Review TRENDS in Plant Science Vol.11 No.7 July 2006

Seeking a way out: export of proteins from the plant endoplasmic reticulum

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The functionality of the secretory pathway relies on the efficient transfer of cargo molecules from their site of synthesis in the endoplasmic reticulum (ER) to successive compartments within the pathway. Although transport mechanisms of secretory proteins have been studied in detail in various non-plant systems, it is only recently that our knowledge of secretory routes in plants has expanded dramatically. This review focuses on exciting new findings concerning the exit mechanisms of cargo proteins from the plant ER and the role of ER export sites in this process.

Uncovering the mechanisms of protein transport in plants

In recent years, many significant discoveries have aided researchers in identifying components of the early secretory pathway that mediate its dynamic behaviour; these findings also highlight how this pathway differs between plants, animals and yeast. Several models have been put forward to reconcile the mobility and organization of the early secretory pathway in plants with mechanisms for efficient ER protein export. We highlight recent advances that clarify the mechanisms used to export proteins from the ER, and discuss the current controversy surrounding models suggesting different distributions of ER export sites (ERES, see Glossary) relative to Golgi bodies. This review comes at an exciting time because researchers are now elucidating the mechanisms involved in the dynamic early secretory pathway in plant cells.

Plants, yeast and mammals: similarities and differences

The endomembrane systems of plants, yeast and mammals all function in a similar capacity: to synthesize and transport secretory cargo molecules to their final destination within the cell or its boundaries. The mechanisms by which proteins are exported from the ER have been studied in considerable depth in yeast and mammalian systems compared with those in plants [1,2]. Although plants appear to use essentially the same protein machinery as other systems for trafficking in the secretory pathway, in some cases they have evolved unique characteristics to serve plant-specific needs. For example, plant cells have established specializations in soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) associated with the vacuolar machinery, which is necessitated by the presence of different types of vacuoles in plant cells [3]. Similarly, the existence of a Golgi-mediated route for protein transport to the chloroplast [4] provides an additional order of complexity that differentiates the plant secretory pathway from those of other kingdoms. Mammals possess an additional organelle termed the ER-to-Golgi intermediate compartment (ERGIC) that has not been observed in yeast and plants [5]. These differences between plants, mammals and yeast suggest that caution is required when forming hypotheses about the secretory pathway of plants based on data from other systems.

Protein transport in the early secretory pathway of mammalian and yeast cells is generally believed to occur via vesicular carriers [6]. Vesicle formation is controlled by small GTPases that regulate the recruitment of cytosolic coat proteins (COPs) to the membrane, as well as their subsequent dissociation. The coat proteins polymerize on the membrane surface, forming complexes that deform the membrane and shape the nascent vesicle. The COP complexes are also thought to play roles in cargo selection for anterograde and retrograde transport between the ER and the Golgi. Two types of COP complex have been identified, designated COPI and COPII [2]. In mammalian cells, COPII vesicles are thought to bud from the ER and travel toward the ERGIC, where they fuse and release their contents [7,8]. It has also been suggested that COPII in mammalian cells mediates the formation of large pleiomorphic carriers to transport cargo molecules that cannot be incorporated into standard COPII vesicles

Glossary

COPI (coatomer): coat protein complex at the Golgi apparatus made up of seven structural subunits (α-, β-, β′-, γ-, δ-, ε- and ζ-COP) plus the GTPase ARF1. COPI vesicles are believed to bud from the *cis*-cisternae of the Golgi and mediate traffic from the *cis*-Golgi back to the ER (retrograde).
COPII: coat protein complex at the ER involved in anterograde protein transport. COPII consists of two heterodimers, Sec13/31 and Sec23/24, plus the GTPase Sar1.
ERES: endoplasmic reticulum export site(s) – region(s) where the transfer of cargo from the ER to the Golgi apparatus occurs.
GEF: guanine exchange factor; protein that stimulates the binding of GTP to GTPases.
GTPase: proteins of the Ras superfamily that regulate the activity of other proteins depending on whether they are bound to GTP (active) or GDP (inactive).
SNARE: soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; a class of proteins necessary for membrane fusion between vesicle (v-SNARE) and target membrane (t-SNARE).

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Available online 16 June 2006

 1360-1385/$ - see front matter doi:10.1016/j.tplants.2006.05.003

because of their size [7]. It appears that although these carriers are not coated with COPII and form adjacent to rather than at ERES, COPII is required for transporting large cargo molecules by this route [9]. It could be that COPII is required for this kind of cargo concentration through some as yet unidentified mechanism. It has been shown in plants that homologues of yeast and mammalian COPII proteins such as Sar1 and Sec12 are involved in ER-to-Golgi transport [10–13]. However, the role of COPII in cargo recruitment and/or concentration has yet to be shown.

On arrival at the ERGIC in mammalian cells, proteins can be recycled to the ER via a COPI-mediated mechanism, or transported to the Golgi. COPI has also been implicated in the anterograde step from ERGIC to Golgi. Similarly, evidence has been presented that COPI-coated vesicles can bud directly from the ER membrane in yeast [1], and it has been postulated that this mechanism is also present in mammals [14]. It has yet to be defined whether COPI is responsible for similar processes in plants, or whether ER–Golgi anterograde transport is mediated solely by COPII [15]. This question arises because dominant negative mutants of ARF1, the GTPase that initiates COPI vesicle formation, have been shown to disrupt ER-to-Golgi transport [15–17]. COPI is associated with retrograde protein trafficking from Golgi to ER, whereby COPI-coated vesicles bud from the *cis*-Golgi and fuse with the ER membrane. The presence of COPI homologues around the *cis*-Golgi in plant cells suggests a similar function in this system [18,19]. Evidence has also been presented for anterograde intra-Golgi transport mediated by COPI in yeast and mammals [20,21]; the possibility that this also occurs in plants cannot be excluded.

At the cellular level, one of the main characteristics that sets the plant secretory pathway apart from those of other systems is the mobility of the ER and Golgi apparatus. In plant cells, the Golgi apparatus exists as multiple stacks distributed throughout the cytoplasm [22] (Figure 1). The use of fluorescent Golgi markers in live cells has shown that plant Golgi stacks are capable of translational movement [23]. This contrasts with the centralized, microtubule-based location of the mammalian Golgi apparatus [24], and also differs from the much slower movements of Golgi elements in yeast [25]. The fundamental mechanism of plant Golgi stack mobility involves actin–myosin motors; this was demonstrated using actin-disrupting drugs and a myosin inhibitor [26,27]. Actin-disrupting drugs also prevent the ER from further remodelling [28], confirming previous indications of a connection between the ER and the actin–myosin system [29]. A recent study has provided evidence that actin filaments interact with a Golgi-localized protein, KAM1/MUR3, thus contributing to proper endomembrane organization in *Arabidopsis* [30]. The putative plant Golgi myosin remains unknown, as does the entity that provides an interface between the ER and the actin cytoskeleton. The identification of additional components is essential to further our understanding of the regulatory mechanisms controlling Golgi mobility.

Golgi bodies retain remarkable stability during their movement; the stacks lose neither structural integrity nor intra-Golgi transport despite the considerable shearing forces generated by the motion [12]. Early electron microscopy data revealed a ribosome-free zone around Golgi stacks as well as the presence of membrane-linking intercisternal filaments in some Golgi [31]. These studies suggested the presence of a Golgi matrix that might play a role in stabilizing the stack [32]. More recent reports in both mammals and yeast have revealed several membrane

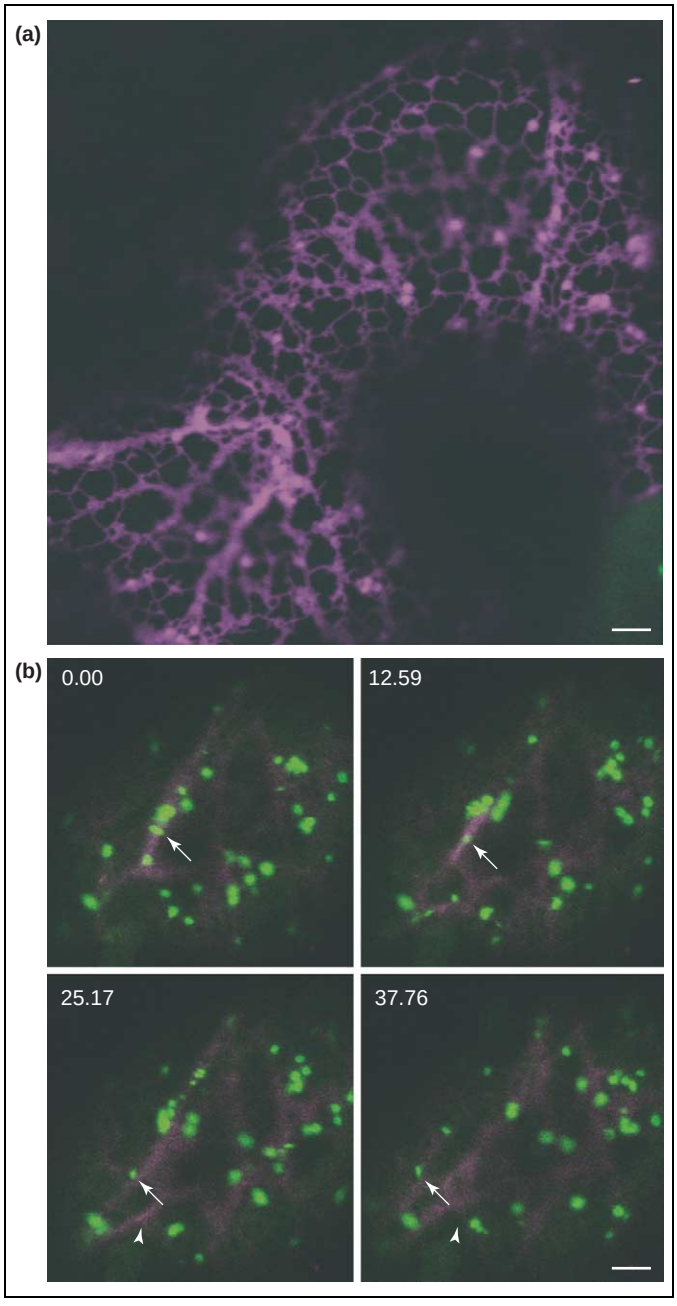

Figure 1. The endoplasmic reticulum (ER) and Golgi apparatus in plants are highly motile. **(a)** Confocal laser scanning micrograph of the ER and Golgi marker AtERD2–YFP [42] expressed in tobacco leaf epidermal cells. The ER is a mesh-like structure of membranes, with the Golgi apparatus present as discrete stacks distributed throughout the cell. **(b)** Dual-colour images demonstrating the mobility of the early secretory pathway in tobacco leaf epidermal cells. The ER is labelled with AtSec12–YFP [12] (magenta, arrowheads) whereas the Golgi apparatus is labelled with AtGONST1–GFP [81] (green, arrows). Images of the same cell were taken at different time points (seconds, shown at top left of each image) to emphasize the mobility of both ER and Golgi. Scale bars = 5 μm.

proteins that might form part of the Golgi matrix [33]. Analysis of sequence alignments has suggested that homologues of these proteins also exist in plants [31]. Furthermore, the recent identification of AtCASP [34] and AtGRIP [35–37], *Arabidopsis* homologues of known mammalian golgins [38,39], has confirmed this prediction. The role of matrix proteins in the maintenance of Golgi integrity remains to be established.

ER export pathways – what are the options?

Export of proteins from the ER to the Golgi apparatus in plants occurs at least in part by a COPII-mediated mechanism [1,10]. Whereas in other systems it has been shown that the COPII coat causes the formation of vesicular intermediates [40,41], no evidence for this has yet been presented in plants. It might be the case that COPII vesicles in plants are unstable entities; however, it is possible that transient tubular connections occur between the ER and Golgi that could mediate protein transport between these two organelles [42,43]. Support for this hypothesis is provided by the close proximity of the two organelles, as well as the observation of ER subdomains (ERES) close to the Golgi apparatus that contain essential proteins for export (COPII and SNAREs) [12]. It is also possible that the COPI coat, which is known to mediate retrograde Golgi–ER transport [18], could play a further role in forward transport [15,42]. Direct evidence for this has been found in yeast and mammals [20,21,44]; in plants, inhibition of COPI-mediated transport reduces the anterograde transport of secretory marker proteins [16]. The COPI machinery is also required for maintaining ERES integrity as well as for exporting proteins from the ER, although it remains to be established whether COPI plays a direct or indirect role [12,15]. Abolition of anterograde ER-to-Golgi protein transport, owing to disruption of the retrograde COPI machinery, might be caused by a reduction in the recycling of components to the ER that are essential for anterograde protein transport [15].

COPII-independence – a vacuolar speciality or widespread phenomenon?

In addition to the mechanisms described above, alternative ER export routes that are thought to be independent of the COPII-mediated ER-to-Golgi pathway have been postulated [45–47]. This suggests a requirement for alternative subdomains of the ER that are specific to these COPII-independent pathways, although the mechanisms by which the pathways operate remain to be identified. The majority of these routes are thought to transport soluble proteins directly to the protein storage vacuole (PSV) by means of large vesicles containing aggregates of storage proteins, avoiding the Golgi apparatus [46,47]. In addition to these aggregates of soluble proteins, various membrane proteins travelling in the same large vesicles have been identified [47,48], some of which might act as receptor proteins to select specific cargo molecules for transport via this route. It has been postulated that there is a pathway between the Golgi apparatus and these intermediates because proteins found on the periphery of the large vesicles can carry Golgi-mediated modifications [49]. A Golgi-dependent pathway to the PSV has also been proposed [50] whereby electron-dense vesicles act as transport intermediates. It might be the case that these dense vesicles fuse with the larger ER-derived vesicles *en route* to the PSV, or that an as yet unidentified mechanism is involved. As well as these routes, which have been analysed mainly by electron microscopy, an alternative COPII-independent route has been proposed based on biochemical data [45]. In this study, the plant-specific insert of the soluble vacuolar protein phytepsin was shown to be important not only for targeting the protein to the vacuole, but also for its transport in COPII intermediates. Deletion of the plant-specific insert resulted in secretion of the protein by a route that is insensitive to overexpression of the guanine exchange factor (GEF) Sec12, which has been shown to inhibit COPII-mediated secretion of cargo proteins [10,12]. It is not clear whether the route proposed for phytepsin is independent of the Golgi apparatus, like those transporting aggregated storage proteins to the PSV. The vacuolar compartment to which the cargo proteins are transported is also unclear because phytepsin has been found in both the PSV and the lytic vacuole [3]. Clarification of these transport routes and their connections is essential to increase our understanding of protein transport at the ER level in plants.

Rabs and SNAREs – elusive effectors

Rab proteins are small GTPases that have been implicated in many transport steps in the secretory pathway [51]. The *Arabidopsis* genome contains a wide diversity of these proteins; more have been identified in *Arabidopsis* than in either mammals or yeast [52]. Little is known regarding the functions of many of these proteins in plant cells, although Rab1 has been implicated in ER-to-Golgi transport [53] and the localization of various other Rabs in the late secretory pathway has been demonstrated by means of fluorescence microscopy [54–57]. It is thought that Rabs interact with SNAREs, which are membrane proteins that are involved in membrane fusion events [58]. Fifteen SNAREs that might function in transport between the ER and Golgi have been identified in plants, six localized at the ER and a further nine at the Golgi apparatus [59]. A recent study on the functions of some of these SNAREs demonstrated that the overexpression of Sec22 and Memb11 resulted in impaired transport of both soluble and membrane-spanning proteins to the Golgi [60], indicating a role in vesicle fusion at the Golgi apparatus. Overexpression of these proteins might result in the titration of other components of the fusion complexes in a similar manner to that of Sec12 in COPII budding events [10]. However, further evidence is required to confirm this hypothesis because transport at the ER–Golgi interface is complex and might involve as yet unidentified factors.

ER Export Sites – from vacuum cleaner to kiss-and-run

COPII-mediated protein export from the ER to the Golgi occurs at the ERES. It is generally accepted that ERES in animal and yeast cells are discrete domains of the ER where COPII-coated membranes and/or vesicles

concentrate after initiation mediated by the GTPase Sar1 [61]. Vertebrate ERES are dynamic structures that exhibit slow, short-range movements [62], whereas in the yeast *Pichia pastoris*, Golgi stacks are immobile and adjacent to discrete ERES that contain COPII coat proteins and Sec12 [63]. *Saccharomyces cerevisiae* has a dispersed Golgi apparatus, where COPII proteins are present in the cytoplasm and Sec12 is distributed throughout the ER [63]. In plants, Sec12 is also found throughout the ER and does not accumulate at ERES; however, the distribution of the soluble COPII components has not been agreed [12,64]. Furthermore, the unique motile nature of the early secretory pathway in plants generates a complicated challenge for the efficient transport of cargo in comparison to other biological systems. Therefore, it is likely that plant-specific mechanisms have evolved to facilitate protein trafficking. For example, it seems reasonable to hypothesize that the mobility of the plant Golgi apparatus allows efficient delivery of cargo molecules by bypassing the hindrance of the large central vacuole, a feature lacking in mammalian cells.

Several models have been proposed that combine the dynamics of membrane movements with protein transport between the ER and Golgi in plants. The 'vacuum cleaner' model suggests that Golgi stacks sweep over the ER, constantly picking up export vesicles [26]. According to this model, a random distribution of ERES results because the whole ER surface is capable of forming the sites. By contrast, the hypothesis underlying the 'stop-and-go' model proposes that Golgi stacks receive cargo that is restricted to defined export sites, which produce a stop signal that temporarily halts Golgi movement over an ERES [27]. This model supports the observation that Golgi movement is not necessary for ER-to-Golgi membrane protein transport [42]; however, it was postulated based on analyses of the movement of GFP-labelled Golgi bodies in the cytosol. The recent advent of multiple fluorescent tags has allowed new ideas to be explored experimentally [65] (Figure 2). Therefore, in addition to the early models for ER export, the theory of a mobile 'secretory unit' has been proposed [15,42]. This model is based on evidence gathered in tobacco leaf epidermal cells, where fluorescent fusions of the COPII components NtSar1, AtSec23 and AtSec24 have been shown to localize at the peri-Golgi area [12,15] (Figure 2a–c). Punctate structures labelled with these markers were found to move in synchrony with the Golgi apparatus [12,15]. Furthermore, NtSar1 cycles on and off ERES, and cargo collection can occur during Golgi movement [12]. These observations have led to the proposal that the transport of secretory cargo from the ER to the Golgi can occur in a continuous manner [12,15]. However, this model raises new questions: what is the mechanism of attachment between the Golgi and ERES and how does this attachment maintain its integrity as the mobile unit travels through the ER membrane? There is evidence of a physical link between the ER and Golgi that is dependent on active secretion [28]. Golgi stacks move at the same rate and in the same direction as photoactivated ER membrane proteins [28]. This suggests that Golgi bodies move with, not over, the surface of the ER, which supports the idea of a continuum between the ER, ERES and Golgi stack as suggested by the secretory unit model. However, further spatial and temporal investigations of the Golgi stack–ERES connection at an ultrastructural level will be needed to gain a complete picture of the carriers (vesicles, tubules or both?) that operate the transport of cargo from the ER to the Golgi in plant cells. In addition to these models, a fourth model termed 'kiss-and-run' has been put forward based on observations in tobacco BY-2 cells [64]. Studies of this system have suggested that several ERES can interact with a single Golgi stack at any one time and that Golgi–ERES associations are not permanent but are continually changing in number and position (Figure 2d). This

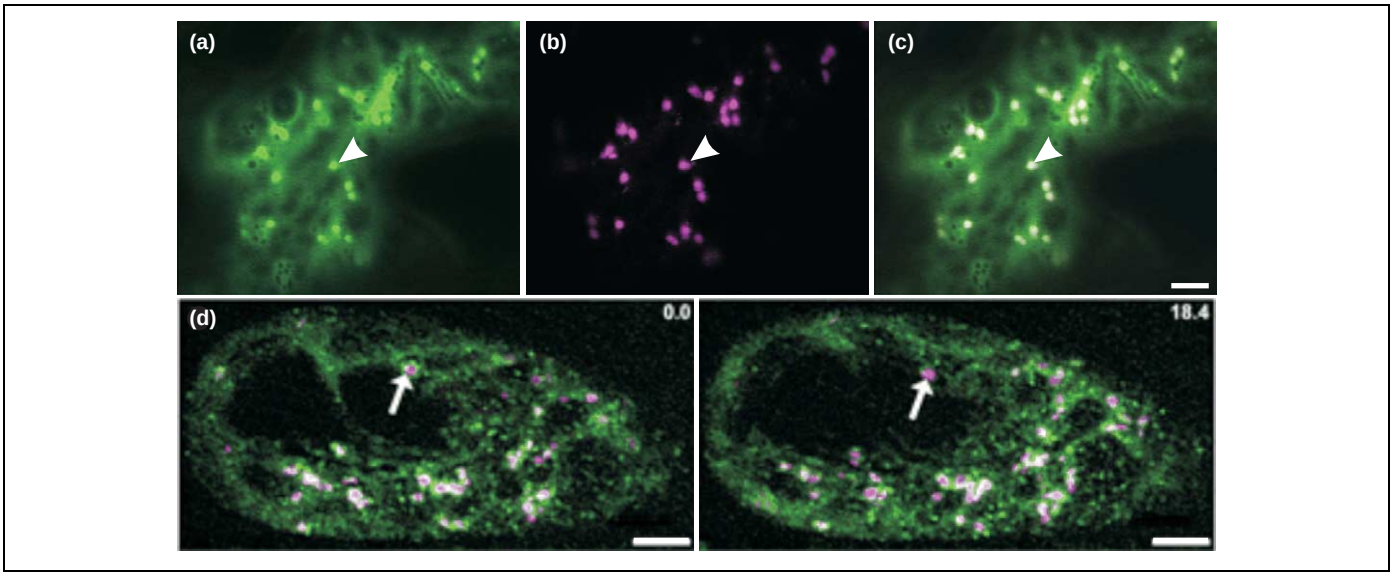

Figure 2. Distribution of ER export sites in different plant systems. **(a–c)** ERES and Golgi are closely associated in tobacco leaf epidermal cells. YFP–AtSec24 (pseudocoloured green) labels cytosol and ERES [15] **(a)** (arrowhead), whereas AtERD2–GFP (pseudocoloured magenta) highlights the Golgi apparatus **(b)** (arrowhead). The merged image **(c)** shows the colocalization of the two proteins at ERES–Golgi. **(d)** In tobacco BY-2 cells, ERES demonstrate differential levels of association with the Golgi apparatus [64]. LeSec13–GFP highlights small ERES that associate transiently with Golgi bodies, labelled by GmMan1–RFP (arrow), over time (seconds, top right of each image). (d) Reproduced, with permission, from Ref. [64]. Scale bars = 5 μm.

model envisages a discontinuous form of ER-to-Golgi protein transport and partially agrees with the two earliest models [26,27], which indicated that ERES outnumber Golgi stacks; however, there are also other considerations. A model such as kiss-and-run raises interesting questions about ERES regulation. What is the driving force behind COPII concentration and ERES formation at a seemingly random area of the ER? One possibility is that there is a signal that directs a Golgi stack to its location and that the stacks do not move randomly but are directed by ERES formation. However, Golgi mobility does not appear to be important for successful ER-to-Golgi transport because transport has been observed in both immobile [42] and moving [66] Golgi stacks. An alternative explanation is that ERES form in response to the presence of a Golgi stack; however, considering the rate of COPII turnover at ERES [67] this seems unlikely.

Finally, it has been shown that inhibiting secretion by disrupting COPI-mediated transport prevents accumulation of COPII markers at the ERES in tobacco leaves, suggesting that the formation of ERES is strictly dependent on a functional retrograde transport route from the Golgi apparatus [12,15]. However, the punctate accumulation of LeSec13–GFP in BY-2 cells was not affected by inhibition of COPI transport mediated by the fungal metabolite brefeldin A (BFA), which targets ARF1 and coatomer [64]. These results indicate that elucidating the biology of ERES will be crucial for understanding the plant secretion mechanisms. The insensitivity of LeSec13-labelled ERES to the inhibition of COPI function in BY-2 cells is similar to that shown in mammalian cells [62], but is in clear contrast to the data obtained in tobacco leaf epidermal cells, where inhibition of COPI function by chemical and genetic agents disrupted ERES [12,15]. We cannot exclude the possibility that the conflicting observations of the distribution and dynamics of LeSec13 in BY-2 cells and NtSar1 and AtSec23/24 in tobacco leaf epidermal cells might have been reached because (i) different experimental systems and/or (ii) different COPII coat markers were used in those studies. Therefore, a comprehensive examination of COPII dynamics based on the expression of structural (Sec23/24 and Sec13/31) and regulatory (Sar1) molecules in various species (e.g. *Arabidopsis*, tobacco leaf cells and tobacco BY-2 cells) is still needed to determine unequivocally the distribution of ERES in plant cells. It has been demonstrated that fluorescent fusions of NtSar1 and AtSec23/24 maintain their biological activity [12,15]. It would therefore be relevant to test whether the fluorescent protein fusion of LeSec13 is efficiently incorporated into a heterodimer with Sec31.

What determines how proteins exit the ER?

Various studies have focused on the factors that determine export from the ER in plants, resulting in many important discoveries; however, many questions remain unanswered. In the case of soluble proteins, it has been shown that a COPII-mediated bulk flow mechanism is responsible for ER exit [10]. By definition, bulk flow results in the export of proteins that are intended to remain in the ER as well as those meant for distal destinations, meaning that a retrieval mechanism from the Golgi apparatus is required to maintain levels of proteins that are resident in the ER. This is achieved by means of a C-terminal H/KDEL signal thought to be recognized by ERD2 [68–70], a receptor protein that cycles between the ER and the Golgi [42] (Figure 3a). This system has been shown to be saturable in cases where increased numbers of H/KDEL-containing ligands are present [71,72]. Perhaps some soluble proteins are 'fast-tracked' out of the ER by means of other receptor molecules; however, no such receptors have yet been identified for transport from the ER to Golgi in plants. BiP, a soluble ER resident chaperone protein, can act as a receptor for misfolded proteins and mediate their transport to the lytic vacuole where they are degraded [71]. The transport mechanism is unclear because transport to the lytic vacuole in plants is generally assumed to be receptor mediated [58].

Transport mechanisms for transmembrane proteins have been the focus of much attention in recent years (Figure 3b). These proteins are generally grouped into three classes: type I, which are oriented in such a way that their C-terminus is cytosolic and their N-terminus is found within the lumen of the secretory pathway; type II, which have the opposite orientation; and multi-spanning, which have more than one transmembrane domain and can therefore have their N- and C-termini in various different alignments relative to the membrane. It is not yet clear whether these different types of proteins are transported by the same mechanism, or whether their orientations dictate distinct modes of transport. It has been shown that type I proteins depend at least in part on the length of their transmembrane domain for transport [73], with those proteins with longer transmembrane domains travelling further through the secretory pathway. It is possible that other types of transmembrane proteins might be transported by similar mechanisms, but no evidence for this has yet been presented. A tyrosine residue within the transmembrane domain of some mammalian golgins has been shown to be important for their transport to the Golgi in mammalian cells [38]. However, a study on a plant homologue of CASP, one of the proteins used in the mammalian study, has shown that the equivalent residue has no effect on the transport of CASP in tobacco [34]. This indicates a significant difference between the two systems, reinforcing the view that assumptions regarding the plant secretory pathway should not be made based on knowledge gleaned from other systems.

Several recent publications have provided crucial insight into the transport of transmembrane proteins from the ER to the Golgi in plant cells. An *in vitro* study has indicated that a dihydrophobic amino acid motif in the cytosolic tail of a p24 protein is able to interact with components of the COPII coat [74]. No *in vivo* evidence has been presented to confirm the biological relevance of these findings, but other studies have demonstrated the functions of different signals *in vivo* [75,76]. A type II prolyl hydroxylase contains a dibasic signal that has been shown to be involved in its ER-to-Golgi transport [75], and

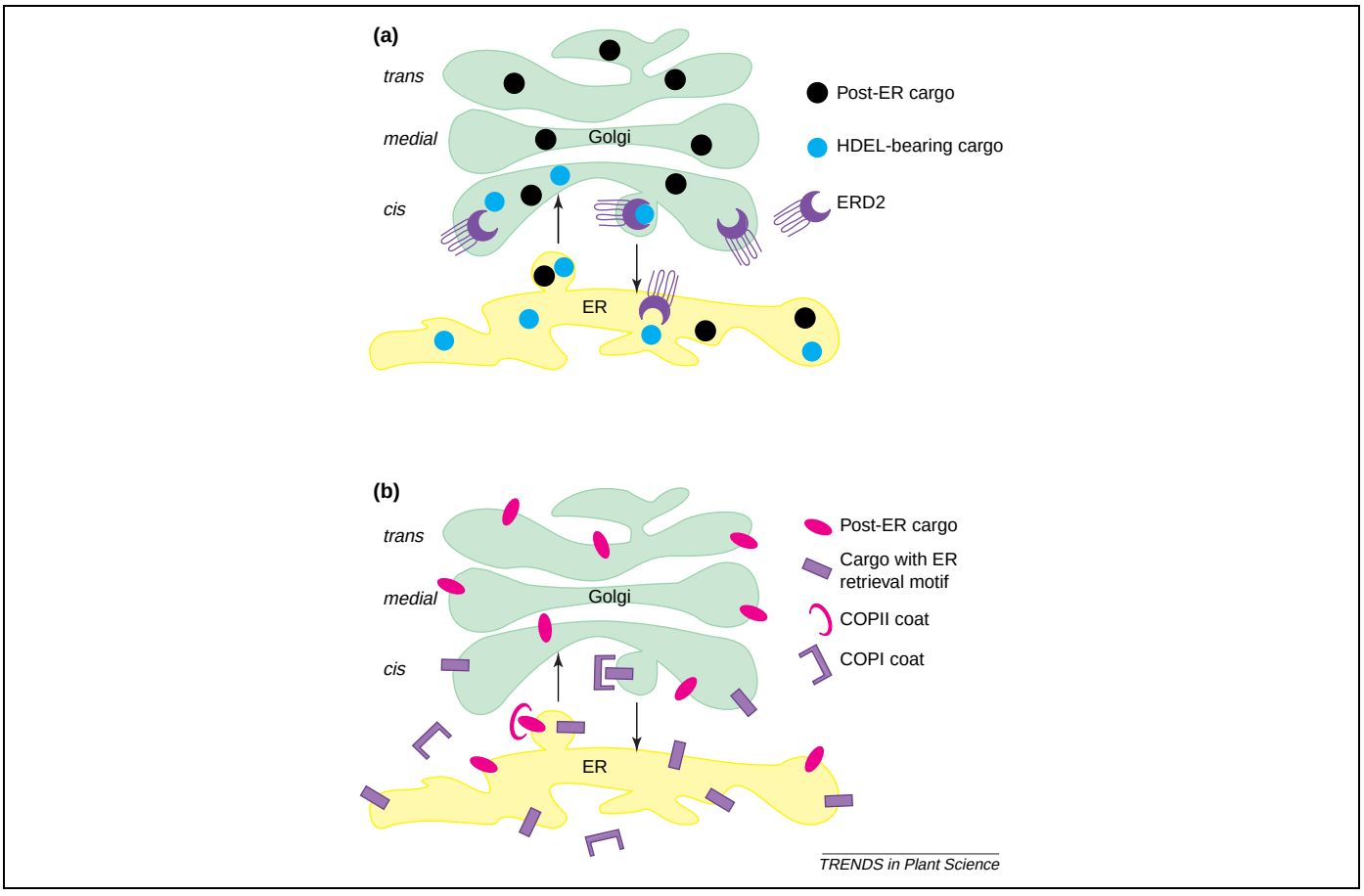

Figure 3. Models for ER export of soluble and transmembrane proteins in plants. **(a)** Soluble proteins intended for transport beyond the ER (black circles) are thought to exit the ER by means of a bulk flow mechanism, whereby cargo molecules diffuse into carriers. However, proteins intended to remain in the ER (blue circles) can also be exported to the *cis*-Golgi as the bulk flow mechanism is not selective. These proteins can be returned to the ER via transport mediated by the receptor ERD2 (purple) that recognizes a C-terminal H/KDEL motif in the cargo protein. **(b)** Some transmembrane proteins exit the ER by signal-mediated transport (magenta ovals), in which specific signals in the cytosolic domain of the cargo protein are thought to interact with COPII coat proteins (magenta crescent). In other cases, the length of the transmembrane domain influences protein export, indicating a form of bulk flow transport. This suggests that ER-resident transmembrane proteins (purple rectangles) can be exported to the Golgi apparatus in a similar manner to that of soluble proteins, and implies that a retrieval mechanism is required. This mechanism is signal-mediated; dilysine signals in the cytosolic domain of the transmembrane protein are recognized by COPI coat proteins [82] (purple brackets). COPI can assemble in the cytosol before binding to cargo, whereas COPII is thought to assemble in a sequential manner on the ER membrane.

a study on various types of transmembrane proteins has demonstrated the importance of diacidic signals in protein transport [76]. The authors also established that a diacidic motif can be dominant over a shortened transmembrane domain that normally causes the retention of the protein in the ER, indicating that there are different levels at which membrane protein transport can be regulated.

Conclusions and future perspectives

Many important advances have been made in recent years relating to the mechanisms that export proteins from the ER in plants. However, in many cases these discoveries have raised new questions that should now be addressed to increase our understanding of the workings of the plant cell, some of which are summarized in Figure 4. For example, although ERES have been visualized in different types of plant cells [12,64], it is unclear what constitutes an ERES, or how ERES relate to Golgi bodies. It appears that Sar1 is recruited to specific parts of the ER [12], but what causes this specific recruitment has yet to be determined. The GEF for COPII formation is localized throughout the ER [12], indicating that another factor must be involved in determining ERES localization of the COPII coat. Cytosolic signals identified in various types of transmembrane proteins [74–76] might interact with COPII coat components, resulting in the formation of ERES. However, this raises the matter of how the cargo proteins are recruited to specific areas of the ER. A recent publication [15] has shown that COPI components are required for maintaining protein export from the ER in plants, meaning that the possibility of COPI-mediated ER export cannot be ruled out. COPI components might also aid in the association of ER and Golgi, which could facilitate anterograde transport.

In addition to these questions regarding cytosolic and transmembrane proteins, the transport of soluble proteins might not be as simple as it initially appeared. Many of the studies on soluble protein transport were carried out on proteins from other systems, for example, fluorescent proteins (such as those from *Aequorea victoria*) [65] and various bacterial enzymes [77], introduced into the plant secretory pathway to avoid targeting signals affecting their transport. However, some endogenous soluble proteins might carry specific signals that target them for

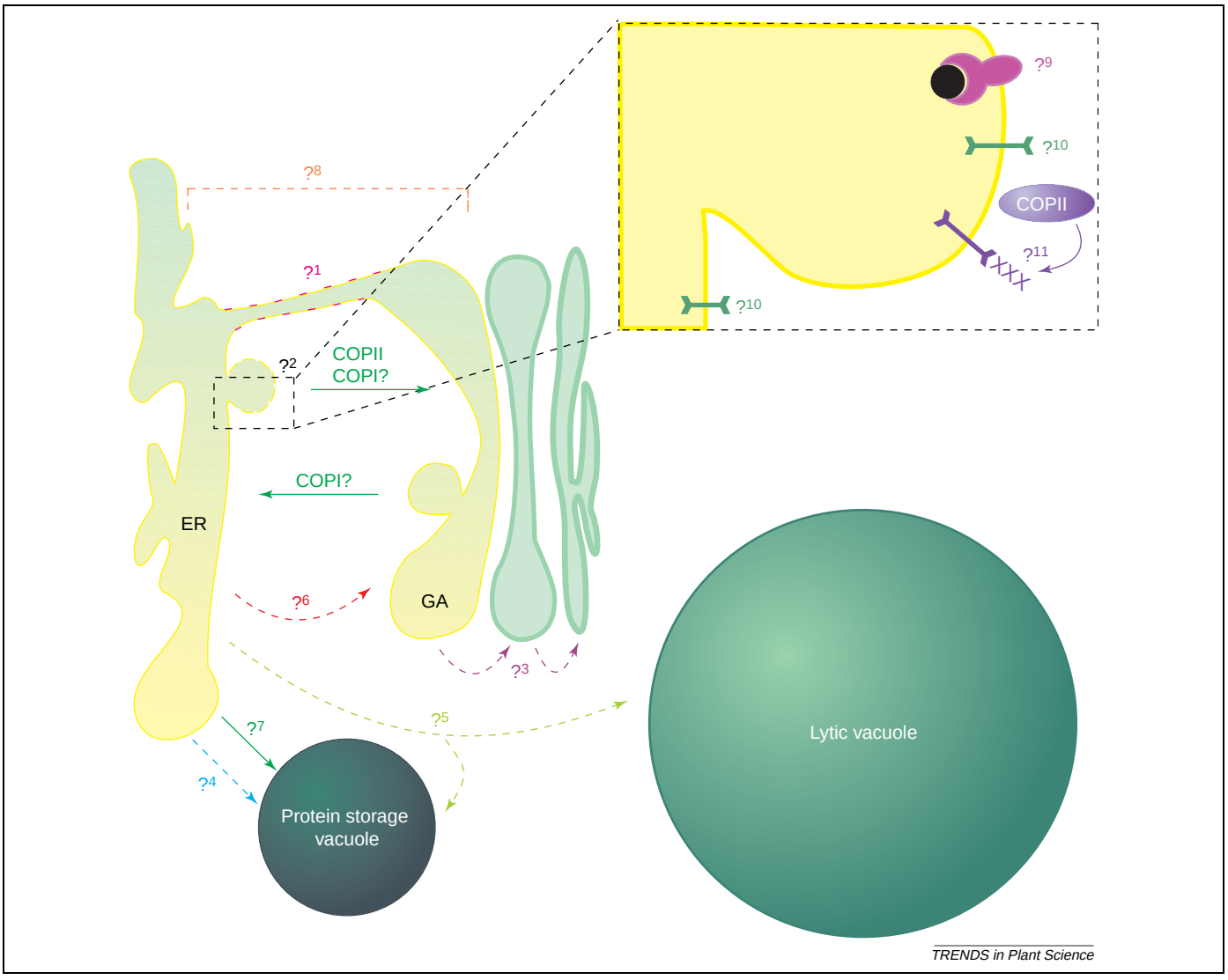

Figure 4. Many questions remain to be answered regarding the plant secretory pathway. The mechanisms controlling protein transport in the early secretory pathway are not well understood in plants owing to the unique organization of the plant cell. We present an inexhaustive list of questions regarding this subject. Each question is represented by a numbered question mark at the appropriate area of the schematic diagram. ?[1] – Data suggest that the ER and Golgi might be physically linked [28,43]; the persistence of these connections has yet to be established. In addition, it is not clear whether the linkage is via membrane connections such as the tubule depicted here or by other means. Finally, it has yet to be determined whether these connections are relevant for the transport of specific cargo proteins. ?[2] – Although COPII has been implicated in anterograde transport from the ER, visualization of transport intermediates in plants has yet to be reported, meaning that we cannot rule out the involvement of other mechanisms. It is also possible that COPII is required for cargo selection rather than for transportation. ?[3] – COPI could be involved in anterograde transport of proteins through the Golgi apparatus; this is another area that requires investigation. ?[4] – Transmembrane proteins that travel via this route to the PSV have been identified [47] but their function as receptors for soluble cargo remains to be demonstrated. ?[5], ?[6] – The COPII-independent transport of the soluble vacuolar protein phytepsin when its plant-specific insert is deleted needs to be investigated further; it has not been shown whether this route is Golgi-dependent or to which vacuole phytepsin is transported. ?[7] – A Golgi-independent route from the ER to the PSV has been indicated; however, several different carrier types have been identified. It is not clear whether these different carriers are specialized for the transport of different cargo proteins or whether they are all forms of the same transport intermediate. ?[8] – The secretory unit model of ER-to-Golgi transport proposes that ERES and Golgi move together [12]; however, the kiss-and-run model describes Golgi–ERES associations that are continually changing in number and position [64]. The nature of ERES is still being debated. ?[9] – Although a bulk flow mechanism has been demonstrated for the export of soluble proteins from the ER in plants, the possibility remains that a receptor exists for certain proteins. ?[10] – The length of the transmembrane domain of type I proteins influences their ability to travel through the secretory pathway in plants, with longer transmembrane domains travelling to more distal locations. However, no data have been published to show whether this type of transport regulation also occurs in type II or multi-spanning proteins. ?[11] – Cytosolic signals have been shown to influence export of different types of membrane proteins from the ER. These signals might interact with components of the COPII coat, leading to the formation of transport intermediates, but further investigations are required to define the specificities of these putative interactions.

ER export through interaction with a receptor. There are also further classes of proteins that have not been discussed in this review, such as the glycosylphosphatidylinositol (GPI)-anchored proteins, which have been shown to play roles in growth, development and cell wall maintenance in plants [78,79]. In other systems it is thought that these proteins are exported from the ER by lipid-mediated mechanisms [80], but little is known about this subject in plants.

It is clear that what we know so far is dwarfed by what is still to be discovered. It is also evident that what is known about the structure and activity of the early secretory pathway in yeast and mammals is not necessarily transferable to a plant system. We therefore need to continue our exploration of the mechanisms that govern the peculiar architecture and dynamics of protein transport along the plant secretory pathway.

Acknowledgements

We apologize to colleagues whose work could only be covered by reference to reviews and discussion in other papers. We thank David G. Robinson (Heidelberg, Germany) for granting permission to reproduce the image shown in Figure 2d. This work was supported by the Canada Research Chair Program and Natural Science and Engineering Research Council of Canada.

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