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Exocytic transport vesicles generated *in vitro* from the trans-Golgi network carry secretory and plasma membrane proteins

(immunoisolation/polymeric IgA receptor/secretory pathway)

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ABSTRACT **We have developed a cell-free assay that reproduces vesicular budding during exit from the Golgi complex. The starting preparation for the *in vitro* system was a rat liver stacked Golgi fraction immobilized on a magnetic solid support by means of an antibody against the cytoplasmic domain of the polymeric IgA receptor. Vesicular budding was ATP, cytosol, and temperature dependent and was inhibited by 1 mM *N*-ethylmaleimide. Budding was maximum within 10 min and originated preferentially from the trans-Golgi. Exocytic transport vesicles immunoisolated from the total budded population were enriched in the mature forms of secretory and membrane proteins destined to the basolateral plasma membrane and were depleted in lysosomal enzymes and galactosyltransferase activity. The finding that a major proportion (>70%) of newly synthesized, sialylated secretory and transmembrane proteins is contained in a single population of post-Golgi transport vesicles implies that, in a constitutively secreting cell, basolaterally destined proteins are sorted and packaged together into the same exocytic transport vesicles.**

The hepatocyte, a polarized epithelial cell, lacks storage granules or a known secretagogue, indicating that in this cell only a constitutive secretory pathway exists (1). This pathway delivers newly synthesized proteins to the blood and involves fusion of carrier vesicles with the basolateral plasma membrane (PM). Transport of proteins into bile, mediated by fusion of vesicles with the apical PM, is by transcytosis rather than by direct traffic from the Golgi complex (2).

Traffic of hepatocyte PM proteins seems to parallel that of secretory proteins. Bartles *et al.* (3) have presented evidence that PM proteins are initially transported to the basolateral PM, irrespective of their ultimate destination to either the basolateral or the apical PM. However, recent data suggest that sorting of apical and basolateral PM proteins can take place either intracellularly, at the trans-Golgi network (TGN), or at the basolateral PM depending on the protein and the cell type (4–6).

Although it is likely that in constitutively secreting cells, PM and secretory proteins are transported from the Golgi to the cell surface in the same transport vesicles, there is currently no direct evidence for this in mammalian cells. Previous electron microscopic studies in hepatoma cells (7) indicated that a secretory protein (albumin) and a viral trans-membrane protein destined to the PM colocalize in structures within the Golgi region, some of which could represent exocytic transport vesicles.

Several defined steps of biosynthetic protein traffic have been reconstituted in cell-free assays. Transport from the endoplasmic reticulum to the Golgi complex (8), between compartments of the Golgi complex (9), and from the Golgi complex to the plasma membrane (10, 11) has been investigated. In all of these studies the reporter molecules followed were viral proteins. We have designed a cell-free system that reconstitutes the formation and release of transport vesicles from the trans-Golgi compartment and followed the distribution of endogenous glycoproteins. From the total population of budded vesicles those destined to the basolateral PM were immunoisolated and characterized.

MATERIALS AND METHODS

Antibodies. We raised rabbit antisera against (*i*) the ectodomain of polymeric IgA receptor (pIgA receptor), (*ii*) rat albumin, and (*iii*) rat transferrin. The hybridoma cells secreting anti-pIgA receptor cytoplasmic domain IgGs (SC 166) were kindly provided by J.-P. Kraehenbuhl (12).

Preparation of Immunoadsorbant (ImAd). Immunoisolation was performed using monodisperse magnetic particles (a gift of J. Ugelstad, University of Trondheim, Trondheim, Norway) as the solid support, prepared as described (13). The linker molecule (affinity-purified sheep antibody against the Fc fragment of mouse IgG) was covalently attached to the solid support. The antibody against the pIgA receptor cytoplasmic domain was bound to the linker-solid support in a ratio of two molecules per linker IgG molecule.

Fractionation and Immobilization. Stacked Golgi (SG) fractions were prepared from rat livers as described (14) and immobilized on the solid support by mixing (3 hr at 4°C) with the ImAd in homogenization buffer adjusted to 0.25 M sucrose plus 0.5% bovine serum albumin. The separation of the bound from the unbound fraction was carried out using a magnet and the immobilized fraction was washed with the same buffer until minimal SG protein was present in the supernatant. The SG was bound to saturation (determined by binding studies and achieved with 0.5 mg of protein per mg of beads) so that vesicles that have budded would not be able to rebind to the ImAd. To determine how much of the pIgA receptor was interacting with the antibody on the solid support, metabolically labeled fractions were solubilized and immunoprecipitated with protein A-Sepharose alone (to quantitate receptor with primary antibody already bound) or with antibodies against pIgA receptor followed by protein A-Sepharose (to quantitate receptor without primary antibody bound), resolved by SDS/PAGE, and quantitated.

Cell-Free System. Generally, 20 mg of ImAd with immobilized SG was suspended in 4205 μl of washing buffer plus 500 μl of a 10× concentrated solution to provide a final concentration of 25 mM Hepes-KOH/25 mM KCl/1.5 mM magnesium acetate, pH 6.7. An ATP-regenerating system

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Abbreviations: ImAd, immunoadsorbant; PM, plasma membrane; pIgA receptor, polymeric IgA receptor; PNS, postnuclear supernatant; SG, stacked Golgi; TGN, trans-Golgi network; NEM, *N*-ethylmaleimide.

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(215 μl; final concentration of 1 mM ATP, 8 mM creatine phosphate, and 0.043 mg of creatine phosphokinase per ml) and 80 μl of cytosol (rat liver homogenate; 150,000 × *g* × 1.5 hr supernatant at 44 mg/ml) were added and incubated at 37°C. The reaction was terminated by simultaneous retrieval of the immobilized SG with a magnet and cooling on ice. The ImAd with remaining SG fraction was washed in ice-cold phosphate-buffered saline/0.5% bovine serum albumin supernatant and washes were retained for analysis. Control experiments were as follows: (*i*) cytosol was replaced with an equivalent volume of washing buffer; (*ii*) ATP-regenerating system was replaced by an ATP-depleting system consisting of 5 mM D-glucose and 0.2 mg of hexokinase per ml; and (*iii*) 1 mM *N*-ethylmaleimide (NEM) was added. To isolate pIgA receptor-containing vesicles, the total budded fraction was mixed with anti-pIgA receptor ImAd and incubated overnight at 4°C. The ImAd with the bound material was separated from the unbound fraction and washed.

Metabolic Labeling. For the long-term biosynthetic labeling, rats were injected in the tail vein with a mixture of 1 mCi each of [^{35}S]cysteine/[^{35}S]methionine (1 Ci = 37 GBq) every day for 4 days, and animals were sacrificed on the fourth day (cysteine, >600 Ci/mmol; methionine, >800 Ci/mmol; Amersham). To label newly synthesized proteins either 1 mCi of [^{3}H]fucose was injected into the saphenous vein 5 min before sacrifice (70–90 Ci/mmol; Amersham) or 1 mCi each of [^{35}S]cysteine/[^{35}S]methionine was injected in the saphenous vein 20 min before sacrifice (15).

Analytical Techniques. Enzyme assays were carried out as in ref. 16. Protein was estimated as in ref. 17. Specific activity of radiolabeled rat liver fractions (cpm/mg of protein) was determined after 5% trichloroacetic acid precipitation. Samples for immunoprecipitation were prepared as in ref. 15. SDS/PAGE was carried out using a 5–15% acrylamide gradient (18). For fluorography, the gels were treated with Entensify (New England Nuclear). Quantitation of immunoprecipitated proteins was carried out by elution of silver grains from autoradiograms (19).

Aliquots of a total microsomal fraction (5-min [^{3}H]fucose label) (20) were solubilized and immunoprecipitated with antibodies against pIgA receptor and rat transferrin. Half of each was treated with 0.1 unit of neuraminidase (*Vibrio cholerae*) in 50 mM sodium acetate, pH 5.5/5 mM $CaCl_2$ and the other half was treated with buffer alone (37°C, overnight) and processed by SDS/PAGE and fluorography.

The fractions were fixed and processed for electron microscopy as described (21).

RESULTS

Preparation of a Golgi Fraction for the Reconstitution of Vesicular Budding. To study vesicular traffic from the trans-Golgi, a SG fraction was prepared (Fig. 1*A*) and immobilized on magnetic beads by an antibody–antigen interaction (Fig. 1*B*). Antibodies against the cytoplasmic domain of the pIgA receptor proved effective in the immobilization step. Differential solubilization with detergents demonstrated that 4% ± 0.8% of the pIgA receptors of the immobilized fraction was interacting with the antibodies on the ImAd and, therefore, most receptors were potentially able to bud during the cell-free assay.

The SG fraction was analyzed for the presence of various reporter molecules (partial data in Table 2) and contained 33% of the postnuclear supernatant (PNS) galactosyltransferase activity (a resident trans-Golgi protein), 5.7% of the β-*N*-acetylglucosaminidase activity (a lysosomal enzyme), 7.7% of alkaline phosphodiesterase I activity (a plasma membrane marker), and 9% of the transcellular pathway (measured by internalization of ^{35}S-labeled pIgA for 10 min) (22). Following the immunoisolation step, the galactosyltransferase and β-*N*-acetylglucosaminidase activities were enriched 6-fold, whereas other reporter molecules were not enriched. The immobilized Golgi fraction provides an ideal starting preparation to reconstitute cell-free vesicular budding because the Golgi stacks remain relatively intact and can be rapidly and efficiently removed from the budded fraction by means of the magnetic properties of the beads.

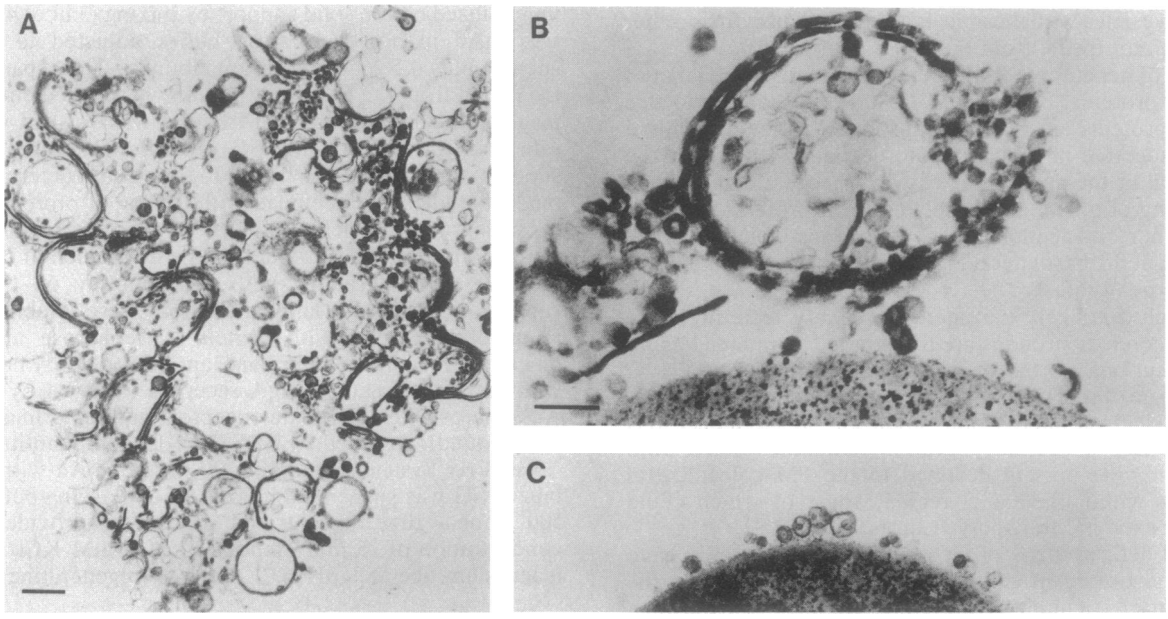

FIG. 1. Morphology of the SG, the immobilized SG, and the exocytic transport vesicle fractions. (*A*) The SG fraction was fixed in suspension, pelleted, and processed for electron microscopy. An overview of the fraction shows a large population of Golgi complexes, similar in appearance to those seen *in situ*. (Bar = 0.5 μm.) (*B*) The SG fraction was incubated with anti-pIgA receptor ImAd and the immobilized fraction was processed for electron microscopy. Thin sections through the fraction show relatively intact Golgi complexes bound to the magnetic bead in many orientations as well as single cisternae and vesicles. (Bar = 0.25 μm.) (*C*) Vesicles immunoisolated from the budded fraction on the anti-pIgA receptor ImAd are 50–200 nm in diameter and have no visible coat.

Cell-Free Budding. The amount of protein recovered at each step was estimated by following the distribution of label provided by long-term biosynthetic labeling (this results in a uniform specific activity of $\approx 0.35 \times 10^6$ cpm/mg of hepatic protein). Immobilized SG fraction was incubated at 37°C in an ATP-regenerating system and cytosol. After 10 min, the beads were retrieved and the distribution of labeled protein was determined. Twenty percent of labeled protein was released under complete budding conditions (Table 1, column 2). When an ATP-depleting system was used or when cytosol was omitted, the budding was reduced to 3.1% and 7.3%, respectively. When the reaction was carried out at 4°C for 1 hr, only 4.2% of the labeled protein was released. Addition of 1 mM NEM reduced the release to 2.3%.

To demonstrate that the release of protein reflects vesicular budding, the ability of the budded material to be pelleted was tested. A SG fraction was isolated and immobilized, the budding reaction was initiated in the presence and absence of cytosol, and the budded fraction was sedimented at 100,000 $\times$ *g* for 2 hr. The distribution of the major secretory protein, albumin, was quantitated in the various fractions (Fig. 2*A*). Incubation in the complete budding medium resulted in release of 15% of the albumin and, of that, 12% was pelleted and 3% was soluble. This indicates that a major portion (80%) of the released secretory protein was vesicular. In the absence of cytosol, 3% of the total albumin was recovered in the soluble fraction and none was pelleted; this represents the background of the reaction.

The kinetics of budding show a maximum release of vesicles in 5–10 min of incubation (Fig. 2*B*).

Isolation of Vesicles Containing pIgA Receptor. Many classes of transport vesicles are expected to bud from the immobilized SG and to be present in the total budded fraction. To recover only those that contain the pIgA receptor, immunoisolation was carried out using the anti-pIgA receptor ImAd. Two populations are expected to bind to the ImAd: (*i*) those en route to the basolateral PM, since the pIgA receptor travels the Golgi-PM pathway, and (*ii*) those moving pIgA receptor from cisterna to cisterna. To distinguish between the two, the molecular forms of the pIgA receptor were analyzed. Of the budded protein, 25% was retrieved by the immunoisolation step (Table 2). The quantitative distribution of the immature and mature forms of the receptor shows that 22% of the immature (105 kDa) form present in the cis and medial Golgi budded and 27% of that was recovered in the immunoisolation step. In contrast, nearly twice as much (43%) of the mature 116-kDa form present in trans-Golgi budded and, of that, 75% was retrieved in the immunoisolation step. Although some intracisternal transport vesicles are present in the final transport vesicle fraction, their concentration is low; the immature form of the receptor is enriched only 6-fold, whereas the mature form is enriched 84-fold. The data indicate that vesicles bud preferentially from a late Golgi compartment and are recovered preferentially.

Table 1. Requirements for budding

Budding conditions	[^{35}S]Cysteine/[^{35}S]methionine*		[^{3}H]Fucose†	
	%	*n*	%	*n*
37°C, + ATP, + cyto	20.0 ± 3	7	70 ± 2	5
37°C, − ATP, + cyto	3.1 ± 0.9	2	4.8 ± 2.1	2
4°C, + ATP, + cyto	4.2 ± 1.2	7	5.8	1
37°C, + ATP, − cyto	7.3 ± 2.5	7	3.0 ± 1.9	5
37°C, + ATP, + cyto, + 1 mM NEM	2.3 ± 0.2	2	2.8	1

Data are presented as the mean ± SD of the % of label released to the supernatant, with *n* = the number of experiments. After the budding reaction, the budded fraction and the fraction remaining on the beads were precipitated with trichloroacetic acid, washed, and assayed for radioactivity. Budding at 37°C was for 10 min and budding at 4°C was for 1 hr. cyto, Cytosol.
*Injected every day for 4 days.
†Injected 5 min before sacrifice.

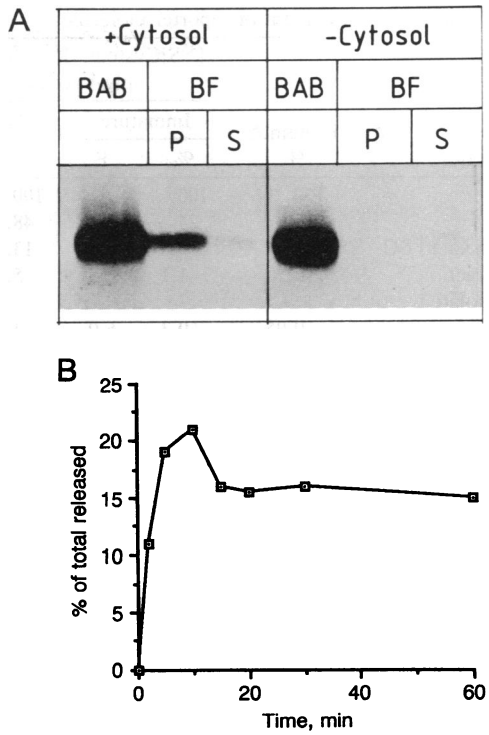

FIG. 2. (*A*) Vesicles are formed during budding. SG was isolated from the liver of an animal sacrificed 20 min after labeling with [^{35}S]cysteine/[^{35}S]methionine and was immobilized using anti-pIgA receptor ImAd. The budding reaction was carried out in the presence or in the absence of cytosol. The budded fraction (BF) was subjected to ultracentrifugation (100,000 $\times$ *g* for 2 hr), resulting in a pellet (P) and a supernatant (S). The beads after budding (BAB), the pellet, and the supernatant were solubilized, immunoprecipitated with antibodies against rat albumin, and analyzed by SDS/PAGE and fluorography. (*B*) Kinetics of budding. Aliquots of SG fraction isolated from a long-term [^{35}S]cysteine/[^{35}S]methionine-labeled animal were immobilized using anti-pIgA receptor ImAd. The beads were incubated in the budding mixture, and at the indicated times the supernatant was removed, solubilized, precipitated with trichloroacetic acid, and assayed for radioactivity. The control contained the complete budding medium but was maintained on ice for 1 hr.

Budding Is Preferentially from the Trans-Golgi. Budding of vesicles exclusively from the trans-Golgi was analyzed directly. Previous data suggested that 5 min after a [^{3}H]fucose pulse, radiolabeled proteins are present in the late Golgi compartments and have not been secreted (15). Therefore, 5-min [^{3}H]fucose labeling could be used as a specific marker for the trans-Golgi compartment. To determine if proteins labeled by this protocol have become terminally glycosylated, neuraminidase sensitivity of two selected proteins was analyzed. Neuraminidase cleaves terminal sialic acid residues that have been shown to be added in the trans-Golgi (23). As shown in Fig. 3, the total population of fucosylated pIgA receptor and transferrin increased in mobility after neuraminidase treatment. These data indicate that the two proteins studied, and probably the other proteins labeled following a 5-min pulse of [^{3}H]fucose, have reached the trans-Golgi compartment.

Comparison of the amount of protein released from the SG fraction (the long-term [^{35}S]cysteine/[^{35}S]methionine biosynthetic labeling) or glycoproteins from the trans-Golgi (the 5-min [^{3}H]fucose labeling) (Table 1) shows that the terminally sialylated glycoproteins are preferentially released, 70% vs. 20%. As in the case of the total protein, the budding of

Table 2. Yield and enrichment of reporter molecules

Fraction	Protein,* %	[35S]Cysteine/[35S]methionine pIgA receptor†				[3H]Fucose transferrin‡		[3H]Fucose protein§		GalTase¶		β-N-AcGlcNase¶	
		Immature		Mature									
		%	E	%	E	%	E	%	E	%	E	%	E
PNS	100	100		100		100		100		100		100	
SG	10.9	24	2.5	48.3	4.4	51.0	4.7	35.0	3.2	33.0	3.0	5.7	0.5
Immunoisolated SG	1.0	5.0	5.0	13.0	13.0	19.8	19.8	15.4	15.4	19.3	19.3	3.5	3.5
Budded fraction	0.2	1.1	5.5	5.6	28.0	14.3	71.5	11.2	56.0	3.1	15.2	1.9	9.5
Immunoisolated vesicles	0.05	0.3	6.0	4.2	84.0	11.1	222	5.4	108	0.03	0.6	0.2	4.0

The yield (%) and enrichment (E) of reporter molecules are calculated relative to the amount or activity in the PNS. Relative recoveries at each step were >95%. Data were collected from 14 experiments.

*Protein was estimated by assaying radioactivity in trichloroacetic acid precipitates (fractions originated from the liver of an animal labeled with the long-term biosynthetic protocol). The budding assay was carried out in medium containing high concentrations of cytosol, and subsequent immunoisolation of the budded vesicles was carried out in 0.5% bovine serum albumin to reduce nonspecific binding and proteolysis; this makes standard protein assays inaccurate.

†[35S]Cysteine/[35S]methionine pIgA receptor was immunoprecipitated (fractions originated from the liver of an animal sacrificed 20 min after the administration of the label), and the immunoprecipitates were quantitated following SDS/PAGE and fluorography.

‡[3H]Fucose transferrin was immunoprecipitated (fractions originated from the liver of an animal sacrificed 5 min after [3H]fucose administration) and processed as above.

§[3H]Fucose protein was determined by assaying trichloroacetic acid precipitates (fractions originated from the liver of an animal sacrificed 5 min after [3H]fucose administration).

¶Galactosyltransferase (GalTase) and β-*N*-acetylglucosaminidase (β-*N*-AcGlcNase) were quantitated by enzymatic activity.

terminally sialylated glycoproteins was ATP, cytosol, and temperature dependent and was inhibited by NEM.

Exocytic Transport Vesicles Carry Secretory and PM Proteins. To determine if pIgA receptor and secretory proteins are released from the TGN in the same vesicles, [3H]fucose-labeled transferrin was quantitated in the appropriate fractions. Seventy-two percent of the fucosylated transferrin was released from the SG fraction during the budding reaction and 78% of that was isolated in the immunoisolation step.

To ensure that the distribution of transferrin in the immunoisolation step is representative of secretory proteins in general, we analyzed the distribution of the cohort of fucosylated secretory proteins {≈95% of the total [3H]fucose label is in secretory proteins (15)}. As in the case of transferrin, 72.7% of the bound [3H]fucose-labeled protein budded and 77.6% of that was retrieved during the immunoisolation step.

The vesicles isolated from the budded fraction on the anti-pIgA receptor ImAd are small smooth membrane vesicles with diameters from 50 to 200 nm (Fig. 1*C*). Clathrin-coated vesicles were seen in the total budded fraction and were isolated on an anti-clathrin ImAd but did not contain the pIgA receptor (data not shown).

Protein Sorting During the Budding Reaction. Transport of proteins into and through the Golgi complex occurs through a common pathway. At the TGN proteins are segregated into distinct vesicles for delivery to their functional destinations (24, 25). Consequently, trans-Golgi resident proteins and proteins en route to other destinations (PM, lysosomal, and secretory proteins) should colocalize in the TGN but will be present in distinct compartments after selective sorting and budding from the TGN had occurred. To establish that exocytic vesicles were formed in the cell-free assay, we analyzed whether vesicle formation was accompanied by a sorting event (Fig. 4). The distribution of various markers was compared in two preparations: (*i*) the SG fraction after total vesiculation of the cisternae by homogenization and (*ii*) the budded fraction. When the SG fraction was vesiculated and subjected to immunoisolation using the anti-pIgA receptor ImAd, >70% of the pIgA receptor, [3H]fucose-labeled proteins, galactosyltransferase, and β-*N*-acetylglucosaminidase activities were retrieved, indicating significant colocalization of these proteins within the Golgi. In contrast, when the budded fraction was immunoisolated, >70% of the pIgA receptor and [3H]fucose-labeled proteins but <1% of galac-

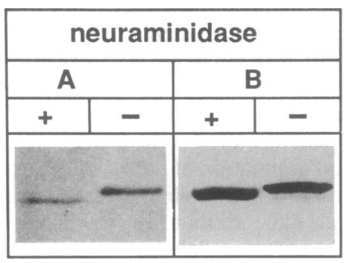

FIG. 3. Five-minute [3H]fucose metabolically labeled proteins are sialylated. An aliquot of a total microsomal fraction from a 5-min [3H]fucose-labeled animal was immunoprecipitated with antibodies against rat pIgA receptor (*A*) and transferrin (*B*). Half of each immunoprecipitate was treated with neuraminidase and neuraminidase-treated (+ lanes) and control (− lanes) were analyzed by SDS/PAGE and fluorography.

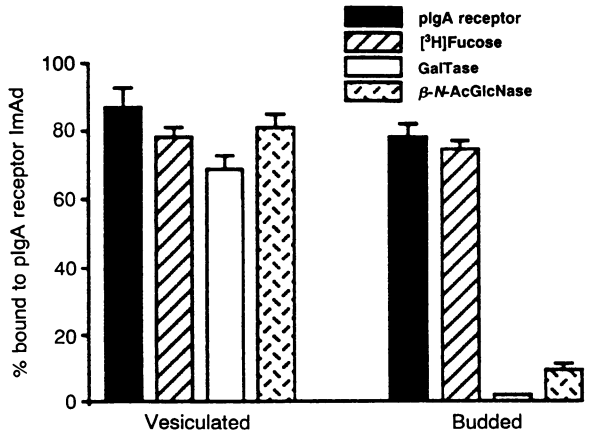

FIG. 4. Sorting of proteins during budding from the TGN. SG fraction was prepared from a 5-min [3H]fucose-labeled animal. An aliquot was vesiculated by homogenization (left) and an aliquot was immobilized and incubated in complete budding medium (right). Homogenized and budded fractions were subjected to immunoisolation using anti-pIgA receptor ImAd. Bound and nonbound fractions were assayed for the distribution of pIgA receptor, [3H]fucose-labeled protein, galactosyltransferase (GalTase), and β-*N*-acetylglucosaminidase (β-*N*-AcGlcNase) activities (recoveries are >95%).

tosyltransferase and 11% of the β-N-acetylglucosaminidase activities were retrieved, indicating segregation of these molecules during vesicle formation.

DISCUSSION

All cells efficiently target specific proteins to their correct intracellular locations. Within the secretory pathway, proteins and lipids move from one subcellular compartment to another by vesicular transport (26). Reconstitution is a powerful approach for defining the components and molecular requirements for specific steps of membrane traffic (e.g., ref. 9). We describe a cell-free assay that reproduces the formation of transport vesicles from the Golgi complex. A SG fraction isolated from rat liver was immobilized on the surface of magnetic beads by an antibody–antigen interaction and incubated under conditions shown to sustain vesicular transport. Transport vesicles were generated, some of which may have fused immediately with their recipient organelle (intercisternal vesicles). Others, for which the acceptor organelle was not present, remained in the supernatant and were isolated and characterized.

Vesicular budding is temperature and ATP dependent, requires cytosol, and can be inactivated by NEM treatment—conditions that influence vesicular transport in other cell-free systems. Whether the cytoplasmic components required for this vesicular budding are related to or distinct from those identified to be important in recognition and fusion events remains to be elucidated.

The budding is vesicular, as implied by the sedimentation of the released material and by the morphology after immunoisolation using antibodies against the cytoplasmic domain of the pIgA receptor. The finding that the released vesicles can be efficiently isolated indicates that the vesicles formed are of the appropriate membrane orientation.

Maximum budding occurs between 5 and 10 min of incubation. The slight decline in released material thereafter suggests rebinding or fusion of intercisternal vesicles with their acceptor cisternae. The budding is preferentially from the trans-Golgi based on the relative enrichment in proteins labeled with [^{3}H]fucose in the budded fraction. Although only 20% of the total protein is released from the Golgi complex in the budding reaction, >70% of fucosylated transferrin is released. Although fucosyltransferase(s) has not been precisely localized to a specific Golgi cisternae, fucose is added to the terminal oligosaccharide chain after either N-acetylglucosamine [this transferase has been localized to the medial Golgi (27)] or galactose [this transferase has been localized to the trans-Golgi (23)] (28). Therefore, the majority of the proteins of the SG fraction labeled after a 5-min labeling protocol would be expected to be in the trans-Golgi. This was confirmed for two proteins, pIgA receptor and transferrin. The total population of the pIgA receptor and transferrin labeled with [^{3}H]fucose increased in mobility on SDS/PAGE after neuraminidase treatment, indicating that all of the labeled molecules were sialylated and therefore had reached the trans-Golgi. Thus, this labeling protocol can be used as a rapid, expedient marker to monitor molecules that have reached the trans-Golgi.

Based on the finding that >70% of transferrin and pIgA receptor present in the trans-Golgi is released during the *in vitro* assay, we conclude that budding from the trans-Golgi is relatively complete. The substrates required for vesicle formation and release are present in the assay and supplied either by the donor membranes, the added cytosol, or both.

To show that a specific sorting event occurs during budding, the vesicles generated from the Golgi complex and destined to the basolateral PM were recovered and characterized. These vesicles were recovered from the total budded fraction with anti-pIgA receptor ImAd and were enriched >80-fold in mature forms of newly synthesized membrane and secretory proteins but were depleted in lysosomal enzymes and galactosyltransferase. If preexisting vesicles were simply released from the ImAd, one would expect them to have the same composition as the homogenized SG fraction. This was not the case, as the vesicle population isolated with the pIgA receptor ImAd after vesicularization contained >70% of the galactosyltransferase and β-N-acetylglucosaminidase activities, whereas the vesicles produced during the ATP-, cytosol-, and temperature-dependent reaction were depleted of these activities.

Our data show that a major portion (>70%) of the mature form of a secretory protein (transferrin) and a membrane protein (pIgA receptor) destined to the basolateral PM of the hepatocyte bud from the trans-Golgi in the same population of transport vesicles. This demonstrates that in constitutively secreting cells, secretory and membrane proteins are sorted in the trans-Golgi into a single population of transport vesicles. We do not know whether the vesicles we have isolated fuse directly with basolateral PM (thus releasing their content by exocytosis) or whether they deliver their cargo to an intermediate organelle, which then fuses with the PM.

The cell-free assay can be used to analyze the distinct steps of protein sorting and budding and will allow the identification of components required to mediate these events.

We are especially grateful to Jean-Pierre Kraehenbuhl for the myeloma cell lines producing pIgA and antibodies against the cytoplasmic domain of the pIgA receptor. The major portion of this work was carried out at the European Molecular Biology Laboratory, Heidelberg, F.R.G. We wish to thank Annette Scharm, Heinz Horstmann, and Ruth Jelinek for skilled technical assistance.

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