Review

Crossing the Divide – Transport Between the Endoplasmic Reticulum and Golgi Apparatus in Plants

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The transport of proteins between the endoplasmic reticulum (ER) and the Golgi apparatus in plants is an exciting and constantly expanding topic, which has attracted much attention in recent years. The study of protein transport within the secretory pathway is a relatively new field, dating back to the 1970s for mammalian cells and considerably later for plants. This may explain why COPI- and COPII-mediated transport between the ER and the Golgi in plants is only now becoming clear, while the existence of these pathways in other organisms is relatively well documented. We summarize current knowledge of these protein transport routes, as well as highlighting key differences between those of plant systems and those of mammals and yeast. These differences have necessitated the study of plant-specific aspects of protein transport in the early secretory pathway, and this review discusses recent developments in this area. Advances in live-cell-imaging technology have allowed the observation of protein movement in vivo, giving a new insight into many of the processes involved in vesicle formation and protein trafficking. The use of these new technologies has been combined with more traditional methods, such as protein biochemistry and electron microscopy, to increase our understanding of the transport routes in the cell.

Key words: COPI, COPII, endoplasmic reticulum, ER export site, Golgi, protein transport

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The secretory pathway is complex system of organelles specialized for the synthesis, transport, modification and secretion of proteins and other macromolecules (1). As such, this system plays a vital role in the life of a cell. It is generally assumed that protein transport through the secretory pathway is controlled by vesicular transport intermediates that carry cargo molecules from one organelle to the next. Vesicular transport can occur in the forward (anterograde) direction, from the endoplasmic reticulum (ER) towards the plasma membrane, or in reverse (retrograde). The entire secretory pathway exists in equilibrium between anterograde and retrograde transport, and any disruption of a part of this balance can result in dramatic changes in the biology of a cell.

Vesicle budding and fusion mechanisms at the ER–Golgi interface are highly conserved between species (2–4). The formation of vesicles is induced by the action of cytoplasmic coat protein complexes (COPs) that polymerize on the membrane surface, capturing both cargo molecules and those that function in vesicle direction, such as soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) in the process. The membrane becomes deformed during the polymerization of the coat, resulting in the formation of a nascent vesicle. Small proteins with GTPase activity regulate the assembly and disassembly of the vesicle coat by cycling between GTP-bound (activated) forms and GDP-bound (inactivated) forms. The activated form initiates the recruitment of coat proteins to the membrane, whereas hydrolysis of GTP to GDP alters the conformation of the GTPase and triggers uncoating of the vesicle. The GTPase activity is tightly regulated by guanine-nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), which prevent unproductive cycles of membrane coating and uncoating.

The Secretory Pathways of Plants and Animals are not Identical

The organization of the organelles that make up the secretory pathway of plants differs greatly from that of mammals and yeast. Despite the identification of plant homologues of proteins that are known to be involved in vesicular transport in other systems, the mechanisms in plants have not yet been fully characterized. Given the differences in the features of the secretory pathway of plants compared with those of other organisms, it seems likely that plants have evolved unique characteristics for achieving efficient protein transport between organelles. We discuss current knowledge of these characteristics, although further study will be necessary to elucidate many of the processes involved in protein transport in plant cells.

The ER in plants is pushed to the cortex of the cell by the large central vacuole, whereas the mammalian ER has no such constraints and can exist throughout the cell, radiating
out from the nuclear envelope. The domains of the ER from which proteins are exported to the Golgi apparatus, termed ER export sites (ERES), are relatively immobile in mammals (5), while in plants, they are highly motile (6). Similarly, the mammalian Golgi apparatus remains relatively stationary in the perinuclear region of the cell and is much larger than the plant Golgi. It has been reported that in various plant cellular systems the Golgi apparatus is present as multiple stacks that are distributed throughout the cytosol and are capable of rapid movement (7–9) (Figure 1). The mammalian secretory pathway contains an additional organelle known as the ER-Golgi intermediate compartment (ERGIC), which does not exist in plants, although the cis-Golgi may play a similar role. In contrast, the plant cell contains one or more vacuolar compartments (10), which can perform different functions such as protein degradation, turgor maintenance and protein storage, depending on the tissue and species. Animal cells contain lysosomes, which perform a similar degradative function to that of the lytic vacuole in plants, but are much smaller and are distributed throughout the cell. The presence of different types of vacuoles in plants necessitates the existence of additional transport pathways that allow cargo molecules to travel to these organelles. Finally, the most distal location in the plant cell is the cell wall, which requires the secretion of a variety of molecules for its generation and maintenance. This transport is one of the driving forces behind the secretory pathway.

Despite these differences in the secretory pathways, proteins in both plants and animals are generally transported in the anterograde direction from the ER to the Golgi apparatus, at which point they are sorted for further transport either forward, in the direction of the cell surface and organelles in the later secretory pathway, or back towards the ER.

The Complexity of Protein Transport in Plants

Figure 2 shows a schematic representation of the secretory pathway in plants, summarizing the main transport pathways that have been established and proposed. Export from the ER to the Golgi occurs via a COPII-mediated mechanism (arrow 1) (6,11,12), but evidence

Figure 1: The endoplasmic reticulum and Golgi apparatus in plants are motile. Confocal laser scanning microscope images showing an epidermal cell from tobacco leaves, coexpressing a GFP-tagged Golgi marker protein and a YFP-tagged endoplasmic reticulum (ER) marker protein. Images were taken at different time-points (seconds; shown at top left of each image) to demonstrate the movement of both ER and Golgi. The white arrowhead indicates a single Golgi body throughout the time series, while the empty arrowhead indicates movement within the ER. Size bar = 2 μm.
for a second anterograde pathway that is not controlled by COPII has also been postulated (arrow 2) (13). To balance these forward transport pathways, at least one retrograde pathway must exist to carry cargo molecules back from the Golgi to the ER. Retrieval of proteins from Golgi to ER is required for a variety of reasons. Some proteins are intended to be resident within the ER, sometimes with a role in the folding and modification of newly synthesized proteins. These proteins can escape from the ER through accidental incorporation into the lumen of export carriers, and then need to be retrieved from the Golgi (12,14). It seems likely that other proteins that are involved in the export machinery itself may also be salvaged so that they can be re-used in a subsequent round of vesicle formation and transport. The retrieval mechanism is based on the presence of one or more signals in the sequence of the protein (15), which can be recognized by a receptor or receptor-like protein. The binding of ligand to receptor is thought to induce the formation of a retrograde vesicle to transport ER-resident proteins back to their destination, as has been shown in mammals (16,17). These vesicles are known as COPI vesicles (arrow 3) (18,19), although COPI-independent retrograde transport pathways have been proposed in mammalian cells (20–22). One or more of these alternative routes may also exist in plants to balance the effects of the COPI-independent anterograde route suggested by Törmäkangas et al. (13).

Later transport in the secretory pathway is mediated by other vesicle types such as clathrin-coated vesicles (arrow 4) (23), which have been proposed to mediate transport from the Golgi apparatus to the lytic vacuole, and dense vesicles (arrow 5) (24,25), which convey cargo proteins to the storage vacuole. In addition to these routes that carry cargo from the Golgi to the vacuoles, a variety of vesicle types that appear to transport storage proteins directly from the ER to the protein storage vacuole (PSV) have been identified in seeds (arrow 6) (26–28). It has been shown in developing wheat grains that aggregates of storage proteins form within the ER bud off to form vesicular structures surrounded by rough ER (27), which are incorporated into the vacuole through a mechanism similar to autophagy. Comparable structures have been identified in germinating seeds, designated KDEL-tailed cysteine proteinase-accumulating vesicles (KVs) (26), and in maturing seeds, termed precursor-accumulating (PAC) vesicles (28). The contents of these vesicles are mainly unglycosylated storage proteins, which do not require Golgi-mediated modifications. However, other storage proteins that carry complex glycan chains have been found on the periphery of the PAC vesicles (28). This may indicate that Golgi-derived vesicles carrying these glycoproteins have fused with the PAC vesicles en route to the PSV. Alternatively, these proteins may be transported to the Golgi for glycan processing to take place, followed by recycling to the ER for packaging into PAC vesicles and transport to the PSV. Further investigation into the precise mechanisms by which proteins are transported to the PSV is required to clarify these ambiguities.

The multitude of transport pathways present in plant cells means that the trafficking of proteins in vesicular intermediates is a vast subject that cannot be properly discussed in a single review. This review therefore focuses specifically on the complex area of transport between the ER and the Golgi in plant cells. We discuss the formation
mechanisms and functions of COPI vesicles and review current knowledge of putative COPII carriers, as well as the various models for the transfer of proteins between ER and Golgi in plant cells.

Anterograde Transport from the ER is Mediated by COPII

Although COPII-mediated export of proteins from the ER is not the only anterograde ER–Golgi route that has been proposed in plants, it has received the most attention and is therefore the best-characterized pathway between these organelles at this time. Transport mediated by COPI and COPII is very similar in many respects. The main difference between the two is that while COPII carriers originate in the ER and export newly synthesized proteins to the Golgi complex, COPI vesicles bud from the cis-Golgi and travel to the ER. In mammalian cells, COPII vesicles transport proteins as far as the ERGIC. After this, it has been suggested that the proteins are repackaged into vesicles that mediate transport to the Golgi apparatus and may be involved in forward transport through the Golgi (29,30). A recent study has shown that protein transport occurs from the ERGIC both forward to the Golgi and back towards to the ER (30), supporting the argument that the ERGIC is a discrete stable compartment. However, this does not preclude the possibility that it eventually develops into the cis-Golgi in an extension of the cisternal maturation model (31–34). It appears that the ERGIC in mammals is similar in function to the cis-Golgi in plants, in that it is a point from which proteins can be returned to the ER or transported forward to distal locations. It has also been reported that COPI vesicles may bud directly from the ER in yeast and mammals (35,36), although no data supporting this possibility in plants have yet been presented.

The formation of both COPII and COPI vesicles requires the activation of a specific small GTPase, which causes the recruitment of structural components of the vesicle coat to the membrane, resulting in the formation of a vesicle that can then bud from the membrane and travel to its destination. COPII vesicles have not yet been isolated from plants, but homologues of several of the components of the COPII coat have been identified (11,37,38) and have been used to study the pathway (6,12). The small, cytosolic GTPase Sar1p mediates COPII vesicle formation in yeast and mammals. Three isoforms of Sar1p have been identified in Arabidopsis (39), at least one of which (AtSar1a) is capable of complementing Saccharomyces cerevisiae mutants (38). This suggests similarities between the mechanisms of vesicle budding in the two systems. The activation of Sar1p is mediated by an ER-localized integral membrane protein called Sec12p, a functional homologue of which has been identified in Arabidopsis (37,38). Sec12p is a GEF, recruiting Sar1p to the ER membrane in order to become activated through the exchange of GDP for GTP, which initiates vesicle formation. Sec12p is not incorporated into COPII carriers (6,40), meaning that activated Sar1p can maintain its membrane association independently of Sec12p. It has been shown in crystallization studies that mammalian Sar1p possesses an amphipathic N-terminal domain that is exposed on binding to GTP and allows direct interaction of Sar1p with the membrane (41). The homology between mammalian and plant Sar1p suggests that this may also be the case in plants, although this has yet to be confirmed experimentally.

It has been demonstrated through in vitro studies of non-plant systems that the coat proteins of COPII vesicles form two heterodimeric complexes that are sequentially recruited to the membrane by activated Sar1p (40,42). Sec23/24p binds to Sar1p, after which the Sec13/31p complex completes the coat. The assembly of the coat proteins on the membrane causes membrane curvature and eventually vesicle budding. After this, the protein coat must be released in order for the vesicle to fuse with the target membrane (in this case the Golgi apparatus). This step is induced by the hydrolysis of GTP by Sar1p, causing a conformational change that allows Sar1p to dissociate from the membrane (41). The hydrolysis of GTP is stimulated by the presence of a GAP, Sec23p (43). The fact that the GAP is a functional part of the COPII coat means that it is in close proximity to Sar1p, which may cause rapid GTP hydrolysis and increase the instability of the vesicular structure. The release of Sar1p from the membrane causes the dissociation of the coat proteins, leaving a membranous vesicle that can fuse with the Golgi apparatus and release its contents. Proteins are then sorted for further anterograde transport or retrograde transport back towards the ER. The instability of the COPII vesicle may account for some of the difficulties faced when attempting to isolate them from plants. However, daSilva et al. (6) demonstrated that recruitment of Sar1p to ERES can be enhanced by overexpressing certain membrane cargo proteins. It may therefore be possible to isolate COPII vesicles using an analogous experimental system to increase the number of vesicles produced.

Disruption of COPII-mediated transport has a dramatic effect on protein transport within the cell (6,9,12,44–47). This demonstrates the importance of the secretory pathway to the normal functioning of the cell. The mutation of a single residue in Sar1p can prevent it from becoming activated, effectively restricting it to the GDP-bound form and preventing the budding of vesicles from the ER in mammals, yeast and plants (45,46,48). Similarly, mutation of another amino acid reduces the GTPase activity of Sar1p, causing it to be confined to the GDP-bound form. This mutation allows vesicles to bud from the ER, but prevents them from uncoating and fusing with the Golgi apparatus in yeast (46,49). Although it has been shown that the GTP-restricted mutant of Sar1p inhibits the export of proteins from the ER in plants (9,12,45,50), the accumulation
of coated vesicles has yet to be confirmed in this system. However, it has been shown that in conditions of low expression, a yellow fluorescent protein fusion to Sar1p (GTP) accumulates at ERES in tobacco leaf epidermal cells (6). This may be taken to mean that the mutant labels a population of transport intermediates that are not capable of fusion with the target membrane.

High expression of these mutant GTPases often results in cell death. COPII-mediated transport can also be inhibited by the overexpression of Sec12p in both plants and yeast (6,12,44), which causes the continual recruitment of Sar1p to the ER membrane and prevents vesicle budding. This titration effect can be reversed by overexpressing Sar1p, in order that some activated Sar1p molecules are present and can form functional vesicles (12,44).

**COPI – the Retrograde Counterpart of COPII**

The existence of COPI vesicles has been demonstrated in mammalian (51), yeast (52) and plant cells (11,18,19). COPI vesicles bud from the cis-cisternae of the Golgi (18) and mediate traffic from the cis-Golgi back to the ER (53,54). This is an essential pathway that continually recycles proteins and lipids from the Golgi to the ER in order to maintain an equilibrium with COPII transport, sustaining the balance between the anterograde and the retrograde transport pathways.

The minimal machinery for the budding of COPI-coated vesicles consists of coatomer, a stable cytosolic complex comprising seven subunits α, β, β’, γ, δ, ε and ζ-COP (51), as well as the small GTPase ARF1p (55,56). Homologues of α-, β-, β’- and γ-COP have been isolated from rice (19), while γ-, δ- and ε-COP have been identified in cauliflower, maize and Arabidopsis (11,18). In addition, ARF1p homologues from Arabidopsis have also been identified (39,57). These findings suggested that COPI vesicles exist in plants, a hypothesis which was confirmed by the in vitro induction of COPI-coated vesicles from cauliflower cytosol (18).

COPI coat assembly is initiated by the exchange of GDP for GTP by ARF1p in a similar manner to that of Sar1p. The GDP-bound form of ARF1p interacts with p23, a Golgi membrane protein, through the myristoylated N-terminus of the GTPase (58). GDP is then exchanged for GTP through interaction with the GEF (56,59), causing a conformational shift that allows direct interaction of ARF1p with the membrane. It has been shown that myristoylation is not required for this direct interaction (60), but rather that the 17 N-terminal amino acids of mammalian ARF1p form an amphipathic structure similar to that of Sar1p that can be inserted between phospholipids in the Golgi membrane (61,62). The activated, membrane-associated ARF1p recruits preassembled coatomer from the cytosol (63). This induces curvature of the membrane, resulting in the formation of a nascent vesicle that can then bud from the membrane.

ARF1p has multiple functions within the cell in addition to its regulatory role in COPII-mediated transport. These functions appear to be defined by different GEFs that can all activate ARF1p (64). For example, it has been shown that ARF1p is involved in the regulation of the BP80-mediated route to the vacuole in plants (50), probably due to the interaction of ARF1p with constituents of the clathrin coat (65–67). The identification of GNOM, an Arabidopsis ARF-GEF that is localized to the endosomes (68), in conjunction with the data presented by Piripl et al. (50), supports the hypothesis that the nature of the GEF determines the action of ARF1p. It is not clear which GEF is responsible for initiating COPI vesicle formation, but the most likely candidates are thought to be Gea1p, Gea2p (69) and ARNO (70). Of these, only the Gea GEFs have been identified in Arabidopsis (71), suggesting that in plants at least, Gea may be responsible for the activation of ARF1p, leading to COPI vesicle formation. Further investigation is required to confirm this, as the existence of an alternative, plant-specific GEF cannot be ruled out.

Uncoating of COPI vesicles requires GTP hydrolysis to allow ARF1p to dissociate from the vesicle membrane. A GAP is involved in this process, and both plants and animals possess several ARF-GAPs, all of which contain a conserved catalytic domain (39,72). It is not clear which of these is responsible for COPI uncoating. Fifteen ARF-GAPs have been identified in Arabidopsis, some containing plant-specific regions. All 15 have homology to one another, but also exhibit considerable diversity within this homology (39). This suggests that the different ARF-GAPs may perform a variety of functions within the cell, which might be expected given that 12 ARF GTPases as well as numerous ARF-related GTPases have been identified in Arabidopsis (39), although their respective functions are not yet clear. It is also possible that different GAPs can act on the same ARF and regulate its function in a similar manner to that of the GEFs.

**Disruption of Retrograde Transport Inhibits Forward Protein Trafficking**

The importance of the COPI pathway in maintaining both retrograde and anterograde transport has been demonstrated in several studies. Brefeldin A (BFA) is a drug that inhibits several ARF1-GEFs including Gea1p and Gea2p (59,73), preventing the activation of ARF1p and the subsequent formation of COPI vesicles. The inhibitory effect of BFA on COPI vesicle formation in plants was demonstrated directly by Piripl et al. (18) using extracts from cauliflower. Various other studies have observed the effects of BFA on the plant-secretory pathway, both in
terms of its functionality (6,50,74,75) and in terms of its morphology (76–79). The morphological effects of BFA on the plant secretory pathway appear to vary depending on the system studied, the concentration of the drug used and the exposure time. In tobacco BY-2 cells, prolonged treatment with 10 μg/mL of BFA results in the sequential incorporation of Golgi membranes into the ER and the formation of a so-called BFA compartment composed of both organelles (76). In contrast, treatment of maize roots with 100 μg/mL of BFA has a much less pronounced effect (77), although the same concentration of BFA in tobacco leaf cells causes the complete disappearance of Golgi bodies (80). Much lower concentrations of BFA have a dramatic effect on the function of the secretory pathway, 0.3 μg/mL being sufficient to reduce the secretion of a marker protein by almost half in tobacco leaf protoplasts (50). This indicates that COPI transport is required in order for anterograde protein trafficking to occur. However, BFA may affect other pathways within the cell (79), causing complete disruption of the protein transport machinery and general collapse of protein trafficking.

A more specific disruption of COPI-mediated transport can be achieved by using mutant forms of ARF1p that are restricted to either the GTP or GDP-bound form. The inhibition of vesicle budding (GDP-restricted form) or fusion (GTP-restricted form) both upset the fine balance between anterograde and retrograde transport, resulting in an inhibition of both pathways (50,78,81,82). These studies have clearly demonstrated the requirement for retrograde transport in order to maintain anterograde transport. When one of the pathways is inhibited, it is likely that a build-up of membrane and vesicle proteins occurs within the target organelle, leaving a depletion of these important vesicle components in the donor organelle that eventually prevents transport from occurring.

What is the Signal?

Studies in mammalian cells have shown that certain membrane cargo molecules are selected for incorporation into COPII transport intermediates and that these are concentrated in specific domains of the ER, termed ERES (83–87). Signals in the cytosolic domains of transmembrane proteins are thought to mediate the recruitment of the COPII coat to the membrane, thereby inducing vesicle formation. It is not clear whether the same mechanism operates in plant cells, although the high degree of homology between the COPII components in plants and other systems (38) suggests that the signals involved may be similar. Co-expression of GFP-tagged membrane cargo proteins and Sar1-YFP has been used to demonstrate the induction of ERES formation in plants (6), and a recent study has shown that a plant p24 protein can interact with both COPI and COPII coat proteins in vitro (88). A similar signalling system also operates for the assimilation of transmembrane proteins into COPI vesicles for transport back from the Golgi to the ER in both mammalian and plant cells (15, 53, 54). However, several different types of export signals have been identified in yeast and mammals (89), and further study is required to demonstrate whether all of these signals are functional in plants or whether certain transport mechanisms are specific to different systems.

It has been shown that soluble proteins are exported from the ER in plants by a COPII-dependent bulk flow mechanism (12,14). This means that proteins that are intended to remain in the ER may be exported as far as the Golgi apparatus along with those travelling to distal locations. The escape of proteins from the ER in this manner is combated by the presence of an H/KDEL motif at the C-terminus of the protein, which is recognized by a Golgi-localized membrane receptor (ERD2p) (90). This receptor induces the activation of ARF1p in mammals by recruiting the GEF (17,91), and thereby causes the formation of COPI vesicles as described above. It is not clear whether soluble proteins simply diffuse into COPII carriers that are created in response to an accumulation of membrane proteins that contain specific signals for export. It is possible that a receptor for specific soluble proteins that need to be exported from the ER exists in plants, and that this could allow a fast-track system of transport from ER to Golgi. Endoplasmic reticulum-export receptors (termed Erv14p and Erv29p) for certain soluble and transmembrane proteins in yeast have been identified (92–95), although it is not known whether homologues of these receptors exist in plants.

A di-acidic or di-basic motif in the cytosolic tail of transmembrane cargo proteins is thought to be important in recruiting the COPII coat in mammals (85,86,96), but other signals that may be involved have also been identified (89). The type I transmembrane protein ERGIC-53, which localizes to the ERGIC in mammals, contains a di-aromatic motif that is reported to be important for its export from the ER (97). This is similar to the signal identified in plants by Contreras et al. (88), although the function of this signal has not yet been analysed in vivo in plants. It has been shown that the length of the transmembrane domain plays an important role in regulating the final destination of single-spanning membrane proteins in plants (98), but export signals may play a role in increasing the rate of export of proteins from the ER. It may also be that export signals can override the length of the transmembrane domain in determining the destination of the protein. Much more investigation is evidently needed in plant cells before any conclusions regarding protein signals required for ER export can be formed.

Mechanisms of Protein Transfer Between ER and Golgi in Plants

It is generally assumed that the function of ERES is to select and concentrate cargo molecules into vesicles for export to the Golgi (6,84,99–101), although non-vesicular carriers have been observed for some cargoes in mammalian cells.
Golgi stacks in plants are found throughout the cytosol and travel along actin filaments in leaves. The ER tubules in plants are also dynamic and are constantly forming and breaking connections, giving rise to an extremely motile system. Protein transport may be challenged by the relatively high mobility of the ER and Golgi, which could have led to the evolution of specific mechanisms in plants in order to permit efficient trafficking.

The mobility of the early plant secretory pathway has suggested three possible mechanisms for protein transport between the ER and the Golgi (summarized in Figure 3). The first of these postulates that Golgi bodies move along the ER to reach vesicles budding from ERES, at which point the cargo molecules can be transferred from the ER to the Golgi. This model was named the ‘vacuum-cleaner model’ and was proposed based on the movement of Golgi bodies along ER membranes. The second model is referred to as the stop-and-go model, and suggests that there is an as yet unidentified signal present on fixed ERES that causes Golgi bodies to become transiently detached from the actin microfilaments, while they acquire cargo proteins from the ER, after which they would reattach to the actin and continue to move. However, neither of these studies included data demonstrating the occurrence of cargo transport during the postulated interactions of Golgi with ERES, meaning that no correlation between cargo transport and Golgi movement has been experimentally determined.

A more recent investigation utilized advanced in vivo imaging techniques to demonstrate that ERES are capable of movement over the ER, and that they are closely linked to Golgi bodies (Figure 4). This would allow continual transfer of cargo between ER and Golgi, allowing transport to go on uninterrupted by Golgi movement. The experimental evidence provided by daSilva et al. demonstrates that the models postulating fixed ERES and motile Golgi do not necessarily reflect the peripatetic nature of the early secretory pathway in plants. The biological function of the actin-mediated movement of this system remains to be established, but does not seem to be related to the transport of cargo molecules between the ER and the Golgi bodies. The exact nature of the connection between the ERES and the Golgi bodies is also not completely understood. It has been demonstrated that the activities of the Sar1p-COPII and ARF1p-coatomer systems jointly serve to form and maintain forward protein transport to the Golgi bodies, whose components continuously circulate through the ER. Similarly, ERES are dynamic structures that exist by virtue of COPI and COPII...
cycling (6). It is difficult to envisage how Golgi bodies and ERES can move together, yet not be physically linked. The movement of the Golgi may be associated with the actin cytoskeleton via a mechanism that has not yet been investigated, while ERES may be differentiated on the ER, as the Golgi bodies travel over the ER membranes due to continuous cycling of COPI and COPII components. It is unclear why the movement of Golgi stacks through the cell should be necessary, if cargo transport can occur while they are stationary. It may be that Golgi movement is required for the efficient transport of proteins to distal locations. Further investigation is required to determine the precise nature of ER–Golgi transport.

Future Perspectives

Although our knowledge of the protein transport between the ER and the Golgi in plants has increased manyfold in recent years, there are several aspects of this process that remain elusive. In addition to specific subjects discussed earlier in this review, such as the unidentified GEF involved in COPI vesicle formation and the reason for Golgi movement on the ER network, there are wider questions to be addressed.

The direction of vesicles to their destinations is mediated by SNAREs, which together with various cytosolic factors form complexes that allow fusion of vesicles with specific target organelles. Genes encoding 54 different SNAREs have been identified in Arabidopsis and are localized on organelles throughout the secretory pathway (105,106). Six of these SNAREs are found in the ER membrane and a further nine in the Golgi membranes (106), but it is not clear whether these proteins exhibit functional redundancy or if they each have a specific function, perhaps in different pathways. Small GTPases called Rabbs are also involved in vesicle trafficking. Plants contain 57 Rab GTPases, some of which have a high homology to mammalian Rabbs, others apparently being unique to plants (107). Although a few of the Rabbs in plants have been studied in some detail (108–113), little is known about the localization and function of most of these proteins. These are just two examples of areas that remain poorly understood within the larger topic of protein transport in plant cells, leaving plenty of scope for further research in the field.

Current research into the subject of ER to Golgi transport in plants increases our knowledge of the topic on an almost daily basis. However, the complexity of the interactions between different proteins and between proteins and membranes requires yet more investigation. The advances in confocal microscopy that allow us to observe the relative rates of transport of different proteins in living cells provide an ideal tool to complement more traditional biochemical approaches. Together, these techniques can provide us with accurate, quantitative information to aid in our understanding of the pathways in the plant cell.

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