Biochemical and Biophysical Research Communications **273,** 176–182 (2000)
doi:10.1006/bbrc.2000.2918, available online at http://www.idealibrary.com on IDEAL®

Characterization of Cop I Coat Proteins in Plant Cells

Inmaculada Contreras, Elena Ortiz-Zapater, Luis Miguel Castilho, and Fernando Aniento[1]
Departament de Bioquímica i Biología Molecular Facultat de Farmacia, Universitat de Valencia, 46100, Burjassot (Valencia), Spain

Received May 15, 2000

Membrane traffic in eukaryotic cells is mediated by COP (coat protein)-coated vesicles. Their existence in plant cells has not yet been unequivocally demonstrated, although coated vesicles (probably with a COP coat) can be seen by electron microscopy. At the gene level, plant cells seem to contain all the components necessary to form COP-coated vesicles. In this paper, we have used antibodies raised against mammalian COPI coat proteins to detect putative homologues in rice (*Oryza sativa*) cells. Using these antibodies, we have found that rice cells contain α-, β-, β′-, and γ-COP, as well as ADP-ribosylation factor (ARF) 1 protein. In addition, we show that antibodies against mammalian β′-COP can immunoprecipitate not only β′-COP but also α-, β-, and γ-COP, suggesting that COPI components in rice cells exist as a complex (or coatomer) in the cytosol, as in mammalian cells. Finally, we show that COP binding to membranes is GTP-dependent, and that ARF1 also binds to membranes in a GTP-dependent manner.

Cytosolic coat proteins regulate membrane traffic in eukaryotic cells. Three classes of coat protein complexes have so far been identified: clathrin and its adaptor proteins (APs), coatomer (COP I, coat protein I), and COP II. Two different clathrin coats containing either AP-2 or AP-1 adaptors mediate, respectively, endocytosis from the plasma membrane and transport from the TGN to endosomes and lysosomes (1). A third adaptor complex, AP-3, has a similar four subunit composition as AP-1 and AP-2, and is presumably involved in transport between Golgi/TGN and endosomes/lysosomes. Whether this complex also associates with clathrin remains controversial (2, 3). Transport between the ER and the Golgi apparatus is carried out by coat protein (COP I- and COP II-) coated vesicles. While it is largely accepted that COP II-coated vesicles are involved in the export from the ER, the role of COP I vesicles, however, is less clear. It is hotly debated whether COP I vesicles are involved in transport from the ER to the Golgi intermediate compartment and the Golgi (4, 5) and/or within the Golgi complex (anterograde transport) (6, 7) and/or from the Golgi back to the ER (retrograde transport) (8, 9). COP I proteins are also involved in transport along the endocytic pathway (10–12). The coat of COP I vesicles consists of eight proteins: seven COPs (13) and ADP-ribosylation factor (ARF) (14). The COPs are assembled in a heptameric protein complex, termed coatomer, that in addition to its membrane associated form it is also present as a soluble form in the cytoplasm (13). All coatomer subunits have been characterized at the molecular level and in mammalian cells are designated α- (135 kD), β- (107 kD), β′- (102 kD), γ- (97 kD), δ- (57 kD), ϵ- (36 kD), and ζ- (21 kD) COP (see (15) for a review).

In plant cells, coats are clearly visible at the electron microscope level, including typical clathrin coated buds and vesicles at the plasma membrane and *trans*-Golgi region and others as yet unidentified coats (16–18). The partially coated reticulum is the paradigm of coated regions (17, 19–21), which are probably involved in sorting events along the biosynthetic and/or the endocytic pathway (see (22) for a review). Coated regions can also be observed in multivesicular bodies, which are endosomal in nature (18, 23, 24). However, the nature of these coated regions remains to be established. Clathrin and adaptins have been identified in plants (18, 25–29). The same is true for proteins of the ARF family (30, 31). In this paper, we have used several antibodies raised against mammalian COP I proteins to identify putative coat proteins in rice (*Oryza sativa*) cells. We have found that these cells contain α-, β-, β′-, and γ-COP, and that these proteins may form a cytosolic complex, as in animal cells. In addition, our data suggest that COP-binding to membranes is GTP-dependent and probably involves ARF1 protein.

Abbreviations used: COP, coat protein; ARF, ADP-ribosylation factor; PMSF, phenylmethylsulfonyl fluoride; PNS, post-nuclear supernatant; SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

[1] To whom correspondence should be addressed at Departament de Bioquímica i Biología Molecular Facultat de Farmacia, Universitat de Valencia, Avgda. Vicent Andrés Estellés, s/n, 46100-Burjassot (Valencia), Spain. Fax: + 34 - 96 3864917. E-mail: Fernando.aniento@uv.es.

0006-291X/00 $35.00

MATERIALS AND METHODS

Antibodies. The M3A5 and maD anti-β-COP antibodies were kindly provided by the laboratory of Dr. Thomas Kreis (University of Geneva, Geneva, Switzerland). The CM1A10 anti β'-COP antibody was a gift of Dr. J. Rothman (Sloan-Kettering Institute, New York). The C1PL anti β'-COP antibody and the antibody against α/γ-COP were kindly provided by Dr. C. Harter (Biochemie-Zentrum Heidelberg, Ruprecht-Karls-Universität, Heidelberg, Germany). The monoclonal anti ARF1 antibody was from Affinity Bioreagents (ABR, Golden, USA).

Isolation of protoplasts. Protoplasts were obtained from rice (*Oryza sativa*) cells in culture, as previously described (32). Briefly, small pieces of rice calli were incubated at room temperature with low shaking in the following digestion mixture: 5 mM MES, 5 mM $CaCl_2$, 1 mM 2-mercaptoethanol, 0.5 M mannitol, 0.5% (w/v) BSA, 2% (w/v) cellulase Onozuka R-10, 1% (w/v) pectinase Macerozyme R-10 and 0.1% pectolyase Y-23, pH 5.7. After 1–2 h of incubation, protoplasts were sequentially filtered through nylon screens of 200, 50, and 30 μm mesh size and washed twice with 5 mM MES, 5 mM $CaCl_2$ and 0.5 M mannitol. Protoplast viability was determined by the methylene blue exclusion test.

Subcellular fractionation. Protoplasts were homogenized by osmotic lysis. Briefly, protoplasts were collected by centrifugation, diluted in a medium containing 25 mM Tris–HCl, pH 7.4, 1 mM DTT, 3 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 20 μM leupeptin, without sucrose, and homogenized by passage through a 22-G needle attached to a 1-ml syringe (six to ten times). After homogenization, sucrose concentration was brought to 0.3 M, to limit damage that may be caused to intracellular organelles, in particular to vacuoles. The resulting homogenate was then centrifuged and the postnuclear supernatant (PNS) was further centrifuged at 100,000*g*, to separate the microsomal membranes from the cytosol. PNS, membranes and cytosol were analyzed by SDS–PAGE followed by Western blot analysis with different antibodies against COP I coat proteins, and alkaline phosphatase-conjugated secondary antibodies.

Precipitation of COPI proteins by neomycin. A cytosolic fraction obtained as described above was diluted in a medium containing 25 mM Tris–HCl, pH 7.4, 1 mM DTT, 3 mM EDTA, 1 mM PMSF and 20 μM leupeptin, to give a final protein concentration of 1.5 mg/ml. To this, neomycin was added to a final concentration of 1 mM, incubated for 2 h at 4°C and then centrifuged at 16,000*g* for 30 min in an Eppendorf centrifuge. Pellets were dissolved in Laemmli sample buffer and subjected to SDS–PAGE followed by Western blot analysis with antibodies against α- and γ-COP or β'-COP.

Immunoprecipitation. Membrane and cytosolic fractions were diluted in immunoprecipitation buffer (IP: 25 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 1 mM PMSF, 20 μM leupeptin), incubated for 1 h on ice and then centrifuged at 13,000 rpm in a microcentrifuge (Heraeus Biofuge 13) for 10 min at 4°C to remove aggregates. 40 μl of protein A-Sepharose (Amersham Pharmacia Biotech; 50% slurry in IP) was added, and the mixture was incubated for 1 h at 4°C on a rotating wheel. The beads were removed by centrifugation and control or specific antibodies were added to the supernatant. After incubation for 2 h at 4°C on a rotating wheel, 40 μl of protein A-Sepharose was added and incubated for further 1 h at 4°C. When using a monoclonal antibody for the immunoprecipitation, a rabbit IgG against mouse IgG was previously bound to the protein A-Sepharose. The beads were collected by centrifugation, and washed 5 × with 1 ml of IP and then 1 × with 1 ml of phosphate-buffered saline. Proteins were eluted from the beads by boiling in Laemmli sample buffer and subjected to SDS–PAGE followed by silver staining or Western blot analysis.

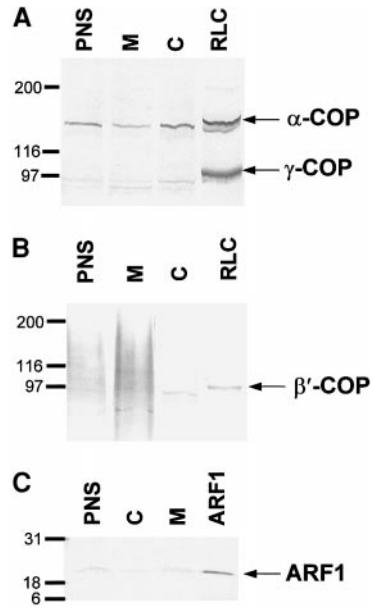

FIG. 1. COPI coat proteins in rice cells. (**A**) Western blot analysis of a postnuclear supernatant (PNS), membrane (M), and cytosolic (C) fractions from rice (*Oryza sativa*) cells with an antibody raised against α and γ-COP (52). Rat liver cytosol (RLC) is shown as a control. 30 μg protein was loaded in each lane. (**B**) Western blot analysis of the same fractions with the C1PL anti β'-COP antibody (35). (**C**) Western blot analysis of the same fractions with an anti ARF1 antibody. 3 μg wt ARF1 were loaded in the last lane.

RESULTS

Rice Cells Contain COP I Coat Proteins

We have used several antibodies raised against mammalian COPI coat proteins in order to look for putative homologues in rice (*Oryza sativa*) cells. Figure 1A shows a Western blot analysis of a post-nuclear supernatant (PNS), membrane (M), and cytosolic (C) fractions from these cells with a polyclonal antibody raised against mammalian α and γ-COP. For comparison, and as a reliable source of both proteins, we used rat liver cytosol (RLC). This antibody recognizes two bands in rice cytosol which have a slightly lower molecular weight (≈144 and 95 kD) than the corresponding bands in rat liver cytosol (≈150 and 100 kD). In the membrane fraction, one or two additional bands may also appear below the one of 95 kD, with an abundance which vary among different preparations. α-COP seems to cross-react better than γ-COP in the rice fractions. Nevertheless, it has to be considered that the latter appears to be also more sensitive to proteolysis, and its abundance also vary among different preparations (data not shown). Both antigens seem to be equally distributed between membranes and cytosol on a relative-protein basis. Figure 1B shows a Western blot analysis of the same fractions with the C1PL anti β'-COP antibody. This antibody recognizes in the cytosolic fraction a band of slightly lower molecular weight

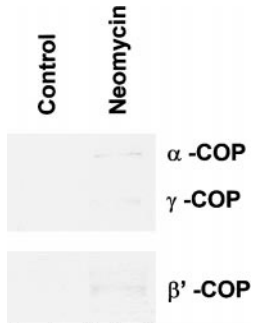

FIG. 2. Precipitation of COPI proteins with neomycin. A cytosolic fraction from rice cells was adjusted to a protein concentration of 1. 5 mg/ml and incubated in the absence (control) or in the presence of 1 mM neomycin for 2 h at 4°C. The mixture was then centrifuged at 16,000*g* for 30 min and the pellets were analyzed by SDS–PAGE (7% acrylamide) and Western blot analysis with an antibody against α- and γ-COP (52) or the C1PL anti β'-COP antibody (35).

(≈95 kD) than the one in rat liver cytosol (≈100 kD), while in the membrane fraction, the antibody gives a very diffuse signal. In addition, we have tested two monoclonal antibodies against mammalian β-COP (M3A5 and maD) which did not cross-react with a putative β-COP protein in rice fractions unless COPI proteins were enriched upon immunoprecipitation with anti β'-COP antibodies (see below, Figs. 3D–3E). Figure 1C shows that a protein with an apparent molecular mass of 21 kD crossreacts with a monoclonal ARF class I antibody prepared against mammalian ARF1. Therefore, rice cells contain also ARF1 protein, which in animal cells mediates the binding of coatomer to membranes (33).

COPI Proteins in Rice Cells May Exist as a Coatomer Complex in the Cytosol

In mammalian cells, COPI proteins are assembled in a heptameric protein complex, the coatomer. Therefore, we decided to investigate whether COPI proteins in rice cells may also exist as a similar protein complex in the cytosol. To this end, we first used the antibiotic neomycin, which has been shown to interact with dilysine binding sites on coatomer, cross-linking it into high molecular weight aggregates (34). Upon treatment of rice cytosol with 1 mM neomycin, several COPI subunits, including α, β'-, and γ-COP, could be pelleted at 16,000*g* for 30 min, conditions that do not sediment soluble coatomer in the absence of neomycin (Fig. 2).

If COPI proteins in rice cells are part of a cytosolic complex, it should then be possible to co-immunoprecipitate all the subunits under native conditions. Several antibodies against COPI subunits have shown their usefulness to immunoprecipitate the whole coatomer complex in mammalian cells. We have used two of those antibodies, the polyclonal C1PL (35) and the monoclonal CM1A10 (36), raised both against mammalian β'-COP, to immunoprecipitate a putative coatomer complex from rice cells. Figure 3A shows a silver stain of the immunoprecipitate of a cytosolic fraction from rice cells with the CM1A10 monoclonal antibody. The specific immunoprecipitate shows bands at around 140 kD (probably α-COP), a band around 95 kD (probably including β-, β'-, and γ-COP), a band around 60 kD (expected molecular weight for δ-COP), a band around 34 kD (expected molecular weight for ϵ-COP) and another band around 21 kD (expected molecular weight for ζ-COP). A very similar pattern was observed when we immunoprecipitated equivalent amounts of a membrane fraction or when the immunoprecipitation was carried out with the C1PL polyclonal antibody (data not shown). We have also analyzed the immunoprecipitates by Western blot analysis with the same antibodies described in Fig. 1. We could see that the immunoprecipitates contain α- and γ-COP, both in membranes and in cytosol (Fig. 3B), β'-COP (Fig. 3C), and β-COP (Figs. 3D–3E). Although we observed a slight heterogeneity in their mobility on SDS gels, the estimated molecular weight for the rice homologues of β-, β'-, and γ-COP, deduced from their mobility in Western blots as the ones shown in Figs. 3B–3E, was around 95 kD for the three proteins. This could explain that only a single band was observed in the silver stain of the immunoprecipitate of rice cytosol at around 95 kD, as compared with the triplet observed in rat liver cytosol for β-, β'-, and γ-COP (Fig. 3A). Therefore, all COPI subunits seem to co-immunoprecipitate with antibodies against β'-COP, of which four (α, β, β', and γ-COP) have been identified by Western blot analysis.

COP and ARF Recruitment onto the Membranes Is GTP-Dependent

We next explored whether the recruitment of COPs onto the membranes fulfilled the same characteristics of COP binding in animal cells. In particular, we assayed the effect of GTPγS, which in animal cells prevents the uncoating of COPI-coated vesicles and therefore enhances the binding of coatomer to membranes (37). We prepared a postnuclear supernatant (PNS) from protoplasts of rice cells, and incubated it in the presence of salts, an ATP regenerating system, and in the absence or the presence of 100 μM GTPγS, for 15 min at 37°C. Membranes and cytosol were then separated by centrifugation and analyzed by SDS–PAGE and Western blot analysis with the anti α/γ-COP antibody, to quantify the relative amounts of COPs in membranes and cytosol. As shown in Fig. 4, the amount of α-COP (Fig. 4A) and γ-COP (Fig. 4B) increased in the membrane fraction by around twofold upon GTPγS treatment (Fig. 4D), while decreasing in the cytosol fraction (data not shown). We then analyzed whether ARF1, which is present on these cells (Fig. 1C), and that in mammalian cells is required for COP

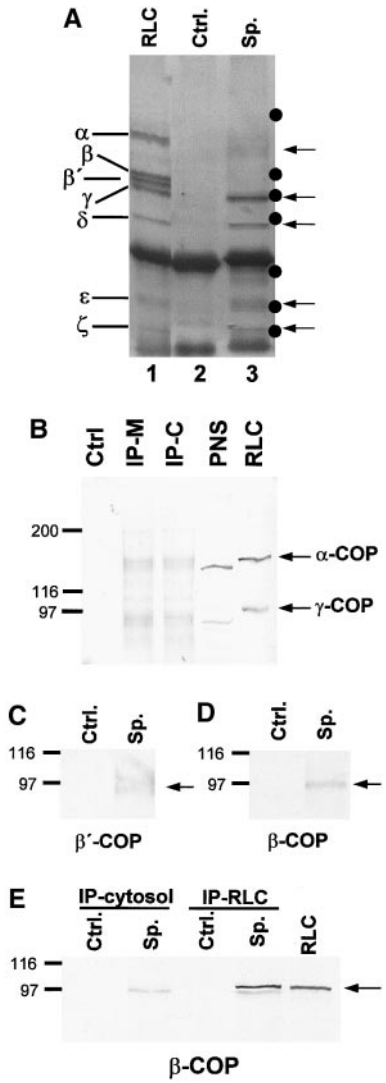

FIG. 3. Immunoprecipitation of a putative coatomer complex from rice cells. We have used two antibodies against β'-COP, the monoclonal CM1A10 (Figs. 2A, 2D, 2E) or the polyclonal C1PL (Figs. 2B–2C) coupled to protein A-sepharose to immunoprecipitate a putative coatomer complex from rice (*Oryza sativa*) cells, as described under Materials and Methods. (**A**) Immunoprecipitation of a cytosolic fraction from rice cells with the CM1A10 anti β'-COP antibody (specific, sp., lane 3) or with a control IgG (ctrl., lane 2), followed by SDS–PAGE (4–16% acrylamide) and silver staining of the immunoprecipitate. As a control, we immunoprecipitated equivalent amounts of rat liver cytosol (RLC, lane 1). Arrows in the right hand side point to bands in the specific immunoprecipitate which are not present in the control. Molecular weight markers are indicated by dots on the right hand side (21. 5, 31, 45, 66, 97, 116, and 200 kD). (**B**) Immunoprecipitation of membrane (IP-M) and cytosolic (IP-C) fractions with the C1PL anti β'-COP antibody, followed by Western-blotting with the anti α/γ-COP antibody. As a control, equivalent amounts of a membrane fraction were used for the immunoprecipitation but in the absence of antibody (Ctrl). The same result was obtained when using a cytosolic fraction for the control immunoprecipitation (data not shown). 30 μg of post-nuclear supernatant (PNS) and 10 μg rat liver cytosol (RLC) are loaded in the last two lanes. (**C**) Immunoprecipitation of a cytosolic fraction with the C1PL anti β'-COP antibody, followed by Western-blotting with the C1PL anti β'-COP antibody. (**D–E**) Immunoprecipitation of a cytosolic fraction with the CM1A10 anti β'-COP antibody, followed by Western-blotting with the M3A5 (**D**) or the maD (**E**) anti β-COP antibodies. As a control, we immunoprecipitated an equivalent amount of rat liver cytosol (RLC). 10 μg RLC were loaded in the last lane.

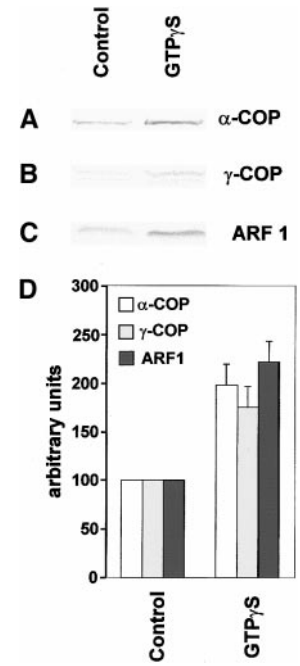

FIG. 4. COP and ARF1 recruitment onto the membranes is GTP-dependent. A postnuclear supernatant (PNS) from protoplasts of rice cells was incubated in the presence of 12. 5 mM Hepes, pH 7. 0, 1 mM DTT, 1 mM MgOAc, 60 mM KCl, an ATP regenerating system (11), and in the absence or the presence of GTPγS (100 μM), for 15 min at 37°C. Membranes and cytosol were then separated by centrifugation (100,000*g*, 30 min) and analyzed by SDS–PAGE and Western blotting with the anti α/γ-COP antibody (**A, B**), or with the anti ARF 1 antibody (**C**). Western blots exposed in the linear range of detection were quantitated using the Scion Image program, to measure the amounts of α- and γ-COP and ARF1 bound to membranes (**D**). To facilitate the comparison, the amount of each of the three proteins in the controls was normalized to 100.

binding, was itself recruited onto the membranes during the assay. As shown in Fig. 4C, ARF1 was also recruited, and recruitment was also GTP-dependent, increasing by about twofold in the presence of GTPγS (Fig. 4D), as it is the case with α- or γ-COP.

DISCUSSION

In mammalian cells, COPI has been proposed to be involved in several steps of membrane traffic, including ER to Golgi anterograde transport, intra-Golgi transport, Golgi to ER retrograde transport and transport along the endocytic pathway (4–12). Although profiles of budding COP-like vesicles (60–90 nm diameter, sometimes with a visible nap-type coat) on the cisternae of plant Golgi stacks have been described and

appear to be coated (38), actual evidence that they do represent COP-coated vesicles is not yet available. Recently, the group of D. G. Robinson has shown the presence in cauliflower (*Brassica oleracea*) inflorescence homogenates of homologues of Sec21p (γ-COP, a 100 kD subunit of the coatomer), and Sec23p (an 85 kD component of the COP II coat) (39). cDNAs corresponding to other COP coat proteins have been also identified in a search of the expressed sequence tag database (40). Thus, at the gene level, plant cells seem to possess coat proteins of the COP I and COP II families.

In this paper, we have used several antibodies raised against mammalian COPI coat components and have found that several of them cross-react with putative homologues in rice cells. Detection of the different COPI coat proteins required an effective inhibition of vacuolar proteases, but gamma-COP was specially susceptible to proteolysis, in particular in the membrane fraction. Indeed, lower molecular weight bands were often observed in this fraction in the γ-COP region (see Fig. 1A). This is consistent with *in vitro* binding studies with coatomer and the cytoplasmic tail peptide of p23 (a member of the p24 family of integral membrane proteins which is highly enriched in COPI-coated vesicles) (41). These studies revealed that the p23 peptide induces specific polymerization of the coatomer complex which is accompanied by a conformational change of the complex. In aggregated, p23-bound coatomer, the gamma-subunit shows an increased susceptibility to protease treatment compared to soluble coatomer.

In support of the idea that all COPI components exist as a complex in the cytosol (coatomer), we could immunoprecipitate them with the C1PL as well as with the CM1A10 anti β′-COP antibodies. Both antibodies have shown their ability to immunoprecipitate the whole coatomer complex in animal cells (35, 36). Silver staining of the immunoprecipitate showed specific bands at the expected molecular weights for the different subunits (α-, β-, β′-, γ-, δ-, ϵ-, and ζ-COP). Western blot analysis confirmed that the immunoprecipitates obtained with anti β′-COP antibodies contain not only β′-COP but also α-, β-, and γ-COP (Fig. 3). Therefore, it is fairly possible that COPI proteins in rice cells also exist as a coatomer complex in the cytosol. In mammalian cells, this coatomer complex has to be recruited *en bloc* to membranes for vesicle formation to occur (42). Further support to this idea comes from experiments using the antibiotic neomycin, which has a coatomer binding site and can effectively cross-link coatomer into large, sedimentable aggregates (34). We could indeed observe that, in the presence of neomycin, α-, β′-, and γ-COP from rice cytosol could be pelleted at very low centrifugal forces, which do not normally sediment soluble coatomer (Fig. 2). In agreement with our observations, Movafeghi *et al.* (39) have shown that AtSec21p (a putative γ-COP homologue in *Arabidopsis*) elutes in a Sepharose column in a fraction with a molecular mass somewhat greater than 700 kD, consistent with the molecular mass of the coatomer in mammalian cells. In addition, the same authors found that the AtSec21p antigen can also be precipitated in the presence of neomycin.

Once established that rice cells contain several components of the COPI coat, we next investigated the mechanism of coat recruitment onto the membranes. In mammalian cells, binding of the COPI coatomer to membranes requires the small GTP binding protein ARF1, and the binding appears to occur sequentially, with ARF1 followed by the coatomer (33). ARF (ADP-ribosylation factor) belongs to the family of the small GTP binding proteins (20 kD) and was originally identified as a cofactor for cholera toxin-catalyzed ADP-ribosylation of the Gs subunit of the trimeric G protein (43). To date, five distinct human ARF proteins have been identified: ARF 1, 3, 4, 5, 6 (44, 45). They are highly homologous (more than 60% identity) and conserved throughout evolution. As for the Ras-related small GTP-binding proteins, ARF has also consensus sequences for GTP binding and hydrolysis (even though its intrinsic GTP hydrolysis activity is very low). All ARFs contain the N-terminal myristoylation consensus sequence MGXXXS/AT, and are myristoylated on the glycine residue (36). The myristoylation is clearly required for efficient GTP-dependent binding of ARF to membrane (33, 46). Not only binding of ARF to membranes is GTP-dependent, but also binding of ARF to the coatomer (47). ARF is activated by a brefeldin A sensitive guanine nucleotide exchange factor (GEF) on the Golgi membrane that catalyzes the exchange of GDP for GTP (48–50). Genes homologous to ARF and Sar 1 (a component of the COPII coat) have been identified from a number of higher plants (31, 51). A cDNA clone encoding an ARF protein has been isolated from *Arabidopsis* (31). Here, we have observed that a putative ARF1 homologue is present in rice cells (Fig. 1C). Since the binding onto the membranes of both α- and γ-COP is stimulated by GTPγS and ARF1 is itself recruited onto the membrane in a GTPγS-stimulated manner (Fig. 4), it is tempting to postulate that ARF1 may mediate COP binding to membranes, as in animal cells. Future studies should concentrate on investigating the precise localization of the COPI coat, as well as other coats, and on their functional characterization.

ACKNOWLEDGMENTS

We would like to thank María Jesús Marcote and Jean Gruenberg for critically reading the manuscript. This work was supported by grants from the Ministerio de Educación y Cultura (Grant PB99-1425) and Consellería de Cultura, Educación y Ciencia (Grant GV99-86-1-05) to F. Aniento. E.O.Z. was a Research Fellow from the Ministerio de Educación y Cultura (Spain).

Note added in proof. Since our manuscript was accepted for publication, the group of D. G. Robinson has submitted a paper to

Plant Cell also dealing with plant COPI coat proteins (Pimpl, P., Movafeghi, A., Coughlan, S., Denecke, J., Hillmer, S., and Robinson, D. G. (2000) *In situ* localization and *in vitro* induction of plant COPI-coated vesicles).

REFERENCES

1. Robinson, M. S. (1994) The role of clathrin, adaptors and dynamin in endocytosis. *Curr. Opin. Cell Biol.* **6,** 538–544.
2. Simpson, F., Peden, A. A., Christopolou, L., and Robinson, M. S. (1997) Characterization of the adaptor-related protein complex, AP-3. *J. Cell Biol.* **137,** 835–845.
3. Dell'Angelica, E. C., Klumperman, J., Stoorvogel, W., and Bonifacino, J. S. (1998) Association of the AP-3 adaptor complex with clathrin. *Science* **280,** 431–434.
4. Pepperkok, R., Scheel, J., Horstmann, H., Hauri, H. P., Griffiths, G., and Kreis, T. E. (1993) Beta-COP is essential for biosynthetic membrane transport from the endoplasmic reticulum to the Golgi complex. *Cell* **74,** 71–82.
5. Scales, S. J., Pepperkok, R., and Kreis, T. E. (1997) Visualization of ER to Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. *Cell* **90,** 1137–1148.
6. Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J. E., and Wieland, F. T. (1991) A coat subunit of Golgi-derived non- clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein beta-adaptin. *Nature* **349,** 215–220.
7. Ostermann, J., Orci, L., Tani, K., Amherdt, M., Ravazzola, M., Elazar, Z., and Rothman, J. E. (1993) Stepwise assembly of functionally active transport vesicles. *Cell* **75,** 1015–1025.
8. Letourneur, F., Gaynor, E. C., Hennecke, S., Demolliere, C., Duden, R., Emr, S. D., Riezman, H., and Cosson, P. (1994) Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. *Cell* **79,** 1199–1207.
9. Orci, L., Stammes, M., Ravazzola, M., Amherdt, M., Perrelet, A., Sollner, T. H., and Rothman, J. E. (1997) Bidirectional transport by distinct populations of COPI-coated vesicles. *Cell* **90,** 335–349.
10. Whitney, J. A., Gomez, M., Sheff, D., Kreis, T. E., and Mellman, I. (1995) Cytoplasmic coat proteins involved in endosome function. *Cell* **83,** 703–713.
11. Aniento, F., Gu, F., Parton, R. G., and Gruenberg, J. (1996) An endosomal β-COP is involved in the pH-dependent formation of tranport vesicles destined for late endosomes. *J. Cell Biol.* **133,** 29–41.
12. Gu, F., Aniento, F., Parton, R. G., and Gruenberg, J. (1997) Functional dissection of COP-I subunits in the biogenesis of multivesicular endosomes. *J. Cell Biol.* **139,** 1183–1195.
13. Waters, M. G., Serafini, T., and Rothman, J. E. (1991) "Coatomer": A cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. *Nature* **349,** 248–251.
14. Serafini, T., Orci, L., Amherdt, M., Brunner, M., Kahn, R., and Rothman, J. E. (1991) ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: A novel role for a GTP-binding protein. *Cell* **67,** 239–253.
15. Harter, C. (1999) COP I proteins: A model for their role in vesicle budding. *Protoplasma* **207,** 125–132.
16. Emons, A. M. C., and Traas, J. A. (1986) Coated pits and vesicles on the plasma membrane of plant cells. *Eur. J. Cell Biol.* **41,** 57–64.
17. Fowke, L. C., Tanchak, M. A., and Galway, M. E. (1991) Ultrastructural cytology of the endocytic pathway in plants. *In* Endocytosis, Exocytosis and Vesicle Traffic in Plants (Hawes, C. R., Coleman, J. O. D., and Evans, D. E., Eds.), pp. 15–40, Cambridge University Press, Cambridge, UK.
18. Robinson, D. G., Hinz, G., and Holstein, S. E. H. (1998) The molecular characterization of transport vesicles. *Plant Mol. Biol.* **38,** 49–76.
19. Pesacreta, T. C., and Lucas, W. J. (1985) Presence of a partially coated reticulum and a plasma membrane coat in angiosperms. *Protoplasma* **125,** 173–184.
20. Hillmer, S., Freundt, H., and Robinson, D. G. (1988) The partially coated reticulum and its relationship to the Golgi apparatus in higher plant cells. *Eur. J. Cell Biol.* **47,** 206–212.
21. Tanchak, M. A., Rennie, P. J., and Fowke, L. C. (1988) Ultrastructure of the partially coated reticulum and dictyosomes during endocytosis by soybean protoplasts. *Planta* **175,** 433–441.
22. Marcote, M. J., Gu, F., Gruenberg, J., and Aniento, F. (2000) Membrane transport in the endocytic pathway: Animal versus plant cells. *Protoplasma* **210,** 123–132.
23. Hillmer, S., Depta, H., and Robinson, D. G. (1986) Confirmation of endocytosis in higher plant protoplasts using lectin-gold conjugates. *Eur. J. Cell Biol.* **41,** 142–149.
24. Robinson, D. G., and Hinz, G. (1997) Vacuole biogenesis and protein transport to the plant vacuole: A comparison with the yeast vacuole and the mammalian lysosome. *Protoplasma* **197,** 1–25.
25. Coleman, J. O. D., Evans, D. E., Hawes, C. R., and Cole, L. (1987) Structure and molecular organization of higher plant coated vesicles. *J. Cell Sci.* **88,** 35–45.
26. Coleman, J. O. D., Evans, D. E., Hosley, D., and Hawes, C. R. (1991) The molecular structure of plant clathrin and coated vesicles. *In* Endocytosis, Exocytosis and Vesicle Traffic in Plants (Hawes, C. R., Coleman, J. O. D., and Evans, D. E., Eds.), pp. 41–63, Cambridge University Press, Cambridge, UK.
27. Holstein, S. E. H., Drucker, M., and Robinson, D. G. (1994) Identification of a β-type adaptin in plant clathrin-coated vesicles. *J. Cell Sci.* **107,** 945–953.
28. Beevers, L. (1996) Clathrin-coated vesicles in plants. *Int. Rev. Cytol.* **167,** 1–35.
29. Robinson, D. G. (1996) Clathrin-mediated trafficking. *Trends Plant Sci.* **1,** 349–355.
30. Memon, A. R., Clark, G. B., and Thompson, G. A. (1993) Identification of an ARF type low molecular mass GTP-binding protein in pea (*Pisum sativum*). *Biochem. Biophys. Res. Commun.* **193,** 809–813.
31. Regad, F., Bardet, C., Tremonsaygne, D., Moisan, A., Lesure, B., and Axelos, M. (1993) cDNA cloning and expression of an Arabidopsis GTP-binding protein of the ARF family. *FEBS Lett* **316,** 133–136.
32. Cornejo, M. J., Luth, D., Blankenship, K. M., Anderson, O. D., and Blechl, A. E. (1993) Activity of a maize ubiquitin promoter in transgenic rice. *Plant Mol. Biol.* **23,** 567–581.
33. Palmer, D. J., Helms, J. B., Beckers, C. J., Orci, L., and Rothman, J. E. (1993) Binding of cotomer to Golgi membranes requires ADP-ribosylation factor. *J. Biol. Chem.* **268,** 12083–12089.
34. Hudson, R. T., and Draper, R. K. (1997) Interaction of coatomer with aminoglycoside antibiotics: Evidence that coatomer has at least two dilysine binding sites. *Mol. Biol. Cell* **8,** 1901–1910.
35. Stenbeck, G., Harter, C., Brecht, A., Herrmann, D., Lottspeich, F., Orci, L., and Wieland, F. (1993) β'-COP, a novel subunit of coatomer. *EMBO J.* **12,** 2841–2845.
36. Orci, L., Palmer, D. J., Ravazzola, A., Perrelet, M., Amherdt, M., and Rothman, J. E. (1993) Budding of Golgi membranes requires the coatomer complex of non-clathrin coat proteins. *Nature* **362,** 648–652.

37. Donaldson, J. G., and Klausner, R. D. (1994) ARF: A key regulatory switch in membrane traffic and organelle structure. *Curr. Opin. Cell Biol.* **6,** 527–532.
38. Hawes, C. R., Faye, L., and Satiat-Jeunemaitre, B. (1996) The Golgi apparatus and pathways of vesicle trafficking. *In* Membranes: Specialized Functions in Plants (Smallwood, M., Knox, J. P., and Bowles, D. J., Eds.), pp. 337–365, BIOS Publishers, Oxford, UK.
39. Movafeghi, A., Happel, N., Pimpl, P., Tai, G.-H., and Robinson, D. G. (1999) Arabidopsis Sec21p and Sec23p homologs. Probable coat proteins of plant COP-coated vesicles. *Plant Physiol.* **119,** 1437–1445.
40. Andreeva, A. V., Kutuzov, M. A., Evans, D. E., and Hawes, C. R. (1998) Proteins involved in membrane transport between the ER and the Golgi apparatus: 21 putative plant homologues revealed by dbEST searching. *Cell Biol. Int.* **22,** 145–160.
41. Reinhard, C., Harter, C., Bremser, B., Brügger, B., Sohn, K., Helms, J. B., and Wieland, F. (1999) Receptor induced polymerization of coatomer. *Proc. Natl. Acad. Sci. USA* **96,** 1224–1228.
42. Hara-Kuge, S., Kuge, O., Orci, L., Amherdt, M., Ravazzola, M., Wieland, F. T., and Rothman, J. E. (1994) *En bloc* incorporation of coatomer subunits during the assembly of COP-coated vesicles. *J. Cell Biol.* **124,** 883–892.
43. Kahn, R. A., and Gilman, A. G. (1984) Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. *J. Biol. Chem.* **259,** 6228–6234.
44. Kahn, R. A., Kern, F. G., Clark, J., Gelmann, E. P., and Rulka, C. (1991) Human ADP-ribosylation factors. A functionally conserved family of GTP-binding proteins. *J. Biol. Chem.* **266,** 2606–2614.
45. Tsuchiya, M., Price, S. R., Tsai, S. C., Moss, J., and Vaughan, M. (1991) Molecular identification of ADP-ribosylation factor mRNAs and their expression in mammalian cells. *J. Biol. Chem.* **266,** 2772–2777.
46. Haun, R. S., Tsai, S. C., Adamik, R., Moss, J., and Vaughan, M. (1993) Effect of myristoylation on GTP-dependent binding of ADP-ribosylation factor to Golgi. *J. Biol. Chem.* **268,** 7064–7068.
47. Zhao, L., Helms, J. B., Brunner, J., and Wieland, F. T. (1999) GTP-dependent binding of ADP-ribosylation factor to coatomer in close proximity to the binding site for dilysine retrieval motifs and p23. *J. Biol. Chem.* **274,** 14198–14203.
48. Donaldson, J. G., Cassel, D., Kahn, R. A., and Klausner, R. D. (1992) ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein beta-COP to Golgi membranes. *Proc. Natl. Acad. Sci. USA* **89,** 6408–6412.
49. Donaldson, J. G., Finazzi, D., and Klausner, R. D. (1992) Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. *Nature* **360,** 350–352.
50. Helms, J. B., and Rothman, J. E. (1992) Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. *Nature* **360,** 352–354.
51. Bar-Peled, M., and Raikhel, N. V. (1997) Characterization of AtSEC12 and AtSAR1. Proteins likely to be involved in endoplasmic reticulum and Golgi transport. *Plant Physiol.* **114,** 315–324.
52. Gerich, B., Orci, L., Tschohner, H., Lottspeich, F., Ravazzola, M., Amherdt, M., Wieland, F., and Harter, C. (1995) Non-clathrin-coat protein α is a conserved subunit of coatomer, and in *Saccharomyces cerevisiae* is essential for growth. *Proc. Natl. Acad. Sci. USA* **92,** 3229–3233.