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Identification of Low Molecular Mass GTP-Binding Proteins in Membranes of the Halotolerant Alga *Dunaliella salina*[1]

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ABSTRACT

A family of specific guanine nucleotide-binding proteins in *Dunaliella salina* was studied. Polypeptides of different subcellular fractions were separated by electrophoresis and transferred to nitrocellulose or Immobilon membranes. Incubation of the transfer blots with [^{35}S]GTPγS or [α-^{32}P]GTP showed no evidence for GTP-binding proteins in the chloroplast and cytosol fractions. However, two GTP-binding proteins with molecular masses of 28 and 30 kilodaltons were present in the plasma membrane and microsomal fractions. An additional 29 kilodalton GTP-binding protein was detected in the plasma membrane. The mitochondrial fraction contained significant amounts of only the 28 kilodalton GTP-binding protein. Binding of [^{32}P]GTP to the protein blots was completely prevented by 10 micromolar GTP or guanosine 5′-*O*-(2-thiodiphosphate) (added in 3 × 10^4-fold excess), whereas ATP or CTP had no effect on the binding. The 28 kilodalton GTP-binding protein was recognized by polyclonal antibodies to the *ras*-related YPT1 protein of yeast but not by the anti-*ras* Y13-259 monoclonal antibody. GTP-binding proteins present in the microsomal fraction could not be solubilized by incubation of microsomes with 1 molar NaCl or 0.2 molar Na_2CO_3, but some GTP-binding activity was solubilized when microsomes were treated with 6 molar urea. These results indicate that *D. salina* GTP-binding proteins are tightly associated with the membranes. The covalent attachment of fatty acids to these proteins was also investigated. Electrophoresis followed by fluorography of delipidated microsomal proteins extracted from [^{3}H]myristic acid-labeled cells showed an intense labeling of a 28 kilodalton protein. We conclude that *D. salina* contains proteins resembling the *ras*-related proteins found in animal cells and higher plants.

The regulation of animal cell metabolism is known to involve a variety of proteins that hydrolyze or bind GTP. Among these are the much-studied trimeric GTP-binding proteins that participate in signal transduction across the plasma membrane (18). Also widely distributed, although less well known with respect to function, is a group of low molecular mass (20–30 kD) proteins that includes several intensively studied *ras* protooncogene products (4). Evidence has been reported suggesting their participation in a number of cellular functions, including secretory processes and signal transduction (12, 16).

Membrane association of *ras* proteins, promoted by post-translational isoprenylation and subsequent palmitoylation, increases the biological activity of these proteins (14). The remarkably high rate of turnover of the palmitate moiety found in p21$^{N\text{-}ras}$ suggests an active cycle of acylation-deacylation that may be involved in its proposed function as a signal transducing protein (20).

In contrast with the voluminous literature on animal GTP-binding proteins, there have been few reports of low molecular mass GTP-binding proteins in plants. In zucchini hypocotyl microsomes, four GTP-binding proteins with molecular masses in the 23 to 28 kD range were identified on the basis of their capacity to bind [^{32}P]GTP on nitrocellulose blots (7), and blots of soybean hypocotyl microsomes and plasma membrane were found to contain [^{35}S]GTPγS-binding proteins with apparent molecular masses of 24 and 28 kD (30). *Arabidopsis thaliana* contains a gene believed to code for a 24 kD GTP-binding protein (21).

We report here the presence and subcellular localization of low molecular mass GTP-binding proteins in the halotolerant green alga *Dunaliella salina*. Data are also presented regarding the probable fatty-acylation of a *D. salina* GTP-binding protein. In this alga, several components of a PIP_2[2]-mediated transmembrane signaling pathway have recently been identified in our laboratory (8, 9), and we suspect that the low molecular mass GTP-binding proteins may participate in a complementary metabolic control system.

MATERIALS AND METHODS

Growth of *Dunaliella salina*

Cells of *Dunaliella salina* (UTEX 1644) were grown at 28°C under white light in 500 mL of a sterile synthetic medium as previously reported (17). Sodium chloride was added to the medium to yield a final salt concentration of 1.7 M. Cell density was measured using a Coulter Counter model ZB, and cultures were used when the cell density reached 10^6 cells/mL.

Subcellular Fractionation

Cells were harvested and disrupted as previously described (17). Typically, cultures totaling 1.5 × 10^9 cells were used. Cells were disrupted in a Parr bomb, and the suspension of

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[2] Abbreviations: PIP_2, phosphatidylinositol 4,5-bisphosphate; PVDF, polyvinylidene difluoride; GDPβS, guanosine 5′-*O*-(2-thiodiphosphate).

broken cells was centrifuged at 2639*g* for 3.5 min to pellet chloroplasts and cellular debris. The supernatant over the chloroplast pellet was centrifuged at 20,000*g* for 15 min to sediment the mitochondrial fraction. The supernatant from the above centrifugation was added to a dextran-PEG aqueous two-phase system to partition the plasma membrane away from the remaining organelles (24). After partitioning cellular membranes by centrifugation at 660*g* for 10 min, PEG- and dextran-rich fractions were centrifuged at 150,000*g* for 1 h, and the resulting pellets were washed free of residual polymers by resuspension in 11 mL of 100 mM Tris-HCl, pH 8.6, and 2 mM PMSF, followed by recentrifugation as above.

In other experiments, microsomal membranes and the cytosolic fraction were obtained after centrifuging the supernatant over the mitochondrial pellet at 150,000*g* for 1 h. All steps of cellular fractionation were carried out at 4°C, and the subcellular fractions obtained were resuspended in 100 mM Tris-HCl, pH 8.6, containing 5 mM benzamidine and 50 mM DTT, and stored at −70°C until use.

Rat brain membranes were isolated as described by Sternweis and Robishaw (29) and assayed for [^{32}P]GTP binding along with *D. salina* subcellular fractions. Proteins were quantified using the bicinchoninic acid assay (27).

SDS-PAGE and Blotting of Proteins

Aliquots of cytosol and different cellular membranes containing 100 μg of protein were subjected to SDS-PAGE in a 7.5 to 15% gradient gel using the buffer system of Laemmli (15). Samples were prepared for electrophoresis by adding 0.67 volumes of 30% sucrose, 5% lithium dodecyl sulfate, and 0.1% bromphenol blue. Samples were then boiled for 2 min, and nonsolubilized material was pelleted by centrifugation. Electrophoresis was performed overnight at 4°C and at a constant current of 16 mA.

After SDS-PAGE, proteins were electrophoretically transferred to Immobilon membranes (filter type PVDF, pore size 0.45 μm, Millipore, Bedford, MA) or, in experiments using [^{35}S]GTPγS, nitrocellulose membranes (pore size 0.45 μm, Hoefer Scientific, San Francisco, CA) in 25 mM Tris and 192 mM glycine, pH 8.3, containing 5% methanol at 12 V and 4°C for 1.5 h.

Molecular mass standards (Bio-Rad, Richmond, CA) were also loaded onto the polyacrylamide gels, blotted along with the samples, and stained with Coomassie blue.

GTP-Binding Assays

The binding assays were modified from Lapetina and Reep (16). The transfer blots were incubated in 10 mL of a binding buffer containing 50 mM Tris HCl, pH 7.5, 200 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.3% Tween 20 at room temperature for 1 h. When the specificity of [^{32}P]GTP binding was studied, 10 μM concentrations of competing nucleotide phosphates (ATP, CTP, GTP, and GDPβS) were also added to the binding buffer to give a 3×10^4-fold excess of nonradioactive nucleotide phosphate. The blots were then incubated for 90 min at room temperature with 10 μCi of [α-^{32}P] GTP (specific activity 3000 Ci/mmol, DuPont-New England Nuclear, Boston, MA) in 10 mL of binding buffer either with or without one of the competitors. The blots were then rinsed four times with binding buffer for 2 h with shaking at room temperature. [^{32}P]GTP binding was visualized by autoradiography at −70°C using Kodak X-AR film and DuPont Cronex intensifying screens.

Extraction of Proteins Loosely Bound to Membranes

The microsomal pellet obtained from 5×10^8 cells was resuspended in 350 μL of 100 mM Tris HCl, pH 8.6, containing 4 mM PMSF and 10 mM benzamidine or, in the case of Na_2CO_3 extraction, in 350 μL of 4 mM PMSF and 10 mM benzamidine. Then 350 μL of 12 M urea, 2 M NaCl, or 0.4 M Na_2CO_3 were added to each tube, and the samples were kept on ice for 30 min with intermittent shaking. Nonextractable material was pelleted by centrifugation at 150,000*g* for 60 min at 4°C. The supernatant was removed by aspiration, and one volume of 20% TCA was added to it. After keeping the preparation at −20°C for 1 h, the proteins were sedimented at 15,000*g* for 15 min at 4°C.

Both solubilized and insoluble microsomal protein preparations were suspended in SDS-PAGE sample buffer and boiled as described above. Electrophoresis was carried out in a 7.5 to 15% acrylamide gradient gel as indicated above. The gels were blotted onto PVDF membranes and assayed for GTP-binding as outlined above.

In Vivo Labeling and Electrophoretic Analysis of Microsomal Fatty-Acylated Proteins

Cultures containing a total of approximately 2×10^9 cells were subjected to centrifugation for 7.5 min, and pelleted cells were resuspended in 500 mL of fresh medium. After 30 min of incubation, cells were labeled by adding 0.5 mCi of [9,10-^{3}H]myristic acid (specific activity 39.3 Ci/mmol, DuPont-New England Nuclear) in 50 μL ethanol. *D. salina* cultures were incubated in the presence of the radioisotope for 4 h, and then the cells were harvested and fractionated as indicated above.

The presence of fatty-acylated proteins was sought in microsomal membranes. Proteins from microsomal pellets were extracted and delipidated as described by Stephenson *et al.* (28). The delipidated protein samples were solubilized in electrophoresis sample buffer, and aliquots containing 100 μg of protein were subjected to SDS-PAGE under the conditions described above. After electrophoresis, gels were prepared for fluorography using Resolution (EM Corp., Chesnut Hill, MA) and were exposed to hypersensitized Kodak X-Omat x-ray film for up to 14 d at −70°C.

Immunoprecipitation and Immunoblotting of Microsomal Proteins with the Y13-259 Anti-*ras* Antibody

D. salina cultures containing 0.8×10^6 cells/mL were transferred to a sulfur-free medium for 15 h. Cells were then pelleted by centrifugation, resuspended in 10 mL of the sulfur-free medium, and labeled for 2 h using 500 μCi of $H_2{}^{35}SO_4$ (specific activity 43 Ci/mg S, ICN Radiochemicals, Irvine, CA). Cells were harvested and fractionated as indicated above. The microsomal pellet was resuspended in 1.5 mL of lysing

buffer (10 mM sodium phosphate, pH 7.4, 0.1 M NaCl, 1% [v/v] Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 0.1% aprotinin [Sigma], 1 mM PMSF, and 0.1% sodium azide). After 10 min on ice, the lysate was centrifuged at 15,000*g* for 15 min and the supernatant incubated overnight at 4°C with 10 μL normal rat serum (Sigma) or with an excess of Y13-259 *ras* rat monoclonal antibody. Immune complexes were precipitated with protein A-Sepharose (Sigma), washed four times with lysing buffer lacking PMSF and aprotinin, and then dissolved in 25 μL of electrophoresis sample buffer. After removing protein A-Sepharose by centrifugation, immunoprecipitates were analyzed by electrophoresis and autoradiography under the conditions previously described.

For immunoblotting with the Y13-259 antibody, *D. salina* microsomal membranes (100 μg of protein) were subjected to SDS-PAGE and western blot preparation as described before. Transfer blots were incubated in a Tris-saline buffer (50 mM Tris-HCl, pH 8.0, 80 mM NaCl, and 0.02% sodium azide) containing 0.05% Tween 20 and 2% BSA as blocking agents. For detection of the antigens, blots were incubated with the Y13-259 antibody (dilution 1:500) for 2 h at room temperature. After two consecutive washes with the Tris-saline buffer, blots were incubated with a second antibody (anti-rat immunoglobulin G alkaline phosphatase conjugate [Sigma], dilution 1:1000) for 2 h at room temperature. Chromogenic substrates (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium, Sigma) were used to visualize the immunolocalized alkaline phosphatase.

Immunoblotting with a polyclonal antibody to the yeast YPT1 protein was generously performed by Dr. Nava Segev and Gregg Jedd of the University of Chicago according to published procedures (26).

RESULTS

Binding of [^{35}S]GTPγS and [α-^{32}P]GTP to *D. salina* Proteins

D. salina cells were fractionated, and proteins of the major fractions were electrophoresed, blotted onto nitrocellulose membranes, and incubated with [^{35}S]GTPγS. A strong band was detected in the 28 kD region of autoradiograms made with the microsomal blots (Fig. 1B). The presence of [^{35}S]GTPγS binding in other fractions was not certain due to a low level of nonspecific binding to proteins.

Similar blots were incubated with [α-^{32}P]GTP (Fig. 1C). Autoradiograms of these blots showed the presence of contaminating radioactivity randomly bound to the nitrocellulose, but the background spots were usually small and only rarely prevented the detection of radioactivity specifically associated with proteins. Under the conditions employed, [α-^{32}P]GTP binding was considerably more sensitive in detecting GTP-binding proteins. Two proteins, of molecular mass 28 and 30 kD, were detected by autoradiography in the microsomal fraction, and the 28 kD component was also present in the mitochondrial fraction. No [^{32}P]GTP binding was detected on blots of chloroplast or cytosolic proteins (data not shown). In cases in which the mitochondrial supernatant was resolved by the aqueous two-phase system into a plasma membrane fraction and a "bottom phase" fraction (mainly endoplasmic reticulum), the plasma membrane was found to contain the 28 and 30 kD GTP-binding proteins. In some experiments, in which the protein loading was greater, an additional 29 kD plasma membrane protein was found to bind [^{32}P]GTP (Fig. 2).

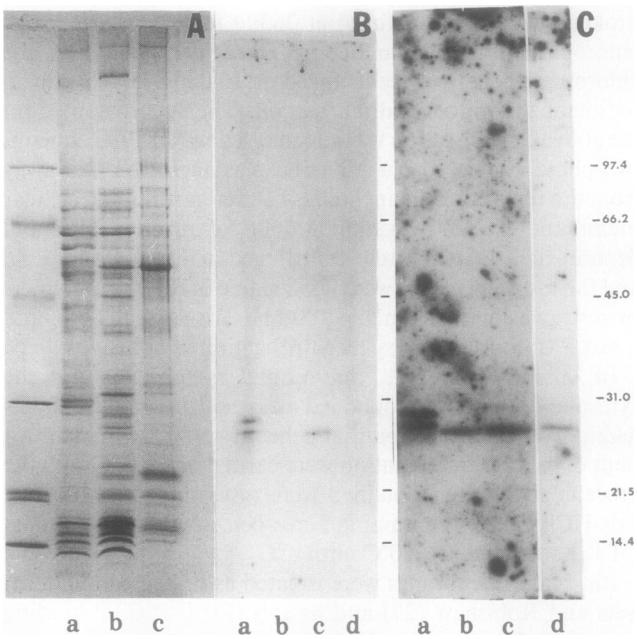

Figure 1. A, Polypeptide profiles of different *D. salina* subcellular fractions after SDS-PAGE and Coomassie blue staining. Lanes: a, mitochondria; b, microsomes; c, plasma membrane. Molecular masses of the proteins used as standards were 97.4, 66.2, 45.0, 31.0, 21.5, and 14.4 kD. B, Detection of GTP-binding proteins in *D. salina* subcellular fractions by [^{35}S]GTPγS binding. Lanes: b, mitochondria; c, microsomes; d, plasma membranes. Equal amounts (100 μg of protein) of individual samples were loaded onto the gels. Lane a shows the binding of [^{35}S]GTPγS to rat brain membrane proteins (20 μg) for comparison. C, Detection of GTP-binding proteins in some subcellular fractions of *D. salina* by [α-^{32}P]GTP binding. Lanes: b, mitochondria; c, microsomes; d, plasma membrane. Equal amounts (100 μg of protein) of individual samples were loaded onto the gels. Lane a in blots B and C shows the binding of [α-^{32}P]GTP to rat brain membrane proteins (20 μg).

When equivalent amounts of proteins were applied to the gels, the microsomal membranes showed a more intense labeling than did the other cellular fractions (Fig. 1). [^{32}P]GTP binding to protein from membranes of rat and *D. salina* fall in the same narrow range of molecular mass, but do not exactly coincide (Figs. 1 and 2)

Specificity of the GTP Binding

The specificity of the nucleotide phosphate binding was studied in the active cell fractions. Individual blots of each fraction were preincubated with 10 μM ATP, CTP, GTP, or GDPβS or without an addition before the usual incubation with [^{32}P]GTP. Autoradiography showed that ATP had no effect on the intensity of [^{32}P]GTP binding to the plasma membrane; CTP had a slight competitive effect against binding to the 30 kD band; and GTP or GDPβS completely

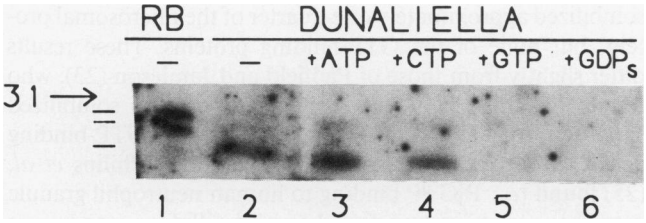

Figure 2. Nucleotide specificity of GTP-binding proteins. Transfer blots of *D. salina* plasma membrane were preincubated in the absence or presence of nucleotide phosphates, each at a concentration of 10 μM, and then incubated with [α-^{32}P]GTP. Equal amounts of plasma membrane proteins (100 μg) were loaded in each lane of the gel. Lanes 2 through 6 represent control (no nucleotide), and proteins incubated with ATP, CTP, GTP, and GDPβS, respectively. Lane 1 shows binding of [α-^{32}P]GTP to rat brain membrane proteins (5 μg).

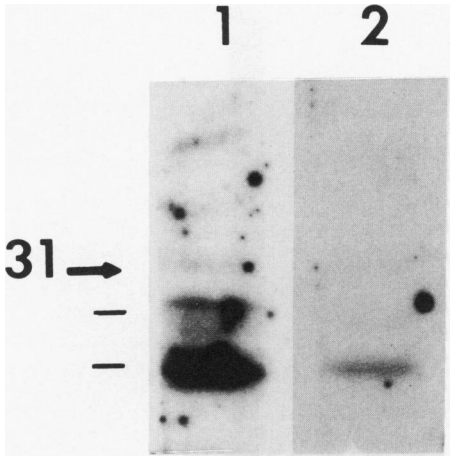

Figure 3. Autoradiogram of 100 μg microsomal proteins resolved by SDS-PAGE, blotted onto a PVDF membrane, and (1) incubated with 10 μCi [α-^{32}P]GTP, or (2) exposed to affinity-purified YPT1 antibody followed by [^{125}I]protein A.

prevented the binding of the radioisotope (Fig. 2). The same results were observed in autoradiograms of mitochondrial and microsomal fractions (data not shown).

Immunological Comparisons

The similarity between the *D. salina* low molecular mass GTP-binding proteins and *ras* proteins identified in yeast and animal cells was investigated in microsomal membranes by immunoprecipitation and immunoblotting techniques using the monoclonal antibody Y13–259. Under our experimental conditions, no *D. salina* protein with molecular mass in the range of the GTP-binding proteins was recognized by the antibody to *ras* proteins (although a large band representing the higher molecular mass free antibody showed some nonspecific binding). Blots of rat brain proteins did give several positive bands in the 20 to 30 kD range.

Immunoblotting was also carried out using an affinity-purified polyclonal antibody directed against the YPT1 protein of yeast (26). That 23 kD GTP-binding protein is associated with yeast Golgi membranes and appears to be involved in secretion. Binding to the 28 kD protein was clearly detected (Fig. 3).

Extractability of the GTP-Binding Proteins from Membranes

Low molecular mass GTP-binding proteins are typically very tightly associated with membranes (25). To investigate this property of the *D. salina* GTP-binding proteins, microsomal membranes were extracted with 6 M urea, 1 M NaCl, or 0.2 M Na_2CO_3. These treatments solubilized 65, 18, and 26%, respectively, of the total proteins. [^{32}P]GTP-binding to blots of electrophoretic gels on which proteins of the solubilized and the residual material had been resolved (Fig. 4) showed that substantial amounts of GTP-binding proteins were extracted from the microsomes by 6 M urea. However, no trace of GTP-binding proteins was detected in the 1 M NaCl or 0.2 M Na_2CO_3 extracts. A companion gel revealed, after silver staining, that the extracting solutions did, as expected, remove certain proteins more effectively than others (data not shown). Only in the case of urea extraction were the protein banding patterns of the soluble and insoluble fractions relatively similar.

Fatty Acylation of the GTP-Binding Proteins

Earlier studies of fatty-acylated *D. salina* proteins in our laboratory produced indications of a fatty-acylated protein in the 20 to 30 kD range (28). These data and the finding of a number of fatty-acylated low molecular mass GTP-binding proteins in animal cells suggest the possible fatty acylation of *D. salina* small GTP-binding proteins. This possibility was investigated by incubating growing cultures of *D. salina* with [^{3}H]myristic acid, which is effectively taken up and elongated to C_{16} and C_{18} fatty acids (5).

The delipidated microsomal proteins extracted from labeled cells were separated by electrophoresis. Fluorography of the electrophoretic gels consistently revealed an intense labeling in a 28 kD protein (Fig. 5). The electrophoretic mobility of this fatty-acylated protein is the same as found for the major microsomal GTP-binding protein. Several additional polypeptide bands were also labeled, in keeping with the observations in animal cells that many proteins of diverse function are modified by fatty acylation (14).

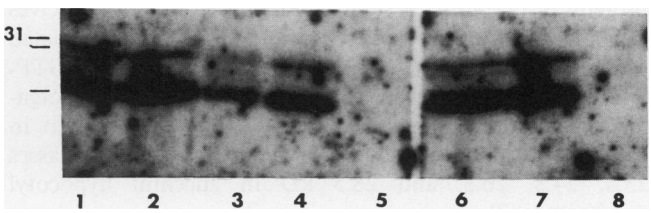

Figure 4. Autoradiogram of fractions obtained by various treatments to partially solubilize *D. salina* microsomal proteins (see text for details). 1, Nonextracted control; 2, urea insoluble; 3, urea soluble; 4, NaCl insoluble; 5, NaCl soluble; 6, nonextracted control; 7, Na_2CO_3 insoluble; 8, Na_2CO_3 soluble. Each lane contained 80 μg protein.

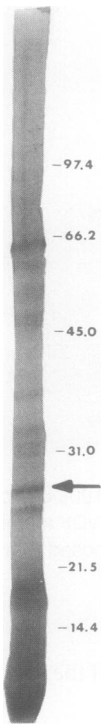

Figure 5. Fatty-acylated microsomal proteins in *D. salina*. Fluorogram of an electrophoretic gel after 4 h of *in vivo* labeling with [^{3}H] myristic acid.

DISCUSSION

We have identified three low molecular mass GTP-binding proteins in *D. salina*. The proteins showed some specificity in their subcellular distribution. Thus, the 29 kD protein could be detected only in the plasma membrane, and the 28 and 30 kD proteins were present in plasma membrane, mitochondria, and microsomes. Membrane localization of low molecular mass GTP-binding proteins in the plasmalemma, endoplasmic reticulum, and Golgi apparatus of animal and yeast cells has been previously reported (12). Cytoplasmic forms of GTP-binding proteins have been also frequently observed in animal cells (12, 16). However, we found no appreciable quantities of these proteins in the soluble cytoplasm.

At 28, 29, and 30 kD, the GTP-binding proteins of *D. salina* match closely in size the two major GTP-binding proteins of rat brain (3) (Fig. 1). *Ras* proteins are themselves generally in the 21 kD mass range, and most of the *ras*-related proteins do not exceed 24 kD in size. However, larger GTP-binding proteins have been identified in a number of organisms, including a major 27 kD GTP-binding protein in platelets (3), and GTP-binding proteins of estimated masses 23.4, 24.8, 26.8, and 28.5 kD in zucchini hypocotyl microsomes (7).

The small GTP-binding proteins are typically associated with membranes (2, 6), although some cytosolic forms have been reported. GTP-binding components of *D. salina* were found only in particulate fractions. Extraction of *D. salina* microsomes with 1 M NaCl or with 0.2 M Na_2CO_3, pH 11.0, solubilized approximately one-quarter of the microsomal protein, but none of the GTP-binding proteins. These results differ slightly from those of Padfield and Jamieson (23), who found that these same extracting solutions each solubilized small (approximately 5–10%) amounts of the GTP-binding activity from rat pancreas zymogen granules. Philips *et al.* (25) found [α-^{32}P]GTP binding to human neutrophil granule membrane proteins transferred to nitrocellulose membranes and showed that all three GTP-binding proteins detected by this procedure were resistant to alkaline extraction. In the report of Philips *et al.* (25), the GTP-binding proteins were solubilized by acid (pH 3.2) extraction, but those conditions may have favored cleavage of an acid-labile fatty acyl bond.

In our hands, some, but not all, of the GTP-binding activity was extracted from the microsomal membranes by 6 M urea. Because of urea's well known tendency to enhance the solubility of nonpolar proteins in water and destabilize phospholipid bilayers, it is likely that large-scale membrane disruption accompanied the GTP-binding protein solubilization. Our general conclusions from the extraction studies are that the *D. salina* small GTP-binding proteins are tightly associated with membranes.

Fatty acylation strengthens the membrane association of GTP-binding proteins as well as other protein classes so modified (14). In our study, covalently bound fatty acid was clearly detected at the 28 kD position of microsomal protein gels, coinciding with the major GTP-binding protein. On the strength of SDS-PAGE analysis alone, it is impossible to be certain that the 28 kD protein linked to fatty acid is the same molecule that binds GTP. Efforts to confirm the identity by two-dimensional gel electrophoresis failed because of the hydrophobic nature of the membrane proteins. Nevertheless, the prevalence of fatty acylation reported on low molecular mass GTP-binding proteins of animal cells lends support to our suspicion that the two features are shared by the same protein in *D. salina* as well.

The failure of *D. salina* proteins to be immunoprecipitated by the Y13-259 anti-*ras* antibody or to cross-react with it on blots was not totally unexpected. The region of the *ras* proteins that binds Y13-259, namely, residues 63 to 73 (11), shows considerable variation in *ras*-related proteins (13). Monoclonal antibodies capable of recognizing all known *ras* p21 proteins did not cross-react with the 27 kD GTP-binding protein of rat brain (3).

On the other hand, the 28 kD *D. salina* protein showed a positive reaction with the polyclonal antibody to the 23 kD *ras*-related YPT1 protein of yeast. This yeast GTP-binding protein and its equivalent, the ubiquitous Rab1 protein of mammals (13), are both believed to mediate membrane fusion events during secretion. One or more of the GTP-binding proteins described here in *D. salina* may participate in the massive fusion of vesicles with the plasma membrane after hypoosmotic shock (19).

The functions of low molecular mass GTP-binding proteins are not clearly understood. Apart from their above-mentioned involvement with secretion, a number of studies have implicated these proteins in cellular signal transduction, particularly by the PIP_2-protein kinase C-mediated pathway (13), but the findings have sometimes been contradictory. The fact that certain low molecular mass GTP-binding proteins are phos-

phorylated during mitosis (1) and in conjunction with inhibition of phospholipase C (22) further suggests that they have an important regulatory function. Because *D. salina* is the most thoroughly characterized algal species with respect to PIP_2-mediated transmembrane signaling (10), it promises to be an excellent model system for use in exploring the properties and regulatory functions of low molecular mass GTP-binding proteins in algae.

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LITERATURE CITED

1. **Bailly E, McCaffrey M, Touchot N, Zahraoui A, Goud B, Bornens M** (1991) Phosphorylation of two small GTP-binding proteins of the Rab family by $p34^{cdc2}$. Nature **350:** 715–718
2. **Balch WE** (1990) Small GTP-binding proteins in vesicular transport. Trends Biochem Sci **15:** 473–477
3. **Bhullar RP, Haslam RJ** (1987) Detection of 23–27 kDa GTP-binding proteins in platelets and other cells. Biochem J **245:** 617–620
4. **Burgoyne RD** (1989) Small GTP-binding proteins. Trends Biochem Sci **14:** 394–396
5. **Cho SH, Thompson GA Jr** (1989) Galactolipids of thylakoid pigment protein complexes separated electrophoretically from thylakoids of *Dunaliella salina* labeled with radioactive fatty acids. Plant Physiol **90:** 610–616
6. **Downward J** (1990) The *ras* superfamily of small GTP-binding proteins. Trends Biochem Sci **15:** 469–472
7. **Drøbak BK, Allam EF, Comerford JG, Roberts K, Dawson AP** (1988) Presence of guanine nucleotide-binding proteins in a plant hypocotyl microsomal fraction. Biochem Biophys Res Commun **150:** 899–903
8. **Einspahr KJ, Peeler TC, Thompson GA Jr** (1988) Rapid changes in polyphosphoinositide metabolism associated with the response of *Dunaliella salina* to hypoosmotic shock. J Biol Chem **263:** 5775–5779
9. **Einspahr KJ, Peeler TC, Thompson GA Jr** (1989) Phosphatidylinositol 4,5-bisphosphate phospholipase C and phosphomonoesterase in *Dunaliella salina* membranes. Plant Physiol **90:** 1115–1120
10. **Einspahr KJ, Thompson GA Jr** (1990) Transmembrane signaling via phosphatidylinositol 4,5-bisphosphate hydrolysis in plants. Plant Physiol **93:** 361–366
11. **Gibbs JB, Marshall MS** (1989) The *ras* oncogene—an important regulatory element in lower eukaryotic organisms. Microbiol Rev **53:** 171–185
12. **Hall A** (1990) The cellular functions of small GTP-binding proteins. Science **249:** 635–640
13. **Hall A** (1990) *ras* and *ras*-related guanine nucleotide binding proteins. *In* MD Houslay, G Milligan, eds, Molecular Pharmacology of Cell Regulation, Vol 1. John Wiley & Sons, Chichester, pp 173–197
14. **James G, Olson EN** (1990) Fatty acylated proteins as components of intracellular signaling pathways. Biochemistry **29:** 2623–2634
15. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
16. **Lapetina EG, Reep BR** (1987) Specific binding of [α-^{32}P]GTP to cytosolic and membrane-bound proteins of human platelets correlates with the activation of phospholipase C. Proc Natl Acad Sci USA **84:** 2261–2265
17. **Lynch DV, Thompson GA Jr** (1982) Low temperature-induced alterations in the chloroplast and microsomal membranes of *Dunaliella salina*. Plant Physiol **69:** 1369–1375
18. **Lochrie MA, Simon MI** (1988) G protein multiplicity in eukaryotic signal transduction systems. Biochemistry **27:** 4957–4965
19. **Maeda M, Thompson GA Jr** (1986) On the mechanism of rapid plasma membrane and chloroplast envelope expansion in *Dunaliella salina* exposed to hypoosmotic shock. J Cell Biol **102:** 289–297
20. **Magee AI, Gutierrez L, McKay IA, Marshall CJ, Hall A** (1987) Dynamic fatty acylation of $p21^{N\text{-}ras}$. EMBO J **6:** 3353–3357
21. **Matsui M, Sasamoto S, Kunieda T, Nomura N, Ishizaki R** (1989) Cloning of *ara,* a putative *Arabidopsis thaliana* gene homologous to the *ras*-related gene family. Gene **76:** 313–319
22. **McAtee P, Dawson G** (1990) Phospholipase C activity in NCB-20 cells is inhibited by protein kinase A-mediated phosphorylation of low molecular mass GTP-binding proteins. J Biol Chem **265:** 6788–6793
23. **Padfield PJ, Jamieson JD** (1991) Low molecular weight GTP-binding proteins associated with zymogen granule membranes from rat pancreas. Biochem Biophys Res Commun **174:** 600–605
24. **Peeler TC, Stephenson, MB, Einspahr KJ, Thompson GA Jr** (1989) Lipid characterization of an enriched plasma membrane fraction of *Dunaliella salina* grown in media of varying salinity. Plant Physiol **89:** 970–976
25. **Philips MR, Abramson SB, Kolasinski SL, Haines KA, Weissmann G, Rosenfeld MG** (1991) Low molecular weight GTP-binding proteins in human neutrophil granule membranes. J Biol Chem **266:** 1289–1298
26. **Segev N, Mulholland J, Botstein D** (1988) The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. Cell **52:** 915–924
27. **Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC** (1985) Measurement of protein using bicinchoninic acid. Anal Biochem **150:** 76–85
28. **Stephenson MB, Ryals PE, Thompson GA Jr** (1989) Fatty acid acylated proteins of the halotolerant alga *Dunaliella salina*. Plant Physiol **90:** 549–552
29. **Sternweis PC, Robishaw JD** (1984) Isolation of two proteins with high affinity for guanine nucleotides from membranes of bovine brain. J Biol Chem **259:** 13806–13813
30. **Zbell B, Paulik M, Morre DJ** (1990) Comparison of [^{35}S]GTPγS binding to plasma membranes and endomembranes prepared from soybean and rat. Protoplasma **154:** 74–79