Living cells are defined by their limiting membrane, known as the cell surface membrane or plasma membrane. Without exception, plasma membranes contain myriad lipid and protein molecules that are synthesized and assembled within the interior of the cell, primarily in the endoplasmic reticulum and Golgi apparatus. The general outlines of the pathway that proteins and lipids follow as they are translocated from their sites of synthesis and assembly within the cell to the cell surface were elucidated from several decades of pioneering studies that culminated in those of Palade and colleagues (Palade 1975). Proteins destined for export are synthesized on ribosomes attached to the endoplasmic reticulum. From there, these proteins are transported to the Golgi apparatus, where they are packaged into secretion granules that detach from the Golgi apparatus and migrate to the cell surface. Secretion granules then fuse with the plasma membrane, discharging their contents to the exterior compartment.

Several considerations suggested that anterograde (forward) transport of membrane material probably parallels the movement of secretory proteins toward the cell surface. First, these proteins are transported to the Golgi apparatus, where they are packaged into secretion granules that detach from the Golgi apparatus and migrate to the cell surface. Secretion granules then fuse with the plasma membrane, discharging their contents to the exterior compartment.

Many recent evidence appears to favor the original flow differentiation model (Figure 1b) over the alternative vesicle shuttle model of intra-Golgi apparatus transport (Figure 1a).

The endomembrane system

An early and, in our opinion, still valid explanation for protein and lipid transport from the endoplasmic reticulum to the plasma membrane was the hypothesis that membranes...
membrane would be returned by compensatory endocytosis. According to the flow differentiation model (b), cisternae are formed at the cis-face of the Golgi apparatus by coalescence of transition vesicles that are derived from the endoplasmic reticulum. The membranes are then modified at successive levels within the Golgi apparatus stack. Eventually, the membrane is discharged through formation of secretory vesicles and the release of a cisternal remnant. Consequently, the entire Golgi apparatus turns over, with the buildup of new cisternae at the cis-face and the release of mature plasma membrane-like units at the trans-face.

Flow through an endomembrane system that includes the endoplasmic reticulum, the Golgi apparatus, and the vesicular and transitional membrane forms that connect these intracellular elements with each other and with the cell surface (Morré and Mollenhauer 1974, Morré et al. 1971b, 1979). The term "membrane flow" was first used to describe membrane migrations that are associated with pinocytosis, a process for import of extracellular materials into the cell (Bennett 1956).

The concept of flow of membrane from endoplasmic reticulum to plasma membrane was elaborated subsequently as a way to explain the kinetics of turnover of membrane-associated liver proteins (i.e., subsequent loss in the "chase" period [where excess nonradioactive amino acids are given] of radioactivity incorporated into proteins during an initial "pulse" with radioactive amino acids administered to animals, isolated organs, tissue explants, or cells; Franke et al. 1971). Evidence from biochemical studies of fractionated cells and tissues, combined with morphological observations, led to the idea of a functional continuum of membranes, in which they extend from the endoplasmic reticulum to the plasma membrane. This continuum was termed the endomembrane system by Morré and Mollenhauer (1974).

Cytological observations of a fungus of the Pythium genus provided crucial support for a central role for the Golgi apparatus in membrane differentiation (Grove et al. 1968). In fungal cells, the Golgi apparatus cisternae at the pole nearest to and in communication with the endoplasmic reticulum (the proximal, forming, or cis face) exhibited ultrastructural characteristics that were indistinguishable from those of the endoplasmic reticulum. Membranes at the opposite pole (the distal, maturing, or trans face), which are involved in formation of secretion vesicles, had ultrastructural characteristics like those of the plasma membrane. Cisternae between the proximal and distal faces of the Golgi apparatus stack had intermediate ultrastructural characteristics, with a progression toward more plasma membrane-like membranes across the stack in the proximal to distal direction. We proposed that as membranes traverse the stacked membranes of the Golgi apparatus en route from the endoplasmic reticulum toward the plasma membrane, their composition was modified by the addition, deletion, or modification of membrane constituents (Morré et al. 1971a, 1979).

Integral to the endomembrane concept was the idea that discontinuous systems of transition elements linked components of the endomembrane system. Small vesicles, known as transition vesicles, were thought to bud from the endoplasmic reticulum, to migrate to the Golgi apparatus region of the cell, and, once there, to fuse with one another or with a forming Golgi apparatus cisterna to deliver new membrane materials as well as secretory products to the Golgi apparatus (Morré et al. 1971b). At the opposite poles of Golgi apparatus stacks, exocytic or secretory vesicles were believed to bud from the mature or plasma membrane-like Golgi apparatus cisternae for delivery of membrane material to the plasma membrane and of secretory products to the cell's exterior.

Vesicle coats were observed and implicated in vesicular trafficking from the early stages of development of the endomembrane hypothesis (Mollenhauer et al. 1976). Transition vesicles in the region of the endoplasmic reticulum–Golgi apparatus interface were covered with a fine, fibrous-appearing coat, whereas exit (secretory) vesicles, formed at the mature face of the Golgi apparatus, frequently appeared to be adorned with spike-like projections (now known to be clathrin coats) over all or parts of their surfaces. Exit vesicles that appeared to be in transit from the Golgi apparatus to the plasma membrane seldom displayed coats, but coats seemingly reappeared prior to or during fusion with the plasma membrane (Kartenbeck et al. 1977).

Much ultrastructural and biochemical evidence in support of the flow differentiation concept was subsequently generated. That evidence, from studies with both plants and animals, led us to suggest that the membrane flow concept was gener-
ally applicable to eukaryotic cells (Morré et al. 1971a, 1971b, 1979).

Endoplasmic reticulum-to-Golgi apparatus transfer

The operation of the vesicle-mediated transit step between endoplasmic reticulum and Golgi apparatus has been demonstrated unequivocally using completely cell-free systems (Baker et al. 1988, Balch et al. 1987, Nowack et al. 1987). Transition vesicles are formed by and released from isolated transitional elements of endoplasmic reticulum in a process that requires energy from nucleotide triphosphate hydrolysis. All three players participating in endoplasmic reticulum-to-Golgi apparatus transfer—the transitional endoplasmic reticulum, transition vesicles, and cis-Golgi apparatus cisternae—have been isolated as purified, functional fractions from rat liver. Transfer and processing of membrane proteins and lipids have been reconstituted in cell-free systems using the purified components (Dunkle et al. 1992, Moreau and Morré 1991, Paulik et al. 1988): Endoplasmic reticulum-to-Golgi apparatus transfer in these cell-free systems exhibited a remarkable fidelity to that in living cells in terms of specificity, ATP requirement, pH optimum, and temperature dependence, as well as sensitivity to sulfhydryl reagents, involvement of monomeric GTP-binding proteins, and efficiency of transfer. Compelling evidence for coated vesicular intermediates between the endoplasmic reticulum and the Golgi apparatus has emerged from other studies as well. Combined genetic and biochemical investigations with yeast (Schekman and Orci 1996) and biochemical studies with permeabilized cells (Peter et al. 1993) have been especially important in leading to a molecular understanding of this transfer event.

The concept of a discontinuous, transition vesicle-mediated system that delivers membrane material from the endoplasmic reticulum to the Golgi apparatus is now widely accepted. A variation of this view of the pathway is the concept that there exists an intermediate compartment between the endoplasmic reticulum and the Golgi apparatus (Mellman and Simons 1992). According to this view, membranes that are ultimately destined for export to the cell surface accumulate in the proximity of the Golgi apparatus before becoming fully integrated into the Golgi apparatus structure. Many, or perhaps all, cell types may exhibit such an intermediate compartment as a stage leading to formation of new cis-Golgi apparatus cisternae. However, such intermediate compartments appear to be more extensive in some cell types than in others.

Golgi apparatus-to-plasma membrane transfer

Also well accepted among cell biologists is that secretory vesicles are formed on distal cisternae, or trans-cisternae, of the Golgi apparatus, or, perhaps, at the trans-Golgi network (TGN; Griffiths et al. 1989). The TGN is a specialized assemblage of cisternae, tubules, and vesicles that are distinct from the Golgi apparatus stacks per se and are located at or near the exit face of the stack. It varies considerably in form and extent among different cell types (Clermont et al. 1995, Rambourg et al. 1979). Vesicles, identified by their contents as secretory vesicles, migrate to the plasma membrane, often from the TGN, to transfer both secretory products and membrane materials to the cell surface. Electron micrographs of many cell types show images of secretory vesicles in the regions between the exit face of the Golgi apparatus and the plasma membrane (e.g., Figure 2). In some micrographs, secretory vesicles may be identified during the process of formation. In others, images representing fusion of secretory vesicles and plasma membrane have been seen (Figure 2).

There is abundant evidence, both morphological and biochemical, for the existence and function of secretory vesicles. Secretory vesicles that are released from the trans-Golgi cisternae have been purified from several different tissues and cells, including rat liver (Merritt and Morré 1973) and mammary gland (Keenan et al. 1979). Such preparations of vesicles have been shown to be en-
Two models for membrane flow

Whereas it is clear that vesicular intermediates exist between the endoplasmic reticulum and the Golgi apparatus and between the Golgi apparatus and the plasma membrane, it is the authors' view that the question of whether vesicles convey materials through the Golgi apparatus remains controversial. The popular vesicle shuttle model (Figure 1a), also known as the multicompartiment discontinuous model or the two-compartment discontinuous model, postulates the involvement of intra-Golgi apparatus transfer vesicles (Dunphy and Rothman 1985). The flow differentiation model (Figure 1b), by contrast, holds that once a Golgi apparatus cisterna is formed, it remains in the Golgi apparatus stack but its constituents can be processed further. Possible processing events include protein glycosylation and phosphorylation; lipid glycosylation; phospholipid interconversion; accretion of cholesterol and other lipids; and lipid exchange. Such processing gradually converts cisternal membranes from endoplasmic reticulum-like to plasma membrane-like.

The flow differentiation model does not include an exclusive role for vesicular traffic between the formation of proximal cisternae and the budding of exit (secretory) vesicles from the distal cisternae. This distinction is indicated in the schematic comparison of Figure 1. In the flow differentiation model (Figure 1b), stacked cisternae of the Golgi apparatus are envisioned as a holding point to allow sufficient time for the biochemical events of differentiation of membranes from endoplasmic reticulum-like to plasma membrane-like to take place. An early article (Frantz et al. 1973) demonstrated that Golgi apparatus isolated from a plant acquired the ability to stain with phosphotungstic acid at low pH after isolation and during a subsequent cell-free incubation. This finding provided the first evidence of cell-free membrane differentiation from endoplasmic reticulum-like to plasma membrane-like within the Golgi apparatus. The reaction with phosphotungstic acid at low pH is otherwise limited to plasma membranes.

The vesicle shuttle model for intracellular flow postulates a third population of vesicles, distinct from transition vesicles and secretory vesicles, that function in intra-Golgi apparatus trafficking (Figure 1a). These vesicles are postulated to shuttle back and forth between adjacent cisternae, gradually enriching the distal face of the Golgi apparatus stack in trans-face constituents and gradually depleting it of cis-face constituents. This vesicle shuttle-mediated enrichment and depletion process is akin to fractional distillation or countercurrent distribution (Dunphy and Rothman 1985). In the discontinuous model, individual Golgi apparatus cisternae are viewed as permanent residents within the stack. A bidirectional flow of membrane constituents mediated by vesicle budding and fusion would achieve membrane differentiation but not necessarily net flow to the plasma membrane. This is because flow to the plasma membrane would be balanced by a return flow of membranes to the Golgi apparatus and because flow of membranes from the endoplasmic reticulum to the Golgi apparatus would be balanced by a recycling of membranes from the cis-face of the Golgi apparatus back to the transitional endoplasmic reticulum.

Basis for the vesicle shuttle model

The vesicle shuttle model originally was advanced as an explanation for observations that were made with an ingenious cell-free system used extensively to study transfer (Rothman 1994, Rothman and Orci 1992, Rothman and Wieland 1996). In this system, Golgi apparatus fractions isolated from two kinds of cells were mixed together, and exchange between them was monitored. Donor Golgi apparatus fractions were isolated from vesicular stomatitis virus (VSV) infected cells that had been mutated so as to lack a functional N-acetylglucosaminyl transferase, a Golgi apparatus enzyme that adds N-acetylglucosamine to growing carbohydrate chains of glycoproteins.

These infected cells synthesize a virus-encoded envelope glycoprotein (VSV-G) that follows the endoplasmic reticulum to Golgi apparatus to plasma membrane route. However, mutant cells could not complete the carbohydrate chain of VSV-G. Accepter Golgi apparatus fractions were isolated from wild-type cells that had not been infected with VSV. After mixing, the Golgi apparatus fractions were incubated with a radioactive sugar nucleotide, UDP-[3H]-N-acetylglucosamine. Radiolabelled VSV-G protein was recovered from the incubation mixture, which indicated that VSV-G protein had been transferred from donor Golgi apparatus to acceptor Golgi apparatus. Golgi apparatus transfer is reduced by nonhydrolyzable analogs of GTP, by protein-alkylating agents, and by the depletion of selected proteins from a cytosolic fraction (e.g., Malhotra 1989, Rothman and Orci 1990).

These results led Rothman and colleagues to propose that transfer from donor to acceptor Golgi apparatus in the cell-free system was mediated by vesicles. They subsequently elaborated a molecular model of events in vesicle budding and fusion (Rothman 1994, Rothman and Wieland 1996). The model envisioned that coated buds were formed on Golgi apparatus cisternae, with the coats being assembled from cytosolic proteins. The coat complex, or "coatamer," was subsequently shown to consist of equimolar amounts of several proteins, one of which is ADP-riboseylation factor (ARF), a monomeric GTP-binding protein. The other proteins are termed COPs (for coat proteins). Coated buds were envisioned to be released as coated vesicles. For coated vesicles to fuse...
with acceptor cisternae, the vesicles would first bind to a fusion protein with ATPase activity (N-ethylmaleimide-sensitive fusion protein, or NSF) and then to cytosolic NSF attachment proteins termed SNAPs. SNAPs bound to the vesicles would then bind to SNAREs, the acronym given to a family of proteins that serve as SNAP receptors. SNAREs, which are related to a family of neuronal proteins involved in the synaptic vesicle cycle (Sudhof 1995), are thought to direct vesicles to the appropriate cellular locations. In the case of vesicle targeting, specific vesicle-associated SNAREs would interact with specific target membrane-associated SNAREs.

Do free vesicles shuttle within the Golgi stack?

Although a large body of evidence seemed to support the vesicle shuttle model, at least one critical assumption on which it is based has not been demonstrated experimentally—that is, the existence of a system of free vesicles associated with intra-Golgi apparatus transport. We believe that such shuttle vesicles do not exist predominantly as free vesicles. We suggest that most of the small, 5‒6 nm diameter vesicular profiles seen in electron micrographs at the periphery of Golgi apparatus are, in reality, cross-sections through Golgi apparatus tubules (see Figure 3). Examples from many different cell types have shown that the central plate-like portions of Golgi apparatus cisternae are surrounded by an extensive network of anastomosing (joining together) tubules (Figure 3; Cunningham et al. 1966, Mollenhauer et al. 1967, Morré et al. 1971a, Rambourg et al. 1979). Thus, in thin-sectioned specimens, what appear to be vesicular profiles are, in reality, cross-sections through such tubules. Profiles of tubular cross-sections can be abundant in electron micrographs. The clear, electron-lucent centers of the profiles and their clearly defined limiting membranes would not be seen in thin sections of 5‒6 nm diameter vesicles. Rather, this appearance is more characteristic of cross-sectioned tubules (Morré et al. 1971a).

Another finding that has been cited in support of the vesicle shuttle model may also represent a misinterpretation. Many years before the vesicle shuttle model was developed, Cunningham et al. (1966) observed that in negatively stained preparations of Golgi apparatus isolated from plants, the ends of Golgi tubules appeared to have coat structures. Proponents of the vesicle shuttle model now interpret these coated ends of tubules as the coated buds that function as intermediates in vesicle formation. These putative intra-Golgi apparatus transport vesicles were suggested to be derived from a system of morphologically distinctive COP-coated buds that were originally visualized by selective staining with tannic acid (Orci et al. 1986), a technique earlier introduced by Mollenhauer et al. (1976) to visualize such coated buds. However, remarkably little evidence has been presented to show that coated buds ever are released, even transiently, as free vesicles (Morré and Keenan 1994).

In the single report of isolation of "coated vesicles" from Golgi apparatus that were incubated in a cell-free system, isolation required a chaotrophic agent, KCl at 0.25 M (Malhotra et al. 1989). Without KCl, the purported "vesicles" remained associated with Golgi apparatus elements. Whether the isolated vesicles were capable of fusing with acceptor membranes was not reported. Although the authors seem to have assumed that KCl released the vesicles from ionic interaction with cisternal membranes of Golgi apparatus, an alternative explanation is that KCl disrupted tubular attachments and permitted shearing of the coated end buds from tubules. We have not been successful in our attempts to "trap" or isolate putative intra-Golgi apparatus vesicular intermediates from cell-free systems, even when we included a nonhydrolyzable analog of GTP (GTPyS), with or without a thiol-alkylating reagent, or subjected cultured cells to reduced temperature in an attempt to arrest intracellular transit.

We used GTPyS to attempt to induce accumulation of the putative transfer vesicles because vesicle uncoating and fusion requires GTP hydrolysis (Malhotra et al. 1989, Figure 3. Negatively stained Golgi apparatus in cliated eartachial cells in situ (a) and after isolation (b). (a) Portion of the Golgi apparatus after in situ negative staining with fixatives containing tannic acid. A traditional cross-sectional view of a Golgi apparatus can be seen at the left. A tangential section is on the right. (b) After isolation of the Golgi apparatus, partial unstacking has occurred, revealing details of individual cisternae. In both panels, arrows point to the complex systems of peripheral tubules surrounding the central plate-like portions of cisternae (CP). These tubules also serve to interconnect adjacent Golgi apparatus stacks, as illustrated in (a). What appear to be vesicles at the peripheries of the cisternal stack are most often cross-sections of these cisternal tubules. Bars = 0.5 μm. Reprinted from Tandler and Morré (1983), with permission of Springer-Wien.
Rothman 1994). However, incubation of Golgi apparatus from Chinese hamster ovary cells in a medium containing GTPyS exaggerated coating at the periphery of cisternae, and the coated structures remained attached at their sites of formation (Weidman et al. 1993). Also, cytosol depleted in the GTP-binding protein ARF—a proposed requirement for the vesicle shuttle system—could still support intra-Golgi apparatus transfer in a cell-free system (Taylor et al. 1994). These results were interpreted as indicating that anterograde transport across the Golgi apparatus probably involved transient tubule-tubule fusion, rather than vesicle formation and fusion (Weidman 1995). Similarly, recent work on the diffusional mobility of Golgi apparatus proteins in living cells is inconsistent with vesicle transport within the Golgi (Cole et al. 1996). Fluorescence loss due to photobleaching was not slowed at 22°C, nor was it affected by energy depletion, as would be expected for a vesicle-mediated process.

If the “coated buds” of Golgi apparatus are not vesicles but rather coated ends of tubules, then how is it possible to explain the experiments of Rothman and colleagues (e.g., Rothman 1994), in which the N-acetylgalcosaminyl transferase enzyme or its substrate appeared to be transferred between Golgi apparatus stacks in a cell-free system? One possibility, as suggested earlier, is that membrane constituents can be exchanged between the Golgi apparatus cisternae but that the interchange occurs between cisternae in adjacent or contiguous stacks via interactions among tubules rather than by the budding and fusion of vesicles (Figure 4; Morré and Keenan 1994).

Indeed, the opportunity for significant interactions among adjacent Golgi apparatus stacks is a well-established feature of the Golgi apparatus (Mollenhauer and Morré 1966). In fact, in several cell types, including lacrimal and pituitary gland cells, motor nerve cells (Tanaka et al. 1986), mucous-secreting cells (Rambourg et al. 1987), spermatids (Clermont et al. 1994), Sertoli cells (Rambourg et al. 1979), and milk-secreting cells (Dylewski et al. 1984), three-dimensional reconstructions of Golgi apparatus have shown that cisternae within stacks have tubular interconnections and that seemingly isolated stacks are also physically interconnected. Moreover, in a study of polykaryons formed by fusing hamster cells, Deng et al. (1992) observed intermixing of resident Golgi apparatus membrane proteins and found that this mixing required direct physical continuity between Golgi apparatus elements.

If the putative transport vesicles function as predicted by the vesicle shuttle model, then they should contain secretory products that are being conveyed across the Golgi apparatus stack. However, an ultrastructural study of the Golgi apparatus of milk-secreting mammary epithelial cells yielded no evidence for product-containing shuttle vesicles (Clermont et al. 1993). Vesicular structures were observed at the margins of cisternae, but these vesicles were devoid of casein micelles and submicelles. (Caseins are a group of milk-specific phosphoproteins that aggregate into micellar structures as they move through the Golgi apparatus. These micelles and their submicellar units stain densely and have a distinctive appearance in the transmission electron microscope; Figure 2.) Micellar structures were distributed throughout intracisternal spaces across the stack of Golgi apparatus cisternae (Clermont et al. 1993). The finding that the vesicles at cisternal margins were devoid of casein submicelles implies that they were not involved in anterograde intra-Golgi apparatus transport. These morphological observations were interpreted as support for the continuous migration of cisternae and as being at odds with predictions based on the operation of vesicle shuttles. In another study (Clermont et al. 1994), the cisternae and associated peripheral tubules of the Golgi apparatus of spermatids were shown to form a single, continuous membrane system. As in mammary epithelial cells, transfer across the Golgi apparatus in glandular epithelial cells of the seminal vesicle appeared not to involve vesicles.

Evidence for the flow differentiation model

The flow differentiation model was based in part on findings that in scale-forming algae, large surface scales made up predominantly of cellulose are assembled within the confines of individual cisternae of the Golgi apparatus (Brown 1969). The assembly appeared to be progressive across the stack, and entire cisternae were discharged at the distal pole to deliver the scales to the cell surface. This process was relatively rapid, with a new scale being discharged every six minutes. The migrating units were large enough to be visualized readily with a light microscope. The scale-forming algae remain as one of the most convincing systems.
for real-time observations of Golgi apparatus dynamics. During scale formation and maturation, cisternae were progressively displaced across the stack, and new cisternae were formed at the proximal face to replace those lost in delivery of scales to the cell surface. In fact, new cisternae must be formed at the same rate as scale discharge, because the number of cisternae per stack remained constant during periods of continuous scale secretion (Figure 5).

Using the ability of the sodium ionophore monensin to induce swelling of distal Golgi apparatus cisternae, additional evidence for cisternal progression has been obtained with both animal and plant cells. As the distal cisterna gains competence to acidify its interior, monensin facilitates the exchange of internal protons for external sodium or potassium ions. This exchange causes the entire cisterna to be released from the stack, and it remains in the vicinity of the Golgi apparatus as a large, swollen vacuole. The number of vacuoles increases in a time-dependent manner for approximately four hours. Quantifications from electron micrographs indicate that one new vacuole appears approximately every 12 minutes in cultured carrot cells (Morré et al. 1983). The time-dependent appearance of swollen Golgi apparatus cisternae with the combination of monensin treatment and glutaraldehyde fixation has been confirmed by direct light microscopic observation in both carrot and mammary epithelial cells (Morré et al. 1992). Results similar to those with carrot and mammary epithelia were obtained with rat hepatoma cells. However, with rat hepatoma cells, cisternae formed at the rate of one every 15 minutes, and the system functioned up to only approximately two hours in the presence of monensin (Morré et al. 1985). According to the flow differentiation model, distal Golgi apparatus cisternae normally would be converted into secretory vesicles at a rate of approximately one every 10–30 minutes to account for the estimated rates of flux of membrane proteins through the Golgi apparatus stack (Morré et al. 1979). Thus, when conversion of distal cisternae into secretory vesicles was blocked by monensin, the number of cisternae predicted to accumulate based on the flow differentiation model actually were observed to accumulate.

**Coats, SNAREs, and SNAPs**

During development of the flow differentiation model, coat structures were suggested to be involved in moving membrane vesicles or tubules from one cellular location to another (Croze et al. 1982, Franke et al. 1976). Coat proteins also may function in shaping, budding, and fusion of transport vesicles (Schekman and Orci 1996). Additionally, coat structures are involved in processes that direct vesicles to the plasma membrane, such as the movement of vesicles from transitional endoplasmic reticulum to the proximal Golgi apparatus, or tubules among adjacent Golgi apparatus stacks, and of secretory vesicles from the distal Golgi apparatus along guide elements (microtubules in animals and microfilaments in plants). Biochemical studies have shown that vesicle and tubule coats may be clathrin or COP based. It also appears that the coat structures must be removed for their membranes to fuse (Rothman 1994).

Coats, SNAREs, and SNAPs hypothesis (Rothman 1994, Rothman and Wieland 1996) predicts that coat proteins carry out specific targeting functions. Docking or interaction of vesicles with target membranes, presumably as a result of specific SNARE–SNARE interactions that may be promoted by interaction of SNAREs with SNAPs and NSF, is followed by uncoating of the vesicle and actual fusion of the lipid bilayers of the vesicle and target membranes.

In yeast, COPs may be involved in selective retrograde transport of membrane proteins from Golgi apparatus to the endoplasmic reticulum as well, by retrieval of endoplasmic reticulum membrane proteins that carry the KDEL or KXXX targeting signal on their cytoplasmic tails (Letourneau et al. 1994). These C-terminal amino acid sequences target proteins to the endoplasmic reticulum and interact with receptors that are postulated to guide the return of proteins from the Golgi apparatus to the endoplasmic reticulum.

Evidence for coatamer-mediated vesicle transport is strongest for the endoplasmic reticulum to the Golgi apparatus step, but a different machinery may be used for delivery from the TGN to the apical plasma membrane (Ikonen et al. 1995). The expression of a recombinant SNAP
in Madin-Darby canine kidney cells stimulated transport from the Golgi apparatus to the basolateral plasma membrane. Additionally, antibodies to NSF and a neurotoxin that cleaves vesicle-associated SNAREs inhibited transport (Ikonen et al. 1995). However, the recombinant SNAP, the anti-NSF antibodies, and the neurotoxin all did not affect transport from the TGN to the apical plasma membrane.

In the absence of convincing evidence for the existence and participation of free shuttle vesicles in the putative intra–Golgi apparatus trafficking process, we suggest that there is no compelling argument in the primary research literature for preferring the vesicle shuffle model of intra–Golgi apparatus transport over the flow differentiation model. In fact, the widespread acceptance of the vesicle shuffle model may have been premature. To accept this model as a valid working hypothesis would require clear demonstration that intermediate shuttle vesicles really do exist as free vehicles and not simply as coated buds or coated ends of tubules. The isolation of the vesicles under conditions that would not fragment tubules, and the direct demonstration of the ability of the isolated vesicles to convey materials among Golgi apparatus cisternae, would be required as well. Such conditions have been fulfilled for the transition vesicles that bud from endoplasmic reticulum to deliver materials to the cis–Golgi apparatus. Here the existence of a vesicular intermediate is much clearer.

Vesicle budding and membrane displacement

In addition to the many studies that establish the operation of transition vesicles between endoplasmic reticulum and Golgi apparatus, progress continues, as well, to understand the processes by which the vesicles form, dissociate from donor endoplasmic reticulum, and acquire coat proteins before migrating to an acceptor compartment at the cis–Golgi apparatus. Formation of transition vesicles requires ATP, and vesicle formation is exquisitely sensitive to N-ethylmaleimide and other thiol-alkylating reagents and is modulated by GTP and monomeric GTP-binding proteins (Balch 1989, Warren et al. 1988). The major ATPase of transitional endoplasmic reticulum of rat liver belongs to the valosin-containing, or AAA, family of ATPases (Zhang et al. 1995). Antisera against this ATPase specifically inhibited ATP-dependent vesicle budding from transitional endoplasmic reticulum in a cell-free system, thus providing direct evidence of a requirement for this ATPase for transition vesicle formation. The reverse pathway, cis–Golgi apparatus to endoplasmic reticulum, remains to be duplicated convincingly in a cell-free system.

There is abundant evidence for recycling from the plasma membrane to different internal endosomal compartments, especially during receptor-mediated endocytosis (e.g., Brown et al. 1983). However, the proportion of plasma membrane that returns to the Golgi apparatus may be small in proportion to total membrane flux through the Golgi apparatus (Thilo 1985). There also is uncertainty about the extent and mechanism of recycling of membrane from the cis–Golgi apparatus to the endoplasmic reticulum. The bulk of the membrane internalized from the plasma membrane during endocytosis appears to enter the endosomal–lysosomal pathway of digestive compartments and to intersect with the Golgi apparatus primarily at the TGN (Griffiths et al. 1989). The TGN may be composed, in part, of structures of endosomal or lysosomal origin. Yet, appreciable transfer of membrane from the TGN to the Golgi apparatus cisternae per se remains to be demonstrated.

The cisternal remnant—a raison d'être for TGN

According to the flow differentiation model of Golgi apparatus function, cisternae are lost at the distal Golgi apparatus pole by conversion into secretory vesicles. In plants, a residual structure, often appearing as a rigid tubular or fenestrated structure with associated clathrin-coated vesicles, is normally discharged periodically from the Golgi stack (Mollenhauer 1971). This remnant may correspond to the partially coated reticulum described by Pesacreta and Lucas (1984). Although the relationship between the cisternal remnant of plants and the TGN of animal cells still is speculative (Geuze and Morré 1990), they may be equivalent structures. Therefore, cisternal release from the distal pole of the Golgi apparatus may involve both the formation of partially or fully clathrin-coated exocytotic vesicles that migrate to the plasma membrane as well as retention of a cisternal remnant that eventually mixes with the endosomal–lysosomal system. This cisternal remnant mixing with the endosome–lysosome system is conceptually reminiscent of what was described originally as the Golgi apparatus–endoplasmic reticulum–lysosome complex (Novikoff et al. 1971), of which the TGN appears to be, at least in some instances, the terminological equivalent. In any event, the existence and functioning of the TGN would be consistent with both the discontinuous and continuous models of Golgi apparatus organization.

What primarily distinguishes the discontinuous and continuous models of membrane movement through the Golgi apparatus is the extent of participation of free shuttle vesicles that operate between or among adjacent Golgi apparatus compartments. Does formation and fusion of shuttle vesicles actually constitute the major route of transfer of membrane materials and/or secretory products among intermediate Golgi apparatus cisternae? Complex systems of peripheral tubules clearly occupy these Golgi regions, but shuttle vesicles appear to be less abundant or even absent. These and other arguments originally led to the development of the flow differentiation model of continuous Golgi apparatus functioning, and they seem to be as relevant today as they were when the model was first formulated.

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