Mobile factories: Golgi dynamics in plant cells

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The plant Golgi apparatus plays a central role in the synthesis of cell wall material and the modification and sorting of proteins destined for the cell surface and vacuoles. Earlier perceptions of this organelle were shaped by static transmission electron micrographs and by its biosynthetic functions. However, it has become increasingly clear that many Golgi activities can only be understood in the context of its dynamic organization. Significant new insights have been gained recently into the molecules that mediate this dynamic behavior, and how this machinery differs between plants and animals or yeast. Most notable is the discovery that plant Golgi stacks can actively move through the cytoplasm along actin filaments, an observation that has major implications for trafficking to, through and from this organelle.

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The Golgi apparatus lies at the hub of membrane transport through the endomembrane system of eukaryotic cells. In addition to the trafficking and sorting function imparted on this organelle by its central location, it also functions as an important biosynthetic compartment that modifies proteins and synthesizes polysaccharides and lipids. The Golgi apparatus of most cell types is well known for its appearance in electron micrographs (EM) as a stack of flattened membrane cisternae surrounded by small vesicles[1–4]. The central biosynthetic function of the Golgi apparatus is that of a complex carbohydrate factory[2]. Thus, the Golgi is the site of modification of *N*-linked glycans on glycoproteins synthesized in the endoplasmic reticulum (ER), *O*-glycosylation of hydroxyproline-rich glycoproteins (HRGPs) and arabinogalactan proteins (AGPs), and *de novo* synthesis of cell wall matrix polysaccharides such as hemicelluloses and pectins. Whereas some of these activities are found in all eukaryotic cells, others, particularly the synthesis of complex cell wall polysaccharides, are unique to plants. Several genes encoding Golgi biosynthetic enzymes have been cloned from plants in recent years (Box 1) and this list can be expected to grow rapidly as analysis of the *Arabidopsis* genome progresses.

The relatively stable distribution of biosynthetic enzymes across Golgi stacks[5,6] is contrasted by the continuous flow of secretory products through the stack. This transport is mediated by membrane bound carriers and requires many regulatory proteins. This review highlights recent advances in identifying elements of this trafficking machinery as well as exciting new findings that illustrate the dynamics of the Golgi apparatus in plant cells.

Vesicular transport is mediated by membrane-associated proteins

Research in cultured mammalian cells and budding yeast has greatly advanced our understanding of the proteins involved in vesicle-mediated inter-organelle transport of macromolecules in the endomembrane system[7]. Although each transport step requires a different set of proteins, the basic mechanisms remain constant: formation of the vesicle at the donor compartment with the help of a protein coat; removal of the coat and translocation to the acceptor (target) compartment; fusion of the vesicle with the acceptor compartment. Numerous proteins associated with these activities have been characterized in animals and yeast[7], and several plant homologs have been identified and investigated[8–10] (Fig. 1). Interestingly, although plants appear to use essentially the same protein machinery for trafficking in the secretory pathway as animals and yeast, in some instances they deploy them in different ways to serve plant-specific needs.

Vesicular transport machinery of plants, animals and yeast is similar

A large-scale search of plant EST sequences for components of the ER–Golgi transport machinery uncovered several genes that show a significant similarity to their animal and yeast counterparts[8], and the high sequence similarity of the encoded proteins suggests that the plant homologs perform similar functions. For several proteins, this assumption is supported by their intracellular localization. In cell fractionation experiments, AtSar1, AtSec12 and the membrane-associated fraction of Sec23 (a component of COP-II coats) have been found to co-migrate with the ER-marker binding protein (BiP), an ER-resident chaperone[11,12]. This is consistent with their role in ER-to-Golgi transport in other systems. In addition, AtSec21 (γ-COP, a coatomer subunit of the Golgi-associated COP-I coat) partially co-fractionated with the Golgi marker RGP (reversibly glycosylated protein)[13] and partially with a soluble form of the coatomer complex[12]. Within the Golgi stacks, several members of the COP-I coat as well as the small GTPASE ARF1 (see Glossary) have been immuno-localized to the rims of cisternae in *Arabidopsis* and maize root tip cells[14]. These results support the conclusion that ER-to-Golgi and Golgi-to-ER transport in plants is mediated by the same set of proteins as in animal and yeast cells, namely COP-II vesicles for ANTEROGRADE TRANSPORT and COP-I vesicles for RETROGRADE TRANSPORT.

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Box 1. Cloning of biosynthetic enzymes of the Golgi

Until a few years ago, no protein involved in the biosynthetic activities of the Golgi apparatus had been isolated from plants. Instead, the existence of these enzymes was only inferred from the presence of their products that could be detected with specific antibodies (e.g. Ref. 47) or by biochemical analysis of secretory products[1]. This situation has changed dramatically and several genes encoding Golgi enzymes have now been identified. However, it should be noted that these important achievements represent at best the tip of the iceberg because the number of distinct enzymatic activities in the Golgi probably number several hundred[48].

Maturation of *N*-linked oligosaccharides

Secreted proteins often carry oligosaccharide sidechains that are added post-translationally in the ER to the NH_2-group of asparagine. These oligosaccharides are modified by Golgi-resident enzymes from their original high-mannose type to the complex type, containing a variety of different sugars in different linkages[49]. The first report of a plant gene involved in this pathway was the isolation of an *Arabidopsis* mutant lacking β1,2*N*-acetylglucosaminyltransferase I (GlcNAc-TI) activity[50]. However, it took several years before the corresponding cDNA could be isolated from tobacco (*Nicotiana tabacum*) based on conserved domains in GlcNAc-TI genes from animals[51]. A similar approach has led to the isolation of cDNAs encoding α-1,2 mannosidase I from soybean (*GmMan1*, Ref. 6), β1,2-xylosyltransferase from *Arabidopsis*[52], and *N*-acetylglucosaminyltransferase II from tobacco[53]. The localization of some of these enzymes to the plant Golgi has been tested by using GFP-fusions of the proteins in heterologous systems at both the light-microscopic[54] and the EM level[6].

Synthesis of complex cell wall polysaccharides

The Golgi apparatus is the site of *de novo* synthesis of hemicelluloses and pectins, which form the bulk of the cell wall matrix. This structurally complex network of polysaccharides and glycoproteins fills the space between cellulose microfibrils and determines in large part the biomechanical properties of primary cell walls[55]. Hemicelluloses have the same β 1,4-glucan backbone as cellulose, but carry additional sugar sidechains in regular intervals, thus providing the molecules with different physical properties[56]. One of the modifications found in several species is the addition of a terminal fucose residue to xylose sidechains. The recent isolation and characterization of a cDNA encoding the enzyme responsible for this modification, xyloglucan fucosyltransferase from *Arabidopsis*[57,58], provides the first molecular foothold in this important biosynthetic pathway. Similarly, cloning of α-galactosyltransferase from fenugreek, which is involved in galactomannan synthesis, might allow better understanding of the synthesis of these important storage polysaccharides[59].

Sugar transporters at the Golgi

For its role as a complex carbohydrate factory, the Golgi apparatus relies on the constant supply of nucleotide sugars as raw materials. These building blocks are synthesized in the cytoplasm and have to be imported into the Golgi lumen where the enzymatic activities take place. To date, the only protein involved in sugar uptake characterized at the molecular level is reversibly glycosylated protein (RGP), which is thought to deliver nucleotide sugars to the cytoplasmic side of Golgi membranes[13]. However, the biochemical characterization of nucleotide transporters, which has progressed substantially over the past few years (e.g. Ref. 60) can be expected to yield more insights and probably the first cloned genes within the near future. A better understanding of the sugar import machinery will also be essential for the goal of manipulating the sugar modifications that occur on plant glycoproteins.

Golgi-to-vacuole trafficking in plants involves unique compartments and molecules

Plant cells contain multiple types of vacuoles, which all appear to receive soluble and membrane proteins from the Golgi. This multiplicity of vacuolar targets requires a variety of sorting and targeting mechanisms that differ from their counterparts in animal or yeast cells[15–18]. Two recent studies on SNARE proteins, which are necessary for targeted-membrane fusion, highlight these sorting and targeting mechanisms[19,20].

Several *Arabidopsis* SNAREs are now known that not only show sequence similarity to yeast genes involved in vacuolar trafficking, but can also complement corresponding yeast mutants (reviewed in Ref. 10). For example, the *AtVAM3* cDNA was isolated based on its ability to complement mutations in the yeast vacuolar t-SNARE Vam3p (Ref. 21). Consistent with this observation, AtVAM3p was found on the tonoplast in cells of the shoot apical meristem[21]. However, a recent study has shown that the AtVAM3 protein localizes to a prevacuolar compartment in root-tip cells[19]. Thus, it appears that plants can use this protein at different stages of vacuolar targeting depending on the tissue it is expressed in.

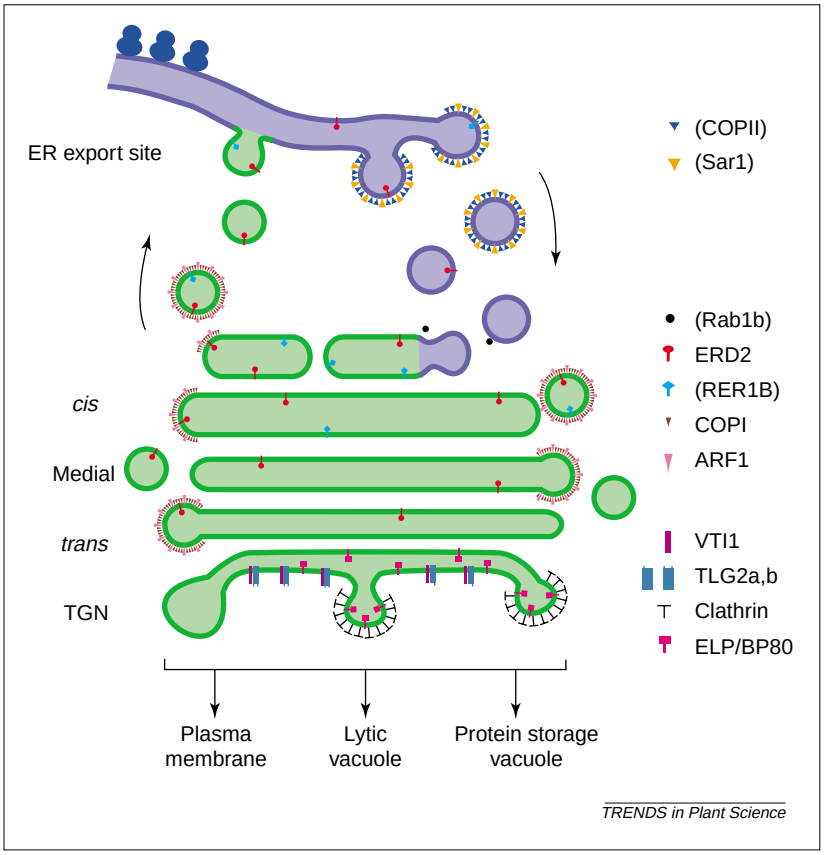

Fig. 1. Organization of vesicular transport at the plant Golgi. Plant proteins involved in various transport steps are indicated as follows: COPII, coat proteins necessary for vesicle formation at the ER (Refs 11,12); Sar1, small GTPase necessary for COP-II coat formation[11]; Rab1b, small GTPase implicated in ER-to-Golgi vesicle fusion at the Golgi; COP-I, coat proteins involved in vesicle formation at the Golgi[14]; ARF1, small GTPase necessary for COP-I assembly[14]; ERD2, RER1b, recycling receptors for escaped ER proteins[28,30]; TLG2a, TLG2b, VTI1, various SNARE proteins[20]; ELP/BP-80, vacuolar-sorting receptor[45,46]; clathrin, component of clathrin-coated vesicles[9]; tER, transitional ER, also known as ER export sites; *cis*, MEDIAL, *trans*, three functional domains of the Golgi; TGN, *trans*-Golgi network. Names in parentheses indicate that the localization of these proteins has not yet been determined by electron microscopy and is inferred by analogy from their putative animal or yeast homologs. References are given only for work on intracellular localization.

Glossary

Anterograde transport: movement from the ER through the Golgi to the final destination (e.g. vacuole, plasma membrane).
Retrograde transport: movement in the opposite direction.
Coat proteins: peripheral membrane proteins that deform the membrane into a vesicle. Often also involved in selection of cargo for vesicle.
COP-I (coatomer): coat protein complex at the Golgi, involved in retrograde transport to the ER and intra-Golgi transport.
COP-II: coat protein complex at the ER, involved in anterograde vesicle formation.
SNARE: *N*-ethylmaleimide-sensitive factor adaptor protein receptor; a class of proteins necessary for membrane fusion on vesicle (v-SNARE) and target membranes (t-SNARE).
tER: transitional ER, ER export site; sub-domain of the ER involved in export of secretory products from this compartment by vesicle formation.
***cis*, medial, *trans*:** sub-compartments of the Golgi apparatus that are characterized by different biochemical activities. Anterograde transport vesicles and their cargo molecules enter the Golgi at the *cis* side and the finished products leave at the *trans* side.
TGN: *trans*-Golgi network; largely tubular network at the *trans* side of Golgi stacks where many secretory products are sorted and packaged into different transport vesicles.
GTPase: proteins of the ras superfamily that can regulate the activity of other proteins depending on whether they have bound GTP (active) or GDP (inactive). Usually required for the assembly of membrane coats.

In addition, plants possess several closely related isoforms of certain t-SNAREs, whereas yeast contains only one. In the case of the t-SNARE Tlg2p, which localizes to the *trans*-Golgi network (TGN) in yeast, two highly similar relatives in *Arabidopsis*, AtTLG2a and AtTLG2b, have been localized to different, non-overlapping regions of the TGN in root-tip cells[20]. This finding suggests that the two isoforms interact with different proteins that associate with different functional subcompartments of the plant TGN. Another line of evidence highlighting the differences between the yeast and plant secretory systems comes from co-precipitation experiments with proteins of the post-Golgi vesicle docking–fusion machinery. This work has revealed that the plant proteins do not always interact with the same proteins as their yeast 'homologs'[20].

Taken together, these and other studies indicate that although plant cells use essentially the same machinery for vesicle transport as mammalian or yeast cells, they have adapted several of these proteins to the unique requirements of their secretory system by using them for transport between membrane compartments not found in other organisms. These examples highlight one of the pitfalls of functional assignments based simply on sequence similarity (or even complementation of mutants) and call for a more cautious use of the term 'homolog'.

A further point worth emphasizing is that plants appear to have evolved additional mechanisms to package secretory products at the Golgi that do not involve the 'generic' model of COAT PROTEIN-mediated vesicle formation. Examples for these other mechanisms are the dense vesicles that transport cargo to the protein storage vacuole (reviewed in Ref. 22) and the large slime-filled vesicles found in root cap cells[23]. Neither of these vesicles carries a discernible coat on their surface, suggesting that the formation of these transport intermediates could be driven by other mechanisms; for example, the aggregation of cargo on their lumenal side. Recent evidence shows that formation of dense vesicles can occur in the *cis* Golgi cisternae[24], but essentially nothing is known about the mechanisms that drive these sorting and packaging events.

GFP reporters are opening up new opportunities for studying Golgi dynamics in living cells

The introduction of green fluorescent protein (GFP) as a tool for studying organelle dynamics in living cells has had a profound impact on our understanding of Golgi dynamics, both in mammalian[25] and plant cells[26,27]. By following the intracellular distribution of marker proteins in individual cells in real time and under a variety of experimental conditions, researchers can now not only obtain snapshots of transient events but also observe response intermediates with high temporal resolution.

A variety of GFP fusions to Golgi-associated proteins have been employed to investigate Golgi-stack dynamics in living plant cells. These include GFP fusions to resident Golgi proteins (mammalian sialyltransferase[28]; soybean α–1,2 mannosidase I, Ref. 6), an artificial secretory product (signal peptide of sporamine coupled to GFP, Ref. 29), recycling receptors for soluble and membrane-bound ER proteins (AtERD2, Refs 28,30; AtRER1B, Ref. 30) and a putative vacuolar sorting receptor (pumpkin PV72, Ref. 31). Although this type of research is just in its infancy, it has already revealed remarkable new insights into the plant secretory system.

Plant cell Golgi stacks move along actin filaments using myosin motors

Arguably the most important discovery coming from the use of live cell markers for Golgi membranes is the observation that plant Golgi stacks display translational movements[6,28]. This is in stark contrast to the fixed localization of the Golgi complex near the centrosomes of mammalian cells[25], and also differs from the much slower movements of Golgi elements in *Saccharomyces*[32]. Plant Golgi stacks therefore not only serve as complex carbohydrate factories[2] and as sorting and packaging stations for the processed molecules (Fig. 1), but also as delivery vehicles of these products within cells. Plant Golgi stacks must be viewed as 'mobile factories' involved in the production and the distribution of secretory products.

The fundamental mechanism of Golgi-stack movement appears to be firmly established, based on studies of two transiently expressed Golgi markers (AtERD2-GFP and STtmd-GFP, Ref. 28) in tobacco-leaf epidermal cells and a stably expressed Golgi marker (GmMan1-GFP, Ref. 6) in transformed tobacco BY-2 suspension cultured cells. Actin-disrupting drugs as well as the myosin inhibitor 2,3-butanedione monoxime (BDM) block Golgi-stack movement indicating that Golgi stacks travel along actin filaments using myosin motors[6,28]. By contrast, microtubule-disrupting drugs have no negative effect on Golgi-stack movement[6]. Golgi-stack movements are not uniform but follow a saltatory, stop-and-go pattern. Analysis of stack movements revealed that even closely associated stacks display highly individualized motions, suggesting that they are not passively dragged along by a general streaming of the cytoplasm, but that they actively track along actin filaments by virtue of a Golgi-associated myosin[6]. Future work should be aimed at identifying this postulated Golgi myosin and elucidating the regulatory mechanisms that control its activity and thereby determine Golgi stack mobility.

Another aspect of Golgi movement that needs to be examined more closely is the remarkable stability of the stacks during movements. They do not seem to lose their structural integrity, and not even the intra-Golgi transport vesicles appear to be lost, in spite of considerable shear forces. EM studies have revealed the presence of membrane-linking intercisternal elements in some Golgi stacks and a ribosome-free 'Golgi matrix' around all Golgi, which might play a role in stabilizing and protecting the stack[1], but their molecular composition is unknown. However, several peripheral membrane proteins have been identified in the Golgi of yeast and mammalian cells that could be part of the Golgi matrix[33,34]. It remains to be seen whether these proteins are also found in plant cells, or whether plants have developed new ways of keeping their highly mobile stacks together.

Is the movement of Golgi stacks coupled to their transport function?

The movement of Golgi stacks has several implications for the transport of products through the secretory system. For example, vesicles involved in ER-to-Golgi transport have a 'moving target', which raises the question of how the vesicles reach their destination. In principle, two scenarios can be envisioned that allow for an efficient anterograde transfer of products between the two organelles. The first (Fig. 2a) assumes that Golgi stacks pick up transport vesicles as they move, a model supported by the observation that Golgi-stack movements along actin cables in leaf epidermal cells follow the prominent ER tubules underlying the plasma membrane[28]. However, tubular ER is mostly smooth and because it does not participate in the production of secretory proteins, it might not release many ER-to-Golgi transport vesicles. The alternative 'stop-and-go' (or 'recruitment') model (Fig. 2b) envisions that Golgi stacks stop transiently at ER export sites and that this is triggered by the export activity of such sites[6]. Thus, the 'recruitment' model proposes a mechanism that could increase the efficiency of ER-to-Golgi transport, whereas the 'moving' Golgi-stack model relies on random encounters between moving stacks and transport vesicles.

There are currently no data to support or refute either of the ER-to-Golgi transport models. However, both make specific predictions that can be tested experimentally. For example, the spatio-temporal relationship between ER export sites and Golgi stack movement might reveal whether the postulated stop signal really exists and whether Golgi stacks are indeed recruited to these sites. A common feature of the two models is that they both assume a role of stack movement in vesicle transport. Recently, this proposed functional coupling has received support from experiments with the small GTPase Rab1b. In particular, overexpression of a dominant negative form of Rab1b, which resulted in a (partial) block of ER-to-Golgi transport, also greatly reduced Golgi-stack movement[35]. Although this result does not allow a distinction between the alternative models, it is suggestive of a role of Golgi stack movement in ER-to-Golgi transport.

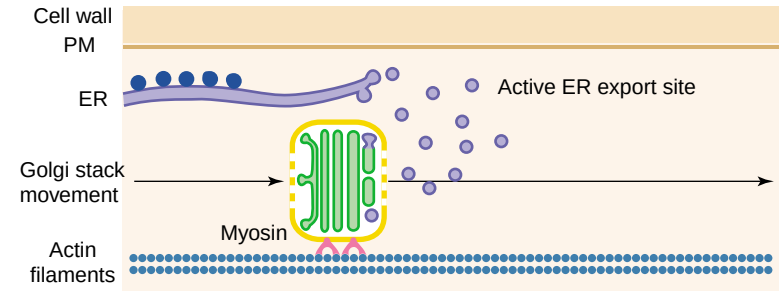

Fig. 2. Models for ER-to-Golgi transport in plant cells. (a) 'Vacuum cleaner' model. Moving Golgi stacks pick up ER-to-Golgi transport vesicles as they move through the cytoplasm past active ER export sites. (b) Recruitment model. Golgi stacks are recruited to active ER export sites by localized stop signals that transiently prevent the stacks from leaving the area, thus increasing the efficiency of ER-to-Golgi transport. Similar signals could also induce the stacks to stop at specific sites for secretion.

The discussion about models of Golgi movement has unexpected connections to another current debate. This concerns the mode of intra-Golgi transport, which in principle can occur either by vesicular shuttles between stable cisternae, or by cisternal progression balanced by recycling vesicles[36,37] (Fig. 3). If intra-Golgi transport occurs by cisternal progression, ER-to-Golgi transport should be coordinated in such a way that an entire cisterna is formed before the supply of vesicles stops. Given an average diameter of 800 nm for Golgi cisternae in BY-2 cells (A. Nebenführ, unpublished) and of 65 nm for COP-II vesicles[7], ~75 transport vesicles are needed to create a new cisterna. Even assuming that half of the cisternal membrane is actually recycled from the next-older cisterna, this still leaves a minimum of ~35–40 vesicles that have to arrive from the ER to form a new *cis* cisterna. Clearly, the acquisition of such a large number of vesicles in a short amount of time would be easier if Golgi stacks interrupted their movement near ER export sites. However, if intra-Golgi transport occurs by vesicle transport, none of these considerations has any relevance because individual transport vesicles could be incorporated at any time.

Golgi stacks are recruited to specific locations during cell division

An intriguing possibility raised by the stop-and-go (or recruitment) model is that Golgi stacks might be recruited not only to ER export sites but also to locations where their products are needed for cell wall formation or growth. One such site where Golgi products are needed in large quantities is the forming cell plate during cytokinesis[38]. It has long been suggested that Golgi stacks accumulate in the vicinity of the phragmoplast, the cytoskeletal structure responsible for cell-plate assembly[39,40]. However, only now, with GFP-tagged Golgi stacks, has it become possible to follow the (re-)distribution of Golgi stacks during cytokinesis in living BY-2 cells, thereby confirming the postulated phragmoplast association[41].

Interestingly, the redistribution of Golgi stacks to specific locations around the future plane of cell division was found to occur long before phragmoplast formation. During early metaphase many stacks accumulate near the spindle poles and in an equatorial belt underlying the plasma membrane. Quantification of the stack distribution shows that these regions have approximately two- to threefold higher stack densities than the rest of the cytoplasm[41]. The concentration of Golgi stacks is specific for this organelle because plastids and to some extent mitochondria are excluded from the areas of highest Golgi stack density. Thus, mitotic plant cells are able to sort their organelles to different locations within the cell. The molecular mechanisms for this segregation remain to be determined. Importantly, Golgi stacks in mitotic cells are positioned to ensure rapid delivery of vesicles both during the initial phase of cell plate assembly and during the centrifugal expansion phase[41]. These observations therefore support the notion that Golgi stacks can act as delivery vehicles for their products and that they can be recruited to positions where they are needed.

Cold shock and brefeldin A block ER–Golgi trafficking

Several research groups are using GFP markers to elucidate the effect of experimental treatments on the secretory pathway. For example, GFP can be converted to a secretory protein by adding a cleavable signal peptide to its amino-terminus (sp-GFP), which directs expression to the ER. Virus-mediated expression of this construct in tobacco-leaf epidermal cells results in accumulation of GFP in the cell walls[29]. The secretion of GFP could be blocked by keeping plants at 4°C for 12 h. Instead of the apoplastic fluorescence found under normal conditions, the treated plants displayed a distinctive ER-like fluorescence pattern in their epidermal cells[29], suggesting that cold shock blocks export of the marker protein from the ER. This conclusion is supported by the observation that cold-treatment leads to a redistribution of the ER-export GTPase Sar1 from membranes into the cytoplasm[11].

The same secreted GFP marker was also used to show the effect of brefeldin A (BFA) on the secretory system of living plant cells. BFA is a fungal

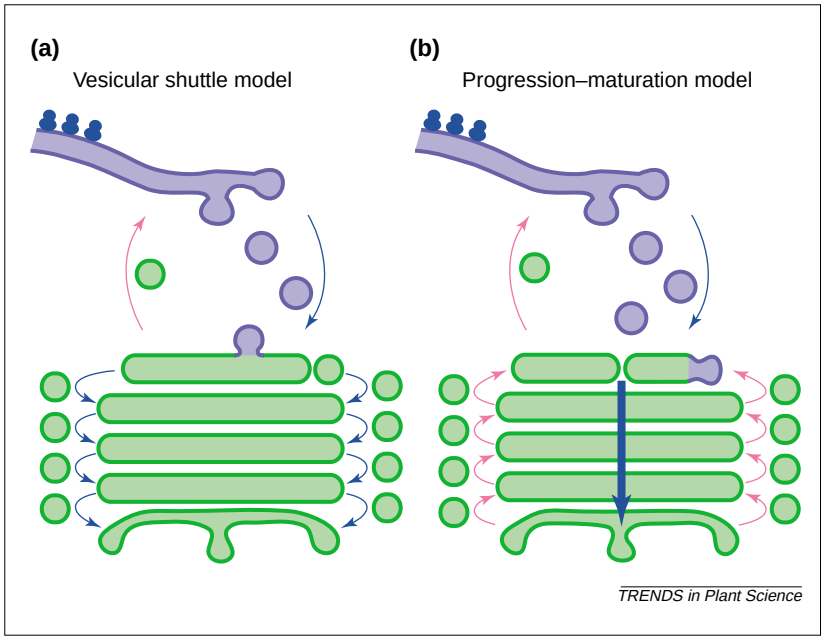

Fig. 3. Comparison of the 'vesicular shuttle' and the 'cisternal progression–maturation' models of intra-Golgi transport. (a) The vesicular shuttle model assumes that cisternae are stable entities containing specific complements of Golgi enzymes. Cargo molecules would travel through the stack by sequential transfer by vesicular shuttles, which travel in the anterograde direction (blue arrows). At the same time, retrograde transport (pink arrows) would carry escaped ER residents back to their proper location. A similar recycling of Golgi residents might occur within the stack (not shown). (b) The cisternal maturation model assumes that cisternae continuously move through the stack. New cisternae are formed on the *cis* side by coalescence of ER-to-Golgi transport intermediates. The newest cisterna receives its complement of Golgi enzymes from the next older cisterna (together with newly synthesized ones from the ER), and so on down the stack. The oldest, enzyme-depleted cisterna (i.e. *trans*-Golgi network) is fragmented into several post-Golgi transport intermediates.

metabolite that disrupts the secretory system by preventing activation of the Golgi-associated ARF1 GTPase (Ref. 42), thus blocking COP-I assembly. Treatment of leaf disks from plants expressing sp-GFP with a low concentration of BFA (10 µg/ml) for 12 h resulted in ER-like fluorescence[29]. One explanation for this effect is that anterograde and retrograde transport between ER and Golgi are functionally coupled so that inhibition of COP-I vesicle formation (and thus retrograde transport) leads to the observed accumulation of sp-GFP in the ER. Alternatively, a BFA-induced fusion of Golgi membranes with the ER, as was observed in animal cells, would effectively remove the target of anterograde transport vesicles, and thus result in the accumulation of secretory products in the ER as a secondary effect. This interpretation is supported by experiments with Golgi markers [AtERD2-GFP, STtmd-GFP (Ref. 28) and GmMan1-GFP, A. Nebenführ and C. Ritzenthaler, unpublished]. Short-term BFA treatments (30 min) of tobacco-leaf epidermal cells or tobacco BY-2 cells redistributed the reporter protein into the ER, suggesting a BFA-induced fusion of the two organelles in these cell types. More work is now needed to establish the primary site of BFA action in plants and to explain why different plant cell types exhibit vastly different sensitivities to BFA (Refs 1,43).

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Small GTPases can be engineered to interfere with specific transport steps

To address the different transport processes between the ER and the Golgi more effectively, it is necessary to use experimental approaches that have better characterized, more specific effects on the experimental system than cold shock or BFA. Recently, three research groups have created experimental tools that allow them to specifically block transport from the ER to the Golgi apparatus. The approach taken by all teams was to create dominant negative mutations of small GTPases by targeted replacement of individual amino acids, which prevent cycling between the GDP-bound and GTP-bound states. Overexpression of these constructs is thought to prevent activation of the endogenous GTPase by sequestration of an essential cofactor necessary for its activation.

Two groups chose Sar1 as a target, the small GTPase necessary for COPII recruitment at the ER (Refs 30,44), the third decided to work on Rab1b which, in other systems, is required for the SNARE-mediated fusion of ER-derived vesicles at the Golgi[35]. Transient expression of mutated forms of Sar1 (mSar1) in plant cells (tobacco-leaf epidermis[44]; tobacco BY-2 suspension culture[30]; *Arabidopsis* suspension culture[30]) together with AtERD2-GFP or AtRER1b-GFP as a reporter resulted in the expected ER-like fluorescence with no signs of the typical punctate Golgi pattern. Similarly, expression of a mutant form of Rab1b in tobacco leaf epidermis cells prevented secreted soluble GFP from reaching its correct localization and instead led to its accumulation in an ER-like network[35]. Interestingly, similar experiments with a membrane-bound Golgi marker (N-ST-GFP) resulted in only a partial block of ER-to-Golgi transport[35], raising the possibility of two different transport mechanisms for the two reporter constructs.

These tools now enable researchers to address the question of protein recycling from Golgi to ER. For this purpose, it will be necessary to first establish a steady-state distribution of the reporter construct assumed to be cycling between the organelles, before the transient expression of mSar1 blocks one leg of the journey. A change in punctate versus reticulate fluorescence will then reveal the extent and kinetics of retrograde transport. It will be interesting to see whether the parameters determined for AtERD2, which is expected to cycle rapidly between ER and Golgi, are different compared with those of typical Golgi residents, such as the *cis*-localized α-1,2 mannosidase I or the a *TRANS*-localized enzyme, such as xyloglucan fucosyltransferase.

Outlook

Recent years have brought exciting new insights into the dynamic properties and the functional organization of the Golgi apparatus in eukaryotic cells. Although most of the initial discoveries have resulted from studies of mammalian and yeast cells, plant

researchers are rapidly gaining ground in identifying the machinery involved in the biosynthetic and transport activities of the Golgi (Box 1 and Fig. 1). Possibly the most important discovery from the perspective of plant researchers is the finding that the plant Golgi apparatus is not a mere carbon copy of other systems, and that its unique features are adaptations to the special requirements of plant cells. At the molecular level this is best illustrated by the specializations associated with the vacuolar sorting machinery (sorting receptors, SNAREs), which is necessitated by the presence of different types of vacuoles. At the cellular level, this uniqueness manifests itself both in the well known dispersed organization and the novel acto-myosin based motility of the stacks, which contrasts with the centralized, microtubule-based location of the mammalian Golgi apparatus.

Future work will have to address the mechanisms of intra-Golgi transport, protein (and lipid) sorting in both the anterograde and retrograde pathways, and the functional role of Golgi-stack movement in plant cell secretion. Most of the tools for these studies are now available and researchers can begin to paint a detailed picture of this dynamic organelle.

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The glycine decarboxylase system: a fascinating complex

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The mitochondrial glycine decarboxylase multienzyme system, connected to serine hydroxymethyltransferase through a soluble pool of tetrahydrofolate, consists of four different component enzymes, the P-, H- , T- and L-proteins. In a multi-step reaction, it catalyses the rapid destruction of glycine molecules flooding out of the peroxisomes during the course of photorespiration. In green leaves, this multienzyme system is present at tremendously high concentrations within the mitochondrial matrix. The structure, mechanism and biogenesis of glycine decarboxylase are discussed. In the catalytic cycle of glycine decarboxylase, emphasis is given to the lipoate-dependent H-protein that plays a pivotal role, acting as a mobile substrate that commutes successively between the other three proteins. Plant mitochondria possess all the necessary enzymatic equipment for *de novo* synthesis of tetrahydrofolate and lipoic acid, serving as cofactors for glycine decarboxylase and serine hydroxymethyltransferase functioning.

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The prime function of the oxidative photosynthetic carbon cycle or C_2 cycle – inappropriately named 'photorespiration' – is to salvage the glycolate-2-P that is produced continuously in the light by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)[1–3]. The C_2 cycle initiated by Rubisco[4] requires a large machinery consisting of 16 enzymes and more than five translocators, distributed over the chloroplast, peroxisome and mitochondrion, in close proximity to each other[3].

The most interesting reaction in the C_2 cycle occurs within the mitochondrial matrix, where glycine molecules formed in the peroxisomes are broken down by a set of proteins (glycine decarboxylase, GDC). By acting in concert, these proteins catalyse the oxidative decarboxylation and deamination of glycine, with the formation of CO_2, NH_3 and the concomitant reduction of NAD^+ to NADH (Refs 5–8). The remaining carbon, the methylene carbon of glycine, is then transferred to 5,6,7,8-tetrahydropteroylpolyglutamate (tetrahydrofolate, THF) to form N^5,N^{10}-methylene-5,6,7,8-tetrahydropteroylpolyglutamate (CH_2–THF). CH_2–THF reacts with a second molecule of glycine in a reaction catalysed by serine hydroxymethyltransferase (SHMT) to form serine. The net reaction catalysed is (Eqn 1):

$$2\ \text{Glycine} + NAD^+ + H_2O \rightarrow \text{Serine} + CO_2 + NH_3 + NADH + H^+ \quad [1]$$

Rates of glycine metabolism by isolated leaf mitochondria from C_3 plants can exceed 1200 nmol of glycine converted to serine per mg of protein, per minute. The rate of CO_2 release from glycine decarboxylation is as much as five times the rate of normal tricarboxylic acid cycle activity. To accomplish these rapid rates of glycine oxidation, the glycine cleavage system and SHMT are present at tremendously high concentrations within the mitochondrial matrix, where they comprise about half of the proteins in mitochondria from pea or spinach leaves[9]. With matrix concentrations approaching 0.3 g/ml, it actually alters the density of the organelles[10]. Conversely, in mitochondria from non-green tissues, such as potato tubers, glycine decarboxylase is present at low concentrations[11]. From a mere trickle in the dark reflecting C_1 metabolism, glycine metabolism becomes the major metabolic reaction in mitochondria of illuminated leaves[5].

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