INVITED REVIEW

Protein Transport in Plant Cells: In and Out of the Golgi†

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In plant cells, the Golgi apparatus is the key organelle for polysaccharide and glycolipid synthesis, protein glycosylation and protein sorting towards various cellular compartments. Protein import from the endoplasmic reticulum (ER) is a highly dynamic process, and new data suggest that transport, at least of soluble proteins, occurs via bulk flow. In this Botanical Briefing, we review the latest data on ER/Golgi inter-relations and the models for transport between the two organelles. Whether vesicles are involved in this transport event or if direct ER–Golgi connections exist are questions that are open to discussion. Whereas the majority of proteins pass through the Golgi on their way to other cell destinations, either by vesicular shuttles or through maturation of cisternae from the cis- to the trans-face, a number of membrane proteins reside in the different Golgi cisternae. Experimental evidence suggests that the length of the transmembrane domain is of crucial importance for the retention of proteins within the Golgi. In non-dividing cells, protein transport out of the Golgi is either directed towards the plasma membrane/cell wall (secretion) or to the vacuolar system. The latter comprises the lytic vacuole and protein storage vacuoles. In general, transport to either of these from the Golgi depends on different sorting signals and receptors and is mediated by clathrin-coated and dense vesicles, respectively. Being at the heart of the secretory pathway, the Golgi (transiently) accommodates regulatory proteins of secretion (e.g. SNAREs and small GTPases), of which many have been cloned in plants over the last decade. In this context, we present a list of regulatory proteins, along with structural and processing proteins, that have been located to the Golgi and the ‘trans-Golgi network’ by microscopy. © 2003 Annals of Botany Company

Key words: Review, Golgi, endoplasmic reticulum, prevacuolar compartment, vacuole, plasma membrane, protein transport, protein sorting, vesicles, SNAREs, small GTPases.

INTRODUCTION

In plants, the Golgi apparatus is central to the synthesis of complex cell wall polysaccharides and of glycolipids for the plasma membrane, as well as the addition of oligosaccharides to proteins destined to reach the cell wall, plasma membrane or storage vacuoles. The Golgi apparatus is also the key organelle in sorting proteins, sending them to their various destinations within the cell. The majority of these proteins are imported into the Golgi from the endoplasmic reticulum (ER), a major organelle of the endomembrane system involved in the folding, processing, assembly and storage of proteins, as well as in lipid biosynthesis and storage (Vitale and Denecke, 1999). The relative importance of the two major Golgi functions in a plant cell, the assembly and processing of oligo- and polysaccharides on the one hand and protein sorting on the other, depends on the cell type and its developmental and physiological state (Juniper et al., 1982). Nevertheless, the two functions cannot be considered as completely unrelated processes; newly synthesized cell wall polysaccharides have to reach the correct target destination and must therefore be appropriately sorted.

The plant Golgi apparatus shares many features with its animal counterpart, but also has unique characteristics. The most important difference concerns its structure. Whereas in animal cells the Golgi apparatus occupies a rather stationary perinuclear position, in plant cells the Golgi is divided into individual Golgi stacks, which are generally considered to be functionally independent (Staehelin and Moore, 1995) (Fig. 1). The number of Golgi stacks per cell and the number of cisternae per stack vary with the species and cell type, but also reflect the physiological conditions, the developmental stage and the functional requirement of a cell (reviewed by Staehelin and Moore, 1995; Andreeva et al., 1998b).

Despite these variations, each individual Golgi stack can be described as a polarized structure with its cisternal morphology and its enzymatic activities changing gradually from the ER-adjacent cis-face to the trans-face (Fitchette et al., 1999). Proteins destined for secretion enter the Golgi at the cis-face and subsequently move towards the trans-face where the majority of proteins exit the stack en route to the plasma membrane or vacuolar system (for exceptions see below). In between the cisternae of the Golgi stack, intercisternal elements can be observed, mainly towards the trans-face (Ritzenthaler et al., 2002) (Fig. 1A). Although no matrix proteins surrounding the plant Golgi stack have yet been identified, the existence of a matrix has been predicted from the appearance on micrographs of ultra-rapidly frozen root cells of a clear zone, excluding ribosomes, around each Golgi (Staehelin et al., 1990). The Golgi matrix...
has been suggested to play an important role in the maintenance of stack organization against the shearing forces during cytoplasmic streaming (Staehelin and Moore, 1995).

Confocal microscopy of Golgi-targeted proteins or peptides fused to the green fluorescent protein (GFP) has revealed that individual stacks are highly mobile within the plant cell, moving over the ER on an actin-network (Boevink et al., 1998; Nebenführ et al., 1999; Brandizzi et al., 2002) (Fig. 2); this has resulted in them being christened ‘stacks on tracks’ (Boevink et al., 1998) or ‘mobile factories’ (Nebenführ and Staehelin, 2001). The fact that the plant Golgi apparatus is divided into highly mobile biosynthetic subunits certainly poses major problems when trying to elucidate mechanisms for controlled protein import into and targeted product export out of the stack. In this Botanical Briefing, we summarize recent findings regarding protein transport from the ER to the Golgi apparatus and sorting of proteins and membranes as they exit the Golgi.

ENTERING THE GOLGI:
IMPORT FROM THE ER

Towards a more dynamic model of ER-to-Golgi protein transport

The discovery that Golgi stacks move in close association with the ER and that this movement is actin-dependent (Boevink et al., 1998) led to the question of whether Golgi movement is necessary for ER-to-Golgi transport. The ‘vacuum cleaner model’ (Boevink et al., 1998) suggested that Golgi stacks move over the surface of the ER picking up products, similar to a vacuum cleaner picking up dust whilst moving over a carpet (Fig. 3A). This implies that the whole surface of the ER is capable of protein export, and that continued formation of cargo vectors occurs. In contrast, the hypothesis underlying the ‘stop-and-go’ or ‘recruitment model’ (Nebenführ et al., 1999) is that Golgi stacks receive cargo from the ER at defined export sites, which produce a local stop signal that transiently halts stack movement (Fig. 3B). This model is supported by the observation that actin-based Golgi movement is not necessary for ER-to-Golgi membrane protein transport (Brandizzi et al., 2002b). Although more attractive, as protein transport out of the ER would be restricted to confined areas, the recruitment model suggests a rather stationary image of the ER surface. A third model might therefore propose that protein export from the ER is restricted to specific export sites, which could be either highly mobile within the ER membrane or mobile due to the movement of the ER surface (Fig. 3C). Thus, Golgi stacks and ER export sites may move together in an actin-dependent fashion, forming discrete ‘secretory units’ (Brandizzi et al., 2002b).

Receptor-mediated protein transport or bulk flow?

Regardless of the physical model of ER-to-Golgi transport, export from the ER and import into the Golgi are two intimately related processes. How proteins are sorted and
concentrated at sites of ER export is therefore a key question. Are they actively sorted with the help of specific ER export signals linking with differential affinity to a common ER export receptor, or is there bulk flow of product with sorting occurring via retention signals by which proteins are deviated from the default route at different levels of the pathway? Support for the second model, at least for soluble proteins, came from the cloning of an ERD2 homologue from *Arabidopsis thaliana* (Lee et al., 1993). This protein acts as a transmembrane receptor that binds to a specific sorting tetrapeptide (H/KDEL) at the carboxyl terminus of ER-resident proteins in yeast and mammals (Lewis and Pelham, 1990; Lewis et al., 1990). The arabidopsis ERD2 is capable of functionally complementing a yeast null mutant (Lee et al., 1993), and its GFP fusion locates to the ER and to Golgi stacks (Boevink et al., 1998). Recent data from quantitative biochemical in vivo assays measuring ER export of well-characterized cargo molecules in *Nicotiana tabacum* protoplasts provided good evidence for the bulk flow theory (Phillipson et al., 2001).

In vivo observations of membrane protein transport from ER to Golgi have recently been facilitated by the use of fluorescent protein chimeras. Selective photobleaching experiments using two fluorescent marker proteins locating to the Golgi (ST-YFP and ERD2-GFP) have permitted confocal imaging of ER-to-Golgi protein transport (Brandizzi et al., 2002b). Fluorescence recovery in individual Golgi stacks after photobleaching of ST-YFP or ERD2-GFP reached 80–90% of the pre-bleach level after only 5 min in cells treated with latrunculin B alone to disrupt actin filaments or in conjunction with colchicine to affect microtubule integrity. This indicated a rapid exchange with pools of the fusion protein from other parts of the cell, independent from an intact actin or microtubule cytoskeleton. The rapid cycling of both fusion proteins was dependent on energy, as could be shown by loss of fluorescence recovery in cells after ATP depletion.

**COPII vesicles vs. direct connections**

In animal cells, protein transport between the ER and the Golgi apparatus occurs through intermediate compartments known as vesicular–tubular clusters (VTCs) (or the ER–Golgi intermediate compartment, ERGIC). These compartments represent the first site of segregation of anterograde (forward from the ER to the Golgi) and retrograde (backwards from the Golgi to ER) protein transport (reviewed by Klumperman, 2000). Transport between the ER and VTCs is supposedly mediated by specialized protein-coated vesicles, the so-called COPII vesicles. Cargo packaging and vesicle formation require sequential binding to the ER membrane of the GTPase Sar1p and two heterodimeric coat protein complexes, Sec23/24p and Sec13/31p (reviewed by Barlowe, 2002). Transport between the VTCs and the Golgi apparatus seems not to be mediated by distinct vesicles but by the fusion of peripheral VTCs to form the cis-Golgi cisternae (Klumperman, 2000). The involvement of another set of coated vesicles (COPI vesicles formed under the influence of the small GTPase Arf1p), normally thought to act as retrograde protein transporters, between the Golgi cisternae and from VTCs back to the ER (see below), in anterograde protein transport

**FIG. 2.** Confocal laser scanning micrographs showing the spatial relationship between Golgi stacks and ER (A) and between Golgi stacks and actin filaments (B). A, 3D-reconstruction (Velocity®) of serial optical sections through the cortical cytoplasm of a tobacco leaf epidermal cell transiently transformed with a GFP-fusion targeted to the ER (GFP-HDEL in green) and a YFP-fusion labelling the Golgi (ST-YFP in red). Golgi stacks are in close association with the ER network. B, Optical section through the cortical cytoplasm of a tobacco BY2 cell stably transformed with ST-GFP (in green). Affinity labelling of actin by rhodamine-phalloidine (in red) reveals that Golgi stacks are aligned with actin filaments (arrows). Bars = 10 μm.
is still hotly debated (Klumperman, 2000; Spang, 2002). Finally, delivery of cargo to the Golgi complex involves the small GTPase Rab1, which controls tethering and fusion events at the Golgi level (Allan et al., 2000).

In plant cells, ER-to-Golgi protein transport might follow a simpler route. To date, no equivalent of the VTCs of animal cells has been identified. On the contrary, recent findings based on GFP expression and transmission electron microscopy (TEM) indicate that direct connections in the form of tubular extensions may exist between the ER and the cis-face of Golgi stacks in tobacco leaf cells (Brandizzi et al., 2002b), suggesting that vesicles might not mediate protein transport between the two organelles. At first glance, this finding seems to contradict the fact that components of the COPII machinery have been identified in plants by EST database searches (Andreeva et al., 1998a), are associated with the ER (Bar-Peled and Raikhel, 1997; Movafeghi et al., 1999), and are necessary for transport of proteins to the Golgi (Andreeva et al., 2000; Phillipson et al., 2001). This has been interpreted as evidence that ER–Golgi protein transport shows structural and functional similarities between animal and plant cells. However, it is conceivable that COPII components simply determine the site of formation of ER-to-cis-Golgi-connections, and that vesicle vectors are not a prerequisite of transport (Hawes et al., 1999). Considering that protein transport in mammalian cells between the VTCs and the cis-Golgi seems to be by fusion of peripheral VTCs to form the cis-cisterna, and that VTCs have not been identified in plant cells to date, direct fusion events between the ER and the Golgi are not implausible.

Similar to its mammalian counterpart, the A. thaliana small GTPase AtRab1b, seems to be involved in protein transport at early steps of the secretory pathway. A dominant-inhibitory mutant of AtRab1b inhibited the secretion of a fluorescent marker protein as well as the Golgi localization of a Golgi-targeted fluorescent marker, leading to fluorescence accumulating in the ER in both cases (Batoko et al., 2000).

Retention and distribution of proteins in the Golgi

The means by which Golgi-resident proteins are retained in the stack is still largely a matter of debate. Whilst many of the ER-resident processing proteins are soluble, Golgi-
FIG. 4. Confocal laser scanning micrographs showing the location of different regulatory and structural proteins of the secretory pathway in relation to Golgi stacks. A, Optical section through the cortical cytoplasm of a tobacco BY2 cell stably transformed with the Golgi marker GmMan1-GFP (Glycine max α-mannosidase 1-GFP, green channel) after fixation and immunolocalization with anti-AtArf1p antibodies (red channel). The merged image clearly reveals that anti-AtArf1p labelling is associated with the Golgi, forming a ring-shaped pattern confined to the periphery of each stack (Ritzenthaler et al., 2002). B, Optical section through the centre of a transgenic GmMan1-GFP BY2 cell after fixation (GFP signal in green channel) and immunolocalization with anti-Atγ-COP antibodies (red channel). As can be seen in the merged image, the COPI coatomer subunit co-localizes with Golgi-associated GFP-fluorescence. As for Arf1p, anti-Atγ-COP fluorescence is restricted to the margins of the Golgi stacks (Ritzenthaler et al., 2002). C, Projection of 15 optical sections (1 μm per section) through a pea root tip cell after fixation and double-immunolabelling with anti-VSR antibodies (17F9, green channel) and JIM 84, a trans-Golgi marker (red channel). As shown in the merged image, more than 90% of the prevacuolar organelles labelled by 17F9 are separate from Golgi stacks (Li et al., 2002). Occasionally, fluorescence labelling by the two antibodies co-localizes (merged image, open arrow). D, Optical section through the cortical cytoplasm of a leaf epidermal cell of a transgenic ST-GFP tobacco plant (GFP signal in green channel) transiently transformed with YFP-AtRab2a (YFP signal in red channel). As can be seen in the merged image, both fluorescent fusion proteins co-localize in Golgi stacks. In addition to the Golgi, YFP-AtRab2a labels small spherical structures (sometimes measuring up to 3 μm in diameter) in which no GFP signal can be detected (merged image, arrows). Bars = 5 μm (A–C, inserting D) and 20 μm (D). Micrographs for A and B kindly provided by Christophe Ritzenthaler and that for C by Liwen Jiang.
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* Literature cited in this table only, not in the main text:
resident enzymes are transmembrane proteins (Table 1). Information regarding the retention of membrane proteins at various points of the secretory pathway has recently been revealed by a study investigating the default pathway of type I membrane-bound proteins (Brandizzi et al., 2002c). In this study, GFP was fused to proteins with transmembrane domains of variable length and the fusion proteins were shown to be distributed along the organelles of the secretory pathway in a transmembrane length-dependent manner. As for animal cells (Munro, 1995), it is expected that the membrane thickness increases in plant cells from the ER to the Golgi and finally to the plasma membrane/tonoplast owing to a sterol gradient in the lipid composition (Moreau et al., 1998). Accumulation in the Golgi occurred when GFP was fused to transmembrane domains of 19 or 20 amino acids (Brandizzi et al., 2002c). Whether this retention mechanism is universally valid for true Golgi resident enzymes remains to be elucidated, although the signal anchor sequence of a rat sialyl transferase targets GFP to the plant Golgi (Boevink et al., 1998; Saint-Jore et al., 2002), and equivalent sequences of plant glycosyltransferases give the same result (Essl et al., 1999; Dinnbinger et al., 2002; Pagny et al., 2003). The residues, suggested to play a role in Golgi retention in mammalian cells, only seem to play a minor role in plants, as indicated by comparison of the number and position of the residues of the transmembrane domain of proteins within the BP80 family (Brandizzi et al., 2002c).

As mentioned earlier, enzymatic activities change gradually from the cis-face to the trans-face of the Golgi stack, reflecting a sequential processing down the stack. Therefore, a logical question is what controls the distribution of resident enzymes across the different cisternae. This has only been addressed experimentally a couple of times in plant cells (Pichette et al., 1999), and models were first formulated for mammalian Golgi enzymes. Again, the bilayer/membrane thickness model provides one explanation. Alternatively, as in mammalian cells, retrograde transport of processing machinery in vesicle vectors may both reduce loss and control positioning of enzymes whilst cisternae continually mature from the cis- to the trans-face (Opal et al., 2001). More evidence is needed before ruling out one or other model for plant Golgi enzymes.

**Intra-Golgi transport and retrograde transport (COPI machinery)**

The fact that each Golgi stack is divided into a number of cisternae leads to the question of how cargo is transported down the stack. In analogy to mammalian cells, two models have been proposed, the ‘vesicle shuttle’ and the ‘cisternal maturation’ models (reviewed by Hawes and Satiat-Jeunemaitre, 1996; NebenfuÈhr and Staehelin, 2001). According to the vesicle shuttle model, Golgi cisternae are stable entities with a specific set of processing proteins, and cargo is sequentially transported down the stack in vesicular shuttles. The second model suggests that cisternae progressively move down the stack and mature from the cis-face to the trans-face of the Golgi. New cis-cisternae are formed by fusion of ER-to-Golgi transport intermediates and retrograde transport vesicles, which shuttle back the processing enzymes from the trans-cisternum. Experimental evidence exists for both models and it cannot be ruled out that either mechanism or a mixture of both is active. In addition to the algal scale argument (Becker et al., 1995), recent data on the ultrastructural morphology of Golgi stacks in BY2 cells and the redistribution of a fluorescent cis-Golgi marker protein after brefeldin A treatment (a fungal toxin widely used to study protein transport along the secretory pathway; NebenfuÈhr et al., 2002) seem to favour the cisternal maturation model (Ritzenthaler et al., 2002). Finally, it cannot be excluded that cisternae of the stack are all joined by interconnecting tubules, necessitating a different model to explain the regulation of cis-to-trans-transport.

In addition to anterograde protein transport from the ER to the Golgi and down the Golgi stack, retrograde protein transport occurs between the Golgi and the ER (e.g. transport of escaped ER residents) as well as inside the Golgi stack, from the trans- towards the cis-face. In mammalian cells, retrograde transport is likely to be mediated by COPI vesicles and regulated by the Arf1p GTPase (Spang, 2002). Components of the COPI machinery have been located to the Golgi by means of immunofluorescence (Ritzenthaler et al., 2002) (Fig. 4A and B). More precisely, a study combining biochemistry with cryosection immunogold labelling techniques, revealed that in plant cells, components of the COPI coat as well as Arf1p mainly locate to the cis-half of Golgi stacks and that COPI coat proteins are present on small vesicles budding off from cis-cisternae (Pimpl et al., 2000). In addition, in vitro COPI vesicle induction from ER/Golgi membranes of transgenic tobacco plants overproducing the soluble secretory marker α-amylase fused to HDEL (the C-terminal sorting peptide of ER residents), showed that COPI vesicles contained the modified secretory marker as well as the ER-resident calreticulin. This was the first indication in plants that COPI vesicles might be involved in retrograde transport from the Golgi (Pimpl et al., 2000). The involvement of AtArf1 in this particular transport event was also deduced from the effect of two dominant negative mutant forms of AtArf1 on the distribution of three fluorescent Golgi markers (Takeuchi et al., 2002). To date, the demonstration of the role of COPI vesicles in intra-Golgi retrograde transport remains a challenge, mainly owing to the lack of marker molecules for this specific transport event.

**Recycling through the Golgi: protein import from destinations other than the ER**

During secretion, vesicle-derived membrane is continuously added to the plasma membrane. To balance this increase in membrane surface area, clathrin-coated endocytic vesicles pinch off from the plasma membrane towards the inside of the cell (reviewed by Holstein, 2002). The different endocytic compartments in plant cells are still not fully characterized. In analogy to mammalian cells, the term ‘endosome’ is often found in the plant literature indicating a compartment containing endocytosed material (Jürgens and Geldner, 2002). Ultrastructurally, the ‘plant endosome’ most likely corresponds to the partially coated reticulum (PCR), a compartment that may originate from the ‘trans-
Golgi network’ (Hillmer et al., 1988). From this compartment, protein might be recycled towards the Golgi or transported towards the lytic vacuole via multi-vesicular bodies (MVBs) for degradation. This was shown by elegant time-course studies following the uptake of cationized ferritin (CF) in soybean protoplasts (Fowke et al., 1991), reflecting vesicle-mediated plasma membrane recycling (and not receptor-mediated endocytosis). This marker is quickly endocytosed through coated pits into coated vesicles. Within the cytoplasm, CF sequentially labelled the following organelles: tubular elements of the PCR; periphery of Golgi cisternae; MVBs; and finally the central vacuole. Labelling of the Golgi by CF almost certainly reflects membrane recycling from the plasma membrane, presumably through the PCR (Fowke et al., 1991). Whether direct plasma membrane recycling to the Golgi can occur in plant cells still has to be elucidated.

In addition to maintaining the plasma membrane surface, protein import into the Golgi through the ‘plant endosome’ could also be important for regulating, by endocytosis at the plasma membrane, the number of ion channels, proton pumps (Crooks et al., 1999) and carrier proteins, such as PIN1 (auxin efflux carrier; see Geldner et al., 2001). Remodelling the distribution of such proteins at the plasma membrane via vesicle trafficking must certainly be regarded as an important way in which a plant can react to changes in environmental conditions (Levine, 2002).

OUT OF THE GOLGI

The major destinations for proteins exiting the Golgi apparatus are the plasma membrane and the vacuolar system. During cytosis, another protein trafficking route leads towards the developing cell plate (reviewed by Bednarek and Falbel, 2002). Here we focus on how sorting of proteins to the plasma membrane or the vacuolar system occurs and where this sorting takes place.

As to the place of sorting, the term ‘trans-Golgi network’ (TGN) from the mammalian ‘endomembrane nomenclature’ is becoming more common in the plant literature. However, in plant cells, no discrete protein-sorting compartment with a characteristic set of proteins, downstream from the trans-face of the Golgi has yet been described. In addition, in plants, protein sorting and secretory vesicle production can take place as early as at the cis-cisternae of the Golgi stack (see below). Therefore, it might be better to suggest that production of clathrin-coated vesicles destined for the lytic vacuolar system is limited to the trans-face of the Golgi. Whether this face of the Golgi, which certainly can comprise a network of tubules and vesicles, is homologous to the mammalian TGN is open for debate.

Secretion: transport towards the plasma membrane and the cell wall

The default destination of soluble proteins and complex carbohydrates has been suggested to be the plasma membrane (Denecke et al., 1990). Secretory proteins usually carry an amino terminal signal peptide for insertion into the ER, which is clipped off upon translocation across the ER membrane (Vitale and Denecke, 1999). Soluble secretory proteins are not known to carry any positive targeting information, which would divert them from the default pathway to the plasma membrane, either to the vacuolar system or back to the ER (Hadlington and Denecke, 2000).

In contrast, the situation with membrane proteins is less clear. Until recently, the tonoplast had been regarded as the default destination (Barrieu and Chrispeels, 1999). However, a VSR-based (vacuolar sorting receptor) GFP-fusion with a lengthened transmembrane domain accumulated on the plasma membrane, indicating that positive sorting information might be necessary to direct membrane proteins from the Golgi towards the tonoplast (Brandizzi et al., 2002c). Nevertheless, transport of proteins to specific areas of the plasma membrane, nicely illustrated by proteins such as the auxin efflux carrier PIN1, which locates to the distal part of the plasma membrane in root cells of Arabidopsis seedlings (Geldner et al., 2001; Jürgens and Geldner, 2002), is difficult to imagine without some form of either positive targeting or retention mechanism.

Transport towards the vacuolar system: different types of vacuoles, vesicles, sorting signals, receptors and sorting sites

As mentioned earlier, sorting signals are necessary for transport to the vacuolar system. In plant cells, two main types of vacuoles [distinguishable by different sets of TIPS (tonoplast intrinsic proteins) and luminal contents] may coexist, and sorting towards either of them depends on different peptide targeting signals and is mediated by different sets of transport vesicles (Paris et al., 1996; Jiang and Rogers, 1998; Hinz et al., 1999).

Transport to the lytic vacuole, characterized by γ-TIPs, occurs via an intermediate compartment known as the prevacuolar compartment (PVC) and relies on amino-terminal, sequence-specific preproteins (NPIR or equivalent), which are recognized by VSRs. The first VSR to be identified was BP80 from Pisum sativum (now called VSRPS-1; reviewed by Paris and Neuhaus, 2002). VCRs are thought to cycle between the Golgi apparatus and the PVC (Mitsuhashi et al., 2000) where they are preferentially located, as was shown by confocal immunofluorescence for several VSRPS-1 homologues (Li et al., 2002) (Fig. 4C). It is thought that VSRs mediate the packaging of cargo destined to the lytic vacuole in trans-Golgi-located clathrin-coated vesicles (Hinz et al., 1999).

In contrast, proteins delivered to the protein storage vacuole (α-TIP vacuoles) reach their destination in vesicles apparently devoid of any specific protein coating and, in legume seeds, have been termed dense vesicles (Hohl et al., 1996). Sorting relies on a carboxyl-terminal propeptide and an as yet unidentified sorting receptor, the existence of which is deduced from the observation that transport towards the storage vacuole can be saturated (Frigerio et al., 1998). Immunogold labelling indicates that sorting of some storage proteins can occur as early as the cis-cisternae of the Golgi in pea cotyledons (Hillmer et al., 2001). Other storage vacuole proteins as well as integral membrane proteins are sorted even earlier, at the ER level, where they...
are packed into so-called precursor-accumulating (PAC) vesicles and transported to the storage vacuole via a route bypassing the Golgi (Mitsuhashi et al., 2001). A vacuolar sorting receptor for this pathway has been identified in pumpkin seeds (Shimada et al., 2002).

REGULATORY PROTEINS AT THE HEART OF THE SECRETORY PATHWAY

In recent years, it has become apparent that the different transport events along the secretory/biosynthetic and endocytic pathway are orchestrated by a plethora of proteins. The functional importance of the plant Golgi apparatus in the secretory pathway is reflected by the fact that numerous regulatory proteins are structurally and functionally linked to the Golgi and the ‘trans-Golgi network’ (Hawes et al., 1999; Sanderfoot and Raikhel, 1999; Nebenführ, 2002; Rutherford and Moore, 2002; see Table 1). For example, small GTPases act as molecular switches involved in the formation, transport and fusion of transport vesicles, while SNAREs are integral membrane proteins involved in determining the specificity of fusion events along the endomembrane system, residing on transport vesicles (R-SNAREs) and target membranes (Q-SNAREs). Considerable effort has been put into comparing various genomes to identify plant homologues of proteins that have been shown to regulate various trafficking events along the secretory pathway in yeast and animals (Andreeva et al., 1998a; Sanderfoot et al., 2000; Jürgens and Geldner, 2002). Statements such as ‘Rab functions are conserved across eukaryotes, such that their subcellular localisation can be inferred from known localisations of members of the same subfamily in other species’ (Jürgens and Geldner, 2002) may be correct as to the functional aspect. However, even though homologous Rab proteins may show the same subcellular location, it cannot be necessarily concluded that they exert the same function. For instance, despite the Golgi-localization of two mammalian splice-variants of Rab6, which differ only in three amino acid residues, Rab6A and Rab6A’ seem to function in different membrane-trafficking events (Echard et al., 2000). Likewise, two plant Rab2 isoforms have been found to locate to Golgi stacks (Fig. 4D) but seem to regulate quite different transport steps. In tobacco pollen tubes, NtRab2 seems to sustain ER-to-Golgi traffic (Cheung et al., 2002), while studies conducted in our group seem to indicate that an arabidopsis Rab2 isoform regulates vesicle traffic between the Golgi and a post-Golgi compartment (U. Neumann, I. Moore, C. Hawes and H. Batoko, unpubl. res.).

It is outside the scope of this current Briefing to consider the putative roles of the various small GTPases and SNAREs that have been identified in plants. A summary of the major regulatory proteins locating to the plant Golgi apparatus identified to date is given in Table 1.

CONCLUSIONS

Immense progress has been made in recent years to elucidate the various transport events along the secretory and endocytic pathways in eukaryotic cells. This is certainly true for the Golgi apparatus, which has seen a renaissance in research popularity since the 100th anniversary of its discovery by Camillo Golgi in 1898. New data regarding the molecular machinery that drives and regulates trafficking events at the Golgi level are published almost weekly. In this context, comparative genomic analyses have helped to identify plant homologues of yeast and animal proteins regulating Golgi-related transport steps. One of the main challenges of the post-genomic era is to provide functional and structural evidence for the specific roles of plant proteins putatively playing a role in the secretory/endocytic pathway. As to structural data at the subcellular level, technological progress in light microscopy such as confocal microscopy and deconvolution technology for improving images, combined with developments in immunolabelling and fluorescent protein technology have revolutionized the study of cell biology (Brandizzi et al., 2002a). However, especially with regard to the Golgi apparatus, confocal microscopy combined with GFP technology in order to locate proteins is not without its pitfalls. It becomes more and more common to assume that a punctate distribution of GFP fluorescence in the cytoplasm is sufficient to establish that a specific protein is located to the Golgi without additional confirmatory evidence such as co-localization with a known Golgi marker at the light microscopical level (e.g. a fluorescent marker of the SNARE complex: programming budding COPII vesicles for fusion. Science 289: 444–448.

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