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Transport from the endoplasmic reticulum to the Golgi in plants: Where are we now?

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Abstract

The biogenesis of about one third of the cellular proteome is initiated in the endoplasmic reticulum (ER), which exports proteins to the Golgi apparatus for sorting to their final destination. Notwithstanding the close proximity of the ER with other secretory membranes (e.g., endosomes, plasma membrane), the ER is also important for the homeostasis of non-secretory organelles such as mitochondria, peroxisomes, and chloroplasts. While how the plant ER interacts with most of the non-secretory membranes is largely unknown, the knowledge on the mechanisms for ER-to-Golgi transport is relatively more advanced. Indeed, over the last fifteen years or so, a large number of exciting results have contributed to draw parallels with non-plant species but also to highlight the complexity of the plant ER-Golgi interface, which bears unique features. This review reports and discusses results on plant ER-to-Golgi traffic, focusing mainly on research on COPII-mediated transport in the model species *Arabidopsis thaliana*.

Introduction

Likely because of the presence of a large central vacuole as well as the need to produce enzymes and critical complex sugars for the synthesis of a cellulosic cell wall, the organization of the endoplasmic reticulum (ER) with respect to the Golgi apparatus is unique in plant cells compared to that of other eukaryotes. For example, in mammalian cells the ER pervades the cytoplasm and is separated from the Golgi apparatus by the presence of an intermediated compartment (ERGIC) or Vesicular Tubular Complex (VTC) that forms through the homotypic fusion of transport carriers originated from the ER [1, 2]. The ERGIC/VTC is responsible for transport of cargo to the Golgi complex along microtubules [3]. In plants no evident ERGIC/VTC has been identified and homotypic fusion of ER export carriers is thought to generate the first subcompartment of the Golgi stack [4, 5]. Indeed, the ER and the Golgi stacks are believed to be physically connected [6].

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Furthermore, the ER is sandwiched between the plasma membrane (PM) and the tonoplast at the cell cortex, and transverses the cell through transvacuolar strands which are normally one or two per cell (Fig. 1A). Similar to the ER of mammalian cells, the plant ER is a network of membranes that assume tubular geometry as well as flattened and enlarged cisternal domains [7]. However, while both in plants and mammalian cells the Golgi apparatus is composed of stacks of flattened stacked sacs called cisternae, in mammalian cells the Golgi stacks are connected and agglomerated at the perinuclear area, while in plants they are dispersed and motile [8] (Fig. 1B). Together, the distribution of the ER and the Golgi at the cell cortex, the motility of the Golgi, the likely attachment of the ER and the Golgi alongside the absence of an intermediate compartment, and the cytoskeletal contribution to ER-Golgi traffic [9] may facilitate fast transport of cargo to distal compartments and bypass the hindrance of the large central vacuole.

Despite the rapid movement of the Golgi with the ER, the membranes of both organelles are continuously remodeled at different temporal and dimensional scales as well as through different mechanisms. The ER appears to be anchored to the PM through ER-PM contact sites, but the ER tubules and cisternae are continuously interconverted through processes of membrane fusion and tubulation that require the cytoskeleton and ER shaping proteins such as the ER membrane shaping reticulons and the fusogens of the Atlastin (mammalian cells) or RHD3 (plant cells) family of GTPase proteins [10–12]. Conversely, the morphology of the Golgi apparatus is, in broad terms, more homogeneous than that of the ER, as it is composed of flattened cisternae. These are identifiable as *cis*-, *medial*- and *trans*- cisternae (Fig. 1C) progressing from the ER towards the trans-Golgi Network (TGN), a largely heterogeneous organelle at the interface of the Golgi and the PM. Nonetheless, the cisternae can appear different with the *cis*-cisternae being generally more enlarged than the *trans*-most cisternae [13]. Functionally, the cisternae are also distinct as they contain enzymes distributed along the stack in a polarized fashion to enable progressive synthesis of modification of substrates [13]. For example, immunoelectron microscopy analyses proposed that the backbone chain of the cell wall component pectin is assembled in the *cis*-Golgi but modified by methylation on the *medial*- and *trans*- cisternae [14]. Furthermore, the set of enzymes dedicated to modification of the high-mannose chain of glycosylated proteins is set progressively across the Golgi cisternae [15]. Despite the polarized organization of the cisternae and the functional compartmentalization of the stack, fluorescence recovery after photobleaching (FRAP) analyses showed that at steady state the integrity of the Golgi apparatus is maintained through remodeling of the cisternae with their membranes being reabsorbed in the ER and subsequently cycled back to the Golgi. Indeed, it was demonstrated in plant cells that fluorescent protein fusions of integral membrane enzymes distributed to the various cisternae can cycle in and out of the Golgi within a relatively short time frame (i.e., within 5 minutes) [9, 16]. These results support that the Golgi cisternae are remodeled continuously. While the organelles' necessity and the cell's benefits for reshaping of the ER and the Golgi are yet unknown, a simultaneous loss of certain RHD3 isoforms is lethal [17], implying that the ER re-shaping process is essential. As the ER maintains the machinery necessary for protein quality control, the reabsorption of Golgi membranes into the ER might ensure functionality of protein complexes in the Golgi, possibly through

quality control processes in the ER, as well as a recycle of proteins and lipids necessary for the homeostasis of membrane export from the ER.

Despite the incessant remodeling of the Golgi membranes linked to import and export of ER membrane and luminal content, the two organelles are functionally distinct. Paradoxically, an active exchange of membrane and soluble cargo between the two organelles is required for their function and their physical separation. Proteins destined to the Golgi are exported from the ER via COPII carriers and those that are required to travel back to the ER from the Golgi are shuttled in COPI vesicles (Fig. 1C). Through expression of dominant negative mutants of COPII or COPI regulators or chemical inhibition of COPI (i.e., implementation of brefeldin A (BFA), which blocks COPI), it has been shown that collapse of either transport route leads to reabsorption of the Golgi membranes into the ER [18–23], thereby compromising not only Golgi membrane integrity but also the molecular composition of the ER. Whether the entire Golgi is reabsorbed in the ER upon disruption of the homeostasis of ER-Golgi traffic is yet unknown. For example, BFA treatment of BY-2 cells over-expressing the cis-Golgi marker GFP-SYP31 leads only to a partial reabsorption of this protein in the ER [24]. These findings suggest that not all the Golgi components may be absorbed in the ER when the homeostasis of the ER-Golgi traffic is impaired, posing that different mechanisms may be in place to support the dynamics and organization of the various components of the Golgi membrane.

This review focuses on findings on COPI and COPII transport routes in the model plant species *Arabidopsis thaliana* and will highlight commonalities and unique aspects of the ER-Golgi membrane traffic across eukaryotes. Because of space limitations, emphasis is given to COPII-mediated transport; the readers are referred to recent reviews for a more in depth discussion on COPI-mediated transport in plant cells [8, 25].

COPI

A conserved set of soluble cytosolic proteins, collectively called coatomer, forms the COPI coat and it is recruited *en bloc* to the Golgi membrane by the small GTPase ADP-ribosylation factor 1 (ARF1) [26–28]. Generally, this is followed by local cargo concentration through recognition of cytosolic-exposed amino acid signals on membrane-associated proteins, deformation of the membrane into nascent vesicles that eventually bud off the membrane and are uncoated by hydrolysis of GTP on ARF1; they are then delivered to the ER where they fuse [29]. In plants, it has been demonstrated that ARF1 also recruits the golgin GDAP1 (GRIP-related ARF-binding domain-containing protein1) to the Golgi [30], implying multi-functionality for this small GTPase on the Golgi membranes.

Activation and de-activation of ARF1 depends on ARF-guanine nucleotide exchange factors (ARF-GEFs) and ARF-GTPase-activating proteins (ARF-GAPs), respectively [31–33]. The *Arabidopsis* genome encodes ARF-GEFs of the Golgi BFA-resistance factor 1 and BFA-inhibited GEF (GBF and BIG) types [34], which exhibit different sensitivity to BFA [35, 36]. For example, as the ARF-GEFs GNOM and GNOM-like1 (GNL1) are sensitive or insensitive to BFA, respectively, the different expression of these GEFs during development at a tissue-level leads to resistance or sensitivity of *Arabidopsis* tissues to the drug during

growth [35, 37, 38], suggesting that analyses of the protein secretory machinery may have to take into account tissue and development expression profiles of the proteins under study.

Unlike the coatomer and GNL1, which are distributed mainly on the Golgi, ARF1 is localized to the Golgi but also to the TGN [20, 23, 35, 37, 39–41]. These results and the evidence that ARF1 recruits GDAP1 on the Golgi and non-Golgi structure (presumably TGN) [30] imply that the activity of ARF1 is spatially regulated and that the selective recruitment of coatomer onto the Golgi membrane requires other factors besides ARF1. Also, based on morphological criteria (i.e., lumen staining, coat architecture and thickness), cisternal budding/origin, and spatial distribution in electron tomography analyses, it has been proposed that Arabidopsis cells have different types of COPI vesicles, specifically COPIa-type and COPIb-type [42]. It has been reported that COPIa-type vesicles localize with COPII vesicles between budding COPII vesicles and the *cis*-Golgi cisternae, whereas the COPIb-type vesicles are confined to the regions around the *medial*- and *trans*-Golgi cisternae and the first TGN cisterna [42]. These data have been interpreted that COPI vesicles may have spatial selectivity for the transport of proteins throughout the stack or from the stack to the ER [42]. However, this is an interesting hypothesis that waits for experimental validation. These microscopy observations imply that mechanisms must be in place for the COPI machinery to differentiate the Golgi cisternae and possibly cargo for incorporation or exclusion into the forming COPI vesicles. Universally-conserved COPI-sorting/binding motifs on the cytosolic portion of membrane cargo (e.g., di-lysine motifs) [43, 44] as well as the H/KDEL tetrapeptide at the C-terminus of soluble luminal proteins [45] have been proven functional in Golgi to ER traffic also in plants. For example, it was shown that phosphinothricin acetyl transferase (PAT), an enzyme that is secreted by the default pathway, is efficiently retained in the ER by the addition of HDEL or KDEL motif at the C-terminus [46]. This observation has proven reproducible in plant cells also with other soluble cargo, including fluorescent proteins [47, 48]. The H/KDEL motif is recognized by the ERD2 (ER retention defective2) receptor, first identified in yeast [49]. The Arabidopsis ERD2, which complements the yeast *erd2* mutant [50], has been localized in the ER and the Golgi as a fluorescent protein fusion [51]. Also, a role of the di-lysine motif in facilitating membrane cargo retrieval from the Golgi has been clearly shown in Arabidopsis using heterologous cargo [52] as well as endogenous proteins, such as the putative cargo receptors p24 proteins [53, 54]. At steady state, p24 proteins of the delta subfamily appear to be localized in the ER, but in the presence of a constitutively active ARF1 mutant (ARF1^{Q71L}), which blocks COPI vesicle budding, they also accumulate in the Golgi [55]. Furthermore, deletion of the di-lysine motif led to missorting of p24s to the prevacuolar compartment and the vacuole [55]. Together, these results strongly support conservation in the role of COPI sorting motifs across eukaryotes.

Fidelity of membrane targeting by COPI and COPII-transport is mediated by tethering factors, which are recruited onto membranes by Rab GTPases [56, 57]. The Arabidopsis genome encodes 57 Rab GTPases [58, 59] and to date only the Arabidopsis RabD and the tobacco RabB have been implicated in ER-to-Golgi protein transport [60, 61]. Tethering factors for retrograde traffic from the Golgi to the ER is mediated by the Dsl1 trimeric complex in yeast [62, 63], which appears to have homologs in mammals and plants [64–67]. The Tip20 component of the Dsl1 has been functionally characterized in Arabidopsis upon

its identification in a forward genetics screen for seed protein storage defects. The protein, dubbed MAG2, was associated with the ER and found to interact with the SNAREs SEC20 and SYP81 as well as the ZW10-domain and SEC39-domain containing MAG2-interacting proteins (MIPs) [66]. The loss of MIPs caused defects in protein storage traffic in seeds [67], supporting that MAG2 and MIPs may function analogously to the proteins of Dsl1 complex. One of the Arabidopsis MIPs proteins, MIP3, contains a SEC1-binding domain [67]. The absence of a SEC1-domain containing protein in yeast and mammalian Dsl1 implicates that, in addition to a largely conserved COPI machinery, plants may have adopted species-specific tethering mechanisms in ER-to-Golgi transport. Finally, to date several Arabidopsis SNAREs have been localized at the ER, including SYP81, SEC22 and VAMP723 as well as the plant-specific SYP71, SYP72 and SYP73 [68–71]. The role of these SNAREs at the ER/Golgi interface is still largely undefined. Inhibition of ER/Golgi traffic has been shown for some of these SNAREs in overexpression conditions [71–73]. However, SYP73 overexpression and loss-of-function studies have recently revealed membrane traffic-independent roles for this SNARE [70], supporting that additional analyses are required to further decipher the precise role of plant SNAREs at the ER and with respect to ER-Golgi traffic.

Similar to mammalian cells, in plant cells the COPI coat and ARF1 cycle on and off the Golgi membranes at different rates [30, 74]. Because the COPI coat appears to stay associated to the Golgi membranes longer than ARF1, it has been proposed that ARF1 may form a membrane domain where cargo is concentrated [30, 74]. This could facilitate efficient use of the GTP-based energy needed for COPI-mediated transport. At the ER-Golgi interface, once budded off the Golgi membrane, COPI vesicles are then delivered to the ER. In plant cells, live cell imaging studies have shown that the ER and Golgi are closely associated [51], and experiments using laser trap technology have demonstrated that individually laser-trapped Golgi stacks can be pulled with associated ER tubules [6]. Based on this evidence and the wealth of electron microscopy analyses demonstrating the close proximity of the ER and the Golgi [75], one would expect that the most energy-efficient and secure path for COPI delivery from the Golgi to the ER would be at the ER-Golgi interface. However, it has been proposed that the sites of arrival of proteins from the Golgi onto the ER, the so called ER import sites (ERIS), may be distributed along the ER and that the Golgi collects the vesicles when the Golgi is stationary [73]. A fluorescent protein fusion to SYP72 was selected as a marker of ERIS based on the criteria that in overexpression in a heterologous system (tobacco leaf epidermal cells) it does not block ER-to-Golgi protein transport and that it forms punctate structures on the ER. Nonetheless, whether SYP72 is implicated in membrane import into the ER is yet to be experimentally tested. Functional analyses may therefore help validate the premise of the proposed ERIS model.

Functional overlap and diversification of COPII in plants

There is consensus in the field that export of cargo from the ER depends largely on the COPII machinery that assembles on the ER [76]. However in plants, COPII is not the only machinery responsible for ER export. This is exemplified by the evidence that through yet-unknown mechanisms proteins like the tonoplast resident protein, VHA-a3, a vacuolar H⁺-ATPase, can reach the vacuole when the ER-Golgi interface is disrupted by the COPI-

inhibitor BFA [77]. Furthermore, none of the known Golgi and post-Golgi trafficking routes appears to contribute to the delivery of this pump to the vacuole [77], implying that some plant proteins harness COPII-independent routes for delivery to their final destination.

COPII is a set of proteins that is highly conserved at a functional level across eukaryotes. The general consensus, largely based on studies in metazoan and yeast cells, is that COPII-mediated export processes at the ER are initiated by the small GTPase SAR1 (for Secretion-associated and ras-superfamily related1), which is activated by the ER-localized transmembrane SEC12, a SAR1-specific guanine nucleotide exchange factor [78–80]. COPII assembly occurs in a step-wise fashion. Specifically, activation of SAR1 leads to extrusion of its N-terminal amphipathic helix that inserts into the ER membrane, and initiates membrane deformation for shaping into budding vesicles and recruitment of the SEC23/24 complexes from the cytosol through interaction with SEC23 [81]. This step leads to capture of COPII cargo by direct interaction of signals exposed on the cytosolic region of the cargo with SEC24 or SAR1 to form a prebudding complex [82, 83]. Subsequently, through an interaction between SEC23 and SEC31, a hetero-tetramer of SEC13/31 is also recruited from the cytosol [84–86]. The SEC13/31 complex forms a cage-like lattice with a cuboctahedral shape that serves to shape the pre-budding complex into COPII-coated vesicles. SEC16 is an additional component of COPII machinery; it is excluded from the coat and it has been proposed to participate in the regulation of SAR1-GTP hydrolysis [87–89]. COPII uncoating is driven by GTP hydrolysis onto SAR1, which is aided by the SAR1-specific GTPase-activating activity of SEC23 [90]. The uncoating can occur in proximity of the Golgi membranes, implying that COPII coated-carriers may be relatively long lived to facilitate correct targeting [91].

In the model species *Arabidopsis thaliana*, three SEC12, five SAR1, two SEC13, two SEC31, seven SEC23, and three SEC24 isoforms [92–94] have been identified through protein homology searches (Table 1). While it is still debated whether COPII vesicles are the only possible form of COPII-carriers for facilitating ER export in plant cells [75], functional dissimilarities of the COPII components across eukaryotes have not been found at a broad scale. For example, similar to metazoan and yeast cells, interference of COPII functioning through overexpression of dominant negative SAR1 mutants leads to collapse of ER export and Golgi integrity in plant cells [5, 21, 22, 95–98]. Furthermore, isoforms of the *Arabidopsis* SEC12, SAR1, SEC24 and SEC13 subunits complement the thermo-sensitivity and secretion phenotypes strains of yeast mutants bearing temperature-sensitive mutations in the analogous COPII subunits [93]. These results support that the plant COPII subunits form a COPII complex with the yeast proteins and therefore indicate an inter-species equivalence of several COPII proteins at a functional level. Indeed, SEC12 and SEC23 were also coimmunoprecipitated SAR1 in total plant cell extracts [93]. This demonstrates that SAR1, SEC12 and SEC23 can form a protein complex, as it would be expected from studies with non-plant COPII proteins. Similarly, an interaction was verified between SEC23A and SEC24A [20], SEC16A and SEC31A, and SEC31A and SEC31B with both SEC13A and SEC13B [99]; an interaction of *Arabidopsis* SEC13A or SEC13B with SEC31A homolog was also demonstrated through interaction studies *in vitro* and *in vivo* [100, 101]. Furthermore, biochemical and imaging studies in plant cells have demonstrated that the recruitment of SAR1 onto the ER is controlled by SEC12 [102] and that overexpression of

SEC12 causes partial collapse of ER export [48, 95], supporting SEC12 involvement in ER-to-Golgi transport also in plants. In addition, similar to non-plant species, loss of SEC16A leads to altered dynamics of binding and release of COPII coat components on and off ER. This phenotype has been attributed to a possible role of SEC16 in regulating COPII assembly at the so-called ER exit/export sites (ERES) [99, 103–105]. Together these results strongly support that the organization and workings of the plant COPII sequence homologs is similar to that of other eukaryotes.

Despite the functional conservation, the plant COPII protein isoforms outnumber those of other species. Such diversification may support functional diversity of COPII machinery at the ER with tissue or development-specific attributes. For example, despite the ubiquitous expression and similar subcellular distribution of SEC24 isoforms [106–108], a knockout of SEC24A is lethal, showing a phenotype so far associated with defective pollen development [107]. Furthermore, partial-loss of function alleles of *SEC24A* cause defects in the size of sepal cells implying a critical role of SEC24A also in post-embryogenesis [109]. However, the functional role of the other two SEC24 isoforms seems different from SEC24A. Indeed, unlike a SEC24C knockdown mutation, a SEC24B knockout showed mild male sterility defects with reduction of pollen germination. Furthermore, reduced expression of both *SEC24B* and *SEC24C* affected female gametogenesis [108]. While these results indicate that SEC24B and SEC24C have an important role in plant reproduction, they also support that unlikely SEC24A their functions are overlapping and unlikely essential. The latter hypothesis may need confirmation through studies of a complete *SEC24C* knock-out. The mammalian SEC24 isoforms recognize amino acid signatures present on the cytosolic tails of the cargo proteins and have preferential binding for certain amino acid signatures over others [110], in support of a degree of specificity for cargo selection exerted by the COPII coat. It is yet to be tested whether plant SEC24 isoforms have similar cargo selection specificity as the mammalian SEC24 isoforms. A pH-dependent binding of plant SEC23/SEC24 to a dihydrophobic (FF or YF) motif in the –7 and –8 position of p24 proteins has been shown *in vitro* [111], and the SEC24 amino acids involved in cargo recognition are conserved across in plants and yeast [107], implying that a direct interaction of SEC24 with ER export signals is likely to occur also in plants *in vivo*. Indeed, fluorescence resonance energy transfer experiments showed that SEC24A interacts with the K⁺-channel KAT1 in a signal-specific manner (i.e., D(X)E motif) [112]. Also in two independent EMS forward genetics screens in Arabidopsis, a SEC24A partial loss-of-function mutant was identified. Due to a missense mutation, the highly conserved Arg 693 residue in the cargo binding pocket of SEC24A [113] was converted to a Lys [107, 114]. The resulting SEC24A mutant (G92, ERMO2) was no longer visibly recruited at the subdomains of the ERES, where wild-type SEC24A accumulates [20, 107, 115]. These results imply that if the cargo recognition by SEC24 proteins functions universally across eukaryotes, it is most likely a prerequisite for the binding of SEC24 to the forming COPII coat. Furthermore, overexpression of neither SEC24B nor SEC24C could complement the phenotype of vegetative cells linked to the SEC24A missense mutation (i.e., formation of ER aggregates where secretory markers partially accumulated) [107], further supporting functional specificity across the SEC24 isoforms. Whether the cargo signals identified to date are the only ones SEC24 recognizes in plants is unknown, as other signals have been shown to be necessary for ER export of plant

N-glycosyltransferases [98]. These may be recognized by other COPII components. For example, SAR1 can recognize directly a dibasic motif [RK](X)[RK] located proximal to the transmembrane domain of Golgi-resident glycosyltransferases in metazoan cells [116]. In plants SAR1B can recognize a similar signal, as demonstrated in a study of the trafficking of bZIP28, an ER stress-responsive membrane tethered transcription factor, which in response to ER stress, is relocated from the ER to the Golgi for activation [117–119]. Overexpression of a dominant negative mutant of SAR1A disrupted bZIP28 export from the ER [120]. Also, it was found that SAR1B interacts with bZIP28 and that the bZIP28 region harboring the pairs of basic residues (i.e., lysine) is critical for the interaction of bZIP28 with SAR1 and its export from the ER [121]. Whether the Arabidopsis SAR1 isoforms are functionally overlapping in recognition of the dibasic region for cargo exit is not known. SAR1A, SAR1B and SAR1C have been localized to Golgi-associated ERES, but SAR1C has been also visualized also at non-Golgi associated ERES [96, 120]. It has also been demonstrated that when overexpressed as fluorescent protein fusions in tobacco leaf epidermal cells, Arabidopsis SARA1A and SAR1B, which share over 90% sequence identity at amino acid level, have different subcellular distribution with SAR1B being associated to the membrane to a larger extent than SAR1A in cellular fractionation analyses and exhibiting different levels of ER export inhibition of the bulk flow marker alpha-amylase [96]. A difference in the influence on ER export of SAR1A has also been detected in comparison to SAR1C, as it was found that overexpression of dominant negative SAR1A exerts less inhibition of ER export of vacuolar aleurain compared to a dominant negative SAR1C mutant [120]. These results support that the cargo-binding requirements of the SAR1 isoforms may be different. Indeed, it was demonstrated that the functional difference of SAR1A and SAR1C is linked to the presence of a Cysteine residue (Cys84) in SAR1A but not SAR1C; in the latter, a Tyr84 is present in place of the Cys84, which is necessary for interaction with SEC23A [120]. The interaction of the cysteine residues in the SAR1A-SEC23A pair appears to be unique across eukaryotes; it has been suggested that it may prevent nonspecific interactions among other SAR1-SEC23 pairs and therefore facilitate functional specificity of the Arabidopsis SAR1A-SEC23A pair [120]. This hypothesis is strongly supported by swapping experiments whereby mutation in SEC23A of the Cys484 to Asp, the amino acid residue present in the other SEC23 isoforms in position 484, led to the ability of overexpressed SEC23A^{C484D} to disrupt aleurain export from the ER, a property that overexpressed wild-type SEC23A does not have [120]. These results suggest that in plants the COPII isoforms have acquired functional specificity to suit not only temporal or developmental stages of vegetative and reproductive tissues, but likely also to facilitate efficient ER-Golgi traffic for plant-specific cargo. Indeed, based on phylogenetic analyses SEC23A is not closely related to the human and yeast SEC23 homologs [94], further supporting that the presence of SEC23A in the evolutionary scale may be the result of a plant-specific adaptation of the COPII machinery. Unlike the other COPII genes, the expression of *SAR1A* and *SEC31A* genes (<20 fold) as well as that of SEC23A (2 fold) is induced in wild type in conditions of ER stress, and the induction appears to be dependent on the presence of bZIP28 and the other ER-stress responsive transcription factor bZIP60 [122]. Given that a dominant negative SAR1A inhibits bZIP28 export from the ER in condition of ER stress [120], the possibility exists that the SAR1A-SEC23A may be responsible for the translocation of bZIP28 to the Golgi in conditions of ER stress. However, SAR1B interacts with bZIP28 and conditions

causing ER stress enhance such an interaction [121]. Therefore, it will be interesting to test the involvement of the other Arabidopsis SAR1 proteins in the export of bZIP28 and other cargo in order to further probe the specificity or cooperativity of SAR1 isoforms and SAR1-SEC23 pairs in this process.

Functional studies have shown differences in expression profiles at a development and a tissue level for the two members of the Arabidopsis SEC13 family, with broad expression in vegetative and reproductive tissues for SEC13A, but SEC13B expression being restricted to young leaves, root apical region, lateral root primordia, and immature stamens [100]. The two SEC13 isoforms also have interesting subcellular distribution [100, 115, 123], which may underlie different cellular roles. In addition to being distributed at ERES and in the cytosol, SEC13A and SEC13B have been detected in the nucleus, suggesting additional roles for these proteins besides ER export. Intriguingly, the two SEC13 isoforms interact with SEC31 in the cytosol but not in the nucleus [100], suggesting that unlike their role at the ERES where they may be part of the COPII cage, their function in the nucleus, if any, may occur through COPII-independent mechanisms. Finally, the evidence that a knock-out of SEC31B causes pollen abortion, most likely through inhibition of secretory activities in the tapetum cells [101], further supports that the diversification of the COPII isoforms in plants have likely evolved to suit the secretory demands during development.

While the identification of most plant COPII components through homology searches is relatively straightforward, the identification of SEC16 is problematic due to the low level of sequence identity with the non-plant counterparts. The non-plant SEC16 proteins contain conserved C-terminal and central regions that have been proposed to mediate the interaction of SEC16 proteins with other COPII components [104]. As detailed earlier [99], the *At5g47480* locus encodes a protein of 1350 amino acids with low sequence similarity to human SEC16A, which is 2357 amino acids long, and yeast SEC16, which is 2195 amino acids long. The Arabidopsis SEC16A contains a central region of 223 amino acids with 31% identity to the central region of human SEC16A, and the C-terminal region of Arabidopsis SEC16A, which is composed of 330 amino acids, has only 25% identity to the C-region of human SEC16A. In a T-DNA-based forward genetics screen for the identification of secretory defects in seeds, Takagi and colleagues (2013) identified a mutation in SEC16A. It was demonstrated that the loss of SEC16A compromises ER-to-Golgi protein transport and cycling of the COPII coat components on and off-ERES. Furthermore, SEC16A was found to be localized at the ERES. Therefore, despite the low levels of similarity at a protein sequence overall, the results support that the *At5g47480* locus encodes a plant SEC16 isoform. Intriguingly, the Arabidopsis genome contains a gene adjacent to the *At5g47480* locus (*At5g47490*) that is expressed at lower levels than SEC16A (ATTED-II database) and encodes a protein with 68% identity and 75% similarity with MAG5/SEC16A, dubbed SEC16B [99]. The loss of SEC16B did not cause a visible secretion phenotype in seeds, and the *SEC16*RNA-mutant null seeds are viable [99], supporting that, unlike the SEC24 family in which at least one member SEC24A is essential, the function of either of the two SEC16 proteins is dispensable in plants. These results imply that either the two SEC16 isoforms function redundantly or that the requirement of SEC16 is important for COPII-mediated ER export but not essential.

Together the characterization of COPII proteins in plants supports a diversification both at a developmental and tissue levels but also at a molecular level. Whether the various COPII isoforms form functional pairs like SAR1A and SEC23A to favor transport of specialized cargo and whether combinations of COPII proteins preferentially accumulate at some ERES over others are yet open questions.

ERES AND TOPOLOGY OF ER EXPORT

In plant cells, COPII coat proteins are mainly distributed in the cytosol and concentrate at ERES that appear in association with motile Golgi stacks; they also accumulate in punctate structures that are not associated with the Golgi (Fig. 2B). The visualization of ERES in continuous association with motile Golgi stacks, the demonstration that COPII protein move in and out the ERES and that cargo moves in and out of the Golgi as the Golgi stacks move led to the formulation of a “secretory unit” model whereby the ERES and the Golgi form a secretory complex [95]. The evidence that the ER and the Golgi appear to be attached [6] supports the possibility that ERES constitute a subdomain of the ER membrane that is relatively stationary to the Golgi and where protein export takes place. The co-localization of cytosolic COPII markers at Golgi-associated ERES has been established in several cell types [75]. Nonetheless, it has been also proposed that the fluorescence of cytosolic COPII component tagged with fluorescent proteins may not represent ERES but rather a temporary accumulation of COPII-coated carriers in the narrow interface between the ER and the overlying Golgi stack [55]. The model was reasoned primarily by the lack of a visible overlap between an ER marker and COPII markers overexpressed in a heterologous system. Indirect support to this view can be found in non-plant systems where the partially uncoated COPII carriers can be long-lived and that tethering proteins on the *cis*-Golgi membrane, such as the TRANSPORT PROTEIN PARTICLE1 (TRAPPI) complexes, interact with the carriers through SEC23 subunits [124]. Nonetheless, a functional characterization of Arabidopsis SEC16 shed light in the subject [99]. A functional fluorescent protein fusion of SEC16A driven by the endogenous promoter in a *sec16a* null mutant was found to be present in cup-shaped punctate structures within the ER network labelled by a bulk marker. The signal of SEC16A was found to partially overlap with the ER marker signal and to a lesser extent with Golgi markers, indicating that the distribution of SEC16 marks high curvature regions of the ER, which are associated with mobile Golgi stacks. Furthermore, it was found that the distribution of SEC16A fusion proteins largely overlapped with fluorescent protein fusions to SEC24A and SEC13A. These imaging results were paired by analyses of protein turnover at the ERES. It was found that SEC16A has different dynamics on and off ERES compared to the COPII coat. Specifically, compared to SEC24A or SEC13A, whose dynamics at the membrane were similar, FRAP analyses showed that the rate of SEC16A protein exchange at the ERES was significantly slower and that the percentage of available SEC16 protein to exchange on and off ERES was also lower. These results support that SEC16A does not accumulate in partially uncoated carriers at the Golgi; if SEC16 had been part of the partially uncoated COPII-coated carriers at the Golgi, then SEC16 dynamics on and off the ERES would be equivalent to those of SEC24 or SEC13. Therefore, it appears that SEC16A protein is largely excluded from the COPII coat and that the structures labeled by SEC16A represent ERES. While these results do not exclude the

possibility that the structures labelled by fluorescent protein fusions to COPII coat components found in association with the Golgi stacks represent also Golgi-associated partially uncoated COPII carriers, the imaging analyses of SEC16A and the FRAP results on the cycling rates of SEC16 and SEC24 support that that ERES and mobile Golgi stacks are associated, consistently with the “secretory unit” model. In this view, Golgi-associated ERES may include carriers being produced by the ER and partially uncoated COPII carriers being consumed by membrane fusion with the *cis*-Golgi upon uncoating (Fig. 1B). This model does not exclude the possibility that non-Golgi associated ERES may exist in plant cell. Indeed, it has been reported that a fluorescent protein fusion to SEC24, SEC13 and SEC23 can be found concentrated in punctate structures that are not associated with the Golgi and are smaller than the Golgi-associated ERES [20, 24, 107, 115]. At least for SEC24A, these structures are unlikely the product of protein overexpression as such they were identified both in overexpression conditions in heterologous systems, but also in complemented cells that contained a non-functional form of SEC24A that is unable to bind ERES [107]. Furthermore, immuno-localization analyses with either SEC13 or SAR1 antibodies identified punctate structures not in association with the Golgi [5, 125]. It was hypothesized that non-ERES associated structures may represent early stages in the formation of new Golgi stacks [20] (Fig. 1B). In addition, a recent study based on the visualization of the SNARE SYP31 using high resolution microscopy has suggested that the ER-associated structures labelled by this marker that are independent from the Golgi stacks may represent a scaffold to build the Golgi stacks, similar to the ERGIC/VTC in mammalian cells [24]. On the other hand, it cannot be excluded that the non-Golgi associated ERES may also contribute to provide cargo to the mobile Golgi stacks, as it has been proposed for a hybrid model [24] between the secretory unit model and a kiss-and-run model, which predicted that Golgi collect cargo when they are stationary [5]. The evidence that ER-Golgi transport occurs while the Golgi moves does not exclude that Golgi stacks also collect cargo as they are stationary. In summary, the evidence that Golgi, ER, COPII coat and SEC16 labelling are in continuous association and that transport occurs as the Golgi moves with the ER supports that the membrane traffic between these two organelles occurs as predicted in the “secretory unit” model. The likely association of COPII carriers being “consumed” by the Golgi as the Golgi moves with the ER and the contribution of cargo delivery to the Golgi from non-Golgi associated ERES are plausible variants of this model. Nonetheless, whether a model ER-Golgi transport developed in one cell type may be universally applicable to other cell types is still an open question. Most topology models for ER-to-Golgi transport have been established in highly vacuolated cells. However, electron microscopy studies in Arabidopsis roots have reported that in columella cells the Golgi stacks can be found in association with any organelle [4]. Furthermore, in meristem cells, tomographic analyses of 14 Golgi stacks identified ten stacks (71%) associated with ERES with the remaining four Golgi stacks lacking the COPII scaffold connections to the ER and located at distances of more than 300 nm from the closest ER cisterna [4]. These results support the possibility that various cell types may have different organization of the ER and the Golgi as well as topology of membrane transport.

Plant ERES are tunable

The first visualization of ERES in live plant cells was reported in an analysis of the distribution of a fluorescent protein fusion of tobacco SAR1 in tobacco leaf epidermal cells [95]. When expressed alone, SAR1-YFP was cytosolic but also distributed to ERES. Interestingly, the intensity of SAR1-YFP fluorescence at the ERES was enhanced in conditions of co-expression of membrane Golgi markers. On the contrary, in cells co-expressing the ER luminal marker GFP-HDEL, the levels of SAR1-YFP fluorescence at ERES were not enhanced compared to cells expressing SAR1-YFP alone, supporting a specific effect of membrane cargo on the recruitment of SAR1 to ERES. A similar effect was later established for the recruitment of YFP-SEC24A onto ERES. Remarkably, not only the fluorescence of YFP-SEC24A was enhanced at ERES in co-expression with membrane cargo, the number of ERES was also increased compared to cells expressing YFP-SEC24A alone [126]. It was demonstrated that this phenomenon depended on the presence of the ER export signal D(X)E on the cytosolic tail of membrane cargo, as mutation of this signal reverted the effect verified for the non-mutated cargo on SEC24A distribution. Later, it was also shown that overexpression of SEC24A increased the distribution of SEC13 at ERES [115], suggesting that the recruitment of SEC24A verified in conditions of increased cargo is paired by the formation of functional COPII carriers. Together these results indicate that ERES intensity and number are tunable in response to cargo synthesis in the ER. As the Golgi stacks and ERES are in close association, it is plausible that, at least in highly vacuolated cells, the number of Golgi stacks increases along with the production of COPII cargo. This hypothesis awaits for experimental validation. In this context, it would be interesting to test whether in conditions of increased cargo production, the number of ERES that are non-associated Golgi changes also, as it would provide insights into the premise of the hypothesis that non-Golgi associated ERESs are implicated in the Golgi biogenesis.

While, at least for SEC24A-labelled ERES, the tuning seems to be dependent on the recognition of signals by SEC24A, the mechanisms that induce the enhanced recruitment of SAR1 to the ERES are yet unknown. It is possible that SAR1 accumulation is responsive to the accumulation/recruitment of cargo adapters at ERES. Members of the Erv14p/cornichon family have been proposed to function as cargo receptors in COPII transport in non-plant species [127, 128]. Whether the Arabidopsis homologs have similar role to the non-plant cornichons is yet unknown. However, it has been recently shown that OsHKT1;3, a Na⁺-transporter, interacts in the ER with a rice cornichon homolog (OsCNIH1) and that ER export of OsHKT1;3 is compromised in a *erv14p* yeast mutant [129]. These results support the possibility that ER export of cargo may depend, at least partially, on adapters that may interact with SAR1 or other components of the COPII machinery. Additional proteins have been implicated in ER export in plants. For example, the ER localized GLUP2/GOT1B (Golgi transport 1B) in rice endosperm, the putative homolog of the Got1p, which in yeast participates to COPII vesicle formation [130, 131], appears to be required for ER export of proglutein and alpha-globulin [132]. Similarly, the loss of putative tethering factors like MAG2 or overexpression of truncated versions of KMS1 and KMS2, the Arabidopsis homologs of the Drosophila TANGO5, a protein identified in a mutant screen for ER-Golgi trafficking defects [133], compromises ER export [66, 67, 134]. These results imply the

existence of additional proteins in traffic at the ER-Golgi interface besides the canonical COPI and COPII machinery. Additional functional characterization of these proteins will aid the understanding of the precise mechanisms underlying their requirement in ER export homeostasis in plant cells.

While an enhanced accumulation of COPII proteins at ERES may be due to the presence of partially uncoated COPII carriers in the ER-Golgi interface, the increased number of ERES is likely dependent on other mechanisms that facilitate the differentiation of ER domains dedicated to protein export. Related to this concept, it is yet unknown how the punctate distribution of the COPII coat proteins at ERES is established. SEC12 is an ER protein distributed in the bulk ER membrane in plant cells [5, 95]. Hence, its distribution alone is unlikely sufficient to determine a punctate localization of SAR1 and initiate a localized recruitment of the COPII coat at ERES. Nonetheless, it has been demonstrated that SEC16 accumulates at ERES that demarcate regions of high curvature of the ER [99], suggesting the possibility that ER membrane curvature-inducing proteins and/or the lipid composition of the membrane may have a bearing on the organization of ERES in plant cells. For example, in metazoans it has been shown that the phosphatase SAC1 shuttles between the ER and the *cis*-Golgi and controls the membrane levels of phosphatidylinositol 4-phosphate/phosphatidylinositol phosphate (PtdIns4P/PtdInsP), which has an effect on membrane traffic in response to growth factor signaling [135, 136]. A recent study of Arabidopsis myotubularins (MTMs), which are 3'-phosphatases that dephosphorylate the lipids PtdIns3 P and PtdIns3,5P2 and generate PtdIns5 P [137, 138], has shown that AtMTM1 localizes at the *cis*-Golgi, while AtMTM2-GFP is distributed at the ER [139]. The interesting hypothesis has been put forward that AtMTM1 and AtMTM2 may coordinate the levels of PtdIns5 P and of PtdIns3P/PtdInsP on the *cis*-Golgi and the ERES, respectively [139]. As PtdInsP isomers have been implicated in the generation of membrane subdomains that respond to signaling clues [138], the function of these proteins in the early secretory pathway may have a bearing on the differentiation of ERES in plant cells. Regardless of the mechanisms that establish the ERES in plants, live-cells analyses of ERES distribution in plant cells have demonstrated that ERES are sensitive to BFA, which leads to disassembly of Golgi stacks into the ER proceeding from *trans*-Golgi cisternae [16]. The reabsorption of Golgi membranes into the ER may alter the local distribution of ER-localized proteins and lipids necessary for the differentiation of ERES, but it cannot be excluded that BFA-induced disruption of COPI-mediated retrograde transport may affect the redistribution of proteins necessary for the maintenance of the ERES. These may include SNAREs and other COPII-cargo or proteins that specifically modify an ER region into a functional ERES.

COPII in stress

The COPII machinery appears to be involved in biotic and abiotic stress responses in plants. For example, it has been shown that Arabidopsis SEC24A interacts in a signal-specific manner with the N-terminal domain 6-kDa viral protein 6K₂, and that a functional SEC24A is required for systemic turnip mosaic virus movement [140]. These results support the possibility that viral movement *in planta* requires ER export of proteins responsible for production of ER-derived viral vesicles through the interaction of SEC24 and 6K₂ [140]. Because the viral vesicles are transported to the plasmodesmata for cell-to-cell movement, it

will be interesting to explore how the COPII carriers are hijacked away from the standard ER-Golgi route for transport of cargo to plasmodesmata. It has been shown that the interaction between bZIP28 and SAR1 can be enhanced in response to ER stress. Thus, under ER stress conditions, bZIP28 becomes available as cargo for transport from the ER to the Golgi as a result of interaction with components of the COPII machinery. Furthermore, COPII proteins have also been involved in resolving ER stress through other means. Specifically, in conditions of elevated temperature a partial loss-of-function of the master regulator of ER stress responses, the ER membrane associated protein kinase and ribonuclease IRE1, is male sterile. It has been shown that overexpression of SEC31A in the *IRE1* mutant background leads to amelioration of the male sterility phenotype [141]. These results suggest that upregulation of the COPII coat may facilitate ER export and ameliorate death-inducing ER stress. Whether the phenotype is specific to SEC31A overexpression or it may be also alleviated by other components of the COPII machinery is yet unknown.

Conclusions

In the last 15 years or so we have witnessed exponential understanding of the mechanisms of ER-to-Golgi transport in plants which has been enabled by the combination of a large number of technical advances such as electron tomography, live cell imaging, genetics and biochemistry. It is clear that all the molecular details of the process have not been figured out yet, but as techniques improve or new ones surface onto the field, new findings, hypotheses and models will be put forward. Implementing new and more established techniques in Arabidopsis loss-of-function mutants as well as in complemented Arabidopsis lines expressing functional markers of COPII and possibly of cargo (e.g., Golgi membranes, bulk flow markers) expressed at endogenous levels appears to be a much needed path forward to draw conclusive models on the function and behavior (e.g., subcellular distribution) of proteins involved in traffic at the ER-Golgi interface. Nonetheless, we have learned much on membrane transport at the ER-Golgi interface thanks to the adaptable nature of the ER-Golgi interface to external (i.e. lab-induced) perturbations. Nonetheless, as highlighted throughout this review, numerous questions on the control and topology of ER-Golgi transport and on the identity of the proteins involved in its regulation in growth and stress are still open. For examples, analyses of ER-Golgi transport have been generally carried out in mature cells; it would be therefore interesting to test ER-Golgi models in elongating cells as well as dividing cells, whose secretory demands are likely different from those of fully-expanded cells. The diversification of the components of plant COPII machinery may only partially account for differences in metabolic activity of the cells during growth. Related to this concept, a promising area of studies relates to the understanding of the intersection of functionally-related yet-distinct pathways that connect with ER-Golgi transport during growth, such as the unfolded protein response, which ensures protein production and is necessary for organ growth [142–144].

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Figure 1. Organization of ER and Golgi in plant cells

A. Diagram depicting the distribution of Golgi stacks (GA) with respect to the ER, which is distributed to the cell cortex beneath the plasma membrane and through the cytoplasm enveloping the vacuole (V) in the transvacuolar strands. B. Confocal image of an ER (ER-YK; red network) and Golgi marker (ST-GFP; green structures) in a tobacco leaf epidermal cell shows the close vicinity of the two organelles (Scale bar = 5 μ m). Image courtesy of Dr. Giovanni Stefano, MSU-DOE PRL. C. Diagram depicting a Golgi stack with *cis*, *medial* and *trans*-cisternae. COPI vesicle deliver cargo from the Golgi to the ER while COPII carriers, whose morphology is still under discussion, facilitate ER protein export to the Golgi.

Figure 2. Models for ERES and Golgi organization in highly vacuolated cells

The kiss-and-run model predicts that Golgi stacks (GA) and ERES are not continuously associated and that ERES are stable. The model also predicts that Golgi stacks pick up cargo from ERES onto which they associate in a non-continuous fashion (blue arrow) (A). The secretory model unit predicts that a Golgi stack and an ERES are continuously associated. The existence of punctate structures labelled by COPII markers that are not in association with Golgi stacks (here indicated as mini-ERES) may be interpreted as sites for the biogenesis of new Golgi stacks or as sites that contribute to ER export to the Golgi. The verified continuous association of COPII coat markers with the Golgi stacks may indicate the partially uncoated COPII carriers at the Golgi. However, as discussed in the main text, the FRAP experiments with SEC16 indicate that the Golgi stacks are associated with *bona-fide* ERES. In the figure the large and small green ribbons at the ERES and Golgi stacks represent COPII labelling.

Table 1
COPII isoforms encoded in the genome of *Arabidopsis thaliana* and reported loss-of-function phenotype

Nomenclature of AGI numbers is based the lists proposed by Robison et al., 2007, deCrane et al., 2010 and Cheung et al., 2016. The list in the table reflects largely the recent nomenclature proposed by Cheung et al., 2016, which does not completely overlap with other lists. KO: knockout mutant; KD: knock-down mutant. Characterization literature refers to publications where the AGI number for the genes/proteins used was indicated

Protein	Gene	Phenotype of loss of function mutations	Characterization reported in:
SAR1			
	<i>SAR1A</i> (<i>At1g09180</i>)	unknown	Zeng et al., 2015, Hanton et al., 2008
	<i>SAR1B</i> (<i>At1g56330</i>)	unknown	Zeng et al., 2015, Hanton et al., 2008
	<i>SAR1C</i> (<i>At4g02080</i>)	unknown	–
	<i>SAR1D</i> (<i>At3g62560</i>)	unknown	–
	<i>SAR1E</i> (<i>At1g02620</i>)	unknown	
SEC23			–
	<i>SEC23A</i> (<i>At4g01810</i>)	unknown	–
	<i>SEC23B</i> (<i>At1g05520</i>)	unknown	–
	<i>SEC23C</i> (<i>At2g21630</i>)	unknown	–
	<i>SEC23D</i> (<i>At2g27460</i>)	unknown	–
	<i>SEC23E</i> (<i>At3g23660</i>)	unknown	–
	<i>SEC23F</i> (<i>At4g14160</i>)	unknown	–
	<i>SEC23G</i> (<i>At5g43670</i>)	unknown	–
SEC24			
	<i>SEC24A</i> (<i>At3g07100</i>)	<i>sec24a</i> KO: pollen lethality	Faso et al., 2009; Conger et al., 2010
		SEC24AP443S: cell size and patterning defects	Qu et al., 2014
		SEC24R693K: ER morphology defects	Faso et al. 2009, Nakano et al., 2009
	<i>SEC24B/Lst1A</i> (<i>At3g44340</i>)	Reduced pollen germination (KO)	Tanaka et al., 2013
	<i>SEC24C/Lst1B</i> (<i>At4g32640</i>)	Male and female gametogenesis defect in <i>sec24b</i> KO <i>sec24c</i> KD double mutant	Tanaka et al., 2013
SEC13			
	<i>SEC13A</i> (<i>At2g30050</i>)	unknown	Hino et al. 2011
	<i>SEC13B</i> (<i>At3g01340</i>)	unknown	Hino et al. 2011
SEC31			
	<i>SEC31A</i> (<i>At1g18830</i>)	unknown	Deng et al., 2016
	<i>SEC31B</i> (<i>At3g63460</i>)	KO: Pollen abortion (tapetum defects); reduced ER export	Zhao et al. 2016
SEC16			

Protein	Gene	Phenotype of loss of function mutations	Characterization reported in:
	<i>SEC16A (At5g47480)</i>	Abnormal accumulation of precursor form of storage proteins in <i>sec16a</i> KO seeds	Takagi et al. 2013
	<i>SEC16B (At5g47490)</i>	unknown	–
SEC12			
	<i>SEC12 (At2g01470)</i>	unknown	DeCrane et al., 2014, Phillipson et l., 2001; DaSilva et al., 2014
	<i>SEC12L (At5g50550)</i>	unknown	
	<i>SEC12L1 (At3g52190)</i>	unknown	

COPII isoforms encoded in the genome of *Arabidopsis thaliana* and description of reported loss-of-function phenotype

Nomenclature of AGI numbers is based the lists proposed by Robison et al., 2007, deCrane et al., 2010 and Cheung et al., 2016. The list in the table reflects largely the recent nomenclature proposed by Cheung et al., 2016, which does not completely overlap with other lists. KO: knock-out mutant; KD: knock-down mutant. Characterization literature refers to publications where the AGI number for the genes/proteins used was indicated.