GTP analogues cause preferential translocation of an 18 kDa cytosolic G-protein to the membrane fraction in the ZR-75-1 human breast-cancer cell line

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Several G-proteins (GTP-binding proteins) were identified by SDS/PAGE in the cytosol (105000 *g* supernatant) and membrane fractions of the oestrogen-dependent human mammary-tumour cell line ZR-75-1. These proteins, with molecular masses in the range 18–29 kDa, specifically bind [α - 32 P]GTP, which can be displaced by unlabelled GTP, GDP and their non-hydrolysable analogues guanosine 5'-[δ -thio]triphosphate (GTP[S]) and guanosine 5'-[β -thio]diphosphate (GDP[S]), but not by GMP, ATP, ADP, AMP and other unrelated nucleotides. The apparent dissociation constant for GTP was approx. 2×10^{-8} M. Homogenization of ZR-75-1 cells in high-salt buffer (1 M-KCl), and successive washing of the membrane fraction, suggested that, among the major G-proteins found, the 18 kDa protein is predominantly soluble, whereas the 27–29 kDa complex is primarily bound to the membrane fraction under the experimental conditions employed. Possible translocation of these G-proteins between membrane and cytosol was analysed. No redistribution of the 27–29 kDa complex was observed, whereas GTP[S] in the presence of Mg^{2+} caused apparent translocation of the 18 kDa protein to the membrane fraction. This effect was specific for GTP and stable GTP analogues, whereas GDP, GMP, ATP, ADP, AMP and other unrelated nucleotides were ineffective. GTP[S] and guanosine 5'-[β -imidol]-triphosphate (p[NH]ppG) were equally potent (apparent $K_d \sim 5 \times 10^{-6}$ M), whereas GTP was rather weak. The nucleotide effect is temperature-, time- and concentration-dependent. The translocation process was reversible, slow, and reached its maximum between 30 and 60 min at 37 °C. The apparent translocation of this small G-protein from the cytosol to the membrane fraction, and the specific effect of GTP analogues, suggest that this process may have functional significance in mammary-tumour cells.

INTRODUCTION

A large family of G-proteins with a heterotrimeric structure of α , β and γ subunits is involved in signal transduction from membrane receptors in mammalian tissues. In these proteins the α subunit, with the characteristic functions of GTP binding and hydrolysis, has a molecular mass around 40 kDa (Ui, 1984; Bourne, 1986; Stryer, 1986; Gilman, 1987).

Recently, it has become apparent that there also exists a large diverse family of lower-molecular-mass G-proteins with masses of 18–30 kDa. In addition to proteins which belong to the *ras* gene family, other small G-proteins have been identified in a variety of tissues, including rabbit liver (Kahn & Gilman, 1984), bovine brain (Kahn & Gilman, 1986; Kikuchi *et al.*, 1988), human placenta (Evans *et al.*, 1986), erythrocytes (Ikeda *et al.*, 1988) and platelets (Bhullar & Haslam, 1987). Bhullar & Haslam (1987) reported that several low-molecular-mass G-proteins in platelet membranes and cytosols can be transferred to a nitrocellulose sheet from SDS/PAGE gels and retain binding activity. Under these conditions, however, high-molecular-mass G-proteins are not detectable. This lower-molecular-mass group of proteins, termed G_n proteins, are present in plant hypocotyls (Jacobs *et al.*, 1988) and rat liver microsomal fractions (Drobak *et al.*, 1988).

Membrane G-proteins are involved in many cell processes, such as the transduction of hormonal signals (Gilman, 1987), the machinery of cellular secretion (Melancon *et al.*, 1987) and regulation of membrane ion channels (Pfaffinger *et al.*, 1985). Significantly less is known about cytosolic G-proteins, although

there are reports relating the activation of soluble phospholipase C by stable GTP analogues bound to these proteins (Baldassare & Fisher, 1986; Deckmin *et al.*, 1986).

We were previously interested in this subject when studying the possible activation of phospholipase C by GTP[S] in mammary-tumour tissue (Segal *et al.*, 1987). In addition, we have reported that cytosolic oestradiol-receptor-related protein, p29, present in mammary tumour and other tissues, specifically binds GTP (Coffer & King, 1988). We considered the possibility that p29 was a member of the G_n family, and studied this further in the oestrogen-dependent human mammary-tumour cell line ZR-75-1. It rapidly became apparent that these cells contained a family of GTP-binding proteins distinct from p29. Their characterization is described here.

MATERIALS AND METHODS

Materials

GTP[S] and GDP[S] were purchased from Boehringer, Mannheim, Germany. All other nucleotides, leupeptin and phenylmethanesulphonyl fluoride were from Sigma Chemical Co., St. Louis, MO, U.S.A. [α - 32 P]GTP (3700 Ci/mmol) was from Amersham International, Amersham, Bucks., U.K. Immobilon-P transfer membranes were from Millipore, Bedford, MA, U.S.A.

Cell culture

ZR-75-1 human breast-cancer cells were in the same passage generation as in previous experiments (Darbre *et al.*, 1983).

Abbreviations used: GTP[S], guanosine 5'-[γ -thio]triphosphate; GDP[S], guanosine 5'-[β -thio]diphosphate; p[NH]ppG, guanosine 5'-[β -imidol]triphosphate; G-protein, GTP-binding protein; G_n , inhibitory subunit of the G-protein.

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Stock cells were grown as monolayer culture in Dulbecco's modified Eagle's medium containing Phenol Red, supplemented with 5% (v/v) foetal-calf serum (Serum Laboratories, Irvine, Scotland, U.K.) in a humidified atmosphere of 10% CO₂ in air at 37 °C, and were subcultured at weekly intervals by suspension with trypsin. Cells used for the preparation of subcellular fractions were harvested with trypsin and stored in liquid N₂.

Homogenization and cell fractionation

Frozen cells were homogenized by hand with 15 strokes of a Teflon/glass homogenizer in 5 vol. of buffer containing 10 mM-Tris/HCl, pH 7.4, 1 mM-EDTA, 2 mM-dithiothreitol, 50 µg of leupeptin/ml and 0.1 mM-phenylmethanesulphonyl fluoride. Subsequent work was carried out at 0–4 °C.

The homogenate was centrifuged at 800 *g* for 10 min, and the pellet was homogenized again in 3 vol. of homogenization buffer and centrifuged as above. The combined supernatant was centrifuged at 105 000 *g* for 65 min in a Beckman ultracentrifuge, with a 50 Ti rotor. The membrane pellet was resuspended in the same buffer, and the supernatant was used as the cytosolic fraction. Protein concentration was measured by the Bradford (1976) method with crystalline BSA as a standard.

Effect of nucleotides on the translocation of G-proteins

The procedure was a modification of a method used for the release of the α subunit of the G-protein from membranes of rat glioma cells (Milligan *et al.*, 1988). Membranes were incubated in a buffer (20 mM-Tris/HCl, pH 7.5, 20 mM-MgCl₂, 1 mM-dithiothreitol, 100 µM-EDTA) for various times at 37 °C in the presence of various nucleotide analogues (100 µM, except where otherwise stated). At the end of the incubation, the samples were separated into supernatant and particulate fractions by centrifugation at 138 kPa (20 lb/in²) at approx. 126 000 *g* for 2 min in an Airfuge (TL-100 rotor) (Beckman Instruments).

SDS/PAGE and blotting of proteins

Sample buffer was added to the supernatant and particulate fractions. The samples were heated at 100 °C for 3 min and analysed by SDS/PAGE (12% acrylamide) (Laemmli, 1970). For apparent-molecular-mass determinations, protein standards were electrophoresed in parallel with the experimental sample after electrophoresis gels were incubated for 15 min in a buffer containing 48 mM-Tris, 39 mM-glycine and 20% (v/v) methanol, pH 9.2, and the proteins were transferred to Immobilon-P [poly(vinylidene difluoride), 'PVDF'] membranes by dry electroblotting (Sartoblot II-S; Sartorius, Göttingen, Germany.) for 1.5 h at 140 mA in the same buffer.

Incubation of blots with [α -³²P]GTP

The procedure was a modification of a method used for the labelling of low-molecular-mass G-binding proteins in platelets and other cells (Bhullar & Haslam, 1987). The Immobilon-P blots were preincubated in incubation buffer (50 mM-Tris/HCl, pH 7.5, 1 mM-MgCl₂, 1 mM-dithiothreitol, 0.3% Tween 20) for 30 min at 37 °C in the presence of ATP (100 µM) to decrease non-specific binding. The membranes were then transferred to a fresh buffer containing 10 µCi of [α -³²P]GTP/ml and ATP (100 µM). After incubation for 75 min at 37 °C, the filters were washed in the same buffer containing no nucleotides for at least 30 min, with multiple washes. The blots were air-dried, and bound radioactivity was detected by autoradiography (4–48 h at –70 °C) by using Amersham Hyperfilm-MP with Cronex intensifying screens. The autoradiographs were scanned by computerized scanner (Ultrascan LKB). The areas of the bands are expressed in arbitrary density units.

Immunological characterization of low-molecular-mass G-proteins

Antisera AS7 and 306R were obtained as a gift from Dr. A. M. Spiegel, Metabolic Disease Branch, N.I.H., Bethesda, MD, U.S.A. (Goldsmith *et al.*, 1987). The AS7 antibody was raised against the C-terminal decapeptide of the α subunit of transducin. These antibodies also recognize the C-terminal sequences of the α subunit of both G₁₁ and G₁₂. The 306R antiserum has a broader spectrum and is able to recognize all α subunits of the heterotrimeric G-proteins. Anti-*ras* antibodies were from Dr. D. Bar-Saghi, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, U.S.A., and the ER-D5 antibody against p29 has been previously described (Coffer & King, 1988).

Immunoblots were prepared essentially as described for the preparation of blots for [α -³²P]GTP binding. The blots were preincubated in phosphate-buffered saline (KCl 0.2 g/litre, KH₂PO₄ 0.2 g/litre, NaCl 8.0 g/litre, Na₂HPO₄·7H₂O 2.16 g/litre)/0.5% Tween, and then made visible with ¹²⁵I-Protein A, as previously described (Coffer & King, 1988).

RESULTS

Low-molecular-mass G-proteins in mammary-tumour cells

Two major and several minor G-proteins were detected in both membranes and cytosols of the ZR-75-1 human mammary-tumour cells. These proteins were detected after gel electrophoresis under denaturing conditions, and therefore do not represent the full complement of such proteins within the cell. The apparent molecular masses (Fig. 1) of these major proteins were 18 kDa and 27–29 kDa, with other minor species also being present. The 27–29 kDa proteins were the most abundant protein in the membrane fraction, whereas the 18 and 27 kDa components predominated in the cytosol.

We investigated whether the identification of similar proteins, albeit in different proportions, in both fractions could be due to cytosol contamination of the membranes. Washing and centrifugation of the membranes (Fig. 1b) removed mainly the 18 kDa G-protein: 70% of this protein was in the cytosolic fraction after homogenization in low-salt buffer (lane 1), and, after three successive washes with 0.15 M-KCl, only 3% remained associated

Fig. 1. Low-molecular-mass G-proteins in ZR-75-1 mammary tumour cells

Autoradiographs of [α -³²P]GTP binding to G-proteins on Immobilon-P sheet transferred from SDS/PAGE are shown. (a) Cells fractionated in the presence of different concentrations of KCl. Cytosols (lanes 1–3), total homogenate (lane 4) or membranes (lanes 5–7) were obtained after homogenization in Tris/HCl buffer, pH 7.4, either in the absence (lanes 1, 4 and 5) or in the presence of 0.15 M- (lanes 2 and 6) or 1.0 M-KCl (lanes 3 and 7); 20 µg portions of protein were separated on 12% (w/v) gels. (b) Cells homogenized in the presence of 0.15 M-KCl were separated into a cytosolic fraction (lane 1) and a membrane fraction, which was submitted to three successive washes with the same buffer: washes 1–3 (lanes 2–4); membranes after washes 1–3 (lanes 5–7). The protein markers (kDa) were as in Fig. 3.

Fig. 2. Effect of various nucleotides on the $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding to 18 and 27–29 kDa G-proteins

Gels were blotted on to the Immobilon-P sheets, which were then preincubated with the indicated unlabelled nucleotides in the range 10^{-9} to 10^{-4} M. Then the sheets were incubated with the same unlabelled nucleotide in the presence of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$. $\blacklozenge$, GTP; $\square$, GDP; $\circ$, GMP. $\diamond$, 'Other' indicates all the non-competing nucleotides, as detailed in Table 1. The binding in the absence of unlabelled nucleotide was 2.3 density units. Similar results were obtained in two additional experiments.

with the membrane fraction. In the presence of 1.0 M-KCl, 91 % of the 18 kDa protein was present in the soluble fraction. In contrast, 75–90 % of the 27–29 kDa complex remained associated with the membrane fraction regardless of the washing procedure. These results suggest that the 18 kDa species is a soluble protein, whereas the 27–29 kDa complex is bound more tightly to the membrane fraction under the experimental conditions employed.

Table 1. Inhibition of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding to the low-molecular-mass G-proteins by various nucleotides

Abbreviation: p[NH]ppA, adenosine 5'-[$\alpha\beta$ -imido]triphosphate.

Nucleotide	Concn. needed for 50 % inhibition of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding (M)
GTP	2×10^{-8}
GDP	2×10^{-8}
GMP	$> 10^{-4}$
ATP*	$> 10^{-4}$
ADP	$> 10^{-4}$
AMP	$> 10^{-4}$
Cyclic AMP	$> 10^{-4}$
Cyclic GMP	$> 10^{-4}$
p[NH]ppA	$> 10^{-4}$
GTP[S]	2×10^{-8}
GDP[S]	2×10^{-8}
p[NH]ppG	2×10^{-8}

* The preincubation was done in the presence of AMP (100 μM) and not with the same concentration of ATP as described in the Materials and methods section.

Characterization of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding to the low-molecular-mass G-proteins

$[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding to these proteins was inhibited progressively by increasing concentrations of GTP, GDP and their stable analogues, but not by other nucleotides such as GMP, ATP and others (Table 1 and Fig. 2). Although Fig. 2 depicts only data for the 18 and 27–29 kDa proteins, the results were essentially the same for all the other minor proteins present in both cellular fractions. The concentration of GTP or other competing nucleotides necessary for 50 % inhibition of $[\alpha\text{-}^{32}\text{P}]\text{GTP}$ binding to all proteins was approx. 20 nM.

Effect of GTP[S] on the translocation of the 18 and 27–29 kDa G-proteins from the supernatant to the membrane fraction

Incubation of crude membrane preparations with increasing amounts of the cytosolic fraction caused preferential translocation of the 18 kDa proteins to the membrane fraction in the presence of GTP[S] (for 1 h at 37 °C) (Figs. 3 and 4). However, when the amount of the cytosolic protein in the incubation mixture was increased above approx. 40 μg , no further effect was noted. These results suggest that the binding sites for the 18 kDa protein on the membranes are limited and saturable (Fig. 4a). Control experiments done in the absence of membranes (results not shown) ruled out the possibility of nucleotide-induced aggregation of 18 kDa protein causing artificial transfer of the protein to the membrane precipitate. No significant effect of the nucleotide was evident on the location of the 27–29 kDa protein complex (Fig. 4b). Ratios of absorbance values with and without GTP[S] were calculated to indicate the nucleotide effect.

The GTP[S]-promoted transfer of the 18 kDa G-protein from

Fig. 3. Effect of GTP[S] on the translocation of low-molecular-mass G-proteins from the soluble to the membrane fraction

Membranes (50 µg of protein) were incubated for 1 h at 37 °C, as described in the Materials and methods section, with increasing amounts of the cytosolic fraction (0–40 µg of protein) with 100 µM-GTP[S] (lanes 2, 4, 6, 8 and 10), or without the nucleotide (lanes 1, 3, 5, 7 and 9): 0 µg of cytosolic protein (lanes 1 and 2), 5 µg of cytosolic protein (lanes 3 and 4), 10 µg of cytosolic protein (lanes 5 and 6), 20 µg of cytosolic protein (lanes 7 and 8) and 40 µg of cytosolic protein (lanes 9 and 10). The supernatant (panel a) was separated from the membrane fraction (panel b) and then processed for [α - 32 P]GTP binding, as described in the Materials and methods section. Prestained protein standards from Gibco/BRL were used for monitoring the migration of proteins on SDS/PAGE and for the transfer of proteins from the gel to Immobulin-P membranes. The protein markers used were: lysozyme, 14300 Da; β -lactoglobulin, 18400 Da; carbonic anhydrase, 29000 Da; ovalbumin, 43000 Da; and BSA, 68000 Da. Similar results were obtained in three additional experiments.

cytosol to membrane was time- and temperature-dependent (Fig. 5), maximal transfer occurring after about 60 min. GDP[S] was ineffective in stimulating the transfer (Fig. 5). When excess of GDP[S] (1 mM) was included in the incubation medium together with GTP[S] (100 µM), partial inhibition of the 18 kDa protein translocation, caused by GTP[S], was observed. However, this effect did not reach statistical significance. The observed inhibitory effect of GDP[S] on the maximal effect of GTP[S] was $20 \pm 10\%$ in 60 min, and $23 \pm 8\%$ in 120 min (means $\pm$ S.E.M. of three experiments). The total amount of [α - 32 P]GTP binding to the 18 kDa protein did not change throughout these experiments (results not shown). The membrane fraction as routinely used did contain small amounts of the 18 kDa protein (Fig. 1a), which

was released into the supernatant on incubation with buffer alone (Fig. 3a, track 1). This release was inhibited by GTP[S] (Fig. 3a, track 2), but not by GDP[S] (Fig. 5). This, together with its salt-extractability (Fig. 1), suggested that it represents trapped or loosely bound cytosolic protein.

The translocation of the 18 kDa protein from the cytosol to the membrane fraction by GTP[S] (100 µM) is fully reversible. After incubation with the nucleotide for 1 h, most of the 18 kDa protein is bound to the membrane fraction (Fig. 5). If the supernatant is removed by fast centrifugation (Airfuge, at 4 °C) and the membranes are re-incubated with fresh medium supplemented with GDP[S] (or without any nucleotide), most of the 18 kDa protein is relocated back to the cytosolic fraction.

The translocation of the 18 kDa protein from the soluble to the membrane fraction is specific for GTP and its stable analogues GTP[S] and p[NH]ppG (Fig. 6). No effect was found with GDP[S], ATP, ADP, GMP, AMP, cyclic AMP and cyclic GMP (results not shown). The effect is dose-dependent (Fig. 6), and GTP[S] and p[NH]ppG are equally potent, with apparent K_m values of $\sim 5 \times 10^{-6}$ M. GTP was 100-fold less potent. The effect of GTP and its stable analogues is specific for the 18 kDa G-protein. The 27–29 kDa protein complex was not affected by a wide range of GTP[S] concentrations (Fig. 6, and results not shown).

The translocation of the 18 kDa protein by GTP[S] is dependent on Mg^{2+} ions. No translocation can be seen in the absence of Mg^{2+} . In order to elicit the cation requirements of the 18 kDa protein translocation, reaction mixtures were incubated with increasing concentrations of Mg^{2+} ions. However, at low cation concentrations there was marked solubilization of the membrane fraction, and this interfered with the accuracy of the measurement. However, it was apparent that concentrations greater than 2 mM- Mg^{2+} are needed for the translocation process (results not shown).

Immunological characterization of the low-molecular-mass G-proteins

None of the major G-proteins in the cytosol migrated with the p29 related to oestrogen receptors. None of the other antisera tested (see the Materials and methods section) reacted significantly with the 18 kDa band.

DISCUSSION

This study reports on several low-molecular-mass G-proteins which are present in the ZR-75-1 mammary-tumour cell line. The original reason for doing this work was the analysis of GTP binding to the cytosolic oestrogen-receptor-related protein p29 (Coffer & King, 1988). This was not possible by this methodology, but it did prove that none of the soluble G-proteins detected here was p29. Furthermore, the question of hormonal regulation was not addressed here, as the cultures were all performed in the oestrogenic environment of Phenol Red plus unstripped serum. The picture presented may therefore not represent the basal condition. We did, however, identify other G-proteins with interesting properties. These proteins were detected after gel electrophoresis under denaturing conditions, and therefore do not represent the full complement of such proteins within the cell. They specifically bind GTP and GDP, but not other nucleotides, as shown previously for other G-proteins. The apparent K_d for GTP is approx. 20 nM, in good agreement with the value reported for the purified 20 and 24 kDa G-proteins from brain membranes (Kikuchi *et al.*, 1988; Yamamoto *et al.*, 1988). Toki *et al.* (1989) have shown that GDP is as potent as GTP[S] and GTP to block [α - 32 P]GTP binding to small G-proteins in rat liver Golgi fraction. One of the ZR-75-1 proteins,

Fig. 4. Effect of the amount of cytosolic protein in the incubation mixture on the GTP[S]-induced translocation of low-molecular-mass G-proteins

The results from the experiment described in Fig. 3 were calculated as the ratio between the density of the 18 kDa protein band obtained in the absence of GTP[S] to that obtained in its presence. Similar results were obtained in three additional experiments. Abbreviations: sn, supernatant; mem, membranes.

Fig. 5. Time course and temperature-dependence of guanine-nucleotide-induced translocation of 18 kDa G-protein from the soluble to the particulate fractions

The experimental design is essentially the same as described in Fig. 3. Membranes (50 μg of protein) were incubated with 5 μg of cytosolic protein with or without the nucleotides indicated (100 μM). After incubation at the indicated temperatures and time periods, the membranes were separated by centrifugation and the 18 kDa G-protein was quantified in the supernatant and particulate fractions, as described in the Materials and methods section. Results are means ± S.E.M. of four experiments.

with apparent molecular mass 18 kDa, is located predominantly (more than 90 %) in the soluble fraction after cell homogenization and membrane washing. However, some 18 kDa protein remains with the membrane fraction under our culture and homogenization conditions. The soluble nature of this protein is further supported by the finding that incubation of membranes even at 0 °C caused further solubilization of the 18 kDa protein bound to the particulate fraction, suggesting that the binding of

this protein to membranes is quite loose. This is in contrast with the 27–29 kDa complex of proteins, which are located primarily in the membranes and whose location is not affected by experimental manipulation. The 18 kDa protein is not the major G-protein found in ZR-75-1 cells by this methodology, and, although it represents more than 50 % of the soluble G-proteins, it is less than 20 % of the total G-proteins.

The most interesting finding in this study is that the binding of

Fig. 6. Dose-dependence of various GTP analogues on the translocation of the 18 kDa protein from the supernatant to the membrane fraction

The experimental design is essentially the same as described in the legend for Fig. 5 and in the Materials and methods section. The incubations include the various nucleotides in the indicated concentrations. Results are means $\pm$ S.E.M. of three experiments.

the 18 kDa protein to the particulate fraction is affected specifically by GTP and its stable analogues. Incubation of crude membranes with small volumes of the cytosolic fraction in the presence of GTP or its stable analogues caused quantitative translocation of this protein to the membrane fraction. The effect of the GTP analogues is reversibly temperature- and dose-dependent. This is a relatively slow process, reaching its maximum in 60 min, and requires the presence of Mg^{2+} .

GDP effects are complex. GDP[S] alone has no effect beyond that of the control in influencing 18 kDa-protein distribution (Fig. 5), but it may inhibit the effect of GTP. High concentrations of GTP are appreciably less effective than GTP[S] in promoting translocation (Fig. 6), which may indicate high GTPase activity. The GDP thus formed could contribute an inhibitory component under these conditions. It seems rather remarkable that physiological concentrations of GTP (~ 1 mM) in the presence of Mg^{2+} , but in the absence of added hormone, were able to mimic (partially) the effect of GTP[S] on 18 kDa-protein translocation, suggesting that a substantial part of the 18 kDa protein in the intact cell might be localized in the membrane.

There was a quantitative recovery of the various G-proteins during the translocation process, as well as in the washing experiments. This, together with their similar ligand-specificities, suggests, but does not prove, that the G-proteins in the two cell fractions are identical. The 18 kDa protein migrates between the soluble and the particulate fractions without significant change in its electrophoretic mobility in SDS/PAGE. This suggests that no proteolytic cleavage of the protein, or other covalent modification such as phosphorylation (Lapetina *et al.*, 1989), coincides with the translocation process, although small changes in the molecular mass would not be detectable by this analytic method. The nature of the interaction of the 18 kDa protein with the membrane fraction caused by GTP analogues is not clear, and needs further study. For example, information on the primary structure of this protein may suggest possible interaction with hydrophobic membrane proteins as with the heterotrimeric G-proteins, or the existence of *N*-terminal-linked myristic acid, as shown for other G-proteins (Buss *et al.*, 1987). An alternative possibility is that GTP[S] binding to p18 may trigger its association with a cytosolic protein such as the GTPase-activating protein ('GAP') (Adari *et al.*, 1988); this protein complex may associate with the membrane in a GTP-independent manner.

No significant effect of nucleotides could be detected on the location of other low-molecular-mass G-proteins, including the 27–29 kDa complex. However, it should be emphasized that these proteins are present mainly in the membrane fraction even

in the absence of GTP analogues, with the result that any translocation may be below the limits of detection.

We are not aware of other reports showing that GTP affects the translocation of G-proteins to the particulate fraction. There are several studies, however, which report that the α subunits of G_o , G_{i2} and G_s (detected immunologically) are solubilized from glioma membranes by stable GTP analogues, suggesting that the α subunit of these heterotrimeric G-proteins is not always a membrane protein (McArdle *et al.*, 1988; Milligan *et al.*, 1988; Milligan & Unson, 1989). Such 'programmable messenger' functions for subunits of G-proteins were suggested by Rodbell (1985). Another study (Audiger *et al.*, 1988) reported on similar effects of GTP[S] on the release of a 41 kDa G-protein from the endoplasmic reticulum of dog pancreas. Unfortunately, we were unable to detect these higher-molecular-mass proteins in our experimental system, presumably because they are more sensitive to the denaturing conditions employed in our assay.

Interestingly, Wang *et al.* (1988) reported that concanavalin A causes a rapid (in seconds) redistribution of part of the GTP-binding activity from the membrane to the cytosolic fraction in mouse thymocytes. Although not directly demonstrated, the study proposed that a low-molecular-mass fraction of G-proteins (23–28 kDa) may be involved in this process, which may be related to the GTP[S]-induced activation of cytosolic phospholipase C. Several low-molecular-mass G-proteins, in the range 18–29 kDa, were found in platelet cytosols and membranes by similar methodology to that used in our study. Interestingly, the most abundant cytosolic G-protein in platelets had a molecular mass around 18 kDa (Lapetina *et al.*, 1989). Although its modulation was not studied, the iloprost-induced phosphorylation of a 22 kDa G-protein was suggested as the mechanism driving its translocation from the particulate to the soluble fraction in the platelets.

The possible activation of cytosolic phospholipase C by GTP[S] in mammary (Deckmin *et al.*, 1986; Segal *et al.*, 1987) and other tissues stimulated Lapetina & Reep (1987) to look for cytosolic G-proteins in human platelets: 21, 27 and 29 kDa proteins were detected by using similar methods to those employed in the present study. Their activity was related indirectly again to enzyme activation. Baldassare *et al.* (1988) focused mainly on the 29 kDa protein as a possible activator of phospholipase C.

It remains to be ascertained whether the translocation process is able to occur within the intact cell, rather than under the conditions *in vitro* that we have employed in this study. The experiments described here offer no information on possible physiological roles of the 18 kDa G-protein, although the

specificity of the effects of stable GTP analogues on the translocation of this protein to the membrane suggests that such role may exist. The time course of the process, however, suggests that it is probably not related to short-term second-messenger-mediated functions.

Attempts were made by using immunoblotting techniques to relate our G-proteins to G_i - and *ras*-related proteins. With the limited number of antibodies available to us, no clear association was evident.

We thank Dr. C. Watts for the critical review of the manuscript, and Dr. A. Coffey for help in the initial stages of this work.

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Received 17 October 1989/29 May 1990; accepted 18 June 1990