Journal of Cell Science 110, 3001-3009 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS4498

COMMENTARY

Post-Golgi biosynthetic trafficking

Patrick Keller and Kai Simons

European Molecular Biology Laboratory, Cell Biology Programme, Postfach 10.2209, Meyerhofstrasse 1, D-69012 Heidelberg, Germany
(e-mail: Keller@embl-heidelberg.de; Simons@embl-heidelberg.de)

SUMMARY

Eukaryotic cells have developed complex machineries to distribute proteins and lipids from the Golgi complex. Contrary to what has originally been postulated, delivery of proteins to the cell surface is not a simple bulk flow process but involves sorting into distinct pathways from the *trans*-Golgi network. Here we describe the various routes emerging from the *trans*-Golgi network in different cell types, and we discuss the mechanisms that mediate sorting into these pathways. While much remains to be learned about these sorting mechanisms, it is apparent that a number of pathways previously believed to be restricted to certain cell types might be used more commonly.

Key words: *Trans*-Golgi network, Sorting signal, Constitutive biosynthetic pathway, Regulated biosynthetic pathway, Raft, Endosome

INTRODUCTION

The *trans*-Golgi network (TGN) is the major sorting station for newly synthesized proteins and lipids in the biosynthetic pathway (Fig. 1). From there a number of different constitutive and regulated routes emerge, that deliver proteins either to the cell surface or to a number of compartments of the endosomal system. These transport events are facilitated by cytoskeletal elements, particularly microtubules and their associated motor proteins (Cole and Lippincott-Schwartz, 1995; Lafont and Simons, 1996).

Epithelial cells have been widely used to study the apical and basolateral routes that deliver newly synthesized proteins to the cell surface. Although these routes are commonly referred to as constitutive pathways, they nevertheless are tightly regulated and may be modulated by extracellular stimuli (Luini and De Matteis, 1993). Most importantly, they are not restricted to polarized cells, such as Madin-Darby canine kidney (MDCK) cells (Fig. 2A) and hippocampal neurons (Fig. 2G), but are present in non-polarized fibroblasts as well (Fig. 2B) (Müsch et al., 1996; Yoshimori et al., 1996). We therefore surmise that our discussion of the different pathways to the cell surface and the signals involved can be generalized to different mammalian cell types.

TRANSPORT TO THE CELL SURFACE

Transport of proteins to the basolateral surface (depicted in blue in Fig. 2) is mediated by cytoplasmic sorting signals. Although these signals are degenerate and somewhat variable they can nevertheless be classified into three groups (Table 1). A first set of signals relies on a critical tyrosine residue placed in the context of at least one large hydrophobic amino acid. The second type consists of a motif grouped around a leucine/leucine or a leucine/isoleucine pair, and the third group contains determinants that are neither tyrosine- nor dileucine-based. As a number of basolateral determinants are very similar to endocytosis signals, they have alternatively also been classified into two groups, one related to clathrin-coated pit localization motifs and another with unrelated motifs (Matter and Mellman, 1994). Since the machinery that mediates basolateral sorting in the TGN has not been identified, it is not known whether one or more vesicular carriers are involved in basolateral transport. If transport is mediated by a single vesicle type, one would expect to find sorting proteins capable of recognizing tyrosine and dileucine signals as well as other unrelated motifs. As these signals differ considerably in their sequence, it is conceivable that the putative sorter would recognize a common structural motif. Whether this would be a β-turn, as suggested for tyrosine-based coated pit localization signals, is uncertain since there is no clear indication that dileucine motifs can adopt a tight-turn conformation (Mellman, 1996). Alternatively, the different basolateral sorting signals could be recognized by distinct machineries (Matter et al., 1992; Reich et al., 1996). In this case, one would expect to find a number of distinct TGN-derived transport vesicles. Whether there are coat proteins functioning like clathrin to encapsulate transport vesicles destined for the cell surface is still unclear, and it remains to be seen whether any of the non-clathrin coatlike proteins identified on the TGN, p62 (Jones et al., 1993), p200 (Narula and Stow, 1995), and p230 (Gleeson et al., 1996), are involved in the generation of basolateral vesicles. A recent report suggests that p200 might be involved in the production of vesicular stomatitis virus glycoprotein (VSV G)-containing vesicles at the TGN (Müsch et al., 1997). However, there are also compelling data excluding a role for that protein in basolateral transport of VSV G (Ikonen et al., 1996). The challenge now is to determine which sorters and

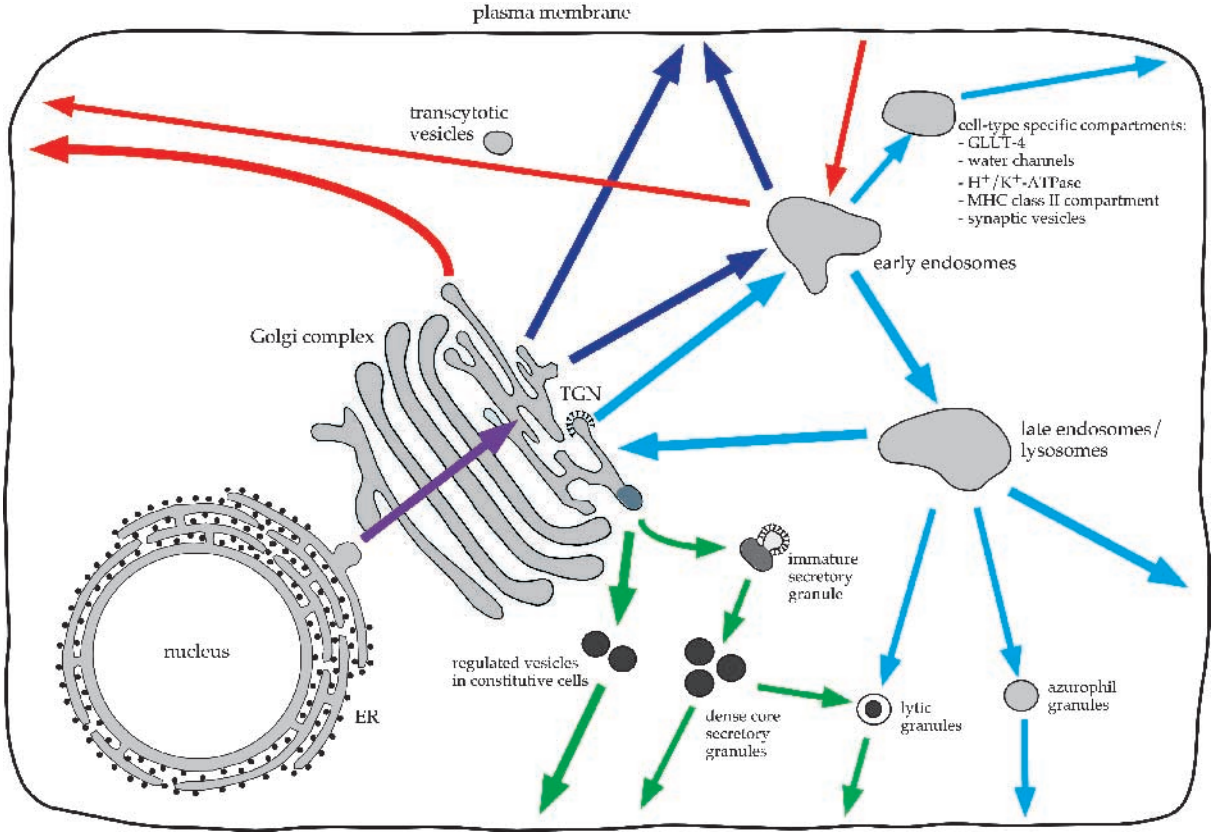

Fig. 1. Hypothetical cell depicting post-Golgi biosynthetic pathways (see text for details). While pathways common to all cells are shown by thick arrows, pathways present in only a subset of cells are represented by thin arrows. The main sorting station of the biosynthetic pathway is the *trans*-Golgi network (TGN), from where direct routes to the apical surface (red), the basolateral surface (dark blue), and the endosomal system (light blue) emerge. In addition, the TGN gives also rise to 'classical' secretory granules, as well as to a regulated secretory pathway in specialized cells (green). The second sorting station in the biosynthetic pathway are early endosomes. They sort apical proteins in cells that use a transcytotic pathway for apical delivery (red) and might in addition be involved in the sorting of some basolateral proteins (dark blue). Early endosomes give also rise to a number of cell-type specific compartments, containing GLUT-4, MHC class II molecules, water channels, H^+/K^+-ATPase, or synaptic vesicle proteins. Azurophil granules of neutrophils are probably derived from late endosomes/lysosomes, which themselves can undergo Ca^{2+} stimulated exocytosis. Lytic granules of natural killer cells and cytotoxic T lymphocytes form a dual-function organelle consisting of regulated secretory components contained within a pre-lysosomal compartment.

coats handle different categories of basolateral cargo. Interestingly, at least three parallel routes mediate TGN-to-surface transport in yeast (Fig. 2I) (Harsay and Bretscher, 1995; Roberg et al., 1997). There is also evidence for two distinct routes to the basolateral surface of hepatocytes (Saucan and Palade, 1994). The three routes in yeast use the GTPase sec4p (Harsay and Bretscher, 1995; C. Kaiser, personal communication), which is the homologue of Rab 8 in mammalian cells. The basolateral pathway for VSV G delivery in MDCK cells, as well as the route to the dendrites in neurons, is Rab 8-dependent and makes use of the NSF-SNAP-SNARE machinery for docking and fusion (Ikonen et al., 1995).

The other major route from the TGN to the polarized epithelial cell surface is the apical pathway (depicted in red in Fig. 2). For the transport of influenza virus hemagglutinin (HA) there is no indication for the involvement of Rabs, NSF, SNAP and SNAREs (Ikonen et al., 1995; Yoshimori et al., 1996), suggesting a fundamentally different transport mechanism than for basolateral delivery. Recent evidence for apical transport of influenza virus HA suggests a model in which sphingolipid-cholesterol rafts form within the exoplasmic leaflet of the Golgi membrane. According to this model (reviewed by Simons and Ikonen, 1997) rafts are closely packed microdomains assembled within the fluid bilayer that in the TGN act as sorting platforms for inclusion of protein cargo destined for delivery to the apical membrane. Using Triton X-100 at 4°C, these rafts can be extracted as detergent-insoluble glycolipid-enriched complexes (DIGs) containing many apical membrane proteins. Such a mechanism would imply the existence of apical targeting determinants that are fundamentally different from basolateral signals (Table 1). Indeed glycosylphosphatidyl inositol (GPI)-anchored proteins use their GPI-anchor to associate with rafts (Brown et al., 1989; Lisanti et al., 1989). While this signal is sufficient for targeting to the apical surface in most epithelial cell lines examined so far, some GPI-anchored proteins are found on the basolateral surface of Fischer rat thyroid cells (Zurzolo et al., 1993). Another apical sorting determinant, used by apical secretory proteins, is constituted by the mannose rich-core part of N-glycans (Scheiffele et al., 1995). Recent results demonstrate that also membrane glycoproteins can use their N-glycan chains as sorting determinants (K. Matter and H.-P. Hauri, personal communication). Raft association can also be mediated

by the transmembrane domains of influenza virus HA (Scheiffele et al., 1997) and neuraminidase (Kundu et al., 1996). It is important to note that removal of cholesterol from cells disrupts the interaction of HA with rafts (Scheiffele et al., 1997) and that this is accompanied by reduced apical transport and basolateral missorting (P. Keller and K. Simons, unpublished). Clearly, not all apical transmembrane proteins are found in DIGs (Danielsen, 1995; Arreaza and Brown, 1995), suggesting the presence of additional apical sorting determinants. These are probably N-glycans in the ectodomain that could, as in the case of apical secretory proteins, interact with the putative receptor VIP-36 (Fiedler et al., 1994) in the TGN. Thus, while some proteins might interact directly with rafts, others might use their carbohydrate chains to interact with the apical sorting machinery, and still others might use a combination of both mechanisms. While all these signals would mediate incorporation into the raft-dependent pathway, there is also the possibility that some proteins may use a raft-independent pathway to reach the apical surface (Mays et al., 1995).

MODULATION OF TRANSPORT PATHWAYS

Taken together, it is likely that there are a number of parallel

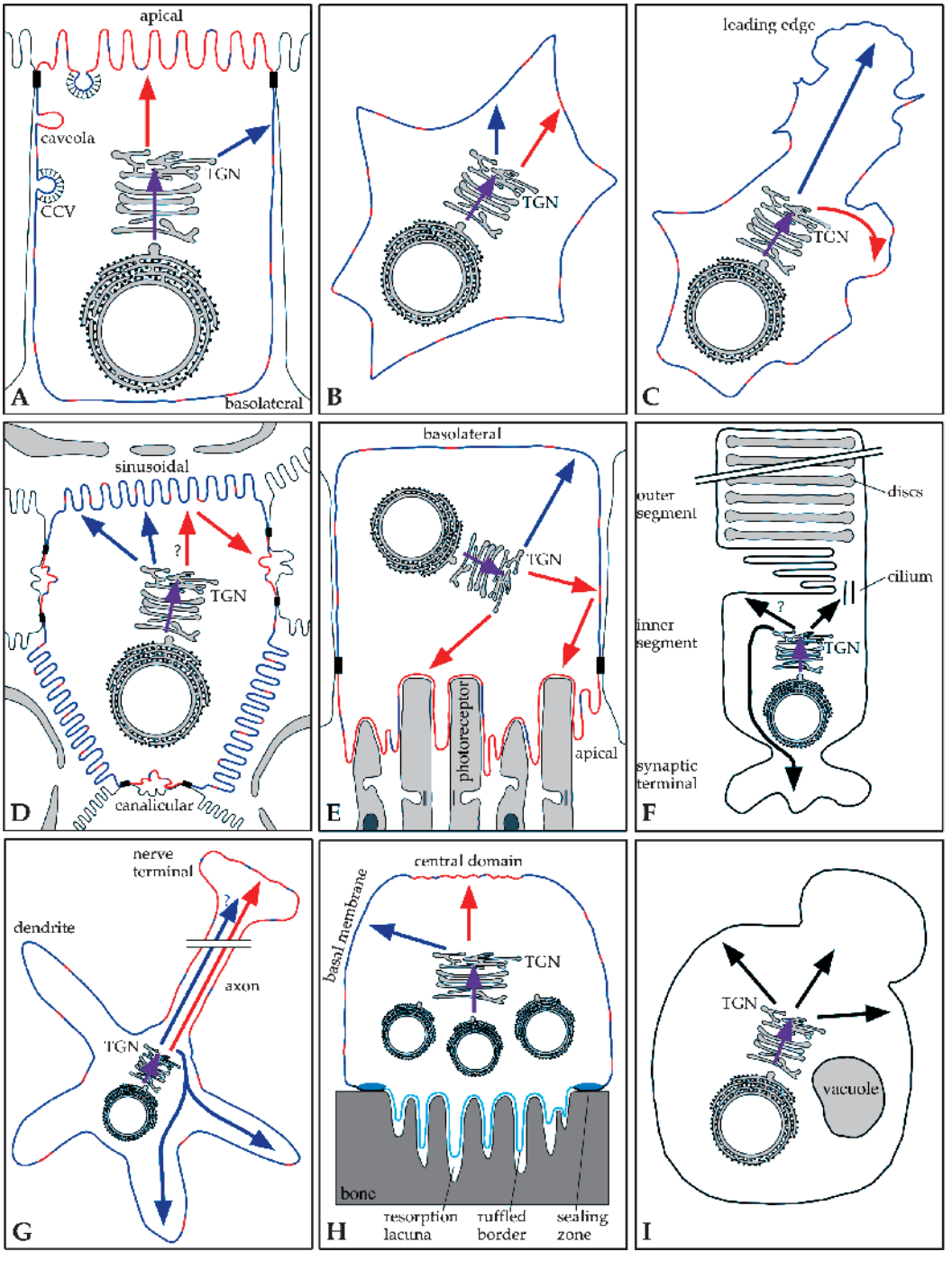

Fig. 2. Apical and basolateral pathways in a number of polarized and non-polarized cells. Membranes equivalent to the apical and basolateral surfaces of MDCK cells (A) are depicted in red and dark blue, respectively. While in a resting fibroblast (B) apical and basolateral pathways reach the cell surface randomly, a migrating fibroblast (C) uses the basolateral route to deliver newly synthesized proteins to the leading edge. Hepatocytes (D) contain at least two pathways to the basolateral surface. They, however, do not have a direct apical pathway, but instead use a transcytotic route. As indicated by the question mark, it is not known whether apical proteins use a raft-dependent mechanism to reach the basolateral surface in hepatocytes. Retinal pigment epithelium cells (E) use a direct as well as an indirect pathway for apical delivery. They are in direct contact with retinal rod photoreceptor cells (F), which contain at least two pathways to different domains of their cell surface. In hippocampal neurons (G) the axon and somatodendritic surfaces are equivalent to the apical and basolateral domains of MDCK cells, respectively. The surface of osteoclasts (H) contains an apical and a basolateral domain, as well as a domain with late endosomal characteristics (depicted in light blue). Finally, yeast (I) contains a least three distinct parallel pathways to the cell surface.

pathways that mediate transport from the TGN to the cell surface. Assuming that these pathways can be modulated separately, a cell would have the flexibility to react to changing conditions in a specific manner. It has for instance been demonstrated that a migrating fibroblast transports 'basolateral' proteins to the leading edge (Peränen et al., 1996; reviewed by Bretscher, 1996) (Fig. 2C), whereas in resting cells the same vesicles arrive randomly at the cell surface (Fig. 2B). This polarization of transport is accompanied by the rearrangement of the actin filament and microtubule systems. Different epithelial cell types target apical and basolateral proteins flexibly to their final destinations. In contrast to MDCK cells (Fig. 2A), hepatocytes have no direct apical pathway (Fig. 2D). Instead, they route all proteins first to the basolateral (sinusoidal) domain, from where apical proteins are transcytosed to the canalicular pole (Wilton and Matthews, 1996). While membrane and soluble proteins are possibly transported in different carriers (Saucan and Palade, 1994), it is not known whether apical and basolateral membrane proteins are included into the same vesicle or whether apical proteins use a raft-dependent mechanism to reach the basolateral surface. In the latter case, the apical docking and fusion machinery would have to be relocated to the basolateral membrane. Also, cells of the retinal pigment epithelium (RPE) use a transcytotic pathway to target influenza virus HA and neuraminidase to the apical surface (Fig. 2E) (Bonilha et al., 1997). Other apical proteins or secretory proteins however appear to follow a direct pathway (Ong et al., 1994; Jawarowski et al., 1995), suggesting that as in CaCo-2 cells (Hauri and Matter, 1991) both direct and indirect routes are used. Thus, whatever pathway is taken by apical proteins, the presence of an indirect pathway illustrates that the TGN is not the only sorting site of the biosynthetic pathway.

What distinguishes the RPE from other epithelia is the fact that its apical surface is not free but is in contact with the photoreceptors of the neural retina, and that some proteins display an inversed polarity (Bok, 1993). Since interactions with the neural retina could be responsible for these observed changes in polarity, these cells may provide a tool to study the role of cell-cell interactions that may modulate trafficking of proteins. One of the main functions of RPE cells is the phagocytosis and degradation of the outer segments of rod retinal photoreceptor cells (Fig. 2F), which provide another system to study polarized transport. In these cells, massive amounts of rhodopsin are transported to the outer segment containing a stack of rhodopsin-laden disks (Deretic and Papermaster, 1991), whereas synaptic vesicle proteins are routed in the opposite direction to the presynaptic terminal (Schmied and Holtzman, 1989). It is not known whether the plasma membrane of the inner segment (Fig. 2F) represents a separate plasma membrane domain and whether it receives transport vesicles directly from the TGN.

Biosynthetic trafficking in neurons and epithelial cells is similar, as both cell types have to deliver newly synthesized proteins to at least two domains of the cell surface. This analogy can be carried one step further since the axonal and somatodendritic domains of hippocampal neurons (Fig. 2G) have been shown to be similar to the apical and basolateral surface of MDCK cells, respectively (Simons et al., 1992; de Hoop and Dotti, 1993). Having this analogy, and knowing that glycoprotein synthesis in neurons is restricted to the cell body, neuronal axons provide a useful system to study post-TGN transport. A wealth of information has been obtained on motors involved in anterograde transport. Apart from kinesin, at least 10 new kinesin superfamily proteins (KIFs) have been identified (Hirokawa, 1996). Most of the cargoes that they move along the axon remain uncharacterized. While KIF1A carries a class of synaptic vesicle precursors, KIF3A/KIF3B, KIF4 and kinesin have been shown to transport vesicles that do not contain synaptic proteins (Hirokawa, 1996). KIF2 transports vesicles containing a subunit of the IGF-1 receptor (Morfini et al., 1997).

While neurons have provided us with a multitude of motors potentially involved in surface delivery, bone matrix-degrading osteoclasts provide another interesting example of how vesicles are targeted to different subdomains of the plasma membrane. In the resorbing osteoclast (Fig. 2H), the plasma membrane is divided into distinct domains, the ruffled border facing the bone (the resorption lacuna), the sealing zone separating the resorption lacuna from the extracellular milieu, and the basal membrane, which contains in its central part a morphologically distinct domain. Influenza virus HA is restricted to this central part while VSV G is confined to the basal membrane (Salo et al., 1996), suggesting that these domains are equivalent to apical and basolateral poles of epithelial cells, respectively. Although the resorption lacuna has been considered generally to be a specialized extracellular lysosome, recent data indicate that the ruffled border facing the resorption lacuna has characteristics of late endosomal membranes. Thus both biosynthetic and endocytic pathways are important in the dynamics and maintenance of this plasma membrane domain in resorbing osteoclasts (Palokangas et al., 1997). Another interesting example of differential surface targeting of influenza virus HA and VSV G is seen during myotube formation. In myoblasts, both marker proteins are transported to the cell surface. However, after myotube formation, VSV G is diverted to two intracellular locations, possibly representing the sarcoplasmic reticulum and a glucose-transporter-containing organelle (K. Metsikkö, personal communication).

INTERACTIONS OF ENDOCYTIC AND BIOSYNTHETIC ROUTES

A number of proteins, such as surface receptors, that reach the plasma membrane by one of the routes discussed above need to be continuously internalized and recycled back to the cell surface. This is accomplished by the endocytic pathway, which, like the secretory pathway, can be thought of as having functionally and physically distinct compartments, although their great plasticity and dynamic nature make it difficult to define a precise relationship between the different stations along the pathway (Fig. 1) (Gruenberg and Maxfield, 1995). Apart from mediating transcytosis, a main function of early endosomes is to discharge internalized ligands from their receptors, which is often followed by transfer of ligands to late endosomes and lysosomes, and by incorporation of free receptors into recycling vesicles and endosomes that transport them back to the plasma membrane (Mellman, 1996). Early endosomes are also involved in the sorting of newly synthesized proteins arriving from the TGN to lysosomes. Soluble lysosomal hydrolases contain mannose-6-phosphate residues as a lysosomal targeting determinant (Table 1). These residues mediate binding to the mannose-6-phosphate receptors (MPRs) in the TGN, where the hydrolase-MPR complexes are segregated into nascent clathrin-coated vesicles (CCVs) destined for the early endosomes

(Ludwig et al., 1991). In late endosomes the MPRs discharge their cargo, which is directed towards lysosomes. The MPRs themselves recycle back to the TGN for another round of sorting. The major late endosomal and lysosomal membrane proteins (lgps/lamps) on the other hand use a conserved cytoplasmic glycine-tyrosine motif followed by a hydrophobic amino acid three residues towards their C-terminus (Table 1) as their lysosomal targeting signal (Mellman, 1996). In many cells, lgps/lamps are transported directly from the TGN to the endocytic pathway, probably by the same CCVs that also mediate transport of the MPRs. However, some missorting of lgps/lamps to the cell surface may occur, which is corrected by the rapid internalization via plasma membrane-derived CCVs. Mutational analysis indicates that the conserved glycine residue is not important for this internalization process, but that it is essential for the efficient delivery of newly synthesized lgps/lamps from the TGN directly to endosomes and lysosomes (Harter and Mellman, 1992). To complicate matters further, the asialoglycoprotein H1 and transferrin receptors have been suggested to traverse endosomes on their way from the TGN to the basolateral surface of MDCK cells (Leitinger et al., 1995; Futter et al., 1995). Whether this transport occurs in the vesicles carrying MPRs or in other transport vesicles is not known.

The ability of endosomes to function in signal-dependent sorting is further illustrated by their capacity to give rise to specialized endosome-derived compartments (Fig. 1), such as the major histocompatibility complex (MHC) class II-containing compartments of antigen-presenting cells. Current evidence suggests that two independent dileucine-based signals of the invariant chain (Ii), which associates with class II molecules in the endoplasmic reticulum, mediate transport to this compartment (Table 1). Dendritic cells are 'professional' antigen-presenting cells, which upon stimulation can reorganize their antigen-presenting apparatus (Cella et al., 1997; Pierre et al., 1997). In immature dendritic cells, newly synthesized MHC class II molecules are routed to lysosomes (Pierre et al., 1997), or to the cell surface from where they are rapidly endocytosed (Cella et al., 1997). Stimulation of dendritic cells leads to an increased synthesis of MHC class II molecules, as well as to the appearance of peripheral endocytic vesicles, resembling the MHC class II-containing compartments found in B cells. In addition there is a dramatic increase in half-life of surface MHC class II molecules due to reduced endocytosis.

Other cells contain specialized, endosome-derived storage compartments (Fig. 1) that allow them to rapidly deliver proteins to the cell surface in response to external stimuli. Examples are the GLUT-4 compartment in adipocytes (Haney and Mueckler, 1994), water channel-containing vesicles in renal duct cells (Brown and Sabolic, 1993), and H^+/K^+-ATPase-containing vesicles in stomach epithelial cells (Urushidani and Forte, 1987). GLUT-3 in platelets on the other hand is stored in α-granules

Table 1. Examples of known transport signals

Type	Location	Representative examples
Basolateral sorting signals		
YXXØ	Cytoplasmic	Influenza virus hemagglutinin (Tyr mutant) Vesicular stomatitis virus glycoprotein
LL/IL	Cytoplasmic	FcRII-B2 receptor
Others	Cytoplasmic	Transferrin receptor LDL receptor distal domain Polymeric immunoglobulin receptor
Apical sorting signals		
GPI-anchor	Membrane, luminal leaflet	Placental alkaline phosphatase Thy-1
N-glycosylation	Luminal	gp-80 Erythropoietin
Transmembrane domain	Membrane	Influenza virus neuraminidase Influenza virus hemagglutinin
Late endosomes/lysosomes		
Mannose-6-phosphate	Luminal	Cathepsin B Cathepsin D
GYXXØ	Cytoplasmic	Lamp-1, Lamp-2 CD63
MHC class II compartments		
LI, ML	Cytoplasmic	Invariant chain (Ii)
Regulated secretory vesicles		
Conformation-dependent motif	Loop stabilized by one disulfide bridge	Chromogranin B Pro-opiomelanocortin
Selective aggregation	Luminal	

Sequences are given in the single letter amino acid code; Ø is a bulky hydrophobic amino acid.

derived from the biosynthetic pathway that can undergo a single round of thrombin-stimulated exocytosis (Heijnen et al., 1997). These vesicles differ from synaptic vesicles formed in neuronal cells (Mundigl and De Camilli, 1994) in that they are storage compartments, while synaptic vesicles in addition contain the machinery to take up neurotransmitters.

REGULATED SECRETORY PATHWAYS

These apparently cell type-specific endosome-derived storage compartments can be considered to be involved in regulated secretion or delivery of membrane proteins to the cell surface. Another form of regulated secretion is the biosynthetic transport employing secretory granules in endocrine, exocrine or neuronal cells. Sorting into this 'classical' regulated secretory pathway occurs in the TGN during the formation of immature secretory granules (Fig. 1) (Thiele et al., 1997). While it is clear that low pH- and Ca^{2+}-dependent aggregation of regulated secretory proteins is important, two models have been proposed to explain sorting into secretory granules. The first model suggests that proteins destined for regulated secretion would bind via a conformation-dependent motif (Table 1) to a sorting receptor in the TGN that would mediate incorporation of either individual proteins or of aggregates of proteins into immature secretory granules. Recently, membrane-bound carboxypeptidase E has been suggested to be this putative receptor (Cool et al., 1997), although it seems unlikely that this would be a generalized receptor for secretory granule sorting (Thiele et al., 1997). This model predicts that signals are required for entry into the secretory pathway, and that proteins lacking these signals would be excluded. The second model proposes that entry into the forming granules is not selective, and that aggregated regulated proteins would be selectively condensed and retained, while others would be progressively removed via CCVs (Dittie et al., 1996) during maturation into dense core secretory granules (Castle et al., 1997; Kuliawat et al., 1997). As many regulated secretory proteins have been shown to associate tightly with the luminal leaflet of the TGN membrane, these membrane-associated forms could be regarded as sorting receptors that would bind to other regulated secretory proteins, due to their ability to engage in homophilic and heterophilic interactions. Such interactions could also serve to concentrate regulated secretory proteins, while preventing others from entering into immature secretory granules. In a way similar to the budding of viral particles from the plasma membrane that involves the interaction of viral transmembrane proteins with the cytoplasmic nucleocapsid, these interactions could provide a simple mechanism for membrane budding around the aggregated secretory proteins (Thiele et al., 1997).

The recent observation that a fraction of newly synthesized glycosaminoglycans can undergo regulated exocytosis in constitutive secretory cells (Fig. 1) suggests that the regulated pathway might be more widespread than was believed previously (Chavez et al., 1996). Although no specific cargo for this cryptic regulated pathway has yet been identified, it was suggested that it could be involved in intercellular communication, or in the transport of a subset of proteins to the cell surface. Since ectopic expression of regulated secretory proteins in CHO and L cells does not lead to sorting into these newly discovered vesicles, it is not clear how they are related to 'classical' regulated secretory vesicles.

Ca^{2+}-regulated exocytosis is also found in cell types involved in cellular defence functions. A major mechanism by which natural killer cells and cytotoxic T lymphocytes kill their targets is by exocytosis of lytic granules (Fig. 1). Although they function as regulated secretory granules, they also have properties in common with lysosomes. Indeed they form a dual-function organelle, where a regulated secretory compartment, the dense core, is contained within a pre-lysosomal compartment (Griffiths and Argon, 1995). Where these organelles originate is not clear, but it has been suggested that they might arise either by crinophagy, the degradation of secretory granules by lysosomes, or more likely by sequential maturation of a specialized secretory compartment (Burkhardt et al., 1990). That secretory granules and lysosomes may be closely related is also indicated by the properties of azurophil granules (Fig. 1) of neutrophils that usually function as degradative intracellular compartments, but nevertheless can be secreted in response to external stimuli. In addition to azurophil granules, neutrophils also contain specific and gelatinase granules, which differ in their contents and are mobilized in a sequential and highly regulated manner. An intriguing question is how neutrophils manage to generate these types of structurally and functionally distinct granules. Based on the observation that the different granules are formed sequentially during the maturation of neutrophils, it was suggested that the heterogeneity of granule subsets might arise by differences in the timing of the biosynthesis of individual proteins rather than by specific sorting signals. Recent data obtained in promyelocytic HL-60 cells support this view (Le Cabec et al., 1997). These cells also appear to lose the ability to form storage granules during maturation, which is accompanied by constitutive secretion of the ectopically expressed azurophil granule protein NGAL (Le Cabec et al., 1997).

More support for the suggested similarity between a class of regulated secretory vesicles and lysosomes comes from the observation that lysosomes can undergo Ca^{2+}-triggered exocytosis (Fig. 1) (Rodriguez et al., 1997). Although the physiological function for an ubiquitous exocytosis of lysosomes is unclear, it is possible that they constitute the intracellular organelles proposed to be responsible for membrane resealing in wounded cells (Bi et al., 1995; Miyake and McNeil, 1995).

SORTING IN THE TGN

One basic issue in biosynthetic trafficking is how cells sort newly synthesized basolateral proteins, endosomal proteins, apical proteins, and regulated secretory proteins into their specific transport carriers. Selective aggregation and condensation of regulated secretory proteins may prevent the entry of other proteins into secretory granules. GPI-anchored proteins and apical transmembrane proteins would preferentially partition into sphingolipid-cholesterol rafts, the proposed sorting platforms for apical delivery in the TGN. Other apical proteins may depend on binding to raft-associated lectins via their N-glycans (Simons and Ikonen, 1997). Since basolateral proteins are usually glycosylated as well, how would they be excluded from the apical pathway? The observation that basolateral proteins become apically sorted when their cytoplasmic tails are removed and that influenza virus HA is sorted basolaterally upon introduction of a tyrosine residue in its cytoplasmic tail argues that a basolateral sorting signal confers a dominant signal, that,

when removed, can allow an apical signal to function. Thus basolateral as well as endosomal proteins would be excluded from rafts by the simple presence of cytoplasmic signals mediating comparatively high-affinity binding to cytoplasmic sorter proteins. While these sorters have not been identified for basolaterally sorted proteins, the AP-1 and AP-2 adaptor complexes of CCVs function as sorters in TGN-to-endosomal transport and endocytosis, respectively (Mellman, 1996). Based on yeast two-hybrid screens and in vitro studies, several tyrosine-based motifs have been shown to interact with the μ subunits of the two complexes (Marks et al., 1997). Consistent with the known ability of lgps/lamps to be included into TGN- and plasma membrane-derived CCVs, their cytoplasmic signals have been shown to interact with both the μ1 and μ2 subunits of the adaptor complexes. In contrast, the signal in the transferrin receptor binds to μ2 but not detectably to μ1, suggesting that basolateral proteins may avoid leaving the Golgi complex via CCVs because of their relative inability to interact with AP-1 adaptors. This inability is probably based on the variability in the flanking residues of tyrosine-based signals, which may determine the affinity and specificity for the interaction with the different sorters. Dileucine-based signals appear to be recognized by intact AP-1 and AP-2 complexes as well (Heilker et al., 1996). However, since tyrosine- and dileucine-based signals do not compete with one another for AP-2 binding, they seem to bind to different sites of the sorting machinery (Marks et al., 1996), and it remains to be seen whether both types of signals mediate incorporation into common or distinct vesicles. A new adaptor complex, AP-3, has recently been identified that interacts with tyrosine-based sorting signals (Simpson et al., 1996). This complex has been shown to be essential for cargo-selective transport to the yeast vacuole (Cowles et al., 1997) and genetic evidence implicates AP-3 in pigment granule formation in *Drosophila* (Ooi et al., 1997).

As mentioned above, some basolateral proteins might traverse endosomes on their way to the cell surface (Leitinger et al., 1995; Futter et al., 1995). Whether this is the case or not, the question of how proteins destined for the endosomal system are distinguished from basolateral ones remains. Furthermore, it also raises the question of how proteins that need to recycle back to the TGN or that have to be transcytosed apically are recognized in endosomes. Targeting of endocytosed polymeric immunoglobulin receptor back to the basolateral surface is ensured by the same amino acids initially used for basolateral sorting in the TGN (Aroeti and Mostov, 1994). Phosphorylation of a serine residue near this determinant results in its inactivation, and, as a consequence, the endocytosed receptor is no longer recycled back to the basolateral membrane but is apically transcytosed (Mostov and Cardone, 1995). Interestingly, mutation of this serine residue to an aspartic acid not only increases transcytosis, but also increases direct TGN-to-apical surface delivery. The mechanism by which proteins are transported back to the TGN is less well understood. TGN38 for instance continuously cycles between the TGN and the plasma membrane, and, although its cytoplasmic domain can bind to both AP-1 and AP-2 adaptors (Ohno et al., 1995), the signal responsible for its transport to the TGN remains unknown.

Finally, another intriguing question is how interactions between cargo, adaptor complexes, and coats are regulated and restricted to specific subcellular sites. Why for instance do AP-1-containing CCVs only assemble on TGN membranes, but not on endosomes, which would also contain suitable cargo molecules? Part of the answer may come from the observation that ARF1 is an essential regulator of clathrin-coat assembly in the TGN (Stamnes and Rothman, 1993; Traub et al., 1993), suggesting that the specific recruitment of ARF1 to the TGN could restrict AP-1 binding and therefore coat assembly to the TGN. In addition, a membrane-bound docking apparatus has been postulated. Although membrane proteins have been identified that bind AP-1 (Mallet and Brodsky, 1996; Seaman et al., 1996), their relevance to AP-1 recruitment has not yet been demonstrated.

Using an in vitro assay reconstituting the ARF1-dependent translocation of cytosolic AP-1 onto TGN membranes, it was also shown that the MPRs are key components for the efficient recruitment of AP-1 (Le Borgne et al., 1996). This is further supported by the fact that the expression level of the MPRs can determine the number of CCVs formed in the TGN (Le Borgne and Hoflack, 1997). Collectively, these data argue that MPR sorting is highly coupled to the first step of coat assembly and that MPRs, ARF1, and possibly other proteins cooperate to create high-affinity AP-1 binding sites. While it is not completely understood how this is accomplished, there are indications that reversible phosphorylation of the MPRs might regulate AP-1-dependent sorting (Chen et al., 1993; Mauxion et al., 1996). In addition, in yeast, a serine/threonine kinase (Vps15p) and a phosphatidylinositol 3-kinase (Vps34p) have been implicated in the proper sorting of vacuolar/lysosomal proteins (Herman et al., 1992), indicating that reversible phosphorylation and phosphoinositides might be important. Obviously, transport vesicle formation is a complicated cooperative process also involving the lipid bilayer in securing correct cargo inclusion, membrane bending and vesicle release.

CONCLUDING REMARKS

It is now apparent that a number of pathways previously believed to be restricted to certain cell types might be used more commonly. Analysis of biosynthetic trafficking to the cell surface for instance has revealed functionally distinct routes, that could be part of two post-Golgi circuits for exocytosis and endocytosis (Simons and Ikonen, 1997). According to the model proposed, one of these circuits would transport membrane proteins containing sorting signals in their cytoplasmic tails (tyrosine-, dileucine-based, and others), while the other would transport proteins associated with sphingolipid-cholesterol rafts. Since these circuits would intersect in different organelles, appropriate sorting and delivery mechanisms would be required at each site. It will be important in the future to compare the sorting machineries at different sites and to understand how similar sorting determinants are distinguished. Although all the sorting events we have discussed are apparently signal-mediated the question whether cell type-specific default pathways exist remains. Would selective blocking of a single pathway, for instance, lead to intracellular accumulation of certain proteins, or would the sorting machineries of other pathways be structured such that, despite of the absence of specific sorting information, default incorporation into vesicles would be possible? Finally, it also remains to be seen whether there is an absolute need for vesicles in order for transport to occur. Analysis of post-Golgi trafficking in living cells using GFP-fusion proteins has revealed the presence of structures larger than single vesicles which move to and eventu-

ally fuse with the plasma membrane (J. White and T. Nilsson, personal communication). Could these structures be TGN tubules (Ladinsky et al., 1994), or specific subdomains of the TGN containing a single type of cargo? The fact that whole Golgi cisternae can be used as vehicles to transport scales to the cell surface in algae (Melkonian et al., 1991) exemplifies the flexibility in handling cargo for post-Golgi trafficking. Clearly much more remains to be learned about the mechanisms responsible for the formation of the transport containers.

We thank Thomas Harder, Peter Scheiffele, Jamie White, and Daniele Zacchetti for critical comments on the manuscript. The work in our laboratory was supported by a Training and Mobility of Researchers grant of the European Community (P. Keller; 83EU-044159) and by a grant from the Deutsche Forschungsgemeinschaft (SFB 352).

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(Accepted 14 October 1997)