The G protein α subunit (GPα1) is associated with the ER and the plasma membrane in meristematic cells of *Arabidopsis* and cauliflower

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Abstract Towards the elucidation of the cellular function(s) of GPα1, we have characterized its subcellular localization using immunofluorescence and cell fractionation. GPα1 is not present in nuclei or chloroplasts. It is a membrane-bound protein, and analysis of isolated endoplasmic and plasma membranes indicates a good correlation between GPα1 in both the plasma membrane and the ER compartment. Interestingly, these results may suggest more different functions for GPα1: it might be involved in transmission of extracellular signals across the plasma membrane and in the cytoplasm, and/or it may also be involved in regulating some aspects of the ER functions or membrane trafficking between both membranes.

Key words: Immunofluorescence; Cell fractionation; G protein; Plasma membrane; ER; *Arabidopsis*; Cauliflower

1. Introduction

Heterotrimeric GTP-binding proteins, also called G proteins, consist of three subunits α, β and γ and are essential components of many signal transduction pathways in animals [1]. They were first identified by their function in transducing hormonal signals arriving at the plasma membrane, and serve as molecular switches between activated cell surface receptors and various effectors inside the cell [2]. For example, the classic stimulatory G_s protein is known to mediate the activation of adenylate cyclase by hormones. In addition, in recent years some G proteins have been shown to also be localized on intracellular membranes and have emerged as important regulators of membrane trafficking (reviewed in [3,4]). For example, both an inhibitory $G\alpha_i$ and the stimulatory $G\alpha_s$ have been shown to cooperate to control the formation of constitutive and regulated secretory vesicles form the trans-Golgi-network in PC12 and MDCK cells [5].

There is biochemical and molecular evidence for the presence of G proteins in plants and a number of studies have implicated G proteins in various plant cell processes such as blue light, phytochrome or auxin signaling, or plant cell defense mechanisms (reviewed in [6]). To date only one gene coding for a G protein α subunit, *GPA1* [7] and one gene coding for a G protein β subunit, *AGB1* [8] have been cloned in *Arabidopsis*, with homologues of these genes isolated from other species (reviewed in [9,10]). No gene coding for a G protein γ subunit has been isolated from plants yet. The apparent uniqueness of these genes [7,8] and the fact that they are highly conserved among plants suggests that they perform important functions.

We have previously described the tissue and cell specific localization of GPα1, the product of *GPA1*, and detected it in all organs and cell types examined [11,12]. In addition, the level of GPα1 was observed to vary between different cell types, in particular, cells of all meristems and organ primordia have high levels of GPα1, while differentiated cells of mature organs have a much lower level of the protein. This suggests the involvement of GPα1 in one or more signal pathways in many, if not all, cells. Furthermore, the high levels of GPα1 protein suggest that signaling processes mediated by GPα1 may be very active in differentiating cells of meristems and organ primordia.

Since some animal G proteins have been shown to localize to more than one cellular compartment and have been reported to have more than one function in the cell, it is important to learn what is the localization of GPα1. In this report we describe the subcellular localization of GPα1. We found that GPα1 is localized not only to the plasma membrane but also to the ER compartment.

2. Material and methods

2.1. Production of anti-GPα1 antibodies

A 0.9 kB *Eco*RI/*Bam*HI fragment of GPA1 cDNA [7] encoding the C-terminal portion of GPα1 was cloned into the pATH2 vector to express a fusion polypeptide with the amino-terminus of the *E. coli* trpE protein. After transformation of this plasmid into TB1 cells and selection for recombinant clones, inclusion bodies isolated from the induced bacterial cultures were purified and used to inject New Zealand White rabbits for anti-GPα1 antibodies. The serum obtained was affinity purified on agarose beads covalently linked to pure recombinant GPα1 using the AminoLink Plus Immobilization kit from Pierce. Pure recombinant GPα1 was obtained by cloning of GPA1 into the pET19b vector to express a recombinant protein with a histidine tag at the N-terminal, allowing the protein to be purified on a metal chelating resin according to the manufacturer's specifications (Novagen).

2.2. Immunofluorescence localization

Arabidopsis root tips were fixed in 8% formaldehyde and 5% DMSO in PME buffer (50 mM Pipes pH 6.9, 5 mM $MgCl_2$, 5 mM EGTA) for 1 h, then rinsed 3 times in PME buffer and treated with 2% Driselase (Sigma) for 20 min. Cells were released by squashing the root tips with a coverslip on poly-lysine coated slides. After the cells were air dried, they were permeabilized with 0.1% Triton X-100 for 5 min and exposed to the affinity-purified primary antibody in PME with 3% BSA for 1 h. After washing to remove unbound antibody, the cells were incubated with anti-rabbit IgG antibody conjugated to fluorescein isothiocynate (1/50 dilution, Amersham) and with concanavalin A conjugated to Texas Red (1/50 dilution, Molecular Probes) in

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PME for 1 h. The cells were washed and the nuclei were stained with 150 ng/ml of DAPI (Sigma) for 1 min and after a final brief wash the slides were mounted in antifade mountant (Vector) and viewed by conventional epifluorescence microscopy with a Zeiss microscope.

2.3. Homogenization of plant tissue

All manipulations in this section and the following sections were carried out at 4°C or on ice. 20–40 g of cauliflower inflorescence or 3-week old *Arabidopsis* plantlets were chopped with a razor blade and homogenized with a Polytron for 2 min at 3000 rpm in 1 ml/g of tissue homogenization buffer (40 mM HEPES pH 7.5, 10 mM KCl, 3 mM EDTA, 1 mM DTT, 5 μM leupeptin, 3 mM *o*-phenanthroline, 3 μM E-64, 0.2 mM bestatin, 0.4 M sucrose, 2.5% Ficoll 400, 2.5% dextran T 250). The homogenate was filtered through 6 layers of miracloth.

2.4. Fractionation by differential centrifugation

The filtered homogenate was centrifuged at 360×*g* for 10 min to yield the pellet P1 and the supernatant S1. P1 was washed 2 times with homogenization buffer containing 1% Triton X100, then rinse twice with homogenization buffer alone. The purity of the nuclei in the washed P1 pellet was checked by light microscopy. The S1 supernatant was centrifuged at 11 000×*g* for 20 min to yield the pellet P2 and the supernatant S2. S2 was centrifuged at 100 000×*g* for 30 min to yield the pellet P3 and the supernatant S3.

Chloroplasts were purified from *Arabidopsis* leaves. Briefly, a filtered homogenate was centrifuged for 15 min at 1000×*g* and the pellet was gently resuspended in homogenization buffer minus the Ficoll and dextran, layered onto a 52/30% (w/w) sucrose step gradient and centrifuged at 110 000×*g* for 30 min. The intact chloroplasts collect at the 52/30% sucrose interface. The sucrose solutions were made in homogenization buffer minus the sucrose, Ficoll and dextran (basic buffer).

2.5. Isolation of ER membranes

ER membranes were isolated by isopycnic centrifugation of a cauliflower homogenate according to a modification of [13]. The homogenate was centrifuged at 6000×*g* for 15 min and the supernatant was layered onto a 50% (w/w) sucrose solution cushion and centrifuged at 100 000×*g* for 30 min to obtain total membranes at the supernatant/sucrose interface. The membranes were diluted with basic buffer to a final sucrose concentration of 10% (w/w) and layered onto a 40%, 35% and 15% (w/w) sucrose solution step gradient and centrifuged at 100 000×*g* for 2 h. Each interface was removed and the 35/40% sucrose interface (enriched in rough ER) was diluted with basic buffer containing 10 mM EDTA and kept on ice for 30 min to strip the ER from the ribosomes. The smooth ER was pelleted by centrifugation at 150 000×*g* for 1 h, resuspended in basic buffer containing 3 mM EDTA and layered onto a 50%, 25% and 15% (w/w) sucrose solution step gradient and centrifuged at 100 000×*g* for 3 h. The smooth ER membranes collect at the 15/25% sucrose interface. All interfaces were collected, diluted with 0.2 M sucrose solution, centrifuged at 100 000×*g* for 30 min and resuspended in basic buffer. All sucrose solutions were made in basic buffer.

2.6. Isolation of Golgi apparatus

The Golgi apparatus was isolated by rate zonal and isopycnic centrifugation of a cauliflower homogenate according to [14]. The homogenate was centrifuged at 6000×*g* for 15 min and the supernatant was layered over a 50% and 45.4% (w/w) sucrose step gradient and centrifuged at 30 000×*g* for 30 min. The supernatant above the particulate material at the 45.4% sucrose interface was removed and aliquots of 43%, 37.5% and 17% w/w sucrose solution were carefully layered on top of the particulate material and centrifuged at 100 000×*g* for 3 h. The 17/37.5% and the 37.5/43% sucrose interfaces are enriched in membranes of the Golgi apparatus. Each interface was collected, diluted with 0.2 M sucrose solution, centrifuged at 100 000×*g* for 30 min and resuspended in basic buffer. All sucrose solutions were made in basic buffer.

2.7. Isolation of plasma membrane

Plasma membranes were isolated by two-phase partitioning of a cauliflower P3 pellet. The pellet was resuspended in PSK buffer (30 mM KH_2PO_4/K_2PO_4 pH 7.8, 1.4 M sucrose, 13 mM KCl) at 2–4 mg protein/ml. 2.75 g of resuspended membranes (5–10 mg protein) were added to a two phase mixture of 6.4 g of a 20% (w/w) stock solution of dextran T500, 6.3 g of a 40% (w/w) PEG-3350 stock solution, 4.75 g of PSK buffer and 4.8 g of distilled H_2O mixed by inverting 20 times and centrifuged at 1500×*g* for 5 min. The upper phase was removed, mixed by inversion with the lower phase of a fresh batch of two-phase mixture, and centrifuged at 1500×*g* for 5 min. The lower phase, the upper phase and the interface were removed, diluted 8–10 fold with 50 mM Tris-HCl pH 7.5, 250 mM sucrose and 1 mM EDTA, pelleted by centrifugation at 100 000×*g* for 30 min and resuspended in basic buffer.

2.8. Enzyme assays

Antimycin A insensitive NADH:cytochrome *c* reductase (CCR), latent inosine 5-diphosphatase (IDPase) and cation-stimulated vanadate-inhibitable H^+-ATPase (ATPase) were measured essentially according to [13], with the exception that the CCR buffer was 20 mM potassium phosphate pH 7, 0.2 mM oxidized cytochrome *c*, 10 mM KCN, 5 μM antimycin A.

2.9. Protein gel blot analysis

Protein concentration was measured using the Bio-Rad dye kit, based on [15]. Proteins of each fraction were separated on a 10% SDS-polyacrylamide gel, electroblotted onto a nitrocellulose membrane and used for immunodetection as described in [11].

3. Results

3.1. Immunofluorescence localization of GPα1

Affinity purified polyclonal anti-GPα1 antibodies detected a single band at 45 kDa on a western blot (see Figs. 2A and B, 3A, 4A and 5A), which corresponds to the predicted size of the product of *GPA1* [7]. We cannot rule out that the antibodies cross-react with an other band of the same size, however this is unlikely because GPA1 appears to be a unique gene in *Arabidopsis* [6]. The antibodies were used to visualize the subcellular localization of GPα1 in *Arabidopsis* root tip cells (meristematic cells). Fig. 1A shows immunofluorescence localization of GPα1 in the plasma membrane. Some fluorescent signal is also detected inside the cells, in particular in the top left cell. The GPα1 staining pattern is coincident with staining by the lectin concanavalin (Fig. 1B) which is known to stain the plasma membrane and the ER [16,17]. The intriguing localization of GPα1, not only on the plasma membrane, but also on cytoplasmic structures prompted us to study its intracellular localization in more detail. Since so few well characterized antibodies for the different plant membrane compartments are available, we opted to use subcellular fractionation procedures.

3.2. GPα1 is a membrane-bound protein

For the purpose of identifying the cytoplasmic structures with which GPα1 is associated, *Arabidopsis* and cauliflower homogenates were submitted to differential centrifugation. Different cellular compartments were identified by characteristic enzymatic activities (see Section 2 for full name of the enzymes): IDPase (Golgi), CCR (nuclei, ER) and ATPase (plasma membranes) (Fig. 2C). In the P1 (*Arabidopsis*) and P1′ (cauliflower) pellets obtained by low speed centrifugation, the CCR marker should indicate the presence of nuclear membrane as the centrifugal force used is not sufficient to pellet other membranes. The results in Fig. 2C indicate that the *Arabidopsis* pellet P1 contains pure nuclei, while the cauliflower pellet, P1′ shows a low level of plasma membrane enzymatic activity. The chloroplasts isolated in a separate experiment from *Arabidopsis* leaves show none of the above activities, while the P2, P2′, P3, and P3′ pellets for both plant extracts show the presence of all three intracellular mem-

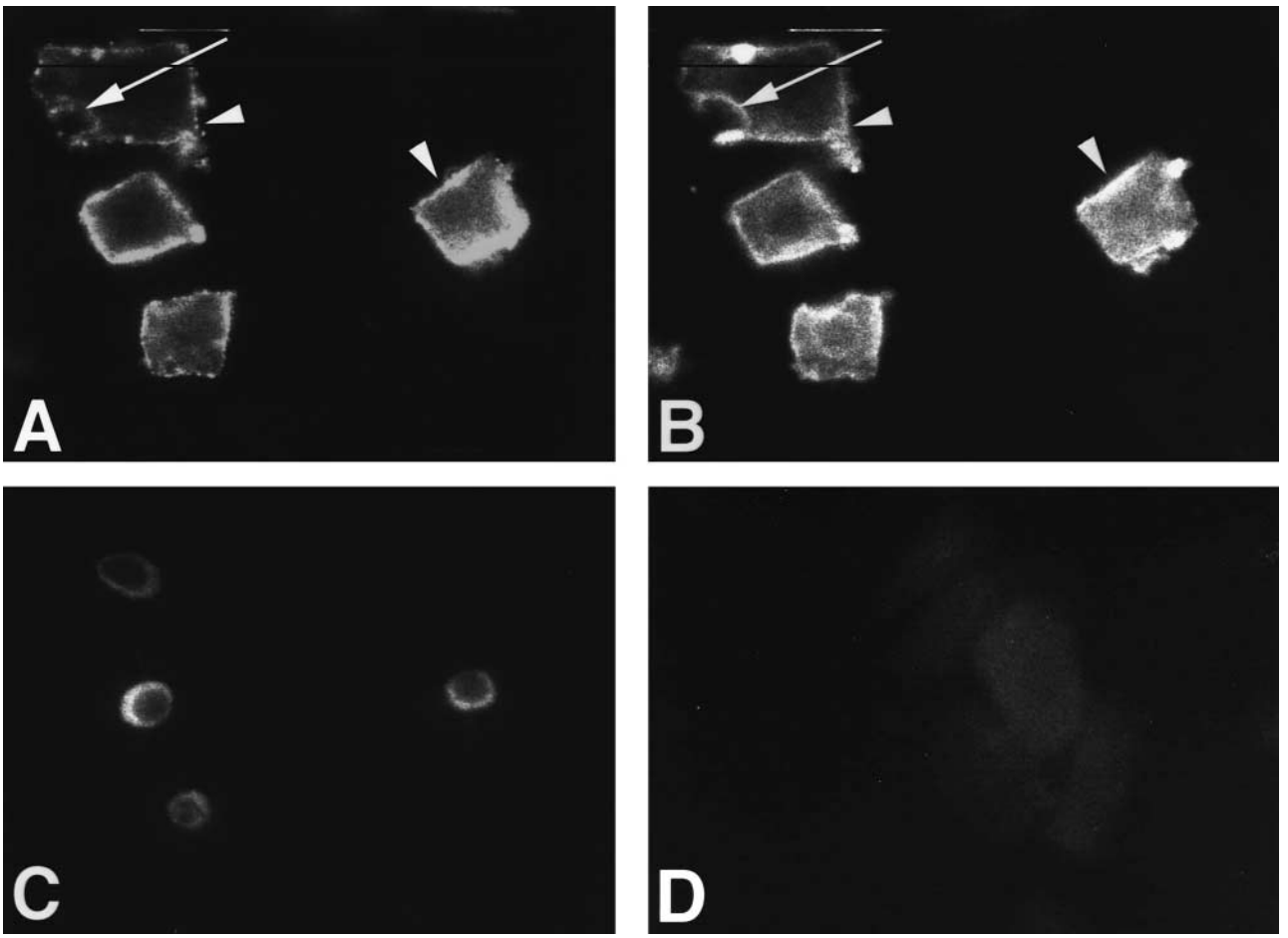

Fig. 1. Immunofluorescence localization of GPα1. Cells isolated from *Arabidopsis* root tips (A–D) were fixed and processed for immunofluorescence. Cells were stained with anti-GPα1 antibody (A), with ConA (B), with DAPI (C) or with normal rabbit serum (D). GPα1 antibody stains the plasma membrane (arrowheads) and ER-like structures inside the cell (arrow), similar to ConA staining pattern.

branes. Most of the plasma membrane is found in the P3 and P3′ pellets and the ER is distributed roughly equally between the P2 and P3; and the P2′ and P3′ fractions (see Table 1 for the % of recovery of each marker in the different cauliflower fractions). The 100 000×*g* supernatant contains most of the IDPase activity, indicating that a large portion of the Golgi apparatus was probably broken in the homogenization process (Fig. 2C, Table 1). The western blot follows the presence of GPα1 in different fractions of *Arabidopsis* (Fig. 2A) and cauliflower (Fig. 2B) extracts and indicates that GPα1 is not detectable in P1 but present at low level in P1′. Since P1′ appears to be contaminated by some plasma membrane, we conclude that GPα1 is most likely not present in nuclei and that the presence of GPα1 in the P1′ pellet is due to plasma membrane contamination. GPα1 is present in the P2, P3, P2′, and P3′ pellets, indicating its presence in membranes, although the data did not allow the distinction between the different cellular compartments. GPα1 is not detected in the

Table 1
Distribution of protein and enzyme activities among fractions of differential centrifugation

Fractions	H[a]	P1′[b]	P2′[c]	P3′[d]	S3′[e]
protein[f] mg/20 g FW	66	7.1	8.8	8	38
% recovery of protein[g]		11	13	12	57
% recovery of IDPase[g]		–	1.7	5	88
% recovery of CCR[g]		24	39	36	–
% recovery of ATPase[g]		2.7	14	80	–

% of recovery from the original homogenate (H) of cauliflower protein and cauliflower enzymatic activity of the membranes markers during differential centrifugation.
[a]Total homogenate.
[b]P1′, 360×*g* pellet.
[c]P2′, 11 000×*g* pellet.
[d]P3′, 100 000×*g* pellet.
[e]S3′, 100 000×*g* supernatant.
[f]Protein homogenate from cauliflower. FW, fresh weight.
[g]Percentage in each fraction from the homogenate; the total is less than 100% because of some loss during fraction collection.

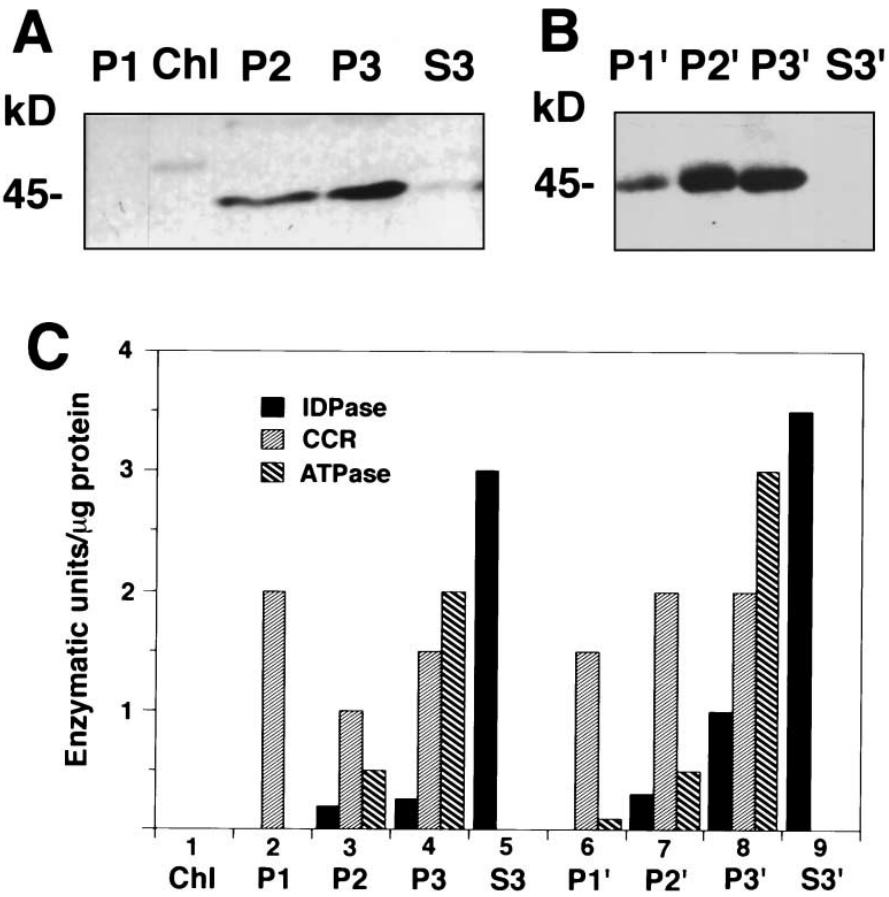

Fig. 2. Distribution of GPα1 in *Arabidopsis* (A) and cauliflower (B) during differential centrifugation. P1, Chl, P2, P3 and S3 represent protein extracts from *Arabidopsis* while P1′, P2′, P3′, and S3′ represent protein extracts from cauliflower. 10 μg of protein of the 360×*g* P1 and P1′ pellet, the 11 000×*g* P2 and P2′ pellet, the 100 000×*g* P3 and P3′ pellet, the 100 000×*g* supernatant S3 and S3′, and the chloroplast (Chl) were subjected to SDS-PAGE electrophoresis, transferred onto nitrocellulose and probed with polyclonal antibody directed against GPα1. (C) Distribution of different marker enzyme in the fractions. IDPase, a Golgi marker, CCR, a nuclear envelope and ER marker, ATPase, a plasma membrane marker. Results are expressed in enzymatic activity per μg of protein. The absence of a column in the graph indicates that the enzyme activity measured is below the level of detection.

soluble fraction (S3 and S3′). Since similar results were obtained with *Arabidopsis* tissue and with cauliflower tissue, the following experiments were done on cauliflower inflorescence which provided a greater source of homogeneous tissue with a high level of GPα1 protein.

3.3. GPα1 is present in the ER

In order to distinguish between the different membranes we performed isopycnic centrifugation on a cauliflower microsomal fraction to isolate the ER (Fig. 3B). In the first gradient, we only detected CCR activity in the 15/35% sucrose interface, while the 35/40% sucrose interface showed the presence of CCR and ATPase activity. After the 35/40% sucrose interface was treated by EDTA to release the smooth ER and centrifuged on the second gradient, the 15/25% sucrose interface showed only CCR activity while the 25/50% sucrose interface showed only ATPase activity. The western blot analysis shows the presence of GPα1 in all four fractions (Fig. 3A). Since we have a pure fraction of rough ER, a pure fraction of smooth ER and a pure fraction of plasma membrane, these results would indicate that GPα1 is present in all these membranes. None of these fractions had any IDPase activity, indicating that they had no contaminating Golgi apparatus.

3.4. Isolation of the Golgi apparatus

To determine if GPα1 was associated with the Golgi apparatus we purified Golgi membranes using rate zonal and isopycnic separation (Fig. 4B). The 37.5/43% and the 17/37% sucrose interfaces were enriched in IDPase activity but they also presented a small amount of ATPase activity. The 43/45.4% sucrose interface has most of the ATPase activity of these fractions and no measurable IDPase or CCR activity. The 45.4/50% sucrose interface has a low level of ATPase activity and no detectable IDPase and CCR activity. The western blot results show the association of GPα1 with fractions containing ATPase activity, pointing to GPα1 being associated with the plasma membrane (Fig. 4A). We cannot rule out, however, that a small portion of GPα1 protein is being associated with the Golgi apparatus, and contributes to the signal observed on the western blot in the 37.5/43% and the 17/37% sucrose interfaces.

3.5. GPα1 is present in the plasma membrane

The plasma membrane was purified by phase partitioning and after the second round of two-phase partitioning, the ATPase activity was found in the interface fraction while the CCR activity was found in the lower phase fraction and

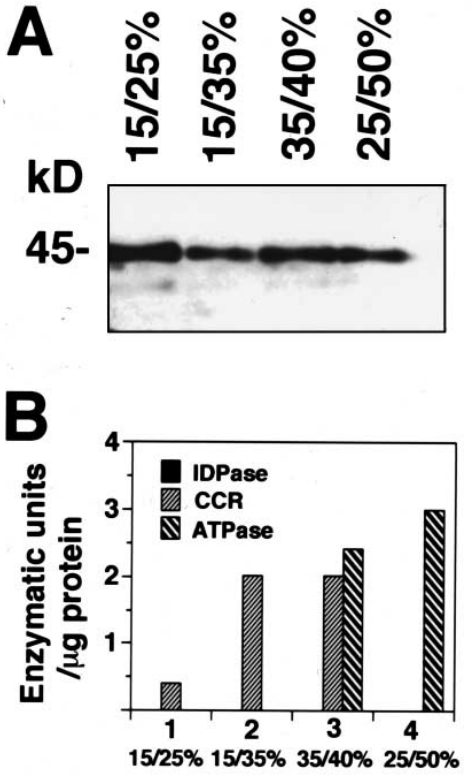

Fig. 3. (A) Distribution of GPα1 during isolation of ER membranes by isopycnic gradient performed on cauliflower tissue. 10 μg of protein from each interface was subjected to SDS-Page electrophoresis and transferred onto nitrocellulose and probed with antibody against GPα1. (B) Distribution of IDPase, CCR and ATPase membrane markers. Results are expressed in enzymatic activity per μg of protein. The absence of a column in the graph indicates that the enzyme activity measured is below the level of detection.

at a low level in the upper phase fraction, while IDPase activity could not be detected in any of these fractions (Fig. 5B). Depending on the phase system used, localization of the plasma membrane at the interface has been reported previously [18]. Western blot analysis clearly indicated that GPα1 is present in the lower phase and interface and at a much reduced level in the upper phase (Fig. 5A). Thus, GPα1 distribution coincides with the distribution of plasma and ER membranes.

4. Discussion

We have characterized the subcellular localization of GPα1, the gene product of *GPA1*, using immunofluorescence microscopy and subcellular fractionation methods on *Arabidopsis* and cauliflower tissue.

Immunofluorescence experiments indicate that GPα1 is associated with the plasma membrane and some structures in the cytoplasm, possibly the ER, as it co-localizes with concanavalin A which can stain the plasma membrane and the ER. The cell fractionation data revealed that GPα1 is not detected in the nuclear pellet, nor in chloroplasts. It is a membrane-bound protein, as indicated by its association with the 11 000×*g* and the 100 000×*g* pellets and absence from the soluble fraction (the 100 000×*g* supernatant). Although the 11 000×*g* pellet contained some ER, Golgi and plasma membranes, it should also contain the bulk of the mitochondria. On the other hand, the 100 000×*g* pellets contained the bulk of the ER, Golgi and plasma membranes, as indicated by marker enzyme activities. Therefore, the observation that GPα1 was detected by western blots to be at a lower level in the 11 000×*g* P2 and P2′ pellets than in the 100 000×*g* P3 and P3′ pellets (Fig. 1A and B) supports the idea that GPα1 is not present in the mitochondria at a high level. However, we were not able to rule out the possibility that a small amount of GPα1 is associated with the mitochondria. In the subsequent isolation of different membranes, the larger and denser particles of the cell (such as the nuclei and the mitochondria) are discarded at an early stage of the procedures which makes it unlikely that our preparations have high contamination by mitochondrial membranes.

Isolation of different membranes showed the association of GPα1 with the plasma membrane and the ER. It is also possible that GPα1 is associated with the Golgi apparatus, but

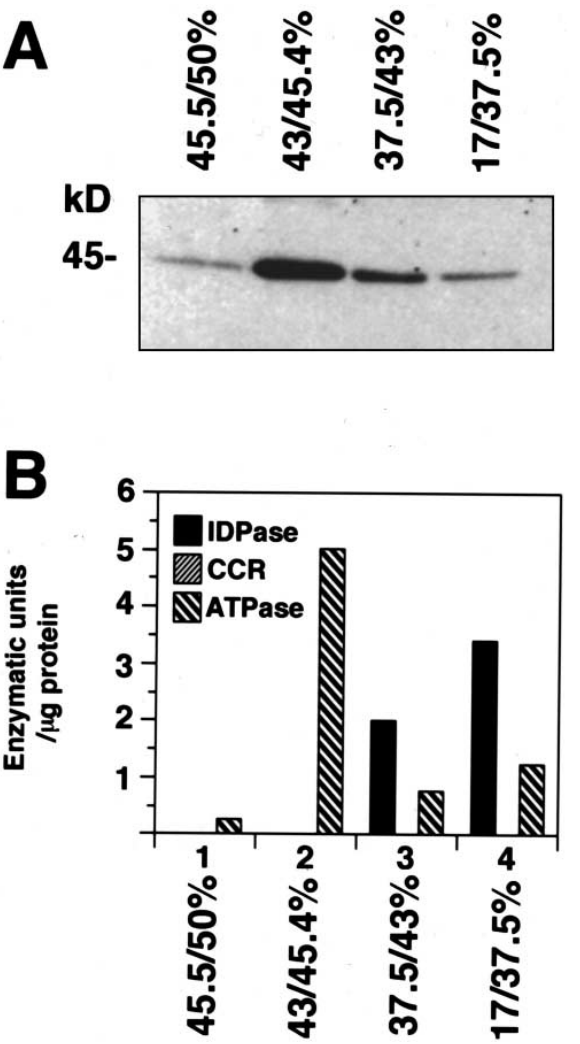

Fig. 4. (A) Distribution of GPα1 during isolation of Golgi apparatus by rate zonal centrifugation followed by isopycnic gradient performed on cauliflower tissue. 10 μg of protein from each interface was subjected to SDS-Page electrophoresis and transferred onto nitrocellulose and probed with antibody against GPα1. (B) Distribution of IDPase, CCR and ATPase membrane markers. Results are expressed in enzymatic activity per μg of protein. The absence of a column in the graph indicates that the enzyme activity measured is below the level of detection.

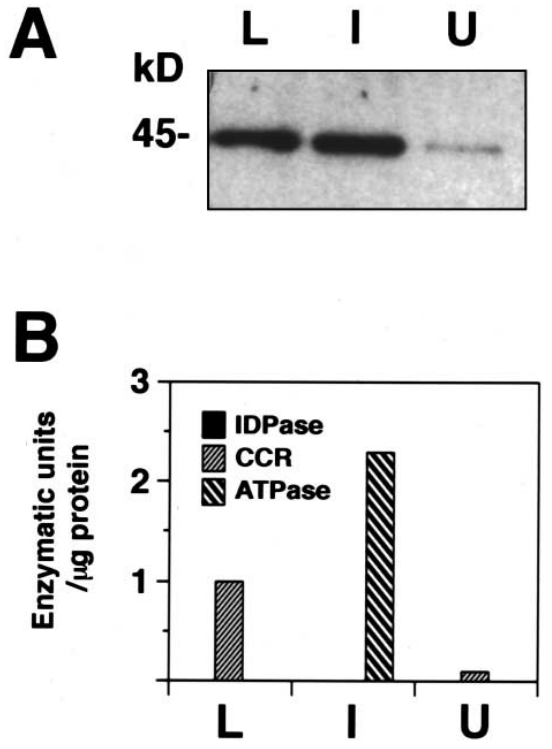

Fig. 5. (A) Distribution of GPα1 during isolation of plasma membrane by two-phase partitioning performed on cauliflower tissue. 1 µg protein from each fraction (U, upper; I, interface; L, lower) of the last round of phase partitioning were subjected to SDS-Page electrophoresis and transferred onto nitrocellulose and probed with antibody against GPα1. (B) Distribution of IDPase, CCR and ATPase membrane markers. Results are expressed in enzymatic activity per µg of protein. The absence of a column in the graph indicates that the enzyme activity measured is below the level of detection.

the level of GPα1 protein on the Golgi, if any, must be much lower than on the plasma and ER membranes. Recently Perroud et al. [19] have detected GTP-binding protein on the tonoplast of mature spinach leaves. One of the GTP-binding proteins can be detected by an antibody raised against the conserved consensus sequence of the GTP-binding site of the animal G α subunit of the heterotrimeric G protein. Since meristematic cells do not have a fully developed vacuole, we have not isolated the tonoplast from cauliflower inflorescence cells and we cannot exclude the possible association of GPα1 with the tonoplast. We have not followed a tonoplast marker in our membrane isolation so it is possible that the membranes are contaminated by tonoplast, but on isopycnic sucrose density gradients, tonoplast vesicles band most frequently at a lower density than even the smooth ER [20], and should not contaminate the other membrane fractions.

Based on the results presented here, we suggest that the plant G protein, GPα1 has a role(s) at the plasma membrane and at least the ER. In the past few years, animal heterotrimeric G proteins have been found to be associated, not only with the plasma membrane but also with endomembranes such as the ER [21] and the Golgi complex [22]. They have also been implicated in regulating the transport of protein from the ER to the Golgi complex, the formation of vesicles within the Golgi complex, the formation of endocytic and transcytotic vesicles, and endosome fusion (reviewed in [23] and [24]).

Little is known in plants at the molecular level about signaling across the plasma membrane and the regulation of the secretory pathway. Although plants have signal perception components at the plasma membrane such as receptor protein kinases (reviewed in [26]) and the two-component receptor for ethylene (reviewed in [25]), it is not known how signals are transmitted from these receptors to intercellular second messengers. The plant secretory system delivers protein to the cell wall and the extracellular space as well as to the vacuole, although much less information is available, the sorting of vacuolar proteins is thought to occur via a trans-Golgi network as in animals cells. There seems to be a high degree of conservation of components that bring about vesicle transport between highly divergent organisms such as mammal, yeast, and plants [27]. For example, of the 24 subclasses of Rab protein that have been identified in animal cells and appear to regulate the docking/fusion processes of membrane trafficking, members of 7 of these classes are known in plants (reviewed in [6]). In one case there is evidence for a role in vesicle-mediated transport where antisense constructs against Rab1 and Rab7 prevented peribacteroid membrane formation in root nodules [28].

By analogy with animal G proteins, the role(s) of GPα1 could be mediating signal perception at the plasma membrane and regulating vesicle transport. But the ER localization of GPα1 also has a second possible implication. It is known that several plant hormones are membrane permeable; in fact, the sequence of an auxin binding protein suggests that it may be localized to the ER [29]. Furthermore, light is the most important environmental signal for plants, and both known types of light receptors, phytochromes for red light, and the *Arabidopsis* HY4 protein for blue light, appear to be in the cytoplasm [30,31]. Because biochemical studies have implicated G proteins in light signaling and auxin signaling, such G proteins could indeed be associated with endomembranes rather than the plasma membrane. An interesting hypothesis is that such signaling cascades would not be across a membrane and that the receptors coupled to the ER-located G protein need not be transmembrane receptors as are all known animal G protein-coupled receptors [32]. Intracellular localization of GPα1 would then not exclude the possibility of its role in mediating extracellular signals at such locations in a way that would be novel in the mechanisms of G protein signaling. Finally the GPα1 located on the ER may be involved in regulating other roles of the ER such as protein synthesis or protein modification in manners that remain to be elucidated or may be involved in membrane trafficking between both the plasma membrane and the ER. Experiments designed to test these exciting possible roles for G protein in plants should bring a new comprehension to plant signaling biology.

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