β-COP Is Essential for Transport of Protein from the Endoplasmic Reticulum to the Golgi In Vitro

Frank Peter, Helen Plutner, Hengyi Zhu, Thomas E. Kreis,* and William E. Balch

Departments of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, California; and *Department of Cell Biology, Science III, University, CH-1211 Geneva, Switzerland

Abstract. Using a novel in vitro assay which allows us to distinguish vesicle budding from subsequent targeting and fusion steps, we provide the first biological evidence that β-COP, a component of non-clathrin-coated vesicles believed to mediate intraGolgi transport, is essential for transport of protein from the ER to the *cis*-Golgi compartment. Incubation in the presence of β-COP specific antibodies and F_{ab} fragments prevents the exit of vesicular stomatitis virus glycoprotein (VSV-G) from the ER. These results demonstrate that β-COP is required for the assembly of coat complexes mediating vesicle budding. Fractionation of rat liver cytosol revealed that a major biologically active form of β-COP was found in a high molecular pool (>1,000 kD) distinct from coatomer and which promoted efficient vesicle budding from the ER. Surprisingly, rab1B could be quantitatively coprecipitated with this β-COP containing complex and was also essential for function. We suggest that β-COP functions in an early step during vesicle formation and that rab1B may be recruited as a component of a precoat complex which participates in the export of protein from the ER via vesicular carriers.

Elucidation of the molecular mechanisms which regulate the formation of carrier vesicles which transport protein from the ER through sequential *cis*-, *medial*-, and *trans*-Golgi compartments is central to understanding the secretory pathway of eukaryotic cells (for review see Rothman and Orci, 1992). Proteins which play a key role in this process are those that serve as soluble coat precursors for vesicle budding. The coatomer complex (Waters et al., 1991*b*), a protein complex (14S) which contains α, β, γ, and δ-COP subunits, has been proposed to be a component of non-clathrin coats found on vesicles mediating traffic between Golgi compartments (for review see Duden et al., 1991*a*; Waters et al., 1991*a*). However, its role(s) as a precursor remains controversial due to lack of a direct demonstration of its biological role in transport (for review see Klausner et al., 1992). A second set of proteins are those found to mediate vesicle budding in yeast (for review see Pryer et al., 1992). They include among others the small GTP-binding protein SAR1 (Nakano and Muramatsu, 1989; Barlowe et al., 1993), SEC23 (a SAR1 GTPase activating protein (GAP)[1] (Hicke et al., 1992; Yoshihisa et al., 1993), and SEC12 (a SAR1 specific guanine nucleotide exchange protein (GDS) (Nakano and Schekman, 1988; d'Enfert et al., 1992). Sec21, the yeast homologue to γCOP, is also required for a step in ER to Golgi transport, although its functional role is presently unknown (Hosobuchi et al., 1992). While many of these proteins can be used to generate partial reactions leading to vesicle budding, direct evidence for their role in supporting a complete round of transport is currently lacking.

We have been interested in understanding the biochemical role of the small GTP-binding protein rab1 in ER to Golgi transport in mammalian cells. Rab1 belongs to a family of proteins which are now considered to serve as GTP/GDP regulated molecular switches governing the function of the exocytic and endocytic pathways. Biochemical and genetic evidence suggests that rab proteins regulate the flow of protein between restricted subsets of intracellular compartments (for review see Goud and McCaffrey, 1991; Pfeffer, 1992). For example, whereas both rab1 (Plutner et al., 1991; Schwaninger et al., 1992; Tisdale et al., 1992; Davidson and Balch, 1993) and its yeast homologue, YPT1 (Schmitt et al., 1988; Segev et al., 1988; Bacon et al., 1989; Baker et al., 1990; Segev, 1991; for review see Pryer et al., 1992). Interestingly, SEC4 is associated with a 19.5S complex containing other proteins (SEC8 and SEC15) recognized to mediate the function of vesicle carriers en route to the cell surface (Bowser et al., 1992). The identity of transport components regulated by rab1 or YPT1, and their role in the different steps of vesicle formation, targeting, and fusion is unknown.

Please address all correspondence to William E. Balch, Departments of Cell and Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037.

1. *Abbreviations used in this paper*: endo D, endoglycosidase D; endo H, endoglycosidase H; GAP, a SAR1 GTPase activating protein; GDS, a SAR1 specific guanine nucleotide exchange protein; VSV-G, vesicular stomatitis virus glycoprotein.

© The Rockefeller University Press, 0021-9525/93/09/1155/13 $2.00

To establish the relationship of rabl to other transport components in the temporal events leading up to vesicle budding from the ER, we have examined the role of β-COP using an in vitro assay which efficiently reconstitutes this state of the secretory pathway in the test tube (Schwaninger et al., 1992; Plutner et al., 1992; Davidson and Balch, 1993). We provide evidence that antibodies and F_{ab} fragments specific for β-COP efficiently inhibit vesicle budding from the ER. The principal targets for these antibodies were soluble components found in rat liver cytosol. Fractionation of cytosol using gel filtration revealed the presence of two biologically active pools. A high molecular weight β-COP containing complex (18-19S), which contained only 10-20% of the total soluble β-COP present in cytosol, promoted efficient export of vesicular stomatitis virus glycoprotein (VSV-G) from the ER. Intriguingly, this complex contained both rablB and β-COP, both of which were required for complex function. We suggest that β-COP and rablB may be recruited coordinately to the ER membrane in a precursor complex during the assembly of coats involved in vesicle budding from the ER.

Materials and Methods

Materials

Assay reagents were obtained as described previously (Plutner et al., 1992). Antibodies specific for rablB (m4D3c, 5C6b and p68) were prepared as previously described (Plutner et al., 1990). Monoclonal and polyclonal antibodies specific for β-COP were prepared as previously described (Duden et al., 1991*b*; Pepperkok et al., 1993). Antibodies were kindly provided by the following individuals: a polyclonal antibody (R37) specific for ARF by R. Kahn, Laboratory of Cancer Treatment, National Cancer Institute, Bethesda, MD; a polyclonal antibody to Sec23p by R. Schekman, University of California, Berkeley, CA; a polyclonal antibody specific for p58 by J. Saraste, University of Bergen, Department of Biochemistry, Bergen, Norway; monoclonal antibodies to NSF by J. Rothman (Sloan Kettering Institute, Department of Cellular Biochemistry and Biophysics, NY), and M. Tagaya (Osaka University, Institute of Scientific and Industrial Research, Osaka, Japan). Recombinant β-COP was prepared from SF9 insect cells by expression from a baculovirus vector containing the coding sequence for β-COP (provided by T. Kreis). Coatomer (Waters et al., 1992) was kindly provided by J. Rothman, Department of Cellular Biophysics and Biochemistry, Sloan Kettering, NY and G. Waters, Department of Molecular Biology, Princeton University, Princeton, NJ.

Incubation Conditions and Analysis of Transport

The ER to Golgi transport assays using permeabilized NRK cells infected with tsO45 VSV were performed as described previously (Beckers et al., 1987; Plutner et al., 1992). Briefly, for biochemical quantitation of transport incubations contained in a final total volume of 40 μl (final concentration): 25 mM Hepes-KOH (pH 7.2), 75 mM KOAc, 2.5 mM MgOAc, 5 mM EGTA, 1.8 mM $CaCl_2$, 1 mM ATP, 5 mM creatine phosphate, 0.2 IU of rabbit muscle creatine phosphokinase, 25-75 μg cytosol, and 5 μl (25-30 μg of protein; 1-2 × 10^5 cells) of semi-intact cells. Transport was initiated by transfer to 32°C. After termination of transport by transfer to ice, the membranes were pelleted by a brief (15 s) centrifugation in a microfuge at top speed. For analysis of processing of VSV-G protein to the Man_5 form, the pellet was subsequently solubilized in an endoglycosidase D (endo D) digestion buffer and digested with endo D as described previously (Davidson and Balch, 1993). Endo D digestions were terminated by adding a 5× concentrated gel sample buffer (Laemmli, 1970) and boiling for 5 min. Where indicated, samples were analyzed for the appearance of the endoglycosidase H (endo H) resistant form as described previously (Plutner et al., 1992). The fraction of VSV-G protein processed to the endo D sensitive or endo H resistant forms was quantitated by densitometry (Beckers and Balch, 1989) or using an AMBIS™ radioanalytic imaging system.

Experimental procedures for digitonin permeabilization, incubation in vitro and indirect immunofluorescence were as described previously (Plutner et al., 1992). In all experiments, NRK cells were permeabilized with 40 μg/ml digitonin.

Transport of VSV-G protein from the ER to the p58 containing punctate pre-Golgi intermediates was quantitated using indirect immunofluorescence (Plutner et al., 1992; Schwaninger et al., 1992; Pind et al., 1993). Briefly, fields of cells were randomly chosen and the number of pre-Golgi punctate structures containing VSV-G determined for each cell in a given field. Generally, 10 fields with an average of 10 cells per field were counted with the total number of punctate structures ranging from none (no transport) to 30-60 per cell under optimal transport conditions. Pre-Golgi intermediates are very distinct in terms of their compact structure and fluorescence intensity compared to the diffuse staining of VSV-G protein when present in the extended ER network (Plutner et al., 1992).

Appearance of VSV-G protein in punctate structures strongly correlates (>75%) with its colocalization with the p58 marker protein or colocalization (>90%) with rablB containing punctate intermediates (Plutner et al., 1992; Schwaninger et al., 1992). The number of punctate structures detected in a given cell is proportional to the concentration of cytosol in the assay, the time of incubation, as well as being sensitive to a broad range of reagents affecting VSV-G transport (Plutner et al., 1992; Schwaninger et al., 1992). These structures consist of clusters of vesicles and small tubular elements as determined by immunoelectron microscopy (Pind et al., 1993). By totaling the number of pre-Golgi punctate structures containing VSV-G for each cell in a field for a given experimental condition, the average number of punctate structures observed per cell over all counted fields was obtained. The statistical variation between cells was routinely within 5-15% of the average value reported in Results for different experiments.

The transport of VSV-G to morphologically distinct intermediates serves as a semiquantitative measure of the extent of export of VSV-G from the diffuse ER network. Results are generally reported as % of total number of punctate structures observed compared to control incubations containing a maximal stimulatory level of rat liver cytosol or the Superose 6 void pool as indicated in Results. This control value was set as 100.

Precipitation of rablB/β-COP-containing Complexes

Rat liver cytosol prepared as described previously (Davidson and Balch, 1992) was centrifuged for 10 min in a microfuge (or 10 min at 150,000 *g* in an Airfuge [Beckman Instruments, Palo Alto, CA] with identical results). The supernate (10-15 mg/ml) of unfractionated cytosol or the Superose 6 void fraction (see below) was combined with the rablB specific m4D3c, or β-COP specific M3A5, or Dlm antibodies (in 25 mM Hepes [pH 7.2], 125 mM KOAc [25/125]) to yield a final concentration of 50 ng antibody per μg cytosol. The antibody containing cytosol was incubated on ice overnight. Subsequently, the cytosol was centrifuged for 10 min in a microfuge to remove pelletable material. Under the conditions used to assay transport as described in Results, the concentration of antibodies remaining in the supernate do not inhibit transport when added separately. Where indicated, precipitates were resuspended by trituration in 25/125 and washed once by centrifugation for 10 min in a microfuge.

To determine the composition of immune complexes formed in the presence of m4D3c or M3A5, 200 μl of the Superose 6 pooled void fractions was incubated for 1 h on ice in the presence of 5 μg of the respective antibody followed by the addition of 5 mg protein A-Sepharose beads (pretreated with 0.1% milk) for 1 h on ice. Beads were subsequently washed five times with 25/125 and bound proteins released using 1× gel sample buffer (Laemmli, 1970). Samples were separated using SDS-PAGE and transferred to nitrocellulose for blotting with either p68 or M3A5 antibodies. Western blotting was performed as described previously (Tisdale et al., 1992).

Gel Filtration

A Superose 6™ column (Pharmacia LKB Biotechnology, Piscataway, NJ) was equilibrated with four column volumes of 25/125 buffer and 0.5 ml of pre-spun rat liver cytosol was fractionated at a flow rate of 0.4 ml/min. Fractions of 0.5 ml were collected and assayed immediately as described above. SDS-PAGE and/or Western blotting was performed as described previously using 1% milk as a blocking reagent (Tisdale et al., 1992). Protein concentrations were determined using a Biorad protein kit (Biorad Labs., Richmond, VA).

Sucrose Velocity Sedimentation

The sedimentation value of the rablB-containing fraction present in the Superose 6 void fraction was determined exactly as described previously (Duden et al., 1991*b*).

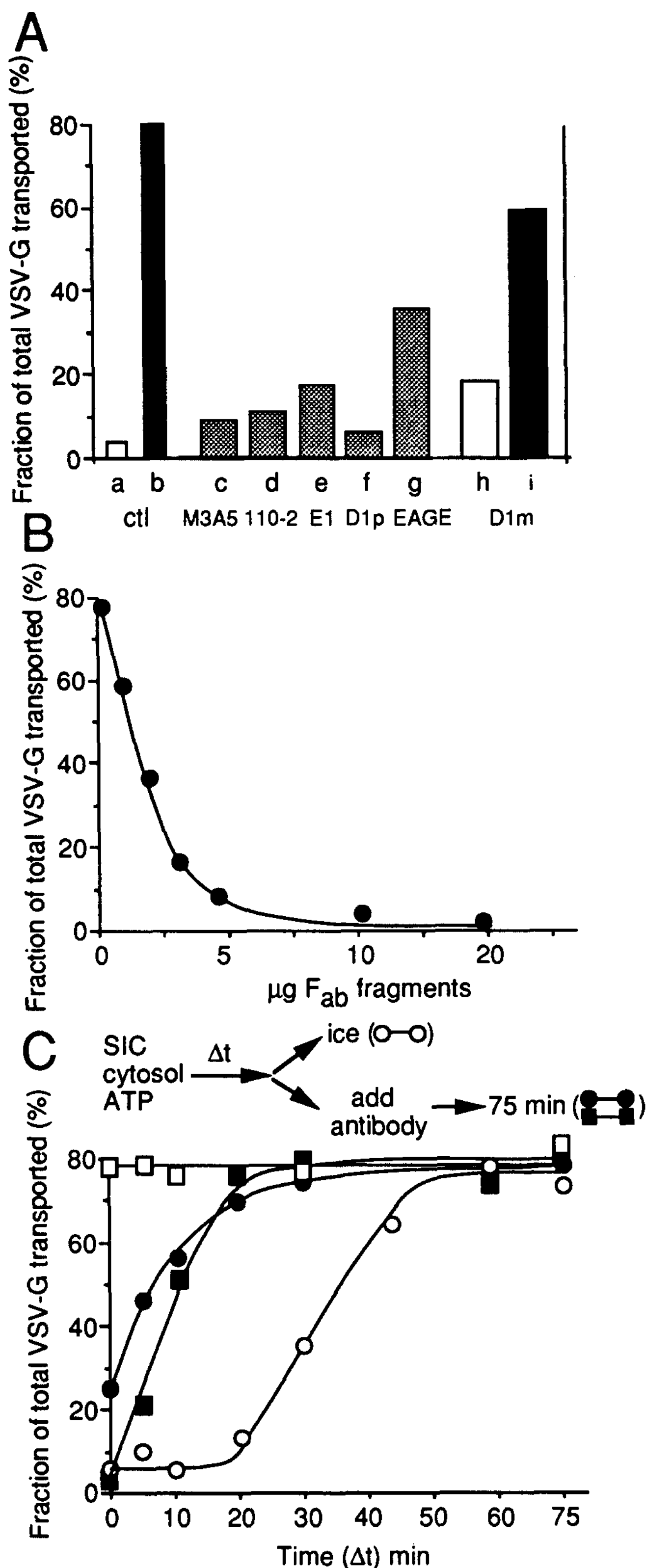

Figure 1. Antibodies specific for β-COP inhibit ER to Golgi transport in vitro. (*A*) Permeabilized cells, cytosol, and ATP were preincubated with the indicated antibodies specific for β-COP for 1 h on ice before incubation in vitro for 75 min. Transport conditions were as follows: (*a*) 75-min incubation on ice; (*b*) 75-min incubation at 32°C (no antibody); pretreatment with either M3A5 (6 μg) (*c*), 110-2 (2 μg) (*d*), El (2 μg) (*e*), polyclonal Dlp (2 μg) (*f*), or EAGE (6 μg) (*g*); (*h* and *i*) pretreatment with monoclonal reagent Dlm (4 μg). In lane *i*, the Dlm containing assay was supplemented with an additional portion (50 μg) of rat liver cytosol to reverse inhibition. (*B*) Permeabilized cells, cytosol, and ATP were pretreated with increasing concentration of M3A5 F_{ab} fragments for 60 min on ice before incubation in vitro for 75 min at 32°C. (*C*) Permeabilized cells were incubated for increasing time at 32°C in the presence of cytosol and ATP before transfer to ice (*open circles*). Cells were subsequently incubated in the presence of the Dlm (4 μg) (*closed circles*), M3A5 F_{ab} fragments (4 μg) (*closed squares*), or in the absence of added antibody (control, *open squares*) for 60 min on ice. Cells were reincubated at 32°C for a total time of 75 min (*closed symbols*). In these experiments, the extent of transport was measured by determining the fraction of VSV-G processed to the endo H sensitive form (biochemical assay) as described in Materials and Methods.

Results

Antibodies Specific for β-COP Inhibit Export of VSV-G from the ER

To assess the functional role of β-COP in ER to Golgi transport, we tested a number of β-COP specific monoclonal and polyclonal antibodies to determine if they inhibit the delivery of VSV-G to the Golgi stack. Transport in vitro using semi-intact or permeabilized cells can be measured by the processing of VSV-G to the endo D sensitive form or to the endo H resistant R_1 form upon arrival in the *cis*-Golgi compartments (Schwaninger et al., 1992; Plutner et al., 1992; Davidson and Balch, 1993). Both assays are indistinguishable in their kinetics of transport and biochemical requirements (Schwaninger et al., 1992; Plutner et al., 1992; Davidson and Balch, 1993).

Six different β-COP specific antibodies inhibited VSV-G transport in vitro (Fig. 1 *A*, *c–h*). All but one strongly inhibited transport when semi-intact cells and cytosol were preincubated with antibody for 1 h on ice before incubation in vitro. Three of these antibodies, designated 110-2, El, and Dlp, were affinity purified polyclonal antibodies prepared against synthetic peptides to different regions of β-COP (Duden et al., 1991*b*; Pepperkok et al., 1993). Two were monoclonal reagents, one prepared against the Dl peptide (Dlm) or the whole protein (M3A5) (Allan and Kreis, 1986; Pepperkok et al., 1993). We were unable to use peptides to neutralize antibody inhibition due to the sensitivity of our assay to the high concentrations required for this purpose (Pepperkok et al., 1993). Antibody inhibition could be neutralized in all cases by preincubation with recombinant β-COP (data not shown); however, neither recombinant β-COP nor partially purified coatomer (Waters et al., 1992) could reverse the inhibition. In contrast, rat liver cytosol was found to efficiently reverse inhibition by Dlm (Fig. 1, *h–i*) and other antibodies tested (data not shown). No inhibition of transport was observed in the presence of antibodies which detect proteins unrelated to vesicular trafficking, as has been demonstrated previously (Plutner et al., 1991).

To insure that inhibition was not simply the result of aggregation of β-COP on the membranes, F_{ab} fragments were prepared from M3A5. As shown in Fig. 1 *B*, F_{ab} fragments inhibited transport in a dose-dependent fashion with close to 90% efficiency. F_{ab} fragments were found to consistently yield a more complete inhibition than intact antibodies and did not require preincubation, suggesting rapid binding to the biologically active forms of β-COP (data not shown).

Given the suggestion that β-COP is likely to be involved in vesicle formation (Waters et al., 1991*b*), we examined the

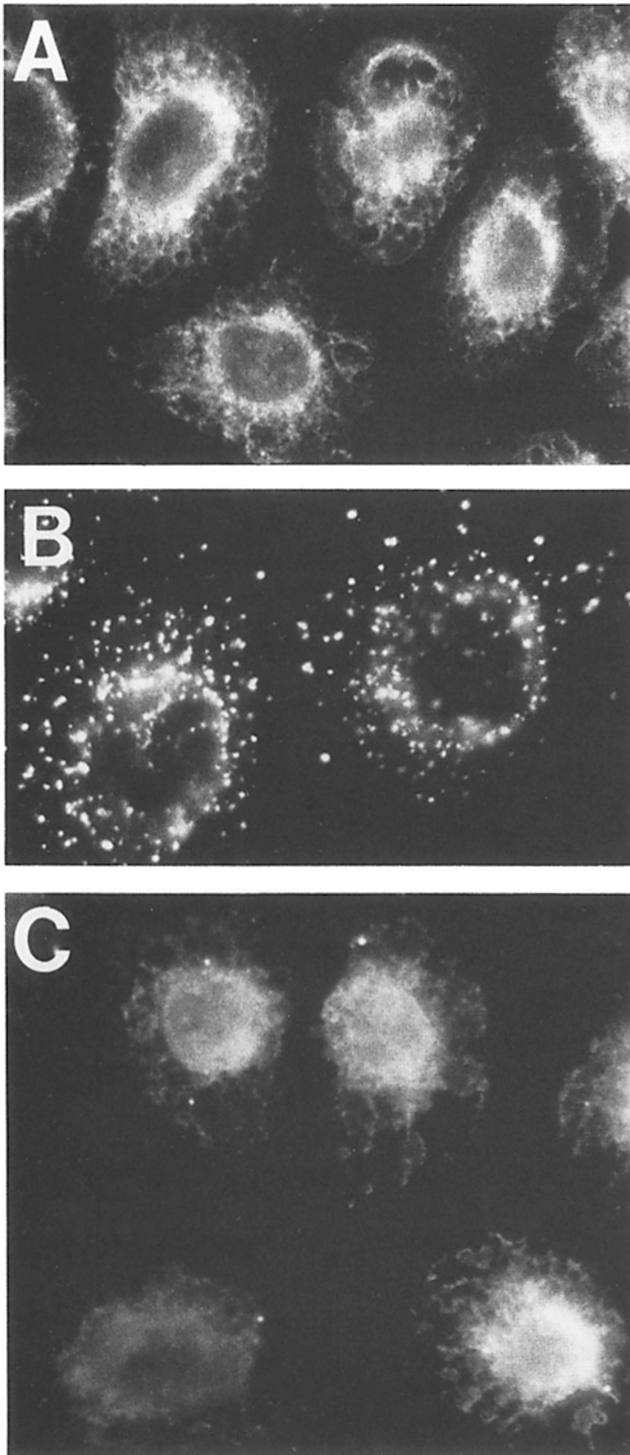

Figure 2. Incubation in vitro in the presence of a β-COP specific monoclonal antibody (Dlm) prevents export of VSV-G from the ER. NRK cells were permeabilized and preincubated in a complete cocktail containing cytosol and ATP for 75 min on ice in the absence (*A* and *B*) or the presence of 4 μg of a monoclonal antibody (Dlm) specific for β-COP (*C*) before incubation in vitro on ice (*A*) or at 32°C (*B* and *C*) for 45 min as described previously (Plutner et al., 1992). The distribution of VSV-G at the end of the incubation was determined using indirect immunofluorescence as described previously (Plutner et al., 1992).

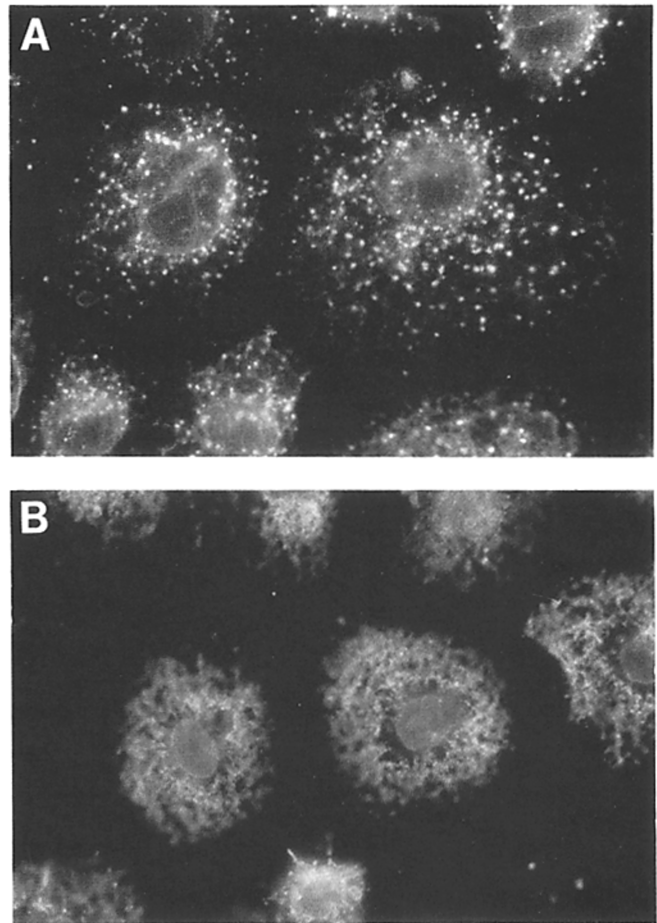

Figure 3. β-COP specific F_{ab} fragments inhibit export from the ER. NRK cells were permeabilized and preincubated in a complete cocktail containing cytosol and ATP in the presence (*B*) or absence (*A*) of 5 μg M3A5 F_{ab} fragments for 60 min on ice before incubation in vitro at 32°C for 45 min. The distribution of VSV-G was determined using indirect immunofluorescence as described previously (Plutner et al., 1992).

temporal sensitivity of VSV-G transport to the β-COP specific Dlm antibody and to M3A5 F_{ab} fragments by adding these reagents after increasing time of incubation of cells in vitro. Both reagents inhibited transport at early, but not late times of addition (Fig. 1 *C*). After only 5–15 min of incubation in vitro in the absence of antibody, time points in which <5% of the total VSV-G was processed to Golgi associated forms (Fig. 1 *C, open circles*), the majority (>80–90%) of VSV-G transport was found to be insensitive to Dlm (Fig. 1 *C, closed circles*) and M3A5 F_{ab} fragments (Fig. 1 *C, closed squares*). These results support the interpretation that β-COP may be required at an early step in vesicle budding from the ER.

A morphological approach was also used to analyze the effects of β-COP specific antibodies on the transport of VSV from the ER to the Golgi in digitonin permeabilized cells grown on coverslips (Plutner et al., 1992). In this assay, we use indirect immunofluorescence and a temperature-sensitive form of VSV-G (strain tsO45) (Plutner et al., 1992). tsO45 VSV-G is found exclusively in the ER when cells are maintained at the restrictive temperature (39.5°C) before permeabilization (Fig. 2 *A*). Upon incubation of permeabi-

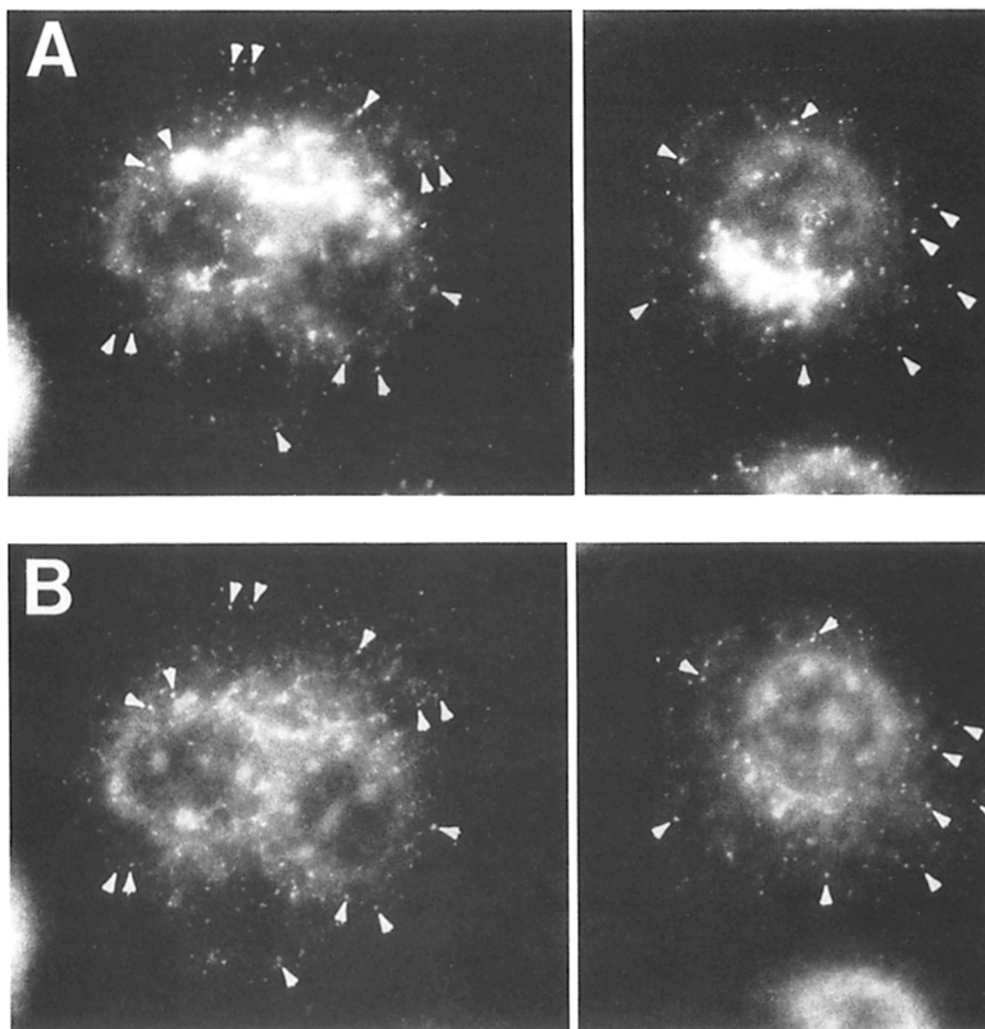

Figure 4. β-COP can be detected on pre-Golgi intermediates. Permeabilized cells were incubated in the presence of cytosol and ATP for 15 min as described in Materials and Methods. The distribution of β-COP (using Dlm) (*A*) and p58 (*B*) were determined as described previously (Plutner et al., 1992). The arrows indicate examples of punctate structures which contain both p58 and β-COP. These pre-Golgi intermediates containing VSV-G do not colocalize with the *cis*/medial Golgi enzyme α-1,2 mannosidase II (data not shown).

lized cells at the permissive temperature (32°C) in the presence of cytosol and ATP, tsO45 VSV-G is transported via punctate, pre-Golgi intermediates, which consist of clusters of carrier vesicles and small tubules (Pind et al., 1993), to the Golgi stack (Fig. 2 *B*) (Plutner et al., 1992; Schwaninger et al., 1992). Incubation in the presence of the Dlm antibody completely inhibited export from the ER. VSV-G was retained in the extensive reticulum observed before incubation in vitro (Fig. 2, compare *A–C*). Export from the ER was also strongly inhibited by the addition of M3A5 F_{ab} fragments (Fig. 3 *B*). No discernible effect of either intact antibody or F_{ab} fragments on Golgi structure could be detected based on the distribution of the α-1,2-mannosidase II, a *cis*/medial Golgi marker protein (data not shown). These results confirm the early effect of β-COP specific antibodies measured biochemically (Fig. 1 *C*), and further indicate that inhibition is not a consequence of blocking either the function of the *cis*-Golgi compartment as an acceptor, or inhibiting indirectly the processing VSV-G to various endo D sensitive and endo H resistant forms.

β-COP Colocalizes with p58 in Pre-Golgi Intermediates

To determine whether the punctate, pre-Golgi transport intermediates observed using indirect immunofluorescence contained β-COP (Fig. 2 *B*), we examined the morphological distribution of β-COP after 15 min of incubation in vitro and compared its distribution to that of the pre-Golgi intermediate marker protein p58 (Saraste and Svensson, 1991). As shown in Fig. 4 *A*, β-COP was detected in the Golgi region and strongly colocalized with p58 containing punctate structures (Fig. 4 *B*). Using confocal microscopy, the extent of colocalization was determined to be nearly 75% (data not shown). We have previously demonstrated that these p58 containing intermediates are enriched in both VSV-G and rab1B after 15 min incubation in vitro (Plutner et al., 1992; Schwaninger et al., 1992). The combined morphological and biochemical analyses are consistent with the interpretation that vesicles mediating ER to Golgi transport acquire both β-COP and rab1B at an early step, presumably during vesicle budding.

Cytosolic Forms of β-COP and Rab1B Are Required for Export from the ER

Efficient inhibition of transport by both the β-COP specific M3A5 and rab1B specific m4D3c antibodies requires preincubation of cells for 45–60 min on ice, presumably to allow for the formation of immune complexes (Plutner et al., 1991). We previously reported (Plutner et al., 1991) that in-

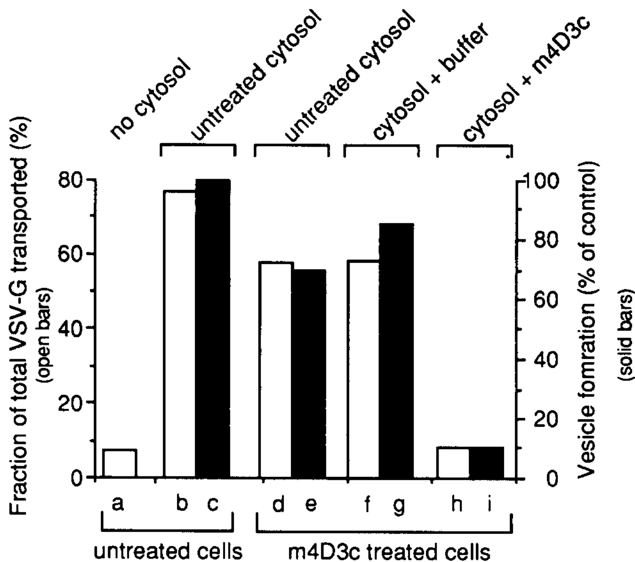

Figure 5. Rat liver cytosol reverses inhibition of transport by rab1B specific antibodies. Semi-intact cells were either not treated (*a–c*) or pretreated (*d–i*) with m4D3c on ice as described in Materials and Methods. Subsequently, cells were incubated in the presence of cytosol which was either not treated (*a–e*), mock-treated (*f–g*), or incubated with m4D3c overnight on ice as described in Materials and Methods. Transport was determined using both the biochemical (endo D sensitivity) (*open bars*) and morphological (*solid bars*) assays as described in Materials and Methods. Transport conditions were as follows: (*a*) incubation in vitro at 32°C in the absence of cytosol; (*b* and *c*) incubation in vitro at 32°C in the presence of untreated cytosol; (*d* and *e*) cells were pretreated with m4D3c for 75 min on ice, washed to remove excess antibody, and incubated in the presence of untreated cytosol; (*f* and *g*) permeabilized cells were pretreated with m4D3c for 75 min on ice, washed, and incubated with cytosol which had been incubated overnight on ice in the absence of antibody; and (*h* and *i*) permeabilized cells were pretreated with m4D3c for 75 min on ice, washed, and incubated with cytosol which had been pretreated with m4D3c overnight on ice.

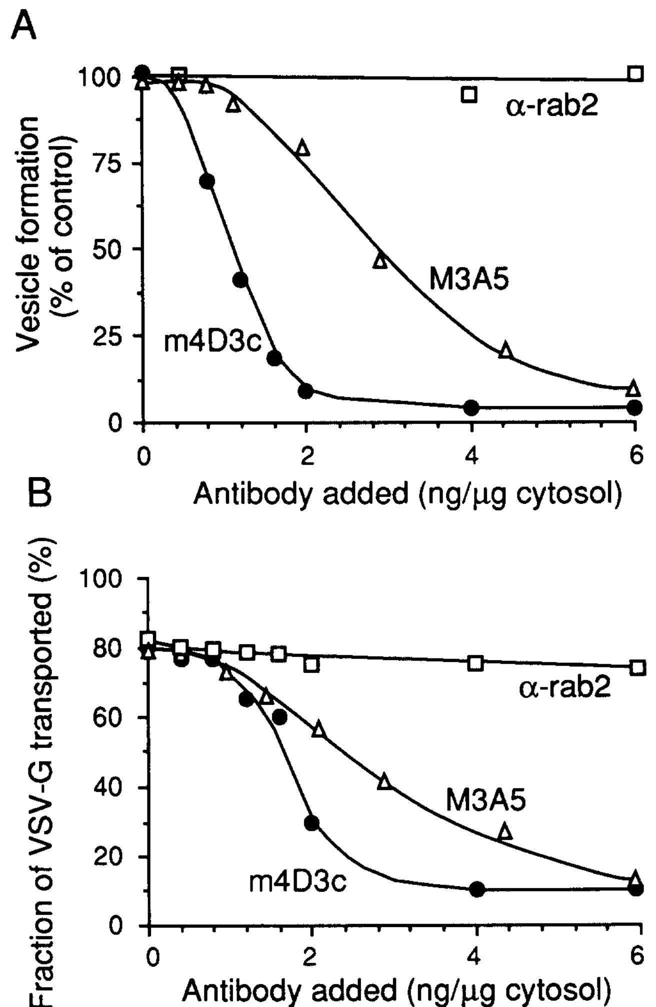

Figure 6. Vesicle budding and transport to the *cis*-Golgi show similar requirements for rab1B and β-Cop. Rat liver cytosol was pretreated overnight on ice with increasing amounts of the rab1B specific (m4D3c) (*closed circles*) or β-COP specific (M3A5) (*open triangles*) monoclonal antibodies as described in Materials and Methods. Supernatants were subsequently assayed either for their ability to support export from the ER using indirect immunofluorescence (morphological assay [*A*]) or processing of VSV-G to the endo D sensitive form (biochemical assay [*B*]) as described in Materials and Methods. A monoclonal antibody specific for rab2 (m2C11a) which does not inhibit transport is shown with the *open squares*.

hibition by m4D3c could not be reversed by the addition of cytosol prepared from CHO cells, but was reversible by components released from CHO membranes, suggesting that a membrane-bound form of rab1B was required. In contrast, we have now found that rat liver cytosol efficiently reverses inhibition as measured biochemically by the acquisition of endo D resistance (Fig. 5, lane *d*) or using indirect immunofluorescence as a semiquantitative method to monitor the maturation of VSV-G to punctate, pre-Golgi intermediates and to the Golgi stack (Fig. 5, lane *e*). Consistent with these results, rat liver cytosol was found to contain a substantial pool of a soluble form of rab1B (see below) which could not be detected in CHO cytosol (Plutner et al., 1991). Since factors involved in vesicle budding must extensively recycle, their distribution between membrane and/or soluble forms is likely to reflect the steady state concentration of various precursor pools in different tissues. As rat liver cytosol can be used to efficiently reverse inhibition by both β-COP and rab1B specific antibodies, it provides us with a soluble source to explore the functional relationships between these two proteins.

To examine the relationship between the function of rab1B and β-COP in the transport of VSV-G to either pre-Golgi intermediates (morphological assay) or to the *cis*-Golgi compartment (biochemical assay), we established conditions to independently inactivate both components by incubating cytosol overnight on ice with specific antibody. In the case of the rab1 specific m4Dc3 antibody, when pretreated cytosol was centrifuged to remove possible immune complexes, the resulting supernatant failed to support processing of VSV-G to the *cis*-Golgi endo D sensitive form (Fig. 5, lane *h*) and the transport of VSV-G from the ER to punctate, pre-Golgi intermediates when quantitated using indirect immunofluorescence (Fig. 5, lane *i*). Cytosol can be inactivated by pretreatment with the rab1B specific antibodies m5C6b and p68, but not with other monoclonal antibodies including one specific for rab2 (m2C11a) (Fig. 6), an IgM specific for NSF (SEC18) (Beckers et al., 1989), nor monoclonal antibodies to antigens unrelated to vesicular transport (data not shown). When the concentration of m4D3c required to inac-

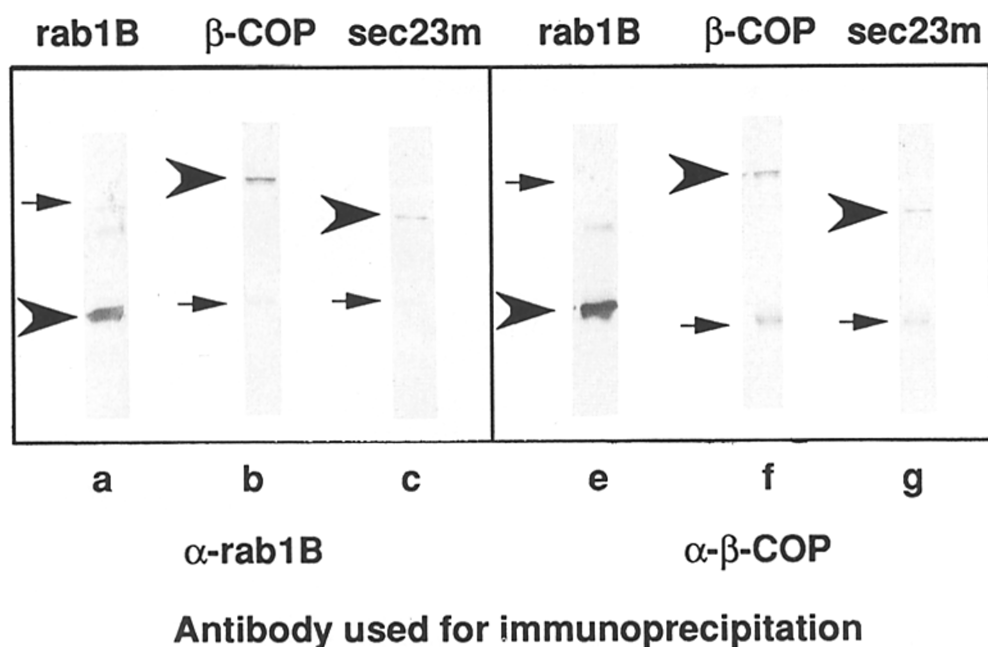

Figure 7. RablB and β-COP can be coprecipitated from rat liver cytosol. Immune complexes formed by incubation of rat liver cytosol in the presence of m4D3c (α-rablB) (*a–c*) or M3A5 (α-β-COP) (*e–f*) were separated by SDS-PAGE, transferred to nitrocellulose and the distribution of rablB (*a* and *e*), β-COP (*b* and *f*) and a cross-reactive species to yeast SEC23 (sec23m) (*c* and *g*) detected using Western blotting as described in Materials and Methods. The separations were performed using either 10% SDS-PAGE for β-COP and sec23m, or 15% SDS-PAGE for rablB. The portion of the gel containing the indicated protein (*large arrowheads*) is shown. RablB and β-COP migrated with the expected molecular weights based on comparison to the recombinant protein. The small arrows indicate the position of the heavy or light chains present in the immune precipitate which are detected by the secondary HRP-conjugated reagent.

tivate cytosol was determined, both budding from the ER and delivery to the *cis*-Golgi compartment were inhibited with similar efficiencies (EC_{50} ~1.5 ng antibody/μg cytosol) (Fig. 6, compare *closed circles* in *A* [morphological assay] to *closed circles* in *B* [biochemical assay]) with maximal inhibition in the presence of ~3–4 ng antibody/μg cytosol. Under these saturating conditions, ~20–30% of the total rablB could be pelleted in the form of immune complexes based on Western blotting (data not shown).

A similar result was obtained using the β-COP specific M3A5 antibody. After pelleting to remove the precipitate, we found that the supernate failed to support either the transport of VSV-G from the ER to pre-Golgi intermediates (Fig. 6 *A*, *open triangles*) or processing of VSV-G to the endo D sensitive form (Fig. 6 *B*, *open triangles*). In this case, the EC_{50} for inhibition of transport was ~2–3 ng antibody/μg cytosol for both assays with maximal inhibition at ~6 ng antibody/μg cytosol. Under saturating conditions only 10–20% of the total rablB could be pelleted from cytosol based on Western blotting (data not shown). For both antibodies, the concentration of M3A5 or m4D3c required to inhibit transport when preincubated with cytosol overnight was ~5–10-fold lower than the amount required when added directly to an assay (~50 ng/μg cytosol), presumably reflecting the kinetics of antibody-complex formation.

The strong correlation between the requirement for β-COP and rablB for vesicle budding (morphological assay) and transport to the *cis*-Golgi (biochemical assay) suggests that the punctate structures observed using indirect immunofluorescence are bona fide carrier vesicles mediating ER to Golgi transport.

A High Molecular Weight Protein Complex Stimulates Export of VSV-G from the ER but Not Transport to the Golgi

The observation that ~20–30% of the rablB forms a pelletable immune complex in the presence of m4D3c is consistent with our previous observation that rablB containing immune complexes can be readily detected using indirect immunofluorescence (Schwaninger et al., 1992). Since m4D3c is a monoclonal antibody, these complexes must contain multiple copies of rablB and possibly other transport components related to rabl function. To explore this possibility, the precipitate formed by overnight incubation on ice was analyzed using Western blotting. We detected not only rablB, but both β-COP and a cross-reacting species to yeast Sec23p (sec23m), a SARl specific GAP involved in export from the ER in yeast (Yoshihisa et al., 1993) (Fig. 7, *a–c*). In contrast, we did not detect NSF or rab2 (data not shown). Conversely, when the β-COP specific M3A5 was added to cytosol and the pelletable immune complex examined using Western blotting, we found β-COP, rablB and sec23m (Fig. 7, *d–f*). Neither rablB, β-COP, nor sec23m were detected in the absence of specific antibody (data not shown).

To ascertain the size of these protein complexes possibly involved in ER to Golgi transport, cytosol, prepared by centrifugation at 150,000 *g* for 1.5 h to insure removal of potential vesicular contaminants, was gel-filtered over a Superose 6 FPLC column. Column fractions were simultaneously assayed for (*a*) export to pre-Golgi intermediates (morphological assay), (*b*) transport to the *cis*-Golgi compartment (biochemical assay), and (*c*) the distribution of rablB, β-COP, ARFl, and sec23m.

Two peaks of activity supporting vesicle budding from the ER were detected based on the morphological assay. These together accounted for 50–70% of the total activity loaded on the column (Fig. 8 *A*, *open circles*). One peak, containing <20–30% of the total activity stimulating export of VSV-G from the ER to pre-Golgi intermediates was found in the included fractions (Fig. 8 *A*, *open circles* [fractions 8–13]). These fractions are likely to contain coatomer (Waters et al., 1992) as indicated by the distribution of β-COP (Fig. 8 *B*).

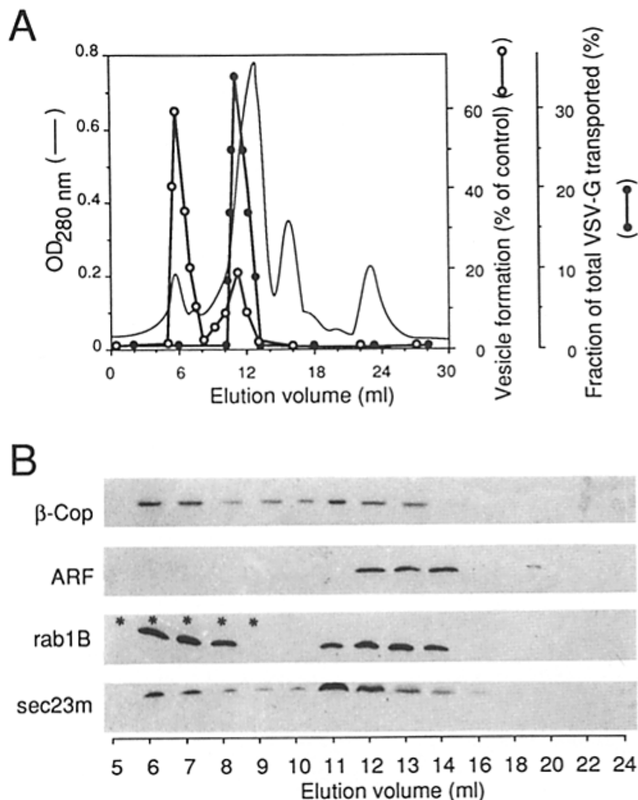

Figure 8. A high molecular weight complex supports export from the ER, but not transport to the *cis*-Golgi compartment. (*A*) Rat liver cytosol was gel filtered on a Superose 6™ column as described in Materials and Methods. Fractions were assayed for both biochemical (endo D sensitivity) (*closed circles*) and morphological (*open circles*) transport as described in Materials and Methods. (*B*) The distribution of β-COP, ARF, rab1B, and sec23m was determined by Western blotting as described in Materials and Methods. Only the portion of the gel containing the relevant protein is shown. The asterisk (*) indicates that the amount of material loaded on the gel in these loads was increased 10-fold over that used in all other lanes to enhance the signal for rab1B.

In addition to vesicle budding, these fractions also supported transport of VSV-G to the Golgi based on both indirect immunofluorescence (data not shown) and the processing of VSV-G to the endo D sensitive form (Fig. 8 *A*, *closed circles*). In contrast, the majority (60–80%) of the export activity detected using indirect immunofluorescence was routinely found in the voided fractions (Fig. 8 *A*, *open circles* [fractions 5–8]), and therefore contains components or complexes with putative molecular weights >1,000 kD. The specific budding activity in the void pool was generally 7–10-fold higher than that found in the crude cytosol. This pool lacked detectable lipid (data not shown), eliminating the trivial possibility that the void fraction contained transport vesicles which could contribute coat precursors. Although this pool supported efficient transport of VSV-G to pre-Golgi intermediates (Fig. 9 *A*, *arrows*), we could not detect transport to the Golgi stack using indirect immunofluorescence (data not shown), nor was VSV-G processed to the endo D sensitive form (Fig. 8 *A*, *closed circles*).

Consistent with the observation that vesicle budding from the ER required β-COP and rab1B, a significant pool (~10–20% based on quantitative immunoblotting) could be

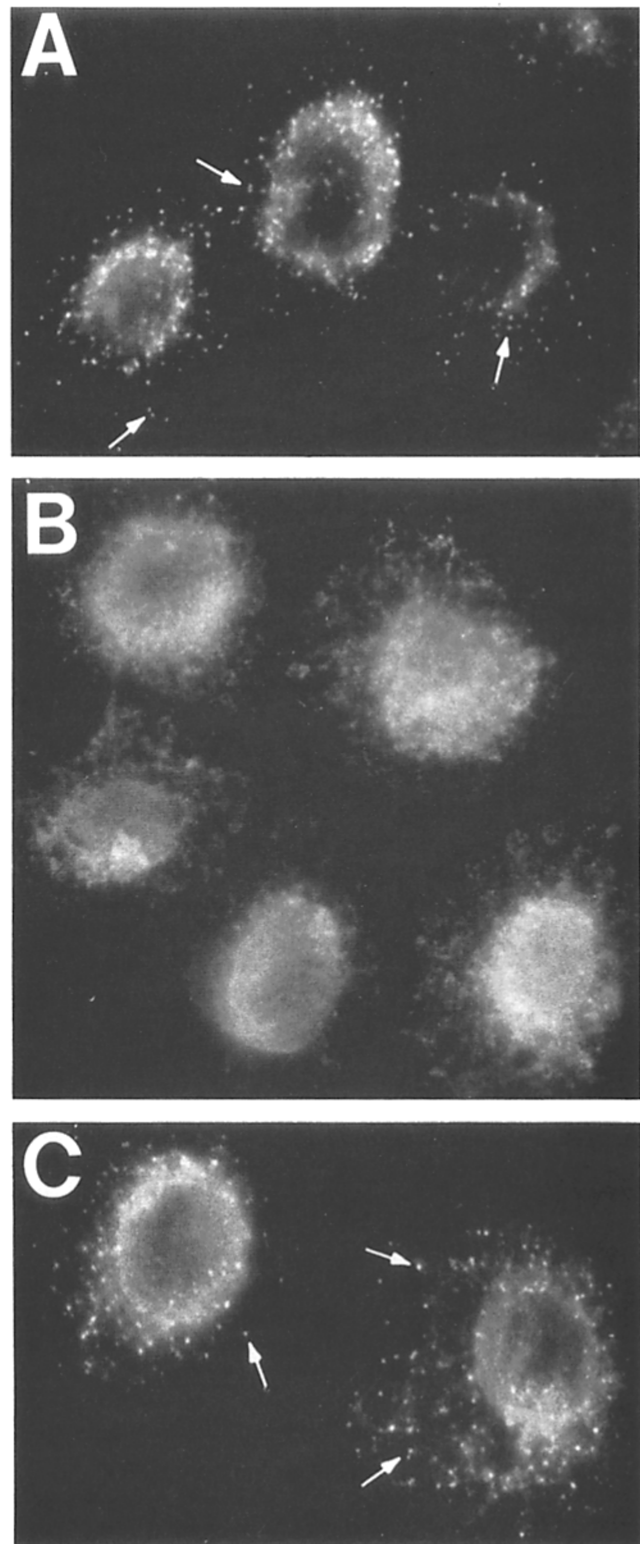

Figure 9. The vesicle budding activity of Superose 6 void fractions requires β-COP. Permeabilized cells and 40 μl of the pooled void fractions were preincubated for 60 min on ice in the absence (*A*) or presence (*B* and *C*) of D1m. In *C*, the assay was supplemented with an additional 40 μl of the void pool before incubation at 32°C. Subsequently, cells were incubated for 45 min at 32°C. Export of VSV-G from the ER to pre-Golgi intermediates was detected using indirect immunofluorescence as described in Materials and Methods. The punctate structures in *A* and *C* colocalize with the pre-Golgi intermediate marker protein p58 (see Fig. 4).

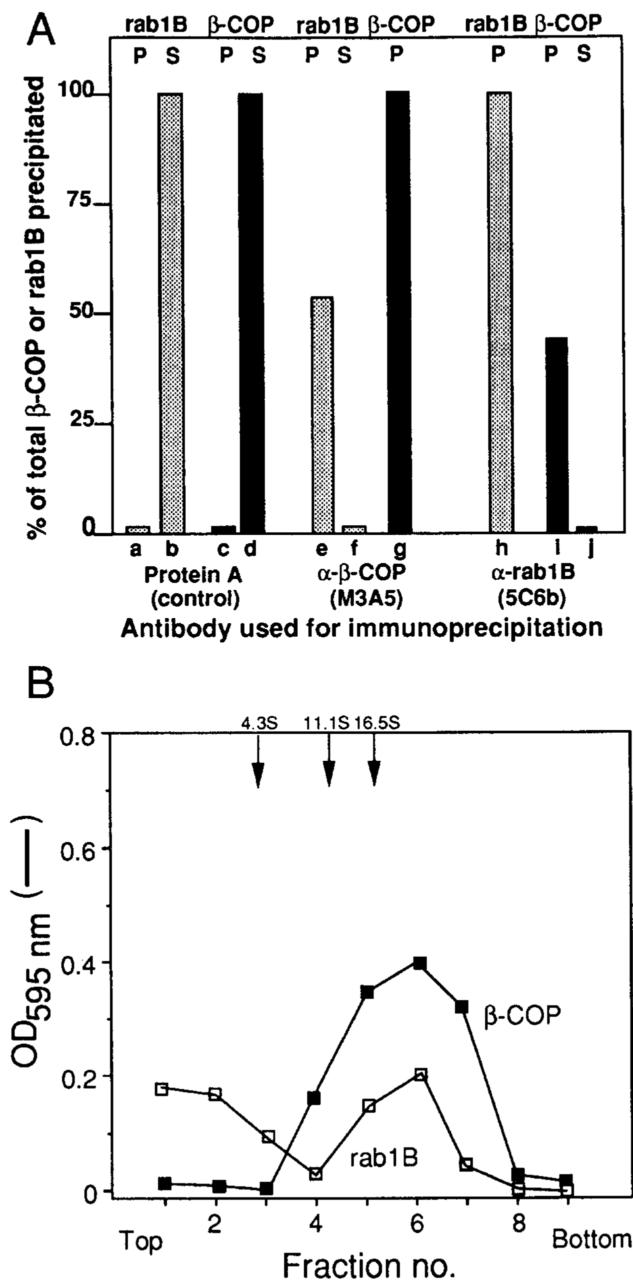

Figure 10. The biologically active protein complex found in the void fractions contains both rablB and β-COP and sentiments as a ~18-19S complex using sucrose density centrifugation. (*A*) The void pool was incubated in the presence of no antibody (*a–d*), M3A5 (β-COP specific) (*e–g*) or m5C6b (rablB specific) (*h–j*) antibodies as described in Materials and Methods. Proteins bound to protein A-Sepharose beads were eluted, separated using SDS-PAGE, transferred to nitrocellulose for Western blotting, and quantitated using densitometry. The values reported are the percent of total rablB or β-COP present in the void pool bound to beads in the presence or absence of antibody (*P*) or, where indicated, the amount of protein remaining in the supernatant (*S*). (*B*) The void pool was prepared and analyzed using sucrose density centrifugation as described in Materials and Methods (Duden et al., 1991*b*). Samples from each sucrose gradient fraction were separated using SDS-PAGE and transferred to nitrocellulose for Western blotting. The distributions of rablB and β-COP are illustrated. The standards used to calibrate the gradient were catalase (*4.3S*), BSA (*11.1S*), and bovine thyroglobulin (*16.5S*) as used previously for determination of the coatomer S value (Duden et al., 1991*b*).

detected in the void fractions. RablB could also be detected in two pools, one corresponding to the high molecular weight void fractions (~10–20% of total based on quantitative immunoblotting [data not shown]), and the remainder in a second pool found in the included fractions (Fig. 8 *B*). Its distribution in the included fraction was coincident with the peak of activity supporting complete transport to the *cis*-Golgi compartment and overlapped in part with the broad distribution of β-COP (Fig. 8 *A*). These results are consistent with the observation that ~20% of the total pools of rablB or β-COP found in crude cytosol form a pelletable precipitate in the presence of specific antibody. In addition, sec23m was detected in the void pool (~10–15% of total based on Western blotting). In contrast, all of the ARF (>98%) (Fig. 8 *B*) and NSF (data not shown) were found in the included fractions.

RablB and β-COP Found in the Void Fractions Form a Functional Complex

We have demonstrated up to this point that the void fractions contain high molecular weight forms of rablB and β-COP, contain a significant fraction of the budding activity eluting from the Superose 6 column and, in addition, have a 7–10-fold increase in specific activity over that found in the crude cytosol. We were curious as to whether the void fractions contained two distinct complexes–one containing multiple copies of rablB and a second containing β-COP, or whether they formed a common complex active in vesicle budding. To determine whether there was a physical association between rablB and β-COP, the void fraction was treated with rablB and β-COP specific antibodies in the presence of protein A-Sepharose beads. Proteins recovered on the beads were analyzed using Western blotting. As shown in Fig. 10, protein A beads incubated in the absence of either m4D3c or M3A5 antibodies failed to bind detectable levels of β-COP or rablB (Fig. 10 *A*; *a* and *c*). In contrast, all the rablB (99%) and ~40% of the total β-COP pool found in the void fraction was associated with the beads in the presence of the anti-rablB antibody (Fig. 10 *A*; *h* and *i*). Addition of recombinant rablB to the mixture before addition of specific antibody blocked coprecipitation of β-COP (data not shown). Conversely, the β-COP specific M3A5 antibody immunoprecipitated >99% of the total β-COP with 50% of the total rablB in the void (Fig. 10 *A*; *e* and *g*). The recoveries observed in the protein A-Sepharose pellets reflect the partial dissociation of complexes during washing steps, as no detectable β-COP or rablB remained in the supernatant of antibody/protein A-treated cytosol (Fig. 10 *A*; *f* and *j*). In contrast, we have observed that neither antibody will coprecipitate rablB or β-COP from the included fractions (data not shown).

Consistent with the ability of rablB and β-COP to form a complex, analysis of the void pool using sucrose density gradient centrifugation demonstrated that both rablB and β-COP cosedimented as an ~18–19S particle, similar in density to the distribution of a SEC4 containing particle (19.5S) (Bowser et al., 1992). RablB could also be detected in earlier gradient fractions, presumably reflecting partial dissociation from the complex during the centrifugation (Fig. 10 *B*).

To demonstrate that the high molecular weight forms of β-COP and rablB were required for vesicle budding, the void fraction was preincubated in the presence of β-COP specific

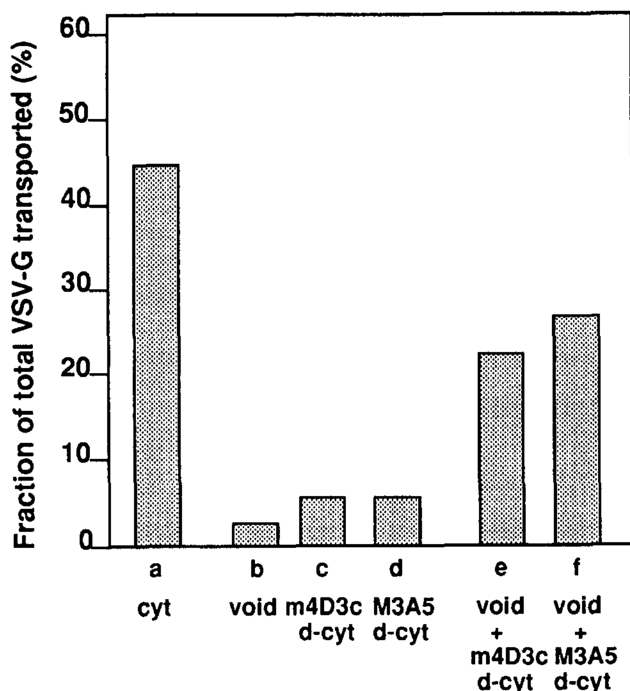

Figure 11. The high molecular pool promotes the formation of functional transport vesicles. Permeabilized cells were incubated in vitro for 90 min at 32°C in the presence of untreated cytosol (lane *a*) or 40 μl of the void pool (lanes *b*, *e*, and *f*) in the absence (lane *b*) or presence of cytosol pretreated with rab1B specific m4D3c (lane *e*), or β-COP specific M3A5 (lane *f*) antibodies by overnight incubation on ice as described in Materials and Methods. The transport activity of m4D3c- or M3A5-treated cytosols when incubated alone is shown in lanes *c* and *d*, respectively. The amount of VSV-G processed to the endo H resistant form was quantitated as described in Materials and Methods.

or rab1B specific antibodies. As expected, both M3A5 (Figs. 6 *A* and 9 *B*) and m4D3c (Fig. 6 *A*) inhibited export of VSV-G from the ER to punctate pre-Golgi intermediates based on indirect immunofluorescence. When the M3A5-treated sample (Fig. 9 *B*) was supplemented with an additional portion of the void pool, transport of VSV-G to punctate, pre-Golgi intermediates was restored (Fig. 9 *C*). In contrast, supplementation of the depleted void pool with purified coatomer (Waters et al., 1992) failed to reconstitute export. Moreover, purified coatomer when added directly to a transport reaction containing the biologically active pool of β-COP found in the void pool, neither stimulated nor inhibited vesicle budding (data not shown).

Pre-Golgi Intermediates Formed in the Presence of the Rab1B/β-COP Containing Complex Transport VSV-G to the Golgi When Supplemented with Late-acting Factors

To provide a final line of evidence that the β-COP/rab1B complex found in the void fraction generates bona fide carrier vesicles, rat liver cytosol was inactivated by pretreatment with either m4D3c or M3A5 antibodies in the presence of protein A–Sepharose (Fig. 11, *c* and *d*). Subsequently, the depleted cytosols were incubated in the absence (Fig. 11, *c* and *d*) or presence (Fig. 10, *e* and *f*) of the void pool. In both cases, transport was restored to a level which was nearly 50% of the control incubation containing an identical amount of non-depleted cytosol (Fig. 11 *a*). These results are consistent with our previous observation that the void pool contains a significant fraction of the total vesicle budding activity and that the included fraction, in addition, contains critical factors to support targeting and fusion (Fig. 8 *A*).

Discussion

β-COP Is Required for ER to Golgi Transport

The major focus of this paper was to establish a biological role for β-COP in ER to Golgi transport in order to begin to analyze its molecular interactions with other known transport components. We provided two lines of evidence based on both biochemical and morphological criteria that β-COP participates at an early step involved in vesicle budding from the ER. First, four antibodies raised against synthetic peptides derived from different regions of β-COP (Pepperkok et al., 1993) as well as F_{ab} fragments generated from M3A5 inhibited export of VSV-G from the ER. Second, the active pools of β-COP found in rat liver cytosol could be depleted or inactivated by overnight incubation on ice in the presence of specific antibodies. In both cases, transport activity could be recovered by addition of fractions containing the active forms of β-COP.

A number of observations are consistent with our observation that β-COP is involved in vesicular traffic. First, β-COP is a component of non-clathrin coats found on vesicles budding from the Golgi compartments (Orci et al., 1991; Serafini et al., 1991*b*; Waters et al., 1991*b*) and accumulates on vesicles when Golgi stacks are incubated in the presence of GTPγS (Melancon et al., 1989). Second, the yeast homologue to γ-COP (Sec21p) is essential for ER to Golgi transport in yeast (Hosobuchi et al., 1992; Novick et al., 1980). Third, a number of reports have now demonstrated the localization of β-COP to the transitional region of the ER, pre-Golgi intermediates and to the *cis* face of the Golgi stack in vivo (Duden et al., 1991*b*; Oprins et al., 1993; Hendricks et al., 1993; Pepperkok et al., 1993). Finally, cells microinjected with β-COP specific antibodies inhibit ER to Golgi transport in vivo, resulting in the accumulation of VSV-G in pre-Golgi intermediates (Pepperkok et al., 1993). These results differ significantly from our results in which β-COP antibodies were found to inhibit vesicle formation and prevent transport of VSV-G to the pre-Golgi intermediates. Given the technical limitations of microinjection, a likely explanation for the observed differences is the inability of antibodies to completely inhibit β-COP function in vivo, allowing for the partial maturation of VSV-G to intermediate carriers, but preventing further transport due to either a more pronounced effect on vesicle uncoating or its potential effect on inhibiting the function of the acceptor *cis*-Golgi compartment in vivo. The combined results demonstrate the use of an in vitro approach to quantitatively deplete or inactivate the functional β-COP pool before incubation to identify its initial site of action.

Our results provide the first direct biochemical demonstration for the biological functionality of β-COP in vesicle budding, in this case, export from the ER. Importantly, they establish a role for β-COP in initiating the anterograde flow of itinerant protein through early steps of the secretory pathway.

Biologically Active Forms of β-COP

A major problem in understanding β-COP function has been to identify biologically active forms since this protein can distribute quite heterogeneously during fractionation of crude rat liver cytosol. The β-COP containing coatomer complex has been postulated to fulfill this function (Waters et al., 1991*b*; Orci et al., 1993), although existing evidence is still indirect concerning the formation of vesicles competent for targeting and fusion. Based on an assay that measured the formation of ER to Golgi carrier vesicles, we found that β-COP could be fractionated from rat liver cytosol into at least two functional pools using gel filtration. One pool was found in the included fractions and contained the majority (>80%) of the β-COP found in cytosol. This fraction had markedly less total activity in stimulating export of VSV-G from the ER to pre-Golgi intermediates based on indirect immunofluorescence. This pool also contained other factors essential for the delivery of VSV-G to the *cis*-Golgi compartment. Given that coatomer is typically prepared from this fraction (Waters et al., 1991), we were surprised to find that even partially purified coatomer failed to reconstitute vesicle budding under a number of different conditions. Our inability to reconstitute export with coatomer suggests that purification of the complex from cytosol results in the loss of essential components required for coat assembly (see below).

A second high molecular weight pool contained only 10–20% of the total β-COP. However, it contained 60–80% of the export activity with a 5–10-fold higher specific budding activity than found in cytosol. This pool promoted efficient export of VSV-G from the ER to punctate, p58 containing pre-Golgi vesicular carriers, but did not support subsequent transport to the Golgi stack. p58 has been shown in a number of cell lines, including the NRK cells used in our studies, to be a marker for pre-Golgi tubular-vesicular intermediates in vivo (Saraste and Svensson, 1991). Using immunoelectron microscopy, we have recently shown that the punctate, pre-Golgi intermediates containing VSV-G observed in vitro are similar in structure to those observed in vivo. They consist of a collection of small 40–80-nm vesicles and small tubular elements whose function is regulated by rab1 (Schwaninger et al., 1992; W. E. Balch, unpublished observations). A role for β-COP in the vesicle budding from the ER is consistent with the striking abundance of β-COP in these vesicular carriers at early time points as shown here in vitro and in vivo (Pepperkok et al., 1993). Since vesicular carriers formed by incubation in the presence of the high molecular weight fraction were efficiently transported to the Golgi after the addition of late-acting factors found in the included fractions, it is clear that the void contains all of the essential ingredients to form functional vesicles. This important result now provides us with a direct assay for late-acting components.

We also detected in the void fraction a mammalian protein of ~70 kD which cross-reacted with an antibody that recognizes the yeast SEC23 gene product. A mammalian homologue to Sec23p has been previously localized to vesicular profiles in the ER/Golgi transitional region in pancreas (Orci et al., 1991*a*). Biochemical and genetic evidence suggests that Sec23p along with a limited number of other proteins is essential for vesicle budding from the ER (Hicke and Schekman, 1989; Kaiser and Schekman, 1990; Hicke et al., 1992; d'Enfert et al., 1992; Barlowe et al., 1993; Yoshihisa et al., 1993). Further experiments using antibodies specific for the mammalian homologue will be necessary to conform and extend the role of this protein in vesicle formation. It is important to point out that we did not detect NSF, rab2, or ARF in the void fractions, proteins we have previously demonstrated to be essential for ER to Golgi transport (Beckers et al., 1989; Balch et al., 1992; Schwaninger et al., 1992; Tisdale et al., 1992). These results make it unlikely that the biologically active void pool represents a non-specific association of cytosolic proteins. The lack of NSF is consistent with its postulated role in a late vesicle targeting or fusion step (for review see Rothman and Orci, 1992). Although ARF was apparently not required in a soluble form for vesicle budding from the ER in vitro, the semi-intact cells used in our assay contain a significant pool of bound ARF (Peter, F., and W. E. Balch, unpublished results). Given previous observations concerning the possible role of ARF in recruitment of β-COP (Donaldson et al., 1992), this pool is likely to contribute significantly to vesicle budding.

β-COP and rab1B Form a Functional Precoat Complex

When we began to analyze the functional basis for the β-COP dependence of ER to Golgi transport, we unexpectedly found a high correlation between transport activity, and the codistribution of β-COP and rab1B in the void fraction. A major concern given current technical limitations was whether the high molecular fraction constitutes a functional precoat complex and not a non-specific mixture of cytosolic proteins. In addition to the observation that the void fraction contains only a subset of transport components, there are several lines of evidence to suggest, but do not prove, that it represents a precoat complex. First, rab1B and β-COP could be coprecipitated from these fractions with concomitant loss of transport activity. In neither case could the two proteins be coprecipitated from the included fractions, although they efficiently inactivated the transport activity found in these fractions. These results attest to the functional importance of both low and high molecular forms of rab1B and β-COP, and in the case of the high molecular weight form, is consistent with its ability to sediment as a 18–19S particle. Rab1 is not the first member of the rab family to be detected in high molecular complexes. SEC4 can be readily detected in a large protein complex which fractionates as 19.5S particle (Bowser et al., 1992). This complex contains SEC8 and SEC15, and possibly other components recognized to mediate vesicle traffic from the Golgi to the cell surface. Rab5 has also been reported to be found in a larger aggregate, but only when cells are depleted of ATP (Kurzchalia et al., 1992). These studies are unrelated both in principle and practice to our current studies since they do not address the question of functionality, involve chemical cross-linking, and the observed protein complexes form only under unusual incubation conditions. In our studies, we have used specific antibodies and direct biological assays to correlate the functional distribution of β-COP and rab1 with other cellular components potentially involved in ER to Golgi traffic under normal incubation conditions.

A second line of evidence which supports our interpretation that the high molecular fraction represents a functionally important precoat complex is the ~5–10-fold increase in specific budding activity found in the void pool over that observed in the cytosol. When combined with restricted ability of this complex to only support vesicle budding and the observation that only 10–20% of the total β-COP resides

in this fraction, we consider it unlikely that the void pool represents a heterogeneous collection of components found in the cytosol. Our evidence suggests that these complexes contain multiple copies of rab1B and β-COP, and that the function of each of these proteins is required for vesicle budding given the ability of F_{ab} fragments to inhibit transport. Consistent with this observation, we have found that the complex can be inactivated by separation of rab1B from β-COP using ion exchange chromatography (Peter, F., and W. E. Balch, unpublished results). Further biochemical characterization of the complex is now in progress.

Functional Significance of a Rab1B/β-COP-containing Complex

What is the functional significance of the association of rab1B and β-COP in a precoat particle? One possible interpretation is that these two proteins may be coordinately recruited during vesicle formation and that rab1B plays an important, but at the present time, unknown role in this process. This would be consistent with our observations that trans dominant mutants in GTP-binding proteins of rab1 inhibit vesicle budding (Nuoffer, C., and W. E. Balch, manuscript submitted for publication). This is also consistent with the ability of F_{ab} fragments to inhibit vesicle formation. Alternatively, β-COP or other components present in the complex are essential for budding, and rab1B, while recruited during vesicle formation, functions at a late step in transport. The latter interpretation is consistent with the role of rab1 GTP-hydrolysis in vesicle targeting or fusion in vivo (Tisdale et al., 1992) and in vitro (Pind et al., 1993), as well as the possible role of YPT1 in ER to Golgi transport in yeast (for review see Pryer et al., 1992). Interestingly, genetic studies have demonstrated that YPT1 and SEC21 (γ-COP) may interact through the action of SLY1 (Dascher et al., 1991; Ossig et al., 1991), suggesting a role for YPT1 in the function of β-COP in yeast. We do not find it surprising that rab1B is associated with larger complexes, particularly those containing components involved in the assembly of vesicle coats. Small GTP-binding proteins such as rab1B serve as molecular switches which interface and control the interactions between upstream and downstream effectors. The potential ability of rab1B to participate in the assembly/disassembly of β-COP containing non-clathrin coats is consistent with its role in ER to Golgi and intraGolgi transport (Schwaninger et al., 1993; Davidson and Balch, 1993).

The role of β-COP in membrane trafficking has been controversial due to the observation that the drug brefeldin A (BFA) causes a global release of the protein from the Golgi stack and the collapse of these compartments into the ER. Such results have led to the suggestion that β-COP primarily serves as a "capping" protein to prevent fusion between membranes (Klausner et al., 1992). In contrast, our results provide the first direct functional evidence for its role in vesicle formation from the ER. Given the likelihood that there is a strong link between organelle structure and vesicular trafficking, it will be interesting to define the more global role of β-COP and rab1 in the maintenance of organelle structure/function in the secretory pathway as the intricacies of their biochemical interactions become unraveled.

Antibodies specific for p58 and Sec23p were generously provided by J. Saraste and R. Schekman; those to National Science Foundation by J. Rothman and M. Tagaya. We thank J. Rothman and G. Waters for providing the coatomer used in these studies.

This work was supported by grants from the National Institutes of Health to W. E. Balch (GM33301 and GM42336), the Fonds Nationale Suisse and the Canton de Genève to T. Kreis and the Deutsche Forschungsgemeinschaft to F. Peter. TSRI manuscript No. 7740-CB.

Received for publication 12 April 1993 and in revised form 23 June 1993.

References

Allan, V. J., and T. E. Kreis. 1986. A microtubule binding protein association with membranes of the Golgi apparatus. *J. Cell Biol.* 103:2229–2239.

Bacon, R. A., A. Salminen, H. Ruohola, P. Novick, and S. Ferro-Novick. 1989. The GTP-binding protein Ypt1 is required for transport in vitro: the Golgi apparatus is defective in ypt1 mutants. *J. Cell Biol.* 109:1015–1022.

Baker, D., L. Wuestehube, R. Schekman, D. Botstein, and N. Segev. 1990. GTP-binding Ypt1 protein and Ca^{2+} function independently in a cell-free protein transport reaction. *Proc. Natl. Acad. Sci. USA.* 87:355–359.

Balch, W. E., R. A. Kahn, and R. Schwaninger. 1992. ADP-ribosylation factor (ARF) is required for vesicular trafficking between the endoplasmic reticulum (ER) and the cis Golgi compartment. *J. Biol. Chem.* 267:13053–13061.

Barlowe, C., C. d'Enfert, and R. Schekman. 1993. Purification and characterization of SAR1p, a small GTP-binding protein required for transport vesicle formation from the endoplasmic reticulum. *J. Biol. Chem.* 268:873–879.

Beckers, C. J. M., and W. E. Balch. 1989. Calcium and GTP: essential components in vesicular trafficking between the endoplasmic reticulum and Golgi apparatus. *J. Cell Biol.* 108:1245–1256.

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.

Beckers, C. J. M., M. R. Block, B. S. Glick, J. E. Rothman, and W. E. Balch. 1989. Vesicular transport between the endoplasmic reticulum and the Golgi stack requires the NEM-sensitive fusion protein. *Nature (Lond.).* 339:397–398.

Bowser, R., H. Muller, B. Govindan, and P. Novick. 1992. Sec8p and Sec15p are components of a plasma membrane-associated 19.5S particle that may function downstream of Sec4p to control exocytosis. *J. Cell Biol.* 118:1041–1056.

Dascher, C., R. Ossig, D. Gallwitz, and H. D. Schmitt. 1991. Identification and structure of four yeast genes (SLY) that are able to suppress the functional loss of YPT1, a member of the RAS superfamily. *Mol. Cell. Biol.* 11:872–885.

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.

d'Enfert, C., L. J. Wuestehube, T. Lila, and R. Schekman. 1991. Sec12p-dependent membrane binding of the small GTP-binding protein Sar1p promotes formation of transport vesicles from the ER. *J. Cell Biol.* 114:663–670.

Donaldson, J. G., D. Cassel, R. A. Kahn, and R. D. Klausner. 1992. ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein β-COP to Golgi membranes. *Proc. Natl. Acad. Sci. USA.* 89:6408–6412.

Duden, R., V. Allan, and T. Kreis. 1991*a*. Involvement of β-COP in membrane traffic through the Golgi complex. *Trends Cell Biol.* 1:14–19.

Duden, R., G. Griffiths, R. Frank, P. Argos, and T. E. Kreis. 1991*b*. β-COP, a 110 kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to β-adaptin. *Cell.* 64:649–665.

Goud, B., and M. McCaffrey. 1991. Small GTP-binding proteins and their role in transport. *Curr. Opin. Cell Biol.* 3:626–633.

Hendricks, L. C., M. McCaffery, G. E. Palade, and M. G. Farquhar. 1993. Disruption of endoplasmic reticulum to Golgi transport leads to the accumulation of large aggregates containing b-COP in pancreatic acinar cells. *Mol. Biol. Cell.* 4:413–424.

Hicke, L., and R. Schekman. 1989. Yeast Sec23p acts in the cytoplasm to promote protein transport from the endoplasmic reticulum to the Golgi complex in vivo and in vitro. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1677–1684.

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.

Hosobuchi, M., T. K. 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 (Lond.).* 360:603–606.

Kaiser, C. A., and R. Schekman. 1990. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. *Cell.* 61:723–733.

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.

Kurzchalia, T. V., J.-P. Gorvel, P. Dupree, R. Parton, R. Kellner, T. Houthaeve, J. Gruenberg, and K. Simons. 1992. Interactions of rab5 with cytosolic proteins. *J. Biol. Chem.* 267:18419–18423.

Melancon, P., B. S. Glick, V. Malhotra, P. J. Weidman, T. Serafini, L. Orci, and J. E. Rothman. 1989. A role for GTP-binding proteins in vesicular transport through the Golgi complex. *Soc. Gen. Physiol. Ser.* 44:175–188.

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.

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.

Novick, P., C. Field, and R. Schekman. 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell.* 21:205–215.

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.

Orci, L., M. Ravazzola, P. Meda, C. Holcomb, H.-P. Moore, L. Hicke, and R. Schekman. 1991*a*. Mammalian Sec23p homologue is restricted to the endoplasmic reticulum transitional cytoplasm. *Proc. Natl. Acad. Sci. USA.* 88:8611–8615.

Orci, L., D. J. Palmer, M. Ravazzola, A. Perrelet, and J. E. Rothman. 1993. Budding from Golgi membranes requires the coatomer complex of non-clathrin proteins. *Nature (Lond.).* 362:648–652.

Ossig, R., C. Dascher, H.-H. Trepte, H. D. Schmitt, and D. Gallwitz. 1991. The yeast SLY gene products, suppressors of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. *Mol. Cell Biol.* 11:2980–2993.

Pepperkok, R., J. Scheel, H. P. Hauri, H. Horstmann, G. Griffiths, and T. E. Kreis. 1993. β-COP is essential in biosynthetic membrane transport from the endoplasmic reticulum to the Golgi complex in vivo. *Cell.* In press.

Pfeffer, S. R. 1992. GTP binding proteins in intracellular transport. *Trends Cell Biol.* 4:57–63.

Plutner, H., R. Schwaninger, S. Pind, and W. E. Balch. 1990. Synthetic peptides of the rab effector domain inhibit vesicular transport through the secretory pathway. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2375–2383.

Plutner, H., A. D. Cox, S. Pind, R. Khosravi-Far, J. R. Bourne, R. Schwaninger, C. J. Der, and W. E. Balch. 1991. Rab1b regulates vesicular transport between the endoplasmic reticulum and successive Golgi compartments. *J. Cell Biol.* 115:31–43.

Plutner, H., H. W. Davidson, J. Saraste, and W. E. Balch. 1992. Morphological analysis of protein transport from the endoplasmic reticulum to Golgi membranes in digitonin permeabilized cells: role of the p58 containing compartment. *J. Cell Biol.* 119:1077–1096.

Pryer, N. K., L. J. Wuestehube, and R. Schekman. 1992. Vesicle-mediated protein sorting. *Annu. Rev. Biochem.* 61:471–516.

Rothman, J. E., and L. Orci. 1992. Molecular dissection of the secretory pathway. *Nature (Lond.).* 355:409–415.

Saraste, J., and K. Svensson. 1991. Distribution of the intermediate elements operating in ER in Golgi transport. *J. Cell Sci.* 100:415–430.

Schmitt, H. D., M. Puzicha, and D. Gallwitz. 1988. Study of a temperature-sensitive mutant of the ras-related YPT1 gene product in yeast suggests a role in the regulation of intracellular calcium. *Cell.* 53:635–647.

Schwaninger, R., H. Plutner, G. M. Bokoch, and W. E. Balch. 1992. Multiple GTP binding proteins regulate vesicular transport from the endoplasmic reticulum to Golgi membranes. *J. Cell Biol.* 119:1097–1116.

Segev, N. 1991. Mediation of the attachment or fusion step in vesicular transport by the GTP-binding Ypt1 protein. *Science (Wash. DC).* 252: 1553–1556.

Segev, N., J. Mulholland, and D. Botstein. 1988. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell.* 52:915–924.

Serafini, T., G. Stenbeck, A. Brecht, F. Lottspeich, L. Orci, J. E. Rothman, and F. T. Wieland. 1991*b*. A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein β-adaptin. *Nature (Lond.).* 349:215–219.

Tisdale, E. J., J. R. Bourne, R. Khosravi-Far, H. W. Davidson, C. J. Der, and W. E. Balch. 1992. GTP-binding mutants of rab1 and rab2 are potent inhibitors of endoplasmic reticulum (ER) to Golgi transport. *J. Cell Biol.* 119: 749–761.

Waters, M. G., I. C. Griff, and J. E. Rothman. 1991*a*. Proteins involved in vesicular transport and membrane fusion. *Curr. Opin. Cell Biol.* 3:615–620.

Waters, M. G., T. Serafini, and J. E. Rothman. 1991*b*. 'Coatomer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. *Nature (Lond.).* 349:248–251.

Waters, M. G., C. J. M. Beckers, and J. E. Rothman. 1992. Purification of coat protomers. *Methods Enzymology.* 329:331–337.

Yoshihisa, T., C. Barlowe, and R. Schekman. 1993. Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. *Science (Wash. DC).* 259:1466–1468.