. W. (1990). *Nature New Biol.* **2,** 66–76.
226. Cleveland, D. W. and Theodorakis, N. G. (1994). In: *Microtubules*. Hyams, J. S. and Lloyd, C. W. (eds.) Wiley-Liss, New York, pp. 47–58.
227. Brostrom, C. O., Bocckino, S. B., and Brostrom, M. A. (1983). *J. Biol. Chem.* **258,** 14,390–14,399.
228. Nurnberg, B. and Ahnert-Hilger, G. (1996). *FEBS Lett.* **389,** 61–65.
229. Webb, B. C. and Wilson, L. (1980). *Biochemistry* **19,** 1993–2000.
230. Pirollet, F., Job, D., Fischer, E. H., and Margolis, R. L. (1983). *Proc. Natl. Acad. Sci. USA* **80,** 1560–1564.