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Identification and localization of low-molecular-mass GTP-binding proteins associated with synaptic vesicles and other membranes

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GTP-binding proteins were studied in synaptic vesicles prepared from bovine brain by differential centrifugation and separated further from plasma membranes using gel permeation chromatography. Following separation by SDS-PAGE of proteins from the different fractions, and transfer to nitrocellulose sheets, the presence and localization of low-molecular-mass GTP-binding proteins were assessed by [α-^{32}P]GTP binding. The vesicle-membrane fraction (SV) was enriched in synaptophysin (p38, a synaptic vesicle marker) and contained low-molecular-mass GTP-binding proteins; these consisted of a major 27 kDa protein and minor components (M_r 26 and 24 kDa) which were trypsin-sensitive and immunologically distinguishable from *ras* p21 protein. GTP-binding proteins of low molecular mass, but displaying less sensitivity to trypsin, were also found in the plasma membrane fraction (PM; enriched in Na^+/K^+-ATPase). In addition, the PM fraction contained GTP-binding proteins with higher M_r ($G_i\alpha$ and $G_0\alpha$), together with another GTP-binding protein, *ras* p21. Putative function(s) of these GTP-binding proteins with low mass are discussed.

Introduction

Synaptic vesicles are storage organelles for neurotransmitters in neuronal cells [1], on stimulation they allow Ca^{2+}-dependent release of transmitters upon interaction with the plasma membrane [1,2] Recent biochemical analysis has detected the presence of several proteins expressed specifically on synaptic vesicles, such as synaptophysin (p38) [1,3,4] However, little is known of the mechanism involved in the exocytotic release of transmitter from synaptic vesicles

Involvement of GTP-binding proteins, named G_e, in exocytosis was suggested from experiments on permeabilized cells using a nonhydrolyzable analogue of GTP [5] It has not been clarified so far whether G_e serves as a substrate of pertussis or cholera toxins which modify the well-known GTP-binding proteins (e g , $G_1\alpha$, $G_o\alpha$, and $G_s\alpha$ with an M_r ranging from 39 to 52 kDa) involved in a variety of cellular signal transduction mechanisms [6,7] On the other hand, recent application of [α-^{32}P]GTP binding assay to nitrocellulose blots of proteins separated by SDS-PAGE revealed the existence in several tissues of a novel class of GTP-binding proteins of low molecular mass (20–29 kDa) and whose function has not been identified [8] As some of these proteins may be involved in the exocytotic process, they might be associated with secretory organelles In the present paper, such GTP-binding proteins were demonstrated in synaptic vesicles purified from bovine brain and their properties examined

Experimental Procedures

Materials

[α-^{32}P]GTP (3000 Ci/mmol), [γ-^{35}S]guanosine 5′-[γ-thio]triphosphate (GTPγS) (1350 Ci/mmol) and monoclonal anti-*ras*-p21 antibody (RAS11, NEI-704) were obtained from Du Pont (Stevenage, U K) Monoclonal anti-synaptophysin (SY38) was obtained from Boehringer Mannheim (Lews, U K) Rabbit antisera against synthetic peptides of carboxy terminals of $G_0\alpha$ subunit (OC1) and $G_{i1}\alpha$ subunit (SG1, this antiserum may recognize also $G_{i2}\alpha$ subunit) [9] were generous gifts from Dr G Milligan (University of Glasgow) Second

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Abbreviations ELISA, enzyme-linked immunosorbent assay GTPγS, guanosine 5′-[γ-thio]triphosphate, SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis, SV, synaptic vesicles, PM, plasma membrane

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antibodies and streptavidin–peroxidase complex were purchased from Amersham International (Amersham, U K)

Preparation of synaptic vesicles

These were isolated from bovine brain according to the method of Hell et al [10] with slight modifications Freshly frozen cerebral cortex (120 g) was crushed and pulverized under liquid N_2 The brain powder was thawed and homogenized at 4° C in 720 ml of buffer containing 0 3 M sucrose, 0 3 mM phenylmethanesulphonyl fluoride and 10 mM Tris-HCl (pH 7 4) and centrifuged at 27 000 × *g* for 10 min The supernatant was centrifuged at 120 000 × *g* for 40 min, an aliquot of the supernatant (50 ml) was layered onto 15 ml of 0 6 M sucrose, 10 mM Hepes-NaOH (pH 7 4) and centrifuged at 235 000 × *g* for 150 min The pellet (P3) was resuspended in 0 3 M sucrose, 10 mM Hepes-NaOH (pH 7 4) and cleared by centrifugation at 27 000 × *g* for 10 min The sample was loaded onto Sephacryl S-1000 column (2 8 × 90 cm) equilibrated with 0 3 M glycine, 10 mM Hepes-NaOH (pH 7 4) and eluted at a flow rate of 0 5 ml/min The fractions enriched in Na^+/K^+-ATPase activity (PM) or p38 (SV) were pooled separately and centrifuged at 235 000 × *g* for 120 min, the two pellets were resuspended in 0 3 M sucrose, 10 mM Hepes-NaOH (pH 7 4) (PM and SV) Protein concentration was determined according to the method of Bradford [11]

Enzyme activities

For measurement of Na^+/K^+-ATPase activity (EC 3 6 1 37), 10 µl of sample was incubated in 140 mM NaCl, 14 mM KCl, 5 mM $MgCl_2$, 1 mM ATP and 50 mM Tris-HCl (pH 7 5) at 30° C for 30 min in a total volume of 0 5 ml The amount of P_i liberated was quantified as described previously [12], the fraction sensitive to 2 mM ouabain was taken as Na^+/K^+-ATPase activity NADPH–cytochrome *c* reductase activity (EC 1 6 2 4) was measured, according to the method of Sottocasa et al [13]

Synaptophysin detection

Synaptophysin was quantified by an enzyme-linked immunosorbent assay (ELISA) Samples (50 µl) were adsorbed to an assay plate (Falcon) for 1 h at 37° C After blocking with 1% (w/v) bovine serum albumin, the plate was incubated sequentially with antibody SY38 (1 µg/ml, 1 h) and horseradish peroxidase coupled to goat anti-mouse IgG (1 500 diluted, 30 min), with three consecutive washings after each incubation Peroxidase reaction was initiated by the addition of 4 mM *o*-phenylenediamine and 0 01% (v/v) H_2O_2 in 50 mM Na_2HPO_4/20 mM citric acid buffer (pH 5 0) and terminated by adding 0 05 M H_2SO_4, the absorbance at 492 nm was determined

Electrophoresis and immunoblotting

SDS-PAGE was performed according to the method of Laemmli [14] Blot transfer on nitrocellulose paper was performed using a semi-dry electroblotter (Sartorius, Gottingen) followed by incubation with bovine serum albumin (10 mg/ml, 1 h) to block nonspecific binding For detection of antigens, blots were incubated with primary antibodies (anti-synaptophysin SY38, 0 1 µg/ml, anti-*ras*-p21, 1 1000 dilution, anti-$G_o\alpha$ subunit OC1, 1 1000 dilution, anti-$G_i\alpha$ subunit SG1, 1 100 dilution) for 1 h at room temperature After three consecutive washes, blots were incubated with a second antibody (biotinylated anti-mouse or -rabbit IgG, 1 500 dilution) for 1 h and subsequently with streptavidin–peroxidase complex (1 400 dilution) for 30 min The color reaction was developed with 0 02% H_2O_2 and 1 3 mM diaminobenzidine

Assay of [α-^{32}P]GTP binding

Binding of [α-^{32}P]GTP to nitrocellulose blots was performed essentially as described by Bhullar and Haslam [8] Nitrocellulose blots treated with bovine serum albumin were incubated in a buffer containing 0 3% Tween 30, 50 µM $MgCl_2$, 50 mM Tris-HCl (pH 7 5) and 1 µCi/ml [α-^{32}P]GTP (1 nM) for 1 h at room temperature, other modifications of the incubation are indicated in legends to the figures All the blots were washed, air-dried and exposed to Fuji X-ray film

Measurement of [^{35}S]GTPγS binding

This was determined essentially by the method of Northup et al [15] The binding mixture contained 1 µM [^{35}S]GTPγS (5 10^5 cpm), 1 mM EDTA, 5 mM $MgCl_2$, 250 mM NaCl, 250 mM $(NH_4)_2SO_4$, 0 1% Lubrol PX and 20 mM Tris-HCl (pH 8 0) in a total volume of 100 µl After addition of a sample (10 µl), the mixture was incubated for 1 h at room temperature, 5 ml of the washing buffer containing 5 mM $MgCl_2$, 100 mM NaCl and 20 mM Tris-HCl (pH 8 0) was added The solution was poured onto a nitrocellulose filter (diameter, 25 mm, pore size, 0 45 µm) and rinsed three times with 3 ml of the washing buffer before counting in a scintillation spectrometer

Results

Purity of synaptic vesicle membrane

Crude synaptic vesicle fractions (P3) were prepared directly from the homogenate of freeze-thawed bovine brain by differential centrifugation according to a rapid and efficient method described by Hell et al [10], this avoids the need of first preparing synaptosomes and the subsequent lysis Vesicles were purified further by gel permeation chromatography using Sephacryl S-1000 column (Fig 1) Plasma membranes, the major contaminant in the P3 fraction, were separated from synaptic

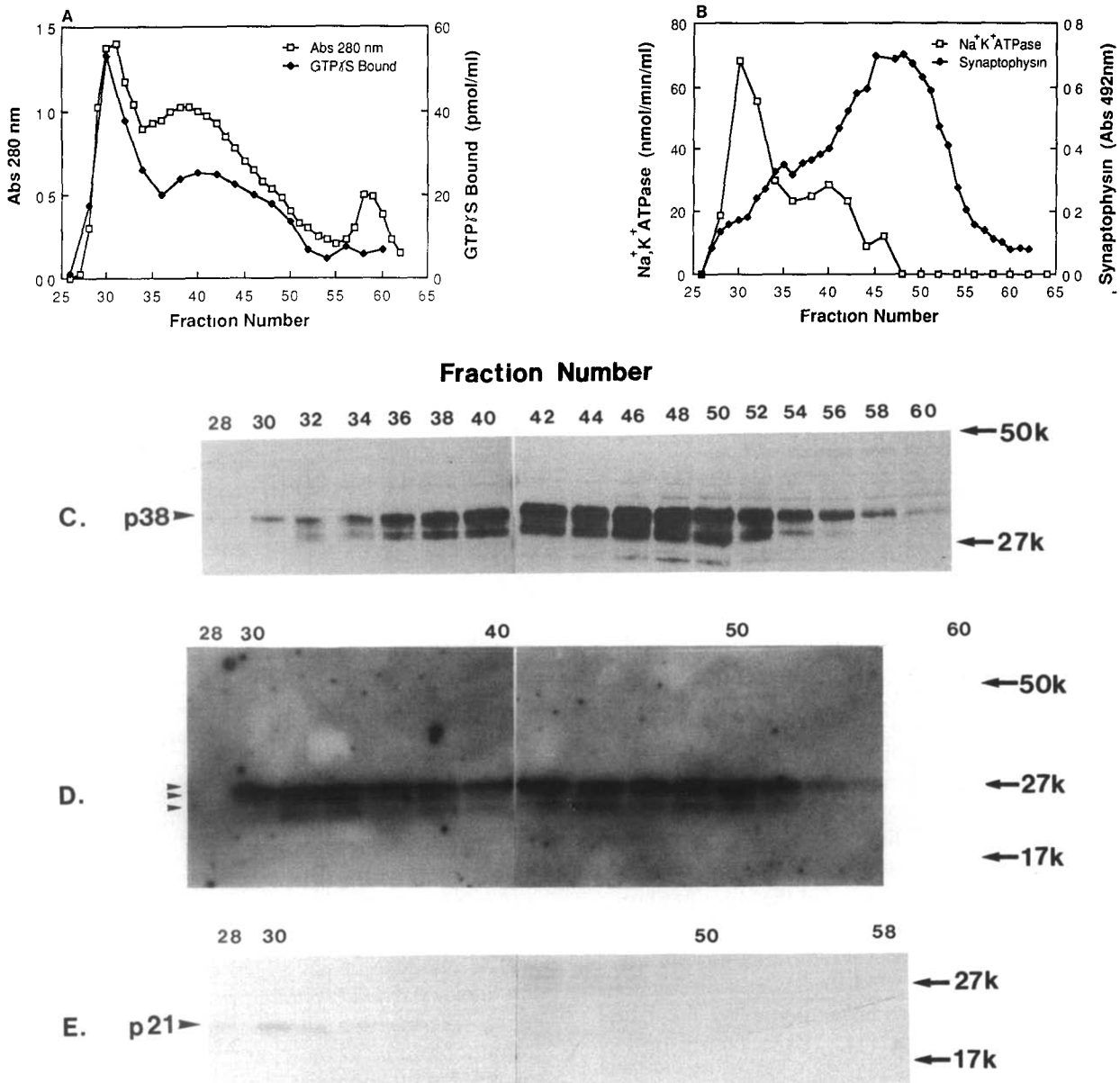

Fig 1 Purification of synaptic vesicles from bovine brain by gel filtration Crude synaptic vesicles (P3, 98 mg protein) was applied onto a Sephacryl S-1000 column as described in Experimental Procedures Fractions collected (8 ml) were subjected to the following assays (A) Absorbance at 280 nm and [^{35}S]GTPγS binding activity (B) Synaptophysin (ELISA) and Na^+/K^+-ATPase activity (C) Immunoblotting of p38 (10% gel) (D) [α-^{32}P]GTP binding after SDS-PAGE (12% gel) and blotting followed by autoradiography (E) Immunoblotting of *ras* p21 (12% gel) Arrows on the right indicate positions of standard proteins

vesicles in this step Thus, Na^+/K^+-ATPase activity, a marker enzyme for plasma membrane, was enriched in a membrane peak eluting with void volume (fraction 32) with a shoulder around fraction 40 (Fig 1B) In contrast, synaptophysin (p38), a marker protein for synaptic vesicles detected by ELISA and immunoblotting, was enriched in a broad peak (fraction 48) included in the gel matrix (Fig 1B and C) Activity of NADPH–cytochrome *c* reductase, a marker enzyme for the endoplasmic reticulum which normally has a larger size than that of synaptic vesicle, eluted in a pattern similar to that of Na^+/K^+-ATPase activity (a broad peak at fraction 32 followed by a shoulder at fraction 35–45, data not shown)

Peak fractions enriched in Na^+/K^+-ATPase activity (fraction 28–35) and p38 (fraction 45–54) were pooled, designated PM (plasma membrane) and SV (synaptic vesicles), respectively, and used in further assessment of their compositions Cross contamination between synaptic vesicles and plasma membrane, in PM and SV fractions, were evaluated quantitatively by p38 ELISA and Na^+/K^+-ATPase activity (Table I) Thus, the content of p38 (per unit protein basis) in SV was 3 4-fold of that in PM, alternatively, specific activity of Na^+/K^+-

TABLE I

Distribution of p38 (synaptophysin) and enzyme activities

Mean ± S D ($n = 3$)

Fraction	p38 (unit/mg)	Na^+/K^+-ATPase (nmol/min per mg)	NADPH–cytochrome-*c* reductase (nmol/min per mg)
P3	3 62 ± 0 15	35 9 ± 2 7	3 92 ± 0 22
PM	2 32 ± 0 05	106 0 ± 16 4	3 90 ± 0 34
SV	7 84 ± 0 33	22 7 ± 6 0	2 27 ± 0 32

ATPase in PM was 4 7-fold of that in SV Specific activity of NADPH–cytochrome *c* reductase, marker enzyme for endoplasmic reticulum, was less abundant in SV fraction than in PM fraction (Table I)

Occurrence of GTP-binding protein with a molecular mass of 24–27 kDa in the vesicles

[^{35}S]GTPγS binding to the membrane fractions from gel permeation chromatography was conducted to assess the distribution of total GTP-binding proteins present As shown in Fig 1A, GTPγS binding activity eluted at two positions, a large peak at fraction 30 and a broad peak at fraction 40 (with a shoulder at fraction 45–50) Based on the results shown below on [α-^{32}P]GTP binding (Fig 1D) and immunoblotting (Fig 2), the elution pattern of [^{35}S]GTPγS binding can be interpreted as follows the first and second peaks consist mainly of high molecular mass GTP-binding proteins, the shoulder to the second peak contains low molecular mass GTP-binding proteins, with the content of the latter in plasma membrane-enriched fractions being much lower than that of high molecular mass GTP-binding proteins

Nitrocellulose blots of SDS-PAGE gels of fractions from the gel permeation chromatography, incubated with [α-^{32}P]GTP, showed (Fig 1D) a family of low

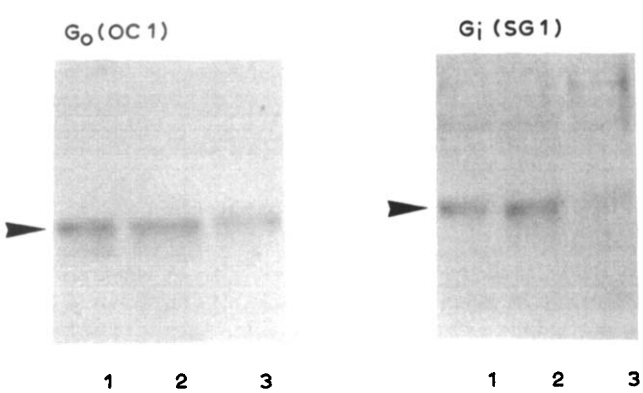

Fig 2 Immunoblotting of synaptic vesicles and plasma membrane proteins Crude synaptic vesicles (3 μg P3, lane 1), plasma membrane (3 μg PM, lane 2), synaptic vesicles (3 μg SV, lane 3) were run on SDS-PAGE slab gels and transferred to nitrocellulose sheets These were probed with anti-$G_o\alpha$ (OC1) and anti-$G_i\alpha$ (SG1) as described in Experimental Procedures Arrowheads indicate the positions of relevant GTP-binding proteins

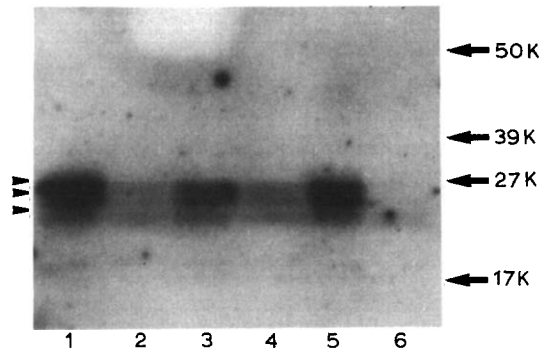

Fig 3 Effect of trypsin treatment of synaptic vesicles on [α-^{32}P]GTP binding Crude synaptic vesicles (15 μg P3, lanes 1, 2), plasma membrane (15 μg PM, lanes 3, 4) and synaptic vesicles (15 μg SV, lanes 5, 6) were incubated in the absence (lanes 1, 3, 5) or presence (lanes 2, 4, 6) of 1 μg trypsin for 30 min at room temperature Then samples were electrophoresed (12% gel), transferred to a nitrocellulose sheet, incubated with [α-^{32}P]GTP as described in Experimental Procedures and subjected to autoradiography

molecular mass proteins consisting of at least three distinct bands (a major 27 kDa and minor 26 and 24 kDa band as indicated by arrowheads in Fig 1D) Examination of the autoradiogram showed that these GTP-binding proteins eluted in two distinct peaks, the first peak (fraction 30–38) corresponded to the peak of Na^+/K^+-ATPase activity (plasma membrane), while the second peak (fraction 44–52) corresponded to the peak of p38 (synaptic vesicles) The binding of [α-^{32}P]GTP to the nitrocellulose blot of pooled fractions (using the same amounts of protein) showed a higher amount of 24–27 kDa GTP-binding proteins in the SV fraction than in the PM fraction (Fig 3, lane 3 and 5)

The presence of *ras* p21 proteins, another family of low molecular GTP-binding proteins, in fractions from the gel permeation chromatography were assessed by immunoblotting using a monoclonal antibody that recognized both the normal and oncogenus *ras* p21 proteins [16] (Fig 1E) Immuno-cross-reactivity was detected at the molecular mass of 21 kDa only with fractions corresponding to the first peak of the [α-^{32}P]GTP binding, and to the main peak of Na^+/K^+-ATPase activity (plasma membrane) However, the amount of *ras* p21 proteins in the plasma membrane-enriched fractions was too small to be detected by [α-^{32}P]GTP binding (see Fig 1D), although they have been reported to bind GTP after transfer to nitrocellulose [17]

The presence of classical GTP-binding proteins in pooled fractions was also examined by immunoblotting (Fig 2) $G_o\alpha$, 39 kDa, was much more concentrated in the PM fraction than in the SV fraction (Fig 2, OC1) $G_i\alpha$, 41 kDa, which is less abundant than G_o in the brain, was also detected mainly in P3 and PM fractions (Fig 2, SG1) Although we did not examine the presence of $G_s\alpha$, another classical GTP-binding protein,

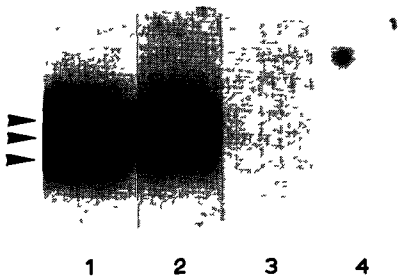

Fig 4 Nucleotide specificity of [α-^{32}P]GTP binding to synaptic vesicles Synaptic vesicles (15 μg of SV) were electrophoresed (12% gel) and transferred to a nitrocellulose sheet Strips containing individual samples were incubated with [α-^{32}P]GTP in the absence or presence of other nucleotides lane 1, no other nucleotides, lane 2, 10 μM ATP, lane 3, 10 μM GTP and lane 4, 10 μM GTPγS

because of its lower abundance in the brain, these observations coincide with the view that classical GTP-binding proteins, serving as substrates of pertussis or cholera toxin, function mainly at the plasma membrane level (but see Ref 18)

Characteristics of low-molecular-mass GTP-binding proteins in synaptic vesicles

Incubation of these fractions (15 μg protein) with trypsin (1 μg) resulted in the disappearance of 24–27 kDa GTP-binding proteins However, 24–27 kDa GTP-binding proteins in the PM fraction were less sensitive to trypsin than those in the SV fraction Although occurrence in the PM fraction of outside-out plasma membrane where trypsin is less accessible to cytoplasmic surface is not estimated, the above observation suggests a difference in the state of 24–27 kDa GTP-binding proteins in plasma membrane and synaptic vesicles

Binding of [α-^{32}P]GTP to 24–27 kDa GTP-binding proteins in the SV fraction was specific in that it was unaffected by the addition of 10 μM ATP, but completely abolished by 10 μM GTP or GTPγS (Fig 4)

Discussion

In the present study, localization of low-molecular-mass GTP-binding proteins on synaptic vesicles as well as on the plasma membrane was demonstrated For this reason, the quality of synaptic vesicles isolated by a simple and efficient method [10], especially cross contamination by plasma membrane, was carefully evaluated by enzyme and protein markers Based on these criteria, the purity of the synaptic vesicles in the present study is quite comparable to that of the original report or that of synaptic vesicles isolated by the classical procedure [10,19] The co-elution in gel permeation chromatography of p38 with [α-^{32}P]GTP binding activity in the second peak (SV) provides evidence that 24–27 kDa GTP-binding proteins are indeed associated with synaptic vesicles Abolishment of the [α-^{32}P]GTP binding activity in the SV fraction after treatment with trypsin, suggests that the 24–27 kDa proteins are associated with the external surface of the synaptic vesicles, assuming that they remain intact based on their rigidity [2]

Co-elution of the first peak of GTP binding activity with Na^+,K^+-ATPase activity (PM) suggests that 24–27 kDa GTP-binding proteins are associated also with the plasma membrane The possibility that the first peak of GTP binding activity is derived from the contamination of synaptic vesicles should be excluded from the following observations (i) the relative amount of p38 in the void volume fraction is much lower than the highest value at the included volume (Fig 1B and C), thus, the contaminating vesicles in the plasma membrane fraction could not account for the content of [α-^{32}P]GTP binding activity in the first peak (void volume) (Fig 1C and D) (ii) [α-^{32}P]GTP binding activity in the PM fraction was less sensitive to trypsin than that in the SV, suggesting some variation in the properties of these proteins, such as their sideness in the membrane However, the possibility that some 24–27 kDa GTP-binding proteins in the PM fraction are derived from endoplasmic reticulum remains to be clarified, since they have been detected in this subcellular fraction from yeast and mammalian cells [20,21] In this context, it is relevant that the PM fraction contained higher amount of NADPH-cytochrome *c* reductase, marker enzyme for endoplasmic reticulum, than the SV fraction

Recently, novel families of low-molecular-mass GTP-binding proteins have been found using different methods, yet their function(s) remain to be clarified [20] *Clostridial botulinum* C_1, D and C3 toxins were shown to ADP-ribosylate 20–26 kDa GTP-binding proteins in mammalian preparations [22–26] One substrate has been identified as a *rho* gene product [27,28] However, the relationship between the ADP-ribosylation and the inhibition of transmitter release by botulinum neurotoxins has been disputed [29,30] Investigations on yeast mutants have revealed the presence of genes coding for low molecular mass GTP-binding proteins involved in a constitutive secretory pathway [20,31] One such gene product, sec4p is a 23 5 kDa GTP-binding protein and is shown to be associated with the secretory vesicles [31] A model of membrane traffic for secretion, in which low molecular GTP-binding proteins interact with unidentified attachment and docking proteins (effector) expressed on vesicles and plasma membrane, respectively, has been proposed [32] Using the [α-^{32}P]GTP binding assay, 18–29 kDa GTP-binding proteins have been shown to be associated with chromaffin granules isolated from bovine chromaffin cells where Ca^{2+}-dependent secretion of catecholamines is readily observed [33,34] In permeabilized chromaffin cells, it has also been shown that the addition of exogenous

guanine nucleotides modulate the Ca^{2+}-dependent release of catecholamines [35] Based on all these collective observations, it is tempting to suggest that the 24–27 kDa GTP-binding proteins associated with synaptic vesicles may also be involved in the Ca^{2+}-dependent exocytotic release of transmitters from these vesicles

Another possible function of the low molecular mass GTP-binding protein is the involvement in cell proliferation and differentiation In rat pheochromocytoma cells, injection of 21 kDa RAS proto-oncogene product induces nerve growth factor-like differentiation [36] Injection of ADP-ribosyltransferase C3 which modifies *rho* gene product, (22 kDa GTP-binding protein) also induces a similar morphological differentiation [26]

To evaluate the function(s) of 24–27 kDa GTP-binding proteins associated with synaptic vesicles in processes such as transmitter release, it could be beneficial to establish their relationship to the above-described gene products and some recently purified low M_r GTP-binding proteins [37,38], identification of the effector protein(s) which these low molecular mass GTP-binding proteins interact with, would also be beneficial

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References

1 Walker, J H and Agoston, D V (1987) Biochem J 247, 249–258
2 Von Wedel, R J , Carlsson, S S and Kelly, R B (1981) Proc Natl Acad Sci USA 78, 1014–1019
3 Timble, W S and Scheller, R H (1988) Trends Neurosci 6, 241–242
4 Wiedenmann, B and Franke, W W (1985) Cell 41, 1017–1028
5 Cockcroft, S , Howell, T W and Gomperts, B D (1987) J Cell Biol 105, 2745–2750
6 Ullrich, S and Wollheim, C B (1988) J Biol Chem 263, 8615–8620
7 Luini, A and De Matteis, M A (1988) Cell Mol Neurobiol 8, 129–138
8 Bhullar, R P and Haslam, R J (1987) Biochem J 245, 617–620
9 Mitchell, F M , Griffiths, S L , Saggerson, E D , Houslay, M D , Knowler, J T and Milligan, G (1989) Biochem J 262, 403–408
10 Hell, J W , Maycox, P R , Stadler, H and Jahn, R (1988) EMBO J 7, 3023–3029
11 Bradford, M M (1976) Anal Biochem 72, 248–254
12 Lynn, T -I and Morales, M F (1977) Anal Biochem 77, 10–17
13 Sottocasa, G L , Kuylenstierna, B , Ernster, L and Bergstrand, A (1967) J Cell Biol 32, 415–438
14 Laemmli, U K (1970) Nature 227, 680–685
15 Northup, J K , Smigel, M D and Gilman, A G (1982) J Biol Chem 257, 11416–11423
16 Carney, W P , DeLellis, R A , Wolfe, H , Cooper, G M and Rabin, H (1987) Proc 78th AACR 28, 397
17 McGrath, J P , Capon, D J , Goeddel, D V and Levinson, A D (1984) Nature 310, 644–649
18 Toutant, M , Aunis, D , Bockaert, J , Homburger, V and Rouot, B (1987) FEBS Lett 215, 339–344
19 Huttner, W B , Schiebler, W , Greengard, P and De Camilli, P (1983) J Cell Biol 96, 1374–1388
20 Burgoyne, R D (1989) Trends Biochem Sci 14, 394–396
21 Goud, B , Salminen, A , Walworth, N C and Novick, P J (1988) Cell 53, 753–768
22 Ohashi, Y , Kamiya, T , Fujiwara, M and Narumiya, S (1987) Biochem Biophys Res Commun 142, 1032–1038
23 Matsuoka, I , Syuto, B , Kurihara, K and Kubo, S (1987) FEBS Lett 216, 295–299
24 Matsuoka, I , Sakuma, H , Syuto, B , Morishi, K , Kubo, S and Kurihara, K (1989) J Biol Chem 264, 706–712
25 Aktories, K and Frevert, J (1987) Biochem J 247, 363–368
26 Rubin, E J , Gill, M , Boquet, P and Popoff, M R (1988) Mol Cell Biol 8, 418–426
27 Yamamoto, K , Kondo, J , Hishida, T , Teranishi, Y and Takai, Y (1988) J Biol Chem 263, 9926–9932
28 Quilliam, L A , Lacal, J -C and Bokoch, G M (1989) FEBS Lett 247, 221–226
29 Adam-Vizi, V , Rosener, S , Aktories, K and Knight, D E (1988) FEBS Lett 238, 277–280
30 Ashton, A C , Edwards, K and Dolly, J O (1988) Trans Biochem Soc 16, 833–834
31 Salminen, A and Novick, P (1987) Cell 49, 527–538
32 Bourne, H R (1988) Cell 53, 669–671
33 Burgoyne, R D and Morgan, A (1989) FEBS Lett 245, 122–126
34 Doucet, J -P , Fournier, S , Parulekar, M and Trifaro, J -M (1989) FEBS Lett 247, 127–131
35 Bittner, M A , Holz, R W and Neubig, R R (1986) J Biol Chem 261, 10182–10188
36 Bar-Sagi, D and Feramisco, J F (1985) Cell 42, 841–848
37 Waldo, G L , Evans, T , Fraser, E D , Northup, J K , Martin, M W and Harden, T K (1987) Biochem J 246, 431–439
38 Kikuchi, A , Yamashita, T , Kawata, M , Yamamoto, K , Ikeda, K , Tanimoto, T and Takai, Y (1988) J Biol Chem 263, 2897–2904