MEMBRANE TRAFFICKING IN PLANTS

Gerd Jürgens
ZMBP, Entwicklungsgenetik, Universität Tübingen, 72076 Tübingen, Germany;
email: gerd.juergens@zmbp.uni-tuebingen.de

Key Words  vacuolar sorting endocytosis, recycling, vesicle fusion, Arabidopsis

Abstract  Plant membrane trafficking shares many features with other eukaryotic organisms, including the machinery for vesicle formation and fusion. However, the plant endomembrane system lacks an ER-Golgi intermediate compartment, has numerous Golgi stacks and several types of vacuoles, and forms a transient compartment during cell division. ER-Golgi trafficking involves bulk flow and efficient recycling of H/KDEL-bearing proteins. Sorting in the Golgi stacks separates bulk flow to the plasma membrane from receptor-mediated trafficking to the lytic vacuole. Cargo for the protein storage vacuole is delivered from the endoplasmic reticulum (ER), cis-Golgi, and trans-Golgi. Endocytosis includes recycling of plasma membrane proteins from early endosomes. Late endosomes appear identical with the multivesiculate pre-vacuolar compartment that lies on the Golgi-vacuole trafficking pathway. In dividing cells, homotypic fusion of Golgi-derived vesicles forms the cell plate, which expands laterally by targeted vesicle fusion at its margin, eventually fusing with the plasma membrane.

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INTRODUCTION

Functional compartmentation of the eukaryotic cell necessitates the exchange of proteins, lipids, and polysaccharides between membrane compartments via transport intermediates. Although many aspects of membrane trafficking are likely to be shared among eukaryotes, the endomembrane system of higher plants displays distinct organizational features that may entail adaptive specializations in membrane trafficking.

Remarkable progress has been achieved in the analysis of plant membrane trafficking over the past several years, using different systems and various approaches. Fluorescent marker proteins have been exploited to visualize endomembrane compartments and the exchange of material between them (Brandizzi et al. 2002a). Such studies were often done in tobacco leaf cells, cell cultures, or Arabidopsis leaf cell protoplasts in which genes of interest were transiently expressed. Cell cultures were also used for biochemical studies of cargo sorting. In-planta studies mainly addressed tissue-specific trafficking processes. Finally, the genetic model Arabidopsis is increasingly being used for functional studies of trafficking components and pathways in mutant and transgenic plants.

This review summarizes current knowledge about plant membrane trafficking. To set the stage, specific features of the plant endomembrane system are first described. This is followed by a brief overview of the vesicle trafficking machinery in plants, as revealed by the Arabidopsis genome sequence. The main part of this review focuses on membrane trafficking pathways and their functional analysis.

Organization of the Plant Endomembrane System

ENDOPLASMIC RETICULUM The endoplasmic reticulum (ER) reaches out from the nuclear envelope to the cortical regions of the cell and may be subdivided into several functionally distinct domains (Staehelin 1997). The ER is the entry point into the endomembrane system for newly synthesized proteins. After removal of the signal peptide by a luminal signal peptidase, soluble proteins are folded by the chaperone BiP (Vitale & Denecke 1999). Improperly folded proteins appear to be recognized by a quality control mechanism and translocated across the ER membrane for degradation in the cytosol (Brandizzi et al. 2003). Both soluble and type I membrane proteins are N-glycosylated with a branched oligosaccharide upon entry into the ER lumen (Vitale & Denecke 1999). Arabidopsis mutants defective in N-glycosylation or lacking α-glucosidase I involved in N-glycan processing are embryo lethal (Lukowitz et al. 2001, Gillmor et al. 2002, Boisson et al. 2001). Type II membrane proteins and those with a C-terminal hydrophobic membrane anchor remain largely exposed to the cytosol. ER-resident proteins are retained in the ER or are recycled back from the Golgi apparatus (Vitale & Denecke 1999). Markers for the ER include GFP-HDEL fused to an N-terminal signal peptide and resident proteins...
such as the SAR1 GDP/GTP exchange factor SEC12, the luminal chaperone BiP, and the calcium-binding proteins calreticulin and calnexin (Bar-Peled & Raikhel 1997, Irons et al. 2003). In addition to exchanging membrane with Golgi stacks, the ER segregates various membrane compartments for oil or protein sequestrations, some of which merge with protein storage vacuoles (Chrispeels & Herman 2000).

**GOLGI APPARATUS** The Golgi apparatus is a major sorting station, delivering cargo proteins to multiple destinations. Plant cells have between several and hundreds of Golgi stacks (Staehelin & Moore 1995, Dupree & Sherrier 1998). Each Golgi stack consists of several morphologically distinct cisternae from the cis to the trans side followed by a trans-Golgi network (TGN), which is often less extensive than in animal cells. Golgi stacks are highly mobile and appear to associate with ER strands (Boevink et al. 1998, Nebenführ & Staehelin 2001).

Plant Golgi stacks glycosylate passenger proteins and synthesize noncellulosic polysaccharides for the cell wall (Staehelin & Moore 1995, Dupree & Sherrier 1998). The functional subdivision of Golgi stacks into cis-, medial-, and trans-cisternae is based on enzyme activities. For example, mannosidase II is thought to modify N-glycans in a medial compartment (Staehelin & Moore 1995, Dupree & Sherrier 1998). Several Golgi markers are green fluorescent protein (GFP) fusions with specific enzymes such as UDP-glucose transporter GONST1, mannosidase I (GmManI), β1,2-N-acetylglucosaminyltransferase I (GnTI or NAGTI), β1,2-xylosyltransferase, and mammalian sialyltransferase (ST) (reviewed by Neumann et al. 2003, Saint-Jore-Dupas et al. 2004). There is no clear sequence for retention in Golgi-resident proteins, but the transmembrane domain and the cytosolic tail seem to be involved (Saint-Jore-Dupas et al. 2004).

**ENDOSOMES** Endosomes are still poorly defined in plants, although early studies described a partially coated reticulum (PCR) as an endosomal compartment (reviewed by Battey et al. 1999). In mammals and yeast, early/sorting endosomes are distinguished from recycling endosomes and multivesiculate late endosomes/prevacuolar compartments by suitable markers (Gruenberg 2001, Raiborg et al. 2003). In plants, some Rab5-type markers appear to label different populations of putative early endosomes, which are numerous and highly mobile (Ueda et al. 2001). Putative early endosomes may correspond to 100–300-nm vesicles that form aggregates and accumulate plasma-membrane proteins in brefeldin A (BFA)-treated Arabidopsis root cells (Geldner et al. 2001). Recently, an endocytic multivesiculate compartment was identified as the prevacuolar compartment (PVC) known to be involved in Golgi-vacuole trafficking (Tse et al. 2004). These putative late endosomes are approximately 200–500 nm in size. Thus plant cells may have at least two distinct endosomal compartments: early endosomes involved in sorting and recycling and late endosomes/PVC en route to the lytic vacuole.
DIVERSITY OF VACUOLES

Plant vacuoles are diverse in shape, size, content, and function (Marty 1999). Even functionally related vacuoles may vary between tissues and plant species. There are two major functional types of vacuoles: lytic vacuoles and protein-storage vacuoles, that can occur within the same cell (Paris et al. 1996). Both types of vacuoles are also regenerated in evacuolated tobacco leaf cell protoplasts, supporting their distinct identities (Di Sansebastiano et al. 2001). Although functionally distinct, the two types of vacuoles can fuse, giving rise to a large central vacuole.

Lytic vacuoles are equivalent to animal lysosomes or yeast vacuoles, functioning as compartments for degradation and waste storage. Distinct markers for lytic vacuoles often used in trafficking studies are the $\gamma$-form of membrane-localized tonoplast intrinsic protein (TIP) ($\gamma$-TIP) and the soluble proteins sweet potato sporamin and acidic cysteine protease aleurain (Matsuoka et al. 1995, Paris et al. 1996). Protein storage vacuoles (PSVs) accumulate proteins that are utilized mainly as nutrients during seed germination, such as 2S albumin, and 7S vicilin-type and 11S legumin-type globulins. Storage proteins are degraded by enzymes that have been sequestered in a lytic compartment (“globoid”) within the PSV (Jiang et al. 2001). Protein storage vacuoles may also store lectin, chitinase, and glucanase (Paris et al. 1996, Jiang et al. 2000). The PSV membrane contains characteristic proteins such as the $\alpha$-form of tonoplast intrinsic protein ($\alpha$-TIP). In vegetative tissues, specialized storage vacuoles ($\Delta$-vacuoles), which are characterized by $\delta$-TIP on their membrane, accumulate vegetative storage protein synthesized in response to developmental or environmental cues (Jauh et al. 1998).

CELL PLATE—A TRANSIENT COMPARTMENT

Dividing somatic cells and cellularizing endosperm form a transient compartment called the cell plate, which originates by homotypic fusion of transport vesicles in the center of the division plane and then grows out to the periphery, eventually fusing with the parental plasma membrane (Samuels et al. 1995, Otegui et al. 2001). In cytokinesis following the male meiotic divisions, the plasma membrane grows in by fusion with tubular networks along the division plane. These tubular networks originate by homotypic fusion of membrane vesicles, which have been transported along microtubules to the division plane (Otegui et al. 2004). Thus the initial phase of cytokinesis is essentially the same in the two systems.

VESICLE TRAFFICKING MACHINERY: INFORMATION FROM THE ARABIDOPSIS GENOME

Vesicle Formation

Vesicle formation from a donor membrane involves activation of a small GTPase by its GDP/GTP exchange factor, resulting in the recruitment of coat proteins. Membrane-bound cargo proteins are sorted into the forming vesicle by interaction with coat proteins, whereas soluble cargo proteins are recognized by cargo receptors that in turn interact with coat proteins.
SAR1, ARF GTPases and Their Interactors. Recruitment of COPII coat proteins requires SAR1 GTPase and its GDP/GTP exchange factor, the ER-localized type II transmembrane protein SEC12. Arabidopsis encodes three SAR1 GTPases and two SEC12 proteins of which one each was isolated by complementation of yeast mutants and shown to associate with the ER (d’Enfert et al. 1992, Bar-Peled & Raikhel 1997, Vernoud et al. 2003).

Small GTPases of the ARF family mediate budding of COPI vesicles from the Golgi complex and budding of adaptor complex/clathrin-coated vesicles in post-Golgi trafficking (Bigay et al. 2003, Zhu et al. 1998, Nie et al. 2003). Arabidopsis encodes between 9 and 12 ARF GTPases (Jürgens & Geldner 2002, Vernoud et al. 2003). Six ARFA1 proteins are closely related to yeast and animal class I ARFs, whereas class II and III ARFs are absent. ARF subgroups A to D are plant-specific. ARF GTPases are activated by specific GDP/GTP exchange factors, ARF-GEFs, which transiently associate with membranes (Jackson & Casanova 2000). Arabidopsis encodes several large, but no small, ARF-GEFs: three of the GNOM/Gea1/2p/GBF1 (GGG) class and five of the BIG/Sec7p class (Jürgens & Geldner 2002). GNOM acts in endosomal recycling, unlike the yeast and mammalian members of the GGG class. The 15 Arabidopsis GTPase-activating ARF-GAPs represent four structurally distinct classes (Vernoud et al. 2003).


COPI vesicles mediating Golgi-ER retrograde traffic have a seven-subunit coat termed coatomer, which consists of four inner (β, γ, δ, and ζ-COP) and three outer (α, β′, and ε-COP) proteins (Bonifacino & Lippincott-Schwartz 2003). Arabidopsis has single genes for γ-COP and δ-COP and multiple genes for the other COPI subunits (Sanderfoot & Raikhel 2003). γ-COP colocalizes with ARFA1c to the periphery of Golgi stacks (Pimpl et al. 2000, Ritzenthaler et al. 2002).

In post-Golgi traffic, vesicle coats often contain heterotrimeric adaptor-protein (AP) complexes surrounded by clathrin triskelion (Bonifacino & Lippincott-Schwartz 2003). Adaptor protein complexes consist of two large adaptins (γ and β1 in TGN-localized AP-1, α and β2 in endocytic AP-2, δ and β3 in endosomal AP-3, and ε and β4 in ill-defined AP-4), a medium and a small adaptin (µ1–4 and σ1–4, respectively). Large subunits of AP complexes interact with clathrin and assembly proteins, µ-adaptins bind cargo via their tail domain (Bonifacino & Lippincott-Schwartz 2003). Arabidopsis encodes all four types of AP complexes (Boehm & Bonifacino 2001). However, their composition has not been clarified and thus Arabidopsis µ-adaptins were designated µA to µD (Happel et al. 2004). Experimental evidence has been obtained for AP-1 and AP-2 complexes as well as clathrin heavy chain (CHC) and light chain (CLC) proteins, and some assembly proteins (reviewed by Holstein 2002; see below). Other
adaptors such as Golgi-localized GGAs or stonins do not exist in *Arabidopsis* (Boehm & Bonifacino 2001). Scission of clathrin-coated vesicles involves large GTPases of the dynamin family (Danino & Hinshaw 2001). *Arabidopsis* encodes 16 dynamin-related proteins (DRPs) of which subgroups DRP1 and DRP2 play roles in endomembrane dynamics and trafficking (Hong et al. 2003).

In yeast, the retromer complex constitutes the coat of vesicles that traffic the vacuolar receptor Vps10p from the prevacuolar compartment back to the trans-Golgi (Pfeffer 2001). Vps35p interacting with the cytosolic tail of Vps10, Vps26p, and Vps29p form the inner subunits, which are coated with Vps17p and Vps5p. *Arabidopsis* encodes eight homologs of four retromer subunits, whereas a Vps17p homolog has not been identified (Sanderfoot & Raikhel 2003).

**SORTING SIGNALS AND CARGO RECEPTORS** Soluble proteins are sorted by binding to the luminal domain of trans-membrane receptors with short cytoplasmic tails. The ER retention signal H/KDEL and various vacuolar sorting signals of soluble cargo proteins have been well characterized in plants (Hadlington & Denecke 2000). Vacuolar sorting signals include specific sequences present in N-terminal or C-terminal propeptides (NTPP, CTPP) as well as internal sequences or overall structures (Matsuoka & Neuhaus 1999). *Arabidopsis* ERD2 H/KDEL receptors recycle cargo back from the Golgi to the ER (Hadlington & Denecke 2000). *Arabidopsis* encodes at least seven vacuolar sorting receptors (VSRs) of which VSR1/ELP has been functionally characterized (Ahmed et al. 2000, Shimada et al. 2003).

Sorting of membrane proteins also requires receptors. In yeast, Rer1p retrieves type II membrane proteins such as Sec12p by interaction with their transmembrane domain (Sato et al. 2001). *Arabidopsis* encodes three RER1 proteins with possibly homologous functions (Sato et al. 1999). Tyrosine motifs to be recognized by µ-adaptins are present on plant vacuolar sorting receptors (Saint-Jore-Dupas et al. 2004). Acidic di-leucine motifs mediate endosomal/lysosomal sorting in mammals (Bonifacino & Traub 2003). The role of this motif in plants was analyzed in targeting of membrane-localized endo-1,4-β-glucanase KOR to the cell plate (Zuo et al. 2000).

**Vesicle Fusion**

Vesicle fusion with the target membrane occurs after the vesicle has shed its coat. Initially, a Rab GTPase on the vesicle membrane interacts with a tethering protein complex on the target membrane. Then SNARE proteins residing on the opposite membranes form a trans-complex, which results in membrane fusion.

**Rab GTPases AND EFFECTORS** Rab GTPases and their effector proteins tether vesicles to target membranes (Whyte & Munro 2002). *Arabidopsis* encodes 57 Rab GTPases grouped into eight subfamilies, A–H, which were assigned tentative roles by sequence homology to yeast and mammalian counterparts (Rutherford & Moore 2002, Vernoud et al. 2003). However, only a few plant Rab GTPases have
been functionally characterized (see below). Several tethering complexes studied in yeast and animals have putative homologs in Arabidopsis, including the exocyst at the plasma membrane, TRAPP at the cis-Golgi, VFT/GARP at the trans-Golgi, C-VPS at the vacuolar membrane, and several components of the Vps34/COG complex involved in Golgi retrograde transport (Jürgens & Geldner 2002, Elias et al. 2003).

SNAREs and Associated Proteins
SNARE proteins initiate membrane fusion by forming a trans-complex via their distinct R- or Q-SNARE motifs (Jahn et al. 2003). As a rule, an R-SNARE on the vesicle pairs up with two or three Q-SNAREs on the target membrane: a syntaxin (Qa-SNARE) plus either a SNAP25-like protein with two SNARE motifs (Qb,c-SNARE) or two SNARE light chains (Qb-SNARE and Qc-SNARE). Each component of a SNARE complex is a member of a protein family, and different SNARE complexes are involved in different trafficking pathways. Arabidopsis encodes at least 54 SNAREs: 18 syntaxins/Qa-SNAREs, 11 Qb-SNAREs, 8 Qc-SNAREs, 3 SNAP25-like/Qb,c-SNAREs, and 14 VAMPs/R-SNAREs. Previously 24 syntaxins of plant (SYPs) were grouped into eight subfamilies by sequence similarity to yeast and mammalian syntaxins (Sanderfoot et al. 2000). All Arabidopsis R-SNAREs are “longins” with a characteristic N-terminal domain (Filippini et al. 2001) and, with the exception of SEC22 and YKT6, are related to mammalian endosomal VAMP7 (Sanderfoot et al. 2000). Sec1/Munc18 (SM) family proteins are cytosolic interactors of SNARE proteins and contribute to the specificity of membrane fusion (Jahn et al. 2003). Arabidopsis encodes 6 SM proteins: SLY1, VPS33, VPS45, and 3 SEC1-like proteins (Sanderfoot et al. 2000). Following membrane fusion, α-SNAP and the AAA-ATPase N-ethylmaleimide-sensitive factor (NSF) assist in the disassembly of cis-SNARE complexes (Jahn et al. 2003). Experimental evidence for NSF and α-SNAP action in plants has been obtained for prevacuolar vesicle fusion and cell plate formation (Bassham & Raikhel 1999, Rancour et al. 2002).

Trafficking Pathways

A secretory default pathway leads from the ER via Golgi stacks to the plasma membrane (Figure 1). Storage proteins can bypass the Golgi altogether or exit from the cis-Golgi en route to storage vacuoles via intermediate compartments. Recycling and sorting takes place in Golgi stacks, at the PVC en route to the lytic vacuole, and at some endosomal compartment. The endocytic pathway converges on the pathway from the trans-Golgi to the lytic vacuole at the PVC.

ER-Golgi Trafficking

There is no evidence for an ER-Golgi intermediate compartment in plants (Neumann et al. 2003). Golgi stacks move over ER strands in an acto-myosin dependent manner (Boevink et al. 1998, Brandizzi et al. 2002b, Saint-Jore et al. 2002, Nebenführ & Staehelin 2001). However, this movement is not required for
ER-Golgi trafficking. Rather, ER and Golgi stacks appear to be one dynamic system for the exchange of molecules (Brandizzi et al. 2002b). Disruption of Golgi stacks by BFA treatment resulted in the redistribution of cis-Golgi, but not TGN, markers into the ER. On removal of BFA, Golgi stacks were reformed in the presence of protein synthesis inhibitors and cytoskeleton-depolymerizing drugs (Saint-Jore et al. 2002). After photobleaching of individual Golgi stacks, cycling of Golgi-localized GFP markers led to rapid recovery of fluorescence (Brandizzi et al. 2002b). Brefeldin A inhibited the recovery of fluorescence, suggesting that the formation of new Golgi cisternae requires the fusion of Golgi-derived COPI membranes with ER-derived COPII membranes (reviewed by Ward & Brandizzi 2004).

ANTEROGRADE TRAFFIC. SAR1 GTPase and its GDP/GTP exchange factor SEC12 mediate COPII-dependent cargo export from the ER (Murshid & Presley 2004). In plants, COPII vesicles have not been observed by electron microscopy. However, GTP-trapped AtSAR1[H74L] caused ER-accumulation of Golgi markers, the vacuolar marker sporamin and the storage protein phaseolin, in transient expression assays (Takeuchi et al. 2000, Sohn et al. 2003, Park et al. 2004). AtSAR1[H74L] also inhibited α-amylase secretion, and the same effect was caused by overexpression of AtSEC12 (Phillipson et al. 2001). Soluble cargo appears to exit the ER by bulk flow in a COPII-dependent manner, with ER proteins being efficiently retrieved from the Golgi complex (Crofts et al. 1999, Phillipson et al. 2001). It is not known whether bulk flow also applies to membrane proteins. In yeast, COPII coat protein Sec24p was shown to select specific types of membrane protein cargo (Barlowe 2003).

Transient expression of dominant-negative AtRAB1b[N121I] resulted in the ER accumulation of secretory GFP (secGFP), which was counteracted by wild-type AtRAB1b but not by AtRAB8c (Batoko et al. 2000). In BFA-treated cells, AtRAB1b[N121I] significantly slowed down the recovery of Golgi-localized

Figure 1 Simplified diagram of plant endomembrane system and trafficking pathways. Secretory cargo is transported from the endoplasmic reticulum (ER) via the Golgi/TGN to the plasma membrane (PM). Cargo destined for the lytic vacuole (LV) is sorted in a BP-80 dependent fashion into clathrin-coated vesicles (CCV) at the TGN and transported to the prevacuolar compartment (PVC/MVB). Cargo destined for the protein-storage vacuole (PSV) is trafficked from the ER to intermediate compartments (DV, PAC, MVB/DIP), from the cis-Golgi via dense vesicles (DV), and from the trans-Golgi via clathrin-coated vesicles (CCV). PSV and LV may fuse to give a large central vacuole (CV). The endocytic pathway involves an early/sorting endosome (EN) for recycling of plasma-membrane proteins and a multivesiculate late endosome corresponding to the PVC. Vacuolar markers, cargo receptors (ERD2, BP-80, PV72), coat proteins (COPI, COPII, AP-1, AP-2), SAR1, ARF, and Rab GTPases, and syntaxins (SYP) are indicated. DIP, DIP organelle; Endo, endocytosis; Exo, exocytosis; MVB, multivesicular body; PAC, precursor-accumulating compartment. For details, see text.
ST-GFP fluorescence (Saint-Jore et al. 2002). Taken together, anterograde traffic involves SAR1/COPII-dependent export from the ER and RAB1-mediated fusion of cargo carriers at the cis-Golgi. The nature of the carriers remains to be determined.

**RETROGRADE TRAFFIC** Both ER-resident calreticulin and secretory α-amylase fused to HDEL were detected in COPI vesicles isolated from tobacco plants (Pimpl et al. 2000). COPI vesicles, ARF1 GTPase, and COPI coat proteins accumulated at the cis-Golgi, suggesting that COPI vesicles mediate retrograde traffic (Pimpl et al. 2000, Ritzenthaler et al. 2002). In BFA-treated BY-2 cells, γ-COP (SEC21) and ARF1 were released into the cytosol (Pimpl et al. 2000, Ritzenthaler et al. 2002). Dominant-negative ARF1 affected ER-Golgi traffic in a manner similar to the action of BFA (Takeuchi et al. 2002, Lee et al. 2002). Thus ARF1 mediates formation of COPI vesicles as carriers in Golgi-ER retrograde trafficking.

Soluble proteins with a C-terminal H/KDEL motif are captured by H/KDEL receptors such as ERD2 for sorting into COPI vesicles. AtERD2-GFP accumulated at Golgi stacks of tobacco cells, as expected for Golgi-to-ER recycling (Boevink et al. 1998). *Arabidopsis* ERD2 proteins have a C-terminal KKXX or KXKXX motif, which mediates recycling of membrane proteins to the ER in yeast and mammalian cells (Jackson et al. 1993). Functionality of the KKXX motif in plants was demonstrated by mutation to NNXX (Benghezal et al. 2000).

**Post-Golgi Trafficking**

The plant Golgi apparatus is a major sorting station from which trafficking pathways diverge to different types of vacuoles and to the plasma membrane. Trafficking to the protein storage vacuole may also involve an ER-derived route that bypasses the glycan-modifying compartment of the Golgi complex.

**PATHWAYS CONVERGING ON THE PROTEIN STORAGE VACUOLE** During pea cotyledon development, PSVs are formed de novo from tubular-cisternal precursors, which surround the shrinking vegetative vacuoles. These precursors accumulate vicilin and legumin storage proteins and contain the PSV membrane marker α-TIP (Hoh et al. 1995). These markers were also detected in ∼150-nm electron-dense vesicles (DV) without a clathrin coat; DVs formed in the cis-Golgi, were carried through the stack and were released from the TGN (Hohl et al. 1996, Hillmer et al. 2001). Isolated dense vesicles lacked γ-COP and the vacuolar sorting receptor BP-80 involved in trafficking to the lytic vacuole (Hinz et al. 1999). Evidence for the two independent trafficking routes to the PSV was obtained in several plant species. In tobacco seeds, TIP-related dark intrinsic protein (DIP) accumulated in 200-nm compound vesicles and in 1–2 µm large-PSV precursors (DIP organelles). These DIP organelles contained α-TIP and a type I membrane protein, RMR, with a Golgi-glycosylated luminal domain (Jiang et al. 2000).
In pumpkin cotyledon cells, membrane-bounded aggregates of 2S albumin and 11S globulin precursors segregated from the ER to form the electron-dense core of precursor-accumulating compartment (PAC) vesicles 300–400 nm in diameter (Hara-Nishimura et al. 1998). However, PAC vesicles also had an electron-translucent outer layer containing glycoproteins with Golgi-modified glycans. In leaf cell protoplasts of three plant species, \( \alpha \)-TIP bypassed the Golgi complex, as indicated by the lack of Golgi-modified glycans and by the failure of dominant-negative Rab1 to inhibit \( \alpha \)-TIP trafficking (Park et al. 2004). In contrast, the storage protein phaseolin from common bean contained Golgi-modified glycans. Furthermore, its traffic was sensitive to BFA and was inhibited by dominant-negative variants of SAR1 and Rab1, indicating that phaseolin is transported via the Golgi complex.

A type I membrane protein, PV72, isolated from PAC vesicles of pumpkin seed was proposed to function as a sorting receptor for storage proteins (Shimada et al. 2002, Watanabe et al. 2002). PV72 is a member of the family of vacuolar sorting receptors (VSRs) and is most closely related to VSR1/AtELP among seven Arabidopsis VSRs analyzed (Shimada et al. 2003). Knockout mutants lacking VSR1/AtELP, but not any other VSR, secreted precursors of 12S globulin and 2S albumin storage proteins into the extracellular space, whereas processing of the aleurain precursor destined for the lytic vacuole was not impaired in vsr1 mutant embryos (Shimada et al. 2003). These results suggest that VSR1/AtELP may act as a VSR for storage proteins in Arabidopsis embryos, which is in conflict with evidence for a role of AtELP in trafficking to the lytic vacuole in vegetative cells (see below). Moreover, the lumenal domain of PV72 fused to HDEL trapped aleurain in the ER of transgenic Arabidopsis leaves, suggesting that PV72 recognizes the same vacuolar targeting signal as the lytic cargo receptor (Watanabe et al. 2004). To clarify the roles of VSR family members, trafficking to both protein-storage and lytic vacuoles should be analyzed in each of the Arabidopsis vsr mutants.

### PATHWAY TO THE LYTIC VACUOLE
Soluble protein cargo destined for the lytic vacuole includes aleurain and sporamin, which were mainly used in vacuolar trafficking studies. The N-terminal sorting determinant (NTTP) of their precursor forms was recognized by the luminal portion of VSRs such as pea BP-80 and AtELP, in contrast to the C-terminal targeting determinant (CTTP) of barley lectin (Kirsch et al. 1996, Cao et al. 2000, Ahmed et al. 2000). AtELP also colocalized with sporamin, but not with barley lectin (Ahmed et al. 2000).

All members of the VSR family share a tyrosine-based sorting motif, YMPL (Saint-Jore-Dumas et al. 2004). YMPL of AtELP bound in vitro more efficiently to mammalian \( \mu_1 \)-adaptin of TGN-localized adaptor complex AP-1 than to \( \mu_2 \)-adaptin of endocytic AP-2 (Sanderfoot et al. 1998). Recently, YMPL of BP-80 was shown to bind in vitro to \( \mu A \)-adaptin from Arabidopsis, which localized to the trans-face of Golgi stacks and thus appears to be a subunit of the Arabidopsis AP-1 complex (Happel et al. 2004). AP-1 specific \( \gamma \)-adaptin interacted in vitro with
the dynamin-related protein DRP2A (ADL6), which localized to clathrin-coated vesicles budding from the trans-Golgi (Lam et al. 2002). Dominant-negative ADL6 inhibited trafficking of sporamin to the vacuole but not trafficking of H\textsuperscript{+}-ATPase to the plasma membrane (Jin et al. 2001). Taken together, these results suggest that AP-1/clathrin-coated vesicles carry cargo destined for the lytic vacuole. Dominant-negative ARF1 inhibited vacuolar trafficking mediated by the BP-80 cargo receptor, implicating ARF1 in the formation of AP-1/clathrin-coated vesicles (Pimpl et al. 2003).

BP-80 and AtELP were localized to both Golgi stacks and the PVC (Ahmed et al. 2000, Paris et al. 1997). Vesicle fusion may involve syntaxin SYP21/PEP12, which localized at the PVC and was shown to reside in a 20S complex (da Silva Conceicao et al. 1997, Bassham et al. 1999). PEP12/SYP21 and syntaxin VAM3/SYP22 formed two related SNARE complexes with Qc-SNARE SYP51 and Qb-SNARE VTI11 (VTI1a) at the PVC (Sanderfoot et al. 2001a). Although PEP12 and VAM3 are closely related, knockouts for each gene were gametophytic lethal, indicating functional diversity (Sanderfoot et al. 2001b). Mutations affecting VTI11 (zig) or VAM3 (sgr3) caused defects in shoot gravitropism, indicating that the VAM3/VTI11 complex mediates vacuolar trafficking in gravity-sensing shoot endodermis cells (Yano et al. 2003). zig mutant plants were viable because VTI12 substituted for VTI11 in SNARE complex formation at the PVC, whereas the zig vti12 double mutant was embryo lethal (Surpin et al. 2003).

In yeast, vacuolar trafficking requires the class C-Vps complex (Peterson & Emr 2001). The Arabidopsis homolog of Vps16, VCL1, is required for vacuole biogenesis. vcl1 mutant embryos were lethal, forming autophagosomes and secreting aperlin into the extracellular space (Rojo et al. 2001). VCL1 formed a complex with VPS11 and VPS33, which are also homologs of yeast C-Vps proteins, and all three proteins localized to the PVC and the vacuolar membrane (Rojo et al. 2003). Co-immunoprecipitation with anti-VCL1 antiserum suggested that SYP21 and SYP22 syntaxins interact with the putative C-VPS complex. Yeast C-Vps interacts with RabG class Ypt7 (Wurmser et al. 2000). A GFP fusion of a RabG class protein, OsRab7 from rice, was recently localized to the vacuolar membrane in Arabidopsis protoplasts (Nahm et al. 2003). Two RabA proteins from pea, RabA3/Pra2 and RabA4/Pra3, may also be involved in Golgi-PVC/endosomal trafficking but their precise roles need to be determined (Inaba et al. 2002).

**RETROGRADE TRAFFIC FROM THE PREVACUOLAR COMPARTMENT** Vacuolar sorting receptors need to be recycled to the trans-Golgi after releasing their cargo into the PVC. Fusion of PVC-derived vesicles may involve SNARE complexes that were localized to the TGN in Arabidopsis (Sanderfoot et al. 2001a). Two of these complexes contained Qb-SNARE VTI12, Qc-SNARE OSM1/SYP61, and the related syntaxins, either SYP41 (TLG2a) or SYP42 (TLG2b). The SM protein VPS45 localized to the TGN and interacted with both TLG2a and TLG2b, but not with
the PVC-localized syntaxins PEP12 and VAM3 (Bassham et al. 2000). Although TLG2a and TLG2b are closely related, knockouts for each gene were gametophytic lethal, indicating functional diversity (Sanderfoot et al. 2001b). A mutation in SYP61 (osm1) caused hypersensitivity to osmotic stress (Zhu et al. 2002). Elimination of VTIL2 accelerated senescence under poor growth conditions, suggesting a possible role of VTIL2 in autophagy (Surpin et al. 2003). VTIL1 substituted for VTIL2 in TGN-localized SNARE complexes of vti12 mutants, the double mutant being embryo lethal.

**Trans-GOLGI TO PLASMA MEMBRANE—DEFAULT PATHWAY** The transport route from the trans-Golgi to the plasma membrane appears to be the default pathway for soluble cargo, as demonstrated for a secreted form of GFP (secGFP; Batoko et al. 2000). The *Arabidopsis* peptide ligand CLV3, which normally spreads into adjacent cells, was diverted to the vacuole when fused to a vacuolar sorting signal (Rojo et al. 2002, Lenhard & Laux 2003). How membrane proteins are targeted to the plasma membrane is not known.

Transport vesicles destined for the plasma membrane have not been unambiguously identified in plants. Nonetheless, several syntaxins and SNAP25 homologs were localized at the plasma membrane, including *Arabidopsis* SYP121/PEN1 and its barley ortholog ROR2 (both of which are involved in nonhost pathogen resistance); their putative tobacco homolog SYR1; SYP122, possibly involved in response to a bacterial elicitor; and *Arabidopsis* SNAP33 and barley SNAP34 (Collins et al. 2003, Leyman et al. 2000, Nühse et al. 2003, Heese et al. 2001). Overexpression of the cytosolic fragment of SYR1 prevented secretion of secGFP, which then accumulated in the ER and Golgi stacks, and this inhibitory effect was abolished by simultaneous overexpression of SYR1 (Geelen et al. 2002).

Several *Arabidopsis* plasma-membrane proteins were localized in specific subdomains. The putative auxin-efflux carrier PIN1 accumulated at the basal surface of vascular cells, the auxin-influx carrier AUX1 at the apical surface, GPI-anchored COBRA protein at the lateral surfaces in inner root cells, and PIN2 protein at the apical surface of root epidermis cells (reviewed by Jürgens & Geldner 2002). Targeted trafficking to plasma-membrane subdomains requires sorting from the general bulk flow, which may occur in Golgi stacks. Alternatively, targeted trafficking may involve recycling from endosomes (see below).

**Endocytosis**

In yeast and animals, receptor-ligand complexes are internalized for signaling, for dissociation of the complex and receptor recycling, or for eventual degradation in the lysosome/vacuole (Clague & Urbe 2001). Information on receptor-mediated endocytosis is still scant in plants, although several receptor kinases and their peptide ligands have been identified (Matsubayashi 2003). SERK1 receptor kinase was internalized on dephosphorylation by a kinase-associated protein phosphatase
A sorting nexin, SNX1 from *Brassica*, interacted in vitro with the kinase domains of SRK and CLV1, suggesting trafficking of these receptors through the endosomal system (Vanoosthuyse et al. 2003).

Early studies of plant endocytosis made use of electron-dense markers such as cationized ferritin; its uptake into protoplasts and intracellular trafficking to the vacuole were analyzed by electron microscopy (reviewed by Battey et al. 1999). More recent studies employed the endocytic tracers FM1-43 and FM4-64 (Emans et al. 2002, Tse et al. 2004). *Arabidopsis* αC-adaptin, a subunit of a putative endocytic AP-2 complex, interacted with clathrin and the assembly factor homolog AtAP180, and AtAP180 was shown to mediate clathrin assembly into regular cages (Barth & Holstein 2004). Thus there is accumulating evidence for endocytosis and its machinery in plants (reviewed by Holstein 2002).

### Trafficking from Endosomes

Early or sorting endosomes are not well characterized in plants. Early studies described a partially coated reticulum (PCR) with clathrin-coated buds (reviewed by Battey et al. 1999). In animals, Rab5 GTPase has been established as a convenient marker for early endosomes, and its *Arabidopsis* homologs RabF1 (Ara6) and RabF2b (Ara7) were shown to colocalize with FM4-64 in endomembrane compartments after brief uptake periods (Ueda et al. 2001). Treatment with BFA led to BFA compartments as vesicle aggregates, which accumulated the *Arabidopsis* BFA-sensitive ARF-GEF GNOM, PIN1, and other plasma-membrane proteins, sterols, and cell-wall pectins (Steinmann et al. 1999, Geldner et al. 2001, Baluska et al. 2002, Grebe et al. 2003). GNOM also colocalized with FM4-64, and Ara7-positive compartments were structurally altered in *gnom* mutant protoplasts (Geldner et al. 2003). Internalized filipin-labeled sterols were colocalized with Ara6 (Grebe et al. 2003). The BFA compartments were clearly distinct from Golgi stacks, as indicated by ultrastructural analysis and double labeling with several Golgi markers (Geldner et al. 2001, 2003; Baluska et al. 2002; Grebe et al. 2003).

**Recycling to the Plasma Membrane** Recycling of plasma-membrane proteins is thought to involve sorting in the early endosome (Woodman 2000). In plants, recycling of plasma-membrane proteins was demonstrated by washing out BFA in the presence of cycloheximide (Geldner et al. 2001). Similarly, internalized sterol was recycled to the plasma membrane in an actin-dependent manner (Grebe et al. 2003). In plants expressing BFA-insensitive GNOM, PIN1 did not accumulate in BFA compartments, whereas PIN2 and H⁺-ATPase were still partially sensitive to BFA (Geldner et al. 2003). H⁺-ATPase-GFP accumulated in Golgi marker-negative BFA compartments on overexpression of dominant-negative AtARFa1c (Lee et al. 2002). BFA compartments of maize root cells were labeled by anti-AtARFa1c antiserum (Baluska et al. 2002). As *Arabidopsis* ARF1 proteins are
almost indistinguishable, the identity of the endosomal ARF1 protein(s) remains uncertain. Taken together, these results suggest multiple recycling pathways.

TRAFFICKING TO THE LYtic VACUOLE In animal and yeast cells, proteins destined for degradation in the lysosome/vacuole are sorted into internal vesicles of the early endosome (Raiborg et al. 2003). The multivesicular body (MVB) thus formed detaches from the endosome and fuses with the late multivesicular endosome/prevacuolar compartment. In plants, endocytic trafficking from the early compartment described above appears to involve a multivesiculate late compartment, which was labeled with both FM4-64 and the vacuolar sorting receptor BP-80 (Tse et al. 2004). In addition, BP-80 colocalized with the prevacuolar syntaxin PEP12 (SYP21), indicating that the late compartment corresponds to the PVC that lies on the trafficking route from the trans-Golgi to the lytic vacuole (see above). Wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3K), blocked FM1-43 traffic to the vacuole (Emans et al. 2002). Wortmannin also affected the morphology of the multivesiculate PVC (Tse et al. 2004). Transiently expressed phosphatidylinositol 3-phosphate (PI3P)-binding FYVE domain from human EEA1 fused to GFP labeled the PVC, as indicated by colocalization with BP-80, and overexpression of the FYVE domain inhibited vacuolar targeting of sporamin (Kim et al. 2001). These results suggest that PI3P plays a role in trafficking at the PVC.

Arabidopsis has three RabF GTPases related to endosomal Rab5 in mammals. RabF1 (Ara6) and RabF2b (Ara7) were localized to different subpopulations of endosomes. Both GTP-locked Ara6 and Ara7 caused fusion of endosomes to form larger structures, whereas GDP-locked Ara6[N147I] resided on the plasma membrane (Ueda et al. 2001). Dominant-negative Ara6 did not interfere with vacuolar trafficking of sporamin, in contrast to dominant-negative RabF2a (Rha1) and Ara7 (Sohn et al. 2003). Thus Ara6 seems to act before the endocytic pathway converges on the vacuolar trafficking pathway. In contrast, a putative Ara6 ortholog from Mesembryanthemum, called m-Rabmc, was predominantly localized to the PVC, and dominant-negative m-Rabmc blocked vacuolar targeting of aleurain presumably at the PVC (Bolte et al. 2004). In addition, m-Rabmc[N147I] was cytosolic, in contrast to the plasma-membrane localization of Ara6[N147I] (see above). Thus the putative orthologs Ara6 and m-Rabmc appear to have different roles in endocytic trafficking. The third Rab5-related Arabidopsis RabF GTPase, Rha1, colocalized with the PVC markers VSR1/ELP and PEP12 (SYP21), and dominant-negative Rha1 blocked delivery of sporamin and aleurain to the lytic vacuole at the PVC (Sohn et al. 2003).

Trafficking During Cytokinesis

Cytokinesis of somatic cells progresses from the center to the periphery and is assisted by a dynamic cytoskeletal array, the phragmoplast, which delivers membrane
vesicles to the division plane, probably along microtubules (Staehelin & Hepler 1996, Otegui et al. 2001). Initially, homotypic vesicle fusion generates a network of tubular membranes, which is then transformed into a disk-shaped continuous membrane compartment, the cell plate, that secretes callose into its lumen (Samuels et al. 1995, Otegui et al. 2001). The tubular membranes are locally constricted by dynamin-related protein DRP1a, which may also localize callose synthase (Otegui et al. 2001; Hong et al. 2001a,b). An *Arabidopsis* double mutant lacking both DRP1a (ADL1A) and related DRP1e displayed defects in cell plate formation (Kang et al. 2001, 2003).

Lateral translocation of the phragmoplast targets later-arriving vesicles to the margin of the cell plate, which thus grows toward the cell periphery, eventually fusing with the plasma membrane. In vacuolate cells, the cell plate appears to fuse with the plasma membrane locally before spreading along the cell margin to the opposite side (Cutler & Ehrhardt 2002). Although unique, plant cytokinesis bears some resemblance to the final stage of animal cytokinesis during which the midbody gap is closed by vesicle delivery and SNARE-mediated vesicle fusion (Low et al. 2003).

 Trafficking to the plane of division appears to be the default pathway in dividing cells, as suggested by the accumulation of plasma-membrane proteins such as PIN1 and KOR, and secretory proteins such as endoxyloglucan transferase (Steinmann et al. 1999, Zuo et al. 2000, Yokoyama & Nishitani 2001). This trafficking probably results from bulk flow and targeted delivery of Golgi-derived vesicles along phragmoplast microtubules.

**CELL PLATE FORMATION BY VESICLE FUSION** Unfused transport vesicles 60–80 nm in diameter accumulated in *Arabidopsis* cytokinesis mutants lacking the cytokinesis-specific syntaxin KNOLLE or its interactor, the SM protein KEULE (Lauber et al. 1997, Waizenegger et al. 2000, Assaad et al. 2001). A KNOLLE-interacting SNAP25 homolog, SNAP33, colocalized with KNOLLE at the cell plate but also accumulated at the plasma membrane in nondividing cells (Heese et al. 2001). Thus the cytokinetic SNARE complex contains a specific syntaxin and a promiscuous SNAP25 homolog. A plant-specific Qb-SNARE, NPSN11, was also localized to the cell plate and appeared to coimmunoprecipitate with KNOLLE (Zheng et al. 2002). An *npsn11* knockout mutant was viable, possibly because of functional redundancy. If NPSN11 formed a complex with KNOLLE and SNAP33, this SNARE complex would be rather unusual, consisting of Q-SNAREs only. Alternatively, KNOLLE may form several SNARE complexes with different partners. Another syntaxin localized to the division plane was the Sed5 homolog SYP31, which labeled Golgi membranes in nondividing cells (Rancour et al. 2002). SYP31 and KNOLLE interacted in vitro with different AAA-ATPases, CDC48 and NSF, respectively. These results were interpreted as evidence for two distinct fusion pathways during cytokinesis (Rancour et al. 2002).

 Syntaxin specificity in cytokinesis was studied by expressing several syntaxins under the control of *KNOLLE cis*-regulatory gene sequences (Müller et al. 2003).
Both prevacuolar PEP12 (SYP21) and plasma membrane-localized PEN1/SYR1 (SYP121) failed to rescue a knolle deletion mutant, although the latter accumulated at the cell plate, whereas the former was detected at the PVC. In contrast, SYP112 of the plasma-membrane group, although normally dispensable, fully substituted for KNOLLE. Thus three factors contribute to syntaxin specificity in cytokinesis: strong expression during M phase, absence of a sorting sequence, and protein function during cell plate formation.

ENDOCYTOSIS AND LOCAL MEMBRANE RECYCLING The cell plate shrinks in surface area and volume by approximately 70% during consolidation, presumably by clathrin-mediated endocytosis (Otegui et al. 2001). During cell plate expansion, KNOLLE disappeared from the center and was concentrated at the margin (Lauber et al. 1997). Evidence for local membrane recycling is scant at present. Brefeldin A treatment of Arabidopsis dividing root cells caused accumulation of KNOLLE and PIN1 in the same BFA compartments (Geldner et al. 2001). In dividing cells expressing BFA-resistant GNOM ARF-GEF, PIN1 no longer accumulated in BFA compartments but KNOLLE did, suggesting that internalized proteins are sorted at endosomes (Geldner et al. 2003). KNOLLE was proposed to accumulate at the margin of the expanding cell plate by local recycling via endosomes (Jürgens & Pacher 2003). It is conceivable that lateral diffusion of proteins within the cell plate is hindered by its lipid composition, which may necessitate membrane retrieval and local recycling. A similar argument has been put forward for the maintenance of the asymmetric localization of plasma-membrane proteins in Arabidopsis and for the maintenance of cell polarity in yeast (Jürgens & Geldner 2002, Valdez-Taubas & Pelham 2003).

FUTURE PERSPECTIVES The analysis of membrane trafficking in plants is coming of age. The increasing use of GFP marker technology in conjunction with transient expression assays in cultured cells has led to remarkable progress in the past several years. However, the current use of different cell types has its limitations. It is not obvious to what extent findings in one system can be transferred to a different system, which is illustrated by the differential effects of BFA. Another concern is the use of heterologous markers and transient expression assays, which may not reflect the physiological situation in the intact organism. These problems could be overcome by studying trafficking processes in the genetic model Arabidopsis. This should also resolve the current conflict about vacuolar sorting receptors.

The results obtained so far support the view that many aspects of membrane trafficking are conserved between plants and nonplant organisms. However, both the endomembrane system and trafficking pathways have plant-specific features that cannot be studied in nonplant organisms. These include not only trafficking to the storage vacuole or during cytokinesis but also rapid recycling between plasma membrane and endosomes. Considering the current rate of progress, it
may not take long until our understanding of membrane trafficking in plants has reached a comparable level of sophistication as in yeast or animals.

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