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The Mammalian Homolog of Yeast Sec13p Is Enriched in the Intermediate Compartment and Is Essential for Protein Transport from the Endoplasmic Reticulum to the Golgi Apparatus

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The role of COPII components in endoplasmic reticulum (ER)-Golgi transport, first identified in the yeast *Saccharomyces cerevisiae*, has yet to be fully characterized in higher eukaryotes. A human cDNA whose predicted amino acid sequence showed 70% similarity to the yeast Sec13p has previously been cloned. Antibodies raised against the human SEC13 protein (mSEC13) recognized a cellular protein of 35 kDa in both the soluble and membrane fractions. Like the yeast Sec13p, mSEC13 exist in the cytosol in both monomeric and higher-molecular-weight forms. Immunofluorescence microscopy localized mSEC13 to the characteristic spotty ER-Golgi intermediate compartment (ERGIC) in cells of all species examined, where it colocalized well with the KDEL receptor, an ERGIC marker, at 15°C. Immunoelectron microscopy also localized mSEC13 to membrane structures close to the Golgi apparatus. mSEC13 is essential for ER-to-Golgi transport, since both the His_6-tagged mSEC13 recombinant protein and the affinity-purified mSEC13 antibody inhibited the transport of restrictive temperature-arrested vesicular stomatitis virus G protein from the ER to the Golgi apparatus in a semi-intact cell assay. Moreover, cytosol immunodepleted of mSEC13 could no longer support ER-Golgi transport. Transport could be restored in a dose-dependent manner by a cytosol fraction enriched in the high-molecular-weight mSEC13 complex but not by a fraction enriched in either monomeric mSEC13 or recombinant mSEC13. As a putative component of the mammalian COPII complex, mSEC13 showed partially overlapping but mostly different properties in terms of localization, membrane recruitment, and dynamics compared to that of β-COP, a component of the COPI complex.

Biochemical assays developed to provide insight into mechanisms regulating vesicular transport between the *cis*- and medial Golgi apparatus have led to a working model of coat protein-mediated budding. In the case of the vesicles coated with the coatomer (COPI) complex, the process is initiated by the sequential binding of the small GTPase, ARF1 (21), and the seven-subunit coatomer complex (19, 66) from the cytosol. Coatomer and ARF1 in fact represent the minimal cytosolic requirement for the generation of Golgi-derived buds and vesicles (41). These proteins assemble into a coat distinct from clathrin that remains stably associated with the surface of vesicles generated in the presence of a nonhydrolyzable GTP analog, GTPγS (49). Coatomer has also been shown to be involved in anterograde endoplasmic reticulum (ER)-Golgi transport. Antibodies against β-COP inhibited the transport of the vesicular stomatitis virus (VSV) temperature-restricted G protein (VSVG) from the ER to the Golgi apparatus both in vivo (43) and in vitro (44). Also, a ε-COP mutant CHO line is defective in ER-Golgi transport at the nonpermissive temperature (16). Homologs for several coatomer subunits have been identified in the yeast *Saccharomyces cerevisiae*. Yeast β-COP, β′-COP, and γ-COP are products of the *SEC26*, *SEC27*, and *SEC21* genes (24, 26, 61), respectively, and all of the corresponding mutants are blocked in ER-Golgi transport. Recent data from work done with the yeast system have suggested that coatomer also plays an essential role in retrograde Golgi-to-ER transport, at least in the retrieval of dilysine-tagged proteins (31). At the ultrastructural level (39), β-COP has been localized mainly to the *cis*-Golgi region that colocalizes with markers of the intermediate compartment (27) and at 15°C to the intermediate compartment by light microscopy (15, 34) and electron microscopy (15). These data lend support to the notion that one of its places of function is at the ER-Golgi boundary.

Combined genetic and biochemical approaches with yeast have identified a distinct set of proteins required for vesicle budding from the ER (2). In the presence of GTP and ATP, the requirement for cytosol in the budding of fusion-competent vesicles from the yeast ER can be satisfied in vitro by Sar1p, the Sec23p complex, and the Sec13p complex (50, 51). Sar1p is a small GTPase (38) that functionally interacts with Sec12p, an ER integral membrane protein (8, 37) which is the GDP/GTP exchange molecule for Sar1p (3). Sec23p is functionally the Sar1p-specific GTPase-activating protein (67) and is found in the cytosol as a 400-kDa complex with a 105-kDa protein, Sec24p, which has no apparent effect on Sec23p's GTPase-activating protein activity but is itself important for ER-Golgi transport (22). The Sec13p complex migrates at 700 kDa and is composed of two proteins with WD-40 (or β-transducin-like) repeats: Sec13p itself (47, 51) and a 150-kDa pro-

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tein, later identified to be Sec31p (68). There is apparent mechanistic similarity in the overall cycle of vesicle budding between that driven by ARF and the coatomer and that driven by the Sec13p-Sec23p complexes identified in yeast. Both are regulated by a small GTPase, and in both cases the cytosolic proteins required for vesicle formation are retained on the vesicles in the presence of nonhydrolyzable GTP analogs. The difference in the molecular compositions of the two coat complexes led to the proposal of the terms COPI for the former and COPII for the latter (2).

Although the function of yeast COPII molecules in ER-Golgi transport has been investigated through the availability of the corresponding mutants, investigations into the function of COPII homologs in mammalian cells have been lacking largely because of technical difficulties in setting up a corresponding vesicle budding assay with posttranslationally inserted cargo marker and the lack of direct molecular markers to study the components involved. Early implications for the participation of mammalian COPII components in ER-Golgi transport came from the localization of the putative mammalian Sec23p (based on immuno-cross-reactivity with the yeast Sec23p) to the transitional elements of the ER (42). Two isoforms of the mammalian Sar1p homolog had recently been cloned, and a *trans*-dominant mammalian SAR1 (mSAR1) mutant with a preferential affinity for GDP is shown to inhibit ER-Golgi transport but not transport between sequential Golgi compartments (28). A human cDNA encoding a protein 53% identical to yeast Sec13p had also been isolated (63), and genetic assays of complementation and synthetic lethality demonstrated that there was conservation of function between this human protein and yeast Sec13p (60), but a direct functional analysis of the role the human Sec13p homolog (mSEC13) in ER-Golgi transport has not been performed.

It is generally understood that ER-Golgi transport represents the first vesicular transport step in the exocytotic pathway (23, 45). However, it is not known with any certainty exactly how many vesicle budding and fusion steps are involved in a single round of cargo transport from the ER to the Golgi apparatus. There is now considerable evidence for the existence of a morphologically defined ER-Golgi intermediate compartment (ERGIC) (23, 27, 54, 58, 59). In studies monitoring ER cargo en route to the Golgi apparatus, the envelope proteins of Semliki Forest virus (52) and VSV (15, 35) can be accumulated during ER-to-Golgi transport by a low-temperature (15°C) treatment in spotty structures which are distributed throughout the cell periphery. At the ultrastructural level, these seem to be made up of clustered vesicles and short tubules (35). The ERGIC is also distinguished by the existence of several markers. A 53-kDa lectin-like (12) molecule, ERGIC53, identified by a monoclonal antibody against Golgi fractions of human CaCo2 cells (56–59) is enriched in a compartment with morphological and sedimentation properties different from those of the ER and Golgi apparatus. A homolog of the primate ERGIC53 in rodent cells has an apparent molecular mass of 58 kDa (30, 53, 54). Both of these proteins, which accumulate in spotty structures upon incubation at 15°C (54, 58, 64), were shown to drastically change their immunofluorescent localization patterns upon a temperature switch from 15 to 37°C. Based on such changes, it has been suggested that these proteins recycle between the ER and Golgi apparatus, although clear biochemical evidence for this model has yet to be obtained. A number of different functions have been attributed to the ERGIC. It has been proposed to be the site of protein palmitylation (6), retrieval of misfolded proteins (18) and ER resident proteins, and a compartment mediating ER-Golgi transport (46, 58). Recent evidence suggests that it may be a compartment where segregation of the ER-Golgi anterograde and retrograde cargo occurs (1, 64).

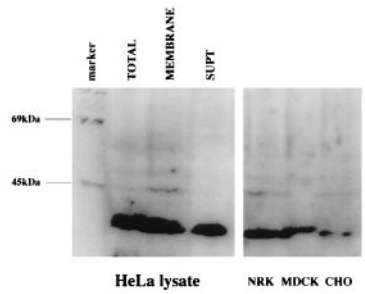

FIG. 1. mSEC13 is found in both membrane-associated and soluble fractions. In the case of total HeLa lysate as well as NRK, MDCK, and CHO cell lysates, cell were harvested by centrifugation and lysed in sample buffer containing 2% SDS. For preparing membrane-associated (MEMBRANE) and soluble (SUPT) fractions, cells were homogenized in phosphate-buffered saline by being repeatedly passed through an 18-gauge needle until more than 95% of cell were disrupted. The lysate was then centrifuged at 100,000 × *g* in a benchtop ultracentrifuge for 30 min. The resulting pellet is the membrane-associated fraction, and the supernatant is the soluble fraction. Total lysates and fractions were separated by SDS-polyacrylamide gel electrophoresis and subjected to immunoblotting.

To morphologically and functionally characterize the mammalian homolog of Sec13p, we have raised polyclonal antibodies against a hexahistidine-tagged recombinant form of the protein. Both the recombinant protein and the affinity-purified antibody inhibited ER-Golgi transport in a biochemical assay. Moreover, we were able to abolish transport activity from the cytosol by the specific immunodepletion of mSEC13 and subsequently restored transport activity with a gel filtration fraction enriched in the 700-kDa mSEC13 complex. The antibody localized the majority of the membrane-associated fraction of mSEC13 to the ER and the ERGIC. Using indirect immunofluorescence, we have also compared the membrane recruitment and subcellular dynamics of mSEC13 to that of β-COP, as well as performed colocalization studies with the anterograde cargo VSVG under various conditions of temperature manipulation.

MATERIALS AND METHODS

Cell culture media and fetal bovine serum were from Gibco Laboratories (Grand Island, N.Y.). Fluorescein isothiocyanate- or rhodamine-conjugated goat anti-mouse immunoglobulin and sheep anti-rabbit immunoglobulin were from Boehringer Mannheim Corp. (Indianapolis, Ind.). [^{35}S]methionine (>1,000 Ci/mmol) was from Amersham Corp. Common chemical reagents were from Sigma Chemical Co. (St. Louis, Mo.). Ni^{2+}-nitrilotriacetic acid resin for metal chelation chromatography was obtained from Qiagen. M1 cells were kindly provided by Victor Hsu and Richard Klausner (National Institutes of Health); other cell lines were purchased from the American Type Culture Collection. A monoclonal antibody against the KDEL receptor was raised against the C-terminal peptide sequence of the bovine KDEL receptor (65). Monoclonal antibodies against β-COP and VSVG were from Thomas Kreis (University of Geneva, Geneva, Switzerland). The mSEC13 cDNA was kindly provided by Anand Swaroop (University of Michigan). A polyclonal antiserum against mSEC13 was obtained by immunizing rabbits with a hexahistidine-tagged recombinant protein and affinity purified with the recombinant protein coupled to CNBr-activated Sepharose. Both the antiserum and the affinity-purified mSEC13 antibody were examined for the ability to detect mSEC13 specifically by immunofluorescence and immunoblotting. In both analyses, the flowthrough fraction of the affinity column was negative for mSEC13 antibody and the observed signals were abolished when incubation was carried out in the presence of recombinant mSEC13.

Immunofluorescence microscopy and confocal microscopy were performed as previously described (64, 65). Cells were cultured as a monolayer on glass coverslips and fixed with 2.7% paraformaldehyde, permeabilized with 0.1% saponin, and then incubated sequentially with primary and fluorescein isothiocyanate- or rhodamine-conjugated secondary antibodies. Fluorescence labeling was visualized by using an Axiophot microscope (Carl Zeiss, Inc., Thornwood, N.Y.) with epifluorescence optics and photographed with Kodak Tri-X 400 film or with

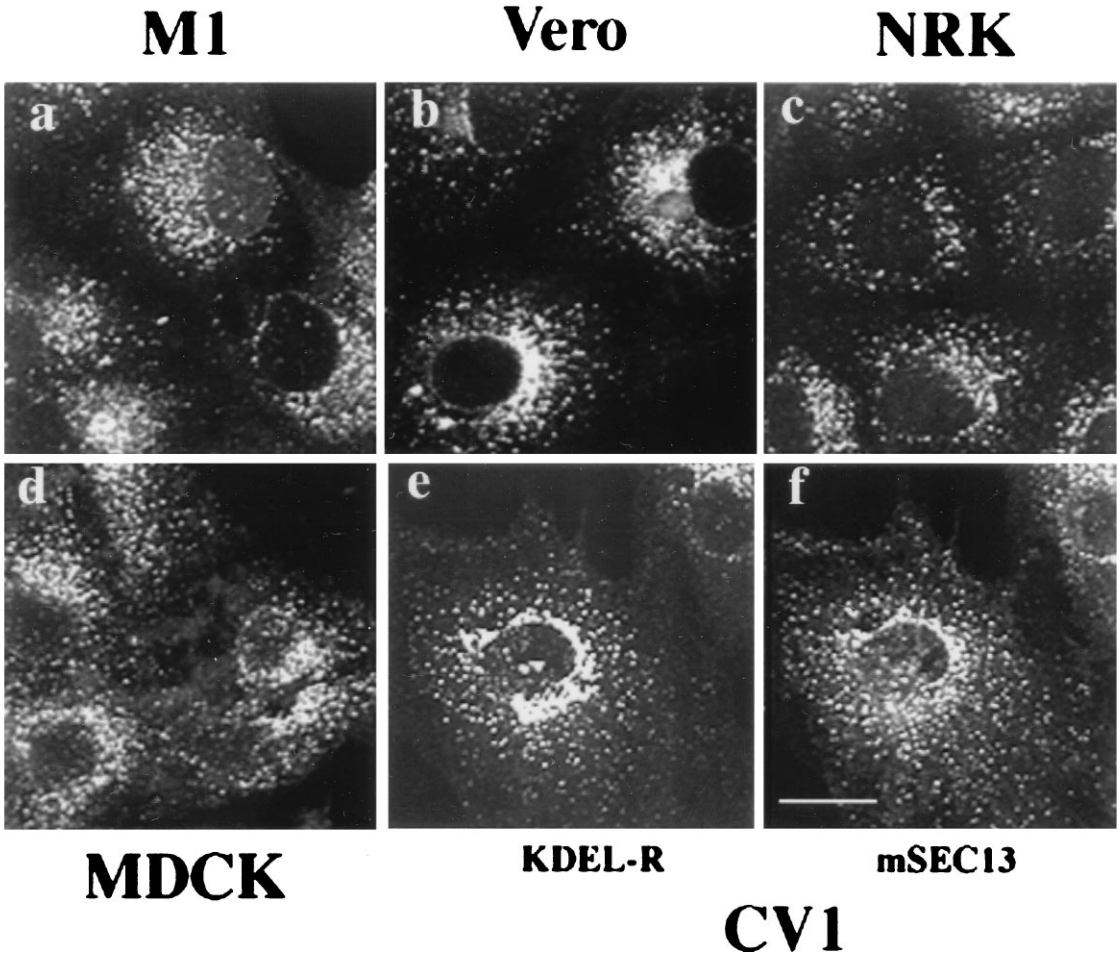

FIG. 2. mSEC13 localization in cells by indirect immunofluorescence. M1 (a), Vero (b), NRK (c), and MDCK (d) cells were fixed and incubated with an anti-mSEC13 antibody followed by a secondary anti-rabbit antibody coupled to rhodamine. CV1 cells were incubated at 15°C for 3 h, fixed, and incubated with an anti-mSEC13 antibody (f) and a monoclonal antibody against the KDEL receptor (e) followed by secondary antibodies. Bar = 10 μm.

a similar microscope connected to a Bio-Rad MRC600 confocal optics system (Bio-Rad Laboratories, Richmond, Calif.).

Mouse L cells were permeabilized with streptolysin O in the presence of the nonhydrolyzable GTP analog GTPγS as previously described (14, 27). GTPγS served to retain, in permeabilized cells, membrane association of molecules whose interactions with the membrane are dependent on the activity of a GTP-binding protein. Immunogold labeling and electron microscopy of thawed cryosections were performed as previously described (13, 14, 27).

Metabolic labeling of cells, subsequent analysis of pulse-labeled protein by immunoprecipitation, and Western immunoblotting were performed as previously described (36, 65).

The biochemical assay to measure transport of VSVG from the ER to the *cis*-Golgi apparatus was performed as described earlier (4, 9). Briefly, NRK cells were grown on 10-cm-diameter petri dishes to form a confluent monolayer and infected with a temperature-sensitive strain of VSV, *ts*045. The cells were pulse-labeled with [^{35}S]methionine at the restrictive temperature and mechanically permeabilized on ice. These semi-intact cells were then incubated in an assay cocktail of 40 μl containing (in final concentrations) 25 mM HEPES-KOH (pH 7.2), 90 mM potassium acetate, 2.5 mM magnesium acetate, 5 mM EGTA, 1.8 mM $CaCl_2$, 1 mM ATP, 5 mM creatine phosphate, 0.2 IU of rabbit muscle creatine phosphokinase, 25 μg of rat liver cytosol (9), and 5 μl of semi-intact cells (ca. 1×10^{-5} to 2×10^{-5} cells). Additional reagents were added as indicated in Results. For a standard assay, samples were incubated for 90 min at 32°C and transport was terminated by transfer to ice. The membranes were collected by a brief spin, solubilized in 0.1% sodium dodecyl sulfate (SDS), and digested overnight with endo-β-*N*-acetylglucosaminidase H (endo H). Subsequently, 5× concentrated gel sample buffer (29) was added, and the samples were separated on an SDS–7.5% polyacrylamide gel. Autoradiographs were quantitated with a PhosphorImager (Molecular Dynamics).

RESULTS

Subcellular localization of mSEC13. Recombinant mSEC13 was expressed in bacteria as a hexahistidine-tagged fusion protein, purified by Ni^{2+} chelation affinity chromatography, and used to immunize animals. Rabbit antisera recognized a 35-kDa band in both the membrane and supernatant fractions of HeLa cell homogenates (Fig. 1) and immunoprecipitated a polypeptide of similar size in [^{35}S]methionine-labeled cells (not shown). Immunoblots with cell lines of other species also revealed a polypeptide band of similar size (Fig. 1), suggesting a broad range of antibody cross-reactivity among mammalian cells.

To ascertain the subcellular localization of mSEC13, indirect immunofluorescence was performed with representative primate and rodent cell lines. As shown in Fig. 2, all cell types examined had similar staining patterns revealed by confocal microscopy. Particularly prominent are vesicular structures which are both perinuclearly localized and also peripherally distributed throughout the entire cytoplasm. These labeling patterns suggest localization to the ERGIC (23, 54, 58, 59). That mSEC13 is indeed localized to the intermediate compartment was given further support by colocalization with the mammalian KDEL receptor (65) at 15°C, shown in Fig. 2 in

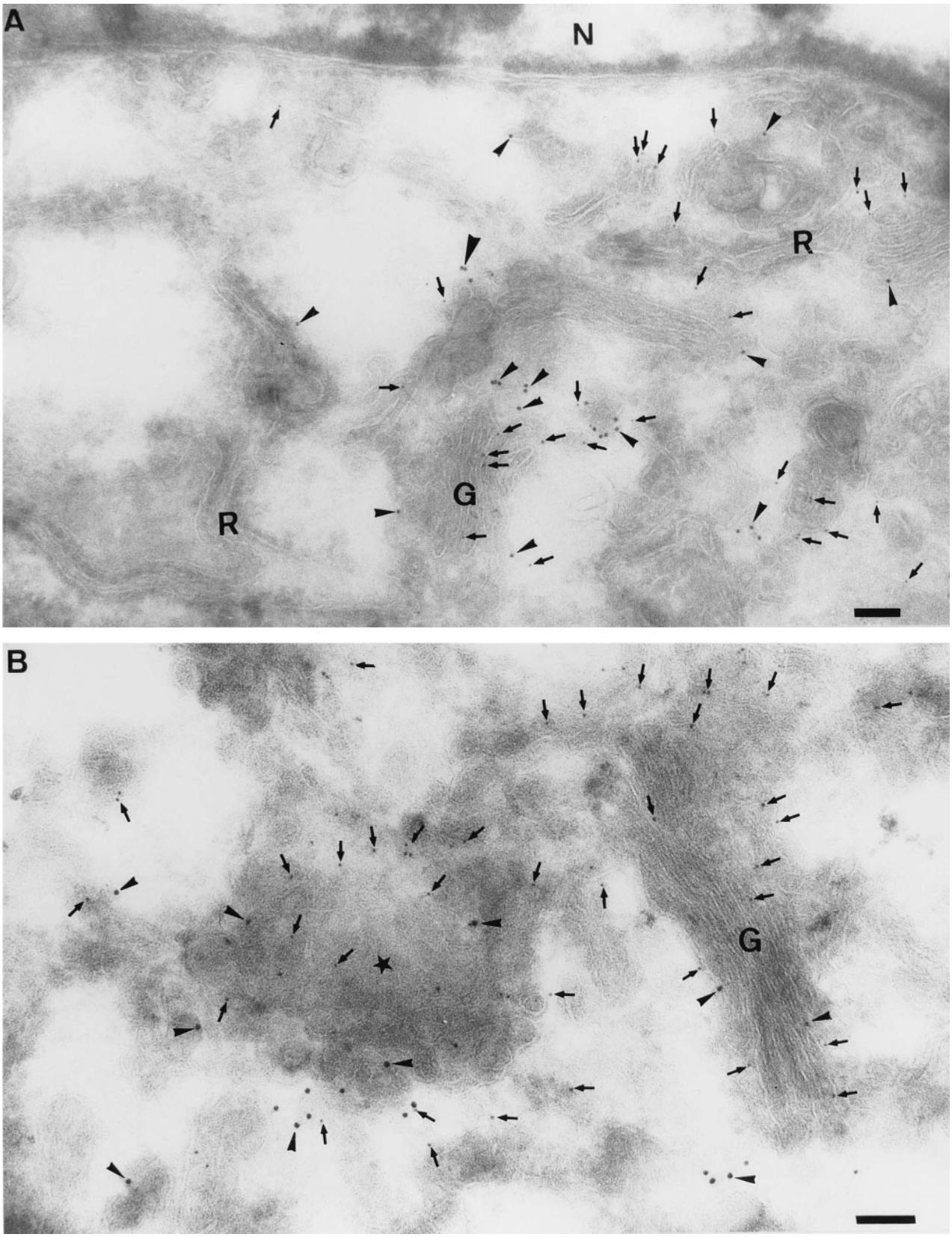

FIG. 3. Ultrastructural localization of mSEC13. Shown are thawed cryosections of mouse L cells treated with GTPγS and double labeled for anti-mSEC13 (10-nm gold [small arrowheads]) and anti-rab1A (5-nm gold [arrows]). The label for both proteins is concentrated on membrane structures close to the Golgi complex (G). In panel A, the rough ER (R) and the nuclear envelope (N) are not labeled with either marker. The large arrowhead indicates mSEC13 labeling of a vesicular bud-like structure. In panel B, the star denotes what is probably an oblique section through the *cis* side of the Golgi complex. Bars = 100 nm.

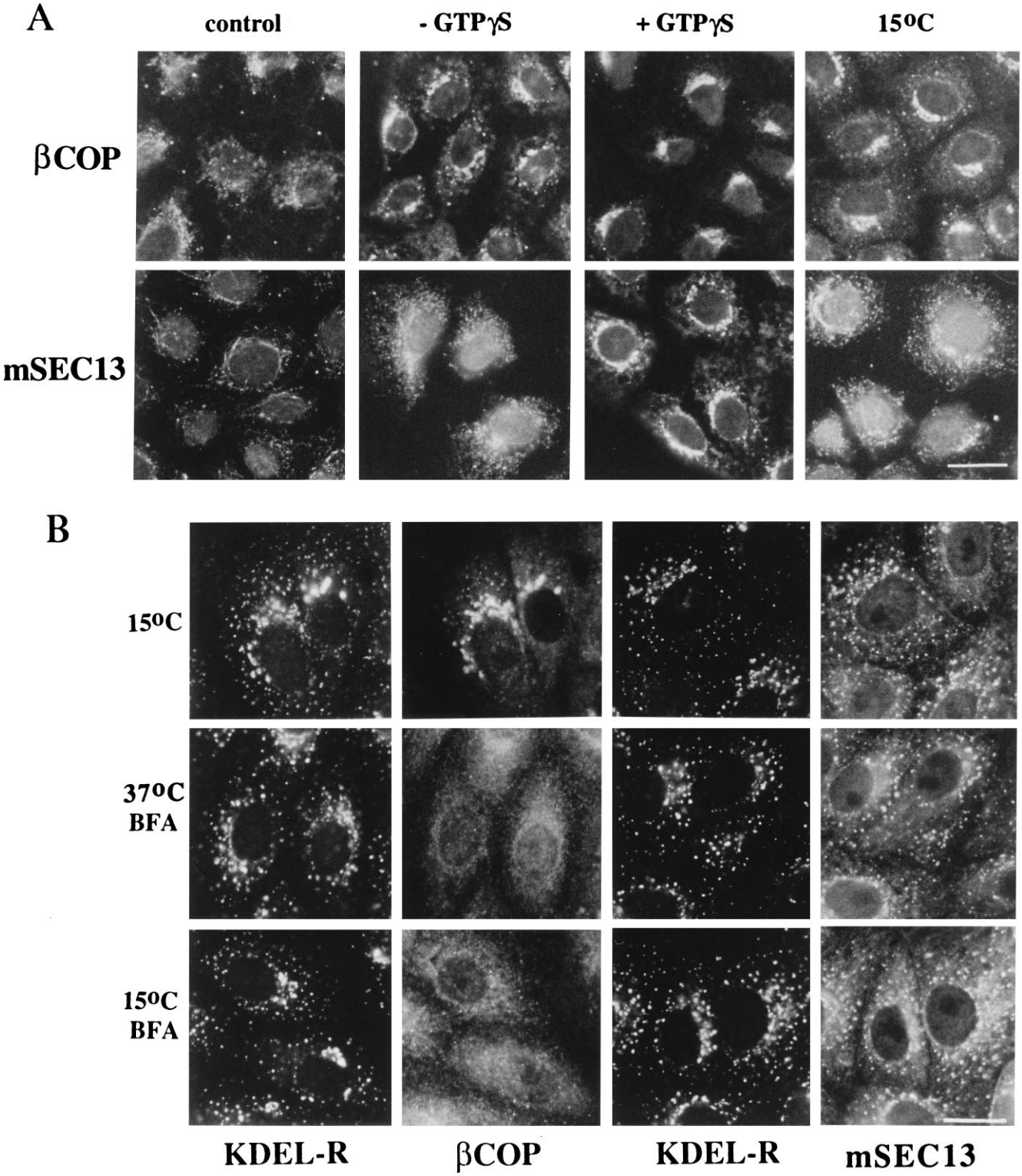

FIG. 4. (A) Membrane recruitment of mSEC13 compared to that of β-COP. Cells grown on coverslips were permeabilized with 20 μg of digitonin per ml on ice and washed extensively in ice-cold washing buffer buffer (20 mM HEPES-KOH [pH 7.2], 110 mM potassium acetate, 2 mM magnesium acetate). Cells were then incubated with buffer alone (control), cytosol (− GTPγS), cytosol with 10 μM GTPγS (+ GTPγS) at 37°C for 20 min, or cytosol with 10 μM GTPγS at 15°C for 20 min (15°C). Cells were then fixed and incubated with antibody against β-COP or anti-mSEC13 antibody. Bar = 10 μm. (B) Membrane association of mSEC13 is not affected by BFA treatment. Cells grown on coverslips were either kept at 37°C or incubated at 15°C for 3 h (15°C) prior to any treatment. Cells were treated with 10 μg of BFA per ml for 10 min at 37°C (37°C BFA) as well as at 15°C (15°C BFA). Cells were then fixed and incubated simultaneously with an antibody against β-COP and a monoclonal antibody against the KDEL receptor or with an anti-mSEC13 antibody and a monoclonal antibody against the KDEL receptor. Bar = 10 μm.

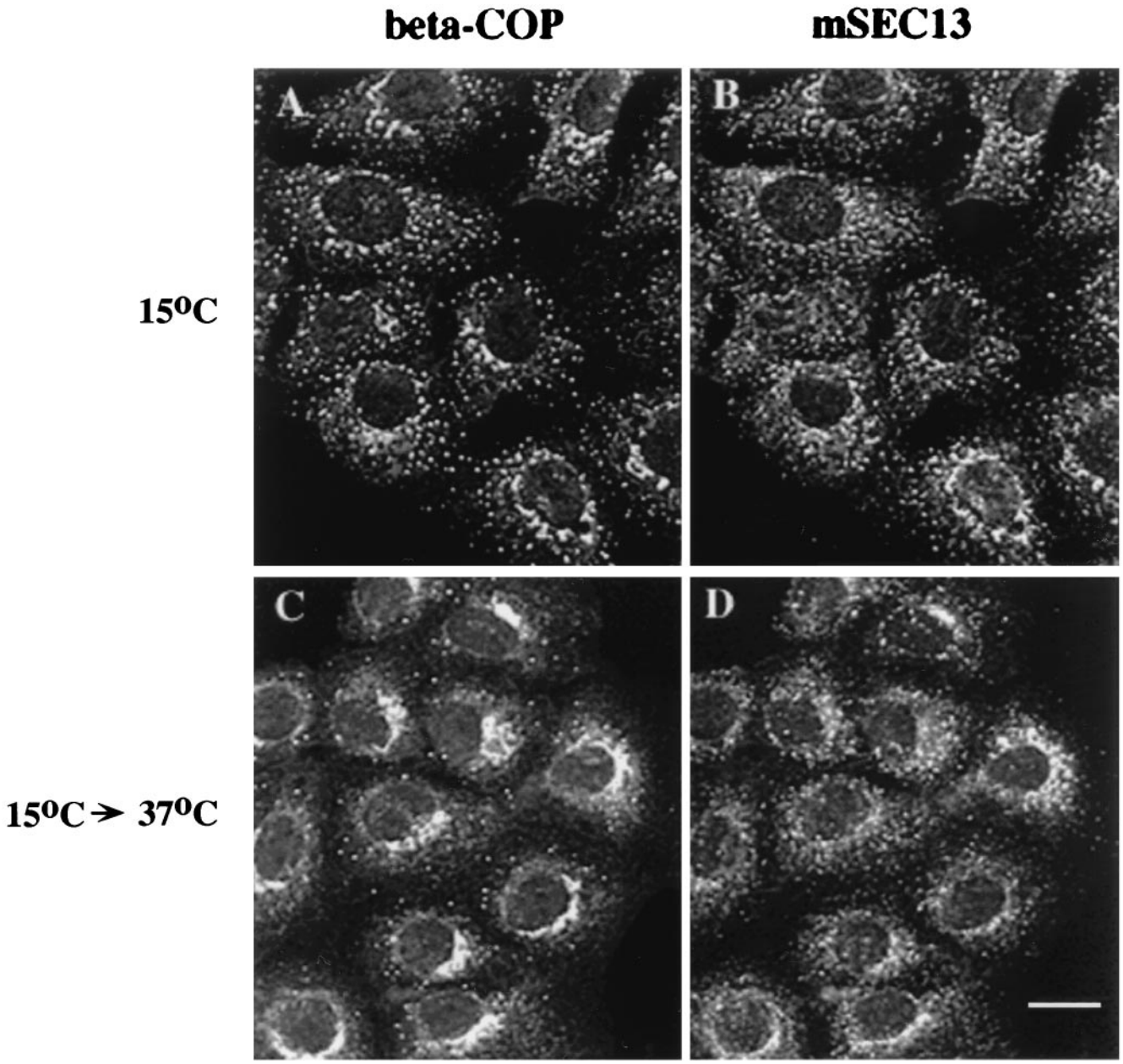

FIG. 5. Movement of mSEC13 compared to that of β-COP after temperature manipulations. Cells grown on coverslips were incubated at 15°C for 3 h (15°C) and shifted to 37°C for 10 min (15°C→37°C). Cells were then fixed and incubated with a monoclonal antibody against β-COP and an anti-mSEC13 antibody. Bar = 10 μm.

CV1 cells. Codistribution was particularly apparent for the peripheral vesicular structures. Aside from the ERGIC, weaker labeling of a reticular structure, probably the rough ER, as well as the nuclear envelope was also observed.

The ultrastructural localization of mSEC13 has been previously demonstrated by Shaywitz et al. in two cell types with well-developed early secretory compartments, the insulin and the acinar cells of the pancreas (60). In these cells, mSEC13 is concentrated in the transitional area of the ER. To determine the ultrastructural localization of mSEC13 in a cell type where the transitional ER elements are less apparent, we performed electron microscopy with immunogold labeling of thawed cryosections of mouse L cells treated with GTPγS (14, 27). Cells were double labeled with anti-mSEC13 and anti-rab1A, a marker of the intermediate compartment (14). As shown in Fig. 3, the label for both mSEC13 and rab1A is concentrated on membrane structures close to the Golgi complex. mSEC13 can also be found in high densities on some peripheral structures. This localization of mSEC13 is in agreement with observations at the light microscopy level. In addition, mSEC13 labeling is often found clustered around bud-like structures.

Membrane recruitment and subcellular dynamics of mSEC13. It is apparent that mSEC13 has a different steady-state membrane localization compared to the COPI component β-COP. We examined whether mSEC13 might also be recruited differently from β-COP in permeabilized cells. As shown in Fig. 4A, addition of cytosol alone to permeabilized cells resulted in partial recruitment of both molecules onto

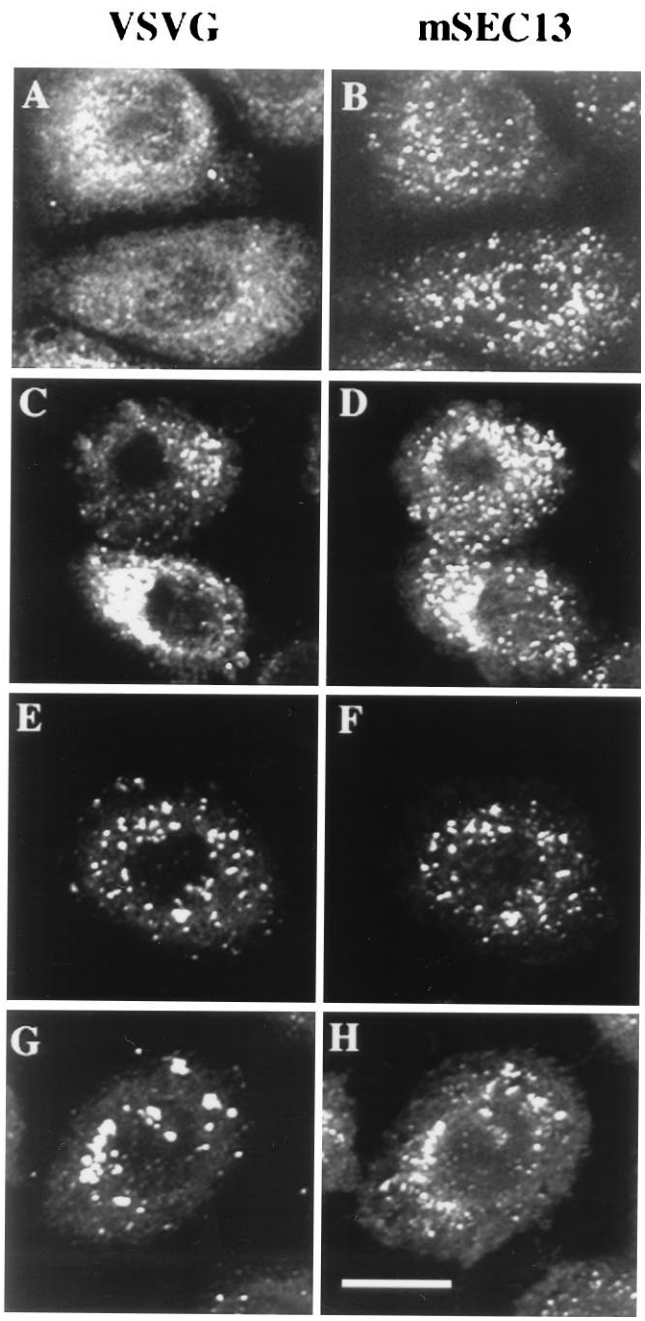

FIG. 6. Movement of mSEC13 compared to that of VSVG after temperature manipulations. Cells grown on coverslips were infected with VSV *ts*O45 for 45 min at 32°C and then incubated for 3 h at 40°C. Cells were then not manipulated further (A and B), shifted to 32°C for 4 min (C and D), or shifted to 15°C for 2 h (E and F) or first shifted to 15°C for 2 h followed by a further incubation at 32°C for 4 min (G and H). Cells were then fixed and incubated with an antibody against VSVG or an anti-mSEC13 antibody. Bar = 10 μm.

membrane structures. Whereas the staining of β-COP is primarily perinuclear, that of mSEC13 is both ER-like and spotty. Addition of GTPγS to cytosol resulted in higher levels of membrane recruitment for both proteins and a concentration of mSEC13 at the perinuclear region. When the incubation was carried out at 15°C instead of 37°C, membrane recruitment for mSEC13 was similar to that seen with the cytosol alone. The recruitment of β-COP at 15°C was comparable to that at 37°C, but with peripheral vesicular structures being more apparent.

One of the striking properties of previously described coat proteins is their dissociation from the membrane upon treatment of cells with the fungal metabolite brefeldin A (BFA) (25, 32, 33, 48). We therefore investigated whether the membrane association of mSEC13 would also be affected by BFA. As shown in Fig. 4B, β-COP codistributes with the KDEL receptor at 15°C (top panels). BFA treatment dissociates β-COP from the Golgi apparatus at both 37 and 15°C. However, under the same conditions, the membrane association of mSEC13 is not affected (middle and bottom panels). Thus, a distinguishing feature of mSEC13 is that unlike β-COP (25) and the Golgi adapter protein γ-adaptin (48), it is unaffected by BFA treatment.

The labeling pattern of β-COP has been shown to change with 15-to-37°C temperature shifts (15, 34). We determined whether mSEC13 also has such properties and if its dynamics is comparable with that of β-COP. As shown in Fig. 5, after incubation at 15°C for 3 h, mSEC13 and β-COP are fairly well colocalized to vesicular structures (although their staining intensities may vary in different structures). When cells were shifted for 10 min to 37°C, the majority of β-COP in all cells shifted to a tight perinuclear cluster. This was not the case for mSEC13, which remained largely dispersed, although in some cells there was also a noticeable increase in perinuclear staining. The noticeable increase in perinuclear staining of mSEC13 in some cells during the shift also differed from that of the perinuclear patch labeled by β-COP, being more vesicular in appearance.

We also examined the dynamics of mSEC13 with reference to the anterograde transport of the G protein of VSV *ts*045 (46). Cells were infected with VSV *ts*045 and incubated for 3 h at 40°C to arrest the G protein in the ER. As shown in Fig. 6A and B, some of the ER-arrested G protein codistributed with elements of ERGIC labeled by mSEC13. When cells were shifted from 40 to 32°C for 4 min to allow for a brief ER exit time, the extent of apparent colocalization of the G protein with mSEC13 increased (Fig. 6C and D). If the cells were shifted from 40 to 15°C for 2 h to arrest anterograde transport in the ERGIC, the G protein and mSEC13 were almost completely colocalized (Fig. 6E and F). However, when the cells were further shifted from 15 to 32°C for 4 min to allow a brief ERGIC exit, a significant fraction of the colocalization between the G protein and mSEC13 was quickly lost as the G protein moved into larger vesicular tubular clusters (Fig. 6G and H). Therefore, in the course of VSVG transport from the ER to the Golgi apparatus, the cargo appeared to colocalize with mSEC13 only at an earlier point.

mSEC13 is essential for ER-to-Golgi transport. Although the involvement of COPII components in ER-Golgi transport is well established in yeast, the involvement of COPII components in mammalian ER-Golgi transport has not been fully characterized. In particular, it has not been clearly demonstrated that COPII components are absolutely required for this particular transport step. We therefore examined the involvement of mSEC13 in ER-Golgi transport by using a well-established assay which measures the transport of pulse-labeled, ER-arrested temperature-sensitive VSVG molecules in semiintact cells as they acquire endo H resistance when transported to the Golgi apparatus. As shown in Fig. 7A, a standard transport incubation with a full complement of energy and cytosol resulted in 70% or more of the pulse-labeled protein acquiring endo H resistance. Addition of anti-β-COP antibody (lane g) previously shown to inhibit ER-Golgi transport (44) inhibited transport almost to the level of the control that had been

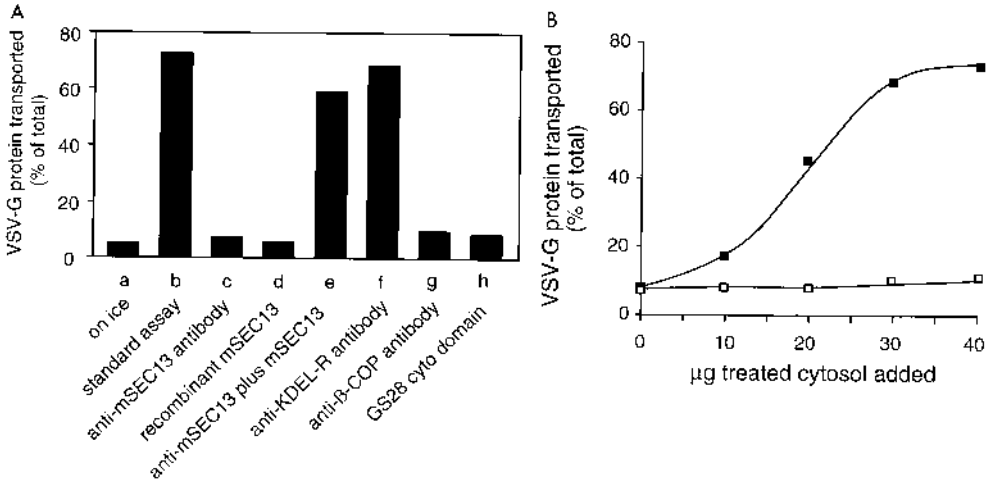

FIG. 7. (A) Both recombinant mSEC13 and the mSEC13 antibody inhibited ER-Golgi transport. Standard transport assays were carried out as outlined in Materials and Methods with different supplements as indicated. Lanes: a, standard transport assay incubated on ice; b, standard transport assay at 37°C; c, with 5 μg of anti-mSEC13 in assay cocktail; d, with 5 μg of recombinant mSEC13 in assay cocktail; e, with 5 μg of anti-mSEC13 plus 5 μg of recombinant mSEC13 in assay cocktail; f, with anti-KDEL receptor antibody in assay cocktail; g, with anti-β-COP antibody in assay cocktail; h, with recombinant cytoplasmic domain of GS28, a SNARE molecule involved in ER-Golgi transport, in assay cocktail. (B) Cytosol depleted of mSEC13 does not support ER-Golgi transport. Standard transport assays were carried out with increasing concentrations of normal cytosol (black squares) or mSEC13-depleted cytosol (white squares).

incubated on ice (lane a). Likewise, addition of affinity-purified mSEC13 antibody (lane c) inhibited transport almost to control levels. In contrast, an antibody directed against a cytoplasmic epitope in the ER-Golgi region (antibody against the cytoplasmic tail of the KDEL receptor) did not inhibit transport (lane f). This result suggests that the inhibitory effect of the antibodies was specific. Furthermore, the addition of the hexahistidine-tagged mSEC13 recombinant protein also inhibited transport (lane d), as well as the cytoplasmic domain of the SNARE protein GS28 (lane h) (62). Inhibition by both mSEC13 antibody and recombinant protein occurred in a dose-dependent fashion (not shown), and the inhibitory effect of each could be neutralized by the other (lane e).

To determine if the mSEC13 present in the cytosol is absolutely required for transport to occur, we immunodepleted mSEC13 from the cytosol by using affinity-purified mSEC13 antibody coupled to Sepharose. Quantitative depletion was achieved, as determined by immunoblot analysis (not shown). When used in a standard transport set up, the mSEC13-depleted cytosol did not support transport (Fig. 7B). At a dose where the mock-depleted cytosol achieved maximal levels of transport, that of mSEC13-depleted cytosol remained at the background level.

To further confirm that it was indeed mSEC13 depletion which resulted in the loss of transport competence of the depleted cytosol, we next attempted to restore transport competence with exogenously added mSEC13. Since the recombinant mSEC13 protein inhibited transport in a standard assay, we reasoned that the monomeric form may not be the functional form of mSEC13 in the cytosol. Such is the case in yeast, in which the monomeric form of Sec13p was inactive in the Sec13-dependent vesicle formation assay (47). In yeast cytosol, Sec13p forms a high-molecular-weight functional complex with a 150-kDa protein (51). To see if such a functional complex also exists in mammalian cytosol, we size fractionated rat liver cytosol by gel filtration on a Sephadex G-75 fast protein liquid chromatography column and analyzed fractions by immunoblotting. As shown in Fig. 8A, mSEC13 was enriched in a high-molecular-weight fraction as well as a low-molecular-weight, monomeric fraction. The high-molecular-weight fraction, although by itself not supporting transport (not shown), restored transport competence of mSEC13-depleted cytosol (Fig. 8B, lane d) in a dose-dependent manner (Fig. 8C), whereas the low-molecular-weight monomeric fraction did not (Fig. 8A, lane e).

DISCUSSION

We have a raised polyclonal antibody against mSEC13, a component of the putative mammalian COPII coat, and used it to investigate several important aspects of the molecule. Firstly, we sought to determine the subcellular localization of mSEC13. Indirect immunofluorescence showed that mSEC13 can be found on distinct membrane structures, primarily appearing as vesicular structures localized both to the perinuclear region and throughout the entire cytoplasm. In addition, mSEC13 was observed in a reticular staining pattern and in the nuclear envelope. These structures are characteristic of ER-GIC and the ER itself. Colocalization of mSEC13 with the KDEL receptor and ERGIC53 (not shown) at 15°C confirmed the ERGIC localization. This finding is in agreement with the previous ultrastructural localization of mSEC13 to the transitional area of the ER in pancreatic acinar cells (60). In mouse L cells, at the ultrastructural level, it appears that mSEC13 is localized to membrane structures resembling the ERGIC and colocalizing with the intermediate compartment marker rab1A (14). Another noteworthy feature of mSEC13 immunogold labeling is that some of the label was obviously clustered around bud-like structures. Although it is not clear without use of other COPII markers to determine whether these are authentic COPII buds, this feature is certainly consistent with the functional nature of mSEC13 as a component of vesicle coats (2).

mSEC13, like its yeast homolog, requires guanine nucleotide exchange for its recruitment to the membrane, as GTPγS enhances its membrane recruitment in permeabilized cells. This requirement probably reflects a recruitment process which is dependent on the mSAR1 (28), as was shown for the mSEC23 (1). It is not certain at present if mSAR1 binding to membranes occurs first, followed by sequential binding of other

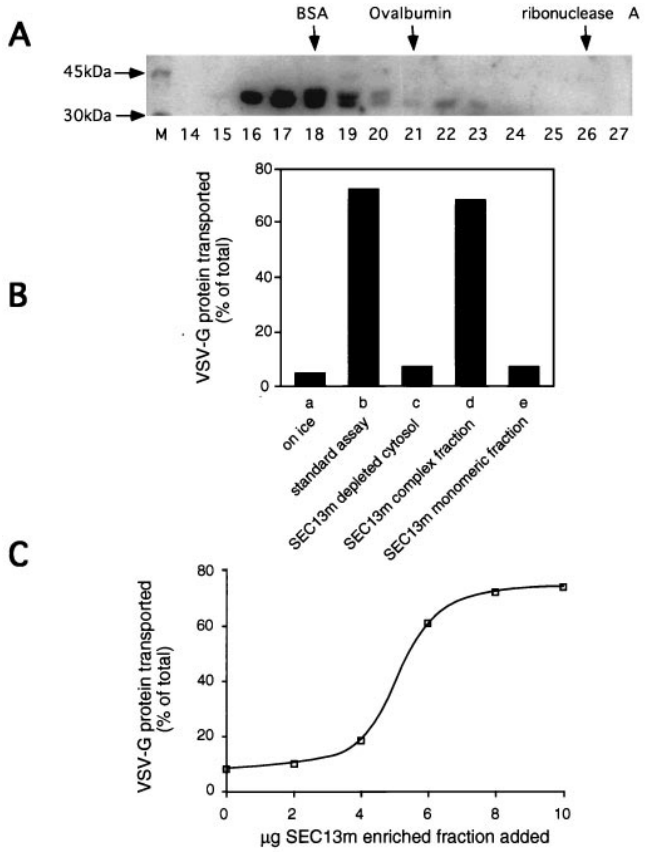

FIG. 8. (A) mSEC13 is found in a high-molecular-weight fraction as well as a low-molecular-weight fraction. Fractions of rat liver cytosol separated by gel filtration chromatography with a Sephadex G-75 column were subjected to immunoblot analysis with an anti-mSEC13 antibody. The size markers used for the gel filtration are bovine serum albumin (BSA; 67 kDa), ovalbumin (43 kDa), and RNase A (13.7 kDa). (B) The high-molecular-weight complex of maSEC13 restores transport competence of mSEC13-depleted cytosol. Both the high- and low-molecular-weight fractions were tested for the ability to restore transport competence to mSEC13-depleted cytosol. Lanes: a, standard transport assay incubated on ice; b, standard transport assay at 37°C; c, transport assay with mSEC13-depleted cytosol; d, transport assay with mSEC13-depleted cytosol supplemented with the high-molecular-weight mSEC13 fraction; e, transport assay with mSEC13-depleted cytosol supplemented with the low-molecular-weight fraction. (C) The high-molecular-weight complex fraction restores transport competence of mSEC13-depleted cytosol in a dose-dependent manner.

components, or whether COPII components dock onto membranes in the form of a preformed complex. It is clear, however, that the guanine nucleotide exchange activity required for mSEC13 binding, unlike that responsible for COPI binding (11, 20), is insensitive to BFA. We have shown that BFA dissociates β-COP from membrane structures both at 37°C and at 15°C, a temperature not previously examined for COPI dissociation but which was shown to be inhibitory to BFA-induced redistribution of Golgi markers to the ER (25). In contrast, BFA had no effect on mSEC13 at either temperature. These results are consistent with previous findings at the ultrastructural level that the distributions of both the mSEC23 (40) and mSEC13 (60) were not affected by BFA treatment and remained excluded from coatomer-rich areas of BFA bodies in pancreatic cells (40).

In a further examination of the steady-state localization of mSEC13, the subcellular dynamics of the molecule was also studied. This was first done with reference to β-COP, a representative of the COPI coat which has long been implicated in ER-Golgi transport, and an anterograde cargo marker, VSVG, by standard temperature manipulations. When cells were cultured at 37°C, the localization of mSEC13 differed markedly from that of β-COP, which is almost exclusively perinuclear. However, when cells were shifted to 15°C for 3 h, there was a tremendous increase in the apparent colocalization of β-COP and mSEC13. This presumably was a result of the accumulation of β-COP in the ERGIC. Interestingly, when cells were shifted back to 37°C, β-COP quickly and completely resumed its localization in a tight perinuclear cluster, presumably shifting back to the region of the *cis*-Golgi apparatus, where it is primarily situated (39). In contrast, although the amount of mSEC13 was apparently elevated in the perinuclear vesicular clusters, it remained largely in vesicular structures. It would appear that mSEC13 and β-COP colocalized only at 15°C, where they are both seemingly found in the ERGIC. Given the evidence that mSEC13 and β-COP are both important for ER-Golgi transport (43, 44), their differential localization is suggestive of their functions in different locations along the pathway.

When the dynamics of mSEC13 was examined together with the anterograde transport of ER-arrested VSVG, the picture was largely in agreement with that of β-COP. Infected cells incubated for 3 h at 40°C showed distinct ER staining of VSVG. Also, a fraction of the VSVG has clearly moved into the ERGIC53/58 compartment to colocalize with mSEC13. This appearance of a fraction of the temperature-sensitive VSVG at the ERGIC53/58 compartment at the nonpermissive temperature has been shown to occur previously (15, 17). When the transport of VSVG into the Golgi apparatus was initiated by shifting the cells to a permissive temperature, a majority of the VSVG moved quickly (in 4 min) into vesicular structures, colocalizing with mSEC13. This colocalization was also observed when the anterograde transport of VSVG was arrested at 15°C. However, when cells were shifted from 15 to 37°C, VSVG quickly moved into structures that no longer colocalized well with mSEC13.

Biochemical evidence for the involvement of COPI in ER-Golgi transport stems from antibody inhibition of VSVG transport both in permeable semi-intact cells (44) and in intact cells microinjected with β-COP antibodies (43). Evidence is presented here that both the affinity-purified mSEC13 antibody and the recombinant mSEC13 protein inhibited VSVG transport between the ER and the Golgi apparatus in an assay using semi-intact cells; the latter may function as a competitive inhibitor for the binding of a functional complex in the cytosol. To prove that mSEC13 is absolutely essential for transport, we immunodepleted the cytosol used in the transport assay and showed that the immunodepleted cytosol is defective in supporting transport. Transport could, however, be restored by a fraction enriched in a high-molecular-weight complex form of mSEC13, which has been previously shown to be the functional Sec13p complex in yeast. A fraction enriched in a low-molecular-weight, monomeric form of mSEC13 was unable to restore transport. We thus provide evidence that mSEC13 is absolutely required for the ER-Golgi transport of VSVG in this assay.

In yeast, mutants in both COPI and COPII components accumulate ER forms of the same secretory precursors (50). In vitro assays suggest that COPII components are the only ones needed for the ER-Golgi transport of several cargo markers (51). Evidence for the involvement of COPI in ER-Golgi transport in yeast is complicated by their involvement in Golgi-ER retrograde transport. Some COPI mutant alleles such as *sec21-1* (γ-COP) and *ret2-1* (δ-COP) show clear defects in ER-Golgi transport, but these may be indirect effects (7). Ves-

icles containing either COPI or COPII could bud directly from the yeast ER (5). Both populations of vesicles carry proteins required for vesicle targeting such as the v-SNARE molecules Sec22p, Bos1p, and Bet1p (5). However, it seems that all other cargoes examined thus far, including the α-factor precursor (2), Gas1p (10), and Emp24p (55), are found only in COPII vesicles. In mammalian cells, there is evidence that COPII and COPI function separately but sequentially to mediate ER-Golgi transport. Aridor et al. (1) have suggested that COPI and COPII complexes are recruited separately and independently to different locations along the ER-Golgi path. By using GDP- and GTP-restricted mutants of Sar1p and ARF1 to control coat recruitment, they showed that COPII is responsible for the initial budding from the ER followed by an as yet mechanistically undefined process of COPI coupling which mediates the segregation of anterograde cargo from p58. This process appears to occur in the ERGIC. We have previously shown that ERGIC53 is indeed segregated from the anterogradely transported VSVG upon exit from the ERGIC (64). Our results suggest that in intact cells, mSEC13 does not seem to move beyond the ERGIC into the Golgi apparatus, whereas β-COP does not seem to move further in from the ERGIC to the ER. However, the otherwise nonoverlapping mSEC13 and β-COP can both colocalize in the ERGIC with a 15°C block. These observations suggest that COPI and COPII functions in ER-Golgi transport may indeed be coupled at the ERGIC.

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REFERENCES

1. **Aridor, M., S. I. Bannykh, T. Rowe, and W. E. Balch.** 1995. Sequential coupling between CopII and CopI vesicle coats in endoplasmic reticulum to Golgi transport. J. Cell Biol. **131:**875–893.
2. **Barlowe, C., L. Orci, T. Yeung, M. Hosobuchi, S. Hamamoto, N. Salama, M. F. Rexach, M. Ravazzola, M. Amherdt, and R. Schekman.** 1994. COPII: a membrane coat formed by sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell. **77:**895–907.
3. **Barlowe, C., and R. Schekman.** 1993. SEC12 encodes a guanine-nucleotide-exchange factor essential for transport vesicle budding from the ER. Nature **365:**347–349.
4. **Beckers, C. J. M., D. S. Keller, and W. E. Balch.** 1987. Semi-intact cells permeable to macromolecules: use in reconstitution of protein transport from the endoplasmic reticulum to the Golgi complex. Cell **50:**523–534.
5. **Bednarek, S. Y., M. Ravazzola, T. Hosobuchi, M. Amherdt, A. Perrelet, R. Schekman, and L. Orci.** 1995. COPI- and COPII-coated vesicles bud directly from the endoplasmic reticulum in yeast. Cell **83:**1183–1196.
6. **Bonatti, S., G. Migliaccio, and K. Simons.** 1989. Palmitylation of viral membrane glycoprotein takes place after exit from the endoplasmic reticulum. J. Biol. Chem. **264:**12590–12595.
7. **Cosson, P., C. Démollière, S. Hennecke, R. Duden, and F. Letourneur.** 1996. δ- and ζ-COP, two coatomer subunits homologous to clathrin-associated proteins, are involved in ER retrieval. EMBO J. **15:**1792–1798.
8. **d'Enfert, C., L. J. Wuestehube, T. Lila, and R. Schekman.** 1991. Sec12p-dependent membrane binding of the small GTP-binding protein Sar1 promotes formation of transport vesicles from the ER. J. Cell Biol. **114:**663–670.
9. **Davidson, H. W., and W. E. Balch.** 1993. Differential inhibition of multiple vesicular transport steps between the endoplasmic reticulum and trans Golgi network. J. Biol. Chem. **268:**4216–4226.
10. **Doering, T. L., and R. Schekman.** 1996. GPI-anchor attachment is required for Gas1p transport from the endoplasmic reticulum in COPII vesicles. EMBO J. **15:**182–191.
11. **Donaldson, J. G., D. Finazzi, and R. D. Klausner.** 1992. Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. Nature **360:**350–352.
12. **Fiedler, K., and K. Simons.** 1994. A putative novel class of animal lectins in the secretory pathway homologous to leguminous lectins. Cell **77:**625–626.
13. **Griffiths, G. (ed.).** 1993. Fine structure immunocytochemistry, p. 137–203. Springer-Verlag, Berlin, Germany.
14. **Griffiths, G., M. Ericsson, J. Krijnse-Locker, T. Nilsson, B. Goud, H.-D. Soling, B. L. Tang, S. H. Wong, and W. Hong.** 1994. Localisation of the KDEL receptor to the Golgi complex and the intermediate compartment in mammalian cells. J. Cell Biol. **127:**1557–1574.
15. **Griffiths, G., R. Pepperkok, J. Krijnse-Locker, and T. E. Kreis.** 1995. Immunocytochemical localization of β-COP to the ER-Golgi boundary and the TGN. J. Cell Sci. **108:**2839–2856.
16. **Guo, Q., E. Vasile, and M. Krieger.** 1994. Distruption in Golgi structure and membrane traffic in a conditional lethal mammalian cell mutant are corrected by ε-COP. J. Cell Biol. **125:**1213–1223.
17. **Hammond, C., and A. Helenius.** 1994. Folding of VSVG protein: sequential interaction with BiP and calnexin. Science **266:**456–458.
18. **Hammond, C., and A. Helenius.** 1994. Quality control in the secretory pathway: retention of a misfolded viral membrane glycoprotein involves cycling between the ER, intermediate compartment, and Golgi apparatus. J. Cell Biol. **126:**41–52.
19. **Hara-Kuge, S., O. Kuge, L. Orci, M. Amherdt, M. Ravazzola, R. T. Wieland, and J. E. Rothman.** 1994. En bloc incorporation of coatomer subunits during the assembly of COP-coated vesicles. J. Cell Biol. **124:**883–892.
20. **Helms, J. B., and J. E. Rothman.** 1992. Inhibition by brefeldin A of a Golgi membrane enzyme that catalyses exchange of guanine nucleotide bound to ARF. Nature **360:**352–354.
21. **Helms, J. B., D. J. Palmer, and J. E. Rothman.** 1993. Two distinct populations of ARF bound to Golgi membranes. J. Cell Biol. **121:**751–760.
22. **Hicke, L., T. Yoshihisa, and R. Schekman.** 1992. Sec23p and a novel 105 kD protein function as a multimeric complex to promote vesicle budding and protein transport from the ER. Mol. Biol. Cell **3:**667–676.
23. **Hong, W., and B. L. Tang.** 1993. Protein trafficking along the exocytotic pathway. Bioessays **15:**231–238.
24. **Hosobuchi, M., T. Kreis, and R. Schekman.** 1992. SEC21 is a gene required for ER to Golgi protein transport that encodes a subunit of a yeast coatomer. Nature **360:**603–605.
25. **Klausner, R. D., J. G. Donaldson, and J. Lippincott-Schwartz.** 1992. Brefeldin A: insights into the control of membrane traffic and organelle structure. J. Cell Biol. **116:**1071–1080.
26. **Kreis, T. E., and R. Pepperkok.** 1994. Coat proteins in intracellular membrane transport. Curr. Opin. Cell Biol. **6:**533–537.
27. **Krijnse-Locker, J., M. Ericsson, P. J. M. Rottier, and G. Griffiths.** 1994. Characterization of the budding compartment of the mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. J. Cell Biol. **124:**55–70.
28. **Kuge, O., C. Dascher, L. Orci, T. Rowe, M. Amherdt, H. Plutner, M. Ravazzola, G. Tanigawa, J. E. Rothman, and W. E. Balch.** 1994. Sar1 promotes vesicle budding from the endoplasmic reticulum but not Golgi compartments. J. Cell Biol. **125:**51–65.
29. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680–685.
30. **Lahtinen, U., B. Dahllof, and J. Saraste.** 1992. Characterization of a 58 kDa cis-Golgi protein in pancreatic exocrine cells. J. Cell Sci. **103:**321–333.
31. **Letourneur, F., E. C. Gaynor, S. Hennecke, C. Demolliere, R. Duden, S. D. Emr, H. Riezman, and P. Cosson.** 1994. Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. Cell **79:**1199–1207.
32. **Lippincott-Schwartz, J., J. G. Donaldson, A. Schweizer, E. G. Berger, H. Hauri, L. C. Yuan, and R. D. Klausner.** 1990. Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. Cell **60:**821–836.
33. **Lippincott-Schwartz, J., L. C. Yuan, C. Tipper, M. Amherdt, L. Orci, and R. Klausner.** 1991. Brefeldin-A's effects on endosomes, lysosomes and the TGN suggests a general mechanism for regulating organelle structure and membrane traffic. Cell **67:**601–616.
34. **Lippincott-Schwartz, J., N. B. Cole, A. Marotta, P. A. Conrad, and G. S. Bloom.** 1995. Kinesin is the motor for microtubule-mediated Golgi-to-ER membrane traffic. J. Cell Biol. **128:**293–306.
35. **Lotti, L. V., M-R. Torrisi, M. C. Pascale, and S. Bonatti.** 1992. Immunocytochemical analysis of the transfer of vesicular stomatitis virus G glycoprotein from the intermediate compartment to the Golgi complex. J. Cell Biol. **118:**43–50.
36. **Low, S. H., B. L. Tang, S. H. Wong, and W. Hong.** 1992. Selective inhibition of protein targeting to the apical domain of MDCK cells by brefeldin A. J. Cell Biol. **118:**51–62.
37. **Nakano, A., D. Brada, and R. Schekman.** 1988. A membrane glycoprotein, Sec12p, required for protein transport from the endoplasmic reticulum to the Golgi apparatus in yeast. J. Cell Biol. **107:**851–863.
38. **Nakano, A., and M. Muramatsu.** 1989. A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. J. Cell Biol. **109:**2677–2691.
39. **Oprins, A., R. Duden, T. E. Kreis, H. J. Geuze, and J. W. Slot.** 1993. β-COP localizes mainly to the cis-Golgi side in exocrine pancreas. J. Cell Biol. **121:**49–59.
40. **Orci, L., A. Perrelet, M. Ravazzola, F. T. Wieland, R. Schekman, and J. E. Rothman.** 1993. "BFA bodies": a subcompartment of the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **90:**11089–11093.

41. **Orci, L., D. J. Palmer, M. Amherdt, and J. E. Rothman.** 1993. Coated vesicle assembly in the Golgi requires only coatomer and ARF proteins from the cytosol. Nature **364:**732–734.
42. **Orci, L., M. Ravazzola, P. Meda, C. Holcomb, H.-P. Moore, L. Hicke, and R. Schekman.** 1991. Mammalian Sec23p homologue is restricted to the endoplasmic reticulum transitional cytoplasm. Proc. Natl. Acad. Sci. USA **88:** 8611–8615.
43. **Pepperkok, R. J., J. Scheel, H. Horstmann, H. P. Hauri, G. Griffiths, and T. E. Kreis.** 1993. β-COP is essential for biosynthetic membrane transport from the endoplasmic reticulum to the Golgi complex in vivo. Cell **74:**71–82.
44. **Peter, F., H. Plutner, T. Kreis, and W. E. Balch.** 1993. β-COP is essential for transport of protein from the endoplasmic reticulum to the Golgi in vitro. J. Cell Biol. **122:**1155–1168.
45. **Pfeffer, S. R., and J. E. Rothman.** 1987. Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu. Rev. Biochem. **56:** 829–852.
46. **Plutner, H., H. W. Davison, J. Saraste, and W. E. Balch.** 1992. Morphological analysis of protein transport from the ER to Golgi membranes in digitonin-permeabilized cells: role of the p58 containing compartment. J. Cell Biol. **119:**1097–1116.
47. **Pryer, N. K., N. R. Salama, R. Schekman, and C. A. Kaiser.** 1993. Cytosolic Sec13p complex is required for vesicle formation from the endoplasmic reticulum in vitro. J. Cell Biol. **120:**865–875.
48. **Robinson, M. S., and T. E. Kreis.** 1992. Recruitment of coat proteins onto Golgi membranes in intact and permeabilized cells: effects of Brefeldin A and G protein activators. Cell **69:**129–138.
49. **Rothman, J. E., and L. Orci.** 1992. Molecular dissection of the secretory pathway. Nature **355:**409–415.
50. **Salama, N. R., and R. W. Schekman.** 1995. The role of coat proteins in the biosynthesis of secretory proteins. Curr. Opin. Cell Biol. **7:**536–543.
51. **Salama, N. R., T. Yeung, and R. W. Schekman.** 1993. The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. **12:**4073–7082.
52. **Saraste, J., and E. Kuismanen.** 1984. Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. Cell **38:**535–549.
53. **Saraste, J., G. E. Palade, and M. G. Farquhar.** 1987. Antibodies to rat pancreas Golgi subfractions: identification of a 58 kD cis-Golgi protein. J. Cell Biol. **105:**2021–2029.
54. **Saraste, J., and K. Svensson.** 1991. Distribution of the intermediate elements operating in ER to Golgi transport. J. Cell Sci. **100:**415–430.
55. **Schimmöller, F., B. Singer-Krüger, S. Schröder, U. Kruger, C. Barlowe, and H. Riezman.** 1995. The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. EMBO J. **14:**1329–1339.
56. **Schindler, R., C. Itin, M. Zerial, F. Lottspeich, and H.-P. Hauri.** 1993. ERGIC-53, a membrane protein of the ER-Golgi intermediate compartment, carries an ER retention motif. Eur. J. Cell Biol. **61:**1–9.
57. **Schweizer, A., J. A. M. Fransen, T. Bachi, L. Ginsel, and H.-P. Hauri.** 1988. Identification, by a monoclonal antibody, of a 53 kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. J. Cell Biol. **107:**1643–1653.
58. **Schweizer, A., J. A. M. Fransen, K. Matter, T. Kreis, L. Ginsel, and H.-P. Hauri.** 1990. Identification of an intermediate compartment involved in protein transport from the endoplasmic reticulum to the Golgi apparatus. Eur. J. Cell Biol. **53:**185–196.
59. **Schweizer, A., K. Matter, C. M. Ketcham, and H.-P. Hauri.** 1991. The isolated ER-Golgi intermediate compartment exhibits properties that are different from ER and cis-Golgi. J. Cell Biol. **113:**45–54.
60. **Shaywitz, D. A., L. Orci, M. Ravazzola, A. Swaroop, and C. Kaiser.** 1995. Human SEC13Rp functions in yeast and is located on transport vesicles budding from the endoplasmic reticulum. J. Cell Biol. **128:**769–777.
61. **Stenback, G., C. Harter, A. Brech, D. Herrman, F. Lottspeich, L. Orci, and F. T. Wieland.** 1993. β′-COP, a novel subunit of coatomer. EMBO J. **12:** 2841–2845.
62. **Subramaniam, V. N., F. Peter, R. Philp, S. H. Wong, and W. Hong.** 1996. GS28, a 28-kilodalton Golgi SNARE that participates in ER-Golgi transport. Science **272:**1161–1163.
63. **Swaroop, A., T. L. Yang-Feng, W. Liu, L. Gieser, L. L. Barrow, K.-C. Chen, N. Agarwal, M. H. Meisler, and D. I. Smith.** 1994. Molecular characterization of a novel human gene, *SEC13R*, related to the yeast secretory pathway gene SEC13, and mapping to a conserved linkage group on human chromosome 3p24-p25 and mouse chromosome 6. Hum. Mol. Genet. **3:**1281–1286.
64. **Tang, B. L., S. H. Low, H-P. Hauri, and W. Hong.** 1995. Segregation of ERGIC53 and the mammalian KDEL receptor upon exit from the 15°C compartment. Eur. J. Cell Biol. **68:**398–410.
65. **Tang, B. L., S. H. Wong, X. L. Qi, S. H. Low, and W. Hong.** 1993. Molecular cloning, characterization, subcellular localization and dynamics of p23, the mammalian KDEL receptor. J. Cell Biol. **120:**325–338.
66. **Waters, G. M., T. Serafini, and J. E. Rothman.** 1991. Coatomer: a cytosolic protein complex containing subunits of nonclathrin-coated Golgi transport vesicles. Nature **349:**248–251.
67. **Yoshihisa, T., C. Barlowe, and R. Schekman.** 1993. Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. Nature **259:**1466–1468.
68. **Zieler, H. A., M. Walberg, and P. Berg.** 1995. Suppression of mutations in two *Saccharomyces cerevisiae* genes by the adenovirus E1A protein. Mol. Cell. Biol. **15:**3227–3237.