Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6413–6417, August 1990
Biochemistry

Octyl glucoside extracts GTP-binding regulatory proteins from rat brain "synaptoneurosomes" as large, polydisperse structures devoid of $\beta\gamma$ complexes and sensitive to disaggregation by guanine nucleotides

(cytoskeleton/detergents/signal transduction)

SHUN-ICHI NAKAMURA AND MARTIN RODBELL

Section on Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Contributed by Martin Rodbell, May 29, 1990

ABSTRACT **GTP-binding regulatory proteins are generally purified from cholate-extracted membranes in the form of heterotrimers (G proteins) consisting of a GTP-binding subunit (α protein) complexed with a tightly interacted heterodimer termed $\beta\gamma$. In this study we extracted the proteins from rat brain "synaptoneurosomes" using the neutral detergent 1-octyl β-D-glucopyranoside (octyl glucoside). Using specific antibodies for detection by immunoblotting and sucrose gradients for analyzing hydrodynamic properties, we found that each species of α protein (α subunits of stimulatory, inhibitory, and brain GTP-binding proteins) exhibited a broad range (4 S to >12 S) of polydisperse structures with peak values (5 S to 7 S) considerably greater than that of heterotrimeric G proteins. The β subunit proteins, for example, appeared as a homogeneous peak at 4.4 S within which only a fraction of the total α proteins can be associated. Incubation of octyl glucose extracts at 30°C rapidly sedimented the α proteins but not the β proteins. Incubation at 30°C with guanosine 5′[γ-thio]triphosphate (10–100 μM) prevented rapid sedimentation. Hydrodynamic analysis revealed that all α proteins were converted to ≈4 S structures by the actions of guanosine 5′-[γ-thio]triphosphate without change in the hydrodynamic properties of the β proteins. Extraction of the membranes with sodium cholate instead of octyl glucoside resulted in complete loss of the large, polydisperse structures of the α proteins; the S values were ≈4 S, in the range for β proteins. These findings suggest that the transducing GTP-binding proteins in synaptoneurosomes exist as polydisperse, possibly multimer, structures of various size that are stable in octyl glucoside but destroyed by cholate. The polydisperse structures are not associated with $\beta\gamma$ complexes and are sensitive to the disaggregating effects of guanosine 5′-[γ-thio]triphosphate.**

Guanine nucleotide-binding proteins comprise a large family of proteins involved in numerous regulatory processes. One class has received considerable attention because its constituent proteins mediate or transduce the actions of many extracellular signals (light, hormones, odorants, neurotransmitters, etc.) acting through specific surface-membrane receptors.

A widely held theory of how the GTP-binding proteins function as transduction elements is based on the properties of purified GTP-binding proteins (termed α proteins) isolated as complexes with two other types of proteins, termed β and γ (for reviews see refs. 1 and 2). With the exception of the light-activated GTP-binding protein, the heterotrimers (G proteins) are soluble only in detergents and usually are extracted and purified in detergents such as sodium cholate and Lubrol. Incubation of the purified G proteins with AlF_4^- or with analogs of GTP—guanosine 5′-[γ-thio]triphosphate (GTP[γS]) and guanosine 5′-[β,γ-imido]triphosphate—that are poor substrates of the intrinsic GTPase of GTP-binding proteins leads to dissociation of the α subunit from the $\beta\gamma$ subunits. From these findings the heterotrimers are generally assumed to be the naturally occurring transducing structures. Receptor-induced dissociation of the heterotrimers into free α subunits and $\beta\gamma$ complexes is thought an essential step in the signal-transduction process that leads to multiple actions of hormones.

Another theory of the structure–function of transducing GTP-binding proteins is not based on the properties of purified proteins. Termed "disaggregation-coupling" (3), this theory is based on target irradiation of hormone-sensitive adenylate cyclase systems in intact membranes (4, 5). Target analysis, a means of determining the functional sizes of structures in native membranes (6), indicated that the ground states of these systems consist of very large structures of ≈1500 kDa; exposure to hormones such as glucagon and GTP reduced the ground state to ≈350 kDa, whereas nonphysiological activating agents such as fluoride ion and guanosine 5′-[β,γ-imido]triphosphate induced the formation of 250-kDa structures. These findings prompted a model in which receptors coupled to GTP regulatory proteins are linked as multimeric or oligomeric structures. Concerted activation of receptors by hormone and of GTP regulatory proteins by GTP disaggregates the multimers to form a receptor–GTP regulatory protein complex. This complex was suggested to serve as the messenger or coupling agent with adenylate cyclase and possibly other effector systems in the cell membrane. The nonphysiological activators, because they bypass the receptor and act directly on the multimeric GTP regulatory proteins, liberate GTP-binding proteins without being linked to receptors.

We have reported (7, 8) that octyl glucoside, a neutral detergent, extracts G proteins from brain membranes in the form of structures that, when incubated at 30°C, sediment along with cytoskeletal proteins, including actin, tubulin, and intermediate filaments; inclusion of nonhydrolyzable analogs of GTP such as GTP[γS] during incubation prevented sedimentation. The sedimented material did not contain the β subunits and could not, therefore, represent heterotrimeric G proteins. These findings raised the possibility that 1-octyl β-D-glucopyranoside (octyl glucoside) extracts cytoskeletal-like structures of the GTP-binding proteins (α proteins) that represent the large sizes of the signal-transduction system

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: α_s, α_i, and α_o, α subunits of stimulatory, inhibitory, and brain, respectively, GTP-binding regulatory protein; α proteins, α_s, α_i, and α_o, which complex with β and γ proteins to form heterotrimeric G proteins; GTP[γS], guanosine 5′-[γ-thio]triphosphate; octyl glucoside, 1-octyl β-D-glucopyranoside.

detected by irradiation analysis. In this study we have investigated the hydrodynamic characteristics of octyl glucoside-extracted α subunits of brain, inhibitory, and stimulatory GTP-binding proteins (α_o, α_i, and α_s, respectively) present in brain synaptoneurosomes. The results offer another perspective on the structures of these proteins.

MATERIALS AND METHODS

Reagents. Octyl glucoside (highest grade) was purchased from Sigma. GTP[γS] was from Boehringer Mannheim. All other chemicals were the highest quality available.

Antisera. Goat anti-rabbit immunoglobulin and rabbit peroxidase–anti-peroxidase were from Organon Teknika–Cappel. Rabbit antisera AS/6 (specific for α_i proteins) and RM (specific for α_s proteins) were gifts from Allen Spiegel (National Institutes of Health, Bethesda, MD). Antiserum reacting specifically with α_o was obtained by injecting rabbits repeatedly with a synthetic peptide spanning the region 284–297 of α_o. Antiserum against the β subunit of G protein was supplied by David Manning (University of Pennsylvania, Philadelphia).

Solubilization and Heat Treatment of "Synaptoneurosomes". Rat synaptoneurosomes were prepared by the method of Hollingsworth *et al.* (9), except that the final pellet was resuspended with buffer A (20 mM Hepes·NaOH, pH 7.4/1 mM EDTA/150 mM NaCl/5 μM dithiothreitol/5 mM $MgSO_4$) and stored at −80°C until use. For extraction purposes, synaptoneurosomes (600 μg) were incubated with buffer A containing 1.5% (wt/vol) octyl glucoside (or sodium cholate, when indicated) in a final volume of 200 μl for 30 min at 4°C or 30°C, as indicated. After extraction, samples were centrifuged in a Beckman TLA 100 rotor at 55,000 rpm (100,000 × *g*) for 10 min at indicated temperatures. After centrifugation, the upper clear half of supernatant was carefully pipetted and used as the supernatant fraction. The pellet was resuspended in 200 μl of buffer A and sonicated for 10 sec (Branson, Sonifier 250, with output 1). Aliquots of both fractions were heated for 3 min at 100°C with Laemmli sample buffer and were subjected to SDS/PAGE on 12.5% polyacrylamide gels (10).

Sucrose Density Gradients. Synaptoneurosomes (0.9 mg) were solubilized as above in buffer A containing 1.5% (wt/vol) octyl glucoside in a final volume of 300 μl for 30 min at 4°C. After centrifugation in a Beckman TLA 100.2 roter at 55,000 rpm (100,000 × *g*) for 10 min, the upper 200 μl of clear supernatant was layered over a gradient of sucrose (5–20%) in 20 mM Hepes·NaOH, pH 7.4/1 mM EDTA/1 mM dithiothreitol/150 mM NaCl/1.5% octyl glucoside. After centrifugation at 50,000 rpm for 15 hr at 4°C in a Beckman SW60 rotor, ≈30 fractions were taken and analyzed by SDS/PAGE and immunoblotting with various antisera. Values of $s^0_{20,w}$ were determined directly from the migration of calibrating proteins, which included cytochrome *c* ($s^0_{20,w}$, 2.1), bovine serum albumin ($s^0_{20,w}$, 4.4), pig immunoglobulin ($s^0_{20,w}$, 7.7), and bovine catalase ($s^0_{20,w}$, 11.2) carried out under identical conditions.

Other Techniques. Electroblotting was done as described (11). Immunostaining was accomplished by a standard peroxidase method (12). Protein concentration was determined by the method of Bradford (13).

RESULTS

Comparative Effects of Sodium Cholate and Octyl Glucoside on Temperature-Dependent Solubility of G Proteins. Both octyl glucoside (1.5%) and sodium cholate (1%) extracted within 30 min at 4°C essentially all G protein subunits in rat brain synaptoneurosomes detectable by immunoblotting with the various specific antisera used. An essential difference between the detergents was that at 30°C the GTP-binding proteins (α proteins) sedimented at 100,000 × *g* when extractions were done with octyl glucoside, whereas with cholate, these proteins were recovered in the supernatant. An example of these findings, typical of all species of the GTP-binding proteins (α_s, α_i, and α_o), is illustrated for α_i in Fig. 1*A*. Note that, in contrast to the α proteins, the β proteins were extracted by octyl glucoside in a nonsedimentable form at either extraction temperature (Fig. 1*B*). Inclusion of GTP[γS] during extraction of membranes with octyl glucoside at 30°C prevented the temperature-dependent sedimentation of α_i (Fig. 1*C*), as reported (8).

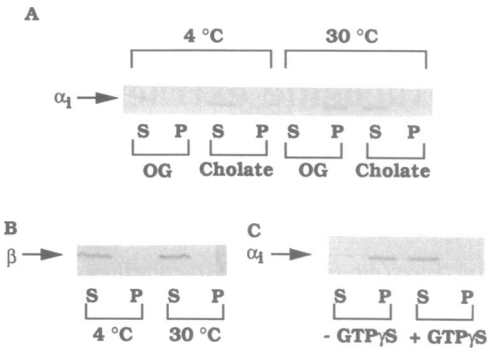

FIG. 1. Effect of temperature and GTP[γS] on solubilization of G proteins in synaptoneurosomes with octyl glucoside (OG) or cholate. Synaptoneurosomes were solubilized with or without 10 μM GTP[γS] with octyl glucoside or cholate under the standard conditions described. For immunostaining, anti-α_i antibody (AS/6) or anti-β antibody was used.

Sucrose Density-Gradient Analysis. The striking differences in the properties of the α and β subunits in octyl glucoside compared with cholate and the preventive effects of GTP[γS] on sedimentation at 30°C prompted examination of the hydrodynamic properties of the G protein subunits on sucrose gradients. In these studies, the proteins were extracted with either octyl glucoside or cholate at 4°C and then subjected to sedimentation-velocity analysis in the same detergent. Effects of GTP[γS] were investigated by incubating the supernatant fractions with or without the nucleotide for 15 min at either 4°C or 30°C before placing the material on the sucrose gradients.

The α_i proteins exhibited polydisperse structures ranging from >12 S to ≈4 S in gradients containing 1.5% octyl glucose at 4°C (Fig. 2). Incubation with 10 μM GTP[γS] at this temperature resulted in conversion toward lower S values (peak ≈4 S). Incubation at 30°C with GTP[γS] resulted in a

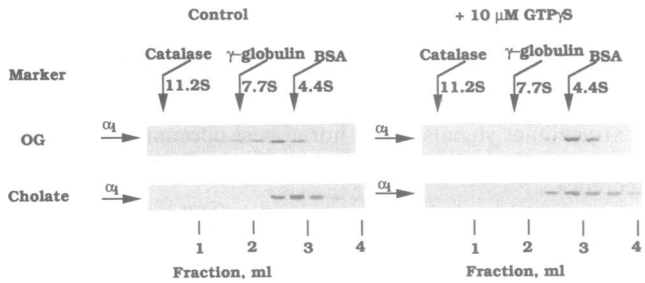

FIG. 2. Sucrose density-gradient sedimentation analysis of α_i extracted from synaptoneurosomes with either octyl glucoside (OG) or with cholate. Synaptoneurosomes were extracted and incubated with or without 10 μM GTP[γS] for 30 min at 4°C and analyzed essentially as described, except that for cholate solubilization, 1% cholate was included in the gradient instead of octyl glucoside and for GTP[γS], 10 μM GTP[γS] was also included in the gradients. For immunostaining, anti-α_i antibody (AS/6) was used. BSA, bovine serum albumin.

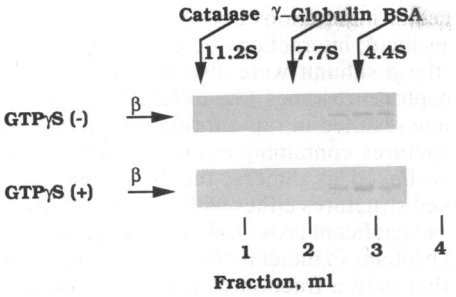

FIG. 3. Sucrose density-gradient sedimentation analysis of β (or $\beta\gamma$) subunits in octyl glucoside-extracted synaptoneurosomes. Samples were prepared under identical conditions to those described in the legend for Fig. 2. Samples were immunostained with anti-β antibody. BSA, bovine serum albumin.

sharper peak of the α_i proteins at ≈4 S (data not shown), indicating that the effectiveness of GTP[γS] was greater at the higher incubation temperature. The hydrodynamic features of cholate-extracted GTP-binding proteins are also shown in Fig. 2. The octyl glucoside-extracted polydisperse structures of α_i were not seen in cholate extracts. Instead, all immunoreactive material appeared in a peak fraction at ≈4 S. Clearly, the much larger structures of the GTP-binding proteins seen in octyl glucoside extracts were destroyed by cholate extraction. Note that incubation of the cholate extracts with 10 μM GTP[γS] resulted in a slight shift to lower S values of the α_i proteins.

The β subunits did not behave as polydisperse structures and sedimented with a relatively sharp peak at 4.4 S; this value was not changed when octyl glucoside extracts were incubated with GTP[γS] (Fig. 3). Densitometric analysis of the immunostained bands in Figs. 2 and 3 of octyl glucoside extracts treated with and without GTP[γS] is graphically presented in Fig. 4. From these data, we estimated that <10% of the α_i proteins can be associated with the 4.4 S peak containing the β (or $\beta\gamma$) subunits.

Brain membranes contain ≈1% of the proteins in the form of α_o, GTP-binding proteins that differ both structurally and functionally from the α_i proteins (2). The α_o proteins distributed on sucrose gradients containing 1.5% octyl glucoside over the same broad range of S values as the α_i proteins (Fig. 5). GTP[γS] (10 μM) also induced a similar conversion to structures of lower S values.

The hydrodynamic behavior of α_s was investigated with an antiserum (14) that reacts with two species of α_s as derived from differential splicing of the same gene (15). As shown for α_i and α_o, 1.5% octyl glucoside readily extracted α_s at 4°C; α_s

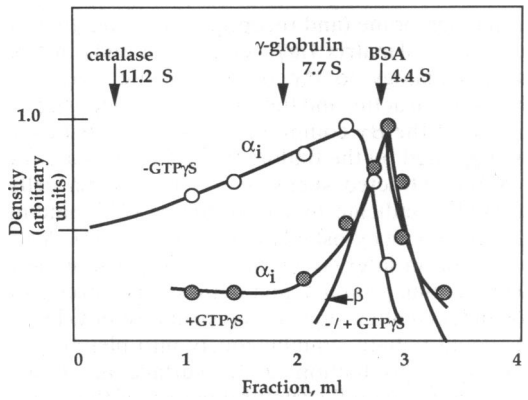

FIG. 4. Effects of GTP[γS] on the hydrodynamic properties of α_i proteins and β proteins. The immunoblots from experiments described in Figs. 2 and 3 were converted to densitometric units by scanning densitometry and plotted as indicated.

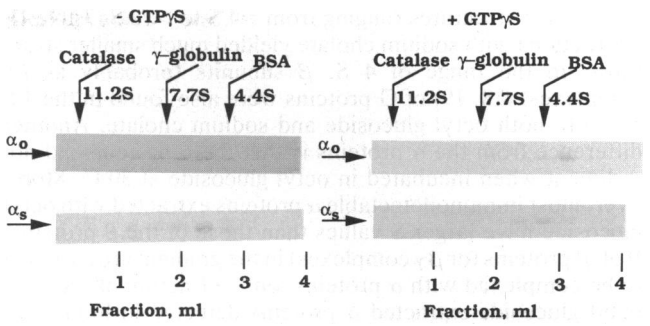

FIG. 5. Sucrose density-gradient sedimentation analysis of various α subunits of G proteins in octyl glucoside-extracted synaptoneurosomes. Samples were extracted with 1.5% octyl glucoside, and the extracts were incubated with or without GTP[γS], essentially as described in the legend for Fig. 2 except that for α_s subunits, the extracts were incubated with 100 μM GTP[γS] for 30 min at 4°C. For immunostaining, anti-α_s antiserum (RM) and antiserum specifically reactive with α_o were used.

sedimented in the detergent when incubated for 30 min at 30°C but remained in the supernate provided that 100 μM GTP[γS], 10-fold higher than required for α_i and α_o, was included in the incubation medium (data not shown). The sedimentation profile on sucrose gradients showed polydisperse structures similar to those of the other α proteins with a peak at ≈6.0 S (Fig. 5); complete conversion of the polydisperse structures to the 4 S structures was obtained when the inclusion of 100 μM GTP[γS] in the incubation medium.

DISCUSSION

Extraction of membrane-bound or membrane-associated proteins with detergents is generally a compromise between obtaining high yields of a given protein and the preservation of the associations that may exist between proteins in their native cellular environment. Sodium cholate is a particularly efficacious detergent for extracting GTP-binding proteins from their associations with membranes and has been the detergent of choice for extraction and purification of heterotrimeric G proteins (16–19). Triton detergents are frequently used to separate membrane proteins from cytoskeletal proteins, the latter as an insoluble matrix. However, as recently shown, extractions of erythrocyte membranes with Triton X-100 disrupts the known interactions between band 3, ankyrin, and spectrin—i.e., between membranes and cytoskeleton. Octyl glucoside, by contrast, preserves the oligomeric structure of band 3 and its association with the cytoskeletal elements (20).

We have shown in this study that octyl glucoside extracts, in high yield, the transducing GTP-binding proteins present in rat brain synaptoneurosomes; extractions at 4°C resulted in soluble preparations that, by sedimentation-velocity analy-

Table 1. S values of various G protein subunits in octyl glucoside extracts of synaptoneurosomes

	Control		GTP[γS]	
	Range	Peak	Range	Peak
α_i	12.0*–4.8	5.8	5.8–4.0	4.7
α_o	9.4*–4.0	5.1	4.9–3.0	4.0
α_s	9.4*–4.8	5.8	5.7–2.8	4.8
β	5.0–3.8	4.4	5.0–3.8	4.4

Data are composite values from several experiments described in text. The concentration of GTP[γS] was 10 μM in all experiments, except those with α_s, which was 100 μM.

*Some immunogenic material completely sedimented; thus, these S values are underestimates of the upper size limit of the α proteins.

sis, yielded structures ranging from ≈4 S to >12 S (Table 1). Extractions with sodium cholate yielded much smaller structures, in the range of 4 S. β subunits (probably as βγ complexes) (18, 19) of G proteins were also found in the 4 S range in both octyl glucoside and sodium cholate. Another difference from the α proteins is that the β proteins did not sediment when incubated in octyl glucoside at 30°C. Moreover, most immunodetectable α proteins extracted with octyl glucoside have larger S values than those of the β proteins. If all β proteins (or βγ complexes) in the gradient are assumed to be complexed with α proteins, only a fraction of the total octyl glucoside-extracted α proteins detected in brain synaptoneurosomes exist as heterotrimeric G proteins. Purified heterotrimeric G proteins prepared from cholate-extracted membranes have values of ≈4 S, similar to that shown here in cholate extracts of brain membranes and are converted to 3 S and 2 S structures by GTP[γS] (21). In this study, GTP[γS] induced conversion of the large polydisperse α_i, α_o, and α_s proteins extracted by octyl glucoside to structures with similar S values (Table 1). These structural changes did not involve linkage of the α proteins to βγ complexes, indicating that heterotrimeric structures are not uniquely required for the actions of GTP[γS] on the GTP-binding proteins. A recent report (22) indicates that α_{i2} exists as a water-soluble protein, uncomplexed with βγ subunits, in the cytosolic fraction of human neutrophils. The sedimentation behavior of the protein (4.12S at 4°C) is consistent with a homodimer; no change in this value was observed on incubation with βγ complexes. Incubation with 10 μM GTP[γS]/10 mM Mg ions resulted in conversion to 2.9 S structures, indicating the formation of monomers. These findings provide further evidence that α proteins can self-associate to give structures susceptible to the actions of guanine nucleotides. In this regard, the GTP-binding protein involved in light activation of phosphodiesterase in the retina also can be extracted as a water-soluble complex not only with βγ subunits but also as homodimers and higher-order structures devoid of βγ complexes (23).

The polydisperse structures of the α proteins seen in octyl glucoside are reminiscent of the properties of cytoskeletal proteins, such as actin and tubulin; these proteins exist in dynamic equilibrium between monomers and multimers of various sizes depending, in part, on temperature and occupation by ATP or GTP (24, 25). We have found that actin extracted by octyl glucoside from synaptoneurosomes also takes a wide range of polydisperse structures and, like the α proteins, rapidly aggregates when incubated at 30°C (unpublished observations). Detailed studies comparing properties of the cytoskeletal proteins and the polydisperse forms of α proteins, including actions of GTP and products of its hydrolysis by the intrinsic GTPase of these proteins, are ongoing.

A fundamental question is whether the polydisperse structures extracted by octyl glucoside represent the native form of the GTP-binding proteins. Thus far, the evidence is inconclusive. As discussed in the introduction, the functional size required for hormone/GTP stimulation of the adenylate cyclase system in liver membranes was ≈1500 kDa, far greater than the sum of the known sizes of the individual components (receptor, α proteins, catalytic component) minimally involved in the activity and regulation of these systems (4). Even larger structures were found for hormone-regulated inhibition (which involves α_i-like proteins) of adenylate cyclase in adipocytes (5). The large, polydisperse structures of the GTP-binding components extracted with octyl glucoside have sizes in the range of those estimated from irradiation analysis.

Cross-linking agents have been used to investigate the structural interactions of the α, β, and γ subunits of the G protein known as transducin (26, 27). By using a photoaffinity azido derivative of NAD^+, a recent study (28) showed intermolecular interactions between three α subunits of purified transducin; interactions between α and γ subunits but not with the β subunit were also indicated. In studies with brain synaptoneurosomes (8), treatment with *p*-phenylenedimaleimide resulted in rapid formation of very large cross-linked structures containing α_i and α_o, whereas the α_s proteins cross-linked as dimers; the β subunits did not form cross-linked structures either with self or with the α proteins. The lack of significant cross-linking of the β subunits with the various α proteins in intact membranes is consonant with our findings that only a fraction of the βγ complexes associate with the large, polydisperse structures of the octyl glucoside-extracted GTP-binding proteins.

None of the evidence cited above shows that heterotrimeric G proteins are either the native or the unique structure of GTP-binding proteins in brain synaptoneurosomes. Although numerous studies with purified G proteins suggest that the heterotrimeric forms of G proteins are involved in signal transduction (1, 2), recent characterization of complexes of muscarinic receptors and G proteins formed in the presence of agonists revealed that the α subunits formed complexes, whereas only substoichiometric amounts of β subunits were associated with the receptors (29). Possibly the heterotrimeric forms of G proteins exist as discrete structures with specialized functions that differ from those of the larger structures of the α proteins extracted by octyl glucoside. There is evidence for distinct compartmentalized states of α_s in human erythrocyte membranes, one of which is associated with the cytoskeletal fraction (30). Immunocytochemical studies with antisera specific for β and α_o proteins indicate selective and unequal distribution of these proteins in different parts of bovine serum (31), in astroglial cells and ependymocytes (32), and in human A-431 cells (33). In ependymocytes, immunofluorescence with α_o antiserum was homogeneously associated with the cytoplasmic matrix. In A-431 cells, the β subunits appeared as punctuate structures throughout the cytoplasm. Recent studies have shown that α_o is a major component, along with cytoskeletal proteins, of the neuronal growth-cone membrane; its structure and function are regulated by the intracellular growth-associated protein GAP-43 (34). Another indication that α_o may be both a structural and regulatory protein is suggested by the high concentration of this protein (≈1% of membrane protein) in brain (35).

An intriguing alternative to independent existence and function of heterotrimeric G proteins and the large polydisperse structures of the α proteins is that both are contained within a single cytoskeletal-like structure differentially disrupted by cholate and octyl glucoside to give the observed products. In this paradigm, the large cytoskeletal-like structures of the GTP-binding proteins may be hybridized both to the plasma membrane (and receptors contained therein) and to the cytoskeletal matrix via βγ complexes and other binding proteins by analogy to the region-specific, cohesive role played by several actin- and tubulin-specific binding proteins. Association of the βγ complexes with the cytoskeletal network is suggested by the finding that β proteins are found in Triton X-100-extracted shells in S49 lymphoma cells (36). Several studies indicate that transducing GTP-binding proteins associate with cytoskeletal proteins (37–44). A compelling feature of an interwoven network of membrane receptors, GTP-binding proteins, and cytoskeletal proteins is that receptor-induced alterations in the structure of GTP-binding proteins could radiate simultaneously into pleiotropic regulation of signal production at the surface membrane and alterations in the overall structural matrix of the cell, culminating in rapid, dynamic changes in cellular structure and metabolism. This mechano–chemical model (8) of the structure and function of the transducing GTP-binding proteins could explain, for example, the multiple effects of chemo-

tactic agents on receptor-coupled G protein action in neutrophils (45).

In conclusion, the major classes of GTP-binding proteins in brain are extracted by octyl glucoside as large, polydisperse structures sensitive to disaggregation by GTP[γS]. These structures are disrupted by sodium cholate, the detergent used for extracting heterotrimeric G proteins, which probably explains the failure to detect such structures in previous studies. Whether or not the large, possibly multimeric, α proteins are responsible for the GTP-dependent transduction processes that link numerous receptor and effector systems remains to be demonstrated.

1. Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56,** 615–650.
2. Neer, E. J. & Clapham, D. E. (1988) *Nature (London)* **333,** 129–134.
3. Rodbell, M. (1980) *Nature (London)* **284,** 17–22.
4. Schlegel, W., Kempner, E. S. & Rodbell, M. (1979) *J. Biol. Chem.* **254,** 5168–5176.
5. Schlegel, W., Cooper, D. M. F. & Rodbell, M. (1980) *Arch. Biochem. Biophys.* **2011,** 678–682.
6. Kempner, E. S. & Schlegel, W. (1979) *Anal. Biochem.* **92,** 2–10.
7. Rodbell, M., Ribeiro-Neto, F., Coulter, S., Haraguchi, K. & Udrisar, D. (1988) in *Progress in Endocrinology*, eds. Imura, H., Shizume, K. & Yoshida, S. (Elsevier, Amsterdam), Vol. 1, pp. 35–45.
8. Rodbell, M., Coulter, S. & Haraguchi, K. (1990) in *Growth Factors—From Genes to Clinical Application*, eds. Sara, V., Low, H. & Hall, K. (Raven, New York), pp. 101–116.
9. Hollingsworth, E. B., McNeal, E. T., Burton, J. L., Williams, R. J., Daly, J. W. & Creveling, C. R. (1985) *J. Neurosci.* **5,** 2240–2253.
10. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
11. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5350–5354.
12. Domin, B. A., Serabjit-Singh, C. J. & Philpot, R. (1984) *Anal. Biochem.* **112,** 195–203.
13. Bradford, J. (1976) *Anal. Biochem.* **72,** 248–254.
14. Simonds, W. F., Goldsmith, P. K., Codina, J., Unson, C. G. & Spiegel, A. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7809–7813.
15. Robishaw, J. D., Smigel, M. D. & Gilman, A. G. (1986) *J. Biol. Chem.* **261,** 9587–9590.
16. Northup, J. K., Smigel, M. D., Sternweis, P. C. & Gilman, A. G. (1983) *J. Biol. Chem.* **258,** 11369–11376.
17. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259,** 3560–3567.
18. Sternweis, P. C., Northup, J. K., Smigel, M. D. & Gilman, A. G. (1981) *J. Biol. Chem.* **256,** 11517–11527.
19. Codina, J., Hildebrandt, J. D., Sekura, R. D. & Birnbaumer, L. (1984) *J. Biol. Chem.* **259,** 5871–5886.
20. Kunimoto, M. & Miura, T. (1989) *J. Biochem. (Tokyo)* **105,** 190–195.
21. Codina, J., Hildebrandt, J. D., Birnbaumer, L. & Sekura, R. D. (1984) *J. Biol. Chem.* **259,** 11408–11418.
22. Rudolf, U., Schultz, G. & Rosenthal, W. (1989) *FEBS Lett.* **251,** 137–142.
23. Baehr, W., Morita, E. A., Swanson, R. J. & Applebury, M. J. (1982) *J. Biol. Chem.* **257,** 6452–6460.
24. Korn, E. D., Carlier, M.-F. & Pantaloni, D. (1987) *Science* **238,** 638–644.
25. Bayley, P., Schilstra, M. & Martin, S. (1989) *FEBS Lett.* **259,** 181–184.
26. Hingorani, V. N., Tobias, D. T., Henderson, J. T. & Ho, Y.-K. (1988) *J. Biol. Chem.* **263,** 6916–6926.
27. Wessling-Resnick, M. & Johnson, G. L. (1987) *J. Biol. Chem.* **262,** 12444–12447.
28. Vaillancourt, R. R., Dhanasekaran, N., Johnson, G. L. & Ruoho, A. E. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 3645–3649.
29. Matesic, D. F., Manning, D. R., Wolfe, B. B. & Luthin, G. R. (1989) *J. Biol. Chem.* **264,** 21638–21645.
30. Skurat, A. V., Yurkova, M. S., Kots, A. Y. A. & Bulargina, T. V. (1989) *Biokhimiya* **54,** 1576–1582.
31. Garty, N. B., Galiani, D., Aharonheim, A., Ho, Y.-K., Phillips, D. M., Dekel, N. & Salomon, Y. (1988) *J. Cell Sci.* **91,** 21–31.
32. Péraldi, S., Dao, B. N. T., Brabet, P., Homburger, V., Rouot, B., Toutant, M., Bouille, C., Assenmacher, I., Bockaert, J. & Gabrion, J. (1989) *J. Neurosci.* **9,** 806–814.
33. Wang, H.-H., Berrios, M. & Malbon, C. C. (1989) *Biochem. J.* **263,** 519–532.
34. Strittmatter, S. M., Valenzuela, D., Kennedy, T. E., Neer, E. J. & Fishman, M. C. (1990) *Nature (London)* **344,** 836–841.
35. Neer, E. J., Lok, J. M. & Wolf, L. G. (1984) *J. Biol. Chem.* **259,** 14222–14229.
36. Carlson, K. E., Woolkalis, M. J., Newhouse, M. G. & Manning, D. R. (1986) *Mol. Pharmacol.* **30,** 463–468.
37. Higashi, K. & Ishibashi, S. (1985) *Biochem. Biophys. Res. Commun.* **132,** 193–197.
38. Sarndahl, E., Lindroth, M., Bengtsson, T., Fallman, M., Gustavsson, J., Stendahl, O. & Andersson, T. (1989) *J. Cell Biol.* **109,** 2791–2799.
39. Jesaitis, A. J., Tolley, J. O. & Allen, R. A. (1986) *J. Biol. Chem.* **261,** 13662–13669.
40. Wang, N., Yan, K. & Rasenick, M. M. (1990) *J. Biol. Chem.* **265,** 1239–1242.
41. Huang, C.-K. & Devanney, J. F. (1986) *FEBS Lett.* **202,** 41–44.
42. Bourguignon, L. Y. W., Walker, G. & Huang, H. S. (1990) *J. Immunol.* **144,** 2242–2252.
43. Painter, R. G., Zahler-Bentz, K. & Dukes, R. E. (1987) *J. Cell Biol.* **105,** 2959–2971.
44. Bengtsson, T., Sarndahl, E., Stendahl, O. & Andersson, T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2921–2925.
45. Baggiolini, M. & Wymann, M. P. (1990) *Trends Biochem. Sci.* **15,** 69–72.