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The Specificity of Vesicle Trafficking: Coat Proteins and SNAREs

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INTRODUCTION

Proteins that are destined for the secretory system usually begin their journey at the endoplasmic reticulum (ER), where proteins are translocated across the ER membrane into the lumen, before being selectively removed from the ER and packaged as cargo into transport vesicles bound for the stacks of the Golgi complex. Protein cargo then passes through the Golgi to the *trans*-Golgi network (TGN), where proteins destined for the vacuole (or, in the case of mammalian cells, the lysosome) are sorted away from cargo intended for secretion or for localization at the plasma membrane. This journey, from the ER to either the cell surface or the vacuole, is referred to as the anterograde pathway. Because protein transport through the endomembrane system is not a one-way trip, however, some vesicles also carry proteins in the reverse direction. This retrograde pathway is essential for the recovery of proteins that may have escaped from other endomembrane compartments and also for the recycling of the machinery involved in anterograde transport (see Battey et al., 1999, in this issue).

Other endomembrane compartments are found as intermediate locations between the well-known organelles. For example, cargo destined for the vacuole may first pass through a compartment between the TGN and the vacuole called the prevacuolar compartment (PVC). The PVC is sometimes called the "late endosome" (especially in mammalian cells) or just "endosome" (especially in yeast). Throughout this review, we use the term PVC for this compartment, reserving the term endosome for the initial sorting compartment of the endocytic pathway.

The first target for vesicles endocytosed from the cell surface is the endosome. Material within the endosome undergoes sorting such that cargo destined to return to the plasma membrane or continue toward the TGN is separated from cargo that will be degraded in the vacuole. The cargo intended for degradation first passes through the PVC, where it mixes with anterograde cargo that is also in transit to the vacuole. In this way, a single compartment can carry simultaneously both anterograde and retrograde cargo, indicating the intricate connections of the secretory system. A diagram of the compartments of the endomembrane system is shown in Figure 1.

With traffic moving in both directions between organelles, how a particular cargo is packaged into the correct transport vesicle and how this vesicle is able to differentiate the correct target membrane from among all the others are important questions. Selection and packaging of cargo at the donor organelle depend on various coat proteins that assemble onto the donor membrane surface and mechanically form the transport vesicle. However, it is not the coat proteins that determine the target of a transport vesicle; instead, this is the role of proteins called SNAREs, which separately reside on the vesicle and target membranes. Together, the coat proteins and the SNAREs coordinate the trafficking of the cargo between the various organelles of the endomembrane system.

CORE TRAFFICKING MACHINERY

Coat Proteins and Vesicle Formation

Transport vesicles are formed at donor organelles through the action of several distinct coat proteins. Each type of coat protein is made of specific "coatomers," and each is somewhat specific for a particular donor organelle, although some coats function at many different donor membranes. For example, a single type of coat, made from the COPII coatomer, is involved in forming vesicles at the ER, whereas a second coat, made from the COPI coatomer, forms vesicles from the Golgi complex (reviewed in Schekman and Orci, 1996). In contrast, many different types of coated vesicles are formed at the TGN, each with a distinct class of cargo destined for the vacuole, the PVC, or the cell surface. Retrograde transport, either from the cell surface, the endosome, or the PVC, also relies on coated vesicles.

Coatomer components generally are recruited to the surface of the donor membrane through the action of a small GTPase that, upon binding GTP, associates with membranes and directs the assembly of the coat. Coatomer assembly mechanically drives the formation of a membrane bud, which subsequently pinches off from the membrane to

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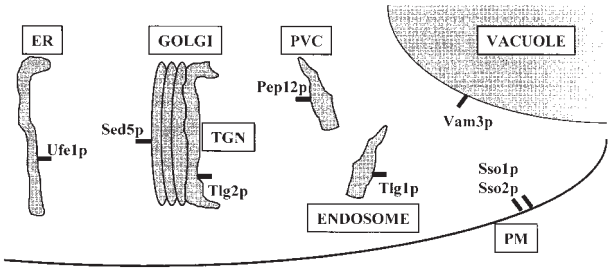

Figure 1. The Endomembrane System of Yeast.

The endomembrane system consists of seven major compartments: the endoplasmic reticulum (ER), the Golgi complex, the *trans*-Golgi network (TGN), the vacuole (or lysosome in mammals), the plasma membrane (PM), the prevacuolar compartment (PVC), and the endosome. In yeast, each of these compartments contains one or more t-SNAREs (thick bars), which function to receive cargo intended for that compartment.

form the transport vesicle. Cargo, which concentrates in the region of the membrane bud in a poorly understood manner, becomes incorporated into the lumen of the transport vesicle. Coated vesicles are not competent for fusion with the target compartments, so the coat must be depolymerized before fusion with the target membrane. This disassembly is believed to be stimulated by hydrolysis of GTP by the membrane-associated GTPase. The disassembly of the coat probably exposes the targeting machinery on the vesicle surface (i.e., the SNAREs) for subsequent delivery of the cargo to the appropriate organelle.

SNAREs and Vesicle Fusion

The term SNARE is used to describe two distinct families of integral membrane proteins that share structural motifs. One type of SNARE, the v-SNARE, is found on vesicle membranes. Members of the second family, the t-SNAREs, are found mainly on the target membrane. Both v- and t-SNAREs are anchored into their respective membranes by either a C-terminal hydrophobic domain or by post-translational attachment of lipids. Both types of SNAREs contain coiled-coil domains that allow interactions between v- and t-SNARE pairs. In fact, pairing of particular v- and t-SNAREs through their coiled-coil domains initially was thought to provide the specificity that is a hallmark of vesicle trafficking.

Each of the organelles in the endomembrane system contains a particular collection of SNAREs that interact in specific ways to coordinate transport among the compartments. The v-SNAREs are normal residents of the donor membranes, but they also are found in transport vesicles and on the target membrane. On the other hand, the t-SNAREs are more stable and can be considered biochemically diagnostic for the target membrane in which they reside. For example, because the entire genome of yeast (*Saccharomyces cerevisiae*) has been sequenced, all of the identifiable t-SNAREs have been characterized (reviewed in Pelham, 1998). Each of the major endomembrane organelles of yeast has been found to contain one or more t-SNAREs (Figure 1). Thus, yeast is likely to have the "basic set" of t-SNARE proteins necessary for traffic through the endomembrane system. Orthologs (i.e., functionally homologous proteins) of some of the yeast t-SNAREs that exist in mammalian cells and those characterized in plants are shown in Figure 2. Mammals and plants appear to have a much greater number of SNAREs or have multiple forms of the single copies found in yeast (Figures 2B and 2C). Some of these extra SNAREs may be cell- or tissue-specific forms. Alternatively, their presence may reflect the more complicated endomembrane system of mammals and plants.

The SNAREs cannot act alone; many other factors are necessary to regulate their function. Two general factors that act ubiquitously throughout the cell to activate SNAREs are the *N*-ethylmaleimide–sensitive factor (NSF) and α-SNAP (for soluble NSF attachment protein; the acronym SNARE stands for SNAP receptor and is derived from one of the functions of these proteins as membrane attachment sites for α-SNAPs). NSF, a large hexameric ATPase, appears to be common to all eukaryotic cells because orthologs exist in animal, yeast, and plant cells (Malhotra et al., 1988; Y. Sato et al., 1997; Eakle et al., 1988).

In addition to these two general factors, two other proteins are required to regulate SNAREs in most targeting steps: (1) a peripheral membrane protein from the Sec1p family, and (2) a small GTPase of the Rab family (reviewed in Rothman and Sollner, 1997). The most recent hypothesis for how SNARE interaction leads to membrane fusion (Hay and Scheller, 1997; Rothman and Sollner, 1997; Weber et al., 1998) is diagrammed in Figure 3. Transport vesicles carry an activated v-SNARE and a GTP-bound form of Rab, whereas the target membrane contains a t-SNARE bound to an Sec1p homolog. The Rab–GTP displaces the Sec1p homolog, freeing the t-SNARE for interaction with the v-SNARE. The v- and t-SNAREs then "zip up" through their coiled-coil domains, an interaction that may provide sufficient energy to allow fusion of the lipid bilayers. The v-/t-SNARE pair then forms a binding site for α-SNAP, which recruits NSF to the SNARE complex. Thereafter, the ATPase activity of NSF disrupts the SNARE complex, releasing the v-SNARE for recycling back to the donor compartment and preparing the t-SNARE for subsequent fusion events.

EARLY SECRETORY PATHWAY

The early secretory system, which includes the ER and the Golgi complex, is the equivalent of an assembly line for newly synthesized proteins. Through a series of quality control steps (see Vitale and Denecke, 1999, in this issue), protein cargo is sequentially folded and modified as it passes

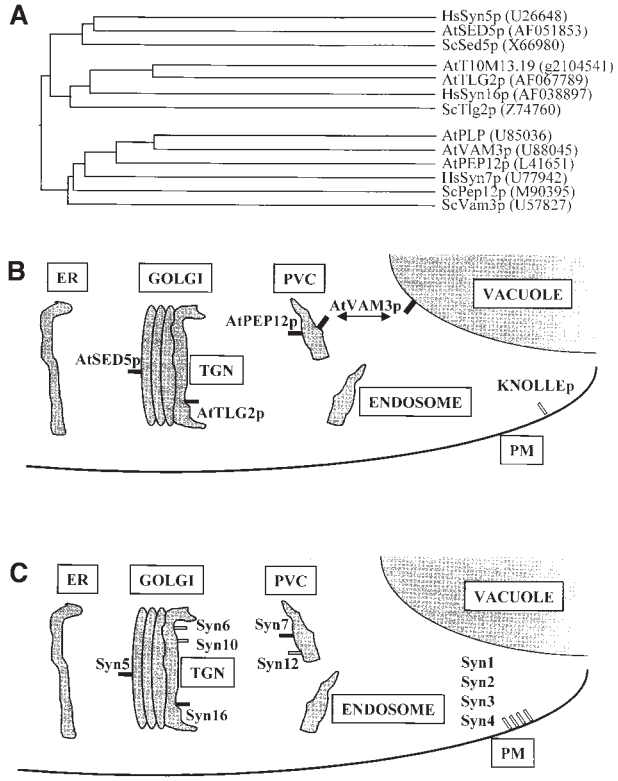

Figure 2. Plant and Mammalian Orthologs of the Yeast t-SNAREs.

(A) On the basis of sequence homology and intracellular localization as well as biochemical and genetic results, potential orthologs of yeast t-SNAREs have been identified in mammalian and plant cells. The phylogenetic tree was made with the MEGALIGN program in the DNASTAR package by using sequences acquired from GenBank (accession numbers indicated). At, *Arabidopsis thaliana*; Hs, *Homo sapiens*; Sc, *Sacharromyces cerevisiae.*

(B) Plant orthologs of t-SNARES (filled bar) generally have been localized to the same compartments as in yeast. KNOLLEp (open bar) represents an additional t-SNARE that has no counterpart in the yeast genome. AtVAM3p may be localized to either the vacuole or the PVC, depending on cell type (indicated by double-headed arrow).

(C) Mammalian orthologs (filled bars) of yeast t-SNAREs. Additional t-SNARES that have no counterpart in the yeast genome are indicated with open bars.

through the ER and the stacks of the Golgi complex. For example, in the ER lumen, many proteins undergo post-translational modifications that can include disulfide bond formation and the addition of a core glycosylation chain to specific Asn residues. In the Golgi complex, this core glycosylation chain may be extended, trimmed, or even removed by various glycotransferases and glycosidases present in the various stacks. Proteolytic modification of some proteins also can occur in the later stacks of the Golgi. Here, we focus on the role of the ER and Golgi in the passage of cargo.

ER-to-Golgi Trafficking

Most proteins destined for the secretory system first enter at the ER. Once in the ER, proteins that are intended for the Golgi complex are believed to be sorted into distinct subdomains of the ER, where they become concentrated over ER-resident enzymes (Aridor and Balch, 1996). The mechanism by which this cargo is packaged into COPII vesicles is covered in detail by Vitale and Denecke (1999) in this issue. In brief, the COPII coatomers are made up of two protein complexes (reviewed in Schekman and Orci, 1996). The coatomer is recruited to the membrane through the action of a small GTPase, Sar1p, which associates with membranes in a GTP-dependent manner. Formation of COPII vesicles can be reconstituted in vitro with synthetic liposomes and the yeast coatomer subunits (Matsuoka et al., 1998). It is thought that uncoating is stimulated by activation of the GTPase activity of Sar1p through the action of additional factors. The GDP-bound form of Sar1p is not able to associate with membranes, which subsequently leads to release of the coatomers from the vesicle. Some components of the COPII machinery, including coatomer components and the SAR1 GTPase, have been found in plant cells (Bar-Peled and Raikhel, 1997; Robinson et al., 1998).

The specificity of ER-to-Golgi trafficking in yeast is directed by four v-SNAREs, Sec22p, Bos1p, Bet1p, and Ykt6p, which are believed to function in anterograde transport to the *cis*-Golgi (Newman et al., 1990; McNew et al., 1997). Each of these v-SNAREs can form a SNARE complex with the *cis*-Golgi t-SNARE Sed5p. Vesicle fusion through the typical SNARE mechanism (Figure 3) effects delivery of cargo to the *cis*-Golgi (see Figure 4A). Potential orthologs of the Rab–GTPase involved in this step have been reported from many plants (e.g., Palme et al., 1992), as has a putative ortholog of the *cis*-Golgi t-SNARE Sed5p from Arabidopsis (AtSED5p; GenBank accession number AF051853; A.A. Sanderfoot and N.V. Raikhel, unpublished data). Because

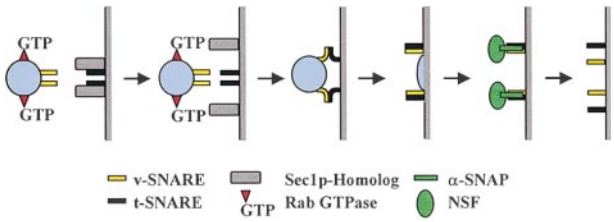

Figure 3. The SNARE Mechanism of Vesicle Fusion.

The v-SNARE and Rab-type GTPase associated with the vesicular membrane recognize the t-SNARE and Sec1p homolog on the surface of the target membrane. Subsequent to this recognition event (docking), the Sec1p homolog is displaced, and specific protein–protein interactions between the v- and t-SNAREs mediate vesicle fusion. α-SNAP and NSF finally work in concert to disassociate the v-SNARE/t-SNARE pair. Additional details are given in the text.

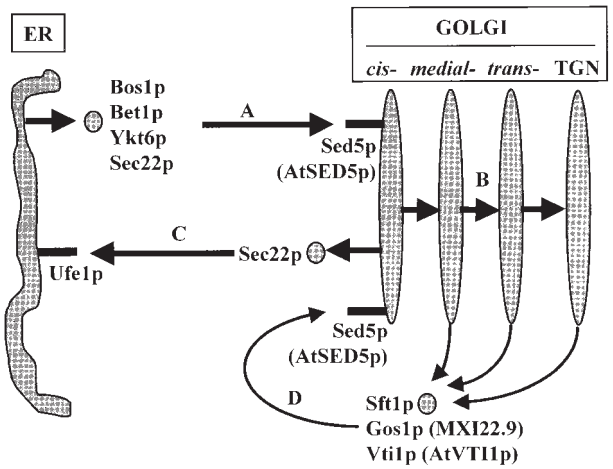

Figure 4. Early Endomembrane System.

The early endomembrane system consists of the ER and the stacks of the Golgi complex. Shown are the SNARE components required in yeast. Plant orthologs of the yeast SNAREs are indicated in parentheses.

(A) Anterograde transport from the ER depends on the COPII coatomer to drive vesicle budding. In yeast cells, these vesicles carry the v-SNAREs Bos1p, Bet1p, Ykt6p, and Sec22p, each of which can complex with the appropriate t-SNARE of the *cis*-Golgi, Sed5p.

(B) Anterograde transport through the Golgi apparatus appears to occur by maturation of the stacks in yeast (*cis*- becomes *medial*-, etc.), although this may not be the case in all eukaryotic cells (see text).

(C) Retrograde transport from the Golgi to the ER depends on the COPI coatomer to form vesicles that carry the v-SNARE Sec22p. Sec22p pairs with the t-SNARE of the ER Ufe1p.

(D) Within the Golgi stacks, COPI vesicles carry one of three v-SNAREs: Sft1p, Gos1p, or Vti1p. Each of these v-SNAREs pairs with the *cis*-Golgi t-SNARE Sed5p.

orthologs of these SNARE components also are found in mammalian cells (Hay et al., 1996), it is likely that the mechanism of ER-to-Golgi transport is conserved among the higher eukaryotes.

Intra-Golgi and Golgi-to-ER Trafficking

The Golgi apparatus is a complex array of stacked membranes that appear to operate in a sequential manner as protein cargo transits progressively from the *cis*- to the *trans*-Golgi stacks. The actual number of stacks in the plant Golgi complex can vary greatly from organism to organism, cell to cell, and even within the same cell, depending upon growth rates and other factors (Zhang and Staehelin, 1992). Despite this structural flexibility, the secretory protein cargo continues to move through the Golgi complex from the *cis* to intermediate to *trans* stacks, acquiring various modifications as it goes. At the same time, material in the retrograde pathway is moving in the opposite direction so that it is retained in specific Golgi stacks or is returned to the ER.

How the cargo actually transits through the Golgi stacks remains controversial. Although the various stacks clearly contain distinct sets of enzymatic activities, strong evidence in favor of a "maturation" model, in which the earlier stacks mature into later stacks while incoming ER-derived vesicles reform the *cis*-Golgi, has been presented by many researchers (e.g., Glick et al., 1997; Pelham, 1998). Other evidence has supported models of intra-Golgi vesicular transport (Orci et al., 1997) or even transient tubular connections (Orci et al., 1998) between stacks as mechanisms that mediate anterograde transport.

Recent work in yeast and animal cells may help to shed some light on this controversy. One clear feature of the Golgi membranes in these organisms is that they are the site of formation for a second type of coated vesicle that carries a COPI (rather than a COPII) coat (reviewed in Schekman and Orci, 1996). The COPI coat was first described in mammalian cells but also has been found in yeast. The COPI coatomer is made up seven subunits and the small GTPase ARF1. Similar to COPII coat formation, the GTP-bound form of ARF1 associates with membranes, leading to recruitment of coatomer subunits and subsequent budding of the coated vesicle. A number of the COPI coatomer components also have been identified in plants (Robinson et al., 1998).

COPI vesicles are required for retrograde transport from the Golgi to the ER, a process essential for recycling of the anterograde trafficking machinery and for retrieval of ER-resident proteins (reviewed in Bednarek and Raikhel, 1992). Such recycling is accomplished in yeast by the receptor Erd2p (Semenza et al., 1990), orthologs of which also are found in mammals and plants (Lewis and Pelham, 1990; Lee et al., 1993). In yeast cells, Golgi-derived COPI vesicles carry the v-SNARE Sec22p (which also plays a role in the anterograde pathway; see above), which interacts with the ER-resident t-SNARE Ufe1p (Lewis et al., 1997; see Figure 4C). No homologs of the ER t-SNARE Ufe1p have been found in mammalian cells; however, recent work has shown that two t-SNAREs, syntaxins 8 and 17, appear to be located on the mammalian ER (Steegmaier et al., 1998). COPI-like vesicles have been observed microscopically in plant cells (see Robinson et al., 1998), but the molecular details of the trafficking machinery have not been reported.

The role of COPI vesicles in anterograde trafficking within the Golgi complex is less clear. COPI vesicles have been suggested to mediate both anterograde and retrograde traffic between the stacks of the mammalian Golgi complex (Schekman and Orci, 1996). Furthermore, distinct populations of COPI vesicles that carry either cargo moving in an anterograde direction or cargo moving in a retrograde direction have been found (Orci et al., 1997). Because coatomers play a role in the collection of cargo, how the same coatomers can distinguish two distinct classes of cargo and designate the resulting vesicles for trafficking in different di-

rections is not clear. Furthermore, although it is likely that intra-Golgi transport occurs via a typical SNARE-mediated mechanism, no t-SNARE that resides on the intermediate Golgi stacks has been found in any cell.

Although it is unlikely that all the t-SNAREs have been identified in mammalian and plant cells, the completed genome sequence of yeast has revealed all of the clearly identifiable t-SNAREs. Each of these yeast t-SNAREs has been biochemically characterized, and none appears to localize to intermediate Golgi stacks (reviewed in Pelham, 1998). It therefore has been suggested that cargo transport from the *cis* to *trans* stacks of the yeast Golgi complex occurs via a maturation-type mechanism, with COPI vesicles only being used for retrograde trafficking (Pelham, 1998). Similarly, microscopic studies of plant cells have indicated that some densely staining cargo can be observed to form at the earliest Golgi stacks and be traced through the Golgi stacks as a unit (Hohl et al., 1996; see Traffic from the TGN to the Protein Storage Vacuole, below).

Elucidating the mechanisms of anterograde intra-Golgi transport requires further study to reconcile these somewhat conflicting results from yeast and animal cells. It is, of course, possible that both mechanisms are used in mammalian cells, or that mammalian and yeast cells have evolved different mechanisms. The eventual elucidation of the pathway utilized by plant cells may reveal which is the "standard" eukaryotic pathway for anterograde cargo transport through the Golgi.

In addition to their presumed role in returning proteins from the Golgi complex to the ER, COPI vesicles also have been implicated in retrograde transport within the Golgi itself (Figure 4D). In yeast, COPI vesicles are formed from the *trans*-most stacks, which carry one of three v-SNAREs: Gos1p, Sft1p, or Vti1p. Each of these v-SNAREs interacts with the *cis*-Golgi t-SNARE Sed5p (Sogaard et al., 1994; Banfield et al., 1995; Fischer von Mollard et al., 1997). Mammalian cells contain the Gos1p ortholog GOS28, which also interacts in vitro with the Sed5p ortholog Syntaxin 5 (Hay et al., 1997). Because a potential homolog of Gos1p has been identified by the Arabidopsis genome-sequencing project (on clone number MXI22.9 from the Kazusa DNA Research Institute), the retrograde intra-Golgi pathway may be common to all eukaryotes.

LATE SECRETORY PATHWAY

The late secretory pathway begins at the loosely defined group of membranes that comprise the TGN. This structure has been observed in plant and animal cells, but it is something of a "hypothetical" compartment in yeast cells, where it has not been as clearly documented microscopically. Proteins departing the TGN travel to one of two principal destinations: the plasma membrane or the vacuole. However, some plant cells possess two functionally distinct vacuoles (a lytic and a protein storage vacuole [Paris et al., 1996]; see Herman and Larkins, 1999, and Marty, 1999, in this issue); thus, there may be three possible destinations of these proteins in plant cells.

Trafficking of proteins to the plasma membrane is believed to represent the default route because vacuolar proteins contain specific sorting information. This sorting information is in the form of post-translational addition of mannose-6-phosphate in animal cells and specific (but different) peptide sequences in yeast and plant cells (reviewed in Bassham and Raikhel, 1997), and it allows vacuolar proteins to be sorted away from proteins destined for the plasma membrane. Several different vesicle types have been observed to bud from the TGN. Each type possesses a morphologically distinct protein coat, but only some of these proteins have been identified (reviewed in Traub and Kornfeld, 1997).

TGN-to-PVC Trafficking

Clathrin-coated vesicles (CCVs) and the protein components of the coat have been found in all eukaryotic cells (Beevers, 1996; Beevers and Raikhel, 1998; see Battey et al., 1999, in this issue). Clathrin consists of two polypeptides, a heavy chain and a light chain, that form a microscopically observable "triskelion" consisting of three heavy and three light chains. Clathrin is recruited to membranes by a specific protein complex, called the adaptor complex (AP). Two distinct clathrin-recruiting APs are found in eukaryotic cells. One complex (AP-1) is involved in formation of anterograde CCVs at the TGN, whereas a second (AP-2) is involved in formation of endocytic CCVs at the plasma membrane. The AP complexes interact with specific protein motifs on the cytoplasmic tails of membrane proteins (Kirchhausen et al., 1997), and in so doing, these proteins become incorporated into the membrane of the CCV.

One important class of AP-interacting membrane proteins comprises the vacuolar cargo receptors. These receptors have been characterized from several eukaryotic cells and include the mammalian mannose-6-phosphate receptor (Kornfeld, 1992), the yeast carboxypeptidase Y receptor (Vps10p; Marcusson et al., 1994), and the putative plant vacuolar sorting receptor (BP-80/AtELP; Kirsch et al., 1994; Ahmed et al., 1997; Paris et al., 1997). Although the overall sequence similarity among these receptors is low, they share common structural features, such as lumenal cysteine-rich repeats, a single transmembrane domain near the C terminus, and a short cytoplasmic tail.

How vacuolar cargo receptors segregate proteins destined for the vacuole from those intended for secretion is shown schematically in Figure 5. Briefly, cargo proteins that contain vacuolar sorting signals are recognized in the TGN by the vacuolar cargo receptor (Figure 5A). The cytoplasmic tail of the cargo receptor is then bound by the AP-1 adaptor complex (Figure 5B). The AP-1 complex then recruits the

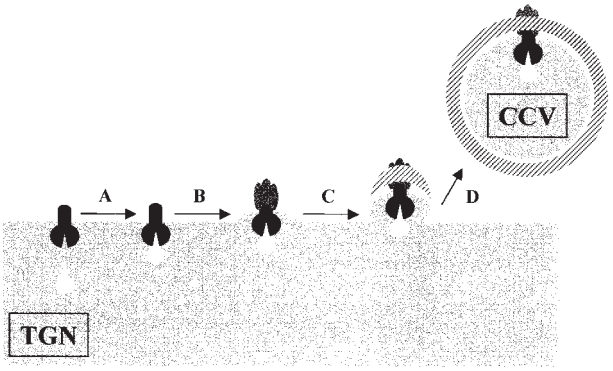

Figure 5. Packaging of Vacuolar Cargo into CCVs at the TGN.

Vacuolar cargo proteins (white circles) contain specific sorting signals (white triangles) that tag cargo for export from the TGN.
(A) The lumenal domain of the vacuolar cargo receptor (shown in black) binds to the sorting signal.
(B) The cytoplasmic tail of the cargo receptor is then bound by the AP-1 complex (shown in gray).
(C) The complex of cargo receptor with AP-1 recruits clathrin coatomers (shown by the hatched area).
(D) Formation of the clathrin coat drives budding of the CCV. See text for details.

clathrin coat (Figure 5C), which eventually results in formation of the CCV (Figure 5D).

Although these TGN-derived CCVs carry cargo destined for the vacuole, evidence from animal, yeast, and plant cells indicates that the PVC is the immediate target for these vesicles (Figure 6A). In yeast, the PVC bears the t-SNARE Pep12p (Becherer et al., 1996), and transport of the Vps10p–carboxypeptidase Y complex to the PVC requires the v-SNARE Vti1p, the Sec1p-homolog Vps45p, the Rab–GTPase Vps21p, as well as the α-SNAP and NSF orthologs (Horazdovsky et al., 1994; Piper et al., 1994; Burd et al., 1997; Fischer von Mollard et al., 1997). Mammalian cells also contain orthologs of Pep12p (syntaxin 7; Wang et al., 1997), Vti1p (Fischer von Mollard and Stevens, 1998), and Vps45p (Pevsner et al., 1996).

Arabidopsis cells also possess an ortholog of Pep12p (AtPEP12p), which resides on the PVC (da Silva Conceição et al., 1997), as well as orthologs of Vps45p (Bassham and Raikhel, 1998) and Vti1p (Zheng et al., 1999b). Consistent with the CCV-mediated TGN-to-PVC trafficking of some vacuolar cargo in Arabidopsis, the putative vacuolar cargo receptor AtELP appears to be able to bind the AP-1 complex and has been colocalized with AtPEP12p on the PVC surface (Sanderfoot et al., 1998).

Despite these apparent functional analogies, however, it seems likely that the mechanisms mediating post-TGN trafficking in plants are likely to be significantly more complex than those in yeast. Not only do some plant cells have more than one type of vacuole (Paris et al., 1996; see Marty, 1999, and Herman and Larkins, 1999, in this issue), but it appears that plants may have more than one t-SNARE involved in TGN-to-PVC trafficking. In addition to AtPEP12p, AtPLP (Zheng et al., 1999a) and AtVAM3p (M.H. Sato et al., 1997) both share significant similarity with AtPEP12p.

AtVAM3 was first characterized as an Arabidopsis cDNA that could functionally complement a deletion of the yeast vacuolar t-SNARE Vam3p, and the corresponding protein has been localized to the tonoplast (i.e., the vacuolar membrane) in the shoot apical meristem of Arabidopsis (M.H. Sato et al., 1997). However, AtVAM3p is more similar to

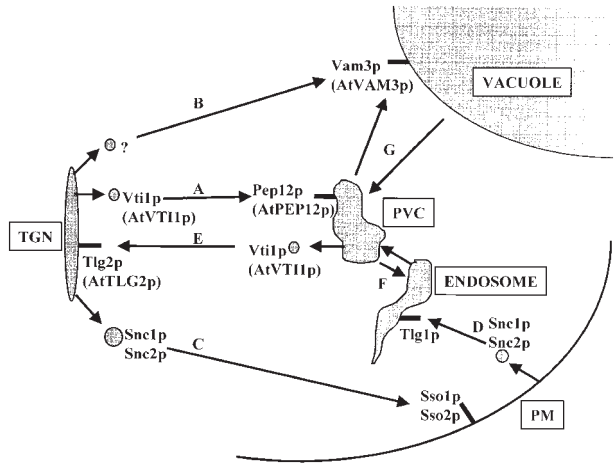

Figure 6. Late Endomembrane System.

The late endomembrane system consists of the TGN, the vacuole, the plasma membrane (PM), the PVC, and the endosome. SNARE components of yeast are as shown, with plant orthologs shown in parentheses.
(A) CCVs formed at the TGN that carry the v-SNARE Vti1p. These vesicles travel to the PVC, where Vti1p pairs with the PVC t-SNARE Pep12p.
(B) Vesicles with an AP-3 coat bud from the TGN and travel directly to the vacuole in a process that requires the vacuolar t-SNARE Vam3p. See text for details.
(C) Secretory vesicles have an unidentified coat and carry the v-SNAREs Snc1p and Snc2p, which pair with the plasma membrane t-SNAREs Sso1p and Sso2p. Secretory vesicles in mammalian cells use a distinct set of SNAREs to deliver cargo to the plasma membrane; it remains unclear which type of SNAREs are used in plants (see text).
(D) Endocytosis from the cell surface may use the v-SNAREs Snc1p and Snc2p and the t-SNARE Tlg1p.
(E) Retrograde transport from the PVC or endosome to the TGN also appears to depend on the v-SNARE Vti1p that interacts with the TGN t-SNARE Tlg2p.
(F) The endosome may deliver cargo destined for the vacuole by maturing into the PVC (see text).
(G) A similar situation is probable for delivery of cargo from the PVC to the vacuole. In Arabidopsis, AtVAM3p resides on the PVC of root cells but may reside on the tonoplast in cells of the shoot apical meristem (see text).

AtPEP12p and to yeast Pep12p than it is to yeast Vam3p (see Figure 2A). Furthermore, recent work shows that AtVAM3p is found on the PVC of Arabidopsis root cells, where it colocalizes with AtPEP12p (A.A. Sanderfoot, V. Kovaleva, H. Zheng, and N.V. Raikhel, manuscript submitted). It is thus possible that AtVAM3p functions on two separate organelles (i.e., the PVC and the vacuole) or performs different duties in different cell types. Further research is needed to differentiate the role of the PVC and vacuolar SNAREs in plant cells.

TGN-to-Vacuolar Transport

Some proteins are able to bypass the PVC and travel directly from the TGN to the vacuole (Figure 6B). In yeast, proteins that follow this "alternate pathway" to the vacuole are packaged into AP-3–dependent vesicles (Vowels and Payne, 1998). The AP-3 complex is a third adaptor complex that has been found in mammals and yeast (Panek et al., 1997; Simpson et al., 1997). AP-3 does not appear to recruit clathrin and is likely to be capable of acting as a coatomer on its own (Stepp et al., 1997; Vowels and Payne, 1998). These vesicles still appear to use a SNARE-dependent mechanism, because the vacuolar t-SNARE Vam3p and other SNARE components are required for delivery of proteins to the yeast vacuole by the alternate pathway (Darsow et al., 1997). Curiously, no v-SNARE partner for Vam3p has been found for this pathway. Because it has been found in yeast that Vam3p and the v-SNARE Vti1p are capable of interacting in vivo (Holthuis et al., 1998a), it may be that the AP-3–dependent pathway also utilizes Vti1p. Interestingly, it has been reported that the mammalian AP-3 coat may recruit clathrin in some cases (Dell'Angelica et al., 1998). The presence of an AP-3 complex in plants has not been examined.

Traffic from the TGN to the Protein Storage Vacuole

As mentioned above, some types of plant cells appear to contain more than one kind of vacuolar compartment (Paris et al., 1996; Di Sansebastiano et al., 1998; Swanson et al., 1998). The lytic vacuole is the typically acidic, proteolytically active compartment usually envisioned as being equivalent to a lysosome. The second type of vacuole serves as a storage compartment for proteins that serve as amino acid and energy reserves for the cell. It is believed that when the protein reserves are needed, the storage vacuole fuses with the lytic vacuole, leading to degradation of the proteins and liberation of amino acids. Proteins targeted to the protein storage vacuole are distinct from those that are targeted to the lytic vacuole (Hohl et al., 1996). Biochemical and microscopic investigations reveal that some of these storage proteins are packaged into a novel type of TGN-derived coated vesicle, a dense vesicle (DV; Hohl et al., 1996).

The storage proteins destined for the DVs have been observed collecting in membrane buds at the *cis*-Golgi of cotyledon cells in pea and pumpkin. These buds progress virtually unchanged through the Golgi complex before being released at the TGN (Hohl et al., 1996; Shimada et al., 1997). DVs appear to contain a protein coat, but the molecular nature of this coat has not been determined. A general trait of most storage proteins is their tendency to aggregate into almost crystalline arrays. It is thought that such aggregation may be the method by which storage proteins are partitioned into the DVs (Vitale and Chrispeels, 1992). It is also possible that specific cargo receptors participate in DV formation, although the vacuolar cargo receptor BP-80 is not found in DVs (Hohl et al., 1996). Whether DVs travel directly to the protein storage vacuole or first pass through the PVC is not clear. Assuming that they do, it would be interesting to determine whether material in DVs passes through a PVC distinct from that involved in trafficking of proteins to the lytic vacuole.

Interestingly, CCVs have been observed budding from DVs before their release from the TGN (Hohl et al., 1996). The budding of CCVs from other types of vesicles has also been observed in mammalian cells, where CCVs have been found to bud from secretory granules (see, e.g., Klumperman et al., 1998). This may represent a mechanism for the recovery of cargo misdirected into DVs. It is possible that the presence of the lytic cargo may perturb the packaging of the DV. Alternatively, it may represent a safety measure to prevent any hydrolases from degrading the storage proteins while they are in transit. The further investigation of these novel vesicles should shed light upon the details of how plants sort cargo into distinct types of vacuoles within the same cell.

TGN-to-Plasma Membrane Transport

At the TGN, proteins intended for secretion from the cell are packaged into yet another type of vesicle. The pathway from the TGN to the cell surface is also important in plant cells for transport of cell wall precursors to the cell surface, although it is not clear whether carbohydrates and secreted proteins travel in the same vesicle. Formation of these vesicles has been studied in many cell types, although only at a microscopic level in plants. Secretory vesicles clearly have a protein coat, but the composition of this coat has not been determined in any system (Traub and Kornfeld, 1997).

After emerging from the TGN, secretory vesicles appear to use a typical SNARE mechanism for delivery of cargo at the plasma membrane (Figure 6C). In yeast, secretory vesicles carry the v-SNAREs Snc1p and Snc2p (Protopopov et al., 1993), which interact with the plasma membrane t-SNAREs Sso1p, Sso2p, and Sec9p (Aalto et al., 1993; Brennwald et al., 1994). In mammalian cells, a distinct set of v- and t-SNAREs, which vary in different cell types, appears to be involved in TGN-to-plasma membrane transport. For

example, in neural cells, the t-SNAREs syntaxin 1 and SNAP-25 and the v-SNARE synaptobrevin (also called VAMP2) are involved in the regulated delivery of neurotransmitter-carrying synaptic vesicles (McMahon and Sudhof, 1995). At least three other syntaxin homologs and several VAMP homologs are believed to function in delivery of various cargo to the plasma membrane in other cell types (Bennet et al., 1993; Advani et al., 1998).

Why do mammalian cells have such a large number of SNAREs for TGN-to-plasma membrane targeting? Some mammalian cells participate in regulated secretion (as opposed to the constitutive secretion of yeast cells), whereas others are polarized. In the latter cells, some proteins are secreted only from the apical plasma membrane, whereas distinct proteins are secreted from the basal-lateral plasma membrane (reviewed in Traub and Kornfeld, 1997). Consistent with this observation, particular syntaxins (t-SNAREs) are restricted to specific domains of the plasma membrane of some mammalian cells, where they may be involved in selecting cargo destined for the apical plasma membrane away from cargo intended for the basal–lateral domains of the plasma membrane (Low et al., 1996). Thus, delivery of cargo to the mammalian cell surface may require a more specialized SNARE mechanism than that used in yeast cells.

Sequences similar to the mammalian v-SNARE synaptobrevin/VAMP2 have been found in plants as expressed sequence tags and during genomic sequencing, but whether the products of these genes function at the plasma membrane is unknown. Furthermore, an Arabidopsis homolog of the mammalian t-SNARE SNAP-25, AtSNAP33p, recently has been characterized and found to be localized to the plasma membrane (GenBank accession number X92419; X. Gansel and L. Sticher, personal communication).

The only other plant t-SNARE known to function at the plasma membrane is KNOLLE. Mutations in the *KNOLLE* gene prevent cell division in the Arabidopsis embryo, and the protein product is found only at the phragmoplast of dividing cells (Lukowitz et al., 1996; Lauber et al., 1997). For this reason, it is believed that KNOLLE functions to receive the TGN-derived vesicles that carry the lipids and cell wall material needed to form the cell plate. These data suggest that KNOLLE does not function in constitutive trafficking of vesicles to the plasma membrane but rather that it has a very specific role in vesicle trafficking during cell growth. Plant cells also undergo regulated and polarized secretion during the rapid growth of pollen tubes and root hairs (Mascarenhas, 1993), although the molecular details of this pathway have not been studied in great detail.

Retrograde Transport and the Endosome

Recovery of the anterograde secretory machinery through the retrograde pathway is essential to support a functional endomembrane system. Along with retrograde transport from the vacuole or PVC, recovery of proteins from the cell surface by endocytosis is used to recycle the various components of the machinery. Endocytosis has a second role in the transport of proteins from the cell surface for degredation in the vacuole, but this is accomplished simultaneuosly with the recycling of the retrograde pathway, as described below.

Vesicles derived from endocytosis at the plasma membrane first fuse with the endosome. Like the PVC, the endosome is a sorting compartment that differentiates proteins intended for return to the cell surface from those that are to be recycled back to the Golgi or to be degraded in the vacuole. In mammalian cells, many types of endosome have been differentiated on the basis of several characteristics, including the type of cell in which they reside. For simplicity, these various compartments are not distinguished here; instead, the collective term endosome is used to describe all these variants (see Introduction).

In yeast, the retrograde pathway, as well as endocytosis, appears to be mediated by the t-SNAREs Tlg1p and Tlg2p (Holthuis et al., 1998a, 1998b). As shown in Figure 6D, Tlg1p appears to reside on the endosome and mediate plasma membrane-to-endosome traffic (Holthuis et al., 1998a, 1998b). The precise localization of Tlg2p is not completely clear, but as shown in Figure 6E, it is likely to represent the equivalent of the TGN in yeast cells (Holthuis et al., 1998b). Supporting this localization is the finding that a mammalian t-SNARE homologous to Tlg2p (syntaxin 16) is found on the Golgi complex (Tang et al., 1998b). Similarly, two homologs of Tlg2p are found in Arabidopsis (A.A. Sanderfoot and N.V. Raikhel, unpublished observations; see Figure 2A). Similarly, Figure 6F shows that the PVC t-SNARE Pep12p also plays a role in endocytosis, most likely receiving the endocytosed cargo that is destined for degradation in the vacuole (Holthuis et al., 1998b).

The compartments of the late endomembrane system interact so extensively that it can be difficult to determine whether a particular endosome is involved in the anterograde or retrograde pathway. It is also possible that some of the interactions among the compartments of the late secretory system do not involve transport vesicles. Some events may occur through direct fusion between two compartments, whereas others may occur by “maturation” of one compartment into another. For example, at steady state, endosome of yeast, animal, and plant cells can contain both endocytosed and TGN-resident proteins, the latter having arrived as a result of anterograde transport (Stoorvogel et al., 1991; Tanchak et al., 1984; Holthuis et al., 1998a, 1998b).

Recycling of proteins back to the plasma membrane or to the TGN (Trowbridge et al., 1994) leaves behind a “depleted endosome” containing only those proteins that are destined for degradation in the vacuole. This material destined for degradation first must pass through the PVC and may do so by “maturation” (Stoorvogel et al., 1991), perhaps by the exchange of the endosomal-type t-SNARE (i.e., yeast Tlg1p or its ortholog) with the t-SNARE of the PVC (i.e., yeast Pep12p

or its ortholog). Because the PVC is also a recycling/sorting compartment, in which components are sorted back to the TGN, the plasma membrane, and even to the endosome (Cooper and Stevens, 1996; Seaman et al., 1997), it too may undergo a similar depletion until all that remains is material destined for the vacuole. Again, the ultimate step, during which cargo travels from the PVC to the vacuole, may occur when the Pep12p-type t-SNARE is exchanged for a vacuolar-type t-SNARE (yeast Vam3p) or may simply occur by direct fusion of these organelles (Griffiths and Gruenberg, 1991; Figure 6G).

Clearly, the concept of compartments slowly changing into one another can be very complicated. Nevertheless, the large number of potential coat proteins required for differentiating transport to and from these many compartments is even more mind-boggling. It is also possible that maturation and vesicular transport could occur in different cells under different conditions. Many mysteries regarding the late secretory system remain to be solved.

FUTURE PERSPECTIVES: DO THE SNAREs HAVE ALL THE ANSWERS?

The complexities of the secretory system are beginning to be unraveled as the molecular details of the protein-trafficking machinery are discovered. The genome sequence of yeast has already pinpointed the exact number of SNAREs in this unicellular organism. However, it is clear that mammals and plants have more SNAREs than do yeast, and although many of the t-SNAREs in these organisms remain to be classified (see Figures 2B and 2C), several t-SNAREs that do not have counterparts in yeast already have been found. For example, mammalian cells contain Syntaxins 6, 10, 11, 12, and 13 (Bock et al., 1996; Advani et al., 1998; Tang et al., 1998c, 1998d), whereas Arabidopsis cells contain AtPLP (Zheng et al., 1999a) and KNOLLEp (Lauber et al., 1997). In addition, mammals and plants appear to have multiple forms of the single v-SNAREs found in yeast. For example, there are two forms of Sec22p in mammalian cells (Hay et al., 1996; Tang et al., 1998a) and two forms of Vti1p in both mammals and plants (Fischer von Mollard and Stevens, 1998; Zheng et al., 1999b). Clearly, yeast has all the SNAREs necessary to run a functional endomembrane system, but mammals and plants probably require many more to run their more complicated multicellular systems. It is also possible that plant and mammalian systems require additional redundancy. The SNARE hypothesis, as it stands now, may turn out to be too simplistic to explain the increased complexity of the secretory systems of multicellular organisms.

Certainly, it is becoming clear that the categorization of v-SNAREs for vesicles and t-SNAREs for target membranes is something of an oversimplification. The t-SNARE syntaxin 1, for example, also can be detected upon the presynaptic vesicles (Walch-Solimena et al., 1995), and the *cis*-Golgi t-SNARE syntaxin 5 also is found on vesicles derived from the ER (Rowe et al., 1998). These results may simply reflect the normal route that newly synthesized t-SNAREs follow to get to their site of action or the accidental packaging of the t-SNAREs into retrograde vesicles. It is also possible that the t-SNAREs could move between compartments as part of their normal activity, which is suggested by studies on the mammalian t-SNARE syntaxin 6. The protein sequence of syntaxin 6 is somewhat related to that of the yeast PVC t-SNARE Pep12p (Bock et al., 1996). However, syntaxin 6 is found at the TGN of mammalian cells, not at the PVC (Bock et al., 1997). Nevertheless, syntaxin 6 is likely to play a role in traffic from the TGN to the PVC, because it has been found to be packaged into AP-1–type CCVs along with the mannose-6-phosphate receptor (Klumperman et al., 1998). Does this packaging into CCVs imply that syntaxin 6 is really a v-SNARE, even though it is much more homologous to t-SNAREs? The probable answer is that not every SNARE can be neatly packaged into a v- or t-functional group.

In addition to their role in vesicular transport, the SNAREs may play a role in organelle assembly. Homotypic fusion of the yeast vacuole, which occurs when small vacuoles fuse to form larger vacuoles, depends upon the pairing between the vacuolar v-SNARE Nyv1p and the t-SNARE Vam3p (Nichols et al., 1997). Other examples of homotypic fusion seem to require only t-SNAREs. In most eukaryotic cells, for example, mitosis leads to disassembly of the nuclear membrane (which can be considered to be a specialized subdomain of the ER). Mammalian cells go further and disassemble the Golgi stacks as part of partitioning the Golgi complex between the daughter cells (Warren, 1993). At the end of mitosis, the organelles must be faithfully reassembled. This homotypic reassembly has been studied for both the yeast ER and the mammalian Golgi complex. The yeast ER is apparently reassembled using only the resident t-SNARE Ufe1p (Rabouille et al., 1998), whereas the mammalian Golgi can be nearly reassembled with just the t-SNARE syntaxin 5 (Patel et al., 1998). Interestingly, this t-/t-SNARE pairing reaction does not require NSF; instead, it uses a related ATPase called p97 (Cdc48p in yeast; Patel et al., 1998; Rabouille et al., 1998). Is this different ATPase required because of the t-/t-SNARE pairing, or does its requirement reflect the need for mitotic control of the process?

It is also unlikely that the one-v-SNARE-to-one-t-SNARE specificity originally suggested for vesicle trafficking holds true in all cases. For example, the yeast v-SNARE Sec22p interacts with the *cis*-Golgi t-SNARE Sed5p on the way to the Golgi complex and with the ER t-SNARE Ufe1p on the way back to the ER (Newman et al., 1990; Lewis et al., 1997). The yeast v-SNARE Vti1p appears to be even more liberal regarding the t-SNAREs with which it will pair. Initially found to be required for delivery of cargo to the PVC through interaction with the PVC t-SNARE Pep12p, Vti1p also was found to interact with the *cis*-Golgi t-SNARE Sed5p (Fischer von Mollard et al., 1997). This was found

also for the Arabidopsis ortholog AtVTI1a, which interacts with both AtPEP12p and AtSED5p (H. Zheng and N.V. Raikhel, unpublished data). In yeast, Vti1p also interacts with the TGN t-SNARE Tlg2p, the endosomal t-SNARE Tlg1p, and the vacuolar t-SNARE Vam3p (Holthuis et al., 1998a). Thus, v-/t-SNARE pairing may not be the only determinant of targeting specificity in the late secretory system.

The lack of SNARE specificity suggests that some form of molecular proofreading might occur before the step mediated by the SNAREs. Research in yeast and mammalian cells indicates that large protein complexes may function to dock vesicles at the appropriate membrane. For example, at the plasma membrane of yeast cells, a large protein complex called the exocyst is required for delivery of secretory vesicles to the cell bud. Genetic studies have suggested that this complex functions upstream of the plasma membrane t-SNAREs (TerBush et al., 1996). This complex also has been found in mammalian cells, where it may be involved in delivery of cargo to the basal–lateral domains of the plasma membrane (Grindstaff et al., 1998). Similarly, a complex called TRAPP has been implicated in the docking of ER-derived vesicles at the yeast Golgi complex (Sacher et al., 1998). Some of the protein components of TRAPP have homologs in mammalian cells (Sacher et al., 1998), suggesting that this complex might be found in mammals as well. Interestingly, a protein called Uso1p in yeast, and p115 in mammals, also has been implicated in the docking of vesicles to the Golgi complex (Waters et al., 1992; Cao et al., 1998). Uso1p/p115 does not appear to be part of the TRAPP complex (Sacher et al., 1998), and whether TRAPP and Uso1p function sequentially or in parallel remains to be determined. Proteins that may function in docking of vesicles at the yeast vacuole (Rieder and Emr, 1997) and PVC (Burd et al., 1997) also have been identified. Perhaps the docking step allows for proofreading between the SNAREs, thereby increasing the in vivo specificity of pairing.

Are SNAREs required for every transport process in the secretory pathway? Evidence suggests that some transport to the apical cell surface of mammalian cells may require neither NSF nor SNAREs (Ikonen et al., 1995). It has been proposed that glycolipids and sterols cluster at the TGN with proteins that contain glycosyl phosphatidylinositol anchors and that these clusters then move as a unit to the plasma membrane in a process called “rafting” (Simons and Ikonen, 1997).

Indeed, the role of lipids in secretory vesicle targeting should not be underestimated. Each organelle in the endomembrane system has a unique lipid composition that is maintained despite the tremendous flux of cargo vesicles that pass through each compartment. It is already clear that the lipid composition of membranes can greatly affect the ability of coatomers to assemble and induce budding (Matsuoka et al., 1998), which raises many questions. Is the lipid composition of vesicles different from that of the donor compartment? If so, does the lipid composition of vesicles determine the lipid composition of the target compartment, or is the lipid composition of organelles maintained by some other mechanism? The variation in lipid content among the organelles can have significant effects on the chemical and physical properties of the membrane, but these questions are only beginning to be asked. It is certain that the lipids of the organelles and the vesicles have a greater role than just a container for the cargo and a place to put the SNAREs.

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