Tansley review

Cell biology of the plant Golgi apparatus

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Summary

The higher plant Golgi apparatus, comprising many individual stacks of membrane bounded cisternae, is one of the most enigmatic of the cytoplasmic organelles. Not only can the stacks receive material from the endoplasmic reticulum, process it and target it to the correct cellular destination, but they can also synthesise and export complex carbohydrates and lipids and most likely act as one end point of the endocytic pathway. In many cells such processing and sorting can take place while the stacks are moving within the cytoplasm and, remarkably, the organelle manages to retain its structural integrity. This review considers some of the latest data and views on transport both to and from the Golgi and the mechanisms by which such activity is regulated.


I. Introduction

We are entering an exciting period in the study of the transport of membrane and cargo within the plant secretory pathway. Over the past few years a range of new tools have been developed that has given much needed impetus to the plant membrane trafficking community and their use is beginning to reap rich dividends. For instance the combination of fluorescent protein technology (Brandizzi et al., 2002a) and the publication of the arabidopsis genome (The Arabidopsis
Genome Initiative, 2000) has resulted in a wide range of new proteins, putatively involved in the secretory pathway being located and their functions analysed (Robinson, 2003 for reviews; Brandizzi et al., 2004).

At the heart of the secretory pathway lies the Golgi apparatus, a collection of stacks of membrane cisternae, which is the site of synthesis of cell wall polysaccharides and various membrane lipids and glycolipids. It is responsible for receiving membrane and soluble cargo from the endoplasmic reticulum (ER), modifying this cargo by further glycosylation, packaging it into transport vectors and targeting it to the correct destination downstream in the secretory pathway. Concomitantly it is likely to receive material from the endocytic pathway and to recycle membranes and protein back to the ER. Figure 1 provides a map of the endomembrane system detailing the various transport pathways identified to date which involve the Golgi apparatus. This wide diversity of function and the myriad of proteins and their interactions that must take place in order to maintain both the structure and function of the Golgi stack make it one of the most fascinating of cellular organelles.

Of course, due to the huge volume of data and literature generated by both the mammalian and yeast secretory pathway community, it is very tempting to equate the function of the various components of the plant secretory pathway and the genes that regulate it with the accepted views perpetuated in the literature from research into members of the other kingdoms. However, this can be a dangerous road to follow, as even untouchable dogmas such as those purporting that the main vector for trafficking secretory cargo around the cell is the ubiquitous vesicle or that the Golgi functions by cisternal maturation are now being challenged (Polishchuk & Mironov, 2004). Therefore, it is pertinent to analyse data fully and interpret experiments carefully on the plant pathway before rushing into assigning function to structures and gene products simply because they have been described in the yeast and mammalian literature.

In this review some of the more recent data exploring the dynamics and functions of the higher plant Golgi apparatus and the putative functions of the structural and regulatory proteins that we now know to be associated with the membrane of the cisternal stacks will be discussed, along with some of the controversies surrounding this enigmatic organelle.

II. What is the plant Golgi apparatus?

Simply put, the Golgi apparatus is the sum total of numerous polarised stacks of membrane bounded cisternae distributed throughout the cytoplasm of plant cells, classically described as such from many electron microscopy studies. The morphology of the stacks has been discussed in many previous articles and reviews and will not be detailed further here (Mollenhauer & Morré, 1980; Staehelin et al., 1990; Staehelin & Moore, 1995; Andreeva et al., 1998a). In more recent years the distribution of the Golgi stacks in a variety of plant cell types has been described from immunocytochemical studies (Satiat-Jeunemaître & Hawes, 1992) and latterly from live cell imaging after expression of Golgi targeted fluorescent protein constructs (Boevink et al., 1998; Essl et al., 1999; Nebenführ et al., 1999; Baldwin et al., 2001; Dirnberger et al., 2002). This ability to study the organelle by fluorescence and confocal microscopy has demonstrated the large number of individual stacks that can exist in a cell (Fig. 2a), running into the hundreds in many cases, and has opened up a new era in the study of the plant Golgi.
1. The plant Golgi apparatus is a dynamic organelle

Until the advent of fluorescent protein technology it had proved impossible to image the plant Golgi apparatus in living cells. It therefore came somewhat as a surprise, when using a viral expression system in *Nicotiana* leaves, that the individual Golgi stacks, expressing a construct of green fluorescent protein (GFP) fused to the carboxy-terminus of the signal anchor sequence of a rat sialyl transferase (ST-GFP), were observed to be mobile within the cortical cytoplasm of the pavement epidermal cells. What was even more surprising was that, using a construct that labelled both the ER and Golgi (AtERD2-GFP – the plant HDEL receptor homologue spliced to GFP), it was noticed that the Golgi was motile over the tubules of the cortical ER network (Fig. 2b; Boevink *et al.*, 1998). This movement was shown to be actin-based and this gave rise to the term ‘stacks on tracks’ describing the Golgi; presumably driven by a myosin motor, travelling over an actin ‘railway track’ which was also overlaid by the ER network. Subsequently the movement of the plant Golgi was confirmed in BY2 cells expressing a plant mannosidase-GFP construct (Nebenführ *et al.*, 1999). Such movement of the Golgi bodies over the cortical ER network is distinct from the rapid movement of the organelle observed in streaming transvacuolar strands of cytoplasm. Direct imaging of Golgi apparently moving on the actin network was observed by the dual expression of ST-GFP and an actin targeted construct consisting of the actin-binding domain of a mouse talin spliced to the yellow fluorescent protein (YFP, Fig. 3; Brandizzi *et al.*, 2002b). Movement of the Golgi has been observed in other tissues such as hypocotyls, petals and root hairs (C. Hawes *et al.* unpublished), although it is too early to say whether this is a general phenomenon for all cell types (Hawes *et al.*, 2003).

III. Out of the ER

1. Can the Golgi be bypassed?

The first organelle in the secretory pathway is the ER, where translocation of proteins into the lumen or incorporation into the ER membrane takes place. Proteins destined for transport in the secretory pathway undergo the early stages of glycosylation (in most cases), N-glycan trimming (Gillmor *et al.*, 2002) and are folded prior to export. Until recently, except in the case of cereals where storage proteins are known to aggregate in the ER and are hived off as storage vacuoles (Levanony *et al.*, 1992), it was assumed that all secretory or cargo proteins passed through the Golgi apparatus. However, in a few cases it has been suggested that the Golgi may be bypassed (Hara-Nishimura *et al.*, 1998; Toyooka *et al.*, 2000).

For example, in maturing pumpkin seeds it has been reported that the ER can bud vesicles containing proprotein precursors of vacuolar storage proteins and transport them directly to storage vacuoles. The so-called precursor accumulating (PAC) vesicles also contained complex glycans indicating that they may also receive material from the Golgi before fusing with the vacuole (Hara-Nishimura *et al.*, 1998).

Another possible Golgi-bypass was reported by Törmäkanagas *et al.* (2001) who showed that an aspartic protease from barley (phytpepsin) contains a 104 amino acid vacuolar targeting domain which is required for COPII mediated exit from the ER (see Section III.2). When this domain is deleted not only
is the protein secreted but its exit from the ER is also insensitive to over expression of the Sec12 guanine nucleotide exchange factor (GEF) for the small GTPase Sar1p and thus probably COPII independent (see Section III.2). However, it has not been investigated whether this protein still passes through the Golgi, indicating the existence of at least two routes of export from the ER to the Golgi, or whether the truncated protein is secreted directly from the ER to the cell surface. Likewise, a chimeric protein, phaseolin-KDEL when highly expressed was shown to escape the ER and reach the vacuole in a seemingly Golgi-independent manner (Frigerio et al., 2001).

2. Current models of ER-Golgi transport

The currently accepted model for ER to Golgi transport in mammalian and yeast cells is based around the construction of transitional ER or exit sites on the ER, which are dependent on the formation of COPII protein coats and buds on the ER membrane and the concentration of cargo at these sites (see reviews Bonifacino & Glick, 2004; Murshid & Presley, 2004). In vitro vesicle reconstitution experiments have resulted in the concept of COPII vesicles budding from these exit sites and transporting material directly to the Golgi (Barlow et al., 1994; Barlow, 2002) or forming an intermediate compartment of varying complexity (Scales et al., 1997; Murshid & Presley, 2004). This compartment, often known as the intermediate compartment or vesicular tubular cluster, can then transport membrane and cargo to the cis-Golgi, whilst also mediating retrograde transport back to the ER.

The COPII coat is comprised of two major protein complexes, Sec13/31p and Sec 23/24p and is initiated by the activity of a small GTPase Sar1p which is regulated by an integral membrane protein, the exchange factor Sec12p (Aniento et al., 2003). Besides its suggested role in producing a transport vesicle, the COPII coat and associated proteins have been shown to mediate the docking, transport and, in some cases, formation of transport vesicles in mammals and yeasts (Barlow et al., 1994; Barlow, 2002) or forming an intermediate compartment of varying complexity (Scales et al., 1997; Murshid & Presley, 2004). This compartment, often known as the intermediate compartment or vesicular tubular cluster, can then transport membrane and cargo to the cis-Golgi, whilst also mediating retrograde transport back to the ER.

3. What happens in plants?

To date, there is little structural evidence of either ER exit sites or ER derived coated vesicles in higher plant cells and certainly it is accepted that there is no structurally visible intermediate compartment between the ER and the Golgi. Indeed in the leaf and suspension culture systems where in vivo imaging has shown an extremely close relationship between the ER and Golgi, it is difficult to envisage the necessity for discrete transport vectors between the two compartments (Boevink et al., 1998; Brandizzi et al., 2002b; Saint-Jore et al., 2002). However, it is clear that plants possess functional genes encoding the majority of the COPII machinery, as reported in yeast and mammals (Bar-Peled & Raikhel, 1997; Andreeva et al., 1998b; Movafeghi et al., 1999; Phillipson et al., 2001). Biochemical studies and in vivo imaging using fluorescent protein technology have shown that over expression of Sec12, the Sar1p guanine nucleotide exchange factor (GEF) inhibits export out of the ER (Phillipson et al., 2001; Törmäkanagas et al., 2001; daSilva et al., 2004), presumably by the titration of Sar1p away from putative ER exit sites. The requirement of the GTPase Sar1p for ER export has been directly demonstrated by the expression of nonfunctional mutant forms of the protein, which resulted in accumulation GFP tagged proteins in the ER which was interpreted as reflecting a block in ER export (Andreeva et al., 2000; Takeuchi et al., 2000; daSilva et al., 2004).

It is therefore becoming clear that the COPII machinery may be required for export of cargo out of the ER. What is not clear is how these proteins are organised to form a functional export competent site on the ER surface. It is however, from the evidence presented to date, premature to suggest that COPII vesicles are functional transport vectors in higher plants. If not, then what is the mechanism of export from the ER?

IV. Regulation of ER to Golgi transport

1. The role of Rabs

The docking, transport and, in some cases, formation of transport vesicles in mammals and yeasts are known to be regulated by a large family of small GTPases, the Rab (Ypt) proteins (Zerial & McBride, 2001), and in the arabidopsis genome 57 loci have been identified that can encode Rab GTPases (Rutherford & Moore, 2002; 2003). Unfortunately functional studies on these proteins in plants are few and far between. However, it has been shown that the plant homologue of Rab1 (At-RabD2a previously AtRab1b) is necessary for normal ER to Golgi transport when used in a GFP-based assay in tobacco leaves (Batoko et al., 2000). Transient expression of a dominant inhibitory mutant form of the protein resulted in the accumulation of a secretory form of GFP in the ER of tobacco leaf epidermal cells, an effect that was rescued by coexpression of the wild type protein. Interestingly, it was subsequently shown that expression of the same mutant delayed the recovery of Golgi reformation after brefeldin A (BFA) treatment of leaves expressing Golgi-targeted ST-GFP (Saint-Jore et al., 2002).
A member of the Rab B group (NtRab2) has also been reported to be involved in the regulation of vesicle trafficking between the ER and Golgi bodies in tobacco pollen tubes as again dominant inhibitory mutants of the protein blocked transport of secretory pathway markers (Cheung et al., 2002). GFP-fusions of the protein were located to the Golgi in the tubes but interestingly not in tobacco leaf epidermal cells after introduction of DNA by particle bombardment (Cheung et al., 2002). However, using Agrobacterium-mediated transient expression in tobacco leaves, efficient localisation of GFP-RabB fusions to the Golgi have been demonstrated (Neumann et al., 2003). Homologues of mammalian Rab6 (AeRabH1b & c) have also been located to the plant Golgi, although no definite function has been assigned (J. Johansen et al., unpublished), whilst Rab 5 homologues have been shown to be involved in the post-Golgi vacuolar pathway but may cycle between the Golgi and a prevacuolar compartment (Bolte et al., 2004; A. M. Kotzer, unpublished).

2. SNAREs at export sites

It is commonly accepted that prior to the fusion of a vesicle with a target membrane, the interaction of a group of membrane proteins, the soluble N-ethyl maleimide sensitive factor adaptor proteins (SNAREs) is necessary. Membrane fusion typically requires the interaction of a complex of three SNAREs (t-SNAREs) on the target membrane and one SNARE (v-SNARE) on the donor membrane via conserved coiled-coil domains. The formation of this SNARE complex eventually results in membrane fusion (Bonifacino & Glick, 2004). Different combinations of SNAREs appear to confer specificity on different membrane fusion events within the secretory pathway. For the post Golgi pathway it is often the case that the v- and t-SNAREs interact with a SNAP protein containing two coiled-coil domains (Bonifacino & Glick, 2004). A large number of SNAREs are found in the arabidopsis genome (Sanderfoot & Raikhel, 1999; Sanderfoot et al., 2000; Blatt & Thiel, 2003) including homologues of those described in other kingdoms that mediate fusion events between the ER and Golgi. These include homologues of Sec22, Bos1, Bet1 (membrin) and Sed5, which all locate to punctate Golgi-like structures when expressed as GFP fusions (Takeuchi et al., 2000, L. Châtre et al., unpublished). The mechanisms by which such proteins may operate in a spatially confined export complex (see Section IV.4) is a major challenge in our understanding of ER to Golgi transport in plants.

3. In vivo imaging reveals transport processes

Initial observations of GFP labelled Golgi in tobacco leaves and tobacco BY2 suspension culture cells resulted in two conflicting models of ER to Golgi transport being proposed, although in neither case was there any experimental data to support the hypotheses. After viral-mediated expression of an ST-GFP construct, Boeving et al. (1998) observed Golgi stacks moving over the tubules of the cortical ER network in tobacco leaf cells. They suggested that the whole of the ER surface may be export competent and the Golgi may simply collect export vesicles in the manner of a vacuum cleaner collecting dirt from a carpet. In contrast Nebenführ et al. (1999) observed that, in BY2 cells expressing an α-1,2 mannosidase-GFP construct, the Golgi appeared to show stop-and-go movements. They suggested that Golgi may come to a halt over ER export sites as the result of an unknown signal and the stacks would collect cargo before resuming movement.

The ‘stop and go’ model was apparently reinforced by the work of Brandizzi et al. (2002b) who used fluorescence recovery after photobleaching (FRAP) to show that in static Golgi tagged with ST-GFP or an AtERD2-GFP construct recovery of fluorescence in bleached Golgi stacks was relatively rapid (80–90% of the prebleach level in 5 mins) indicating transport of new fluorescent protein to the Golgi (Fig. 4). This was the first in vivo demonstration of the transport of a protein to the Golgi and as the constructs did not appear to label any compartments downstream of the Golgi it was assumed that new protein was being delivered from the ER. The FRAP technique assumes that recovery of fluorescence due to transport of fluorescent molecules into the bleached area must be matched by an equivalent loss of bleached molecules from the same area. Thus, it is likely that the ST-GFP construct is cycling out of the Golgi either back to the ER or to a downstream compartment. This hypothesis was confirmed by the complete inhibition of fluorescence recovery after energy depletion and a 50% inhibition of recovery after treatment with the secretory inhibitor BFA. In order to carry out these FRAP experiments it was necessary to inhibit Golgi movement with actin depolymerising drugs first. Thus, the conclusion was made that ER to Golgi transport can take place when Golgi stacks are stationary as proposed by Nebenführ et al. (1999).

More recently the FRAP technique has been refined to permit analysis of fluorescence recovery in moving Golgi stacks. By targeting two different fluorescent proteins to the same Golgi stacks it was possible to photobleach with one laser wavelength and observe recovery whilst tracking the individual Golgi stack through fluorescence using the laser line at the second wavelength (Brandizzi & Hawes, 2004; L. daSilva et al., 2004). This proved that ER to Golgi transport can take place whilst the Golgi are motile, whilst confirming that the bleaching process does not destroy the Golgi stack.

4. Motile export sites

It has been suggested that in certain plant tissues, due to the close proximity of Golgi and ER (Brandizzi et al., 2002b), that the individual Golgi stacks may be directly associated with the
ER, thus forming a motile secretory complex that can move over the surface of, or within the ER membrane (Neumann et al., 2003). Recently we have shown that a Sar1p fluorescent protein chimera when expressed in tobacco leaves locates on the ER surface together with individual Golgi stacks and moves in tandem with the Golgi over the ER (daSilva et al., 2004). Interestingly, a GTP-bound mutant of Sar1p tagged with GFP, which is predicted to cycle more slowly from the export sites, located to motile punctate structures associated with the ER and colocalised with coexpressed Golgi targeted constructs. Over expression of this construct both inhibited export of a secretory form of GFP from the ER and also resulted in the disappearance of fluorescent protein labelled Golgi, again suggesting that membrane proteins in the Golgi cycle from the organelle.

Being the first protein to associate at sites of COPII coat formation, Sar1p is a good indicator of export sites which appear to be spatially located in the vicinity of the Golgi. The conclusion from this work is that perhaps in plants COPII coat components are needed for cargo accumulation and maybe for regulating export out of the ER, but not necessarily for forming discrete transport vesicles. However, the exact molecular architecture of the ER/Golgi interface has yet to be determined.

5. The making of a Golgi stack

The events at the ER exit site may become clearer if consideration is given to the nature of Golgi stack formation. The biogenesis of individual Golgi stacks and the Golgi apparatus as a whole has, to say the least, been a controversial topic. Does the Golgi divide prior to mitosis and cell division or can the cell generate new Golgi from the ER? A detailed study of exit sites on the ER has provided us with some clues.

In mammalian cells two schools of thought exist as to the partitioning of the Golgi during mitosis. It is clear that the perinuclear Golgi ribbon is dispersed around the cell, but whether this is as vesicles distributed in the cytosol or whether the whole structure is reabsorbed into the ER is still a contentious issue (Shorter & Warren, 2002). The latter theory then depends on the Golgi reforming from the activity of specific exit sites on the ER which produces vesicular tubular clusters which in turn migrate in a microtubule dependent manner to the perinuclear region where they form the new Golgi ribbon (Zaal et al., 1999). However, in plants it is clear that the individual stacks remain intact during all phases of the cell cycle and that they partition between daughter cells during mitosis (Nebenführ et al., 2000), although it is not clear at what stage during the cell cycle the Golgi stacks duplicate or increase in number.

The ability of the mammalian ER to generate Golgi de novo, has become clear from experiments based on the disruption of the Golgi ribbon with the microtubule depolymerising drug nocodazole (Cole et al., 1996; Yang & Storrie, 1998). On depolymerisation of the cytoplasmic microtubules it is well known that the mammalian Golgi ribbon breaks down and appears to become dispersed throughout the cytoplasm in the form of mini-cisternal stacks, resembling the plant Golgi apparatus. However, it has been demonstrated that these mini-stacks are not directly formed from the breakdown of the perinuclear Golgi ribbon. In an elegant experiment, Zaal et al. (1999) photobleached the Golgi ribbon in CHO cells expressing a galactosyl transferase-GFP (GalTase-GFP) soon after nocodazole treatment. Under these conditions fluorescent mini-stacks formed at ER exit sites and there was no recovery of fluorescence of the main Golgi ribbon. This suggests that Golgi can assemble de novo at ER exit sites by the production of Golgi intermediates and in the absence of microtubules these can no longer be transported to the perinuclear ribbon.

De novo biogenesis of Golgi stacks has also been observed in the yeast Pichia pastoris where ER exit sites marked by the COPII coat protein Sec13 tagged with GFP were observed to grow from small fluorescent spots on the ER. It is thought that new Golgi cisternae are formed from budding vesicles at these exit sites (Bevis et al., 2002). It has also been shown that in the

![Fluorescence recovery after photobleaching shows transport of ST-GFP into a bleached Golgi stack in a tobacco leaf epidermal cell.](image-url)
absence of the actin cytoskeleton, ER in a young *P. pastoris* bud can generate a Golgi stack (B. Glick, pers. comm.). Thus partitioning of the existing Golgi apparatus is not necessary for successful bud formation in this yeast. It is interesting to note that in *P. pastoris* the proteins necessary for the formation of ER exit sites, including the Sar1p exchange factor Sec12p, collocate near the Golgi stacks which are relatively immobile in the cell.

In higher plants, as described previously, the ER has the capacity to regenerate Golgi after BFA treatment, even in the absence of any cytoskeleton and protein synthesis (Saint-Jore *et al.*, 2002), and the Golgi appear to be intimately associated with the ER export sites (daSilva *et al.*, 2004). The main difference between the yeast *P. pastoris* and the plant Golgi appears to be the high mobility of the latter, which is perhaps reflected in the distribution of a transiently expressed Sec12-GFP construct over the whole surface of the ER, so that Sar1p activation can occur when necessary (daSilva *et al.*, 2004). Interestingly, in the yeast *Saccharomyces cerevisiae*, which has dispersed individual Golgi cisternae, Sec12 is also distributed over the whole ER network (Rossanese *et al.*, 1999). As in yeast and mammalian cells Golgi can arise de novo from the ER, it is tempting to speculate that the biogenesis of plant Golgi stacks may be by a similar mechanism. Thus, when a cell needs to increase its secretory activity or double its Golgi stack complement premitosis, new stacks can be generated from the ER by the formation of new exit sites.

V. Retrograde transport – Golgi to ER – the role of ARF and COPI

Since the arabidopsis homologue of the yeast HDEL receptor (Lewis *et al.*, 1990) was first reported (Lee *et al.*, 1993), considerable evidence has been published confirming the existence of a retrograde pathway between the Golgi and ER in plant cells (Denecke, 2003). Indeed, a range of carboxy terminal ER retrieval/retention motifs have now been described in plants (Crofts *et al.*, 1999; Denecke, 2003; McCartney *et al.*, 2004). Recently it has been shown in *vitro* that dilysine motifs in the cytosolic tail of some type 1 ER membrane proteins interact with the COPI vesicle coat, providing more evidence for a COPI mediated retrieval pathway (Contreras *et al.*, 2004). Evidence also exists that the ER chaperone calreticulin cycles from the ER and returns from the Golgi, most likely in COPI vesicles (Pimpl *et al.*, 2000; Phillipson *et al.*, 2001; Contreras *et al.*, 2004).

Understandably, much of the evidence for a retrograde Golgi to ER pathway in plants has come from the use of the secretory inhibitor BFA and its ability to disrupt COPI vesicle coat formation. Indeed, the COPI coat was first described due to the action of BFA on releasing the protein complex from Golgi membranes (Donaldson *et al.*, 1990). The COPI complex (coatamer) was initially found on the intracisternal transport vesicles identified in the pioneering *in vitro* Golgi transport assay developed by Rothman (Waters *et al.*, 1991). Since this work in the early 1990s the exact role of COPI vesicles has been a matter of much controversy (Murshid & Presley, 2004).

The first reports on the effects of BFA on plants showed that in root cells the Golgi aggregated in to large compartments and appeared to vesiculate from the trans-face (Satiat-Jeunemaitre & Hawes, 1992). These so-called ‘BFA compartments’ accumulated both a Golgi marker glycoprotein (JIM 84, Fitchette *et al.*, 1999) and secretory material (Satiat-Jeunemaitre & Hawes, 1993). More recently both the Golgi stacks and the BFA compartments have been shown to contain COPI proteins and the ADP-ribosylation factor (ARF1), the small GTPase that in its GTP configuration associates with Golgi membranes and initiates COPI coat formation (Fig. 5; Couchy *et al.*, 2003). BFA compartments have also been described in Arabidopsis root tip cells by immunolabelling of a mammalian sialyl transferase which was located in the Golgi of transformed plants (Wee *et al.*, 1998).

It is now known from mammalian and yeast cells the prime target for BFA is in fact the Sec7 domain of GEFs that control the GDP/GTP exchange on AFR1 (Chardin & McCormick, 1999; Robineau *et al.*, 2000; Nie *et al.*, 2003). Interestingly, although BFA has been shown to release ARF1 and COPI components from plant Golgi (Ritzenthaler *et al.*, 2002), the only plant GEF that has to date been reported to be BFA sensitive is the arabidopsis GNOM protein (Geldner *et al.*, 2003). GNOM has been shown to mediate BFA-induced accumulation of PIN1, the plasma membrane located auxin efflux carrier, in BFA compartments via an endocytic pathway (Geldner *et al.*, 2001). The authors concluded that the BFA compartments were formed from a BFA induced aggregation of endosomal-like compartments, although they did not dismiss the possibility of a contribution of vesicles from the Golgi. Further evidence of the endosomal nature of BFA compartments came from the suggestion that partially esterified pectins in maize roots meristematic cells are endocytosed into large aggregates (Baluška *et al.*, 2002). However, in this study it was not conclusively demonstrated that the accumulation was not due to blockage of secretion and ARF1 was shown to accumulate in the BFA compartments, as also reported by Couchy *et al.* (2003; Fig. 5). Surprisingly, a *cis*-Golgi marker revealed by immunofluorescence did not appear to redistribute either into the ER or into the BFA compartments on BFA treatment, contradicting the electron microscopy of Satiat-Jeunemaitre & Hawes (1993) who clearly demonstrated BFA induced Golgi aggregation, vesiculation and disappearance of cisternal stacks in maize root meristem cells. It is therefore still unclear as to the exact effect of BFA in root meristems, although it is likely that the BFA compartment is a hybrid containing both Golgi derived membrane and cargo plus endocytosed material.

In leaf and suspension culture cells the physical manifestation of BFA treatment has been more akin to, although much
slower than that reported for mammalian cells. Various fluorescent protein constructs of Golgi targeted membrane proteins have been reported to be redistributed into the ER network on exposure to BFA (Fig. 6; Boevink et al., 1998; Ritzenthaler et al., 2002; Saint-Jore et al., 2002) resulting in an almost total loss of Golgi fluorescence in the cells. Saint-Jore et al. (2002) demonstrated that this process can occur, in the absence of any cytoskeletal elements and also when protein synthesis had been blocked, indicating that ER fluorescence was not due to synthesis of new fluorescent protein. On removal of the drug, fluorescent Golgi bodies slowly reappeared and this process could also take place in the absence of protein synthesis and any cytoskeletal elements, highlighting the close proximity of the two organelles to each other. Such experiments indicate that there must be a retrograde pathway for membrane proteins which is stimulated or otherwise modified by BFA. It is now apparent that such a pathway may also exist for soluble cargo proteins (L. Frigerio, pers. comm.).

To date there is little data on the prime target of BFA which induces dispersal of the Golgi in plants. Immunofluorescence and biochemical studies on BY2 cells have indicated that both (γ-COP and ARF1 are displaced from the Golgi within 5 min of BFA treatment (Ritzenthaler et al., 2002). However, all the (γ-COP, but not all the ARF1 was removed from the Golgi. This is in contrast to the very rapid removal of ARF1 from Golgi stacks in mammalian cells, prior to displacement of the COP1 proteins (Donaldson et al., 1992). Interestingly, a recent report has suggested that BFA increases the binding of plant ARF1 to the cytosolic tail of a plant P24 protein, which may be a putative cargo receptor, suggesting that this binding is dependent on the GDP-bound form of ARF which would then be predicted also to be associated with the Golgi mem- 

brane (Contreras et al., 2004). The loss of coatomer in BY2 cells treated with BFA, was followed by a subsequent loss of Golgi cisternae starting at the cis-face and the formation of a so-called ER/Golgi hybrid compartment through fusion of Golgi cisternae with the ER (Ritzenthaler et al., 2002). Whether the loss of COP1 coat components is directly linked to the fusion of the Golgi with the ER or whether this reflects two different BFA effects has yet to be determined. Studies with secretory GFP certainly indicate a block of fluorescent protein in the ER (Boevink et al., 1999). Brandizzi et al. (2002b) using photobleaching have demonstrated inhibition of ST-GFP transport from the ER to Golgi on BFA treatment before reabsorption of Golgi membranes into the ER, indicating a possible site of action at the ER export site. However, even if the exact molecular mechanisms of BFA on the plant ER/Golgi complex have still to be determined, the use of the
drug has highlighted the existence of a retrograde Golgi-ER pathway in several cell types.

A possible role of ARF1 in regulating exit out of the ER emerged from the fact that various AtARF nucleotide binding mutants, when expressed in tobacco and Arabidopsis cultured cells, changed the location of AtErD2 from the Golgi to the ER and inhibited secretion of a soluble vacuolar protein sporamin-GFP (Yahara & Nakano, 2002). Likewise a GDP locked form of ARF1 induced relocation of ST-GFP to the ER and retargeted an H1-ATPase-GFP construct away from the plasma membrane to another organelle (Lee et al., 2002). A dominant-negative GTP-restricted ARF1 mutant has also been shown to inhibit the secretion of α-amylase in a biochemical assay whilst simultaneously inducing the secretion of the vacuolar protein phytase to the culture medium (Pimpl et al., 2003). Interestingly, the induced secretion observed was restricted to proteins which were expected to be sorted in a BP80-dependent manner (i.e. namely is those destined for the vacuole in clathrin-mediated vectors). Thus, the authors suggested that ARF1 is required for the default secretory pathway to the cell surface and for vacuolar sorting by interacting with clathrin associated adaptors. Therefore, the growing body of evidence indicates that ARF1 may play a role in both anterograde and retrograde pathways in the Golgi. However, it is worth noting that the possibility exists that the COPI and COPII pathways are interdependent on each other due to the necessity for recycling the regulatory machinery of the secretory pathway such as the various SNARE proteins involved in membrane fusion (Ward et al., 2001). Thus, inhibition of either an anterograde or retrograde transport step may result in the collapse of an opposing route.

VI. Transport down the stack

A quick perusal through the Golgi literature over the past ten years or so will reveal a remarkable volte-face over the accepted model explaining transport through the Golgi stack from cis- to trans-face. The well-accepted vesicle shuttle model was superceded by the cisternal maturation model when it became clear that perhaps the major role for COPI vesicles was in mediating retrograde transport back up the stack to the ER (Murshid & Presley, 2004). These two models have often been discussed in relation to the operation of the plant Golgi stack (Staehelin & Moore, 1995; Hawes & Satir-Jeunemaitre, 1996; Nebenführ & Staehelin, 2001; Nebenführ, 2003). Traditionally the plant community have favoured the cisternal progression model due to the convincing argument put forward showing exclusion of algal scales from peripheral Golgi vesicles (Becker et al., 1995). Evidence from BFA treatment of BY2 cells expressing α-1,2 mannosidase-GFP also supported the cisternal maturation model (Ritzenthaler et al., 2002). There is now evidence that, at least in some storage tissues such as developing pea cotyledons, protein sorting into dense vesicles can take place as early as the cis-cisternae (Hillmer et al., 2001), implying that complete processing of a protein may not require transport through all of the cisternae of the stack. However, in this system it now seems likely that such dense vesicles stay attached to the Golgi and follow the cisternal maturation pathway to the trans-Golgi, as it has recently been shown that sorting of sucrose-binding-protein homologues into legumin and vicilin containing dense vesicles occurs in the medial and trans-Golgi (B. Wenzel et al., unpublished).

Recently, other models have been introduced which help explain some of the data from live cell imaging of secretion in cultured mammalian cells and from EM tomographic reconstructions of Golgi. Thus, it has been suggested that there may be direct tubular connections between cisternae or that such connections may be transient permitting rapid transit of cargo through the stack (Nebenführ, 2003; Polishchuk & Mironov, 2004). These models are appealing in that they permit rapid bulk transport of secretory material and there are various examples in the literature where the plant Golgi stack has been shown to be interconnected by tubules of varying width (Juniper et al., 1982; Lockhausen et al., 1990; Harris & Oparka, 1983). No matter what model ultimately is proved to be correct, or whether different models and combinations thereof predominate in different tissues at different physiological states, it is still necessary to explain the maintenance of the biochemical identities of the various Golgi compartments, especially with respect to processing enzymes and sugar transporters.

VII. Targeting within the Golgi

One key feature that distinguishes the Golgi apparatus is the polarity of the stack from cis to trans-faces. Such polarity not only reflects putative entry and exit points from the organelle but must also reflect the distribution of the processing machinery throughout the stack. At this point it is also worth mentioning that each cisternum exhibits radial differentiation becoming fenestrated at the margins, although nothing is known in plants about the biochemical differentiation within individual cisternae. Initially the putative distribution of processing enzymes was deduced from differential immunogold location of their product in the various Golgi cisternae (Fitchette-Lainé et al., 1994). Clues as to the targeting information required to retain various transferases in the cisternal stack and similarities with the mechanisms in mammalian cells came from the expression of heterologous fluorescent protein chimeras. It was shown that the signal anchor sequence of a rat sialyl transferase (52 amino acids of the transmembrane domain and the cytoplasmic tail) and the equivalent sequence from a human galactosyl transferase was sufficient to target GFP to the Golgi stacks in tobacco leaf cells and BY2 suspension culture cells (Boeving et al., 1998; Saint-Jore et al., 2002). Subsequently the equivalent sequences from recently cloned plant glycosyl transferases gave the same
result (Essl et al., 1999; Dirnberger et al., 2002; Pagny et al., 2003). These results suggest that the mechanisms of targeting and retention of glycosyl transferases may be conserved across kingdoms.

Various models have been proposed in the literature to explain the retention of Golgi processing enzymes in their correct spatial positions and it is likely that a combination of these may finally be required. The length of the hydrophobic trans-membrane domain (TMD) has been implicated as a general indicator of retention at the correct organelle (Munro, 1995, 1998) as exemplified by the results from Saint-Jore et al. (2002). Generally, the thickness of the membranes of the organelles of the secretory pathway increases from the ER to the Golgi and finally the plasma membrane owing to a gradient in the sterol composition of the lipid bilayers (Moreau et al., 1998). Targeting related to TMD length was revealed in a study varying the length of TMDs in GFP fusion proteins made from the human lysosomal membrane protein LAMP1 and expressed in tobacco leaves (Brandizzi et al., 2002c). A fusion protein containing the native 23 amino acid TMD located to the plasma membrane. When the TMD was shortened to 20 and 17 amino acids the GFP chimeras were restricted to the Golgi and ER membranes, respectively.

However, such experiments do not determine spatial location within the Golgi stack. In the early nineties the ‘kin recognition model’ based around the oligomerisation and aggregation of processing enzymes within cisternal membranes was postulated (Nilsson et al., 1993), as such enzymes were not thought to be incorporated into anterograde transport vesicles. Whilst attractive, this model does not explain the capacity for mammalian transferases to recycle continually via the ER (Ward & Brandizzi, 2004), a situation that probably also occurs in plants (Brandizzi et al., 2002b). A model proposing retrograde transport of processing machinery in vesicle vectors is attractive as this could both explain the positioning of enzymes and also the control over their loss as product is delivered towards the trans-face of the Golgi (Opat et al., 2001).

VIII. Holding it all together

It is difficult to believe that the integrity of the Golgi stack and its relationship with the ER and the proteins of the ER exit site is simply maintained by interactions between the component membranes. Indeed, in mammalian cells a complex series of interacting Golgi matrix proteins have been identified which may control the integrity of the Golgi ribbon (Klumperman, 2000; Ward et al., 2001); tether incoming and percolating vesicles (Barr & Short, 2003); and maintain interactions with the cytoskeleton (Murshid & Presley, 2004). These include the Golgins, a family of coiled-coil proteins which function in a variety of membrane to cytoskeleton and membrane to membrane tethering events at the Golgi. These are in turn regulated by small GTPases of the Rab and Arl (ARF-like) GTPase families (Barr & Short, 2003). The coiled-coil regions of Golgins form the so-called GRIP domain which confers Golgi targeting plus Rab6 and Arl binding (Munro & Nichols, 1999; Barr & Short, 2003). Although a few homologues of Golgins can be found in the arabidopsis genome, to date no information exists as to their function. Recently, an arabidopsis gene AtGRIP1, which encodes for a putative GRIP protein has been shown to target the plant Golgi (Gilson et al., 2004). Rab6, involved in retrograde transport in the mammalian Golgi stack (Sannerud et al., 2003), is thought to mediate vesicle tethering and also binds GRIP domains and the dynactin complex. Interestingly, the arabidopsis homologue of Rab6 (RabH1b) also locates to the Golgi stack, although a specific role in organisation of the matrix has not yet been shown (J. Johansen et al., unpublished).

The presence of matrix-like proteins in the plant Golgi stack has, however, been known since the early electron microscopic studies of Mollenhauer (1965), and Turner & Whaley (1965) revealed the presence of intercisternal elements (Fig. 7). These have been reported to be more prevalent between trans-cisternae (Staehelin et al., 1990; Andreeva et al., 1998b; Ritzenthaler et al., 2002) and have still to be characterised either biochemically or at the molecular level. It has also been suggested that each Golgi stack is surrounded by a matrix as there is an area of exclusion of ribosomes around each stack (Staehelin & Moore, 1995).

**Fig. 7** Golgi structure as revealed by high pressure freezing and freeze-substitution of a tobacco BY2 suspension culture cell. Notice the well-defined intercisternal element between trans-cisternae. Bar, 100 nm.
IX. Exit from the stack

On exit from the Golgi stack, cargo is predominantly transported in vesicle vectors to either the lytic/storage vacuole or to the cell surface, with the latter considered to be the default pathway (Denecke et al., 1990). Transport of Golgi derived vesicles to the developing cell plate in the phragmosome at cytokinesis can be classified as a third distinct destination for secretory cargo and membrane (Bednarek & Falbel, 2002).

1. To the cell surface

One of the key functions of the Golgi apparatus is the production of the complex polysaccharide component of the cell wall (see reviews Staehelin & Moore, 1995; Hawes et al., 1996). Little is known about post-Golgi targeting of such matrix polysaccharides (if any occurs), although it is clear that secretory vesicles can carry mixed cargos, for example of xyloglucans and pectins (Sherrier & Vanden Bosch, 1990). However, there are many instances of cells showing asymmetry in composition and thickness of their walls as well as polar distribution of plasma membrane proteins such as the auxin efflux carrier PIN1 (Steinmann et al., 1999), so it has to be assumed that some form of preferential targeting or post-deposition sorting to domains at the cell surface occurs.

Certainly it is relatively easy to demonstrate that the cell surface appears to act as a default destination for secretory proteins. Such proteins carry an amino terminal signal sequence that mediates translocation across the ER membrane and which is cleaved before subsequent processing of the peptide chain and transport to the Golgi (Vitale & Denecke, 1999). Such secretory proteins appear not to contain any positive targeting information which directs them to the plasma membrane (PM) (Hadlington & Denecke, 2000), although it is possible that their carrier vesicles/vectors contain targeting signals. This pathway has been demonstrated in vivo by the expression of secretory GFP constructs (Boevink et al., 1999; Batoko et al., 2000; Zheng et al., 2004) and inhibition of such secretion with BFA efficiently traps the secretory markers in the ER.

Targeting of membrane proteins to specific domains of the plasma membrane has been demonstrated. For example, PIN1 locates to the distal region of the plasma membrane in root cells of arabidopsis seedlings (Geldner et al., 2001; Jürgens & Geldner, 2002). However, such a distribution can be re-established after release of the protein from BFA induced vesicular compartments, indicating that direct involvement of the Golgi apparatus may not be required for successful targeting.

2. To the vacuoles

Due to the economic importance of many vacuolar proteins, considerable effort has been directed at uncovering the mechanisms by which such proteins are sorted to the two main classes of vacuoles, lytic and storage (Paris et al., 1996; Jiang & Rogers, 1998; Hinz et al., 1999; Jiang & Rogers, 2003). These vacuolar types are distinguished both by the tonoplast intrinsic protein (TIP) component and by their luminal contents (Jauh et al., 1999; Hinz & Herman, 2003; Jiang & Rogers, 2003). It is clear that targeting depends on different peptide signals in the proprotein or mature proteins and is mediated by structurally different transport vesicles (Paris et al., 1996; Jiang & Rogers, 1998; Hinz et al., 1999). Therefore, it has been assumed that there may be various families of receptors located on the Golgi that are responsible for sorting different vacuolar proteins into specific vesicle types.

Transport to the lytic vacuole, which is characterised by (γ-TIPs on the tonoplast, is receptor mediated, utilises clathrin-coated vesicles and occurs via a prevacuolar compartment (PVC) characterised by a SNARE PEP12/AtSYP21 (Sanderfoot et al., 1998; 2000). The first vacuolar sorting receptor (VSR) to be characterised was BP80 from Pisum sativum (VSRPS-1, Kirsch et al., 1994; Paris & Neuhaus, 2002) and homologues have been identified in various other species (Ahmed et al., 2000). VSRs recognise sequence specific NPIR motifs on amino terminal propeptides, on the C-terminus or internally and cycle between the trans-Golgi and PVCs in clathrin coated vesicles (Hinz et al., 1999; Mitsuhashi et al., 2000; 2001; Jiang & Rogers, 2003). These VSRs are integral membrane proteins which have been shown to recruit the clathrin coats through a classic adaptin-binding tyrosine motif in their cytosolic tails (Paris & Neuhaus, 2002). However, the preferential location for VSRs appears to be on the PVC as shown by immuno-cytochemical studies and the expression of fluorescent protein constructs (Li et al., 2002; Tse et al., 2004). Recently, it has been shown that the multivesicular bodies, previously described as part of the endocytic pathway (Tanchak et al., 1984; Tanchak & Fowke, 1987), also function as PVCs (Tse et al., 2004). Scission of the trans-Golgi associated clathrin coated vesicles from the parent membrane may be mediated by a dynamin-like protein (Arabidopsis dynamin-like 6, Jin et al., 2001) acting as a so-called 'pinchase'.

Proteins such as the legume globulins and legumins destined for storage vacuoles (Hillmer et al., 2001), characterised by various combinations of α, δ, and γ-TIPs (Jiang & Rogers, 2003) are transported in noncoated Golgi-derived vesicles, which have sometimes been termed ‘dense vesicles’ due to the osmiophilic nature of their contents (Hohl et al., 1996; Hinz & Herman, 2003). Classic electron microscopy and immunogold labelling has shown that sorting into these vesicles can take place as early as the cis-Golgi (see Regulation of ER to Golgi transport). Sorting relies on a carboxy-terminal propeptide and it is assumed that this is in some way receptor mediated as transport to the storage vacuole is saturable (Frigerio et al., 1998).

The rather neat compartmentation of storage protein exit from the ER may, however, not be as simple as initially imagined. Recent data suggests that the vacuolar sorting receptor may also be involved in sorting proteins destined for the
storage vacuole as well as lytic vacuole. A knock out of AtVSR1 (an arabidopsis isoform of VSR) resulted in partial secretion of the precursors of all major classes of storage proteins in arabidopsis seeds (Shimada et al., 2003). Jolliffe et al. (2004) demonstrated both biochemically and by immunogold cytochemistry that in the endosperm of developing castor bean seeds that proricin and pro2SA albumin pass through the Golgi and colocalise in large post-Golgi vesicles which probably fuse to generate a mature PSV. Interestingly, in vitro experiments demonstrated that both proricin and pro2SA bind to proteins that are similar to members of the VSR/ArEPL/BP-80 vacuolar sorting receptor family, indicating that VSR function may not be restricted to sorting proteins destined for the lytic vacuole.

X. Roles for lipids

It is well known that the lipid component of the various organelles in the endomembrane pathway is important in conferring certain physical properties on the membrane. Thus, the high sterol content of the plasma membrane in eukaryotic cells avoids transition of the highly saturated membrane to the crystalline state. One feature of the Golgi apparatus is that it is here that the sterol and sphingolipid content of membranes increases as the thin membrane of the ER is converted into the thicker tougher plasma membrane (van Meer, 1998). Unlike that in mammalian cells, the plant PM is characterised by a range of sterols. These have been shown to be synthesised on the ER and transported to the PM, presumably via the Golgi and colocalise in large post-Golgi vesicles which probably fuse to generate a mature PSV. Interestingly, in vitro experiments demonstrated that both proricin and pro2SA bind to proteins that are similar to members of the VSR/ArEPL/BP-80 vacuolar sorting receptor family, indicating that VSR function may not be restricted to sorting proteins destined for the lytic vacuole.

XI. Conclusions

This review has concentrated on the more recent ideas on the dynamics and molecular mechanics of the plant Golgi apparatus. It has not been possible to consider the huge volume of literature on the synthesis and transport of cell wall material nor the mechanisms of glycosylation of proteins as they traverse the Golgi stack. The number of questions still to be answered on the functions and dynamics of this intriguing organelle probably still out number those that have been answered. For instance, the molecular organisation of the ER/ Golgi interface still has to be mapped, the mechanisms of both anterograde and retrograde vesicle transport (where it occurs) have to be elucidated and nothing is known about the many proteins that must be involved in maintaining the integrity of the cisternal stack. How can a one micron diameter stack of membrane discs motor around the cytosol at speeds of over one micron a second while receiving material from the ER, generating different classes of transport vesicles and targeting them to different destinations and still remain intact? An interesting few years in plant Golgi research lie ahead.

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Note added in proof

Since the submission of this article the following paper has been published, detailing the location of 15 fluorescent protein tagged SNARE molecules that are most likely involved in transport between the ER and the Golgi apparatus.


References


Review


Shimada T, Fuji K, Tamura K, Kondo M, Nishimura M, Hara-Nishimura I. 2003. Vacular sorting receptor for seed storage proteins in...


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