The *trans* Golgi Network: Sorting at the Exit Site of the Golgi Complex

GARETH GRIFFITHS AND KAI SIMONS

The Golgi complex is a series of membrane compartments through which proteins destined for the plasma membrane, secretory vesicles, and lysosomes move sequentially. A model is proposed whereby these three different classes of proteins are sorted into different vesicles in the last Golgi compartment, the *trans* Golgi network. This compartment corresponds to a tubular reticulum on the *trans* side of the Golgi stack, previously called Golgi endoplasmic reticulum lysosomes (GERL).

PROTEINS THAT ARE INSERTED INTO, OR TRANSLOCATED across, the membrane of the rough endoplasmic reticulum (ER) must contain information that determines their final destination (*1*). This destination may be intra- or extracellular. For some membrane proteins the target organelle is the ER itself, and they are not transported further (*2*). All other proteins are carried to the Golgi complex, which is a series of membrane compartments through which proteins in transit pass sequentially (*3–7*).

Most proteins that are synthesized in the ER are glycoproteins. The elaborate series of covalent modifications that glycoproteins undergo in the ER and subsequently in the Golgi complex are now well established (*8*). The structure of the oligosaccharide moiety at any one time after synthesis indicates which enzymes of the processing pathway the protein has been exposed to. In many cases this information can be used to indicate the compartment reached during transport. However, this characterization is not absolute, because our knowledge of the molecular composition of the compartments in the pathway is still rudimentary.

Newly synthesized proteins that pass through the Golgi complex can be classified into three groups characterized by their exit pathway from the Golgi. The first group consists of plasma membrane proteins and proteins that are constitutively secreted. The second group consists of secretory proteins that are packaged into secretory granules that are, in turn, released in response to an external signal. Lysosomal enzymes constitute the third group. These three classes of proteins seem to be transferred through an identical pathway until the last compartment of the Golgi complex, because (i) proteins of all three classes are found in all Golgi cisternae by immunocytochemistry (*9*) and (ii) the oligosaccharide moieties of glycoproteins in all three classes (with the exceptions of some oligosaccharides of lysosomal enzymes) are covalently modified by ER and Golgi enzymes in a similar fashion (*8*). Therefore, the sorting of proteins into separate pathways occurs either in the last Golgi compartment or after exit from the Golgi. We now discuss the structure and function of the intracellular site where these sorting events are proposed to occur.

The authors are at the European Molecular Biology Laboratory, Postfach 10.2209, 6900 Heidelberg, Federal Republic of Germany.

Current Models of the Golgi Complex

In the simplest possible model, the Golgi stack consists of three discrete cisternae, or groups of cisternae, which can be distinguished on the basis of distinct cytochemical reactivities (Fig. 1). Although this interpretation may be an oversimplification (*10*), these cytochemically defined zones have greatly influenced most current models of Golgi functions (*3–7*) (Fig. 1). In present models, the different cytochemical reactions in the Golgi cisternae are thought to reflect the presence of three different functional compartments through which proteins pass vectorially. Various functions of the Golgi have been proposed to occur in each of these three compartments, although most of these assignments are still tentative.

Another feature of these models is that selective transfer of lipid and proteins from one compartment to the next occurs through vesicular transport (*7, 11*). Vesicles are thought to enter the system by budding off specialized regions of the ER, termed the transitional elements, and then to fuse with the *cis* compartment of the Golgi stack. After a succession of sequential budding and fusion events that transport the proteins from one cisterna to the next, the proteins exit by budding off the *trans* cisterna or cisternae (Fig. 1). The vesicles that have been proposed to mediate transport between the subcompartments have proven elusive. There is still no direct evidence for their existence.

The current models of the Golgi complex usually do not incorporate the Golgi endoplasmic reticulum lysosome (GERL) structure (*12, 13*). Its role has remained controversial despite documentation of the existence of this Golgi-associated structure in many morphological studies of different cell types.

The GERL Concept

The recognition of the importance of the GERL structure began in the 1960's with Novikoff's pioneering morphological studies of lysosomes, in which acid phosphatase was used as the cytochemical marker (*12*). Novikoff saw reaction product not only in lysosomes, but also in a reticular structure in the perinuclear region of spinal neurons. The location of this structure in the light microscope corresponded precisely with the cytochemical reaction for thiamine pyrophosphatase, which was already an accepted marker for the Golgi region in light microscopic studies. Subsequent electron microscopic observations led him to propose that this acid phosphatase–reactive structure was a specialized region of smooth ER that was directly continuous with the rough ER, as well as with dense bodies (lysosomes). He believed that this structure was specialized for the biogenesis of lysosomes, and he introduced the acronym GERL to indicate that this structure is "intimately related to the Golgi saccule (G), that it is part of the ER, and that it forms lysosomes (L)" (*12*, p. 358). He then suggested that after lysosomal enzymes were synthesized by the ER, they bypassed the Golgi stack via the GERL. The same claim was later made for secretory proteins

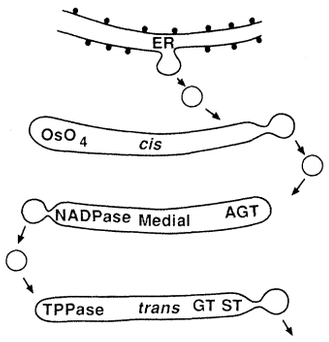

Fig. 1. Prevailing model of the Golgi complex. The major feature is the presence of three compartments through which transit proteins move sequentially. The cytochemical activities and the specific functions ascribed to the compartments are shown (*6, 7*). The model proposes that proteins move from one station to the next by vesicular transport. Abbreviations: AGT, *N*-acetylglucosamine-transferase I; GT, galactosyl transferase; ST, sialyl transferase; OsO_4, reduced osmium tetroxide; NADPase, nicotinamide adenine dinucleotide phosphatase; TPPase, thiamine pyrophosphatase.

when it became clear that immature secretory granules were budding off the GERL (*14*).

The morphological and cytochemical differences between GERL and the cisternae that made up the Golgi stack are well documented (*15*). In most cell types, the GERL structure that stained for acid phosphatase had morphological features distinct from the Golgi cisternae. However, part of the GERL often appeared to be cisternal and, in the absence of a cytochemical reaction, was difficult to distinguish from the other cisternae on the *trans* side of the Golgi stack. The membranes forming the GERL were usually more tubular, were thicker in cross section, and had luminal contents that often reacted differently with electron-opaque strains from Golgi cisternae. The presence of budding, coated vesicles and extensive coated membrane regions further distinguished GERL from other Golgi cisternae (*12, 13, 16*). Electron microscopy showed that it was the *trans* Golgi cisternae adjacent to GERL that contained thiamine pyrophosphatase and that, in most cases, the GERL structure itself did not (*14, 17*).

GERL was always a strictly morphological concept, and, although biochemists often referred loosely to the "Golgi-GERL region" of the cell, the GERL itself was not generally considered part of the exocytic pathway. Morphologists were divided between those who accepted Novikoff's theories and those who believed that GERL was the last (*3*), or most "mature," cisterna of the Golgi stack (*18*). In fact, little evidence corroborated its continuity with the ER. Several lines of evidence argue strongly against such continuities (*19*). For this reason the acronym GERL no longer seems to be appropriate for this structure, which has been given many other names, including the "boulevard peripherique" (*20*), the *trans* tubular network (*21*), and the *trans* Golgi reticulum (*22*). To avoid confusion with the ER or with the original GERL concept, we propose to call this structure the *trans* Golgi network (TGN). This is a modification of the terminology used by Rambourg *et al.* (*21*) and emphasizes that the TGN belongs to the Golgi complex.

The TGN—the Exit Compartment of the Golgi Complex?

In the model we are proposing, the TGN is a specialized organelle on the *trans* side of the Golgi stack that is responsible for routing proteins to lysosomes, secretory vesicles, and the plasma membrane from the Golgi complex (Fig. 2).

Novikoff used acid phosphatase as a marker for the TGN. This cytochemical reaction is probably produced by phosphatases in transit to the lysosomes. Recently, other markers labeling the TGN have been identified. Within the Golgi complex, the TGN appears to be the main, and perhaps exclusive, site of clathrin localization (*23, 24*). Although coated, presumably budding structures on the rim of the cisternae in the Golgi stack are frequently observed; these could not be labeled with antibodies against clathrin (*23, 24*), which suggests that a different protein forms these structures in the Golgi stack. Sialyl- and galactosyltransferase have also been localized in the TGN in hepatocytes (*25, 26*). These enzymes, which have well-defined functions at the terminal stages of nitrogen-linked glycosylation, may be present in the *trans* cisternae of the Golgi stack (*27*). The distinction between the *trans* cisterna and the TGN in Fig. 2 is a tentative one, based only on the nonoverlapping localizations, in many cell types, of TPPase (thiamine pyrophosphatase) reactivity (*trans*) and acid phosphatase (TGN) by cytochemistry (*15*).

Exit of Plasma Membrane Proteins from the Golgi Complex

A significant step in defining the TGN as a sorting organelle for outgoing membrane traffic was the observation that, at 20°C, the transport of newly synthesized viral glycoproteins from the ER to the cell surface was blocked (*28*). The proteins were terminally glycosylated and sialylated (*29*), but remained inside the cell. On warming the cells, these viral proteins were transported rapidly to the cell surface, which indicated that the block was fully reversible. Vesicular stomatitis virus (VSV) G protein accumulated in the TGN, which was apparent on one side of the Golgi stack as a tubular network that reacted for acid phosphatase (*24*). The large amount of G protein that accumulated in this structure caused the protein to be packed into regular arrays. This facilitated the identification of the compartment, even in the absence of additional markers. During the low-temperature block, the TGN had many budding structures with cytoplasmic coats. Although many of these coated structures reacted immunocytochemically with antibodies to clathrin, others unexpectedly failed to do so. Both types of coated, budding structures failed to react with antibodies to the G protein, in agreement with earlier studies (*30*). Thus, clathrin-coated vesicles do not seem to transport newly synthesized plasma membrane proteins from the Golgi to the cell surface, in contrast to the proposal by Rothman *et al.* (*31*). Recent studies in yeast have also provided compelling evidence against a role for clathrin in exocytosis. Deletion of the clathrin heavy-chain gene had little influence on the rate of intracellular transport and secretion of newly synthesized invertase (*32*).

Nonpolarized cells such as fibroblasts and macrophages are thought to exocytose plasma membrane proteins uniformly over their surfaces, although, when they are carefully examined, preferen-

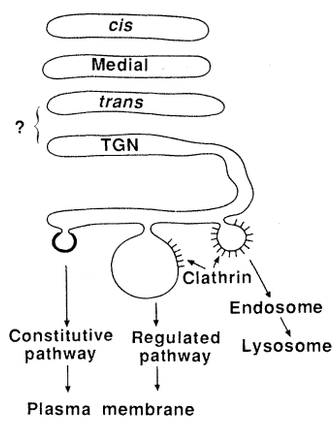

Fig. 2. Model for sorting in the TGN. The distinction between the compartment labeled "*trans*" and the TGN is a tentative one. It is based only on the consistent colocalization in many cell types of TPPase reactivity (*trans*) and acid phosphatase (TGN) by cytochemistry (*15*). A part of the TGN may appear as a cisterna and be difficult to distinguish from other Golgi cisternae [see, for example, figure 4b in (*24*)]. The mechanism of transport between the Golgi compartments has not been considered in this scheme (Fig. 1). In epithelial cells, it appears likely that two types of vesicles bud off the TGN and carry apical and basolateral plasma membrane proteins, respectively, to their surface domains (*35*).

tial exocytosis has been observed at the leading, motile edge of some cells (*33*). Polarized epithelial cells, which make up the bulk of the cell types in tissues, contain two different plasma membrane domains, apical and basolateral, which are separated from each other by tight junctions (*34, 35*). In the Madin-Darby canine kidney (MDCK) cell line, which is polarized in culture, membrane proteins targeted to the apical domain are sorted from those destined for the basolateral domain at the Golgi complex (*36*). The site of sorting is probably the TGN because the viral membrane proteins that are differentially targeted to the two domains can be colocalized by immunocytochemistry throughout the Golgi stack (*37*), and they remain in contact with each other when their intracellular transport is blocked at 20°C (*29*).

Those secretory proteins that are constitutively secreted by eukaryotic cells are presumably transported by a mechanism similar, if not identical, to that responsible for the transport of plasma membrane proteins. The two groups of proteins may be carried to the cell surface in the same vesicles (*38*). Although this mechanism is likely, the only direct data in its support are the colocalization of VSV membrane glycoprotein with albumin and transferrin in putative transport vesicles (*39*).

Exit of Secretory Proteins from the Golgi Complex

Regulated secretory cells concentrate proteins destined for export into storage granules. In the early stages of formation these are clearly continuous with the TGN (*14*). Secretory cells can carry out both constitutive and regulated secretion simultaneously, using separate carrier vesicles (*38*).

Proteins that have moved through the Golgi complex and are destined for the regulated secretory pathway seem to condense in the lumen of the TGN before they are sorted into a developing secretory granule (*38, 40*). These developing secretory granules all contain patches of clathrin (*41*). Because the patches do not cover the entire cytoplasmic surface of the budding granule, it is unlikely that clathrin is necessary for the budding of the secretory granules. The clathrin remains bound even after the secretory granule has been released from the TGN, but dissociates as the granule matures and becomes more electron-opaque. This bound clathrin may recycle excess membrane back to the TGN (*38*). The proteolytic cleavage of prohormones such as proinsulin occurs in these coated granules (*42*). Whether this proteolytic processing starts while the granule is still attached to the TGN membrane is not known. Another posttranslational modification of secretory proteins, sulfation of tyrosine residues, probably occurs in the TGN (*43*), although this has yet to be confirmed by immunocytochemical localization of the sulfotransferase responsible for this reaction.

Exit of Lysosomal Proteins from the Golgi Complex

The biochemical changes that lysosomal enzymes undergo during their transport from ER to lysosomes are now being elucidated (*8, 44, 45*). After the enzymes are transferred from the ER to the Golgi complex, a specific subset of high-mannose oligosaccharide chains are phosphorylated at the 6-carbon position of mannose by the concerted action of two early Golgi enzymes. This alteration serves as an address tag that allows these proteins to bind to the 215-kD mannose-6-phosphate (M6P) receptor. Further biochemical events in the pathway are a low *p*H-mediated dissociation of the ligands from the receptor and two other, relatively late changes, a proteolytic cleavage and a dephosphorylation. The latter changes probably occur in the lysosome itself. Phosphorylation of lysosomal enzymes is thought to occur in the *cis* Golgi compartment (*46*), although conclusive evidence is lacking. Exactly where the lysosomal enzymes bind to the receptor is not yet clear. In many cases, the oligosaccharide chains of lysosomal enzymes that are not phosphorylated undergo the same modifications seen in other glycoproteins, including the trimming of mannose residues and the addition of the terminal sugars, *N*-acetylglucosamine, galactose, fucose, and sialic acid. Thus, those lysosomal enzymes which become sialylated must move through the whole Golgi stack into the TGN. This conclusion is supported by cytochemical data (*19, 47*) and by recent immunocytochemical studies (*26, 48*), both of which reveal low levels of the enzymes throughout the Golgi stack and a higher concentration in the TGN.

There is still some dispute as to whether the lysosomal enzymes in the TGN are bound to the M6P receptor and whether this receptor is at all present in this compartment. The receptor may be concentrated in TGN and in coated membrane buds of this structure in Chinese hamster ovary (CHO) cells (*49*). In this study, however, no markers were used to distinguish the TGN from endocytic structures (Fig. 3). In human hepatoma cells (Hep G 2) (*26*), albumin was used as a specific marker to distinguish the exocytic pathway from the endocytic one. In these cells, double-labeling experiments with frozen-section immunocytochemistry showed that the M6P receptor as well as lysosomal enzymes were concentrated in TGN and included in coated buds. Whether these label with antibodies to clathrin is not yet known.

Other studies, based on immunoperoxidase localization, have suggested that the M6P receptor is concentrated in the *cis* compartment of the Golgi complex and that it is absent from the *trans* side (*50*). We believe that these differences are technical in nature (*50*) and that the most reasonable interpretation of all the available data is that the M6P receptor and lysosomal enzymes are present in low concentrations throughout the Golgi stack, but are concentrated in the TGN before they exit from the Golgi complex (Fig. 2). The vesicles transporting the receptor-bound lysosomal enzymes from the Golgi complex have not yet been characterized. Clathrin-coated vesicles leaving from the TGN are the most attractive candidates at present (*51*).

There is also an alternative mechanism for lysosomal enzyme sorting by the M6P signal that depends on another receptor, a 46-kD protein (*52*). Pathways even exist in which nonphosphorylated lysosomal enzymes seem to be correctly targeted to lysosomes (*45*). To what extent these alternative pathways involve the TGN is not known. It is also not known how lysosomal membrane proteins reach the lysosomes. The few that have been characterized are heavily sialylated (*53*), which indicates that they have passed through the TGN. However, these proteins are not phosphorylated, suggesting that the mechanism of targeting does not depend on an M6P recognition system (*53*).

The Size and *p*H of the TGN

The TGN seems to be able to vary in size in response to the cell's requirements. A "hypertrophy" of TGN in secretory cells, for example, occurs after cells are stimulated to secrete (*54*). Stereological studies of the TGN in baby hamster kidney (BHK) cells indicate that this compartment almost doubles in size after VSV infection at 20°C; within minutes of reversing the temperature block, it returns to its normal size (*55*). Moreover, in a number of pathological conditions that require more lysosomal enzyme production, there is also a hypertrophy of the TGN (*15*).

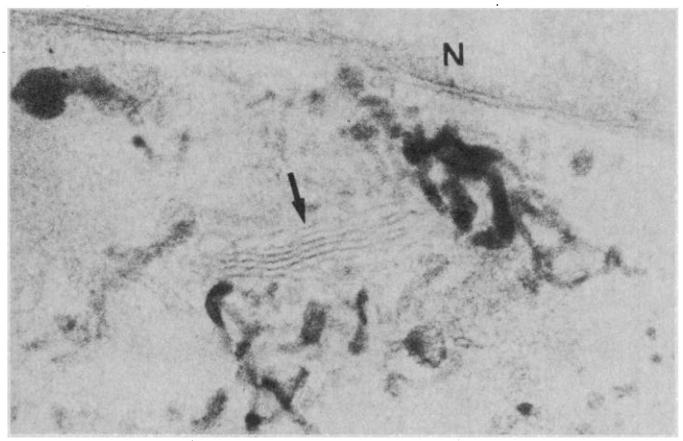

Fig. 3. Endosomes labeled by endocytosed peroxidase. Thick section (0.5 μm) through the Golgi region of a BHK cell that had endocytosed horseradish peroxidase for 2 hours at 20°C. Stained endosome structures are very close to the Golgi stack (arrow), and the two sets of structures would not be distinguished from each other in the absence of a marker. The two compartments are, however, functionally distinct (*24*) [from the study described in (*63*)]. Abbreviation: N, nucleus. ×42,120.

Recent studies indicate that the TGN may be mildy acidic. This was shown with the lysosomotropic agent 3-(2,4-dinitroanilino)-3′-amino-*N*-methyl-dipropylamine (DAMP), which accumulates selectively in acidic organelles (*56*). In the protonated form, DAMP can be visualized in the electron microscope either by indirect immunoperoxidase staining or by the use of antibodies to DAMP marked by colloidal gold. In fibroblasts this reagent accumulates in membrane structures on the *trans* side of the Golgi stack. Although not unequivocally identified in these studies, we suggest that this labeled structure is the TGN. An acidic *p*H in this organelle could explain the effects of lysosomotropic agents on exocytic sorting (*57*).

If the TGN is acidic, its *p*H would not be expected to fall below 6 for several reasons. Because many of the lysosomal enzymes undergo a proteolytic cleavage at a late stage of transport and probably in the lysosome itself, these enzymes are likely to be in the precursor, uncleaved form in the TGN. Biochemical and cytochemical evidence suggests, however, that these precursors would be active at low *p*H (*58*). It may, therefore, be important for the *p*H in the TGN, where lysosomal enzymes are mixed with other transit proteins, to be above the *p*H optimum for lysosomal enzyme activity, that is, above *p*H 6.0. Further, because the M6P receptor and lysosomal enzymes seem to be bound to each other in the TGN, the *p*H must be above 6. At lower *p*H's the ligands would dissociate from the receptor (*8, 44, 45*). Finally, a number of viral spike proteins function as fusogens at *p*H's around 6 or below (*59*). Even brief exposure to low *p*H causes irreversible loss of the fusogenic activity essential for infectivity. Thus, these proteins are probably not exposed to a *p*H significantly below 6 during their transport to the cell surface (*60*).

Target Organelles for Proteins Exiting from the TGN

The vesicles that carry newly synthesized plasma membrane proteins from the TGN have not yet been characterized, although preliminary immunocytochemical data suggest potential candidates (*24, 28, 61*). Those vesicles containing newly synthesized VSV G proteins seem to fuse directly with the plasma membrane rather than with an intermediate organelle, such as an endosome compartment (*62*). During the low temperature–dependent accumulation of VSV G protein in TGN (*24*), exogenously added horseradish peroxidase was simultaneously endocytosed by the same cells. At 20°C, this marker selectively fills endocytic compartments without being transported to the lysosomes (*63*). In a double-labeling procedure, both G protein and peroxidase were visualized in the same sections and endosome structures and the TGN, although often adjacent, were nevertheless distinct (Fig. 3). No G protein could be detected in endosome structures at any time during exocytosis. The same conclusion has been recently obtained in a similar double-labeling procedure with exocytosing G protein for the outward pathway and recycling transferrin for the inward one (*64*).

Of the three classes of proteins that exit from the TGN, the lysosomal proteins are those whose sorting is best understood at the molecular level. The key processes in this sorting are a neutral *p*H binding of phosphorylated lysosomal enzymes to the M6P receptor shortly after leaving the ER and a low-*p*H dissociation of ligand and receptor at a later step in transport, which allows the receptor to recycle for further rounds of sorting. The precise cellular pathway that the lysosomal enzymes follow from the ER to lysosomes is still not clear.

Both biochemical (*8, 44, 45*) and immunocytochemical (*26, 48, 49*) data suggest that the M6P receptor is in the TGN, in the endosomes, and on the plasma membrane, where it seems to be concentrated in coated pits. Both approaches have failed to detect the receptor in lysosomes. The receptor on the cell surface can bind exogenous lysosomal enzymes with high affinity and target them to lysosomes. However, this pathway is likely to be a relatively minor one because an excess of M6P in the culture medium (which effectively removes any bound ligand from the receptor) failed to alter the correct targeting of newly synthesized enzymes (*65*). The most likely target of TGN-derived vesicles carrying lysosomal enzymes seems to be an endosome compartment. Since there are compelling arguments against the presence of significant acid hydrolase activity in the total endosome population (*62*), lysosomal enzymes bound to the M6P receptor may be directed to a specific subset of endosomes where their dissociation occurs. A likely candidate is a class of multivesicular bodies, referred to as a multivesicular endosome (*66*), commonly found in the Golgi region of cells. The fusion of the TGN-derived vesicles with a multivesicular endosome may be the event that converts a "late" endosome compartment into a lysosome.

How does the presence of the M6P receptor on the cell surface fit into the above scheme? It may be a subset of M6P receptor that has been missorted from the TGN. Another, perhaps more likely, possibility is that unoccupied receptor or a fraction of it, after releasing its ligand in a late endosome compartment, may recycle to the Golgi complex via the plasma membrane. This model has, in part, already been proposed (*44*). In both cases exogenous ligand, when present, could be targeted correctly to lysosomes by receptor-mediated endocytosis.

M6P receptors in their different cellular locations are in dynamic equilibrium with each other. When antibodies to the M6P receptor were added to the culture medium of fibroblasts, the bulk of the cellular receptors became bound to antibodies and were inactivated within 2 hours (*44, 67*). This treatment led to an aberrant secretion of lysosomal enzymes, presumably because functional receptors became depleted from the Golgi complex.

TGN and Membrane Recycling

If the TGN is sorting and segregating proteins into separate carrier vesicles for delivery to different post-Golgi destinations, the

loss of TGN membrane in these sorting processes must be compensated for by membrane recycling. The pathway for the return of membrane components to the Golgi complex is not yet clear. Various electron-opaque tracers or exogenous ligands that bind to cell-surface receptors have often been shown to be endocytosed into tubular elements on the *trans* side of the Golgi stack. In most of these studies, however, it has been difficult to conclude whether uptake had occurred into endosome structures in the vicinity of the Golgi complex or whether the tracers had actually moved into the TGN or other Golgi compartments (Fig. 3). Double-label electron microscopy can distinguish between these alternatives, but such studies have been rare. Endocytosed ligands may be localized, at least transiently, in structures with the cytochemical properties of TGN (*68*). Cell-surface markers have been shown in a few cases to enter into the cisternae of the Golgi stack and even into the newly formed secretory granules (*6, 69*). It has also been shown that in CHO cells, endocytosed transferrin, initially found in a *p*H 5.5 endosomal compartment, later moved into tubular structures in the vicinity of the Golgi stack that had a *p*H of 6.4 (*70*). Whether the latter is the TGN or a late endosome compartment is not yet clear [see also (*64*)].

Both transferrin and the transferrin receptor, if desialylated in vitro, seem to be resialylated during membrane recycling (*71*). This resialylation presumably occurs in the TGN. The half-time for resialylation—2 to 3 hours in the case of the receptor—is much slower than that needed for the movement of receptors from the plasma membrane to the endosomes and back, a process requiring only minutes. The transferrin receptor might have to make many endocytic rounds on average, before it is diverted into the TGN. This could explain why it has often been difficult to observe membrane recycling into the TGN.

More work is necessary to elucidate how the exocytic and the endocytic pathways are linked. The phenotype of some yeast mutants suggests that endocytosis may be coupled obligatorily to the last steps of protein secretion (*72*). Furthermore, mutants of CHO cells that are defective in acidification of endosomes had abnormal terminal glycosylation of some viral spike glycoproteins and of several endogenous secretory glycoproteins (*73*). Although the molecular nature of the defects in these mutants is unknown, one possibility is that endosomes and the Golgi complex share gene products necessary for the function of both organelles (*57*).

Conclusions

We propose that the sorting of three distinct classes of proteins into separate pathways is accomplished by the *trans* Golgi network. This structure is defined as the last station along the Golgi pathway involved in the final processing steps of *N*-linked glycosylation in the cell. The TGN must now be further characterized in different cell types to establish its precise role in sorting of plasma membrane, lysosomal, and secretory proteins.

The pathways that connect the TGN with endosomes, lysosomes, and plasma membrane must be studied in well-defined systems. This task will best be accomplished by combining immunocytochemical and biochemical approaches under conditions in which a defined transit protein is blocked in a specific and identified site, then released and followed kinetically in a quantitative manner. The elucidation of molecular mechanisms of membrane sorting will have to await a reconstitution of TGN functions in the test tube, an approach already in use for studying transport between Golgi compartments (*7*).

REFERENCES AND NOTES

1. G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1496 (1980).
2. R. Brands *et al.*, *J. Cell Biol.* **101**, 1724 (1985); A. Yamamoto, R. Masaki, Y. Tashiro, *ibid.*, p. 1733; L. Liscum *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 7165 (1983).
3. M. G. Farquhar and G. E. Palade, *J. Cell Biol.* **91**, 77 (1981).
4. J. E. Rothman, *Science* **213**, 1212 (1981).
5. A. M. Tartakoff, *Trends Biochem. Sci.* 7, 174 (May 1982).
6. M. G. Farquhar, *Annu. Rev. Cell Biol.* **1**, 447 (1985).
7. W. G. Dunphy and J. A. Rothman, *Cell* **42**, 13 (1985).
8. R. Kornfeld and S. Kornfeld, *Annu. Rev. Biochem.* **54**, 631 (1985).
9. J. P. Kraehenbuhl, L. Racine, J. D. Jamieson, *J. Cell Biol.* **72**, 406 (1977); H. J. Geuze, J. W. Slot, K. T. Tokuyasu, *ibid.* **82**, 697 (1979); J. Green, G. Griffiths, D. Louvard, P. Quinn, G. Warren, *J. Mol. Biol.* **152**, 663 (1981); J. E. Bergmann, K. T. Tokuyasu, S. J. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1746 (1981); R. Brands, J. W. Slot, H. J. Geuze, *Eur. J. Cell Biol.* **27**, 213 (1982); H. J. Geuze, J. W. Slot, G. J. A. M. Strous, A. Hasilik, K. von Figura, *J. Cell Biol.* **98**, 2047 (1984).
10. Although much insight into the three-dimensional structure of the Golgi stack has been obtained from an excellent serial-section reconstruction study (*21*), the number of discrete compartments in the Golgi complex is not known. This approach is limited by the diameters of the cisternae and many of the tubular connections between adjacent structures, which are near the thickness of the section. Small connections can therefore be missed. A complete three-dimensional model of the Golgi stack would be possible only if each compartment could be selectively and completely filled with different electron-opaque markers prior to serial thin-section reconstruction. This has not yet occurred. Current models assume that the different cytochemical reactions do not overlap, although no two of the three reactions have ever been localized simultaneously in the same Golgi stack. In fact, there is evidence in some systems that the different cytochemical reactions may overlap (*17, 19*).
11. G. Palade, *Science* **189**, 347 (1975).
12. A. B. Novikoff, *Biol. Bull.* **127**, 358 (1964).
13. ———, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2781 (1976).
14. ———, M. Mori, N. Quintana, A. Yam, *J. Cell Biol.* **75**, 148 (1977); A. R. Hand and C. Oliver, *ibid.* **74**, 399 (1977).
15. A. R. Hand and C. Oliver, in *Cell Biology of the Secretory Process*, M. Cantin, Ed. (Karger, Basel, 1984); A. B. Novikoff and P. M. Novikoff, *Histochem. J.* **9**, 525 (1977).
16. E. Holtzman, A. B. Novikoff, H. Villaverde, *J. Cell Biol.* **33**, 419 (1967); R. Pictet, L. Orci, A. E. Gonet, C. Rouiller, A. E. Renold, *Diabetologia* **3**, 188 (1967); D. S. Friend and M. G. Farquhar, *J. Cell Biol.* **35**, 357 (1967).
17. Although the distinction between these two structures is usually clear cytochemically, the acid phosphatase and thiamine pyrophosphatase reactions overlap or mix in a few cases. This is true in some tissue culture cells [G. Griffiths, P. Quinn, G. Warren, *J. Cell Biol.* **96**, 835 (1983)] and in cells during particular developmental stages or in response to different physiological or pathological conditions [C. Oliver and A. R. Hand, *J. Histochem. Cytochem.* **31**, 1041 (1983); A. I. Doine, C. Oliver, A. R. Hand, *ibid.* **32**, 477 (1984)].
18. During this period the "maturation" model of the Golgi stack had support [D. J. Morré, J. Kartenbeck, W. W. Franke, *Biochim. Biophys. Acta* **559**, 71 (1979)]. This theory proposed that the cisternae themselves "moved" across the stack, maturing in the process. For arguments against this model, see (*7*).
19. S. Goldfischer, *J. Histochem. Cytochem.* **30**, 717 (1982).
20. D. J. Morré and L. Ovtracht, *Int. Rev. Cytol.* **5** (suppl.), 61 (1977).
21. A. Rambourg, Y. Clermont, L. Hermo, *Am. J. Anat.* **154**, 455 (1979).
22. M. C. Willingham and I. H. Pastan, *Int. Rev. Cytol.* **92**, 51 (1984).
23. G. Griffiths, G. Warren, I. Stuhlfauth, B. M. Jockusch, *Eur. J. Cell Biol.* **26**, 52 (1981); M. C. Willingham, J. H. Keen, I. H. Pastan, *Exp. Cell Res.* **132**, 329 (1981); L. Orci, M. Ravazzola, M. Amherdt, D. Louvard, A. Perrelet, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5385 (1985).
24. G. Griffiths, S. Pfeiffer, K. Simons, K. Matlin, *J. Cell Biol.* **101**, 949 (1985).
25. J. Roth *et al.*, *Cell* **43**, 287 (1985).
26. H. J. Geuze, J. W. Slot, G. J. A. M. Strous, A. Hasilik, K. von Figura, *J. Cell Biol.* **101**, 2253 (1985).
27. Galactosyl transferase is colocalized with TPPase in HeLa cells [J. Roth and E. Berger, *ibid.* **93**, 223 (1985)]. The TGN was not identified in this study, and thiamine pyrophosphatase might react in HeLa cells with both the *trans* compartment and the TGN, as it does in other undifferentiated cells (*17*). Galactosyl transferase is present in the TGN of hepatoma cells and liver hepatocytes (*26*). H. J. Geuze (personal communication) has also consistently found low concentrations of this enzyme in one or two cisternae on the *trans* side of the Golgi stack. These results suggest that galactosyl transferase may be present in the preceding Golgi compartment (*trans* in Fig. 2) as well as in the TGN. Further data are needed to clarify this point both for this enzyme and for sialyl transferase.
28. K. Matlin and K. Simons, *Cell* **34**, 233 (1983); J. Saraste and E. Kuismanen, *ibid.* **38**, 535 (1984).
29. S. D. Fuller, R. Bravo, K. Simons, *EMBO J.* **4** , 297 (1985).
30. J. Wehland, M. C. Willingham, M. G. Gallo, I. Pastan, *Cell* **28**, 831 (1982).
31. J. E. Rothman, H. G. Pettegrew, R. E. Fine, *J. Cell Biol.* **86**, 162 (1980).
32. G. S. Payne and R. Schekman, *Science* **230**, 1009 (1985).
33. P. Marcus, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 351 (1963); M. Bretscher, *ibid.* **66**, 707 (1981); A. A. Rogalski, J. E. Bergman, S. J. Singer, *J. Cell Biol.* **99**, 1101 (1984).
34. E. Rodriguez-Boulan, *Mod. Cell Biol.* **1**, 119 (1983).
35. K. Simons and S. D. Fuller, *Annu. Rev. Cell Biol.* **1**, 243 (1986).
36. K. Matlin and K. Simons, *J. Cell Biol.* **99**, 2131 (1984); D. E. Misek, E. Bard, E. Rodriguez-Boulan, *Cell* **39**, 537 (1984); S. Pfeiffer, S. D. Fuller, K. Simons, *J. Cell Biol.* **101**, 470 (1985).

37. M. J. Rindler, I. E. Ivanov, H. Plesken, E. Rodriguez-Boulan, D. D. Sabatini, *J. Cell Biol.* **98**, 1304 (1984).
38. R. B. Kelly, *Science* **230**, 25 (1985).
39. G. J. A. M. Strous *et al.*, *J. Cell Biol.* **97**, 1815 (1983).
40. J. Tooze and S. Tooze, *ibid.*, in press.
41. L. Orci *et al.*, *Cell* **39**, 39 (1984).
42. L. Orci *et al.*, *ibid.* **42**, 671 (1985).
43. W. B. Huttner, *Nature (London)* **299**, 273 (1985); R. W. H. Lee and W. B. Huttner, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6143 (1985); D. B. Baeuerle and W. B. Huttner, in preparation.
44. G. G. Sahagian, *Biol. Cell.* **51**, 207 (1984).
45. G. E. Creek and W. S. Sly, in *Lysosomes in Biology and Pathology*, J. T. Dingle, R. T. Dean, W. S. Sly, Eds. (North-Holland, Amsterdam, 1984).
46. D. Goldberg and S. Kornfeld, *J. Biol. Chem.* **258**, 3159 (1983).
47. D. F. Bainton and M. G. Farquhar, *J. Cell Biol.* **45**, 54 (1970).
48. H. J. Geuze *et al.*, *ibid.* **98**, 2047 (1984).
49. M. C. Willingham, I. H. Pastan, G. G. Sahagian, *J. Histochem. Cytochem.* **31**, 1 (1983).
50. Using detergent permeabilization and immunoperoxidase, W. Brown and M. G. Farquhar [*Cell* **36**, 295 (1984)] have concluded that, within the Golgi complex, the M6P receptor was observed mainly in the *cis* compartment. These different results could be due to differences in cell type or to different immunocytochemical techniques (*6*). With the peroxidase technique, a quantitatively minor amount of antigen may, however, in some cases, be amplified by the enzymatic reaction.
51. E. Schulze-Lohoff, A. Hasilik, K. von Figura, *J. Cell Biol.* **101**, 824 (1985); C. A. Campbell and L. H. Rome, *J. Biol. Chem.* **258**, 13347 (1983); G. C. Sahagian and C. J. Steer, *ibid.* **260**, 9838 (1985).
52. B. Hoflack and S. Kornfeld, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4428 (1985).
53. V. Lewis *et al.*, *J. Cell Biol.* **100**, 1839 (1985); J. W. Chen, T. L. Murphy, M. C. Willingham, I. Pastan, J. T. August, *ibid.* **101**, 85 (1985); J. Lippincott-Schwartz and D. M. Fambrough, *ibid.* **102**, 1593 (1986); I. Mellman and A. Helenius, personal communication.
54. A. R. Hand and C. Oliver, *J. Histochem. Cytochem.* **32**, 403 (1984).
55. G. Griffiths *et al.*, in preparation.
56. R. G. W. Anderson and R. K. Pathak, *Cell* **40**, 635 (1985).
57. I. Mellman, R. Fuchs, A. Helenius, *Annu. Rev. Biochem.* **55**, 663 (1986).
58. A. Frisch and E. F. Neufeld, *J. Biol. Chem.* **256**, 8242 (1981). That the cytochemical reactions for acid phosphatase and aryl sulfatase occur at *p*H 5 but not at neutral *p*H's also suggests that the enzymes are potentially active.
59. J. White, M. Kielian, A. Helenius, *Q. Rev. Biophys.* **16**, 151 (1983).
60. To become active, many of these spikes, such as the hemagglutinin (HA) of influenza virus, require a proteolytic cleavage late in their transport to the plasma membrane. The precise location of this cleavage is not clear, but the TGN is a likely candidate. Even the uncleaved precursors of HA undergo an irreversible conformational change at *p*H's about 6.0 (R. Doms and A. Helenius, personal communication).
61. H. Arnheiter, M. Dubois-Dalcq, R. A. Lazzarini, *Cell* **39**, 99 (1984).
62. Endosomes are defined here as the total population of endocytic prelysosomal acidic organelles. This term actually comprises a heterogeneous set of structures that have an uncharacterized relation to each other [D. A. Wall and A. L. Hubbard, *J. Cell Biol.* **90**, 687 (1981); A. Helenius, I. Mellman, D. Wall, A. Hubbard, *Trends Biochem. Sci.* **8**, 245 (1983)].
63. In this, as well as in the previous study (*24*), endosomes were defined operationally as those structures that accumulated horseradish peroxidase at 20°C for 2 hours [M. Marsh, G. Griffiths, G. E. Dean, I. Mellman, A. Helenius, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2899 (1986)]. These conditions probably do not resolve different subcompartments of endosomes (*62*).
64. K. Hedman, K. L. Goldenthal, A. V. Rutherford, I. Pastan, M. C. Willingham, *J. Cell Biol.* **101**, 422a (1985).
65. W. S. Sly and H. D. Fischer, *J. Cell. Biochem.* **18**, 67 (1982).
66. C. Harding, J. Heuser, P. Stahl, *J. Cell Biol.* **97**, 329 (1983).
67. K. von Figura, V. Gieselmann, A. Hasilik, *EMBO J.* **3**, 1281 (1984); C. Gartang, T. Braukke, A. Hasilik, K. von Figura, *ibid.* **4**, 1725 (1985).
68. N. K. Gonatas, S. U. Kim, A. Stieber, S. Avrameas, *J. Cell Biol.* **73**, 1 (1977); E. Essner and H. B. Haimes, *ibid.* **75**, 381 (1977); H. B. Haimes, R. J. Stockert, A. G. Morell, A. B. Novikoff, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6936 (1981); R. D. Broadwell and C. Oliver, *J. Histochem. Cytochem.* **31**, 325 (1983).
69. A. Patzak and H. Winkler, *J. Cell Biol.* **102**, 510 (1986).
70. D. Y. Yamashiro, B. Tycko, S. F. Fluss, F. R. Maxfield, *Cell* **37**, 789 (1984).
71. E. Regoeczi, P. A. Chindemi, M. T. Debanne, P. A. Charlwood, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2226 (1982); M. D. Snider and O. C. Rogers, *J. Cell Biol.* **100**, 826 (1985).
72. H. Riezman, *Cell* **40**, 1001 (1985); M. Makarow, *EMBO J.* **4**, 1861 (1985).
73. A. R. Robbins *et al.*, *J. Cell Biol.* **99**, 1296 (1984).
74. We thank the following for their help with this review: B. Burke, S. Fuller, H. Geuze, A. Helenius, K. Howell, A. Hubbard, E. Hughson, W. Huttner, S. Kornfeld, D. Louvard, M. Marsh, K. Matlin, I. Mellman, L. Orci, P. Quinn, L. Roman, J. Slot, J. Tooze, and G. Warren.

What Has Happened to Productivity Growth?

MARTIN NEIL BAILY

The collapse of U.S. productivity growth is the most severe and persistent of recent economic problems. Unless there is an increase in growth, American living standards will remain stagnant and problems such as the budget deficit will plaque policy-makers. Why has this happened? Among the important reasons are a failure to innovate, changing demographics, and disruptions to the economy, including oil price increases and inflation.

A PERSISTENT DECLINE IN PRODUCTIVITY GROWTH IN THE U.S. economy has prevailed since the late 1960's, and it intensified after 1973. Multifactor productivity in nonfarm business grew at 1.75% per year from 1953 to 1968. This rate dropped after 1968 and fell to only 0.32% a year by 1973 to 1979 (Fig. 1). The cumulative effect of this decline on the output of the economy was substantial. Had the pre-1968 growth rate continued, output in 1979 would have been 12% higher than it actually was, with no additional capital or labor used in production. This amount of additional output is much larger than that needed to solve many of today's economic problems, notably the budget deficit.

The nonfarm business sector of the U.S. economy includes everything except government operations, agriculture, and nonprofit organizations. Figure 1 also shows productivity growth in manufacturing, which differs from that of the aggregate economy (*1*). Productivity growth in manufacturing actually accelerated from 1968 to 1973, before slumping from 1973 to 1979.

Although the difference between manufacturing and the aggregate economy is important, a disaggregated view of the slowdown shows the pervasiveness of this decline. Productivity growth has decreased in almost all of the major sectors of the economy. Within the major sectors, the Bureau of Labor Statistics (BLS) looked at specific industry-level performance and found that three-quarters of the industries in their sample had declines in productivity growth (*2*).

The Dimensions of the Slowdown

Multifactor productivity growth is the concept favored by most economists, and now by BLS also. To calculate this measure of

The author is a senior fellow in the Economic Studies Program, Brookings Institution, Washington, DC 20036.