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The Secretory System of Arabidopsis

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Over the past few years, a vast amount of research has illuminated the workings of the secretory system of eukaryotic cells. The bulk of this work has been focused on the yeast *Saccharomyces cerevisiae*, or on mammalian cells. At a superficial level, plants are typical eukaryotes with respect to the operation of the secretory system; however, important differences emerge in the function and appearance of endomembrane organelles. In particular, the plant secretory system has specialized in several ways to support the synthesis of many components of the complex cell wall, and specialized kinds of vacuole have taken on a protein storage role—a role that is intended to support the growing seedling, but has been co-opted to support human life in the seeds of many crop plants. In the past, most research on the plant secretory system has been guided by results in mammalian or fungal systems but recently plants have begun to stand on their own as models for understanding complex trafficking events within the eukaryotic endomembrane system.

I. INTRODUCTION

While many different plants have been used in studies of the plant secretory system, many labs have focused their attention on the model plant *Arabidopsis thaliana*. Because of the amount of sequence information, both prior to and (especially) after the recent completion of sequencing of the genome, Arabidopsis was an excellent subject for identifying the molecular machinery required for the operation of the secretory system. With the availability of sequence data for other models, as well as crop plants, other plants may serve as useful comparisons to the Arabidopsis model that has been developed. In the following work, we will attempt to provide an updated review of the secretory system as found in Arabidopsis (using work from other plants as needed). We will report the details of the molecular mechanisms for secretory system function, highlighting the tools available from the many labs working on Arabidopsis. We will also attempt to provide a road map for future research that will help us to better understand the function of the system in Arabidopsis and other plants.

II. THE VESICLE TRAFFICKING MACHINERY

The endomembrane system is composed of many organelles, each of which must maintain a unique composition of membrane and cargo proteins (Figure 1). Traffic flows in two main ''default'' pathways through the secretory system: secretory and endocytic (Figure 2). The secretory pathway represents the major biosynthetic route (the ''anterograde'' direction) that starts with newly synthesized proteins that have been targeted to the endoplasmic reticulum (ER) membrane, then flows first to the Golgi, and subsequently to the plasma membrane (PM)/extracellular matrix (ECM). Conversely, the endocytic route represents a counter flow of cargo endocytosed from the PM/ECM, which travels by way of endosomes to the vacuole. Aside from these default pathways, there are several ''signal-mediated'' pathways that redirect specific proteins from either default pathway (Figure 2). So-called ''retrograde'' pathways serve as recycling mechanisms to retrieve material back from later steps in either pathway, or serve to direct newly synthesized cargo destined for the vacuole from the default secretory pathway. In addition to these bulk-trafficking methods, recent evidence has indicated that peroxisomes may also be a semi-autonomous part of the endomembrane system (Titorenko and Mullen, 2006), and evidence has even suggested that some plastid proteins may travel through the endomembrane system as well (Nanjo et al., 2006).

It is widely believed that all of these organelles exchange information through the use of small membrane-enclosed transport vesicles. These vesicles bud from a donor compartment using a complex and ordered system of coat proteins that nucleate on the site of vesicle formation to collect cargo and deform the local membrane until a vesicle is freed by scission (Figure 3). Once free, these vesicles then travel through the cytosol by association with cytoskeletal motors or other docking factors. Finally, through the action of docking and tethering factors as well as members of the SNARE family of proteins, these vesicles must identify their target compartment among all the other possibilities and then fuse their lipid bilayer with the target to deliver their cargo (Figure 4).

Clearly, some manner of regulation is required at each of these steps to maintain order. One would expect each donor compart-

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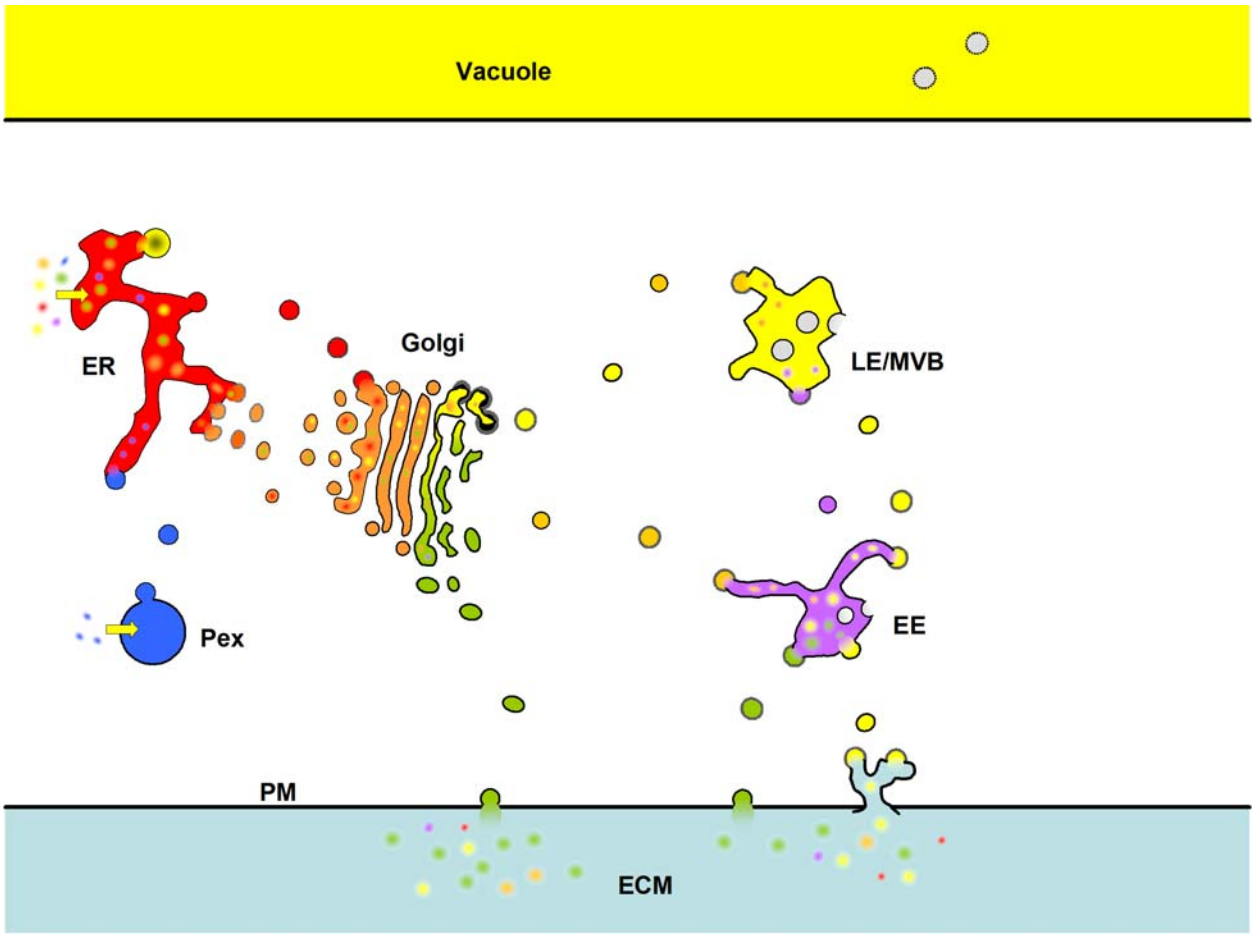


Figure 1. The Secretory/Endomembrane System of Arabidopsis

The endomembrane system of all eukaryotes consists of those membrane-bound organelles that exchange lipid and cargo by vesicle trafficking. Despite the constant exchange of vesicles, each organelle/compartment has a relatively constant array of resident membrane and lumenal proteins. In the figure, resident proteins of the endoplasmic reticulum (ER) are red, those of the Golgi are orange, those of the vacuole are yellow, those of the endosomes (EE) are purple. Secreted proteins (green) end up either in the plasma membrane (PM) or are released into the extracellular matrix (ECM; equivalent to the "cell wall" or "apoplast"). Peroxisomal (Pex) proteins, some of which are first trafficked through the ER, are indicated in blue.

ment to have a peculiar array of coat proteins regulated by specific coat GTPases that would create a particular transport vesicle (or equivalent membrane-bound carrier). In turn, each target compartment should be uniquely labeled by Rab GTPases, arrays of docking and tethering factors and a distinct collection of SNAREs. In the following sections, we will discuss these particular parts of the vesicle traffic machinery.

II.A. Coat GTPases and Coats: Making a Vesicle

Formation of a transport vesicle is not a spontaneous event. First, a mechanism for cargo selection and concentration is required to decide what belongs in the vesicle. Secondly, some signal must be sent from the lumen to the cytoplasm (where a coat must form) to indicate a site for vesicle formation. Most importantly, some method of membrane deformation and scission from the donor compartment is needed to actually produce the vesicle. This is the job of a large collection of proteins that are referred to as coat proteins. Each type of coat is distinct, though some are related, and a particular coat is responsible for vesicle formation at a particular type of organelle. An overall similarity in the coating mechanism is found (See Figure 3), even though the protein content of the coats may vary. The first step requires recruitment of the cargo to a site on the donor membrane. The details of such a step are still a matter of debate, however it most likely involves the cytoplasmic tails of integral membrane proteins. These proteins may be themselves cargo, or may serve as cargo receptors, or may serve simply in signaling the site of budding. Coincident with this step is the GTP-cycle of a coat-GTPase which helps to coordinate the coating process. This coat-GTPase differs depending on the particular coat proteins, and may be of the ARF-family or of the

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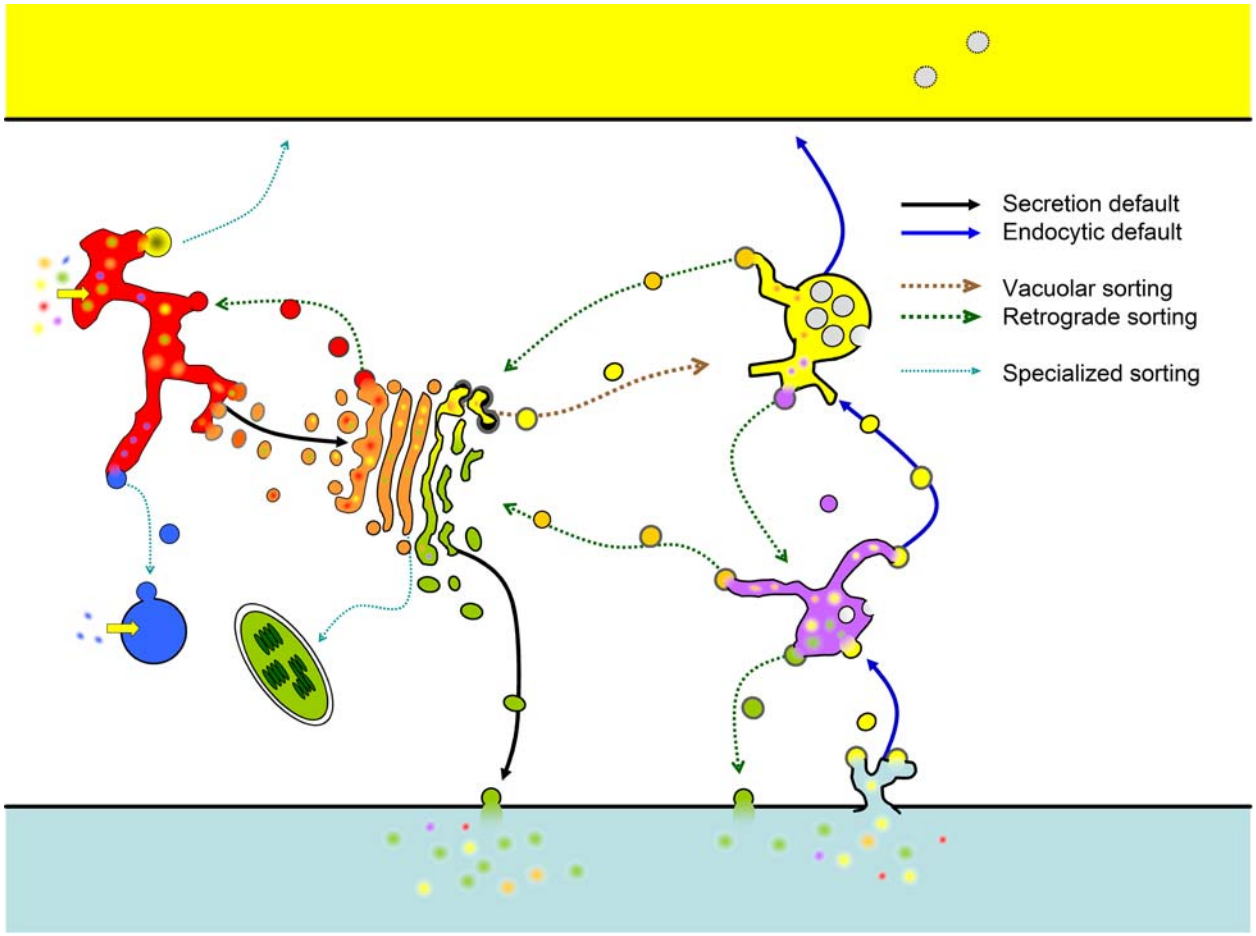


Figure 2. The Secretory Pathway, Endocytic Pathway and Retrograde/Recycling Pathways.

The traditional secretory pathways begins by translocation of proteins (yellow arrow) into the endoplasmic reticulum (ER), followed by transport in vesicles to the Golgi by bulk flow (default secretion, solid black line). From the Golgi, secretory proteins are transported to the Plasma Membrane (PM) or extracellular matrix (ECM, "cell wall"). Also in the Golgi, vacuolar proteins are re-directed to the vacuole (by way of the late endosome) due to specific sorting signals (vacuolar sorting, dashed brown line). Cargo that is endocytosed from the PM/ECM is transported through the endosomes by bulk flow (default endocytosis, solid blue line), first to the early endosomes, then to the late endosomes where endocytic cargo meets vacuolar cargo, and finally the cargo arrives in the vacuole. Many signal-mediated retrograde pathways operate to recycle specific cargo at most compartments (dashed green lines). Finally, several specialized sorting pathways serve to transport peroxisomal proteins from the ER to peroxisomes or to transport some glycosylated proteins from the Golgi to the plastid (dashed cyan lines; see text).

SAR1 family of small G-proteins. The coat-GTPase for the COP-II coat is called SAR1, and is a single copy gene in yeast, though mammals and plants have multiple SAR1-homologues (TABLE 1). The ARF family of G-proteins is required for both COP-I and clathrin-type coat formation. Consistent with these expanded roles, the ARF-family is large in most eukaryotes, and may have as many as 21 members in Arabidopsis (TABLE 1).

The coat-GTPases typically exist in a soluble GDP-bound state while inactive. A GTP-exchange factor (GEF) localized to the coating site serves to recruit the coat-GTPase to the donor membrane and then triggers nucleotide exchange, such that the coat-GTPase assumes its active GTP-bound state. The GEF for the Sar1p-type G-protein is a member of the SEC12-family, a group of ER-integral membrane proteins found in all eukaryotes. The Arabidopsis SEC12 is somewhat atypical in that both SEC12-like genes are smaller than the orthologues found in other eukaryotes, though each seems to function as normal. The GEF for the ARF-type G-protein varies somewhat, since the ARF-type coat-GTPase is involved in many different coating steps. Typically, the ARF-GEF will contain a Sec7-like domain, which is named for the Sec7p protein that is an ARF-GEF used for budding vesicles at the yeast Golgi (reviewed in Jackson and Casanova, 2000). The Sec7-domain proteins fall into 3 main classes. The Gea/GNOM/GBF class contains the Arabidopsis protein GNOM (as well as other proteins related to GNOM; Geldner et al., 2003), mutants of which are embryo lethal due to defects in the polar transport of auxin transporters, and other developmental and physiological defects (Busch et al., 1996; Steinmann et al., 1999). The Sec7/BIG class contains a fam-

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ily of Arabidopsis proteins that show high homology to the metazoan members, though their function has yet to be examined in Arabidopsis. Finally, the ARNO class of proteins is found only in mammals. The interface between the Sec7-domain and the ARF is the target for brefeldin A, a drug used commonly in the study of Golgi function in eukaryotes (Peyroche et al., 1999; Robineau et al., 2000). Treatment of eukaryotic cells with brefeldin generally leads to a breakdown of the Golgi stacks and a complete block in most vesicle transport, illustrating the importance of the ARF-cycle to vesicle transport.

Once activated at the budding site, the active coat-GTPase then functions in the recruitment of "effector"-proteins as well as "cargo-selective" subunits of the coat complexes from the cytoplasm (Figure 3). Effectors can function as enzymes to alter the lipid-characteristics of the budding site, or simply recruit other factors (i.e.: v-SNAREs, Rab-type GTPases, etc.) into the budding vesicle. Finally, the "cage" subunit of the coat complex is nucleated onto the bud site (Figure 3), and functions as a mechanical device to drive membrane deformation (Stagg et al., 2007). Scission of the membrane to free the vesicle is either driven by the coats themselves, by the peculiar lipid composition of the vesicle "neck" region, by other molecular machines such as the dynamin family of large GTPases, or by a combination of all three. Subsequently, a GTPase Activation Protein (GAP) triggers the intrinsic GTPase

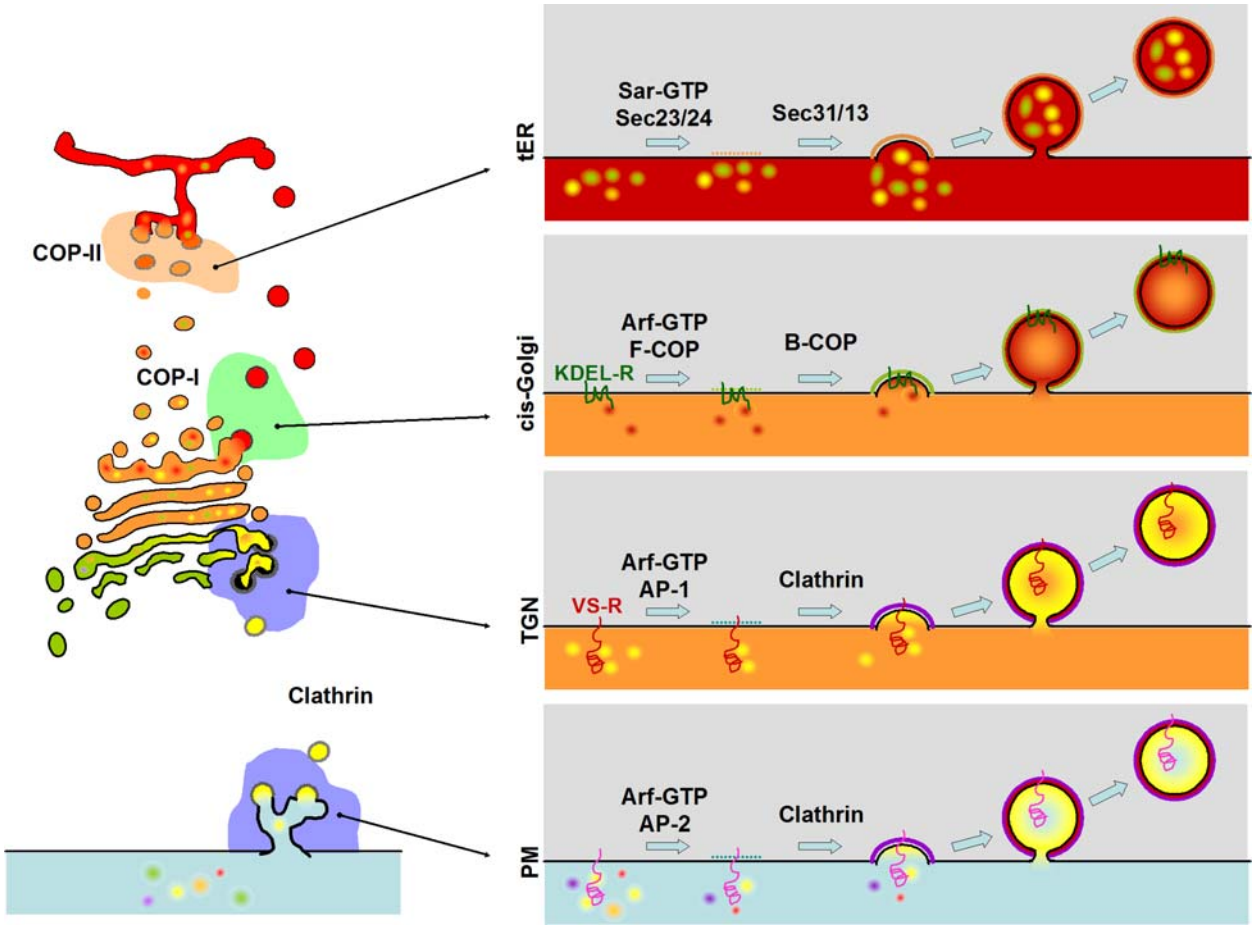


Figure 3. Vesicle Formation at the Donor Compartment

Vesicle formation is accomplished by a similar process at each compartment, though the individual protein machinery varies at the different compartments. The basic process is the same at each compartment: a coat-GTPase cooperates with a cargo-selective coat subunit to collect cargo molecules at the bud site, next a "cage" subunit is recruited from the cytosol to physically deform the lipid bilayer into a vesicle. Vesicle formation at the transitional ER (tER) is accomplished by the COP-II coat system (top panel at right). At the cis-Golgi, vesicle formation is accomplished by the COP-I coat system (second panel at right). Recovery of ER-resident proteins is accomplished by a cargo receptor called the KDEL-Receptor (KDEL-R, encoded by *ERD2*) which is a membrane protein that binds to ER-retrieval signals (KDEL-COOH) on the lumenal side, and recruits coat components on the cytosolic side. At the TGN, vacuolar cargo is selected by a cargo receptor (VS-R; see text) which recognizes vacuolar sorting signals on the lumenal side and recruits the clathrin adaptor complex (AP-1) on the cytosolic side (third panel at right). Clathrin coats (in this case using AP-2 adaptor) also operate at the PM to accomplish the first step of endocytosis (lower panel at right). In other organisms (i.e., mammals) there are many cargo receptors which serve to internalize extracellular cargo but little is known about such proteins in plants.

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activity of the coat-GTPase. This step occurs either directly after vesicle formation (COP-II), or can be triggered by some other event (COP-I or clathrin). The GDP-bound G-protein now triggers uncoating of the vesicle, releasing the coatomers back to the cytoplasm for future coating steps. The uncoated vesicle is now free to travel to its target membrane where the SNARE-mediated fusion process occurs.

COP-II Coats

The COP-II coat is conserved in all eukaryotes and is involved in export of secretory cargo from the ER in vesicles (See Figures 3 and 5). In most organisms, ER-exit sites (ERES) are discrete points along the ER membrane, although in some organisms (like budding yeast) the entire ER membrane seems capable of acting as an ER-exit site (reviewed in Glick, 2001). The exit sites are points at which the COP-II machinery is concentrated, and also where anterograde cargo is collected. How cargo becomes concentrated remains unclear, with some arguing for a "bulk-flow" model, whereas others invoke specific cargo receptors. In a bulk-flow model, all cargo not specifically retained in the ER is subject to COP-II-directed transport to the Golgi. On the other hand, some propose that a large class of conserved proteins is involved in clustering cargo for packaging at exit sites. Evidence for both models has accumulated, and it is possible that both occur depending on the cell-type or developmental state or particular cargo molecule.

The coat-GTPase for COP-II is SAR1, which is found in all eukaryotes. SAR1 serves to recruit the "cargo selective" subunit which is made of two highly-conserved proteins (SEC23 and SEC24; Figure 5). The "cage" sub-unit of COP-II is made from two proteins (SEC13 and SEC31; Figure 5) and assembles onto the nucleation site as directed by the Sec23/24 subunit. The Arabidopsis COPII coat subunits are each encoded by multiple genes (TABLE 1), though whether this indicates redundancy has yet to be investigated. The COP-II coated vesicles are quite unstable as the GAP for SAR1 turns out to be a component of the coat itself (SEC23), thus the vesicles are uncoated soon after budding. This may be significant since in most eukaryotes, the COP-II-derived vesicles are short lived. These vesicle quickly fuse with each other into larger vesicles which (according to the predominant "cisternal maturation" model for Golgi transport) forms a de novo cis-Golgi stack upon docking with the Golgi matrix. Upon uncoating, Sar1p and the coat proteins are released into the cytoplasm for further rounds of vesicle budding.

COP-II mediated sorting has been recently been examined in plants, with all indications that it is similar to that found in other eukaryotes. Takeuchi et al. (2000) showed that dominant negative mutants of the SAR1-GTPase block ER-to-Golgi trafficking. Philipson et al (2001) later showed that transient overexpression of SEC12 (which would presumably prolong the GTP-bound state of SAR1, mimicking the dominant negative) had a similar effect. Interestingly, this SEC12-overexpression effect was not noted previously by Bar-Peled and Raikhel (1997) when they overexpressed SEC12 in Arabidopsis. Since Bar-Peled and Raikhel

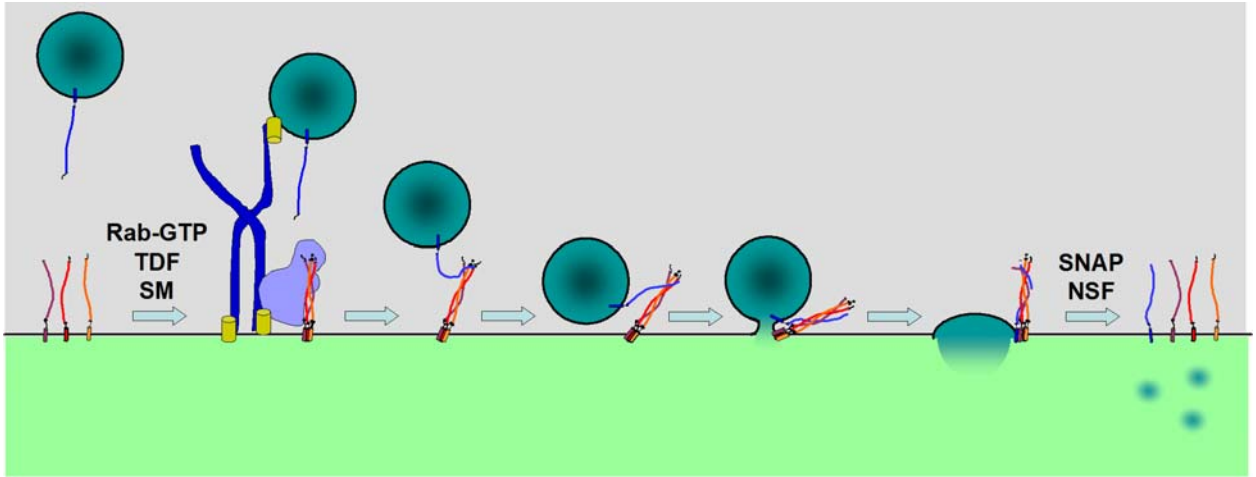


Figure 4. Docking and Fusion of a Vesicle at the Target Compartment.

Docking and fusion of a vesicle is also accomplished by a similar process at each trafficking step, again the individual protein machinery varies. Identification of the target compartment for a particular vesicle is mediated by a member of the Rab family of small GTPases which acts on both the vesicle and target membrane to recruit tethering and docking factors (TDF) and other effectors that serve to attach the vesicle to the target membrane. Once a vesicle is tethered, a member of the SM family of proteins mediates assembly of the target-(t)-SNAREs into a three SNARE-helix bundle that serves as a binding site for the vesicle-(v)-SNARE helix. Mutual twisting of the SNARE helices pulls the membranes into close proximity and drives fusion of the bilayers. (Only one set of SNAREs is shown, fusion is likely mediated by multiple SNARE complexes surrounding the fusion site.) Following fusion of the vesicle, lumenal cargo is delivered into the target compartment lumen and vesicle membrane proteins and lipids are now part of the target membrane. The final four-helix SNARE complex is resolved by the action of a complex of proteins (SNAP and NSF) that use ATP energy to unwind the helices of the individual SNARE proteins for subsequent rounds of fusion (t-SNAREs) or for recycling back to the donor compartment (v-SNARE).

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Table 1. Arabidopsis Coat Complexes and Coat-GTPase

Clathrin		
Triskelion (cage):	Heavy chain	At3g11130, At3g08530
	Light chain	At2g40060, At3g51890
AP1 (cargo rec.):	γ	At1g60070, At1g23900
	β1/2[1]	At4g11380, At4g23460
	μ1	At1g60780, At1g10730
	σ1	At2g17380, At4g35410
AP2 (cargo rec.):	α	At5g22770, At5g22780
	β1/2[1]	see above
	μ2	At5g46630
	σ2	At1g47830
AP3 (cargo rec.):	δ	At1g48760
	β3	At3g55480
	μ3	At1g56590
	σ3	At3g50860
AP4 (cargo rec.):	ε	At1g31730
	β4	At5g11490
	μ4	At4g24550
	σ4	At4g24550
COP-I		
B-COP (cage):	α	At1g62020, At2g21390
	β'	At1g52360, At3g15980, At1g79990
	ε	At2g34840, At1g30630
F-COP (cargo rec.):	β	At4g31480, At4g31490
	γ	At4g34450
	δ	At5g05010
	ζ	At1g60970, At3g09800, At1g08520
COP-II		
Sec13/31 (cage):	Sec13	At3g01340, At2g30050
	Sec31	At1g18830, At3g63460
Sec23/24 (cargo rec.):	Sec23	At5g43670, At3g23660, At4g14160, At1g05520, At2g21630
	Sec24	At3g07100, At3g44340, At4g32640, At2g27460
Retromer		
PI3P-binding:	Vps5/SNX	At5g06140, At5g07120, At5g58840
Cargo rec.:	Vps26	At4g27690
	Vps29	At3g47810
	Vps35	At2g17790, At3g51310, At1g75850
ESCRT		
ESCRT-I	VPS23	At3g12400
	VPS28	At4g05000, At4g21560
	VPS37	At3g53120
ESCRT-II	VPS22	At4g27040
	VPS25	At4g19003
	VPS36	At5g04920
ESCRT-III	VPS2	At2g06530
	VPS20	At5g63880
	VPS24	At5g22950
	SNF7a	At5g09260
	SNF7b	At4g29160
	SNF7c	At2g19830
	DID2	At1g73030
Misc. Class E:	VPS31/Bro1	At1g15130
	VPS4	At2g27600
	Hrs/VPS27	At1g61690

(Continued)

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Table 1. (continued)

Coat-GTPases		
ArfA Group (Arf1):	ARFA1a	At1g23490
	ARFA1b	At5g14670
	ARFA1c	At2g47170
	ARFA1d	At1g70490
	ARFA1e	At3g62290
	ARFA1f	At1g10630
ArfB Group (none):	ARFB1	At2g15310
ArfD Group (none):	ARFD1a	At1g02440
	ARFD1b	At1g02430
ArfB2 Group (Arf6?):	ARFB2a	At5g17060
	ARFB2b	At3g03120
Sar1-like:	SARA1a	At1g09180
	SARA1b	At1g56330
	SARA1c	At4g02080

[1]Like many organisms, plants have only one type of β1/2 protein that presumably acts in both the AP1 and AP2 complexes (Dacks et al., 2008).

used stably-transformed plants, some compensation mechanism during the growth of the plants may have occurred and it would not be noted in the transient assays. Some interesting hints concerning the role of vacuolar targeting signals in export from the ER has been reported by Törmäkangas et al. (2001), and surely future research will reveal more interesting observations about the ER in plants.

Plant COP-II proteins have a unique subcellular distribution. Fluorescent protein fusions of a tobacco Sar1 and most of the Arabidopsis COPII coat proteins have been found restricted to mobile ER exit sites localized in the near vicinity of Golgi stacks (Stefano et al., 2006; Hanton et al., 2007; Hanton et al., 2008). However, similarly to mammalian Sar1 and Sec12, in addition to a distribution to ER exit sites, two isoforms of Arabidopsis SAR1 have been found to be associated extensively with ER membranes (Hanton et al., 2008) where SEC12 is also localized (daSilva et al., 2004; Bar-Peled and Raikhel, 1997).

It has been shown that increased synthesis of membrane cargo destined to the plant Golgi induces a signal-mediated recruitment to ER exit sites of a tobacco SAR1 isoform (daSilva et al., 2004) and SEC24 (Hanton et al., 2007). However, soluble proteins that are secreted by bulk-flow do not appear to up-regulate membrane recruitment of COPII, showing that the availability of membrane cargo influences the level of COPII recruitment to ER exit sites. Furthermore, the assembly of COPII coat proteins, such as SEC23 and SEC24, at the plant ER exit sites depends on retrograde protein transport from the Golgi (Stefano et al., 2006). In tobacco leaves, disruption in the activity of Arf1, the GTPase that regulates the retrograde protein route from the Golgi apparatus to the ER (see below), leads to redistribution of SEC23 and SEC24 COPII coat to the cytosol (Stefano et al., 2006). Disruption of plant ERESs due to blockage of the retrograde pathway is reversible as recruitment of COPII coat proteins at the ER exit sites can be re-established by release from Arf1 inhibition (Stefano et al., 2006). This effect suggests that the plant ER is capable of accommodating reversible changes in COPII assembly at ERESs. Gene depletion experiments have demonstrated that ER exit site-associated proteins such as Sec16 contribute to differentiation of ERESs and to efficient ER protein export in mammals and yeast (Connerly et al., 2005; Watson et al., 2006). A role of plant proteins with analogous functions to Sec16 has yet to be established.

COP-I Coats

COP-I coats are typically associated with Golgi trafficking (See Figures 3 and 5), mainly in the retrograde sorting pathway (though in the past it has been argued that anterograde COP-I vesicles act to move cargo forward in Golgi stacks). COP-I coats may also be involved in forming coated vesicles at the ER and endosomal compartments (Kirchausen, 2000). The coat GTPase that drives COP-I coat formation is ARF1, which is typically represented by a large gene family (e.g., 6 ARFA genes in Arabidopsis, see TABLE 1) that also function in other coating processes (See clathrin below). ARF1 appears to have multiple roles in plant cells. It has been shown that ARF1 is distributed to the Golgi and to post-Golgi compartments that bud from the Golgi apparatus and may be involved in endocytosis (Xu and Scheres, 2005; Stefano et al., 2006; Matheson et al., 2007). ARF1 appears to be involved in the recruitment of a golgin, GDAP1, on these Golgi-derived compartments (Matheson et al., 2007), but the functional role of this protein is yet to be determined.

The COP-I coatomer is made up of two multi-subunit complexes called F-COP and B-COP that are conserved among all eukaryotes (Figure 5). F-COP serves as the cargo-selective subunit and is made from four proteins (γ-COP, β-COP, δ-COP, and ζ-COP), while the B-COP forms the "cage" complex and consists of (α-COP, β'-COP and ε-COP) As with COP-II coat proteins, the COP-I- proteins are encoded by multiple genes (except for γ and δ) in Arabidopsis (TABLE 1); whether this represents redundancy, or may help to explain the morphologically distinct types of COP-I vesicles observed on plant and algal Golgi by Donohoe et al. (2007) remains to be investigated.

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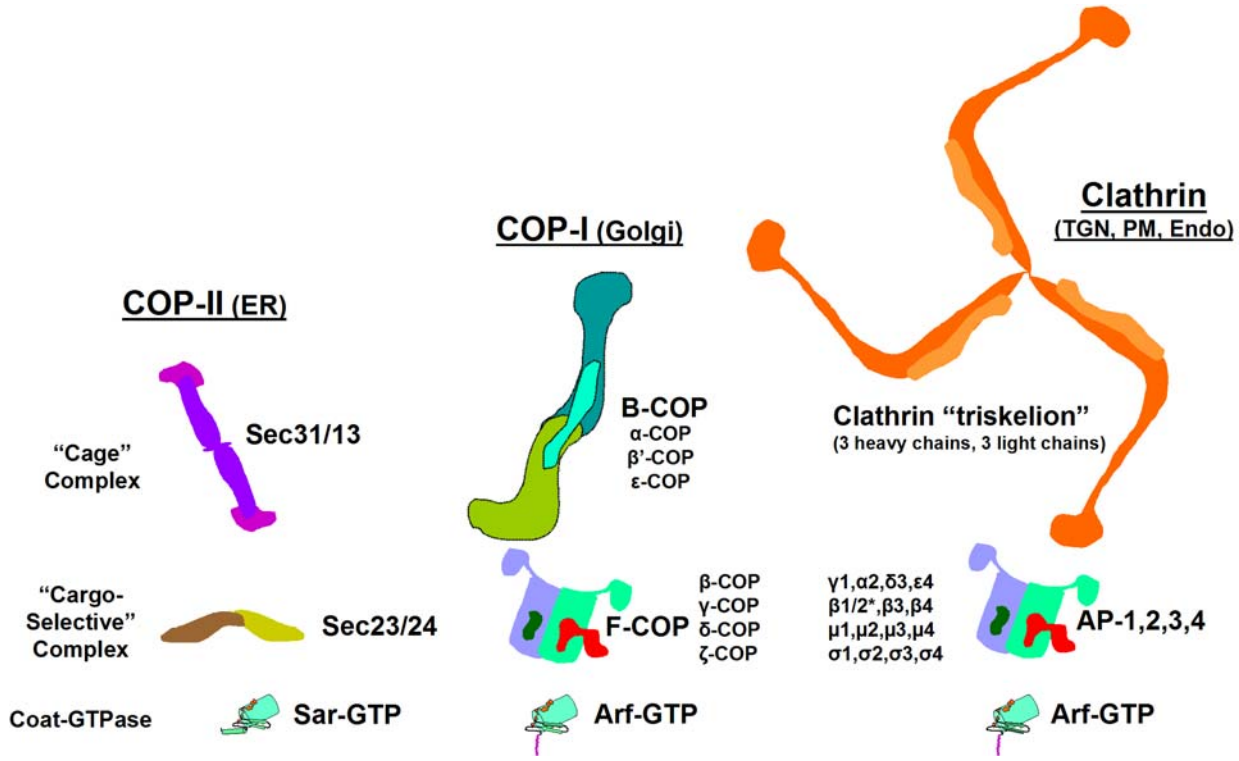


Figure 5. The Sub-Complexes of the Three Major Coat Systems.

Each coat system can be considered to represent three major sub-complexes: a coat-GTPase, a "cargo-selective" complex and a "cage" complex. In some cases, these individual subunits have a clear evolutionary relationship: The Sar and Arf GTPases share a common ancestor among the small GTPases, and the subunits of the four clathrin adaptors are clear examples of gene duplication and also share a common ancestor with the components of F-COP. The three cage subunits also share many common protein folds like the α-solenoid and WD-40 motifs, but this state may be convergent rather than ancestral (see Stag et al., 2007).

Clathrin

Clathrin coated vesicles (CCVs) were the first to be described, and are found in all eukaryotes (Reviewed in Kirchausen, 2000). Coats containing clathrin are found on two major compartments in most organisms, the TGN and the PM, and are involved in sorting cargo into various endosomes from both organelles (See Figure 3). Aside from a role in vesicle formation, clathrin-coated pits (membranes coated with clathrin coatomers but not forming a vesicle) also are essential for signal transduction pathways, and for defining distinct membrane patches both at the PM and the TGN. The clathrin coatomer, made of two protein chains (heavy and light), forms a microscopically visible structure called a triskelion named for its three-legged shape (Fotin et al., 2004). Some organisms, such as yeast, have only a single type of light chain, whereas mammals can have two different light chains (type A and B) in a single triskelion. Evidence from purified plant CCVs suggests that plants may also have more than one type of light-chain peptide (see Robinson et al., 1998), and the fact that two genes are found to encode light-chain like proteins (TABLE 1) supports this hypothesis. Clathrin triskelions are the cage subunit for CCVs, and they require a cargo-selective subunit which is typically a member of the adaptor protein (AP)-family, the GGA-family, or another type of protein (Figure 5). In addition, CCVs require the action of a coat-GTPase from the ARF family.

The AP-complexes were the first clathrin-coordinating complex described, and come in four different types (reviewed in Boehm et al., 2001). The AP-complexes function as the cargo-selective subunit for CCVs, but in some cases these adaptors can act to form coated vesicles without clathrin. Interestingly, the individual subunits of the AP-complexes are evolutionarily related not only to each other, but also to the subunits of F-COP, indicating a "duplication and specialization" mechanism for creating new coatomer specificities (Boehm et al., 2001). In mammals, there are four distinct AP-complexes (AP-1, to -4), each made of 4 subunits: two large subunits (one of α, γ, δ, or ε and one of β1- β4; note that these subunits are distinct from the similarly named COP-I components), one medium subunit (μ1- μ4), and one small subunit (σ1- σ4). While mammals have distinct β1 and β2 proteins, many other organisms, including plants, have only a single "β1/2" subunit that probably functions in both the AP-1 and AP-2 complexes (Boehm et al., 2001; Dacks et al., 2008). The AP-complexes recognize specific peptide motifs in the cytoplasmic tails of cargo proteins. These motifs fall into two classes, tyrosine motifs and di-leucine motifs. The tyrosine motif consists of a Yxxφ sequence (where Y is tyrosine, x is any amino acid, and φ is any bulky hydrophobic residue). Different AP-complexes have different preferences for variants of the tyrosine motif depending on the nature of the x residues, the context of the signal, and even the phosphorylation state of nearby

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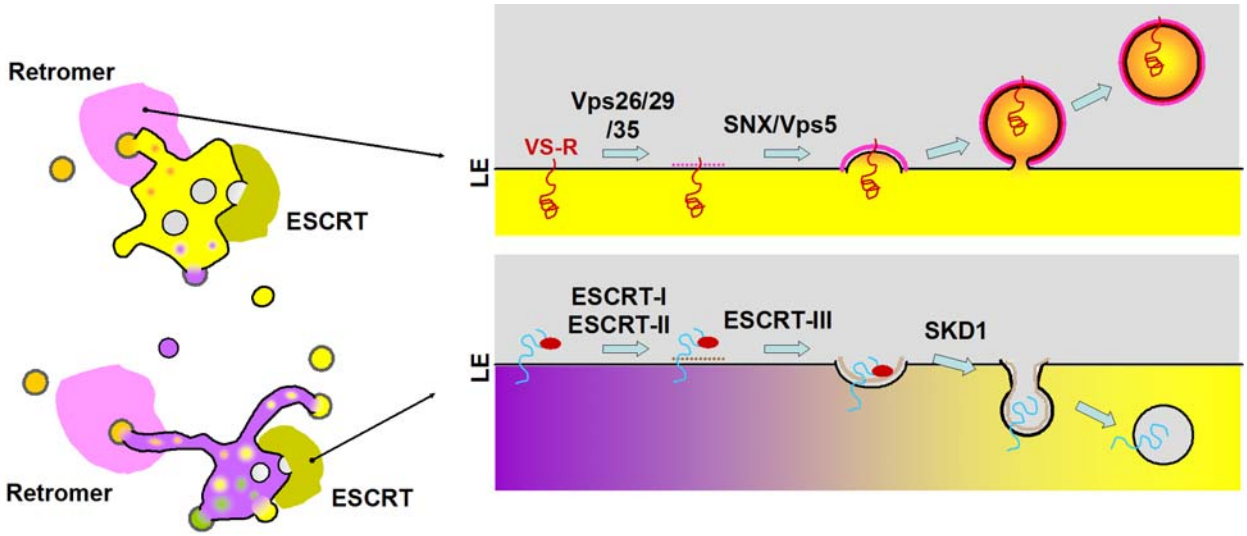


Figure 6. Retromer and ESCRT Coat Machinery

The retromer coat is responsible for recycling of proteins (e.g., the vacuolar cargo receptor) from the endosomal compartments back to the Golgi. Coat formation is superficially similar to that of the other coat complexes (see Figure 3), with the complex of Vps26, Vps29 and Vps35 functioning to bind and collect cargo proteins and the sorting nexin/Vps5 subunit functioning as a cage, but the mechanisms appears to be distinct. Similarly, the ESCRT system has subunits that function in cargo recognition and some variation of a "cage", but it is topologically reversed to create a vesicle inside the lumen of the endosomal compartments (though evidence of the protein coat having a role in membrane deformation as shown in the figure is controversial). The ESCRT-I, -II and –III sub-complexes act sequentially to identify cargo and form a "cage" that leads to the invagination in the endosomal membranes. A large ATPase (SKD1) then acts to release the ESCRT complexes back to the cytosol prior to enclosure of the lumenal vesicle. These internal vesicles are ultimately delivered to the vacuolar lumen where their contents are degraded.

residues (Ohno et al., 1998). The di-leucine motif contains some variant of two adjacent bulky hydrophobic residues (i.e.: LL or LV, etc.), and different AP-complexes respond differently to the context of these residues (e.g.: Rodionov and Bakke, 1998; Honing et al., 1998; Rapoport et al., 1998). These motifs are recognized mainly by either the μ or the β subunit of the complexes, though other subunits may also contribute to binding. Upon binding cargo, some of the AP-complexes are capable of recruiting the clathrin triskelion through a clathrin-binding domain in the large subunits; however, some AP-complexes seem able to form coated vesicles in the absence of clathrin.

The AP-1 complex (γ, β1, μ1, and σ1) is a clathrin-interacting complex that functions in coated vesicle formation at the TGN and endosomal compartments in many eukaryotes (reviewed in Hirst and Robinson, 1998). Arabidopsis encodes two γ, μ1, and σ1 subunits (and a single β1/2-orthologue that probably also acts in the AP-2 complex; Dacks et al., 2008; TABLE 1), suggesting that there may be different AP-1-isoforms present, though this is speculative. Mammalian cells have multiple isoforms of some AP-1 subunits, with some tissues having specific subunits (Meyer et al., 2000). The AP-1/clathrin complex was generally believed to be involved in packaging vacuolar-bound cargo into CCVs at the TGN in most eukaryotes. In Arabidopsis, it has been reported that the AP-1 complex interacts with the cytoplasmic tail of the vacuolar sorting receptor ELP (Sanderfoot et al., 1998), and may thus play a role in sorting of vacuolar cargo. Recent research has indicated that this step may actually be mediated by the GGA-type adaptors in yeast, and perhaps mammals, although a role for AP-1 has not been ruled out. Other roles for AP-1 in recycling back from endosomes to the TGN have also been proposed.

The AP-2 complex (subunits α, β2, μ2, and σ2) is intimately involved in the endocytosis of many (though not all) cargo proteins from the PM (reviewed in Hirst and Robinson, 1998). Arabidopsis encodes single genes for most of these subunits (except for the β2, which is probably substituted for by the β1/2-type gene; Dacks et al., 2008), though genes for the α-type are found as a tandem repeat on chromosome 5 (TABLE VI.A). The AP-2 complex, like the AP-1, requires clathrin for coat formation. To direct endocytosis of a vesicle, many other proteins are important either for recruiting AP-2, or for recruiting clathrin directly to the site of vesicle formation. The AP-2-associated mammalian proteins such as AP180, epsin, auxilin, and perhaps synaptojanin have homologues in the Arabidopsis genome (see table), whereas amphiphysin, endophilin, intersectin, stonin, and synaptotagmin do not. The mammalian AP-2 complex appears to be essential, though the homologous complex in yeast is not essential for endocytosis in these cells (Yeung et al., 1999). The importance of the AP-2 complex in plants, and of the potential orthologues of some of its associated proteins, has yet to be investigated.

The AP-3 complex (δ, β3, μ3, and σ3) is found in most eukaryotes (reviewed in Ordoizzi et al., 1998), though the ability to interact with clathrin has only been shown for the mammalian complex (and even there, this is controversial; Drake et al., 2000; Le Borgne

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et al., 1998; Dell'Angelica et al., 1998). It is possible that the AP-3 complex forms vesicles in the absence of clathrin. The role of the AP-3 complex is restricted to the TGN and endosomes, and seems to be essential for the targeting of membrane proteins directly to the vacuole/lysosomal membrane in a step that bypasses the late endosome/MVB (Stepp et al., 1997). So, for example yeast enzymes like alkaline phosphatase, or mammalian enzymes like tyrosinase are trafficked directly from the Golgi to the vacuolar/lysosomal membrane. Mutations in these subunits lead to inheritable defects in mammalian cells, and have been associated with known diseases in humans (Dell'Angelica et al., 2000a), indicating the importance of this pathway. Plants encode single genes for each of the subunits (TABLE 1). This is different than mammals, which again have brain-specific isoforms of many AP-3 subunits. The AP-3 complex seems to have a preference for a particular kind of di-leucine signal, a so-called acidic di-leucine, where 3-5 residues upstream are found either aspartic or glutamic acid residues. In addition, the AP-3 complex can recognize many kinds of tyrosine motifs, at least in vitro.

The AP-4 complex (ε, β4, μ4, and σ4) has been described in mammalian cells (Dell'Angelica et al., 1999; Hirst et al., 1999), though it is not found in all fungi. Arabidopsis encodes a single gene homologous to each subunit (TABLE 1). In mammals, AP-4 is found on the TGN, and may or may not be associated with clathrin. A clear role has not been assigned for the AP-4-mediated trafficking, though it seems likely it may be responsible for a sub-set of Golgi-to-endosomal transport.

Other Coats

Another novel coat complex has been described in yeast as being essential for recycling of membrane proteins from the late endosomes to the Golgi, and is called the "Retromer complex" (Seaman et al., 1998; Figure 6). This complex consists of five subunits, Vps5p, Vps17p, Vps26p, Vps29p, and Vps35p. These coatomers interact with the cytoplasmic tails of membrane proteins (i.e.: Vps35p interacts with the tail of Vps10p, the vacuolar sorting receptor) and lead to their packaging into vesicles bound for the Golgi complex (Notwehr et al., 2000). In the absence of this complex, membrane proteins are not recycled, and instead accumulate in the LE before eventual degradation in the vacuole. Orthologues of most of these coat components (except Vps17p) are found in mammals, and it is likely that they function in a similar manner. In fact, the mammalian Vps5p-homologues are members of the sorting nexin group of proteins, a group that is involved in sorting of membrane proteins in the endosomal system. Arabidopsis also contain orthologues of all but Vps17p, in fact having 3 each for Vps5p and Vps35p (TABLE 1). The Arabidopsis retromer has been implicated in recycling of the VSR vacuolar cargo receptors and also in the localization of the auxin efflux carriers PIN1 and PIN2 (Jaillais et al., 2006; Oliviusson et al., 2006; Shimada et al., 2006; Jaillais et al., 2007; Yamazaki et al., 2008) (See **III.D**).

Though many coat complexes are well conserved among all eukaryotes, this is not universally the case. Yeast and mammals also have a coatomer called the GGA-type that has recently been shown to be essential for Golgi to endosomal traffic. The GGA stands for Golgi-localized, Gamma (γ)-ear-containing, ARF-binding proteins (Hirst et al. 2000; Dell'Angelica et al., 2000b). The

Table 2. Docking and Tethering factors: Rab-GTPases and SM-Proteins

Rab-GTPases		
RabA group (Rab11)	RABA1a	At1g06400
	RABA1b	At1g16920
	RABA1c	At5g45750
	RABA1d	At4g18800
	RABA1e	At4g18430
	RABA1f	At5g60860
	RABA1g	At3g15060
	RABA1h	At2g33870
	RABA1i	At1g28550
	RABA2a	At1g09630
	RABA2b	At1g07410
	RABA2c	At3g46830
	RABA2d	At5g59150
	RABA3	At1g01200
	RABA4a	At5g65270
	RABA4b	At4g39990
	RABA4c	At5g47960
	RABA4d	At3g12160
	RABA4e	At2g22390
	RABA5a	At5g47520
	RABA5b	At3g07410
	RABA5c	At2g43130
	RABA5d	At2g31680
	RABA5e	At1g05810
	RABA6a	At1g73640
	RABA6b	At1g18200
RabB Group (Rab2):	RABB1a	At4g17160
	RABB1b	At4g35860
	RABB1c	At4g17170
RabC Group (Rab18):	RABC1	At1g43890
	RABC2a	At5g03530
	RABC2b	At3g09910
RabD Group (Rab1):	RABD1	At3g11730
	RABD2a	At1g02130
	RABD2b	At5g47200
	RABD2c	At4g17530
RabE Group (Rab8):	RABE1a	At3g53610
	RABE1b	At4g20360
	RABE1c	At3g46060
	RABE1d	At5g03520
	RABE1e	At3g09900
RabF Group (Rab5):	RABF1[1]	At3g54840
	RABF2a	At5g45130
	RABF2b	At4g19640

(Continued)

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Table 2. (continued)

RabG Group (Rab7):	RABG1	At5g39620
	RABG2	At2g21880
	RABG3a	At4g09720
	RABG3b	At1g22740
	RABG3c	At3g16100
	RABG3d	At1g52280
	RABG3e	At1g49300
	RABG3f	At3g18820
RabH Group (Rab6):	RABH1a	At5g64990
	RABH1b	At2g44610
	RABH1c	At4g39890
	RABH1d	At2g22290
	RABH1e	At5g10260
Other small GTPases		
Arl1-like[2]:	ARL1 [ARFE1[3]]	At2g24765
ArfRP1-like[2]:	ARFRP1 [ARLB1[3]]	At5g52210
Arl2-like[4]:	ARL2 [ARLC1[3]]	At2g18390
Arl5-like[4]:	ARL5 [ARFC1[3]]	At3g22950
Arl8-like[4]:	ARL8a [ARLA1a[3]]	At5g37680
	ARL8b [ARLA1b[3]]	At3g49860
	ARL8c [ARLA1c[3]]	At3g49870
	ARL8d [(ARLA1d[3]]	At5g67560
SM-family proteins		
PM:	KEULLE	At1g12080
	SEC1a	At1g02010
	SEC1b	At4g12120
TGN:	VPS45	At1g77140
LE/Vac.:	VPS33	At3g54860
ER-Golgi:	SLY1	At2g17980

[1]RabF1 is myristoylated at the N-terminus rather than prenylated at the C-terminus.
[2]Arl1 and ArfRP1 are involved in vesicle tethering at the TGN rather than coat formation, (see Kahn et al., 2006)
[3]In Vernoud et al. (2003) these were given the names in parenthesis, but here new names are given to better identify the homology to well-known Arl proteins (Kahn et al., 2006).
[4]Arl2, Arl5, and Arl8 are probably involved in cytoskeletal or nuclear processes, and may not play a role in vesicle trafficking (see Kahn et al., 2006)

name describes their localization, the presence of the clathrin-interacting γ-ear (from the AP-1 γ-subunit) domain found in their carboxy-terminus, and their ability to interact with the ARF-GTPases mentioned earlier as coat-forming GTPases. Based upon this description, it should not be surprising that these proteins play an essential role in the binding of the cytoplasmic tails of cargo proteins, and then triggering clathrin coat formation through the ARF and clathrin-interaction domains (reviewed in Tooze, 2001). Yeast contain two partially redundant genes encoding Gga1 and Gga2p, whereas mammals contain three orthologues (GGA1-3) (Hirst et al. 2000; Dell'Angelica et al., 2000b). Arabidopsis and other plants have no obvious homologues to GGA-type coatomers. It is possible that this role has been usurped by other coats (i.e.: AP-1 adaptors?), or that some unknown protein may have a GGA-like function.

A third class of "coat-like" components are represented by the ESCRT complex, which function "in reverse" to make the internal vesicles of the MVB (see Figure 6 and III.D). Arabidopsis (like other plants) encodes all of the expected subunits of the ESCRT complexes (TABLE 1). Other coats are certain to exist in cells. One clear example is the coat that likely envelops the "Dense Vesicles" (DVs) which carry the storage proteins to the PSV in many plant cells. No molecular details of such a coat have been established. Other potential coats have been suggested by researchers working in various organisms, but none are widely studied and such studies are not as well characterized at this point. It is also important to note that the molecular details concerning the type of coat (if there is even such a coat required) that forms secretory vesicles (i.e.: the Golgi-to-PM pathway) have not been reported in any eukaryote. Clearly many experiments remain to be done.

II.B. Rab-GTPases and SM-proteins: Preparing the Membranes for Fusion

Formation of a vesicle through the action of the coat proteins is only the first step of vesicle trafficking. Once freed from the cage of the coat through uncoating, the vesicle now must traverse the cytosol in search of a unique target compartment that must be distinguished from all the other endomembranes of the cell. These steps are the role of the Rab-GTPases and a variety of tethering and docking proteins. Though there is much recent work concerning these tethering and docking complexes in mammals and yeast, very little work on these proteins has been done in plants. A recent study by Koumandou et al. (2007) has examined the evolutionary conservation of these proteins across eukaryotes, and we will not repeat such analysis here. Instead, we will focus on the two classes of proteins that have been examined in plants, the Rab-GTPases and the SM-family of proteins.

Rab-GTPases

Rabs represent a large subgroup of the small G-protein family, and have a standard G-nucleotide binding cycle. In general, Rabs are prenylated at a C-terminal Cys-motif (although one type of plant specific Rab is instead myristoylated at the N-terminus), and through this lipid modification, can cycle on and off of membranes in a G-nucleotide-specific manner. Like the rest of the vesicle trafficking machinery, Rabs are a eukaryotic specific protein family, and are found in all eukaryotes examined thus far (e.g., see Pereira-Leal et al., 2003). Through genomic analysis, there are thought to be eight types of Rab proteins that are conserved among the majority of eukaryotes, and are thought to represent the "minimal set" of Rab proteins (Pereira-Leal and Seabra, 2001;

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Field et al., 2006). Appropriately enough, plants have members of each of these groups, and the groups have been named A-H (i.e., "RabA"; Pereira-Leal and Seabra, 2001; Vernoud et al., 2003; TABLE 2.). Though this nomenclature suffers in comparison to that of mammalian Rabs, it has the advantage of instantly identifying the different ancestral Rab groups. It is thought that each of these ancestral groups would mediate a particular vesicle trafficking event, and this has tended to be the case based on research in disparate eukaryotes (reviewed in Vernoud et al., 2003; Field et al., 2006). Some organisms have additional types of Rabs, while almost all have multiple members of each type. Plants, for instance, tend to have small gene families of two-to-nine members in addition to sub-groups within each ancestral type.

In eukaryotes, it is known that the Rab/Ypt-family is involved in cargo selection, recruitment of molecular motor proteins, and interactions with docking factors (Figure 4). Some work has indicated a similar function in plant cells. Arabidopsis contains 57 members of the Rab-type of small GTPase (reviewed in Vernoud et al, 2002; TABLE 2). Like the coat-GTPases, the Rabs also have an essential G-protein cycle. While in the GDP-bound state, the Rabs are inactive and are typically found in the cytoplasm bound to a protein called RabGDI (GDP disassociation inhibitor). When recruited to a particular membrane by a Rab-GEF (Guanine Exchange Factor), the GDP is exchanged for GTP, and the Rab becomes associated with the membrane. The activated Rab then further recruits other effectors to the bud site, including SNAREs, so called docking factors, as well as molecular motors that will tether the vesicle to the cytoskeleton. Such effectors have been recently reviewed elsewhere for yeast and mammalian cells (Pfeffer, 1999; Lowe, 2000), and since very little is known about plant factors, we will not discuss these further at this time.

Studies in Arabidopsis have generally found the members of the various Rab groups to localize to the compartments expected based on homology, and often Rabs are used as markers for a particular compartment. For example, members of the RabB and RabD groups label the ER and Golgi compartments (Batoko et al., 2000; Cheung et al., 2002) as expected from homology (to the Rab2 and Rab1 type of mammalian/fungal Rabs, respectively; TABLE 2). The Golgi itself is marked by members of the RabH group (Bednarek et al., 1994; Latijnhouwers et al., 2007) as expected by the homology with mammalian Rab6. The members of the RabG family label the vacuolar compartments (e.g., Nahm et al., 2003) as expected from their homology to the lysosomal/vacuolar Rab7 proteins from mammals and fungi. The other groups of Rabs (RabA, RabC, RabE and RabF) all label endocytic compartments of some kind, which is expected from homology to the mammalian orthologues (Rab11, Rab18, Rab8, and Rab5, respectively; see TABLE 2). Importantly, their differential localization among the endocytic compartments may greatly influence the "maturity" of a particular endosome or of a specific domain within an endosomal membrane (for example, see Ueda et al., 2004; Preuss et al., 2004; Haas et al., 2007; Chow et al., 2008). However, because most Rabs are encoded by large gene families in Arabidopsis, only in a few cases has a particular Rab been functionally implicated in targeting to a given compartment (for example, see Sohn et al., 2003; Zheng et al., 2005; Preuss et al., 2006). "Stacking" of mutants, together with other molecular genetic techniques may eventually reveal individual functions for Rab isoforms.

Table 3. Arabidopsis SNARE Proteins (see Sanderfoot, 2007)

Qa-SNAREs (Syntaxins)		
ER:	SYP81	At1g51740
Golgi:	SYP31 (AtSED5)	At5g05760
	SYP32	At3g24350
TGN:	SYP41 (AtTLG2a)	At5g26980
	SYP42 (AtTLG2b)	At4g02195
	SYP43 (AtTLG2c, synt5)	At3g05710
LE/Vac:	SYP21 (AtPEP12)	At5g16830
	SYP22 (SGR3/AtVAM3)	At5g46860
	SYP23 (AtPLP)	At4g17730
	SYP24 (pseudo)	At1g32270
CP:	KNOLLE (SYP111)	At1g08560
	SYP112	At2g18260
PM1:	PEN1 (SYP121/SYR1)	At3g11820
	SYP122 (Synt4)	At3g52400
PM2:	SYP123	At4g03330
	SYP124	At1g61290
	SYP125	At1g11250
PM3:	SYP131	At3g03800
	SYP132	At5g08080
Qb-SNAREs		
ER?:	SEC20	At3g24315
ER/Golgi:	MEMB11	At2g36900
	MEMB12	At5g50440
Golgi:	GOS11	At1g15880
	GOS12	At2g45200
TGN/Vac.:	VTI11 (ZIG1/VTI1a)	At5g39510
	VTI12 (ZIP1/VTI1b)	At1g26680
	VTI13 (pseudo)	At3g29100
	VTI14 (pseudo)	At5g39630
TGN/PM:	NPSN11	At2g35190
	NPSN12	At1g48240
	NPSN13	At3g17440
Qc-SNAREs		
ER?:	USE11	At1g54110
	USE12	At3g55600
ER/Golgi:	BET11 (BS14a)	At3g58170
	BET12 (BS14b)	At4g14445
Golgi?:	SFT11	At4g14600
	SFT12	At1g29060
TGN/Vac:	SYP51	At1g16240
	SYP52	At1g79590
TGN/Endo:	SYP61 (OSM1)	At1g28490
PM:	SYP71	At3g09740
	SYP72	At3g45280
	SYP73	At3g61450
Qb+c-SNAREs (SNAP25)		
PM:	SNAP33	At5g61210
	SNAP29	At5g07880
	SNAP30	At1g13890

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Table 3. (continued)

R-SNAREs (VAMPs)		
ER-Golgi:	SEC22	At1g11890
Golgi/Vac.?:	YKT61	At5g58060
	YKT62	At5g58180
TGN/Vac.1:	VAMP711	At4g32150
	VAMP712	At2g25340
	VAMP713	At5g11150
TGN/Vac.2:	VAMP714	At5g22360
TGN/PM1:	VAMP721	At1g04740
	VAMP722	At2g33120
	VAMP723	At2g33110
	VAMP725	At2g32670
	VAMP726	At1g04760
	VAMP728 (pseudo)	At3g24890
TGN/PM2:	VAMP724	At4g15780
TGN/PM3:	VAMP727	At3g54300
PM?:	TYN11 (Tomosyn)	At5g05570
	TYN12	At4g35560

SM (Sec1-Munc) Family Proteins

The SM-family of proteins are known to interact with the syntaxin (Qa) family of SNAREs (reviewed in Hanson, 2000). This interaction is believed to trigger a change of conformation in the syntaxin (from "closed" to "open") allowing formation of SNARE complexes (e.g.: Dulubova et al., 1999). Arabidopsis has 6 members of the SM family and other plants have similar numbers (Sanderfoot et al., 2007; TABLE 2). This number is many-fold lower than the number of syntaxins in Arabidopsis, so clearly, the Sec1-family members must interact with more than one syntaxin, or other mechanisms for triggering the conformational change in syntaxins must exist. Three members of the Arabidopsis Sec1- family have been investigated. VPS45 is a Sec1-family member that is localized to the TGN in Arabidopsis (Bassham et al., 2000). Interestingly, while it is able to complement a defect in a homologous gene in yeast (also called VPS45; Bassham and Raikhel, 1998), in Arabidopsis its localization and the syntaxins with which it interacts (Bassham et al., 2000) suggest that it plays a distinct role in plant cells (discussed in Bassham and Raikhel, 2000). VPS33 of Arabidopsis is found on late endosomes and the vacuole, and seems to interact as part of a "Class-C-Complex" similar to the orthologues in yeast and mammals (Rojo et al., 2005). Whereas the other types of SM-family proteins appear to exist in single-copy, the PM-type of SM-protein is represented by three genes in Arabidopsis (KEULLE, SEC1a, and SEC1b). Of these three, only one has been investigated thoroughly: KEULLE interacts with the cytokinesis-specific syntaxin KNOLLE (Assaad et al., 2001), and likely regulates the function of this syntaxin since keule mutants have similar phenotypes to knolle, and double mutants display an additive phenotype (Waizeneggar et al., 2000).

II.C. SNAREs: The Machines of Membrane Fusion

The various members of the SNARE family of proteins are distributed among the organelles in a specific way. Particular SNAREs label unique target membranes, while others are incorporated into vesicles from only certain donor compartments. Of course, since v-SNAREs must travel between compartments, they will be found on more than a single organelle. In fact, due to flexibility in SNARE-SNARE interactions, different combinations of SNAREs can lead to additional levels of organization among the organelles. SNARE proteins have been reviewed many times recently, including for several plants (Sanderfoot, 2007). The basic aspects of the SNARE hypothesis (see above) have been well supported, and all results are consistent with SNARE proteins operating as both target-specificity and membrane-fusion determinants in vesicle trafficking.

Examining the sequences available from many eukaryotes has indicated that most eukaryotes have a common set of SNARE proteins, with the SNARE helices of these proteins falling into 4 main types (Qa-, Qb-, Qc- and R; Table 3). Moreover, through such genomic analysis, one can also identify a "core set" that likely represents the genes from the last common ancestor of the extant eukaryotes (Sanderfoot, 2007). This core set would include five Qa-SNAREs, four Qb-SNAREs, four Qc-SNAREs and three R-SNAREs. In addition to these 16 putatively ancestral SNAREs, a few others are shared across several major clades, but appear to have been lost in others (Sanderfoot, 2007). For example, only animals and fungi seem to have a class of R-SNAREs called "brevins"; while most eukaryotes outside of the Animal + Fungi clade have a type of Qb-SNARE and Qc-SNARE that probably was missing in the opisthokont ancestor. There is also a double-SNARE-helix type (Qb+Qc-SNAREs; e.g., mammalian SNAP25, fungal Sec9p or plant SNAP33) that has a very spotty appearance among eukaryotes, appearing in mainly the multi-cellular lineages, but absent from many of the unicellular organisms (Sanderfoot, 2007). It has previously been noted that land plants have an extreme surplus of SNARE-encoding genes (Sanderfoot et al., 2000). While this surplus can partially be explained by the presence of small gene families, it seems that the plant gene families are not simply examples of redundancy, and may actually represent a large degree of complexity in vesicle trafficking.

Qa-SNAREs

Qa-SNAREs ("syntaxins") are the thought to be the anchor point of the SNARE complexes. Members of each of the other classes of SNARE have been proposed to act as vesicle-(v)-SNAREs, but the Qa-SNAREs seem to always act on the target membranes, and have been considered to be useful markers for particular organelles on which they reside. For example, the various groups of Qa-SNAREs in land plants seem to mark a non-redundant set of organelles (see below).

The Qa-SYP8 family is the likely syntaxin of the plant ER system, represented in Arabidopsis by a single gene, SYP81 (Sanderfoot, 2000). Little work has been done in plants, though fusing a fluorescent protein to SYP81 and expressing the fusion in protoplasts results in localization to the ER (Uemura et al., 2004). Since green plants tend to have a very distributed ER which has many

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essential functions in plant cells, members of the SYP8 family should be a useful target for research in the future.

Members of the Qa-SYP3 family have been localized to the ER and Golgi in many plants (Rancour et al., 2004; Uemura et al., 2004), and their homology to the mammalian syntaxin 5 and fungal Sed5p support this role. In Arabidopsis, the two members of the gene family (SYP31 and SYP32) may serve distinct functions (Rancour et al., 2004), though this needs further investigation.

Later in the Golgi system, at the TGN, the Qa-SYP4 family takes over. Research has indicated that members of the SYP4 family are found on the trans-face of the Golgi stacks (Bassham et al., 2000; Geldner et al., 2003, Uemura et al., 2004) and participate in several SNARE complexes at that point (Bassham et al., 2000; Sanderfoot et al., 2001b; Chen et al., 2005). Evidence indicates that these proteins are essential in Arabidopsis (Sanderfoot et al., 2001a), indicating the importance of vesicle trafficking at this organelle.

Among the endosomes, the Qa-SNARE is from the Qa-SYP2 family. Proteins in this family have been shown to localize to the TGN, late endosomes and to the vacuolar membrane (Conceição et al., 1997; Sato et al., 1997; Sanderfoot et al., 1999; Rojo et al., 2005), and form several SNARE complexes in this region (Bassham et al., 2000; Sanderfoot et al., 1999). Members of this gene family also seem to have major physiological roles in plants, since mutants in these genes are either lethal, or greatly alter plant morphology (Sanderfoot et al., 2001b; Yano et al., 2003).

The numerous members of the Qa-SYP1 family represent the PM-syntaxin in the plant kingdom. This group is made from four smaller clades which likely represent distinct aspects of PM-vesicle trafficking (Sanderfoot, 2007). KNOLLE (SYP111) is expressed in a cell-cycle-specific manner and localizes to the cell plate during cytokinesis, a role that is essential for seedling survival (Lukowitz et al., 1996; Lauber et al, 1997). The ROR2/PEN1-group (PEN1/SYR1/SYP121 and SYP122 in Arabidopsis) seems to specialize in "point defense" against fungal invaders (Collins et al., 2003; Assaad et al., 2004), though it likely also plays roles in general secretion (Kargul et al., 2001). The SYP124-group (SYP123, 124 and 125 in Arabidopsis) plays an unknown role, though their expression is very high in the male gametophyte (Sanderfoot, 2007). The SYP13-group (SYP131 and 132 in Arabidopsis) probably represents the more ancient clade (Sanderfoot, 2007) with roles in general secretion (Catalano et al., 2007; Kalde et al., 2007).

Qb-SNAREs

Qb-SNAREs generally operate as t-SNAREs at many essential parts of the endomembrane system. Though some have been used as markers, they are not as restricted to individual compartments as the Qa-SNAREs.

The Qb-SNARE of the ER-complex is a member of the SEC20 group in animals and fungi (see Dilcher et al., 2003), and this type of SNARE is also found in plants (Sanderfoot, 2007). However, no study of this protein has been reported from plants.

Two different Qb-SNAREs operate in the Golgi, similar to animals and fungi. Qb-MEMB1 (MEMB11 and 12 in Arabidopsis), which is homologous to mammalian Membrin or fungal Bos1p, has been shown to localize to the ER-to-Golgi in plants (Uemura et al., 2004; Chatre et al., 2005; Latijnhouwers et al., 2007). Later in the Golgi, the Membrin/Bos1p-SNARE is replaced by a Qb-GS29/Gos1p-SNARE in mammals and fungi (Hong, 2005), and the equivalent protein in plants is Qb-GOS1. In Arabidopsis, this group of SNAREs is represented by a two member gene family (GOS11 and GOS12), both of which reside in the Golgi as expected (Uemura et al., 2004).

The fourth Qb-SNARE (VTI11-to-14 in Arabidopsis) seems to function in the endosomes and vacuole. Arabidopsis and other plants have multiple paralogues that have been found to take on several overlapping roles in the Golgi/endosomal/ vacuole SNARE complexes (Bassham et al., 2000; Sanderfoot et al., 2001b; Zheng et al., 1999; Kato et al., 2002; Surpin et al., 2003). Like the Qa-SYP22 group, members of this group seem to play larger roles in plant physiology, including response to gravitropism (Kato et al., 2002). They also represent an example of partial redundancy among gene family members: individual mutants of *VTI1* gene family members are viable yet have distinct physiological phenotypes and SNARE-partner specificity (Kato et al., 2002; Niihama et al., 2005; Surpin et al, 2003).

The fifth kind of Qb-SNARE, NPSN1, was first identified among the green plants (Sanderfoot et al., 2000), though it is now clear that many other eukaryotes (outside of animals or fungi) also have a member of this family (Sanderfoot, 2007). This SNARE seems to operate as part of the endosomal/secretory pathway, and likely plays a role in cytokinesis, at least in Arabidopsis (Uemura et al., 2004; Zheng et al., 2003).

Qc-SNAREs

Qc-SNAREs operate as v-SNAREs in the early endosomal system, and as likely t-SNAREs in the later secretory pathway (Hong, 2005). Among eukaryotes, they are found to form four main groups, with some additional groups seen in some lineages.

A Qc-SNARE that operates in the ER is the Qc-USE1-like SNAREs recently identified by several labs in fungi (Burri et al., 2004; Dilcher et al., 2003). Due to an annotation error early in the Arabidopsis genome annotation, this gene was misidentified with a cation exchange transporter that was adjacent to the Arabidopsis USE11 gene, and a second family member is also found elsewhere in the genome (Sanderfoot, 2007).

The Qc-BET1-homologue of the green plants probably operates as the v-SNARE for ER-derived vesicles in transit to the cis-Golgi (Uemura et al., 2004) and is represented by two genes in Arabidopsis (BET11/Bs14a and BET12/Bs14b). Like both mammals and fungi, green plants have a second BET1-like SNARE that presumably operates in the later stacks of the Golgi. Called the plant-SFT1 clade (Sanderfoot et al., 2000; Sutter et al., 2006), it is represented by two genes (SFT11 and SFT12) in Arabidopsis (Sanderfoot, 2007). Outside of the green lineage, no obvious equivalents to the plant SFT1 clade are found, similar to the fungal Sft1p and the mammalian GS15 being lineage specific expansions of the BET1-group (Hong, 2005).

Three other related groups of Qc-SNAREs are found in the endosomal system. Qc-SYP6 (SYP61/OSM1 in Arabidopsis) has been localized to the TGN and endosomes in Arabidopsis (Uemura et al., 2004; Bassham et al., 2000). Mutations in the Arabidopsis *SYP61* gene lead to defects in resistance to changes in osmotic pressure (Zhu et al., 2002), suggesting that this protein

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may have roles in aspects of secretion similar to mammalian syntaxin 6 (reviewed in Hong, 2005). The Qc-SYP5 group (SYP51 and 52 in Arabidopsis), is probably the equivalent to mammalian syntaxin 8 and fungal Syn8p (Sanderfoot et al., 2000; Sanderfoot, 2007). As with SYP6, SYP5 is found on the TGN, the endosomes and on the vacuole (Uemura et al., 2004; Bassham et al., 2000; Carter et al., 2004). It has also been shown to interact with the SNARE complexes of the late endosome and vacuole (Bassham et al., 2000). Qc-SYP7 (SYP71 and 72 in Arabidopsis) was first identified in the green plants (Sanderfoot et al., 2000), but has since been identified in many other eukaryotes, except in animals and fungi (Sanderfoot, 2007). Members of the Qc-SYP7 family have been found on the PM in several plants (Marmagne et al., 2004; Mongrand et al., 2004; Morel et al., 2006). Arabidopsis SYP71 interacts with Qb-NPSN11 and Qa-KNOLLE as a part of cell plate formation during cell division, and is also found specifically at the basal domain of the PM in non-dividing root cells (L.C. Conner and A.A. Sanderfoot, unpublished data), suggesting a role in specialized secretory processes.

Qb+Qc-SNAREs

The SNAP33-type proteins are an example of a Qb+Qc-SNARE, and have been shown to be essential in such roles as general secretion, pathogen defense, and for cell plate formation during cytokinesis (Sanderfoot et al., 2000; Collins et al., 2003; Heese et al., 2001; Kargul et al., 2001). Compared to animal/fungal Qb+c-SNAP25, the land plant SNAP33 proteins have an N-terminal extension (which does not bear any strong resemblance to the extensions of the mammalian SNAP29, SNAP47 or the fungal Sec9p N-termini), and lack the palmitoylation site of the animal SNAP25-like proteins (Sanderfoot, 2007). Interestingly, this type of SNARE is missing from many unicellular eukaryotes, and instead only seems to be found in multicellular organisms (Sanderfoot, 2007).

R-SNAREs

The classic R-SNAREs are the so-called "Brevins" that are found in both mammals and fungi (for example, Synaptobrevin/VAMP1 in mammals and Snc1p in yeast; Hong, 2005), but the more prevalent type of R-SNARE are the "longin" type (Rossi et al., 2004). Green plants do not appear to have any brevin-type proteins; like most other eukaryotes, they only have the "longin" types of R-SNARE (Sutter et al., 2006; Sanderfoot, 2007). R-SNAREs are most often considered to be v-SNAREs, and thus reside on multiple compartments.

The R-SEC22 type of SNARE (SEC221 and 222 in Arabidopsis) seems to operate in several ER/Golgi SNARE complexes in many eukaryotes, including plants (Chatre et al., 2005; Sanderfoot, 2007). A second type of longin-R-SNARE is the YKT6-type of SNARE, which is somewhat unique in being attached to membranes by lipid modification rather than through a transmembrane domain like other SNAREs (Hong, 2005). YKT6-group members have been found on several membranes in plant cells, and have been reported to form several SNARE complexes with Golgi and endosomal SNAREs (Chen et al., 2005).

A third group of longin-type R-SNAREs similar to mammalian VAMP7 are found in all eukaryotes (Sanderfoot, 2007). Plants have two major clades of VAMP7-like R-SNAREs: One clade, the VAMP71 clade (VAMP711-to-714 in Arabidopsis) is found in all green plants and is phylogenetically more similar to the VAMP7-SNAREs found in other eukaryotes (Sanderfoot, 2007). The members of this group are found on organelles in the late endosomal and vacuolar system (Uemura et al., 2004; Carter et al., 2004), similar to the yeast and mammalian VAMP7 SNAREs (Pryor et al., 2004; Wen et al., 2006). The VAMP72 group (VAMP721-to-728 in Arabidopsis) is only found in green plants (Sanderfoot, 2007), and is involved in secretion (Kwon et al., 2008).

In addition to those SNAREs that operate as fusion machinery, there are also some SNARE proteins that seem to operate in regulation. For example, an evolutionarily conserved family of proteins related to the mammalian protein Tomosyn have a C-terminal R-SNARE domain, but lack a transmembrane span (or any other mechanism for direct attachment to the membrane) and have a large N-terminal domain with several WD-40 repeats (Gerst et al., 2003; Sutter et al., 2006; Sanderfoot, 2007). In Arabidopsis, this is represented by two genes (TYN11 and TYN12; Sutter et al., 2006), but whether this protein regulates SNARE complex formation in plants like it does in animals and fungi has not yet been examined.

III. THE ORGANELLES AND COMPARTMENTS

III.A. ER-to-Golgi Trafficking

The endoplasmic reticulum (ER) is continuous with the nuclear membrane as well as ramifying throughout the cytoplasm as a reticulated sheet (Figure 7). It is likely that some portion of the ER is in close contact with every organelle in the cell, performing various roles with each contact. The ER also has many biochemical and metabolic roles, though its role in initiating the bulk of the secretory system is of most relevance to this chapter. How the ER

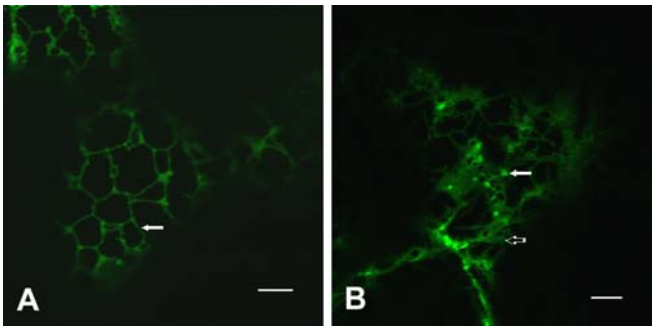


Figure 7. The Early Endomembrane System: ER and Golgi.

(A) The ER is typically observed as a reticulate pattern that ramifies throughout the cytosol, here visualized by confocal microscopy in a leaf epidermal cell expressing GFP-Calnexin (see: Irons et al., 2003). Early Golgi markers, such as GFP-ERD2 (the KDEL-receptor; see Boevink et al., 1998) show bright spots corresponding to the Golgi stacks (arrow), but also have a small percentage of protein that is also found in the ER (open arrow).

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maintains its reticulate appearance is not known. Novel studies suggest that reticulons may be involved in this aspect of the ER, similarly to other systems (Tolley et al., 2008).

Proteins intended for the endomembrane system typically contain N-terminal signal peptides, or have analogous transmembrane domains at other places within the protein that engage the ER-translocation machinery in a similar way (reviewed in Martoglio and Dobberstein, 1998). The overall mechanism and the proteinaceous machinery is remarkably conserved across eukaryotes. Most eukaryotes are capable of both co- and post-translational translocation, and often similar proteins may use either method depending on the species in which they are expressed (reviewed in Kalies and Hartmann, 1998). Regardless, signal peptides can be successfully exchanged between different organisms (Gierasch, 1989), indicating the overall conservation of the process.

Once engaged by the translocation machinery, proteins that lack membrane-spanning domains are released into the lumen of the ER, whereas the membrane proteins are threaded into the membrane (often multiple times) depending upon the folding information within the peptide sequence of the membrane protein. In either case, chaperones and other factors assist in the folding, disulfide bond formation, core-glycosylation, and oligomerization of the proteins. During this process, misfolded proteins are selectively retained by a quality control process which either completes the folding and releases the protein, or marks it for destruction (see review by Vitale and Denecke, 1999).

It has been suggested that the ER has many distinct domains based upon morphology and other criteria (Staehelin, 1997). The domain of most relevance to this review is the transitional ER (tER). The tER (also called ERESs) is the domain where secretory cargo proteins become concentrated for packaging into the COP-II transport vesicles which will carry them to the Golgi stacks. The tER is best studied in mammals and fission yeast, where clear morphological and cytochemical evidence for tER "exit sites" is found (Hammond and Glick, 2000, Rossanese et al., 1999). Structures with similar morphology have been reported in plant cells (Staehelin, 1997). The tER-type domain may not be strictly necessary, as budding yeast does not appear to produce specific tER sites, and instead, it is believed that cargo may exit from any point in this yeast (Rossanese et al., 1999). Packaging of cargo in the tER still remains somewhat controversial. While some of the proteinaceous components of the vesicle forming machinery have been characterized (see COP-II, section **II.A**), whether there is a selective packaging or simple "bulk-flow" of lumenal contents towards the Golgi in many cell types is unclear. Many so-called ER-to-Golgi "cargo receptors" have been identified in yeast and mammals suggesting specific packaging (e.g.: Lavoie et al., 1999, Denzel et al., 1999, etc.). Whether a bulk-flow or specific mechanism exists has been investigated in plant cells (Phillipson et al., 2001; Törmäkangas et al., 2001), and further work will be required to implicate one over the other. Despite some level of specificity in packaging, ER-residents are often reported to escape as far as the trans-Golgi network, and an efficient Golgi-to-ER recycling system (KDEL-receptor) is found for their retrieval (reviewed in Vitale and Denecke, 1999). Thus, it is likely that some selective process is functioning for particular proteins, whereas some non-selective sampling of the lumenal contents also occurs. Selection of membrane proteins for packaging is likely more selective and is probably dependant on peptide signals within the membrane protein sequence (see COP-II, section **II.A**).

The Golgi is not the only destination for cargo exiting from the ER. Many plant species create unique Protein Bodies (also called "Precursor Accumulating", PAC vesicle; Hara-Nishimura et al., 1998) of seed storage proteins by selective distention of an ER subdomain (reviewed in Herman and Larkins, 1999). Seed storage mRNAs are translated upon this portion of the ER membrane leading to quick delivery of the proteins into the forming protein body. When "full", the protein body buds away from the ER, and may eventually fuse with a Protein Storage Vacuole. Some evidence of a direct (non-Protein Body) pathway from the ER to the Vacuole has also been reported (Jiang and Rogers, 1998), though mechanistically, little is known about this type of trafficking. It has recently become clear that peroxisomes may also be formed (at least partially) by budding from a sub-domain of the ER (reviewed in Mullen et al., 2001).

Since the ER is also a major biosynthetic source of lipids, the ER also must exchange material with other organelles such as plastids, mitochondria, and the plasma membrane, though the mechanics of these exchanges are unclear. Storage of lipids (as an energy source) during seed development occurs in a similar process (reviewed in Galili et al., 1998). Triacylglycerols are funneled between the leaflets of the ER membrane, creating an oil body that is surrounded by a single monolayer. Eventually, this oil body recruits a particular set of proteins, and subsequently buds off the ER and exists free in the cytoplasm. Further studies into these areas may lead to a new understanding of how the ER functions in intracellular traffic apart from the traditional vesicle-mediated secretory pathway.

III.B. The Golgi: Central Sorting Station for the Secretory Pathway

The Golgi apparatus is the common destination for most secretory proteins after departing the ER. It is typically formed of 5-20 individual cisternae which are stacked in a stereotypic manner producing a polarized organelle of cis-to-trans orientation. In Arabidopsis (as in most plants), many individual Golgi bodies (called Dictysomes in some older literature) are found dispersed throughout the cytoplasm, moving quickly throughout the cell by cytoplasmic streaming (for example, see Nebenführ et al., 1999; Boevink et al., 1999; Nebenführ et al., 2000; Figure 7). This type of Golgi distribution is found in many eukaryotes, though is in contrast to the mammalian pattern of a single "super" Golgi stack in the perinuclear region, or to the budding yeast pattern of unstacked Golgi cisternae. The cisternae are distinguished not only by morphological criteria, but also by diagnostic metabolic and enzymatic activities, as well as by the order in which secretory cargo passes. Cargo fresh from the ER first arrives in the cis-Golgi network, passing sequentially through cis-, medial-, and trans-cisterna before arriving at the trans-Golgi network (TGN).

How the cargo passes through the cisternae is still a matter of some controversy (see Pelham and Rothman, 2000). Some evidence suggests that the cisternae are static organelles, and that all traffic between the stacks is vesicle-mediated by COP-I coated vesicles. Other evidence indicates that the stacks may simply be transient structures (i.e.: cisternal maturation models), that are

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formed new from ER-derived vesicles (or collections of fused vesicles called VTCs in mammalian cells) at the cis-side, then sequentially mature into medial- and trans-cisternae. New cis-stacks form behind the maturing cisternae creating an "assembly-line"-like formation of stacks. Proteins which are specific to an earlier cisterna are collected into COP-I vesicles which remove these proteins back to the earlier stacks, while the maturing stack receives its own specific proteins by collecting COP-I vesicles from a later stack. Finally, at the TGN, the stack is partitioned into secretory vesicles and endosomal compartments. As you may see, the major difference between these two models is the role of the COP-I coated vesicles: In the first case, the COP-I coat caries cargo both forward (trans-wise) and backward (cis-wise), whereas in a cisternal maturation-type model, COP-I coats only move in a retrograde manner. Most recent evidence has supported cisternal maturation, especially in plants (e.g. see Otegui et al., 2006; Donohoe et al., 2008), but these models are not necessarily mutually exclusive, and may vary between different organisms at different times of development.

The cis-Golgi is the first cisterna encountered following the ER. The ER-derived vesicles fuse with the cis-Golgi delivering their contents to the lumen or limiting membrane. Within the cis-stack, modifications to the core N-glycosylation of proteins begin. Enzymes such as α-mannosidase remove the terminal mannose residues, creating substrates for other glycosyltransferases which act in the later stacks to produce the unique glycosylation patterns found on many proteins. One of these enzymes, α -mannosidase, has been fused to green fluorescent protein (GFP) by the Staehelin group, and has been used as an *in vivo* marker for the Golgi stacks, revealing some interesting behaviors in dividing cells (Nebenführ et al., 2000).

The cis-cisternae are also the site of recapture of escaped ER residents. The KDEL-receptor is a transmembrane protein whose lumenal domain specifically recognizes a C-terminal K/HDEL motif found at the C-terminus of ER-resident soluble proteins (Pelham, 1996). Upon binding of proteins with these signals, the KDEL-receptor recruits the COP-I machinery and mediates the return of these proteins to the ER. Recent work has indicated that the plant KDEL receptor appears to exceptionally efficient, and rarely allows any ER residents to escape past the early Golgi stacks (Phillipson et al., 2001). Experiments in mammalian cells have indicated that ER residents may sometimes be allowed passage as far as the TGN before recapture. The passage of some ER residents into the Golgi stacks may be beneficial for the acquisition of Golgi-specific modifications that may be needed in some cases, however the functional significance of this difference is unclear.

Since the number of cisternae in a Golgi apparatus can vary significantly, definition of a "medial" stack is equally variable. Enzymatically, these stacks can often have activities that differ from that of the earlier and later cisternae. Certainly, the spectrum of glycosyltransferases must be somehow distinct, but how this distinction is set-up and maintained remains unclear. Some evidence suggests that synthesis of some cell wall glycans (rhamnogalacturans and pectins) is initiated in the medial stacks, whereas the trans-cisterna is the typical site of xyloglucan assembly (reviewed in Dupree and Sherrier, 1998). Some of the molecular details of the glycosyltransferases involved in these processes have begun to be worked out, though much remains to be done.

At some point within the trans-cisterna (perhaps earlier), particular cargo proteins have been selected based upon their final destinations. At some level, the cisternae at the TGN are segregated into domains. This has been observed directly in only a few cases; for example, when the localization of two related TGN-localized syntaxins SYP41 and 42 (see SNAREs) were examined they were found to be at distinct locales at the TGN (Bassham et al., 2000). Since that time, using these markers, other proteins have been found to segregate in similar manners: The vacuolar cargo receptor ELP was found to preferentially localize with SYP42 at the TGN (Bassham et al., 2000), the syntaxin SYP51 was also found with only SYP42 (Sanderfoot et al., 2001b), and CTPP-type vacuolar cargo was found to segregate away from ELP at the TGN (Ahmed et al., 2000). Similar results showing a partition of some cargo away from the vacuolar cargo receptor has also been reported by Robinson and co-workers using expanding cotyledon cells of pea as a model system (Hohl et al., 1996; Hinz et al., 1999). Vacuolar cargo (of all types) must also be segregated away from secreted cargo by the Golgi-resident vacuolar sorting receptors (reviewed in Vitale and Raikhel, 1999). These receptors lead to a concentration of the vacuolar cargo into at least one domain of the cisterna. In doing so, secretory cargo is excluded and ends up in distinct regions of the stack. Clearly, the work of various coat proteins and Golgi matrix components are essential for this process, though we are still early in the identification of these factors.

The ELP/BP-80 class of vacuolar sorting receptors (VSR) are found concentrated in the trans-cisternae and the TGN (Paris et al., 1997; Sanderfoot et al., 1998). The VSR proteins are typically represented by large protein families that may exhibit distinct signal specificity. Evidence has indicated that members of this family can specifically recognize both N-terminal and C-terminal vacuolar sorting signals (Kirsch et al., 1994; Cao et al., 2000; Ahmed et al., 2000; Shimada et al., 2003; Otegui et al., 2006), though it remains unclear whether this can be accomplished by the same kind of VSR. It does appear that at least one member of the VSR family is capable of recruiting a clathrin-type coat to the membrane (perhaps through the AP-1 type of coatomer), and directs the packaging of the NTPP-type cargo into CCVs (Ahmed et al., 2000). These CCVs travel onto the late endosomes/prevacuolar compartment (see **III.E**). Other VSR family members also seem to be able to recognize CTPP-type vacuolar cargo and direct this cargo to a protein storage vacuole (Shimada et al., 2003). In cells that lack a storage vacuole, the specificity of the cargo receptors must be regulated in some way such that NTPP and CTPP cargo can become segregated at the TGN (Ahmed et al., 2000; Otegui et al., 2006). Other potential cargo receptors (i.e., AtRMR; Jiang et al., 2000) have been suggested for targeting of various vacuolar cargo from within the Golgi, but the mechanisms remain elusive and controversial (Otegui et al., 2006; Hunter et al., 2007; Olbrich et al., 2007).

In the absence of other sorting signals, soluble cargo proteins are directed into secretory vesicles and targeted to the plasma membrane. It remains unclear in any eukaryote as to the nature of the coat proteins involved in packaging secretory vesicles. Regardless, some measure of specificity must be involved, especially in polarized cells. Certain proteins are restricted to particular portions of the cell membrane (see **III.C**), indicating that secretion need not be a non-specific process. However, very little is known at this time about how a plant cell produces or maintains the polarization of its plasma membrane.

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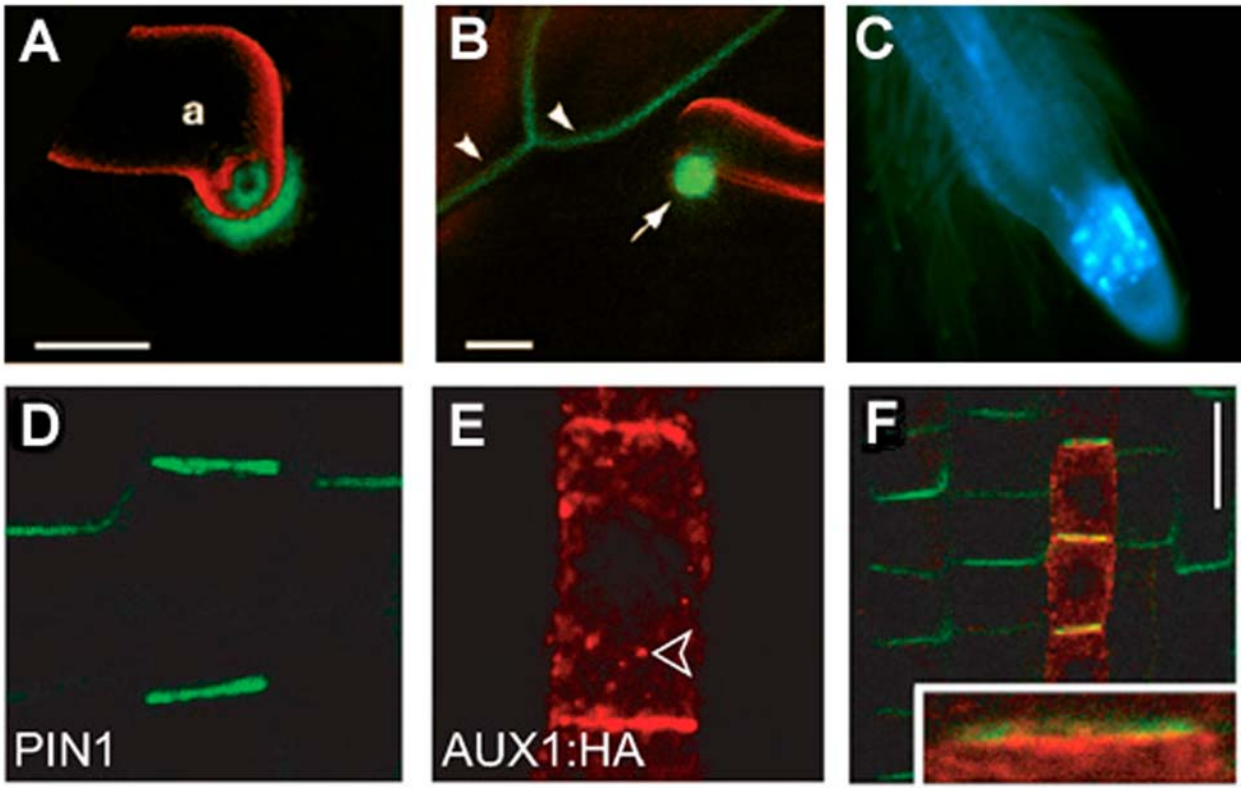


Figure 8. The Plasma Membrane in Polarized Cells

In addition to simply forming the limiting membrane of the plant cell, the PM has recently been found to have multiple kinds of specialized sub-domains in cell that are polarized or that are responding to particular stresses or developmental signals. For example, in studies reported by Assaad et al (2004), an epidermal cell responds to infection by powdery mildew (Erysiphe cichoracearum; red) by establishment of a particular PM structure called papillae. Two PM-SNAREs, Qa-PEN1 **(A)** and Qa-SYP122 **(B)**, visualized by confocal microscopy in cell expressing GFP- or CFP-fusions (respectively), become enriched in these domains and are essential for resisting the fungal infection. In a similar way, the Qa-SNARE KNOLLE is also important for establishment of the cell plate in dividing cells **(C)**,here visualized in the root tips of plants expressing KNOLLE::CFP-KNOLLE. The best studied examples of polarization in plant cells has been shown by the auxin efflux (PINs) and influx (AUX1s) transporters. For example, in work reported in Kleine-Vehn et al. (2006) the authors performed whole-mount immunofluorescent microscopy to show how the protophloem cells of the root have independent apical and basal domains enriched in AUX1 and PIN1 (respectively **(D-F)**). Images **(A)** and **(B)** are from Assaad et al., 2004; images **(D-F)** are from Kleine-Vehn et al., 2006.

Another aspect of Golgi mediated targeting occurs during cytokinesis (reviewed by Jürgens, 2005). Plants have a unique method of cell division whereby a novel membrane structure (the cell plate) is synthesized by Golgi-derived vesicles at the point of cytokinesis. The cell plate is a site of furious vesicle fusion and formation, and eventually, a new plasma membrane is formed by the fusion of the phragmoplast with the maternal plasma membranes (Seguí-Simarro et al., 2007). It seems likely that this organelle is derived from a novel form of regulated secretion, since the components of the vesicle fusion machinery involved in this step (see **II.C**) are related to the factors involved in general secretion.

III.C. The PM is the Default Destination, but why are the Different Sides Different?

The limiting membrane of the cell is the target for cargo both intended for secretion and for incorporation into the membrane. Thus, secreted proteins and the components of the extracellular matrix are delivered to the apoplast where they can become incorporated or diffuse away. Membrane proteins become incorporated into the plasma membrane where they act as ion channels or membrane transporters, ligand receptors and signaling complexes, or even as physical contact points for both the intracellular cytoskeleton network or for the extracellular matrix. A useful example of a receptor complex is the Clavata system that controls cell fate in the Arabidopsis shoot apical meristem (reviewed in Clark, 2001). In this system, a multi-member transmembrane receptor kinase complex (CLV1/2) is assembled across the plasma membrane and coordinates binding to the extracellular peptide ligand CLV3 with signal transduction through a cytoplasmic kinase cascade. Disruptions of any of the genes in this pathway prevent signal transduction, resulting in overproliferation of the shoot apical meristem. The secretory system is essential to functioning of this system as well, both in positioning the receptor complex at the PM, and in delivery of the secreted CLV3 ligand. Redirection of

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the ligand to the vacuole (Rojo et al., 2002) blocks signal transduction, indicating the importance of proper targeting of proteins in the secretory system.

The lipid of the plasma membrane is quite different from that of the other membranes, being enriched in specific lipids and sterols. This lipid composition is unlikely to homogeneous, rather, similar to what is found in other eukaryotes, there are likely to be distinct patches which are highly enriched in particular lipids. These patches serve to concentrate particular types of proteins like receptor/signaling complexes, cytoskeleton attachment points, and endocytic machinery. Most work on the compartmentalization of lipid domains has been done in mammals, where proteins like caveolin are essential for stability of these domains. Although plants lack caveolin (no homologous gene is found in the Arabidopsis genome), it is still likely that similar domains exist, though little work has been reported on such things in plant cells.

The limiting membrane itself also is likely to show sidedness in the sense that the upper (or apical) portion of the cell may contain proteins not found in the lateral or basal (bottom) portion of the cell. This polarity is accomplished both by specific targeting to a particular domain, by specific retrieval though endocytosis, and by peculiar characteristics of the lipid patches found in the domains. This polarity is essential to cell function and to signaling. For example, transport of auxin through the root is accomplished by specific influx transporters in the apical membrane which transport the neutralized auxin into the cytoplasm. In the cytoplasm, the auxin ionizes due to the near neutral pH. At the basal membrane, an active efflux transporter carries the auxin ion back to the apoplasm (reviewed in Jones, 1998). Without the specific localization of the transporter to particular regions, polarity of the auxin transport could not be accomplished. The probable auxin efflux carrier PIN1 is localized specifically to the basal part of root cells (Galweiler et al., 1998; see Figure 8). This specific polarized localization of PIN1 is accomplished through Golgi-mediated trafficking and requires the action of the product of the GNOM gene, a Sec7-domain-containing protein that likely regulates vesicle budding through interaction with an ARF-GTPase at the trans-Golgi (Steinmann et al., 1999; Geldner et al., 2003). Other members of the PIN1-like family are found on the apical or lateral faces rather than basal portion of the PM, suggesting further polarized domains exist in plants (Müller et al., 1998; Friml et al., 2002; Friml et al., 2003; Klein-Vehn et al., 2006). How these domains are established and maintained remains unknown in plants, and continues to be an area of intensive investigation.

III.D. Endosomes as a Central Sorting Station for the Late Endomembrane System

Endosomes are organelles that transport and sort cargo from both the biosynthetic pathway (for example proteins coming from the Golgi) and the endocytic pathway (endocytosed plasma membrane molecules). There are several types of endosomes in eukaryotic cells and they differ in function, structure, and biochemical composition (Figure 9). Endocytosed plasma membrane proteins are transported in vesicles to the “early endosomes.” Early endosomes may have recycling functions and send back to the plasma membrane some of the endocytosed cargo. In mammalian cells, there is a distinct set of endosomes called recycling endosomes that play this recycling role. Other endocytosed proteins that have been targeted for degradation remain in the endosomal system where they are recognized by the ESCRT machinery and sorted into internal vesicles (reviewed by Hurley 2008 and see Coat Proteins). These endosomes containing luminal vesicles are called late endosomes or multivesicular bodies. Multivesicular bodies, frequently called prevacuolar compartments in plants, finally fuse with the vacuolar membrane, releasing the internal vesicles into the vacuolar lumen where they are degraded by hydrolyases.

Endosomes also receive Golgi-derived vesicles carrying newly synthesized vacuolar proteins (Kotzer et al. 2004, Otegui, et al. 2006, Tse, et al. 2004) that are delivered to the vacuole. In particular, multivesicular endosomes act as prevacuolar compartments in developing seeds, where they carry storage proteins and processing proteases and deposit them in protein storage vacuoles (Figure 9).

The recycling of vacuolar cargo receptors back to the TGN also happens at the endosomes (Figure 6). The endosomal protein complex called Retromer (see Coat Proteins), that in mammals and yeast is responsible for the recycling of vacuolar cargo receptors, has been identified in Arabidopsis (Jaillais et al. 2006, 2007, Oliviusson et al. 2006, Shimada, et al. 2006). The Arabidopsis retromer subunits VPS35, VPS26 and VPS29 have been shown to localize to Rab5- positive multivesicular endosomes, together with the vacuolar sorting receptor VSR1 (Oliviusson et al., 2006; Jaillais et al, 2006). vps35 mutants exhibit dwarfism, early leaf senescence, mis-sorting of vacuolar seed storage proteins to the extracellular space, and increased accumulation of VSR receptors (Yamazaki et al., 2008). In fact, VPS35 co-immunoprecipitates with VSR1 (Oliviusson et al., 2006). Similar mis-sorting of seed storage proteins has also been reported in the vps29/mag1 retromer mutant (Shimada et al. 2006). In addition, vps29 mutants show abnormal embryo development, seedlings with multiple cotyledons, and reduced primary root growth with fewer lateral roots and altered gravitropic response (Jaillais et al., 2007). Some of these phenotypic alterations seem to be related to a role of the retromer in the subcellular localization of PIN auxin efflux carriers and the subsequent alteration of auxin transport in retromer mutants (Jaillais et al., 2007).

A number of important plant proteins continuously cycle between the plasma membrane and endosomes in a mechanism called constitutive cycling (reviewed by Murphy et al. 2005). Examples of constitutively recycled proteins include the PIN proteins, (putative auxin efflux regulators), PIP2 (a plasma membrane ATPase thought to be a water channel), BRI1 (brassinosteroid receptor), and KAT1 (a K^+-channel). This mechanism appears to allow dynamic changes in the distribution of plasma membrane proteins. A key player in constitutive cycling of PIN1 is GNOM, a GDP/GTP exchange factor for the ARF GTPases (Steinmann et al. 1999).

Although it is clear that the endosomal system is well developed in plant cells, the identity of the plant early endosomes has remained elusive despite having been a target of study for decades (see Joachim and Robinson, 1984; Tanchak et al, 1984). Recent studies suggest that a TGN-related compartment received endocytosed material from the plasma membrane, acting as an early endosome (reviewed by Lam et al. 2007).

The sorting of plasma membrane proteins into endocytic vesicles and subsequently into the internal vesicles of multivesicular

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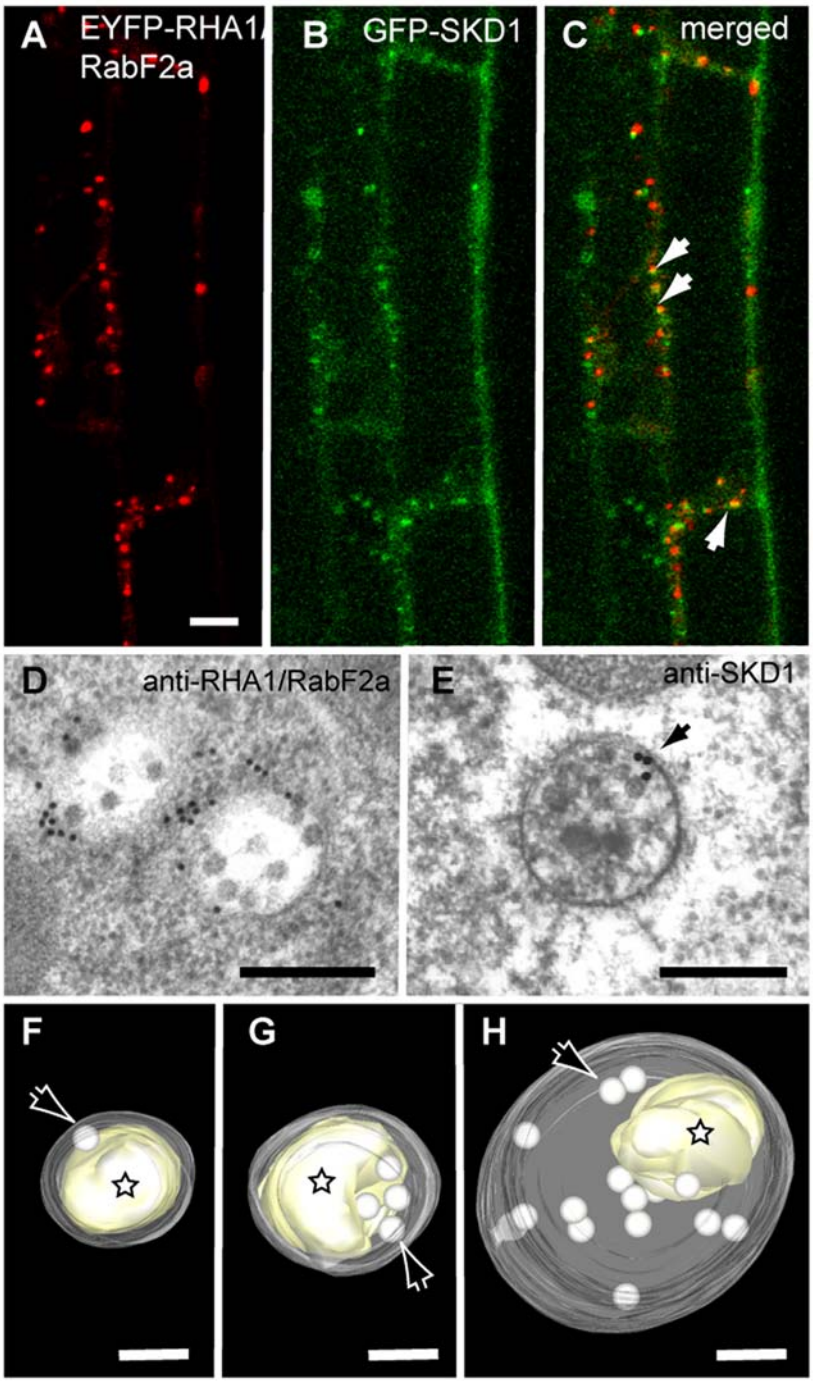


Figure 9. Endosomal Compartments in Plants

Endosomal compartments in plants have long been under examined due to a lack of suitable marker proteins, however, new marker proteins have recently been developed allowing observation of these compartments through both fluorescent and electron microscopy. **(A)-(C)** Partial co-localization of GFP-SKD1 and a fluorescent fusion protein of the endosomal Rab GTPase RHA1/RabF2a in Arabidopsis roots cells. Arrows indicate areas of colocalization. Bars = 10 m. **(D)-(E)** Immunogold localization of endosomal proteins in high-pressure frozen/freeze-substituted wild type Arabidopsis roots. Gold labeling shows localization of RHA1/RabF2a **(D)** and SKD1 **(E)** on multivesicular bodies or MVBs. Bars = 200 nm. **(F)-(H)** Tomographic models of prevacuolar compartments/MVBs during formation of protein storage vacuoles in the Arabidopsis embryo reconstructed from electron tomograms. The limiting membranes of MVBs have been made translucent in these tomographic models to visualize the internal vesicles (arrows) and electron dense aggregates of vacuolar storage proteins (stars). Images **(A)** to **(E)** are from Haas et al. (2007); images **(F)-(H)** are from Otegui et al. (2006).
Bars= 100 nm

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bodies are crucial steps in the down-regulation of receptors involved in development, signaling, and cell differentiation as well as in general turnover of plasma membrane proteins in eukaryotes. Ubiquitination of membrane-bound receptors is now well established as a signal for both endocytosis at the plasma membrane and sorting into internal vesicles of multivesicular bodies (reviewed by Mukhopadhyay and Riezman 2007). When the ubiquitinated receptors reach the late endosome, flat clathrin coats highly enriched in HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) form on the endosomal membranes. Three multi-subunit complexes called ESCRT-I, -II, -III (endosomal sorting complexes required for transport) are also required for protein sorting into MVB internal vesicles in yeast and mammalian cells.

Once the three ESCRTs are assembled onto the endosomal membrane, the AAA ATPase Vps4p (yeast)/SKD1 (mammals) is required for endosomal membrane invagination, presumably by releasing the ESCRTs from the membrane (Figure 6). The binding of Vps4p/SKD1 to the membrane is regulated by its ATPase cycle, being membrane-associated in its ATP-bound form and cytoplasmic in its ADP-bound form. Vps4p/SKD1 is a crucial player in the MVB pathway: when Vps4p/SKD1 is knocked out or a dominant negative (ATPase deficient) form of Vps4p/SKD1 is expressed, aberrant endosomes form, causing disruptions in the secretory and endocytic pathways (Babst et al. 1998). Whereas there is some variation in the organization of the ESCRT complexes in different organisms, the function of Vps4p/SKD1 appears to be highly conserved in all eukaryotic cells. However, whereas yeast and mammalian cells are able to survive expressing ATPase deficient versions of Vps4p/SKD1, the expression of such a form of AtSKD1 in plant cells causes dramatic alterations in the endomembrane system and cell death, suggesting an essential role of the endosomal sorting machinery in plants (Haas et al. 2007). Fluorescent fusion proteins of AtSKD1 and ELC, a Vps23p/TSG101 homolog that is part of the ESCRT machinery, have been shown to localize to endosomes in *Arabidopsis* (Figure 9) (Haas et al., 2007; Spitzer et al., 2006). In addition, AtSKD1 has been shown to partially localize with Rab5-like endosomal GTPases (Figure 9).

III.E. Vacuoles: More than Just a Lysosome

The most conspicuous organelle in a mature plant cell is the large central vacuole, which can occupy as much as 90% of the cell volume (Figure 10). Plant vacuoles play many essential roles for the cell. A major role is storage: of water, ions, sugars, hormones, secondary metabolites, metals, pigments, toxins, and even proteins in specialized types of vacuoles. The vacuole is also the equivalent of the lysosome of mammals, in that it contains the various hydrolyases which are used to degrade and recycle proteins, lipids, carbohydrates, etc (reviewed by Muntz 2007). In concert with the plasma membrane, the vacuole also has the essential role of maintaining turgor pressure in the cell. This turgor is essential for homeostasis and also for growth and development. It is generally thought by expansion of a large water filled vacuole held tight with turgor pressure, a plant cell can grow quickly and become quite large without an equivalent increase in the volume of energy-intensive cytoplasm. The essential importance of the vacuole to plant growth and development was shown by Rojo et al. (2001) with the *vacuoleless* mutant of Arabidopsis. Plants where the *VACUOLELESS* gene is disrupted by a T-DNA were found to completely lack a central vacuole, and die as embryos.

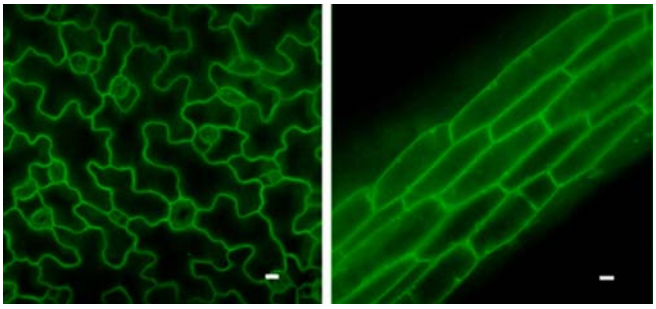


Figure 10. Vacuolar Compartments in Plants

In most mature vegetative cells, the majority of the cell volume is occupied by the large central lytic vacuole. This can be visualized by confocal microscopy of cells expressing *35S::GFP-δ-TIP* (J. Zouhar, E. Avila, and N.V.R., unpublished) in either epidermal cells of the cotyledon **(left)** or hypocotyl cells **(right)**.

Vacuoles differ in their functions and contents and some cell types may contain more than one type of vacuole depending on the developmental stage and environmental conditions (Hoh et al., 1995; Paris et al., 1996; Di Sansebastiano et al., 1998; Swanson et al., 1998; Otegui et al. 2005). Protein storage vacuoles (PSVs) in seed tissues accumulate large amounts of storage proteins, processing proteases, and phytic acid crystals called globoids. In Arabidopsis and legumes, the seed storage proteins destined for the PSV begin to condense in the early stacks of the Golgi and form "dense vesicles" (DVs; Hohl et al., 1996; Hinz et al., 1999; Hillmer et al., 2001, Otegui et al. 2006). The DV expands as additional cargo is packaged within, until finally budding free from the TGN. The DVs are subsequently trafficked to multivesicular bodies where their initial proteolytic processing starts until they are released into the PSV upon multivesicular bodies-PSV fusion. In other species (cucurbits), some storage proteins are packaged into a DV-like organelle (called a PAC vesicle) at the ER (Hara-Nishimura et al., 1998). The ER distends until a PAC buds free into the cytoplasm. A single PAC vesicle may fuse with other PAC vesicles, receive additional cargo delivered from the Golgi, eventually form an multivesicular body-like organelle, and then fuse with a PSV.

The lytic functions of vacuoles serve multiple purposes: degradation of storage proteins for amino acid recycling during seed germination, degradation of plasma membrane proteins (see **III.D**), degradation of cytoplasmic material sent to the vacuole via autophagy (see **III.F**), defense against pathogens (reviewed in van der Hoorn and Jones, 2004), senescence, and programmed cell death (reviewed by Jones 2001).

The tonoplast or vacuolar membrane contains numerous transporters and channels that mediate the transport of different compounds and ions between the vacuolar lumen and the cytoplasm (Carter et al, 2004; Jaquinod et al., 2007, Schmidt et al., 2007). Most vacuoles seem to contain at least vacuolar H^+-ATPases, vacuolar H^+-translocating pyrophosphatases and TIP (tonoplast intrinsic protein) aquaporins. The proton gradient generated by both

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types of proton pumps provides the force that allows other transporters to translocate ions across the tonoplast against their concentration or electrochemical gradient. For example, Na^+, Ca^{2+}, NO^{3-}, Zn^{2+} and several other ions enter the vacuoles through proton antiporters (Hirschi et al. 2004; de Angeli, 2006; Kawachi et al., 2008). In fact, transgenic Arabidopsis plants overexpressing the vacuolar H^+ pyrophosphatase AVP1 show higher salt tolerance, likely due to an increased pH gradient across the tonoplast which could facilitate accumulation of ions inside the vacuole (Gaxiola et al. 2001). The vacuole is a major site for Ca^{2+} storage in the form of free Ca^{2+}, calcium oxalate, or chelated with organic acids. In addition to the Ca^{2+}/H^+ antiporter, at least one vacuolar Ca^{2+} pump and several Ca^{2+} efflux channels have been identified in the vacuole (Geisler et al, 2000).

As mentioned above, the pressure exerted by the vacuole is important for keeping cell turgidity and allowing changes in cell volume during growth. Rapid transient changes in cell volume, such as in guard cells during stomatal opening/closure or during closure of Venus fly traps to capture insects and pulvinus-mediated leaf movement in *Mimosa*, also depend on water fluxes through both the plasma membrane and the tonoplast. These water fluxes are facilitated by water channels called aquaporins, which are found in both the plasma membrane and the tonoplast. Arabidopsis contains approximately 35 genes encoding for aquaporins, from which 10 genes seem to have vacuolar functions. The mechanism by which aquaporins are regulated is not completely clear but it seems to depend on phosphorylation, pH, and osmotic gradients (reviewed by Maurel 2007 and Katsuhara et al. 2008). The water fluxes observed during rapid transient changes in cell volume are induced by changes in osmotic pressure associated with ion fluxes through the plasma membrane and the tonoplast.

In addition, vacuoles play an essential role in plants with CAM (crassulacean acid metabolism)-type of photosynthesis. These plants open their stomata during the night to minimize water loss. The CO_2 is incorporated into organic compounds and stored in the vacuole as malic acid. During the day, the CO_2 is released from the organic acid to enter the Calvin cycle in the chloroplast. It has been shown that an equivalent of 17% of the total dry mass of leaf cells is transported through the tonoplast in CAM species in a day (Pucher et al., 1949; Holtum et al., 2005).

III.F. Autophagy: Last Desperate Step or just a Normal Part of Life?

Macroautophagy, termed simply autophagy herein, meaning 'self-eating', is a pathway for the degradation of proteins and other macromolecules by transport into the vacuole (Gupta, 1988; Aubert et al., 1996; Moriyasu and Ohsumi, 1996). Autophagy is a somewhat unique vesicle trafficking pathway in that double-membrane transport vesicles form *de novo* in the cytoplasm, engulfing and enclosing cytoplasmic components before being transported to the vacuole (Bassham, 2007). After fusion between the outer autophagosome membrane and the tonoplast, the inner membrane-enclosed contents are deposited in the vacuolar lumen and degraded by resident hydrolases. Unlike most trafficking pathways in plants, autophagy is inducible, with a barely detectable level of transport under normal growth conditions, presumably performing housekeeping functions (Inoue et al., 2006; Xiong et al., 2007a), and a massive induction under stress or senescence conditions (Yoshimoto et al., 2004; Contento et al., 2005).

A number of genes have been identified as required for autophagy, primarily in yeast through genetic screens (Tsukada and Ohsumi, 1993; Thumm et al., 1994; Harding et al., 1995). Most of these genes are conserved throughout eukaryotes (Meijer et al., 2007), and can be classified based on their function at different stages of the autophagy process. Proteins have been classified as involved in (1) ATG9 cycling, which may be required for membrane recruitment (2) phosphatidylinositol 3-kinase complex, functioning in formation of PI3P at the autophagosome initiation site and (3) ubiquitin-like conjugation systems, required for autophagosome formation and expansion (Xie and Klionsky, 2007).

Plant genes involved in autophagy have been identified by sequence similarity to known yeast autophagy genes (Bassham et al., 2006; Xie and Klionsky, 2007). Arabidopsis knockout mutants have been characterized for representative genes of each of the functional classes described above. The first knockout mutations in predicted Arabidopsis autophagy components, from classes (1) and (3) above, revealed requirements for autophagy in carbon and nitrogen starvation responses (Doelling et al., 2002; Hanaoka et al., 2002). The mutants also showed early senescence, suggesting, as predicted, a role for autophagy in nutrient recycling both under nutrient deficiency and during the senescence program. Knockouts in additional genes of these functional classes show similar phenotypes (Yoshimoto et al., 2004; Suzuki et al., 2005; Thompson et al., 2005; Xiong et al., 2005). Further phenotypic analyses of these mutants may reveal roles for autophagy in additional physiological processes; a function for autophagy in degradation of oxidized proteins during oxidative stress has already been demonstrated in this manner (Xiong et al., 2007b).

Analysis of an Arabidopsis homolog of a class (2) gene, *ATG6*, has produced very different results. Arabidopsis knockout mutants in *ATG6* are gametophyte lethal, with defects in pollen function that preclude the generation of homozygous knockouts (Fujiki et al., 2007; Qin et al., 2007; Harrison-Lowe and Olsen, 2008). Yeast Atg6 has a dual function in autophagy and vacuolar trafficking (Seaman et al., 1997; Kametaka et al., 1998); the Arabidopsis *atg6* mutant phenotype may therefore be due to defects in vacuolar trafficking rather than an autophagy defect.

It has long been suggested that, in addition to autophagy being a survival response during environmental stress conditions, programmed cell death in plants may also occur via activation of autophagy (Bozhkov and Jansson, 2007). Cell death certainly involves the vacuole, and vacuolar proteases have been implicated in activation of cell death pathways in Arabidopsis (Hatsugai et al., 2004; Rojo et al., 2004; Kuroyanagi et al., 2005). However, most of the characterized Arabidopsis autophagy mutants show no developmental abnormalities and are indistinguishable from wild-type plants under normal conditions, suggesting that at least some types of cell death are independent of the autophagy machinery. Research in tobacco provided intriguing evidence for a role for autophagy in regulation of programmed cell death during the hypersensitive response to pathogen attack. Upon exposure to tobacco mosaic virus, autophagy was induced at the infection site, and also systemically in non-infected leaves. Silencing of a tobacco *ATG6* homolog prevented this induction and also led to unrestricted spread of the cell death to uninfected regions of the leaf (Liu et al., 2005). This suggests that autophagy is required for re-

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stricting programmed cell death, rather than functioning in the cell death process itself.

Recent evidence indicates that this function of autophagy is also conserved in Arabidopsis. To circumvent the lethality of Arabidopsis *atg6* mutants (Fujiki et al., 2007; Qin et al., 2007; Harrison-Lowe and Olsen, 2008), *ATG6* antisense lines with reduced expression were generated. In addition to the typical starvation and senescence phenotypes of autophagy mutants (Doelling et al., 2002; Hanaoka et al., 2002), these plants showed unrestricted cell death upon pathogen infection during both a resistance response and during disease (Patel and Dinesh-Kumar, 2008), consistent with results in the tobacco system. While the precise mechanism behind the regulation of programmed cell death by autophagy remains unknown, this is expected to be a fruitful area of future research.

IV. CONCLUSIONS

In the past few years, Arabidopsis has been a major model system for the study of many aspects of plant growth and development, as well as the intricacies of cell biology. In this chapter, we have described the recent results concerning the operation of the secretory system of Arabidopsis. As noted many times in the text, what we know is dwarfed by what is left to be discovered. This is, of course, a good thing, and continuing research using Arabidopsis as an essential model will help to greatly expand our knowledge – both of the secretory system of plants, but also of all eukaryotes.

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