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The Golgi Apparatus – A Key Organelle in the Plant Secretory Pathway

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ABSTRACT

The secretory pathway in plants mediates the transport of cargo molecules from their site of synthesis to other compartments within the pathway as well as to the cell wall. At the centre of this system is the Golgi apparatus, a transport hub that is responsible for receiving, modifying and sorting proteins for transport to their destinations. Several recent publications have investigated the routes and transport mechanisms that connect the Golgi apparatus with other secretory organelles, including exciting new links with non-secretory organelles such as plastids. This review therefore focuses on the multiple roles of the Golgi apparatus in the secretory pathway and its connections with other organelles within the plant cell.

Keywords: Protein transport, imaging, endoplasmic reticulum

CONTENTS

INTRODUCTION

The secretory pathway of plants is essential to the transport, sorting and modification of proteins destined for delivery to different organelles within the cell or to the extracellular space (**Fig. 1**; Matheson *et al*. 2006). The various secretory organelles are linked together by different processes, starting with protein synthesis at the endoplasmic reticulum (ER) membranes and ending with storage or degradation in specific vacuoles, or secretion outside the cell via the plasma membrane. At the centre of this transport system lies the Golgi apparatus. The Golgi apparatus not only mediates the sorting and packaging of proteins received from other organelles so that they can be delivered to their final destinations, but is also responsible for the modification of glycan moieties attached to these proteins, which may affect their eventual function within the cell.

THE ENDOPLASMIC RETICULUM

The ER consists of a network of membranes that are continuous with the nuclear envelope and undergo constant movement and remodelling (**Fig. 2A**; Irons *et al*. 2003; Hanton *et al*. 2005a; Runions *et al*. 2006). The dynamic behaviour of the ER in plants is dependent on an actin-myosin system, which has been shown to drive cytoplasmic streaming, causing the movement of other organelles in the cell (Knebel *et al*. 1990; Liebe and Menzel 1995; Hawes and Satiat-Jeunemaitre 2001).

Proteins destined for the secretory pathway are synthesised on the ER membranes: soluble proteins pass through the membrane into the lumen, while membrane proteins are inserted into the membrane and locked in by specific motifs in the amino acid sequence (reviewed by Vitale and Denecke 1999). Proteins known as chaperones within the ER are involved in a variety of quality-control processes to ensure that misfolded proteins are not released or able to form aggregates (reviewed by Vitale and Denecke 1999; Vitale and Ceriotti 2004). If a protein is irretrievably misfolded, degradation occurs in order that the constituent amino acids can be recycled. Some proteins are retro-translocated to the cytosol, where they are degraded in the

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Invited Review

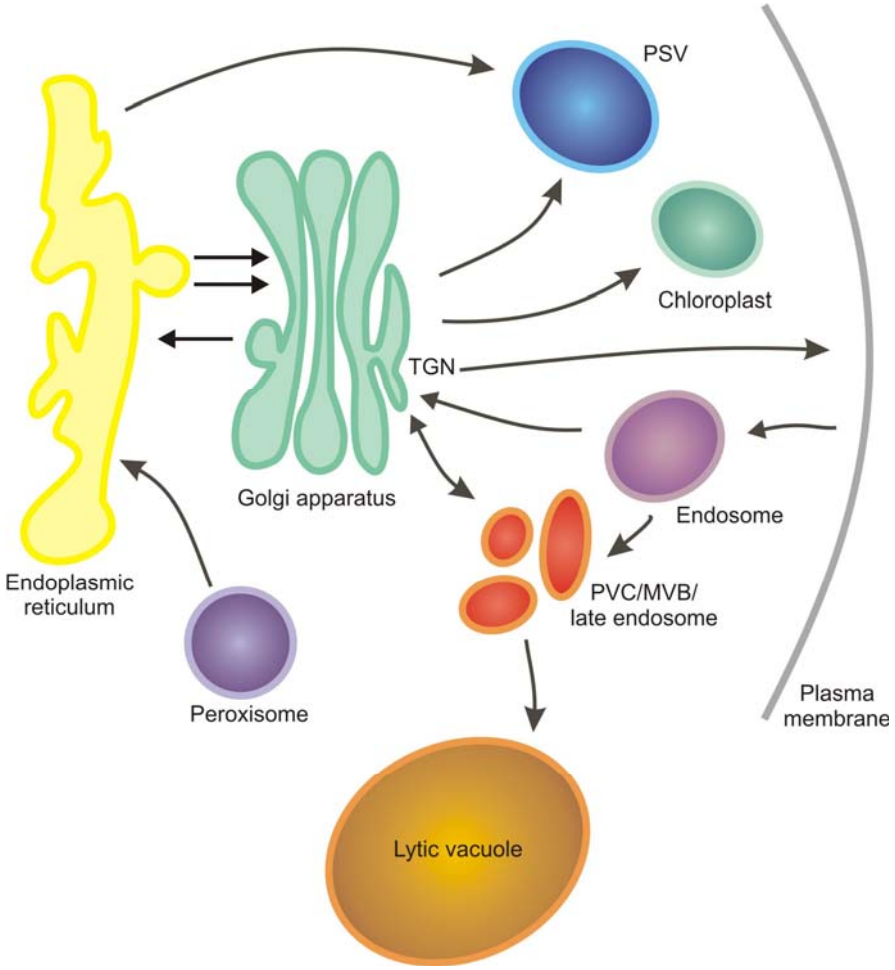

Fig. 1 Schematic representation of the plant secretory pathway and transport routes connecting the constituent organelles. The secretory pathway in plants is made up of various different organelles and transport routes between them (represented by arrows). Organelles such as the chloroplast and peroxisome have recently been shown to have connections with the secretory pathway, although they were not previously thought of as "secretory" organelles. This may result in the expansion of the system termed the secretory pathway to include many more parts of the cell. Proteins can exit the ER and be transported directly to the protein storage vacuole; Golgi modified proteins can also travel to the protein storage vesicle via dense vesicles. Cargo can be transported between the ER and Golgi by either COPII-mediated or COPII-independent mechanisms, while transport from the Golgi to the ER takes place in a COPI-dependent fashion. Post-Golgi transport can be directed to the plasma membrane, chloroplast, lytic vacuole or prevacuolar compartment (PVC)/ multivesicular body (MVB)/late endosome via clathrin-coated vesicles. Additional routes from the PVC/MVB/late endosome to the TGN and the lytic vacuole also exist. The endocytic pathway carries cargo from the plasma membrane to the early endosome at which point it is thought to be transported to the TGN or passed to a PVC/MVB/late endosome.

cytoplasmic proteasome (Pedrazzini *et al*. 1997; Brandizzi *et al*. 2003; Muller *et al*. 2005). A possible alternative to this cytosolic degradation was put forward by Pimpl *et al*. (2006), who showed that the ER-resident chaperone BiP can be transported to the lytic vacuole, presumably in order that misfolded proteins to which it is bound can be degraded.

ER-TO-GOLGI TRANSPORT

One of the most important transport routes from the ER is that to the Golgi apparatus. The early secretory pathway of plants differs from that of mammals in several ways (reviewed by Hanton *et al*. 2005a), including the absence of the ER-Golgi intermediate compartment (ERGIC) that is found in the mammalian secretory pathway (Schweizer *et al*. 1990; Appenzeller-Herzog and Hauri 2006). The mechanisms and machinery by which transport between ER and Golgi occurs in plants remain the subject of much debate (Hanton *et al*. 2006).

Two anterograde pathways exist: export from the ER to the Golgi can occur via a COPII-mediated mechanism (Phillipson *et al*. 2001; da Silva *et al*. 2004), or by a COPII-independent mechanism (Törmäkangas *et al*. 2001), although less is known about the latter. To balance these routes, a COPI-mediated mechanism retrieves cargo molecules from the Golgi to the ER (Pimpl *et al*. 2000; Stefano *et al*. 2006a; Donohoe *et al*. 2007). COPI-independent retrograde routes have been identified in mammals (Girod *et al*. 1999; White *et al*. 1999; Chen *et al*. 2003), homologues of which may also exist in plants to balance the two anterograde routes.

The COPII machinery is composed of a group of proteins that are highly conserved between species (reviewed by Lee *et al*. 2004; Hanton *et al*. 2006): a small GTPase known as Sar1, and two heterodimeric structural subunits: Sec23/24 and Sec13/31. These components are recruited to the ER membrane at specific domains known as ER export sites (ERES, Watson and Stephens 2005), from which transport intermediates are thought to bud, carrying cargo to the Golgi apparatus. In plants, ERES were first visualised in tobacco leaf cells using a fluorescent fusion of a tobacco isoform of Sar1, which is recruited to ERES upon over-expression of membrane cargo proteins destined for the Golgi (da Silva *et al*. 2004). These structures travelled in continual association with Golgi bodies, leading to the proposal of the secretory unit model for ERES-Golgi interaction. This model was supported by a later study using fluorescent fusions of Sec23 and Sec24, which label ERES in both the presence and absence of membrane cargo (Stefano *et al*. 2006a). However, a study in tobacco BY-2 cells using Sec13 as a protein marker indicated that multiple ERES could associate transiently with a single Golgi body, described as a "kiss-and-run" mechanism (Yang *et al*. 2005). The use of different marker proteins in different expression systems makes it difficult to compare these studies directly, leaving this topic open to debate.

A recent study presented evidence for the existence of COPII vesicles in plant cells through electron microscopy (Donohoe *et al*. 2007), some seven years after the isolation of COPI vesicles from plants (Pimpl *et al*. 2000). Although this is an important breakthrough in the study of ER-Golgi transport, the existence of direct connections between the ER and Golgi suggested earlier cannot be excluded at this stage (Juniper *et al*. 1982; Harris and Oparka 1983). Further study is required to characterise the transport intermediates responsible for traffic between ER and Golgi.

ER EXPORT SIGNALS

It has been shown that soluble proteins can travel by a non-selective bulk flow route (Denecke *et al*. 1990; Phillipson *et al*. 2001). However, the transport of membrane proteins appears to be significantly more complex. Studies of type I

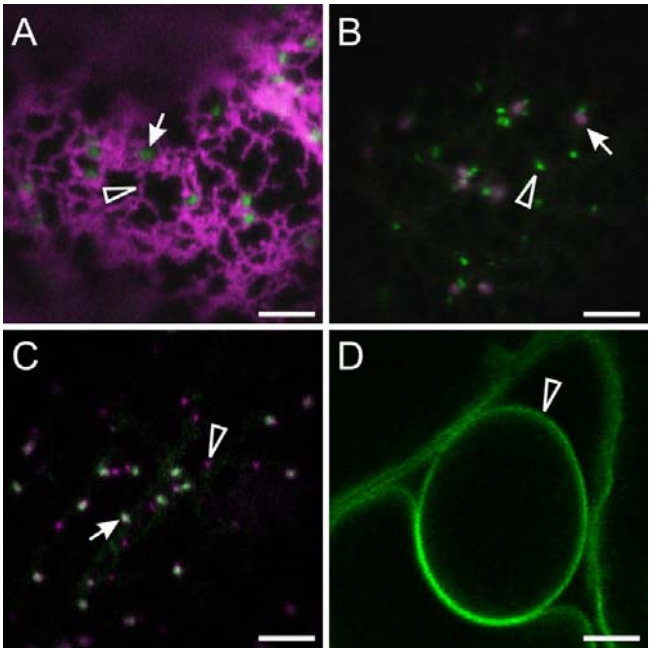

Fig. 2 Fluorescent proteins are an important tool in visualising parts of the secretory pathway. Various proteins have been established as markers for different organelles in the secretory pathway. These micrographs demonstrate the usage of several markers alone or in combination to show the distribution of some of the organelles. (**A**) The ER labelled by spYFP-HDEL (Irons *et al.* 2003; Hanton *et al.* 2005a) exists as a mesh-like network of membranes distributed throughout the cell cortex (arrowhead). Golgi bodies (labelled by TMcCCASP, Hanton *et al.* 2005b) can be seen as dots within this network (arrow). (**B**) Both the Golgi apparatus and prevacuolar compartments are distributed throughout the cytoplasm in plant cells. In this image spGFP-BP80 (Hanton and Brandizzi 2006) was used as a PVC marker (arrowhead), also faintly labelling Golgi bodies (arrow), which are visible due to the use of ERD2-YFP (Brandizzi *et al.* 2002b) as a marker. (**C**) The GTPase ARF1, which plays roles in both Golgi-ER transport and trafficking to the lytic vacuole, is distributed at the Golgi apparatus (arrow) along with the Golgi marker ST-GFP (Boevink *et al.* 1998). ARF1-YFP (Stefano *et al.* 2006a) also labels additional dots (arrowhead), which may be involved in the endocytic pathway (Xu and Scheres 2005). (**D**) The marker BobTIP26-1-GFP (Reisen *et al.* 2003) labels the vacuole membrane, or tonoplast. Membrane-bound structures within the vacuole are frequently observed (arrowhead), although their function is not yet known. Bars = 5 μm.

and type II membrane proteins showed that the length of the transmembrane domain plays a role in regulating their transport through the secretory pathway (Brandizzi *et al*. 2002a; Saint-Jore-Dupas *et al*. 2006). In addition to these findings, three different types of export signals have been identified in the cytosolic domains of plant transmembrane proteins: di-hydrophobic, di-acidic and di-basic (Contreras *et al*. 2004b; Hanton *et al*. 2005b; Yuasa *et al*. 2005). Analysis of a di-acidic motif in a type I protein indicates that the presence of this functional export signal is sufficient to overcome transport limitations incurred by a short transmembrane domain (Hanton *et al*. 2005b). Evidence for recognition of a di-hydrophobic motif by COPII components *in vitro* has also been presented (Contreras *et al*. 2004b), indicating that selection of membrane cargo proteins bearing export signals may be mediated directly by COPII. This would then allow membrane cargo to be packaged into transport carriers for export to the Golgi apparatus, whereas soluble proteins might diffuse into the carriers and thus travel via bulk flow.

COPI-MEDIATED RETROGRADE TRANSPORT

By its very nature, bulk flow anterograde transport is not a selective mechanism. This means that some soluble proteins that are not intended to leave the ER may accidentally be exported to the Golgi apparatus, and then have to be retrieved. In addition, certain membrane proteins such as SNAREs reach the Golgi as part of the transport machinery between ER and Golgi, and must therefore be recycled to the ER to maintain efficient transport of proteins out of the ER. A mechanism for retrieval of proteins to the ER is therefore essential to the cell. This retrieval is mediated by carriers coated with the COPI protein coat, consisting of the small GTPase ARF1 and a heptameric complex of coat proteins termed coatomer (Malhotra *et al*. 1989).

Certain membrane proteins have a selective retrieval system in place that is similar to that which mediates their export from the ER. A signal in the cytosolic tail of the protein interacts with components of the COPI coat (Contreras *et al*. 2004a; McCartney *et al*. 2004), causing the cargo to be incorporated into the transport carrier for trafficking to the ER. In the case of soluble proteins, direct interaction with cytosolic coat components is impossible. Therefore, soluble ER resident proteins possess a tetrapeptide H/KDEL motif at their extreme C-terminus (Denecke *et al*. 1992), which is thought to interact with the receptor protein ERD2 (Lee *et al*. 1993), as has been shown in yeast (Lewis *et al*. 1990; Lewis and Pelham 1992). This interaction allows receptor-mediated retrieval of the soluble protein to the ER.

NON-GOLGI ER EXPORT ROUTES

In addition to the transport routes between ER and Golgi discussed above, various other routes from the ER exist that do not appear to involve the Golgi apparatus. For example, a variety of vesicle types appear to transport storage proteins directly from the ER to the protein storage vacuole in seeds from different species (Levanony *et al*. 1992; Hara-Nishimura *et al*. 1998; Toyooka *et al*. 2000; Takahashi *et al*. 2005). These vesicles are then thought to be incorporated into the vacuole through a mechanism similar to autophagy (Levanony *et al*. 1992). It may be the case that these routes exist only in seeds, in order to cope with the large amounts of storage proteins that must be stockpiled in the protein storage vacuole. It is thought that the majority of proteins that travel by these routes do not require Golgi-mediated modifications, making it unnecessary to expend energy on transport to the Golgi followed by repackaging into a second type of carrier for subsequent transport to the protein storage vacuole. In support of this hypothesis, glycosylated proteins have been identified around the periphery of some of the Golgi-independent vesicles (Hara-Nishimura *et al*. 1998), suggesting that a second transport route for Golgi-modified proteins may exist between the Golgi apparatus and the large transport vesicles themselves.

The early secretory pathway has also been implicated in protein transport to peroxisomes, although it is not clear whether the Golgi apparatus is involved. It appears that several peroxisomal proteins accumulate in specific subdomains of the ER (Lisenbee *et al*. 2003; Flynn *et al*. 2005; Karnik and Trelease 2005), termed peroxisomal ER (pER, Mullen and Trelease 2006). A transport route from the peroxisome to the ER has also been identified (McCartney *et al*. 2005), indicating the potential for protein cycling between the two organelles, although the ER to peroxisome pathway remains to be characterised. It has been suggested that peroxisomes may differentiate from the ER, although they can also be generated through division of pre-existing peroxisomes (Lingard and Trelease 2006). It may therefore be the case that some proteins are incorporated into nascent peroxisomes as they differentiate from the ER, while others may be transported to pre-existing peroxisomes from pER subdomains. Evidence has been presented indicating potential involvement of the Golgi in at least one peroxisomal transport pathway (Mullen *et al*. 1999), but it is apparent that further study is required to elucidate the transport mechanisms that link the early secretory pathway with peroxisomes.

THE PLANT GOLGI APPARATUS

The active exchange of cargo between the ER and the Golgi by means of balanced anterograde and retrograde transport routes maintains the integrity of Golgi membranes and the distribution of Golgi membrane proteins (Hanton *et al*. 2005a; Hawes and Satiat-Jeunemaitre 2005). This central organelle of the plant secretory pathway is the sum of many polarised stacks of membrane-bounded cisternae distributed throughout the cytoplasm of plant cells (**Fig. 2A-C**). The Golgi has functional roles in the glycosylation of proteins and lipids, and in the production of polysaccharides for cell wall biosynthesis. In transit through the organelle, proteins and lipids are processed by Golgi-resident enzymes that modify attached glycan chains. The products either take up residence in the Golgi apparatus or are sorted at the *trans* Golgi network (TGN) and packaged into vesicles for transport.

The fact that many individual mobile Golgi stacks exist in a single plant cell sets the plant Golgi apparatus apart from the secretory pathway of several other systems, including mammals and yeast (reviewed by Hanton *et al*. 2006). Each stack possesses polarity from the ER-adjacent *cis*-face to the *trans*-face. Such polarity not only reflects putative entry and exit points from the organelle, but also dictates the distribution of the processing machinery throughout the stack. It has been shown that the enzymes of the N-glycan processing pathway are spatially arranged in the order in which individual enzymes act sequentially on their glycoprotein substrates (Saint-Jore-Dupas *et al*. 2006). Studies in mammalian cells have suggested that the organisation of Golgi-resident enzymes is based on a COPI-independent, Rab-dependent mechanism (Bannykh *et al*. 2005); however, very little is known in plants regarding the mechanisms of enzyme recruitment to individual cisternae. Furthermore, how the complex architecture of the Golgi apparatus contributes to its efficient operation remains to be fully elucidated.

GOLGI STACK BIOGENESIS

Contrary to the mammalian Golgi apparatus, which dissipates during the process of cell division (Shorter and Warren 2002), the plant Golgi stacks remain intact during mitosis (Nebenführ *et al*. 2000). Interestingly, it was suggested that plant and algal Golgi stacks multiply by division (Hirose and Komamine 1989). The signals that initiate the scission of Golgi membranes and the specific mechanisms that lead to constriction and separation of the stacks are unknown. The evidence that Golgi stacks can divide does not exclude the possibility that Golgi membranes can form *de novo*.

It is tempting to speculate that the biogenesis of plant Golgi stacks occurs in response to the need of a cell to secrete. For example, ER export can be reversibly disrupted by drugs, such as brefeldin A (BFA, Driouich *et al*. 1993; Ritzenthaler *et al*. 2002). This drug affects the integrity of the Golgi membranes and of ERES (da Silva *et al*. 2004). Upon removal of BFA, the plant ER regenerates Golgi stacks and associated ERES, and ER export is reconstituted (Saint-Jore *et al*. 2002; da Silva *et al*. 2004; Stefano *et al*. 2006a). In addition, in *Arabidopsis* apical meristem cells, the number of stacks doubles in number immediately prior to the organelle partitioning between daughter cells that occurs during mitosis (Segui-Simarro and Staehelin 2006). The duplication of Golgi stacks during division may be needed to deal with the increased necessity for delivery of materials to the nascent cell wall. A recent report analysed the response of plant cells to an increased need for secretion, demonstrating that the plant ER is able to respond to an increase in the number of membrane cargo proteins destined for the Golgi by recruiting COPII machinery onto existing ERES as well as by generating new ERES (Hanton *et al*. 2007). Both mechanisms were found to be dependent on the presence of an active ER export motif in the membrane cargo. This signal-mediated mechanism for the *de novo* formation of ERES strongly suggests the existence of a tightly-regulated feedback between cytosolic factors involved in cargo export and differentiation of membrane subdomains.

STRUCTURE: GOLGI MATRIX

Several suggestions have been put forward as to how the Golgi apparatus maintains its structural integrity and its identity from the ER. A group of proteins, collectively known as the Golgi matrix, has been identified in mammalian and yeast cells. It is predicted that some of these proteins form the inter-cisternal elements, thus playing a role in the regulation of traffic, whereas others tether and guide vesicles (Mogelsvang and Howell 2006). Understanding the Golgi matrix, and how its components work together to promote stack stabilisation, is expected to be crucial in gaining a global appreciation for the function and organisation of the Golgi apparatus in plants.

Golgins, a family of coiled-coil proteins, are thought to be part of a Golgi matrix (Latijnhouwers *et al*. 2005a; Short *et al*. 2005). The *Arabidopsis* genome encodes homologues of mammalian and yeast peripheral and integral membrane golgins (Latijnhouwers *et al*. 2005a). The first plant golgin identified was AtCASP (for CCAAT displacement protein alternatively spliced product), but its functions at the Golgi apparatus have yet to be characterised (Renna *et al*. 2005). A number of peripheral golgins contain a carboxy-terminal GRIP domain, which is sufficient for Golgi targeting (Munro and Nichols 1999). One such golgin from *Arabidopsis* has been characterised and fluorescent fusions of the GRIP domain as well as the full length protein (AtGRIP) target the Golgi apparatus through a mechanism that is dependent on the small GTPase, ARL1 (Gilson *et al*. 2004; Latijnhouwers *et al*. 2005b; Stefano *et al*. 2006b). The existence of other putative *Arabidopsis* homologues to animal and yeast golgins has been suggested but these have yet to be investigated experimentally (Latijnhouwers *et al*. 2005a). In addition, it is possible that the plant genome contains other plant-specific, golgin-type proteins that support the unique nature of the plant Golgi apparatus. For example, the existence of a plant-specific coiled-coil protein, MAF1 from tomato (LeMAF1), which associates with the nuclear envelope and Golgi apparatus, has been reported (Patel *et al*. 2005). It is possible that proteins like LeMAF1 contribute to the establishment of unique features of the plant Golgi apparatus. Organelle specific proteomics may become a useful strategy to identify and isolate Golgi matrix proteins that might be otherwise undetectable through bioinformatics (Rose *et al*. 2004; Dunkley *et al*. 2006).

POST-GOLGI TRANSPORT

In addition to the roles of the Golgi described above, a major function of this organelle is to mediate the sorting and export of proteins that are destined for onward transport to distal organelles in the cell. These include the lytic and storage vacuoles and their prevacuolar compartments, the plasma membrane and the chloroplast. In recent years, much progress has been made in elucidating aspects of the transport to these compartments.

TRANSPORT TO THE LYTIC VACUOLE

Proteins that are transported to the lytic vacuole are thought to travel first to the prevacuolar compartment (PVC), and thence to the vacuole (**Fig. 2D**). It has been shown that the PVC exists as multivesicular bodies (MVB) in plants, which can be stained by the dye FM4-64, indicating that this compartment also has an endocytic function (Tse *et al*. 2004). Emerging evidence indicates that the PVC in plants may be equivalent to the late endosome in other systems (Bolte *et al*. 2004b; Tse *et al*. 2004; Lam *et al*. 2007). Soluble proteins rely on sorting signals to be targeted to the lytic vacuole; it

is generally believed that the so-called sequence-specific sorting signals enable interaction of the cargo molecule with vacuolar sorting receptors (VSRs) such as BP80 (Kirsch *et al*. 1996; Ahmed *et al*. 2000). It is not known at which transport step this interaction occurs, but the cargo-receptor complex is thought to traffic from the Golgi to the PVC, where dissociation occurs due to the lower pH of that compartment (Kirsch *et al*. 1994). Recent studies have provided information on the mechanisms by which BP80 carries out its function within the cell (da Silva *et al*. 2005, 2006); disruption of the receptor cycling between PVC and Golgi results in its delivery to the vacuole membrane and secretion of its ligands out of the cell (da Silva *et al*. 2005), demonstrating the importance of this cycling in vacuolar transport. Signals within the cytosolic tail of BP80 were also shown to be important in its transport, with a conserved YXXΦ motif being essential for the correct delivery of cargo molecules to the vacuole (da Silva *et al*. 2006). Similar motifs interact with components of the clathrin coat to induce the formation of clathrin-coated vesicles at the *trans*-Golgi; indeed, an interaction between a μ-adaptin and a VSR, both from *Arabidopsis*, has recently been demonstrated (Happel *et al*. 2004). da Silva *et al*. (2006) also provided evidence for an alternative BP80 route to the PVC, via the plasma membrane and apparently involving endocytosis. This corresponds to findings in yeast by Deloche and Schekman (2002), where the VSR Vps10 cycles between the plasma membrane and late endosome.

In addition to the information summarised above, several recent studies have identified regulatory molecules that are involved in the transport steps between the Golgi and lytic vacuole. The precise roles of these molecules are not known as yet, but their identification opens the way for in-depth characterisation studies. For example, a dominant negative mutant of the Rab protein m-$Rab_{mc,}$ which localises at both the Golgi and PVC, results in reduced transport of the soluble vacuolar cargo protein aleurain to the vacuole at a post-Golgi step (Bolte *et al*. 2004a). Similarly, a GTPase-activating protein (GAP) from *Oryza sativa*, which interacts specifically with Rab proteins, mediates transport from the Golgi to the lytic vacuole (Heo *et al*. 2005). However, this Rab-GAP also appears to be involved in transport from the Golgi to the plasma membrane (Heo *et al*. 2005), suggesting that it may control export from the Golgi apparatus rather than specific transport routes to certain organelles. The involvement of the actin cytoskeleton in vacuolar trafficking has also been studied (Kim *et al*. 2005). The authors showed that a functional actin network is necessary for the transport of soluble cargo molecules to the lytic vacuole. It appears that these molecules accumulate at the *trans*-Golgi network, indicating that the Golgi-PVC transport step is disrupted. It may be the case that Golgi movement, which relies on actin filaments (Boevink *et al*. 1998; Nebenführ *et al*. 1999), is necessary for the efficient transfer of proteins to the PVC, although this remains to be demonstrated directly. Finally, it is still unknown how the transport step between PVC and vacuole occurs; it is not clear whether this step involves fusion of the two organelles, or vesicular transport between them. However, the putative molecular machinery involved in this step is beginning to be unravelled. A SNARE protein (SYP21) has been implicated in PVC to vacuole transport (Foresti *et al*. 2006), providing a starting-point for future investigations. Over-expression of SYP21 results in the accumulation of both soluble and membrane proteins in the PVC, as well as increased secretion of soluble vacuolar cargo. This accumulation of protein in the PVC is accompanied by an increase in the size of these organelles, although their number is reduced. These data indicate that SYP21 plays a role in PVC-vacuole transport, although the details of this mechanism remain unknown.

TRANSPORT TO THE PROTEIN STORAGE VACUOLE

The complexity of the secretory pathway of plants is increased by the presence in some cell types of a second vacuole with a storage function (Paris *et al*. 1996). Transport of proteins to the protein storage vacuole has been shown to occur by a number of pathways, both via the Golgi and direct from the ER (reviewed by Vitale and Hinz 2005). These routes may be specific to different proteins; those that require Golgi-mediated modification travel from the Golgi to the protein storage vacuole via dense vesicles (Hohl *et al*. 1996), while those travelling direct from the ER to the protein storage vacuole utilise a variety of different vesicle types (Levanony *et al*. 1992; Hara-Nishimura *et al*. 1998; Toyooka *et al*. 2000; Takahashi *et al*. 2005). Evidence has been presented for the involvement of receptor proteins in the transport of cargo to the protein storage vacuole, regardless of the route taken (Shimada *et al*. 1997, 2002; Jolliffe *et al*. 2004; Park *et al*. 2005); however, vacuolar sorting receptors have not been detected in dense vesicles (Hinz *et al*. 1999), suggesting that multiple mechanisms may be involved. Vacuolar sorting receptors that recognise storage proteins have homology to BP80 (Shimada *et al*. 1997), but it is not clear how the distinction is made between cargo destined for the lytic vacuole and that intended for the protein storage vacuole, nor how the mechanisms of transport ensure the correct delivery of these cargo molecules. Evidence has also been presented for the recycling of storage protein receptors, in a similar manner to that of BP80 at the PVC and Golgi (Shimada *et al*. 2006). These data together suggest that a PVC-like compartment that is specific to the protein storage vacuole may exist in addition to the PVC of the lytic vacuole; it is also possible that the multi-vesicular nature of the PVC (Tse *et al*. 2004) enables it to segregate proteins destined for the different types of vacuole from one another and to function in a dual capacity serving both vacuole types (Jiang *et al*. 2002).

TO AND FROM THE PLASMA MEMBRANE

Transport toward the plasma membrane has not been fully characterised in plants. For soluble proteins without targeting signals, it has been shown that the default destination is secretion from the cell (Denecke *et al*. 1990), indicating that this process is not receptor-mediated. A recent study showed that a di-acidic motif present in the cytosolic tail of a plasma membrane-localised membrane protein is required for its transport from the ER to the plasma membrane (Mikosch *et al*. 2006); however, it seems likely that this motif operates at the level of ER export, as has been shown for other di-acidic motifs (Hanton *et al*. 2005b). Furthermore, it has been shown that the length of the trans-membrane domain of type I proteins strongly influences their arrival at the plasma membrane (Brandizzi *et al*. 2002a). However, little is known about the mechanisms that mediate transport between the Golgi apparatus and plasma membrane in plants. Clathrin-coated vesicles have been identified both at the plasma membrane and the TGN (Lam *et al*. 2001), but these might be involved in other processes such as vacuolar sorting or endocytosis, rather than trans-port to the plasma membrane. Various effector molecules have been identified that appear to mediate transport to the plasma membrane from the TGN (Preuss *et al*. 2004; de Graaf *et al*. 2005; Zheng *et al*. 2005), but more information is required in order to establish a model for transport from the Golgi to the plasma membrane.

A major area of controversy is the identity of the endosomal compartments; these were initially labelled with the Rab5 homologues Ara6 and Ara7 (Ueda *et al*. 2001), but later studies showed that Ara7 can colocalise with PVC markers such as BP80 (Bolte *et al*. 2004a; Kotzer *et al*. 2004). Two populations of endosomal compartments have been reported (Ueda *et al*. 2004), giving rise to the possibility that the PVC may act as an endosomal compartment

as well as a vacuolar sorting station. A V-ATPase that is localised at the TGN and tonoplast was recently shown to influence the process of endocytosis (Dettmer *et al*. 2006), supporting this hypothesis. The importance of the PVC in the life of the plant cell becomes more apparent as more is known of post-Golgi trafficking.

CONCLUSIONS AND FUTURE PERSPECTIVES

Our knowledge of protein trafficking in the plant secretory pathway, the organelles and transport mechanisms involved, increases almost daily. The influence of the secretory pathway on non-secretory parts of the cell has become more evident as new pathways are discovered. In recent years, evidence for Golgi-mediated transport to the chloroplast has been presented, indicating that even "non-secretory" organelles are involved in this transport system (Villarejo *et al*. 2005; Nanjo *et al*. 2006; Radhamony and Theg 2006). An understanding of the many pathways and processes surrounding the Golgi apparatus will undoubtedly help us to form a complete picture of how the plant secretory pathway contributes to cellular and tissue regulation.

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