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Protein Sorting by Transport Vesicles

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Eukaryotic life depends on the spatial and temporal organization of cellular membrane systems. Recent advances in understanding the machinery of vesicle transport have established general principles that underlie a broad variety of physiological processes, including cell surface growth, the biogenesis of distinct intracellular organelles, endocytosis, and the controlled release of hormones and neurotransmitters.

All eukaryotic cells contain numerous membrane-bounded compartments whose specialized functions require distinct protein compositions. These proteins originate predominantly in the endoplasmic reticulum (ER), and must then be sorted and distributed by transport vesicles (*1*) to their correct destinations (Fig. 1). How these compartments acquire the correct set of proteins is now understood in general terms based largely on recently discovered machinery responsible for formation, targeting, and fusion of transport vesicles. Aspects of these pathways also explain the related process of regulated exocytosis, by which hormones and neurotransmitters are released.

Any one vesicle shuttle allows travel between a pair of membrane-bounded compartments. One member of the pair (the donor compartment) produces the transport vesicle and the other (the acceptor compartment) receives the vesicle and its cargo. The set of all such shuttles constitutes the vesicle flow pattern in a cell. Individual proteins are transported within the pattern according to intrinsic signals that dictate their ability to enter or avoid the various shuttles (*2*). Transit via each shuttle typically takes one to several minutes and occurs by simple diffusion—unless the vesicle must travel a great distance across the cytoplasm to reach the acceptor compartment, in which instance its journey is speeded by movement along cytoskeletal fibers (*3*).

The core protein machinery that underlies vesicle transport includes coat proteins, which sculpt a vesicle out of a donor membrane (Fig. 2A); the vesicle- and target-specific identifiers v-SNAREs and t-SNAREs (vesicle- and target-specific SNAP receptors, respectively), which bind each other and thereby dock the vesicle to the acceptor membrane (Fig. 2B); and NSF (*N*-ethylmaleimide–sensitive fusion protein) and SNAP (soluble NSF attachment protein) proteins, which bind to the SNARE complex and initiate fusion when NSF hydrolyzes adenosine triphosphate (ATP) (Fig. 2C). Although additional pieces of machinery and important mechanistic details likely remain to be uncovered, enough has been learned for the general principles of vesicle transport to be clear in outline, as will be described in the following three sections.

Vesicle Budding

In all instances examined in detail, a coat is used as a mechanical device to bud off vesicles. Coats are spherical protein shells (analogous to virus capsids) of fixed composition, consisting of many copies of the same subunits, and are typically from 50 to 100 nm in diameter. Subunits are recruited from the cytosol to the donor membrane, where they are assembled stepwise, incrementally deforming the attached segment of membrane in the process. The vesicle is released encased in the coat, which is then discarded to allow the vesicle to fuse with the acceptor membrane (Fig. 2A). Four types of coats have been characterized—two clathrin coats and two COP coats (Table 1)—which are largely used by different organelles. This catalog of well-characterized coats will surely expand.

Budding is currently most fully understood for two distinct COP coats that form from ER (*4*) and Golgi cisternae (*5*). In both instances, a guanosine triphosphatase (GTPase) (ARF protein for COPI coats and the closely related SAR protein for COPII coats) controls the budding process: Guanosine triphosphate (GTP)–bound ARF (ARF[GTP]) (*6*, *7*) or SAR[GTP] (*4*) triggers coat assembly, whereas guanosine diphosphate (GDP)–bound ARF (ARF[GDP]) (*6*, *8*) or SAR[GDP] (*4*) triggers release of the coat. ARF and SAR proteins in the cytosol are bound to GDP. An enzyme localized to the donor compartment (*9*) catalyzes the exchange of GTP for GDP, and the resulting

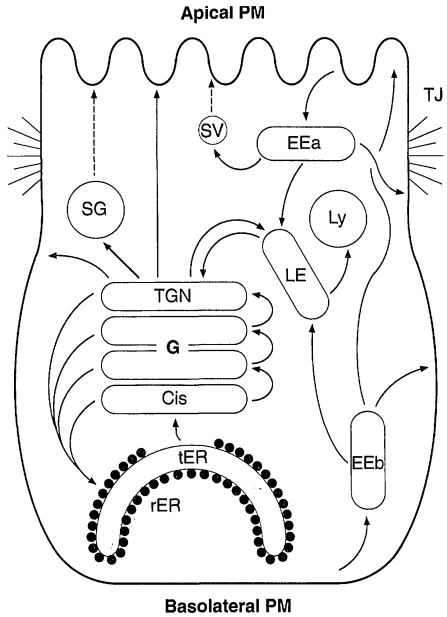

Fig. 1. Pattern of vesicle flow in a representative animal cell, with distinct apical and basolateral plasma membranes (PM), that is present in a sheet of cells connected by apical tight junctions (TJ). Each arrow represents a one-way vesicle shuttle that either has been directly demonstrated through identification of the carrier vesicle or can be clearly inferred to exist. Not all shuttles indicated will be present in all cell types, and additional shuttles are present in certain specialized cell types. Dashed lines refer to vesicle shuttles whose fusion step is known to be physiologically regulated in certain cell types, in which case the carrier vesicle is a metastable organelle. In one such instance, a secretory granule (SG) fuses with apical plasma membrane in the process of exocytosis, the main mechanism for the release of peptide hormones and proteins in endocrine and exocrine physiology. In neurons, the "dense core" vesicle, typically containing peptides, is closely equivalent to a secretory granule (*81*). Another such instance concerns transport from apical early endosomes (EEa) to apical plasma membrane. The metastable carrier vesicle in this case in a neuron is the synaptic vesicle (SV), containing nonpeptide neurotransmitters such as acetylcholine. Cell types that store a plasma membrane protein and deliver it only after a physiological stimulus use a close equivalent of a synaptic vesicle for this purpose. For example, muscle and fat cells store glucose transporters in such vesicles, which fuse with the plasma membrane in response to insulin stimulation (*82*). Ly, lysosome; LE, late endosome; EEb, basolateral early endosome; rER, rough ER with attached ribosomes; tER, transitional ER with few if any ribosomes; G, Golgi stack with cis (entry) and trans (exit) faces; TGN, trans-Golgi network, a special term for the trans-most (exit) cisterna of the stack. Organelles whose contents are not known to be delivered by vesicle shuttles (mitochondria, chloroplasts, nucleus, and peroxisomes) are not shown.

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ARF[GTP] or SAR[GTP] molecules then bind to the membrane (*10*), which remains flat (*11*). Next, cytosolic coat subunits (*12*) bind to the membrane-bound ARF[GTP] or SAR[GTP] and assemble together into spherical coats, budding off vesicles in the process (*4*, *6*, *7*).

After budding, the bound GTP is hydrolyzed, causing ARF or SAR to dissociate and leave behind an unstable coat, which then itself dissociates in a reversal of the coat assembly process (*4*, *8*). Although additional factors may be involved, it is likely that GTP hydrolysis is triggered when the ARF or SAR proteins contact one of the coat proteins in the coat (*13*); the rate of stimulated hydrolysis appears to be sufficiently slow to allow budding to occur before much GTP is hydrolyzed.

The clathrin coats are less well understood but appear to bud according to a generally similar mechanism. Indeed, it was the discovery of the regular polyhedral structure of these coats on purified vesicles (still not evident in electron micrographs of COP coats) that led to the seminal suggestion 27 years ago (*14*) that budding is driven by the stepwise formation of an adherent spherical coat. This concept was supported by the simple composition of these coats (*15*), and the intrinsic capacity of these coats to self-assemble in the absence of membranes (*16*).

ARF is also required for the assembly of clathrin coats on membranes (*17*). In contrast to COP coats, clathrin coats are intrinsically stable (*16*) and thus can persist after ARF[GDP] dissociates. An additional uncoating mechanism is therefore required, which includes a specialized DNA J homolog and an uncoating 70-kD heat shock protein adenosine triphosphatase (ATPase) that act cooperatively (*18*).

Important aspects of budding mechanisms remain obscure. Little is known of the membrane molecules to which ARF and coat proteins attach. However, linkage of the coat to cargo, receptors for cargo, and docking machinery components such as v-SNARES must occur to ensure that these molecules are packaged, and may be important for polymerization of the coat and for targeting the correct coat subunits to the correct membrane. Substantial but indirect evidence also indicates that phosphatidylinositol derivatives may be required for budding (*19*). It is possible that coat proteins attach noncovalently to polyphosphoinositides or phosphatidic acid (*20*), in addition to ARF (or SAR), in a multivalent interaction—which could allow the rate of vesicle budding to be regulated according to signaling pathways, or provide a means by which a vesicle could select its own specific lipid composition.

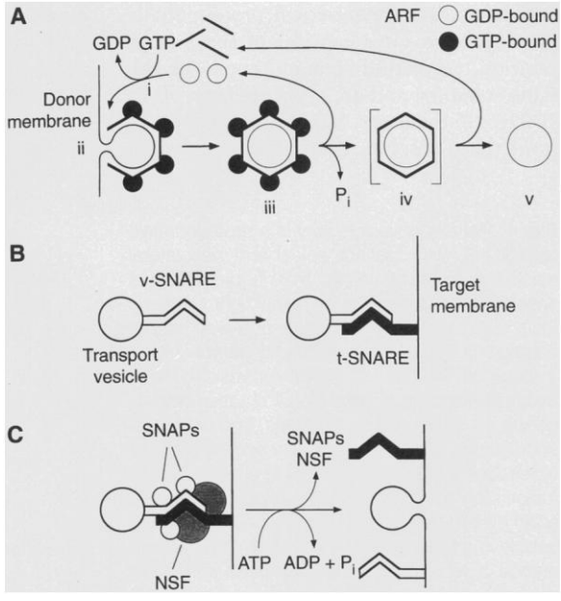

Fig. 2. Stages and components of a vesicle shuttle (see text for details). (**A**) Vesicle budding driven by the progressive assembly from cytosolic subunits of a protein shell termed a coat, resulting in the sculpting of a vesicle out of the donor membrane. Step (i): Assembly is triggered when the GTP-binding protein ARF (or a similar protein; there are multiple species of ARFs and related GTP-binding proteins) is activated by binding GTP. Cytosolic ARF is bound to GDP, and the exchange for GTP is a catalyzed process. The resulting ARF[GTP] binds to the donor membrane and then recruits coat proteins (line segments) to assemble the coat, resulting in the formation of a bud. (ii) The bud pinches off in a process termed periplasmic fusion, which, for COPI-coated vesicles, Ostermann *et al.* (*7*) showed requires fatty acyl coenzyme A, and for clathrin coats requires the GTPase dynamin (*83*). (iii) When the bound GTP is hydrolyzed, liberating inorganic phosphate (P_i), ARF dissociates to yield a still-coated vesicle (iv) that is less stable (indicated by brackets). In some instances (COPI-coated vesicles), the remaining coat proteins dissociate immediately after ARF, so that the ARF-free coated vesicle is not ordinarily observed. In other instances (clathrin-coated vesicles), the coat proteins are more stably associated, and the ARF-free coated vesicle becomes the most prominent species. The remaining coat proteins are then rapidly dissociated or are removed to yield the product of the pathway, the uncoated and fully formed transport vesicle (v). (**B**) Targeting of vesicles. Uncoating exposes the vesicle's identifiers, termed v-SNAREs, which can now bind to cognate t-SNAREs present on the targeted acceptor membrane, resulting in the formation of a SNARE complex. v-SNAREs are a family of proteins with structural similarity to the synaptic vesicle protein VAMP (synaptobrevin) (*84*). t-SNARES generally comprise two types of subunits; one is a homolog of the neuronal plasma membrane protein syntaxin (*85*), the other is related to the synaptic protein SNAP-25 (*86*) (no relation to the fusion protein SNAP, see below). (**C**) Fusion mediated by NSF and SNAPs. SNAP proteins (of which there are at least three subtypes) bind to the SNARE (SNAP receptor) complex at the attachment site of the vesicle and its target. Hydrolysis of ATP by NSF disrupts the SNARE complex and initiates membrane fusion. Whether additional proteins are required for complete bilayer fusion is not known.

Docking and Fusion

Specific docking of a transport vesicle at the intended acceptor (target) compartment requires that it be endowed with distinct "pilot" proteins to encode its destination. Corresponding receptors must exist on target membranes to capture the vesicles.

The class of cytoplasmically oriented integral membrane proteins termed SNAREs (*21*) appears to fulfill these functions. There are two categories of SNAREs, each consisting of broad families of related proteins: v-SNAREs are localized to transport vesicles (and their donor membranes) and have the properties predicted for pilot proteins, whereas t-SNAREs are localized to the target membranes and have the properties predicted for vesicle receptors (*22*, *23*). The cytoplasmic domains of both v- and t-SNARE proteins have extended regions predicted to form α-helical coiled coils, and the compartment-specific pairing of v-SNAREs with t-SNAREs that mediates the docking of vesicles (Fig. 2B) involves these regions (*24*).

The role of SNAREs in compartment-specific docking is most fully understood for the distinct shuttles that connect the ER to the Golgi and the Golgi to the plasma membrane (*22*). The ER and the Golgi each express their own species of v-SNAREs that are efficiently packaged into departing transport vesicles during budding. Mutation of these v-SNAREs prevents docking of the corresponding transport vesicles in cells. The Golgi and plasma membrane also express specific t-SNAREs required for docking of the ER-derived and Golgi-derived transport vesicles, respectively. The v- and t-SNARE proteins specialized for the ER-Golgi interface assemble into a stable, stoichiometric complex that accumulates in cells when fusion is blocked in an NSF mutant. The complex is free of the v- and t-SNAREs specialized for the Golgi–plasma membrane interface, illustrating the high degree of specificity and lack of promiscuity of v-SNARE–t-SNARE interactions (*23*). The latter SNAREs form a distinct complex that docks Golgi-derived vesicles to the plasma membrane (*25*).

A similar but more complex situation is apparent at nerve endings, where synaptic vesicles, which store neurotransmitters, are docked to the presynaptic plasma mem-

brane before Ca^{2+}-activated fusion. The synaptic vesicle v-SNARE (VAMP/synaptobrevin) specifically binds to the two-subunit plasma membrane t-SNARE (syntaxin and SNAP-25) to form a stable, trimeric complex in vitro (*26*). However, although deletion of either VAMP or syntaxin genes prevents transmitter release, it does not prevent docking of synaptic vesicles, which accumulate at the presynaptic plasma membrane (*27*). Most likely, these vesicles remain docked by the binding of a second v-SNARE, synaptotagmin, to the t-SNARE subunit SNAP-25 (*28*).

Although v- and t-SNAREs assemble spontaneously in vitro, their assembly in cells is critically influenced (in a manner that is still obscure) by other families of proteins that may act by controlling the exposure of SNAREs to each other. In so doing, Rab and Sec1 proteins may confer an additional level of specificity. Rab proteins (*29*) are GTPases that allow assembly of SNARE complexes (*23, 25*), perhaps proofreading them for accuracy; Sec1 proteins (*30*) bind to specific t-SNARE subunits and can prevent their assembly (*31*). Because both Rab and Sec1 proteins cycle on and off membranes, neither can encode primary information for specificity in docking.

In sum, SNARE proteins are central for compartment-specific docking, and have the correct properties to encode specificity. Whether SNARE proteins are the sole determinants of vesicle targeting to membranes is not known, and is a proposition that would be difficult to establish formally. Additional docking mechanisms may exist, and SNAREs may play an additional role in lipid bilayer fusion after docking.

General fusion machinery derived from the cytosol can function at many compartments to help fuse specifically docked vesicles without compromising specificity. For this purpose, SNAP proteins (*32*) and the ATPase NSF (*33*) assemble at docking sites [some fusion processes may be mediated by homologs of NSF (*34*)]. Each v-SNARE–t-SNARE complex binds between three and six SNAP proteins and then binds NSF (*26, 35*). NSF hydrolyzes ATP and uses the energy made available to disrupt the docking site by dissociating the coiled-coils binding v-SNAREs to t-SNAREs and releasing SNAP proteins (*26*). Although the precise role of this reaction is not known, it is required for the process of fusion after vesicle docking. It is likely that this process alters the conformation of one or more of the participating proteins, because disrupted complexes do not efficiently reassemble (*36*). Presumably, a state is thereby created that represents an irreversible step toward bilayer fusion.

Whether the physical fusion of bilayers follows the NSF ATPase reaction as the direct result of the action of conformationally switched SNAP or SNARE proteins or both (without the involvement of additional proteins) is not known. However, this is the simplest possibility, by analogy to viral envelope fusion proteins, which are activated for fusion by proton-triggered disruption of quaternary structure and an accompanying conformational switch involving coiled coils (*37*).

As for SNARE proteins, the expectation that NSF and SNAP proteins are required for the Ca^{2+}-regulated fusion (exocytosis) of synaptic vesicles in the release of neurotransmitters has been confirmed in vivo (*38*). Hormones, growth factors, and other mediators stored in similar vesicles can be assumed to be released by similar mechanisms. How the general fusion machinery is held in check until a signal for exocytosis is received is unclear, but it almost certainly involves the assembly of a stable complex with the Ca^{2+}-binding protein synaptotagmin (*39*), introduced above as a v-SNARE. This complex would form after ATP is hydrolyzed but before bilayer fusion is complete (*40*). Binding of Ca^{2+} to synaptotagmin might be the switch that allows fusion to proceed to a rapid completion. Synaptotagmin has been shown to bind the brain-specific β isoform of SNAP protein (but not α-SNAP, the general isoform) (*28*), linking this Ca^{2+} receptor to the known fusion machinery.

Table 1. Coat proteins of vesicle shuttles. The four well-characterized types of coated vesicles are indicated. Evidence suggests the existence of other types of coats that function in a similar manner. SAR is closely related to ARF. TGN, trans-Golgi network. See (*4–18*).

Type of coated vesicle	Subunits of coat	GTPase	Origin-destination
AP-1 clathrin	Clathrin, AP-1 adapter	ARF	TGN-prelysosomes
AP-2 clathrin	Clathrin, AP-2 adapter	ARF?	Plasma membrane–endosomes
COPI	Coatomer	ARF	ER-Golgi; bidirectional within Golgi; Golgi-ER
COPII	COPII proteins	SAR	ER-Golgi

Selection of Cargo: Protein Sorting

A structural feature of a given protein that is responsible for the choice among potential fates (entering or not entering a given vesicle shuttle) is termed a sorting signal. Such signals are most often discrete peptide domains of 4 to 25 residues (Table 2), but may also be conformationally determined epitopes (*41*). A given protein can have multiple sorting signals, each specifying the fate of that protein at successive stages and collectively determining its itinerary.

Because sorting signals must specify either movement or lack of movement, there are only three potential fates for a given protein with respect to a given type of transport vesicle budding from a given compartment:

Table 2. Examples of known transport signals. An additional layer of complexity may be introduced by the fact that some signals overlap, are operational in combinations, or show reversible posttranslational modifications. K, Lys; D, Asp; E, Glu; L, Leu; R, Arg; Y, Tyr; Q, Gln; N, Asn; P, Pro; X, any amino acid. GPI, glycosylphosphatidylinositol. See (*79, 80*).

Signal	Location in protein and with respect to membrane	Fate specified
KDEL	COOH-terminus, luminal	Retrieval of proteins from Golgi to ER
KKXX	COOH-terminus, in cytoplasm	Retrieval of membrane proteins from Golgi to ER
XXRR	NH_2-terminus, in cytoplasm	Retrieval of membrane proteins from Golgi to ER
Propeptide	NH_2-terminus, luminal	Transport from Golgi to endosomes or lysosomes
Mannose 6-phosphate	Asn-linked saccharides, luminal	Transport from Golgi to endosomes or lysosomes
Tyrosine-rich dileucine	Cytoplasmic domain	Transport from Golgi to endosomes or lysosomes
YQRL	Cytoplasmic domain	Transport from cell surface to Golgi
NPXY (and similar)	Cytoplasmic domain	Transport from cell surface to endosomes
GPI anchor	COOH-terminus, luminal	Transport from Golgi to apical cell surface in polarized cells

1) The protein is concentrated in a departing vesicle as cargo if it has a sorting signal—termed a transport signal—that specifies movement (Fig. 3A). Transport signals function in one of two closely related ways depending on the side of the donor membrane on which the signal resides. Transport signals on the cytoplasmic side (in membrane-bound cargo) bind directly to a coat protein (*42*). Transport signals on the luminal side (as in secretory proteins or proteins taken up from outside a cell by endocytosis) bind to the luminal domain of an intermediary protein—a specialized transmembrane cargo receptor—whose short cytoplasmic domain in turn contains a transport signal that binds to the coat. The classic example of a transmembrane cargo receptor is the low density lipoprotein (LDL) receptor, which is packaged into clathrin-coated vesicles that bud from the plasma membrane and carries LDL particles into the cell (*43*).

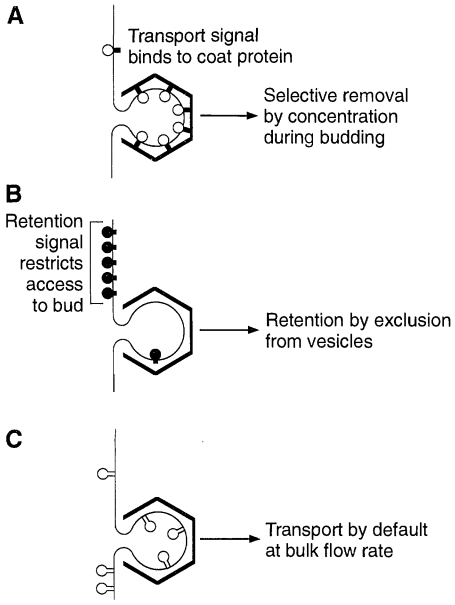

Fig. 3. The three possible fates of a given protein with respect to a given vesicle shuttle departing a given location, as determined by which particular signals the protein possesses. A single protein can contain multiple transport signals that are sequentially executed in successive compartments (Fig. 1), allowing complex itineraries to be planned. (**A**) If the protein has a transport signal that binds to a given coat directly or indirectly, then that protein will become concentrated in vesicles whose budding is mediated by that coat, and will be efficiently transported out at a concentration above its prevailing, bulk value. (**B**) If the protein instead has a retention signal that triggers (bracket) patching of the protein or its partitioning into a separate domain of the lipid bilayer, access to the vesicle will be physically restricted. Such retention is inefficient, because slow leakage of the protein into departing vesicles (and thus downstream compartments) occurs (indicated by the sole protein in the bud). (**C**) If a protein does not contain a signal, it diffuses into buds until it reaches its prevailing concentration, and it will then be transported out by default at this bulk concentration, in a process termed bulk flow.

The simple concept that the coat (directly or indirectly) selects cargo can explain why cells have multiple types of coats. A single type of coat could, in principle, suffice for the mere act of budding vesicles from all compartments, but then cargo would have to be selected in the same manner everywhere; by definition, protein sorting could not then occur. A repertoire of coats used differentially by the various compartments (Table 1) allows different cargo selection rules in different locations, and thus different itineraries for different proteins.

2) The protein is restricted from entering a departing vesicle by a sorting signal—termed a retention signal—that specifies lack of movement (Fig. 3B). Retention signals are compartment specific, with distinct, tranplantable retention signals specifying ER versus cis-, medial-, and trans-Golgi compartments. Retention signals are generally (but not always) localized to membrane-spanning segments of integral membrane proteins (*44*). The mechanism of retention is unknown, but in the case of membrane proteins is thought to involve either the formation of patches or aggregates of retained proteins that are too large to enter a vesicle (*41*), or the selective partitioning of retained proteins into the favored compartment based on an energetically favorable match between their membrane-spanning segments and the lipid composition of that compartment (*45*). Retention of soluble proteins in the lumen of the ER, by definition, cannot occur by lipid partitioning.

3) The protein lacks both transport and retention signals and enters a budding vesicle at its prevailing (bulk) concentration in the donor compartment (Fig. 3C). Such a default pathway for proteins that lack signals is termed bulk flow (*46*). Transport of cargo across the Golgi stack appears to occur by bulk flow (*47*).

In summary, the overall framework of vesicle budding, cargo selection, docking, and fusion is well established. However, the detailed manner in which these principles apply is far from understood in many instances.

Combinatorial Code for Export from ER to Golgi?

Transport between the ER and the Golgi as well as the functional organization of the Golgi stack itself are subjects of intense investigation because of the central role of these organelles in the propagation of the three-dimensional organization of the cytoplasm. The remainder of this article will therefore focus in a more detailed and speculative manner on this topic.

Whether cargo is carried from ER to Golgi by transport signals (Fig. 3A) or by bulk flow (Fig. 3C) has long been debated, but recent experiments (*4*, *48*) have revealed that a variety of cargo molecules in transit from ER to Golgi are concentrated in COPII-coated vesicles during their budding from the ER, indicating that these molecules possess transport signals.

One reason that this basic point has been so difficult to establish is that experiments in the mid-1980s indicated that artificially translocated proteins with little or no possibility of having a transport signal (*49*), and even glycopeptides (*46*), are rapidly transported, consistent with a bulk flow mechanism. However, the quantitative aspects of these experiments, when reinterpreted in light of the time now known to be required for proteins to fold in the ER after translocation, imply that many folded proteins exit the ER in vesicles at 5 to 10 times their bulk concentration (*50*). Although it is unlikely to be the major mechanism, bulk flow out of the ER may be physiologically important for some proteins or act as a backup mechanism for others.

Another reason for this uncertainty is that, despite two decades of intensive effort, not one clear-cut, transplantable transport signal for ER export has been identified. Why has this been such a problem, when analogous signals have been identified for many other transport steps (Table 2)? The obvious explanation is simply that export from the ER is not mediated by a single signal that binds with high affinity to a single receptor but rather relies on a combination of less efficient signals that must bind simultaneously to multiple low-affinity receptors. Any one element of this combined signal would not suffice for export, explaining the failure to detect such signals in transplantation experiments.

Why might such a combinatorial code be necessary in the special case of export from the ER, in contrast to the other known cases (Table 2)? Proteins must fold up after translocation across the membrane of the ER, and a mechanism termed quality control ensures that folding is completed before export to the Golgi can occur (*51*). Precisely how this works is unknown, but freshly translocated proteins continue to interact with the chaperone system that facilitates their folding until folding is complete. However, it is also possible that a positive selection of completely folded proteins by cargo receptors of transport vesicles, operating in tandem with chaperone-mediated retention of incompletely folded proteins, is required to ensure quality control.

Transport signals consisting of conformationally determined epitopes would enable folded proteins to be preferentially selected as cargo. Because most proteins are

composed of multiple folding units, each such unit would, in general, need its own epitope; only when the protein is completely folded would all epitopes be present. Selective packaging of fully folded proteins would occur if simultaneous binding of most or all of the assembled epitopes to cognate "epitope receptors" were needed to achieve high-affinity, multivalent attachment to budding vesicles, which would be the case if the epitopes, individually, have low affinity for their receptors.

Such a combinatorial coding mechanism for export need only use a relatively small number of species of epitope receptors to enable the selective export of proteins from the ER. Cargo would be delivered vectorially from the ER to the Golgi—despite the fact that epitope receptors must themselves be returned to the ER—if the various epitope receptors differed in their efficiency of retrograde transport, so that the sequential cisternae of the Golgi stack would gradually break up the combinations of receptors used for export out of the ER (by use of the distillation principle, discussed below).

If combinatorial coding is used, epitope receptors will be difficult to detect by binding assays or by genetic approaches because of their low affinity and possible redundancy. On the other hand, epitope receptors as a group should be major proteins of COP-coated vesicles budding from ER and Golgi.

Recently, a family of more than a dozen transmembrane proteins has been recognized (*51*), with members found in both COPI- and COPII-coated vesicles (*51*, *52*). These p24 proteins are excellent candidates for cargo receptors. They have a highly variable NH_2-terminal domain and a more conserved, membrane-proximal domain with a predicted propensity to form coiled coils in the vesicle lumen, suggestive of a receptor domain mounted on a stalk. The short, COOH-terminal cytoplasmic domains of p24 proteins bind to coatomer, the subunit of the COPI coat (*53*). Deletion of a p24 protein found in COPII-coated vesicles did not affect cell viability, but selectively slowed both vesicle budding and the export of some (but not all) species of cargo tested (*51*, *52*), as would be expected for a combinatorial code. Further studies are warranted to explore whether these proteins may be the missing link in understanding export from the ER (and possibly also cargo transport across the Golgi). Establishing whether there is a combinatorial code for export awaits the identification of specific epitopes recognized for transport.

Retrograde Transport

The ER and Golgi compartments retain their own distinct sets of proteins, as needed for their distinct functions. Residency is not permanent, however, but is the overall result of retention (Fig. 3B) and a process—termed retrograde transport—of selective retrieval by vesicles that compensates for the fact that retention is not completely efficient. Integral membrane proteins of ER and Golgi, as well as soluble proteins in the lumen of the ER, all escape to other compartments in departing transport vesicles (Fig. 3B) many times during their lifetimes, and are then subject to retrograde transport (*54*, *55*). Inefficient retention of membrane proteins is expected in the lipid partitioning model, because preference for one lipid phase over another is unlikely to be absolute.

Luminal residents of the ER characteristically bear the sequence KDEL (Lys-Asp-Glu-Leu) at their COOH-terminus (*56*), which constitutes the transport signal for retrieval from the Golgi to the ER by a cargo receptor (the KDEL receptor) (*57*). Eliminating the KDEL signal from these proteins results in their slow secretion (*54*), confirming that they also have effective, but imperfect, retention signals.

A transport signal for retrograde movement of membrane proteins of the ER is a cytoplasmically disposed tetrapeptide that contains two basic residues, such as KKXX at the COOH-terminus (*58*) or XXRR at the amino-terminus (*59*) (R, Arg; X, virtually any amino acid).

Indirect but substantial evidence suggests that COPI-coated vesicles are responsible for retrieving escaped ER residents from the Golgi. Coatomer binds to KKXX and related peptides, and certain mutations in coatomer selectively reduce retrograde transport of membrane proteins with KKXX at the COOH-terminus—but only under partially restrictive conditions that do not eliminate anterograde transport of these same proteins to the cell surface (*60*).

Direct morphological evidence (*5*, *61*, *62*) as well as genetic (*63*) and biochemical data (*61*, *64*) also suggest that COPI-coated vesicles carry cargo in the anterograde direction. Furthermore, it was recently shown that COPI-coated vesicles can bud from the ER, carrying different cargo than COPII-coated vesicles, suggesting that these two types of coated vesicles constitute parallel and synergistic anterograde pathways for export from ER (*48*).

Taken together, the existing data thus imply that COPI-coated vesicles are responsible for steps in both anterograde and retrograde transport in the ER-Golgi system. In light of the principle that the coat selects cargo, how could a single species of coatomer-containing COPI coat mediate bidirectional transport yet still allow protein sorting in the Golgi stack, sending (for example) ER resident proteins backward and plasma membrane precursors forward?

One possibility is that the coatomer, with its seven subunits, is a conformationally complex protein machine, and that different conformational states are used for retrograde and anterograde transport (*53*). In such a mechanism, cargo and v-SNAREs would have to switch the conformation of the coat according to their destinations. Some form of conformational switching of the KKXX-binding site must occur to explain how anterograde coated vesicles budding from the ER (*48*) avoid draining the ER of its many KKXX-bearing residents. It is also possible that the coatomers that participate in retrograde transport differ in subunit composition or in posttranslational modifications from those that mediate anterograde transport (*65*).

Possible Flow Patterns in the Golgi Stack

A combination of morphological (*66*, *67*), biochemical (*68*), and genetic (*69*) evidence implies that anterograde traffic flows sequentially from cisterna to cisterna, from the cis to the trans face, across the Golgi stack (Fig. 4). Although the exact number of vesicle transfers required to traverse these compartments remains unclear (*70*), this basic concept is well established.

The pattern of retrograde flow in the stack is less clear. In this regard, it may be useful to distinguish the retrieval of escaped

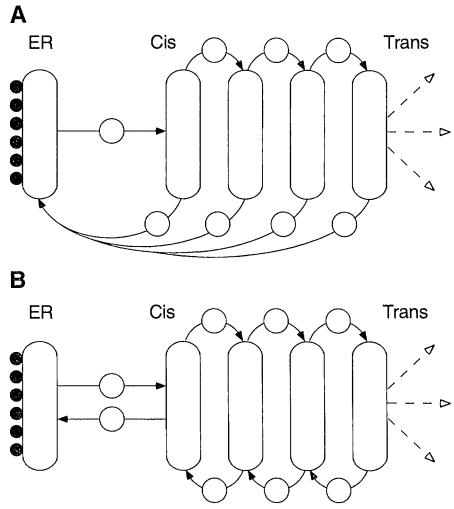

Fig. 4. Two likely membrane flow patterns in the Golgi stack. (**A**) Crosscurrent flow, a variation of countercurrent flow (*74*), may be used in the Golgi stack for multistage retrieval of ER resident proteins and hence their separation from exported proteins. (**B**) Countercurrent flow may be used by the Golgi stack to distribute residents among its own compartments. Each solid arrow represents a vesicle transfer step (anterograde arrows to the right, retrograde arrows to the left). The dashed arrows indicate multiple species of vesicles exiting the trans face of the Golgi stack for destinations such as the plasma membrane, endosomes, and secretory granules (Fig. 1).

resident proteins from another type of retrograde transport, termed recycling, that is necessary for every donor compartment to retrieve components of the transport machinery, such as v-SNAREs and cargo receptors, from acceptor compartments. The vesicles responsible for recycling transport machinery components have not been identified, and it is therefore not known whether these proteins are recycled in the same COPI-like vesicles that may retrieve escaped ER residents.

Although native residents of the ER are almost always retrieved from the first Golgi (post-ER) compartment and penetrate no further than this into the stack (*54, 71*), the capacity to retrieve escaped ER residents exists at every level of the Golgi stack. The KDEL receptor is present in all cisternae (*72*), and KDEL-containing peptides introduced into the trans-most cisterna can be returned to the ER via the KDEL receptor (*73*). Artificially constructed ER residents containing a KKXX signal but lacking a retention signal penetrate to trans-Golgi cisternae before returning to the ER (*55*).

Does this retrograde traffic go directly back to the ER from every level of the Golgi stack, a flow pattern termed crosscurrent (Fig. 4A)? Or must a KDEL-tagged protein being retrieved from a distal cisterna percolate backward, layer by layer, until it finally reaches the first cisterna, and only then enter a retrograde vesicle that can fuse with the ER—a flow pattern termed countercurrent (Fig. 4B)?

Although crosscurrent flow is apparently the more efficient choice for the retrieval of escaped ER proteins, it would appear to be an inefficient manner in which to recycle transport machinery components from acceptor to donor compartments within the Golgi stack; crosscurrent flow would force these Golgi residents back to the ER every time they are used. In contrast, countercurrent flow would allow the recycling of Golgi residents with the minimum number of steps. Indeed, recent evidence (*69*) strongly implies that countercurrent flow of v-SNAREs occurs within the confines of the Golgi stack.

In sum, it is possible that crosscurrent and countercurrent flow patterns may coexist in the Golgi stack, serving different purposes for different types of proteins.

Golgi Cisternae: Plates in a Distillation Tower?

The capacity to retrieve escaped ER proteins at every level of the Golgi stack (*55, 72, 73*) implies that cells possess a multistage recapture mechanism. If a protein avoids retrieval in the first cisterna, it can then be captured in the next one, and so on. This principle is precisely that of distillation, the method used by chemists to separate the components of a liquid mixture, in which the same fractionation step is applied over and over again to increase the efficiency of separation in an exponential manner (*74*). Liquid and vapor phases are repeatedly and sequentially equilibrated at each of the stacked plates that constitute a distillation tower. Vapor enriched in more volatile components rises to the plate above, whereas condensed liquid enriched in less volatile components drips downward, either to the previous plate (countercurrent method) or directly to a reservoir at the bottom of the tower (crosscurrent method).

With either of the two flow patterns discussed (Fig. 4), the Golgi stack would be operating as a distillation tower. The cisternae would function as the plates, anterograde-directed transport vesicles as the rising vapors, and retrograde-directed transport vesicles as the falling condensates. The cisternae, like plates, would merely serve as passive way stations in which the differentially selected contents of the two oppositely directed mobile phases (anterograde- and retrograde-moving vesicles) can mix and then be separated again.

Although the demonstrated capacity for multistage retrieval of ER proteins makes it clear that the Golgi has the potential to "distill" proteins, unless it can be shown that flow patterns such as those shown in Fig. 4 are used physiologically for the traffic of native proteins, distillation can only remain an appealing analogy.

The hallmark of a distillation mechanism is its immediate product: steady-state concentration gradients of the protein species that are distilled. A protein preferentially selected by anterograde-directed vesicles (and that does not leave the stack) will be concentrated toward the trans face in a gradient whose steepness is determined by the relative probabilities of its entering anterograde-directed versus retrograde-directed vesicles. Proteins preferentially selected by retrograde-directed vesicles will be concentrated at the cis face, with decreasing concentrations in each successive cisterna. For example, a twofold preference over five cisternae will generate a 32-fold (2^5) gradient in concentration across the stack.

The KDEL receptor is distributed in a gradient across the Golgi stack, being most concentrated at the cis face (*72*). Such a distribution is predicted by the distillation hypothesis: By its very nature as a cycling retrograde cargo receptor, the KDEL receptor must be able to enter both anterograde-directed and retrograde-directed vesicles but to prefer the latter—which establishes the principle that the Golgi can distill its own residents (for all practical purposes the KDEL receptor is a Golgi resident because little is present in the ER at steady state). Recent evidence suggests that cis- and medial-Golgi residents are in a rapid, dynamic equilibrium with the trans-Golgi (*75*), even though they remain at their highest steady-state concentration in the cis-Golgi—implying that they, too, are distributed in a gradient concentration across the stack. Thus, it appears that distillation is a primary mechanism by which membrane proteins are localized within the stack. It is possible that even components of the transport machinery (such as SNAREs) that defines the internal compartment boundaries of the Golgi stack may be localized by distillation.

Although it is readily apparent how distillation can concentrate protein species at either end of the stack, the situation with Golgi residents, such as glycosyltransferases, and possibly SNAREs, that localize to central cisternae is more complex. These proteins are typically distributed in a series of overlapping peaks across the stack, each occupying mainly two cisternae (*67*), and generally have transmembrane, hydrophobic segments that function as retention signals, conferring this pattern of localization (*44*). Such intermediate peaks cannot be explained solely by distillation, which can only concentrate proteins toward one face or the other, but can be explained by an interplay of the retention signals with distillation. For example, a lipid compositional gradient across the stack could be established either directly by distilling the lipids themselves (*45*) or indirectly by distilling various lipid biosynthetic enzymes toward either end of the stack. Intermediate peaks could result from the selective partitioning of different hydrophobic segments into those cisternae that contain a preferred lipid mixture; that is, according to the lipid partitioning model for retention (*45*). A gradient of cholesterol concentration likely exists (*76*). The binding of specific lipids, such as polyphosphoinositides, to coat proteins could direct coat assembly to compositionally distinct lipid bilayer domains and provide one of many possible mechanisms for vesicles to select nonrandon samples of lipid species to permit the establishment of lipid gradients by distillation.

As we learn more about the details of transport machinery and its localization in the Golgi stack, the tools will emerge to define precise flow patterns (Fig. 4) and to test the distillation hypothesis more definitely in the process. But, as we have indicated, evidence suggests that distillation may be a fundamental principle by which the Golgi operates, the overall selectivity of this organelle being the consequence of joining a series of less selective steps. We might even regard the ER as the first plate in this distillation tower, greatly expanded in surface area as compared with the other

plates in order to optimize its special role in importing proteins from the cytosol.

The distillation hypothesis was originally proposed 15 years ago to explain the then-postulated process of retrieval of escaped ER proteins by retrograde transport (*77*). Although direct evidence now exists for the distillation of Golgi residents, and ER residents are now known to escape and be retrieved as was predicted, missing is any demonstration of a declining concentration gradient of native ER proteins across the Golgi stack to indicate the physiological use of multistage retrieval. There is simply no evidence that retrieval of native ER proteins occurs beyond the first cisterna (*78*). There are many possible explanations. Perhaps the gradient is too steep to detect but is still important. Even traces of certain ER proteins in later compartments may be toxic, with multistage retrieval needed to prevent that trace accumulation. Alternatively, an unidentified subset of ER residents may not be efficiently retrieved from the first cisterna and the expected gradients would readily be detected if we knew which proteins to look for. It is unlikely that the Golgi of both yeast and animals contains all of the elaborate machinery necessary for retrieval of ER proteins throughout its stack and never once uses it.

Future Perspectives

Although the conceptual framework of vesicle transport and protein sorting now seems clear, the particular manner in which these principles are applied throughout the cell requires further study. To understand protein sorting fully, we will need to acquire a complete inventory of the cell's content of and the specificities of coat proteins, SNAREs, cargo receptors, and other participating proteins, and to learn to precisely which steps of transport each contributes. Only then will we know definitely how the transport pathways are integrated to serve cellular and organismal physiology, and obtain answers to such elementary questions as, why does a cell need a Golgi apparatus and why does it have the form of a stack? The possibility that the Golgi stack functions as a distillation tower remains an appealing (and still the only posited) explanation.

The very real prospect of obtaining a full inventory is both daunting, and exciting, and will no doubt challenge the field for some time to come. Fortunately, given that many examples of transport machinery components are already known, the process of discovering new or analogous proteins should be ever easier, especially with the soon-to-be-completed full genome sequence of yeast and the eventual completion of a mammalian genome. The necessary weapons—central concepts and appropriate techniques—are now in hand for a final assault to yield a complete picture of the flow of proteins in cells, including, for each new piece of transport machinery, molecular mechanism (with the use of biochemistry and structural biology), site of action (by demonstrating localization with microscopy), and confirmation of biological relevance (with genetic and physiological approaches).

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87. We apologize to many authors whose notable contributions could not be cited because of space limitations, restricting citations to the first or most definitive examples, or to reviews.

RESEARCH ARTICLE

Global Observations of Oceanic Rossby Waves

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Rossby waves play a critical role in the transient adjustment of ocean circulation to changes in large-scale atmospheric forcing. The TOPEX/POSEIDON satellite altimeter has detected Rossby waves throughout much of the world ocean from sea level signals with ≤10-centimeter amplitude and ≥500-kilometer wavelength. Outside of the tropics, Rossby waves are abruptly amplified by major topographic features. Analysis of 3 years of data reveals discrepancies between observed and theoretical Rossby wave phase speeds that indicate that the standard theory for free, linear Rossby waves is an incomplete description of the observed waves.

One of the major breakthroughs in the development of a theoretical understanding of the large-scale circulations of the ocean and atmosphere was Carl Gustave Rossby's discovery in the 1930s (*1*) of a special class of waves that owe their existence to the spherical shape of the Earth. These planetary Rossby waves (*2*) are easily observed in the atmosphere as the large meanders of the mid-latitude jet stream that are responsible for the prevailing seasonal weather patterns and their day-to-day variations. Rossby waves have been much more difficult to detect in the ocean because of their small sea-surface signature (height variations of order 10 cm or smaller), slow propagation speeds (of order 10 cm s^{-1} or less), and long wavelengths (hundreds to thousands of kilometers). We present here a summary of global observations of oceanic Rossby waves from the joint United States–French TOPEX/POSEIDON satellite altimeter mission and a comparison of the observations with predictions based on the standard theory for freely propagating, linear Rossby waves.

Rossby waves are central to all modern theories of large-scale ocean circulation. They are responsible for establishing the most fundamental feature of the large-scale circulation: the westward intensification of circulation gyres (*3*). In the North Atlantic, this is manifest as the intense Gulf Stream western boundary current. There are counterparts in the other major ocean basins. Rossby waves are also the dynamical mechanism for the transient adjustment of the ocean to changes in large-scale atmospheric forcing. In concert with coastal-trapped waves along the eastern boundary of an ocean basin, Rossby waves are a mechanism for transmitting information from the tropical ocean to the middle and high-latitude interior ocean (*4*). It has recently been suggested (*5*) that Rossby waves generated by El Niño events (*6*) may account for ocean circulation anomalies a decade later in the mid-latitude North Pacific. Such ocean changes might significantly influence weather patterns over North America.

Theoretical background. Rossby waves are the large-scale dynamical response of the ocean to wind forcing and buoyancy forcing (heating and cooling) at the eastern boundaries and over the ocean interior. They can also be generated by perturbations along the eastern boundaries associated with coastal-trapped waves originating at lower latitudes. Although it is possible for Rossby wave characteristics (amplitude and propagation speed) to be altered by wind or buoyancy forcing that is coherent with the wave at precise wave numbers and frequencies (*7*), there is no evidence at present to indicate that such resonance exists over the broad ranges of wave numbers and frequencies and the global geographical domain over which sea level signals with Rossby wave–like characteristics are observed in

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