A Membrane Protein Enriched in Endoplasmic Reticulum Exit Sites Interacts with COPII*

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Although all mammalian COPII components have now been cloned, little is known of their interactions with other regulatory proteins involved in exit from the endoplasmic reticulum (ER). We report here that a mammalian protein (Yip1A) that is about 31% identical to S. cerevisiae and which interacts with and modulates COPII-mediated ER-Golgi transport. Yip1A transcripts are ubiquitously expressed. Transcripts of a related mammalian homologue, Yip1B, are found specifically in the heart. Indirect immunofluorescence microscopy revealed that Yip1A is localized to vesicular structures that are concentrated at the perinuclear region. The structures marked by Yip1A co-localized with Sec31A and Sec13, components of the COPII coat protein complex. Immunoelectron microscopy also showed that Yip1A co-localizes with Sec13 at ER exit sites. Overexpression of the hydrophilic N terminus of Yip1A arrests ER-Golgi transport of the vesicular stomatitis G protein and causes fragmentation and dispersion of the Golgi apparatus. A glutathione S-transferase fusion protein with the hydrophilic N terminus of Yip1A (GST-Yip1A) is able to bind to and deplete vital components from rat liver cytosol that is essential for in vitro vesicular stomatitis G transport. Peptide sequence analysis of cytosolic proteins that are specifically bound to GST-Yip1A revealed, among other proteins, mammalian COPII components Sec23 and Sec24. A highly conserved domain at the N terminus of Yip1A is required for Sec23/Sec24 interaction. Our results suggest that Yip1A is involved in the regulation of ER-Golgi traffic at the level of ER exit sites.

Molecular and biochemical characterization of the cellular components (for recent reviews, see Refs. 1 and 2) responsible for the formation of transport vesicles from the ER is important for the understanding of the mechanisms underlying protein export from the ER. Three cytosolic components: Sar1p, the Sec23p-Sec24p complex, and the Sec13p-Sec31p complex, represented the sole cytosolic requirements for vesicle formation from yeast microsomal membranes reconstituted in vitro (3). Together, these proteins form the COPII coat complex (1, 4). The COPII coat complex is responsible for vesicle budding from the ER. It is structurally and functionally distinct from the coatamer or COPI coat complex (5). The primary function of the latter is believed to mediate retrograde transport in the early secretory pathway (6–8), but has also been shown to be essential for ER-Golgi transport (9, 10).

An outline of the events leading to COPII vesicle budding from the ER is now known (11, 12). In the initiation of COPII vesicle budding, the small GTPase Sar1p (13) is first recruited onto the ER membrane, at least in part, through a guanine nucleotide exchange reaction catalyzed by the ER membrane protein Sec12p (14). Activated, GTP bound Sar1p on the membrane then recruits the Sec23p-Sec24p complex (15). Sec23p is catalytically a GTPase activating protein of Sar1p (16). Sec24p apparently does not influence the GTPase activating protein activity of Sec23p and although essential, its exact function is unclear (15). The resulting Sar1p/Sec23p-Sec24p complex appears to be important for cargo packaging (11, 12). The subsequent recruitment of the Sec13p-Sec31p complex (3, 17–21) drives the final steps of vesicle formation through a yet undefined mechanism. COPII components, on their own, are sufficient to drive vesicle formation from chemically defined liposomes (22, 23).

All the corresponding mammalian homologues of yeast COPII components have now been cloned (17, 19–21, 24–28). Antibodies against the mammalian COPII components (17, 19–21, 25) provided the first morphological definition of COPII-budding sites, or ER exits sites (ERES) (29), on specific regions of the ER. These appear as vesicular “spotty” structures by light microscopy. The ERES are located in discrete positions of the ER reticulum and are found with the highest density at the perinuclear region, presumably where the ER membranes are in close apposition to the Golgi apparatus. At the ultrastructural level, immunogold labeling of COPII components can be found in distinct vesiculotubular structures devoid of ribosomes. These structures appear to be similar to structures where several viral proteins accumulate upon a low temperature (15 °C) treatment and which had been defined in terms such as vesicle-tubular structures (30) and the ER-Golgi intermediate compartment (31).

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The abbreviations used are: ER, endoplasmic reticulum; ERES, endoplasmic reticulum exit sites; PCR, polymerase chain reaction; FITC, fluorescein isothiocyanate; NRK, normal rat kidney; VSVG, VSV G protein; EST, expressed tag sequence; PAGE, polyacrylamide gel electrophoresis.
Although a rough picture of the process of COPII budding has now emerged as outlined above, the mechanistic details are still missing. It is not known, for example, how each step of complex binding and interaction are regulated or coordinated. The COPII components are after all, cytosolic, and regulatory functions are probably executed by membrane components of the budding machinery. Little is known about the membrane components of the ERES, other than the expected presence of the mammalian homologue of the Sar1 GEF, the mammalian Sec12. We now show that mammalian membrane protein Yip1A, whose corresponding yeast homologue interacts with the yeast transport GTPase Ypt1p (32), is found at the ERES. Interestingly, Yip1A interacts with the Sec23-Sec24 complex of mammalian COPII.

MATERIALS AND METHODS

Clones and Constructs—Data base searches were performed with the various Basic Local Alignment Search Tools (BLAST) algorithms (33, 34) available at the National Center for Biotechnology (NCBI) World Wide Web server. Human and mouse expressed sequence tag (EST) clones were generated by the Washington University-MERCK EST projects, and were obtained from the IMAGE consortium via Research Genetics Inc.

Polymerase chain reactions (PCR) were carried out using high fidelity Fidelity polymerase Strategene. Fragments of the human Yip1A were generated by PCR and cloned into pGEX-4T vector (Amersham Pharmacia Biotech) for the production of fusion proteins in bacteria or into a modified mammalian expression vector based on pCMx (Promega) for Myc-tagged constructs.

DNA and Protein Work—Library screening, cloning, and DNA sequencing were performed using standard methods as described (35). Northern blot analyses were performed using mouse multiple tissue Northern blot from CLONTECH (Palo Alto, CA). 2 μg of poly(A)+ RNA was present in each lane of the blot. DNA fragments corresponding to the divergent N-terminal regions of Yip1A and Yip1B were generated by PCR and used as probes. Peptide microsequencing was carried out using the QSTAR system (PerkinElmer Life Sciences) based on electrospray ionization mass spectrometry.

Antibodies—Polyclonal antibodies against mammalian Sec13 (20) and Sec31 (21) have been described previously. Antiserum against human Sec23A was kindly provided by Dr. Jean-Pierre Paccaud (University of Geneva Medical Center, Switzerland) (25). Polyclonal antibodies against Yip1A in this study were obtained by immunization of mice and rabbits with a soluble glutathione S-transferase fusion construct corresponding to the hydrophilic N-terminal cytoplasmic region of Yip1A (GST-Yip1Acya).

Immunofluorescence Microscopy—Cells were maintained in RPMI medium supplemented with 10% fetal bovine serum. Immunofluorescence microscopy was performed as described previously (21). Cells plated on coverslips were fixed with 4% paraformaldehyde followed by sequential incubation with the primary antibodies and FITC or rhodamine-conjugated secondary antibodies. Fluorescence labeling was visualized using an Axioskop microscope (Carl Zeiss, Inc., Thornwood, NY) with epifluorescence optics or MRC1024 (Bio-Rad) confocal laser optics.

Electron Microscopy—Cryosectioning and immunogold labeling for electron microscopy were performed as described previously (21, 36).

ER-Golgi Transport Assay and ER Budding—The ER to Golgi transport assay using semi-intact cells was performed as described previously (20, 21). Briefly, NRK cells grown to confluency were infected with VSVts045 for 1 h at 32 °C and then shifted to 40 °C for 2 h to accumulate the coat from a strain with a dominant negative ERES protein in the ER. VSV-infected NRK cells were harvested and homogenized using the Balch’s homogenizer in 0.375 M sorbitol in 20 mM Hepes KOH, pH 7.2. The homogenate was centrifuged briefly to remove nuclei and large debris, adjusted to 0.25 mM sorbitol, and centrifuged at top speed for 5 min to pellet the microsomes. Microsome were resuspended in the transport assay mixture as above and the budding reaction initiated by the addition of cytosol and incubation at 32 °C for 20 min. The reaction mixture was then adjusted to 0.25 mM sorbitol and 150 mM potassium acetate, and centrifuged at 20,000 rpm (TLA 100 rotor, Beckman Instruments) for 1 min to generate the medium-speed pellet. The medium-speed supernatant was then centrifuged at 60,000 rpm with the same rotor to generate the high-speed pellet.

RESULTS

Molecular Characterization of Yip1p-like Mammalian Proteins—Several mammalian (human and mouse) Yip1p-like sequences exist in the data base as EST clones. Complete DNA sequencing of some of these EST clones revealed two mammalian genes encoding proteins that share about 31% overall identity with yeast Yip1p (32) and are about 61% identical to each other. We have named them Yip1A and Yip1B, respectively. An alignment between Yip1p, mouse Yip1A and mouse Yip1B is shown in Fig. 1A.

Data base search using the Yip1A sequence revealed a family of homologues in Caenorhabditis elegans (F32D8.4, T21659), Drosophila melanogaster (CG12404, AAF53235), Schizosaccharomyces pombe (T41464), and Arabidopsis thaliana (AAD21436) (Fig. 1B). These proteins do not contain any discernable known functional domains. However, that they are members of a conserved family is also clearly illustrated by the similarity of their respective hydrophilicity profile (Fig. 1C). In addition to their overall homology, all of them have a hydrophilic N-terminal half followed by a hydrophobic C-terminal half with multiple potential membrane spanning domains. Yeast Yip1p had been shown to be a membrane protein with the hydrophilic N terminus facing the cytoplasm (32).

Northern blot analysis with a cDNA fragment specific for Yip1A revealed two transcripts of about 1.8 and 2.8 kilobases in length with a fairly ubiquitously expression (Fig. 1D). Yip1B, on the other hand, is encoded by a small transcript of about 1 kilobase in length and is found specifically in the heart.

Subcellular Localization of Yip1—To investigate the subcellular location of Yip1A, we first expressed Myc-tagged Yip1A and Yip1B in cells. Indirect immunofluorescence with Myc antibody revealed that both Myc-tagged Yip1A and Yip1B are localized to membrane structures with a reticular ER-like background but are mainly concentrated at the juxtanuclear region (Fig. 2, upper panels). The ER-like reticular background is more obvious for highly expressing cells. To investigate the subcellular localization of the endogenous protein, antibodies against the N-terminal hydrophilic portion of Yip1A were raised. Affinity purified rabbit antibodies detected a specific protein band of about 29 kDa in cell lysates (data not shown). Detection of the endogenous Yip1A in both primate (HLa and Vero) and rodent (NRK) cells by indirect immunofluorescence
**Fig. 1. Mammalian Yip1-like proteins.** A, alignment of the amino acid sequences of mouse Yip1A and Yip1B with the yeast Yip1p (MegAlign program of DNASTAR). Residues identical in two of the three proteins are shaded. B, table indicating the percentage of similarity and divergence between Yip1 homologues in several species aligned by the MegAlign program of DNASTAR. Shown here are the *Sacharomyces cerevisiae* Yip1p (32) and homologues from *C. elegans* (F52D8.4, T21659), *D. melanogaster* (CG12404, AAF53235), *S. pombe* (T41464), and *A. thaliana* (AAD21436). C, Kyte-Doolittle hydrophilicity plot of some of the Yip1 family members, generated by the Protoblast profiling of DNASTAR. D, tissue distribution of mouse Yip1A and Yip1B by Northern blot analysis. DNA fragments corresponding to the divergent N-terminal regions of Yip1A and Yip1B were generated by PCR and used as probes.
using Yip1A antibodies revealed a pattern of vesicular structures. Although these structures can be found throughout the cell, they are concentrated at the juxtanuclear region (Fig. 2, lower panels).

The labeling pattern of Yip1A is reminiscent of markers of the early secretory pathway. There is considerable co-localization between Yip1A with the KDEL receptor (38) at the light microscopy level (Fig. 3, upper panels). Furthermore, like that of the KDEL receptor, the staining of Yip1A became more vesicular and scattered upon BFA treatment (Fig. 3, middle panels), a property characteristic of proteins in the early secretory pathway (38–41). Similarly, treatment with the microtubule disrupting drug, nocodazole, scattered the juxtanuclear labeling of both Yip1A and the KDEL receptor (38) into smaller membrane islets with a more peripheral distribution.

The labeling pattern of Yip1A is also highly reminiscent of that of mammalian COPII components at the ERES (20, 21, 25). Double labeling of Yip1A and the COPII component Sec31A indeed revealed a high degree of co-localization between these two proteins on membrane structures (Fig. 4A). This suggests that Yip1A is localized to the ERES. We further examined the subcellular localization of Yip1A at the ultrastructural level. Immunogold labeling of Yip1A can be found around but not within the Golgi stacks (Fig. 4B) and are particularly enriched in structures with a vesiculated membrane profile around the Golgi apparatus. These structures are also

**Fig. 2. Subcellular localization of Yip1.** Localization of Myc-tagged Yip1A and Yip1B transiently expressed in HeLa cells (upper panels) and endogenous Yip1A in HeLa (human) or Vero (simian) or normal rat kidney (rat) cells by indirect immunofluorescence microscopy. Cells were fixed with 4% paraformaldehyde and incubated with an anti-Myc mouse monoclonal antibody (clone 9E10) (upper panels) or affinity-purified rabbit anti Yip1A antibody. This is followed by incubations with FITC-labeled anti-mouse or anti-rabbit IgG, respectively. Bar, 10 μm.

**Fig. 3. Co-localization of Yip1A with the KDEL receptor (KDEL-R) in normal, brefeldin A-treated, or nocodazole treated cells.** Vero cells grown on coverslips were either untreated (control) or treated with 10 μg/ml brefeldin A (BFA) or 10 μg/ml nocodazole (Nocodazole) for 1 h. Cells were then fixed with 4% paraformaldehyde and incubated with mouse monoclonal antibody against KDEL-R and rabbit polyclonal antibodies against Yip1A, followed by incubation with FITC-labeled anti-rabbit IgG and Texas Red-labeled anti-mouse IgG, respectively. Bar, 10 μm.
Therefore, consistent with the results obtained at the light microscopy level, Yip1A is enriched the ERES.

In view of the extensive co-localization of Yip1A with both the KDEL receptor and COPII components and bearing in mind that the KDEL receptor exhibits dynamic recycling between the ER and the Golgi, we examined to what extent is Yip1A distributed between the ERES and the cis-Golgi. A low temperature treatment (15°C for 3 h) had been known to result in a block in transport at the ER-Golgi boundary, in the so-called “15°C compartment” (31, 42, 43). We have previously shown that upon release from this 15°C block, the KDEL receptor can be observed to move toward the juxtanuclear Golgi region (38). Incubation of cells at 15°C resulted in excellent co-localization of Yip1A with the KDEL receptor and Sec31 in structures that appeared less juxtanuclear and more scattered throughout the cell periphery as compared with untreated cells (Figs. 5, A and B, upper panel). 10 min upon transfer of the cells to 37°C, the majority of the labeling of all three proteins became more concentrated at the juxtanuclear region (Fig. 5, A and B, middle panel). The concentration occurs to differing degrees for the three proteins. Whereas the peripheral labeling for the KDEL-R almost exclusively became concentrated at the juxtanuclear region, a substantial number of peripheral spotty structures remained labeled with Sec31 and Yip1A (Fig. 5, A and B, lower panels). Yip1A is therefore behaving more like...
Sec31 than the KDEL receptor in terms of its dynamics upon release from the 15 °C block. We have previously shown that structures labeled with the COPII component Sec13 become delocalized from those labeled with the anterograde cargo VSVG not long after its exit from the ER (20). To follow the labeling of Yip1A with an anterograde cargo, we double-labeled vesicular stomatitis virus (VSV)Vts045 infected cells for Yip1A and the VSVG at various time points after transferring cells incubated at 40 to 32 °C. As shown in Fig. 6, the best co-localization between Yip1A and VSVG occurs at 4 min after release from the temperature restriction (upper panel). At this time point a majority of the VSVG appeared to be co-localized with Yip1A at the ERES. There is already fairly apparent segregation of Yip1A and VSVG 7 min after release (second panel). At 15 min after release, VSVG is found exclusively at the juxtanuclear Golgi region (third panel). On the other hand, a significant number of peripheral spots with Yip1A labeling alone were observed. Even at the juxtanuclear region where the Yip1A labeling are more intense, Yip1A and VSVG labels do not appear to coincide. The former appeared to be a continuous large membranous structure resembling the Golgi apparatus, while the latter remained vesicular, very much like its peripheral counterparts. The segregation between Yip1A and VSVG is obvious at the 40-min time point. These results are consistent with the notion that Yip1A resides preferentially at the ERES.

Yip1A Is Involved in ER-Golgi Transport—Mutant yeast cells depleted of Yip1p and conditionally lethal yip1 mutants accumulate ER membranes and are defective in protein secretion (32). In view of its homology to Yip1p and the fact that it is enriched in the ERES, we sought to determine if Yip1A is involved in ER-Golgi transport. We reasoned that the N-terminal hydrophilic cytoplasmic domain of Yip1A might have a dominant negative effect when expressed in cells through its ability to compete for important interactions intrinsic to the endogenous Yip1A. We have therefore made an expression construct, mycYip1Acyto, that would express the cytoplasmic domain of Yip1A when transfected into cells.

We first asked if the expression of mycYip1Acyto would in any way affect the transport of the VSVG from the ER to the cell surface. Cells transiently transfected for 15 h were infected with VSVVts045 at 32 °C and viral protein synthesis and trans-

![Image](http://www.jbc.org/content/early/2018/07/25/JBC.201800431F.large.jpg)
As shown in Fig. 7A, double labeling for VSVG and mycYip1Acyto in two different cell types revealed that while there is significant cell surface labeling of VSVG 5 h post-infection in untransfected non-expressing cells, VSVG in cells expressing the cytoplasmic portion of Yip1A is accumulated at a juxtanuclear structure. It is apparent that efficient transport of VSVG to the cell surface is inhibited in cells expressing mycYip1Acyto at the ER-Golgi boundary.

We further observed that overexpression of mycYip1Acyto results in the disruption of the Golgi apparatus. Although not apparent in cells with low expression (not shown), cells expressing moderate to high levels of the cytoplasmic portion of Yip1A at 36–48 h after transfection do not have an intact Golgi apparatus (marked by the Golgi marker galactosyl transferase) (Fig. 7B). The inhibition of VSVG transport and the disruption of the Golgi apparatus by overexpression of the cytosolic portion of Yip1A implicate the involvement of the protein in regulating ER-Golgi transport.

**Yip1A Binds to the Sec23-Sec24 Complex and Is Efficiently Incorporated into COPII Vesicles in Vitro**—The observed effect of myc-Yip1Acyto on the ER-Golgi transport and Golgi structure prompted us to further investigate the molecular basis of this dominant negative effect. We first examined whether the cytoplasmic domain of Yip1A could be interacting with a factor(s) in the cytosol that is important for ER-Golgi transport. To establish this possibility, we incubated rat liver cytosol with either GST bound to glutathione beads or GST-Yip1Acyto bound to glutathione beads and their ability to support ER-Golgi transport was assessed using the permeabilized cell assay (“Materials and Methods”). Transport setups with a complement of 5 µl of untreated cytosol was used either as a positive control (Normal cytosol) or kept on ice as a negative control (on ice). A dose range of 1, 3, 5, 7, and 9 µl of the depleted cytosols were compared in the assay. The upper bands in the autoradiogram represent the endo-H-resistant Golgi forms of Sec23 and Sec24. Molecular sizes indicated on the left are in kDa. B, GST-Yip1Acyto and GST bound to glutathione beads were incubated with rat liver cytosol (2–3 mg). Bound proteins were eluted (beads) and 1/10 of the respective depleted cytosols (cytosol) were resolved on SDS-PAGE gel and blotted onto a nitrocellulose membrane. The membrane was then processed for Western immunoblot with anti-Sec23 antibody (20).

![Fig. 8](image-url)

**Fig. 8.** GST-Yip1Acyto depletes the ER-Golgi transport activity of rat liver cytosol. Rat liver cytosol is incubated with either GST bound to glutathione beads (GST-depleted cytosol) or GST-Yip1Acyto bound to glutathione beads (GST-Yip1Acyto depleted cytosol) and their ability to support ER-Golgi transport was assessed using the permeabilized cell assay (“Materials and Methods”). Transport setups with a complement of 5 µl of untreated cytosol was used either as a positive control (Normal cytosol) or kept on ice as a negative control (on ice). A dose range of 1, 3, 5, 7, and 9 µl of the depleted cytosols were compared in the assay. The upper bands in the autoradiogram represent the endo-H-resistant Golgi forms of Sec23 and Sec24. Molecular sizes indicated on the left are in kDa. B, GST-Yip1Acyto and GST bound to glutathione beads were incubated with rat liver cytosol (2–3 mg). Bound proteins were eluted (beads) and 1/10 of the respective depleted cytosols (cytosol) were resolved on SDS-PAGE gel and blotted onto a nitrocellulose membrane. The membrane was then processed for Western immunoblot with anti-Sec23 antibody (20).

![Fig. 9](image-url)

**Fig. 9.** Yip1A binds to the Sec23-Sec24 COPII subcomplex. A, GST-Yip1Acyto and GST-VAP33Bcyto bound to glutathione beads were incubated with rat liver cytosol and bound proteins were eluted and resolved on a 7% SDS-PAGE gel. Distinct bands were cut out and subjected to peptide microsequencing. Arrows indicate bands corresponding to mammalian Sec23 and Sec24. Molecular sizes indicated on the left are in kDa. B, GST-Yip1Acyto and GST bound to glutathione beads were incubated with rat liver cytosol (2–3 mg). Bound proteins were eluted (beads) and 1/10 of the respective depleted cytosols (cytosol) were resolved on SDS-PAGE gel and blotted onto a nitrocellulose membrane. The membrane was then processed for Western immunoblot with anti-Sec23 antibody (20).
be explained by the defective transport activity of GST-Sec24 COPII subcomplex from the cytosol may, at least in part, with GST-Yip1Acyto suggests that the depletion of the Sec23-Res13 and Sec24, respectively.

It was revealed that two of these bands correspond to mammalian Sec23 and Sec24, respectively. As shown in Fig. 9A, several distinct proteins were specifically retained by GST-Yip1Acyto. In doing this, we scaled up the binding was performed as above and identical sets of the respective medium speed pellets (M) and high-speed pellets (H) were resolved on SDS-PAGE and processed for Western immunoblot with a monoclonal antibody against VSVG. B, COPII budding was performed as above and identical sets of the respective medium speed pellets (M) and high-speed pellets (H) were resolved on SDS-PAGE and processed for Western immunoblot with a monoclonal antibody against VSVG or affinity-purified polyclonal antibody against Yip1A.

depleting property is specific to GST-Yip1Acyto. GST fusion protein of the cytoplasmic domain of VAP33B (GST-VAP33B)cyto, another protein that is localized to the ER-Golgi boundary (also named ERG30 (44), did not exhibit any ability to deplete the cytosol of components important for ER-Golgi transport (not shown).

We next investigated the components in the cytosol interacting with GST-Yip1Acyto. In doing this, we scaled up the binding reaction of cytosol to GST-Yip1Acyto beads and resolved the bound proteins by SDS-PAGE. Proteins specifically retained by GST-Yip1Acyto were then subjected to peptide microsequencing. As shown in Fig. 9A, several distinct proteins were specifically eluted from GST-Yip1Acyto that are not present in the eluate from GST-VAP33Bcyto. Upon peptide microsequencing, it was revealed that two of these bands correspond to mammalian Sec23 and Sec24, respectively.

Sec23 and Sec24 form a subcomplex of the COPII coat that is responsible for vesicle budding from the ER. Their interaction with GST-Yip1Acyto suggests that the depletion of the Sec23-Sec24 COPII subcomplex from the cytosol may, at least in part, be explained by the defective transport activity of GST-Yip1Acyto preincubated cytosol. We confirmed this interaction by immunoblotting. As shown in Fig. 9B, GST-Yip1Acyto, but not GST itself, binds to Sec23, resulting in a corresponding depletion of Sec23 in the cytosol. Our preliminary mapping of the region of Yip1A that interacts with Sec23 indicates that a region corresponding to amino acid residues 75–106 is important for Sec23 interaction (not shown). Interestingly, this region is also the most conserved region among Yip1 homologues from different species, suggesting a conserved biochemical property underlying Yip1 function.

The interaction between Yip1A and COPII components raised the question as to whether it is incorporated into COPII vesicles. To investigate this, we performed an in vitro COPII budding assay according to Rowe et al. (37) using microsomes prepared from a VSVts045-infected NRK cell line expressing elevated amounts of Yip1A (see “Materials and Methods”). The infected cells were kept for 2 h at the non-permissive temperature of 40 °C to accumulate the mutant VSVG in the ER. As shown in Fig. 10A, the appearance of VSVG in the high-speed pellet (which is indicative of vesicle budding) is dependent on cytosol. Furthermore, the appearance of VSVG in the high-speed pellet is also largely dependent on COPII as it is inhibited to a significant extent by antibody against mammalian Sec13, but not control IgG. Like VSVG, Yip1A also appears in the high-speed pellet in a cytosol-dependent manner (Fig. 10B). This result suggests that Yip1A is incorporated into COPII vesicles generated by the microsome.

**DISCUSSION**

All components of the mammalian COPII coat complex have now been identified, and a rough picture of the sequence of events that occur during COPII vesicle formation has recently emerged. However, the exact mechanistic action of each of the components and their regulation by other components remained largely unknown. In an in vitro system, vesicle budding can be reconstituted using chemically defined lipids and purified COPII components (22, 23). The tremendous simplicity of the system provided important insights to the processes of transport vesicle formation, such as the notion that there is no obligatory requirement for a membrane protein or cargo protein for a vesicle to form. The process of COPII vesicle formation reconstituted as such, however, occurs at a much lower efficiency as compared with vesicle budding in vivo. Therefore, although the basic requirement may have been satisfied, it is unlikely to reflect the myriad of protein-protein interactions that occur physiologically during COPII vesicle budding.

Recent findings from several different laboratories have highlighted several interactions between the soluble COPII components and other proteins. It is well known that in yeast, the membrane protein Sec12p serves as the guanine nucleotide exchange factor for Sar1p and is largely responsible for the recruitment of Sar1 onto the membrane (14). In the in vitro system mentioned above, the requirement for Sec12p can be bypassed by using activated Sar1p. Furthermore, the interaction between activated Sar1p and the vesicle SNARE Bet1p is essential for the formation of a priming complex that initiates the subsequent steps of COPII component recruitment (22, 45). Similarly, Sec16p, a large multidomain protein that may have a docking function, interacts with Sec23p, Sec24p, and Sec31p (46, 47). Mammalian Sec23A has been recently shown to interact directly with p125, a protein with homology to phospholipid-modifying proteins (48). Yeast Sec24p has also been shown to interact specifically with the cis-Golgi syntaxin Sed5p (49). Although the significance of the last two interactions is not yet known, they have been predicted to serve important regulatory functions.

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2. B. L. Tang and W. Hong, unpublished results.
We now report a membrane protein that is enriched at the ERES and binds to Sec23/Sec24. We have named this protein Yip1A because of its homology to yeast Yip1p. Interestingly, the yeast Yip1p was discovered in a two-hybrid screen as a protein that interacts with Ypt1p, the yeast equivalent of mammalian Rab1 (50, 51). We have not been able to show unambiguously a direct interaction between Yip1A and Rab1 (data not shown). On the other hand, evidence for the interaction between Yip1A and the Sec23-Sec24 complex is rather compelling. First of all, Yip1A co-localized well with COPII components at the ERES. Second, GST-Yip1Acyto was able to bind and pull down in a quantitative manner the Sec23-Sec24 subcomplex from rat liver cytosol. This ability is corroborated by the ability of GST-Yip1Acyto to deplete an activity in the cytosol that is essential for ER-Golgi transport. Furthermore, Sec23/24 interaction is mapped to a region of Yip1A that is highly conserved among Yip1-like proteins from different species.

Our morphological data strongly suggest that Yip1A (at least a large fraction of it) is found in the ERES. The ERES has always been mentioned in the same light as morphological intermediates in ER-Golgi transport, such as the ERGIC (31) or the vesicular-tubular clusters (30). The availability of antibodies against COPII components further defined the ERES as discrete ER subdomains marked by these components (20, 21, 25). Recent observations with GFP-labeled COPII components in live cells have revealed that these are relatively long-lived and immobile structures (52, 53), that give rise to mobile anterograde cargo containers that may move from the cell periphery to a juxtanuclear position in a microtubule-dependent manner (54, 55). We have previously noted that although Sec13 containing structures may change in terms of overall cellular distribution with various treatments, they remained discrete and co-localized with the anterograde cargo VSVG only at the early time points upon ER exit (20). It would appear that, based on its behavior after released from the 15 °C block and its gradual delocalization from VSVG upon release from the temperature arrest, Yip1A primarily resides in the ERES. If so, it would be the first documented resident membrane protein of the ERES. The specific interaction of Yip1A with COPII components also supports this notion.

The yeast Yip1p, on the other hand, appears to be localized to the Golgi apparatus (32). It is possible that there exists other Yip1 isoforms in mammalian cells that are Golgi-localized. Another possibility, which warrants further investigation, is that the Yip1p staining pattern seen in yeast may actually be that of the yeast ERES. The S. cerevisiae ER-Golgi boundary is not well defined morphologically. Unlike the single, compact, perinuclear stack found in mammalian cells, the yeast Golgi apparatus takes the form of several small stacks that are distributed to different regions in the cytoplasm. Since the ERES is most concentrated in regions in close apposition to the Golgi, the Yip1p staining pattern may therefore resemble that of yeast Golgi markers.

A further point to note is that Yip1A appears to be efficiently incorporated into COPII vesicles, at least in vitro. The efficiency of Yip1A incorporation (~60% of total) is even higher than the anterograde cargo VSVG (~15% of total). This is in agreement with the results of Otte et al. (56), who showed that the yeast Yip1p is very efficiently incorporated into yeast COPII vesicles in vitro, much more so than several ER vesicle (Erv) proteins, ER-Golgi SNAREs, and an early Golgi marker. However, our morphological data have indicated that there appear to be no bulk movement of Yip1A with the anterograde cargo from the ER to the Golgi. One possible explanation for this is that Yip1A incorporated into COPII vesicles are very quickly recycled back to the ERES instead of following the mobile cargo containers to the Golgi. This property may have important implications with regards to the physiological function of Yip1A.

What may the physiological function of Yip1A be? The presence of homologues of the protein from yeast to human and its conservation throughout evolution would suggest that it performs an essential function. Its enrichment at the ERES and its interaction with Sec23-Sec24 complex strongly suggest that it may serve to modulate COPII-mediated transport. The first evidence for this is the ability of the cytoplasmic domain of Yip1A to inhibit the surface expression of VSVG when overexpressed in cells. VSVG appeared to be blocked in the region of the ER-Golgi boundary. Furthermore, high levels of expression of Yip1Acyto also caused the disruption of the Golgi apparatus. This phenomenon is reminiscent to that of other perturbations that result in inhibition of ER-Golgi transport, such as microinjection of Rab1 mutants (57) or a negative dominant form of Sar1 in cells (58).

On the other hand, Yip1A may not be essential for COPII vesicle formation per se. It may, for example, serve as an adaptor that recruits specific soluble or peripheral components into COPII vesicles. In this case, its role may be simply that of a cargo adaptor and its interaction with Sec23 will then be of limited significance in as far as regulation of COPII budding is concerned. Consistent with this possibility, antibodies generated against GST-Yip1Acyto did not inhibit VSVG transport in the permeabilized cell assay to any significant extent (data not shown). However, it is possible that the Yip1A antibody does not interfere with the binding of the Sec23-Sec24 complex.

Although we were not able to demonstrate any direct binding between Yip1A and Rab1 in vitro, it is still possible that the interaction occurs, nonetheless, in vivo. If Yip1A does interact with both COPII components and Rab1, the speculative implications would be of great interest. In as far as interaction with Rab1 alone, Yip1A may function simply by recruiting Rab1 to membranes, as either a guanine nucleotide exchange factor or, reminiscent to the case of Rab9, a GDP-dissociation inhibitor displacement factor (59). Its dual interactions with both Rab1 and COPII, however, may provide a mechanism whereby Rab1 can be incorporated into COPII vesicles.

There are several documented cases where Rabs have a role in vesicle formation (60). Rab5 was recently demonstrated to be required for ligand sequestration into clathrin-coated pits (61). A dominant negative GDP-binding form of rab9, rab9 S21N affects accumulation of the mannose 6-phosphate receptor in transport vesicles, resulting in a reduction of mannose 6-phosphate receptor recycling (62). Of particular relevance is, as mentioned above, that the Rab1-GDP binding mutant inhibits ER-Golgi transport (57). Rab1 may not necessarily function in COPII vesicle budding per se, but there is accumulating evidence that suggest that Rabs must be incorporated during vesicle formation. This is presumably a regulatory measure to ensure such that a vesicle has all the necessary machinery in place for the downstream docking and fusion process. Balech and colleagues (63) have recently shown that Rab1 recruits the tether protein p115 into a cis-SNARE complex associated with COPII vesicles, thus programming the vesicle for docking and fusion. Yip1A may thus serve to recruit Rab1 into COPII vesicles via its interaction with Sec23-Sec24 subcomplex.

In any case, the Sec23-Sec24 subcomplex may well not be the only components required for ER-Golgi transport that can interact with Yip1A. We have not been able to replenish transport activity of the depleted cytosol with a high molecular weight cytosolic fraction enriched in the Sec23-Sec24 subcomplex (data not shown), indicating that GST-Yip1Acyto may

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have also depleted some other components essential for transport that is not found in the fraction.

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