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REVIEW PAPER

Endomembrane mediated-trafficking of seed storage proteins: from Arabidopsis to cereal crops

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Abstract

Seed storage proteins (SSPs) are of great importance in plant science and agriculture, particularly in cereal crops, due to their nutritional value and their impact on food properties. During seed maturation, massive amounts of SSPs are synthesized and deposited either within protein bodies derived from the endoplasmic reticulum, or into specialized protein storage vacuoles (PSVs). The processing and trafficking of SSPs vary among plant species, tissues, and even developmental stages, as well as being influenced by SSP composition. The different trafficking routes, which affect the amount of SSPs that seeds accumulate and their composition and modifications, rely on a highly dynamic and functionally specialized endomembrane system. Although the general steps in SSP trafficking have been studied in various plants, including cereals, the detailed underlying molecular and regulatory mechanisms are still elusive. In this review, we discuss the main endomembrane routes involved in SSP trafficking to the PSV in Arabidopsis and other eudicots, and compare and contrast the SSP trafficking pathways in major cereal crops, particularly in rice and maize. In addition, we explore the challenges and strategies for analyzing the endomembrane system in cereal crops.

Keywords: Cereal, endomembrane system, protein body, protein sorting, protein storage vacuole, seed storage proteins, trafficking pathways.

Introduction

During seed maturation, flowering plants store their long-term nitrogen reserves mainly in the form of seed storage proteins (SSPs), which are then remobilized during germination to support the growing seedling. Once synthesized in the endoplasmic reticulum (ER), SSPs can either remain inside the ER as protein bodies (PBs), or be exported to specialized protein storage vacuoles (PSVs) (Müntz, 1998). To be able to accommodate such a massive production of different types of SSPs, the endomembrane system in developing seeds undergoes drastic changes to elevate its synthetic and trafficking capacity.

Depending on the species, SSPs accumulate either in the embryo or in the endosperm, a specialized tissue that resulted from a double fertilization event. Arabidopsis embryo cells develop PSVs by remodeling the pre-existing embryonic vacuole during seed maturation (Feeney *et al.*, 2018). Very few endosperm cells remain in the mature Arabidopsis seed and, therefore, they are not a major site for SSP accumulation. In cereals, different types of SSPs are synthesized in embryo and endosperm tissues, and their subcellular storage compartments also differ among cell types. For example, the SSPs in the epidermal layer (aleurone) of the maize and sorghum endosperms are mainly stored in PSVs, whereas the SSPs in the inner endosperm (starchy endosperm) accumulate exclusively in PBs. In contrast, rice SSPs predominantly accumulate in the subaleurone layer (1–2 layers underneath the aleurone) in both PBs and PSVs, also referred to as type-1 PB (PB-I) and PB-II, respectively (Fig. 1). Embryo cells in the cereal grains form exclusively PSVs.

At least three major SSP trafficking pathways to the PSVs have been identified in flowering plants (see Fig. 2; Vitale and Hinz, 2005; Xiang *et al.*, 2013; Ashnest and Gendall, 2018). All SSPs bear an N-terminal signal peptide that mediates their translocation into the ER lumen during protein synthesis. Once vacuolar SSPs are synthesized, folded, and assembled inside of the ER lumen, they are most commonly transported to the Golgi, to the *trans*-Golgi network (TGN), and to prevacuolar compartments (PVCs)/multivesicular endosomes (MVEs) that fuse with PSVs, releasing their content into the vacuolar lumen. Some proteins are packed into ER-derived precursor-accumulating (PAC) vesicles and delivered directly to PSVs in a Golgi-independent manner (Hara-Nishimura *et al.*, 1998). The third pathway delivers portion of the ER containing SSP aggregates to the vacuole through a poorly characterized autophagic mechanism (Reyes *et al.*, 2011).

Besides serving as a major repository of nitrogen for plants, SSPs in cereal crops represent a major protein source in the human diet, as they provide nearly 50% of the global food protein (faostat.fao.org). Moreover, the capacity to accumulate large amounts of proteins makes cereal grains an attractive platform for the production of recombinant proteins of pharmaceutical or industrial value. In this review, we discuss the main endomembrane routes involved in SSP trafficking to the PSV, highlighting studies performed in Arabidopsis and in major cereal crops, particularly in rice (*Oryza sativa*) and maize (*Zea mays*). We also explore and contrast the known regulatory factors that control SSP trafficking pathways. Finally, we discuss challenges and strategies in the analysis of endomembrane trafficking pathways in cereal crops.

Seed storage proteins in flowering plants

Based on their solubility in various solvents, SSPs have been traditionally classified as albumins (soluble in water), globulins (soluble in saline solution), glutelins (soluble in diluted acids or alkali), and prolamins (soluble in water/alcohol mixtures) (Osborne, 1924). The 2S albumins are widely present in dicotyledonous plants and have been extensively studied in the model plant Arabidopsis, whereas prolamins and glutelins are only present in grasses, including major cereals.

Globulins are widely present in flowering plants and even in some fern spores (Templeman *et al.*, 1987). They can be further divided into two subgroups: the trimeric and predominantly glycosylated 7S vicilin-type globulins and the hexameric and mostly non-glycosylated 11–12S legumin-type globulins.

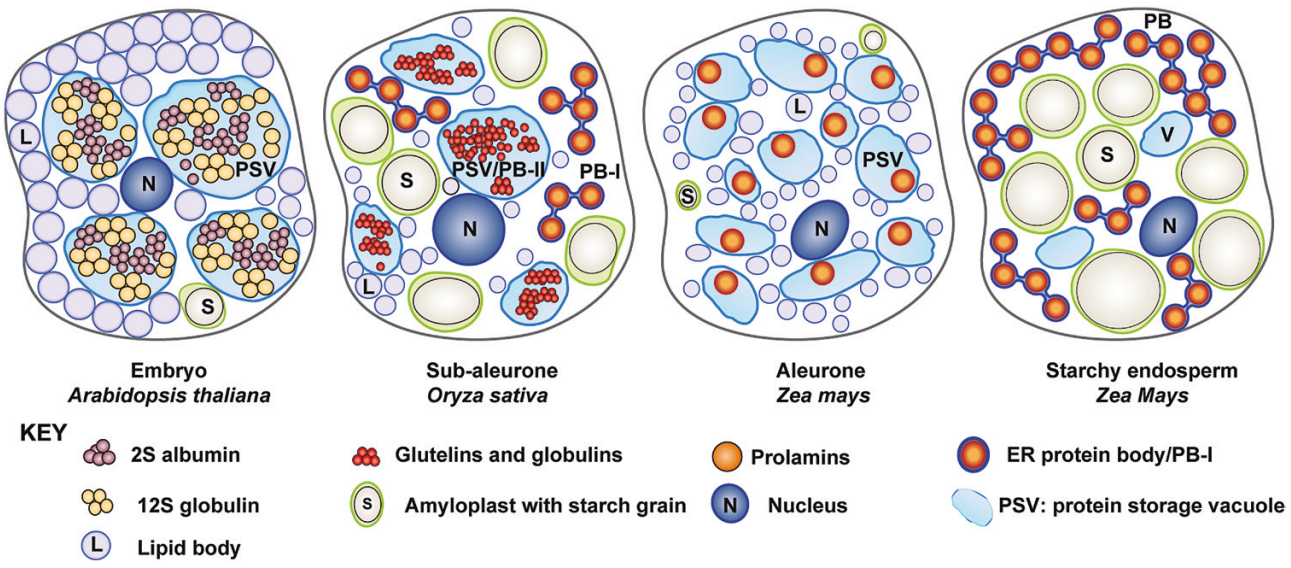

Fig. 1. Schematic comparison of the seed storage organelles found in Arabidopsis embryonic tissues, rice subaleurone layer, and maize aleurone and starchy endosperm tissues.

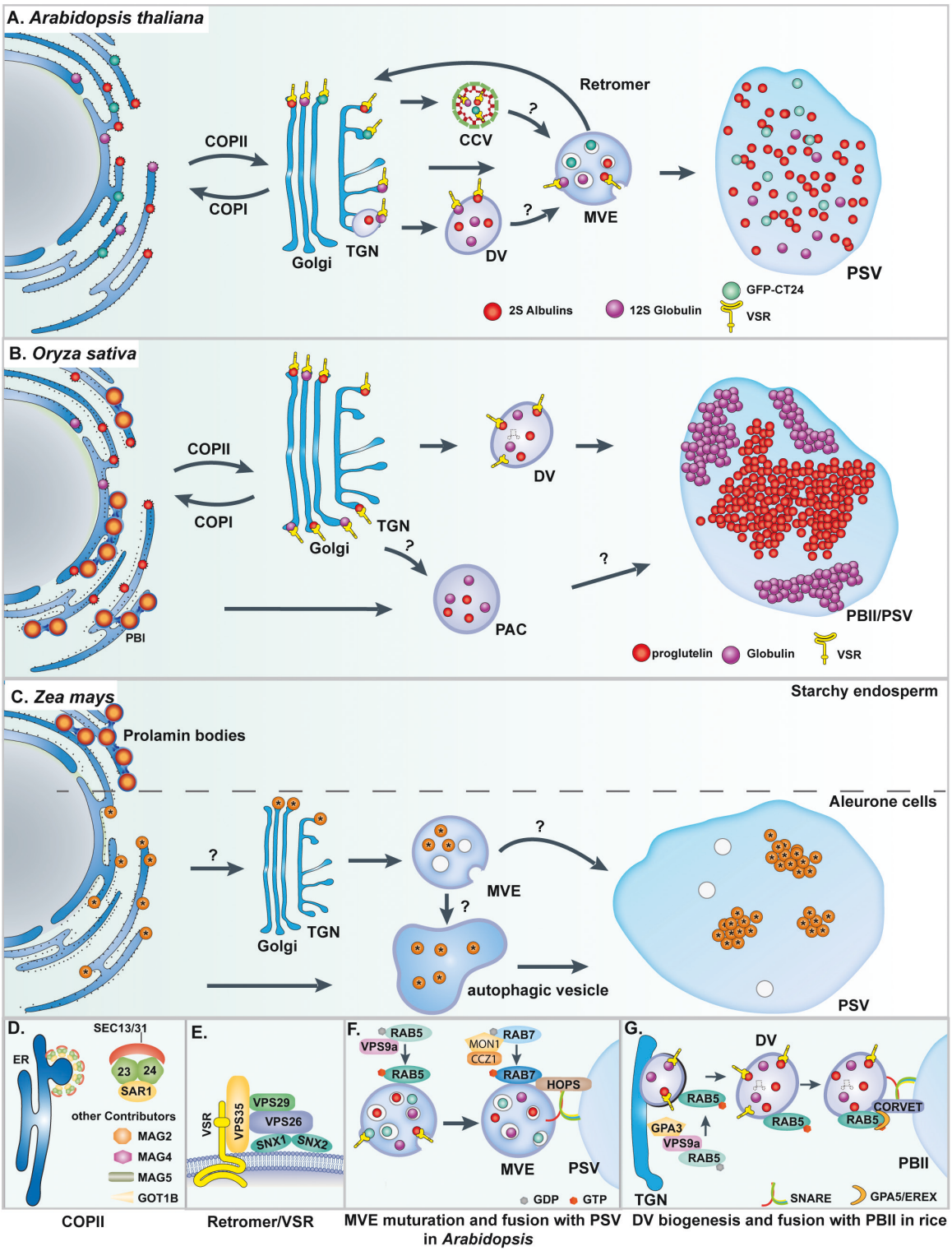

Fig. 2. Model depicting the sorting and trafficking of storage proteins in Arabidopsis, rice, and maize. In Arabidopsis (A), precursors of 2S albumins and 12S globulins are transported to the protein storage vacuole (PSV) via several Golgi-dependent routes. During Golgi-dependent trafficking, storage proteins are firstly transported from the ER to the Golgi via COPII vesicles (D), and then targeted to the PSV via clathrin-coated vesicles (CCVs) or transported via dense vesicles (DVs). Vacuolar sorting receptors (VSRs) recognize and bind storage proteins at early endomembrane compartments to promote their trafficking, and dissociate at late endomembrane compartments and recycle back by retromer (E) for a new round of sorting. The maturation of the multivesicular endosome (MVE) and its fusion with the PSV is regulated by a Rab5 and Rab7 cascade (F). In rice (B), prolamins directly aggregate inside the ER lumen to form type I protein bodies (PB-I), whereas glutelins are targeted to the type II protein bodies (PB-II) or PSVs through a DV-mediated pathway, which is regulated by a Rab5 cascade (G). In developing maize endosperm (C), prolamins in the ER lumen gradually deposit to form PBs, and an ATG8-independent autophagy route was found to be involved in the trafficking of zeins in aleurone cells.

In Arabidopsis and other crucifers, the predominant SSPs are the 2S albumins and 12S globulins (Heath *et al.*, 1986). Interestingly, Arabidopsis mutants lacking either of these canonical major SSPs show no significant differences in seed size, seed germination, or seedling vigor compared with the wild type (Withana-Gamage *et al.*, 2013), indicating that, at least in some conditions, either SSP can act as the energy source for germination.

Prolamins represent a large superfamily of related SSPs with different amino acid composition and molecular weight. In rice, prolamins can be further classified into three subgroups (10, 13, and 16 kDa prolamins) based on their composition and molecular weights (Shewry *et al.*, 1995). Prolamins from maize (zeins) and related panicoid cereals such as sorghum (*Sorghum bicolor*) and pearl millet (*Pennisetum glaucum*) are usually classified in four groups (α, β, γ, and δ) (Shewry *et al.*, 1995). Although zeins represent 50% of the total seed protein content, they are deficient in several essential amino acids, such as lysine, tryptophan, and methionine, which reduce their nutritional value.

Glutelins are related to the 11–12S globulins and represent a major component of the protein mixture called gluten, commonly used in the making of bread and malts. Glutelins, just like prolamins, accumulate in the endosperm but not in the embryo of cereal grains. The rice grain contains glutelins (60–80%) and prolamins (20-30%) as the most abundant storage proteins (Kawakatsu *et al.*, 2010). In the maize kernel, >60% of the total SSP in the endosperm consist of zeins, whereas 10–20% of the total proteins in the embryo are globulins-1 and -2 (Larkins *et al.*, 2017).

SSPs are also different in their sorting, post-translational processing, and trafficking pathways. Prolamins in the cereal starchy endosperm directly aggregate inside the ER lumen as protein granules that eventually develop into PBs (also known as PB-I in rice) of 1–3 μm in diameter (Larkins and Hurkman, 1978; Krishnan *et al.*, 1986). Prolamin synthesis and accumulation are restricted to specialized ER subdomains (Li *et al.*, 1993; Choi *et al.*, 2000). In the maize starchy endosperm, PBs consist of a core region containing a 19 kDa and 22 kDa α-zein-rich middle layer, and a γ-zein-rich outermost layer (Holding, 2014). The composition and segregation of prolamins within maize PBs are important not only for the stable accumulation of PBs but also for the texture of the endosperm and, therefore, for grain yield and quality (Holding, 2014). The prolamin PB-Is in rice endosperm also are spherical, with a concentric arrangement of proteins and the 10 kDa prolamin at the core surrounded by layers of 13b, 13a, and 16 kDa prolamins (Saito *et al.*, 2012).

Unlike prolamins, the other three groups of SSPs are initially synthesized as long and sometimes oligomeric precursors at the ER and sequentially undergo post-translational cleavage by vacuolar proteases at PSVs or while en route to the PSVs (Gruis et al., 2002, 2004; Otegui *et al.*, 2006; Wang *et al.*, 2009; Mylne *et al.*, 2014). For example, the Arabidopsis 12S globulin precursors are first synthesized and translocated into the ER lumen where disulfide bonds are formed between the α- and β-chains (Chrispeels *et al.*, 1982; Mylne *et al.*, 2014). After reaching MVEs via an ER–Golgi–TGN pathway, 12S globulin precursors are proteolytically processed by vacuolar processing enzymes (VPEs) to promote the formation of intra- and inter-chain disulfide bonds and the subsequent formation of 12S hexamer or octamer complexes (Adachi *et al.*, 2003; Gruis *et al.*, 2004; Otegui *et al.*, 2006). Proteolytic processing of Arabidopsis 2S albumins is more extensive and more complex, and is initiated at the MVEs before reaching the PSV. The 2S albumin precursors undergo four rounds of cleavage events that result in two disulfide-linked mature polypeptides (Mylne *et al.*, 2014). Like Arabidopsis 2S albumins and 12S globulins, rice glutelins are also initially synthesized as 57 kDa precursors at the ER (Yamagata *et al.*, 1982) and sequentially delivered to the PSVs through the Golgi and TGN (Krishnan *et al.*, 1986; Yamagata *et al.*, 1986). Proglutelins are proteolytically processed by VPEs to form irregular PB-II/PSVs that also store globulins (Yamagata *et al.*, 1982; Krishnan *et al.*, 1992). Thus, the trafficking pathways controlling SSP deposition varies substantially depending on plant species, cell types, and the biophysical properties of the SSPs.

Golgi-independent routes to the protein storage vacuole

The classical route (ER–Golgi–TGN–MVE), described above for Arabidopsis 2S albumins and 12S globulins, is generally considered as the most prevalent pathway for delivering SSPs to the PSVs in seed plants. However, other alternative routes have been reported to deliver SPPs from the ER to the PSV bypassing the Golgi (as summarized in Fig. 2). Basically, the routes rely on the budding of ER vesicles that carry SSPs to the vacuole directly or on different autophagy modalities.

Precursor-accumulating (PAC) vesicle

PAC vesicles were first discovered in the developing cotyledons of pumpkin (*Cucurbita maxima*) and the endosperm of castor bean (*Ricinus communis*) (Hara-Nishimura *et al.*, 1998), and later in other plant species, such as soybean (*Glycine max*) (Mori *et al.*, 2004) and rice (Takahashi *et al.*, 2005). PAC vesicles contain densely packed aggregates of storage proteins, such as albumins, globulins, and glutelins, together with ER chaperones (Levanony *et al.*, 1992; Hara-Nishimura *et al.*, 1998; Takahashi *et al.*, 2005). They are 200–400 nm (up to 1 μm in rice) in diameter, and frequently observed in close proximity to the rough ER, sometimes surrounded by ribosomes. For these reasons, PAC vesicles are thought to derive from the ER and transport SSPs directly to the PSVs. The presence of Golgi-derived glycosylated vacuolar sorting receptors (VSRs) in PAC vesicles, however, suggests that PAC vesicles may not

carry SSPs solely from the ER. For example, PV72, a VSR in pumpkin seeds, recruits 2S albumin precursors and is present in PAC vesicles (Shimada *et al.*, 2002). Moreover, PV72 molecules isolated from PAC vesicles contain Golgi-processed, complex oligosaccharides, indicating that PAC vesicles may recruit at least a small proportion of 2S albumin precursors at the Golgi apparatus (Vitale and Hinz, 2005). Therefore, the origin and nature of PAC vesicles and the contribution(s) of the Golgi in this pathway still needs to be investigated.

The mechanisms mediating SSP aggregation inside the ER and sequestration into PAC vesicles are still unclear. We do know, though, that the quality and quantity of SSP cargo proteins influence their propensity to aggregation. For example, developing soybean cotyledons containing higher amount of glycinin (11S globulin) tend to form PAC-like vesicles more frequently than wild-type cotyledons (Mori *et al.*, 2004). There is abundant evidence that the prevalent SSP transport route is determined not only by the SSP itself but also by other factors specific to developmental stages, cell types, and species. For example, wheat (*Triticum aestivum*) prolamins generally follow conventional Golgi-dependent traffic to the PSVs at early stages of seed development, while they aggregate inside the ER and are prominently transported to the PSVs by PAC vesicles at later stages (Shy *et al.*, 2001; Tosi *et al.*, 2009). The identity of the machinery involved in the formation, budding, and fusion of PAC vesicles is as yet unknown, but it has been suggested that PAC vesicles may be engulfed by PSVs through an autophagy-like process (see below) or fuse with PSVs directly (Hara-Nishimura *et al.*, 1998; Robinson *et al.*, 2005).

Autophagy

Autophagy (meaning self-eating) is a highly conserved catabolic pathway in eukaryotes, by which unwanted or dysfunctional cytoplasmic materials are degraded by the vacuole/lysosome to maintain cellular homeostasis (Yang and Bassham, 2015; Masclaux-Daubresse *et al.*, 2017; Marshall and Vierstra, 2018). Thus far, studies with Arabidopsis and other plant species have identified three distinct types of autophagy: micro-, macro-, and mega-autophagy (Marshall and Vierstra, 2018). In microautophagy, cytoplasmic materials are engulfed directly by invaginations of the vacuolar membrane. During macroautophagy, cargo becomes sequestered into a double-membrane organelle called an autophagosome, which subsequently fuses to the vacuole releasing its internal cargo-containing core also called an autophagic body. When these two autophagic pathways converge to degradation and recycling, the engulfed cargoes are degraded by a set of vacuolar hydrolases so breakdown products can be then exported back to the cytoplasm for nutrient recycling (Marshall and Vierstra, 2018). The third type of autophagy in plant cells, mega-autophagy, represents an extreme form of massive degradation at the end of programmed cell death (PCD) that occurs during plant development or during biotic stress [e.g. the hypersensitive response (HR)]. During mega-autophagy, the vacuolar hydrolases are released into the cytoplasm through permeabilization or rupture of the tonoplast, where they break cytoplasmic material directly (van Doorn and Papini, 2013).

Besides its role in degradation and recycling, autophagy seems to play a role in the deposition of other metabolites such as anthocyanins (Chanoca *et al.*, 2015) and SSPs. The first indication of such a role came from a study in developing wheat endosperm (Levanony *et al.*, 1992). Wheat prolamins aggregate inside the ER in small PBs that are then sequestered into PSVs through a process reminiscent of microautophagy (Levanony *et al.*, 1992). A later study in developing maize endosperm employing electron tomography showed that whereas maize prolamins (zeins) accumulate in ER PBs in both aleurone and starchy endosperm cells, PBs in aleurone cells are delivered to the PSV through a autophagy-like mechanism (Reyes *et al.*, 2011), possibly related to microautophagy, that does not depend on canonical autophagy factors such as ATG12 (Li *et al.*, 2015). Thus, whereas starchy endosperm cells accumulate large quantities of prolamins in PBs, aleurone cells only form prolamin-containing PBs transiently and then sequester them into PSVs. This is also the case when maize zeins are expressed in transgenic tobacco (*Nicotiana tabacum*) seeds, which do not naturally accumulate prolamins. The tobacco plants form ER-derived PBs containing zeins but they are ultimately delivered to PSVs (Coleman *et al.*, 1996), presumably by a microautophagic mechanism similar to that reported in the wheat endosperm cells. Interestingly, similar microautophagic mechanisms seem to mediate the vacuolar sequestration of some specialized metabolites, such as rubber (Backhaus and Walsh, 1983) and anthocyanins (Chanoca *et al.*, 2015). These observations have led to the interesting speculation that microautophagy may be a highly efficient method for vacuolar deposition of various bulky compounds, including PBs.

Whereas several lines of evidence support a role for microautophagy in the vacuolar deposition of SSPs, the participation of macroautophagy in SSP trafficking is less clear. Arabidopsis mutants deficient in macroautophagy (e.g. *atg5*) accumulate fewer storage proteins, more specifically mature forms of 12S globulins, than the wild type. This reduction seems to be due, at least in part, to the incomplete processing of the 12S globulin precursors (Di Berardino *et al.*, 2018). The proteolytic processing of the Arabidopsis SSPs has been documented to start in MVEs and to be completed inside PSVs (Otegui *et al.*, 2006), thus how exactly macroautophagy controls SSP processing is unclear. However, many mutants affected in different aspects of endomembrane trafficking result in the abnormal mis-sorting of SSP precursors (see below), and thus the abnormal accumulation of SSP precursors in macroautophagy mutants could be due to a general disruption in endomembrane fluxes.

Golgi-dependent routes to the protein storage vacuole

Vacuolar protein sorting machine: determinants and receptors

The default location for ER-synthesized proteins is released to the cell exterior through exocytosis. For vacuolar delivery, most SSPs are recognized by membrane-bound sorting receptors in the early secretory pathway, directed to the Golgi, TGN, and MVEs, and ultimately reach the vacuole. Such receptor-mediated sorting depends on signals in the cargo amino acid sequences known as vacuolar sorting determinants (VSDs). Three different types of VSDs have been described in plants; sequence-specific VSDs, C-terminal VSDs, and physical and structural VSDs.

The sequence-specific VSDs were first identified in barley (*Hordeum vulgare*) aleurain and sweet potato (*Dioscorea esculenta*) sporamin as sorting signals for lytic vacuoles (Holwerda *et al.*, 1992; Nakamura *et al.*, 1993). They contain the core signal sequence Asn–Pro–Ile–Arg (NPIR) or a similar motif. Similar signal sequences were also later described in SSPs targeted to PSVs, such as the castor bean storage protein ricin and 2S albumins (Frigerio *et al.*, 2001; Brown *et al.*, 2003).

The C-terminal VSDs are normally comprised of 8–20 highly hydrophobic amino acids (Pereira *et al.*, 2013) and were first discovered in barley lectin and tobacco chitinase (Bednarek and Raikhel, 1991; Neuhaus *et al.*, 1991). Similar to the sequence-specific VSDs, the C-terminal VSDs are sufficient to target a cargo protein to the vacuole. As their name implies, the C-terminal VSDs must be placed at the C-terminus of a cargo protein to function as sorting signals, whereas the sequence-specific VSDs can function independently of their position within the protein (Koide *et al.*, 1997). C-terminal VSDs have been reported for many SSPs, including the 7S globulin β-conglycinin α-subunit from soybean (Nishizawa *et al.*, 2003), the 11S globulin amaranthin from *Amaranthus*, and the rice glutelin β-subunit (W. Li *et al.*, 2013). Although the sorting mechanism of the Arabidopsis 2S albumins has not been analyzed in detail, they bear hydrophobic C-terminal sequences similar to those of soybean β-conglycinins, and may function as C-terminal VSDs (Fuji *et al.*, 2007).

The physical and structural VSDs are not well conserved at the amino acid sequence level and often consist of multiple internal sorting determinants within the storage protein, which probably form higher structures to promote sorting (Saalbach *et al.*, 1991; Hegedus *et al.*, 2015). For example, Arabidopsis 12S globulins contain both a C-terminal VSD plus internal sequences needed for vacuolar sorting (Shimada *et al.*, 2003; Hegedus *et al.*, 2015). Thus, the Arabidopsis 12S globulins were first shown to interact with VSR1 through their C-terminal region in a Ca^{2+}-dependent manner (Shimada *et al.*, 2003), while a more recent study revealed the presence of multiple internal sorting determinants within the 12S globulin AtCruA that are required for its vacuolar deposition (Hegedus *et al.*, 2015). These internal sorting sequences are hydrophobic with a Leu or Ile residue at the core and usually within or adjacent to a surface-exposed hydrophilic loop (Hegedus *et al.*, 2015).

Thus far, two different types of vacuolar receptors have been described in plant cells: the BP-80/VSR family and the receptor homology domain–transmembrane sequence–RING-H2 (RMR) protein family. The BP-80/VSR family are type I integral membrane proteins, with a large lumenal ligand-binding domain, a single transmembrane domain, and a short cytosolic C-terminal tail (Robinson and Neuhaus, 2016). The lumenal region, which recognizes and binds the VSD in cargo proteins, consists of an N-terminal protease-associated (PA) domain, a central domain, and three epidermal growth factor repeats. The PA domain has been shown to bind the residues preceding the NPIR motif of barley aleurain, favoring the binding of the NPIR motif to the central domain (Luo *et al.*, 2014). The cytosolic C-terminal domain of VSRs contains a Yxxϕ (where x is any amino acid and ϕ is a hydrophobic amino acid) motif that interacts with a clathrin adaptor complex (see below) and mediates their incorporation into vesicles (Gershlick *et al.*, 2014). Although first isolated as sorting receptors for lytic vacuolar cargo, growing biochemical and genetic evidence later revealed their roles in SSP trafficking to the PSV (Kirsch *et al.*, 1994; Robinson and Neuhaus, 2016). VSRs can recognize the VSDs of several SSPs from different dicotyledonous species, including 2S albumins of castor bean and pumpkin (Cao *et al.*, 2000; Shimada *et al.*, 2002; Brown *et al.*, 2003), 7S β-conglycinins of soybean (Fuji *et al.*, 2007), and 12S globulin of Arabidopsis (Shimada *et al.*, 2003).

The VSR family in *Arabidopsis thaliana* is encoded by seven genes (*VSR1*–*VSR7*) that show some level of functional specialization (Shimada *et al.*, 2003; Zouhar *et al.*, 2010). Genetic studies have shown that a significant proportion of 2S albumin and 12S globulin precursors are mis-sorted to the apoplast in the *vsr1* mutant, but not in the *vsr2*-7 mutant (Shimada *et al.*, 2003; Delgadillo *et al.*, 2020), indicating that AtVSR1 is the major player responsible for proper sorting of the SSPs to the PSVs in developing seeds. In addition, processing defects in the *vsr1vsr3* and *vsr1vsr4* double mutants are more severe for 2S precursors than for 12S precursors, suggesting specialization in cargo selection within the VSR family (Zouhar *et al.*, 2010).

RMR proteins were originally identified based on the sequence similarity to the PA domain of VSRs, suggesting a role as sorting receptors (Cao *et al.*, 2000; Wang *et al.*, 2011). Although molecularly and functionally distinct, RMRs have a domain structure similar to that of VSRs, as they consist of a lumenal ligand-binding domain, a single transmembrane domain, and a long cytoplasmic tail containing a typical C3H2C3 RING-H2 domain (Wang *et al.*, 2011). Genome analysis showed that RMR proteins generally are encoded by a small gene family in higher plants, with five members in Arabidopsis (AtRMR1–AtRMR5, Park *et al.*, 2005) and two in rice (Shen *et al.*, 2011).

The exact role of RMRs in the vacuolar delivery of SSPs is controversial. Immunofluorescence and immunoelectron microscopic studies showed that the AtRMR1 receptor mainly localizes to the PSVs of developing embryos (Park *et al.*, 2005), whereas AtRMR2 was detected not only in the PSV, but also in the Golgi apparatus and storage protein-loaded dense vesicles, coinciding with the distribution of the 12S globulin cruciferin (Hinz *et al.*, 2007). OsRMR1 was also found in the PB-II/PSVs of the developing rice endosperm, as well as in the Golgi apparatus, TGN, and MVEs (Shen *et al.*, 2011). In addition, *in vitro* binding assays showed that Arabidopsis RMR proteins are able to interact with the C-terminal VSDs of several PSV-resident proteins, including barley lectin, bean phaseolin, and tobacco chitinase (Park et al., 2005, 2007). Moreover, when co-expressed with AtRMR1 deletion mutants lacking either the luminal domain or the cytosolic tail, bean phaseolin partially failed to reach the vacuoles of Arabidopsis leaf protoplasts (Park *et al.*, 2005). These observations suggested a role for RMRs as SSP receptors in vacuolar sorting. However, the genetic studies by Zouhar *et al.* (2010) showed that the processing of 2S or 12S precursors and the morphology of PSVs were not affected in either *rmr* single or double mutants, indicating that RMR receptors in Arabidopsis either function redundantly with VSRs or do not have significant roles in the SSP trafficking. Further investigation of the SSP processing phenotype and PSV dynamics of mutants eliminating all *VSR* or *RMR* genes, or combination of *vsr rmr* mutants through CRISPR/Cas9 genome editing, would be useful to address the question.

ER and Golgi trafficking

ER-synthesized proteins are transported to the Golgi apparatus via a coat protein complex II (COPII)-dependent route, whereas ER-resident proteins are retrieved from the Golgi back to the ER via COPI vesicles. COPII consists of five components: secretion-associated RAS-related 1 (Sar1), Sec23, Sec24, Sec13, and Sec31 (Fig. 2D; Brandizzi, 2018). Sar1 is a small GTPase responsible for switching on and off the assembly of COPII coats, while the heterodimers Sec23/24 and Sec13/31 form the membrane-bound inner layer and the cage-like outer layer of the COPII coat, respectively, to promote COPII vesicle budding (Barlowe *et al.*, 1994). The participation of Sar1 in ER export of SSPs has been experimentally shown in rice, where the simultaneous suppression of three of the four rice *Sar1* genes (*OsSar1a/b/c*) impaired the export of glutelin precursors and α-globulin from the ER, leading to their accumulation in abnormal ER PBs (Tian *et al.*, 2013).

Critical contributions to elucidating the role of the ER to Golgi transport in SSP trafficking came from the analysis of the *maigo* (*mag*, means a stray or lost child in Japanese) mutants in Arabidopsis, which are deficient for accessory factors involved in vesicle budding, tethering, and fusion (Li *et al.*, 2006; Takahashi *et al.*, 2010; Takagi *et al.*, 2013). In three of these *maigo* mutants, *mag2*, *mag4*, and *mag5*, SSP precursors overaccumulate in electron-dense, ER-derived structures, instead of being released to the extracellular space like in other mutants defective in later steps of the conventional secretory pathway (Li *et al.*, 2006; Takahashi *et al.*, 2010; Takagi *et al.*, 2013). MAG2 interacts with ER-localized SNAREs and with MIP1–MIP3 (MAIGO2-interacting proteins 1–3) to form an ER-localized tethering complex, similar to the yeast Dsl1p complex, which is required for retrograde transport from the Golgi apparatus to the ER (L. Li *et al.*, 2006, 2013). *MAG4* encodes a *cis*-Golgi-localized tethering factor, with similarities to the animal bovine vesicular transport factor p115 and yeast Vso1p required for the anterograde transport from the ER to the Golgi (Takahashi *et al.*, 2010). MAG5, an ortholog of yeast and human Sec16, was recently shown to mediate protein export from the ER through controlling COPII coat turnover (Takagi *et al.*, 2013).

Another important regulator of COPII vesicle assembly required for SSP export was characterized in rice. GPA4 (glutelin precursor accumulation 4) is a conserved membrane protein homologous to the yeast Golgi transport 1B (GOT1B), also known as GLUP2 (Fukuda *et al.*, 2016; Y. Wang *et al.*, 2016). In rice *gpa4/glup2/got1b* mutants, the subcellular distribution of Sar1 is drastically altered, and the ER export of glutelin precursors is repressed. Rice GPA4 interacts with the COPII component Sec23 and is required for the proper assembly of the COPII vesicles and, therefore, export of SSPs from the ER (Y. Wang *et al.*, 2016).

Given that the ER–Golgi anterograde transport represents the first step of SSP trafficking along the secretory pathway, it was assumed that VSRs engage their SSP cargo at the ER and release them in later compartments, such as the TGN or MVEs. However, where exactly VSRs bind their cargo proteins has remained controversial (Niemes *et al.*, 2010a). The expression of ER-retained VSR mutant proteins results in the accumulation of soluble vacuolar proteins in the ER, suggesting that VSR can bind to their cargoes at the ER or *cis*-Golgi (daSilva *et al.*, 2004; Watanabe *et al.*, 2004; Niemes *et al.*, 2010a). However, more recent studies by Gershlick *et al.* (2014) and Früholz *et al.* (2018) showed that VSR–ligand binding seems to occur at the *cis*-Golgi, leaving the open question of how SSPs are sorted for export at the ER.

Receptor recycling

SSPs move through the Golgi bound to their VSRs but, before reaching the PSV, at least some of the receptors disengage from the cargo and are recycled for new rounds of cargo binding and sorting. The VSRs have been shown to be recycled by the retromer, an evolutionarily conserved complex involved in retrograde transport and cargo/receptor recycling (Oliviusson *et al.*, 2006; Yamazaki *et al.*, 2008; Niemes *et al.*, 2010b; Kang *et al.*, 2012). It consists of a 'core' complex which recognizes the VSR cytoplasmic tails, and the peripheral sorting nexins, which sense and/or induce membrane curvature to facilitate

vesicle formation (Fig. 2F; and see review by Heucken and Ivanov, 2018). In Arabidopsis, the retromer is composed of three core proteins: VPS26 (VPS26A and VPS26B), VPS29, and VPS35 (VPS35A, VPS35B, and VPS35C). Additionally, three nexin-encoding genes have been identified in the Arabidopsis genome (*AtSNX1*, *AtSNX2a*, and *AtSNX2b*) (Jaillais *et al.*, 2006; Shimada *et al.*, 2006; Yamazaki *et al.*, 2008).

The retromer complex is required for the normal transport of SSPs to the PSVs, as abnormal SSP processing and trafficking are often found in Arabidopsis retromer mutants at the late stage of seed development. For example, the *vps29* and *vps35a vps35b* mutants deliver 2S and 12S precursors to the apoplast instead of the PSV. This leads to the accumulation of SSP precursors and the formation of smaller PSVs in embryos (Shimada *et al.*, 2006; Yamazaki *et al.*, 2008; Durand *et al.*, 2019). Accumulation of SSP precursors in mature seeds has also been reported in the mutants for *VPS26* and the sorting nexins, *SNX1* and *SNX2* (Yamazaki *et al.*, 2008; Pourcher *et al.*, 2010; Zelazny *et al.*, 2013), suggesting that all retromer components are required for vacuolar trafficking of SSPs. However, multiple genetic evidence suggests functional diversification of some of the retromer subunit isoforms in Arabidopsis. For example, *vps35a* single mutants deliver SSP to the PSVs, but fail to traffic plasma membrane proteins such as the auxin efflux carrier PIN1 (Nodzynski *et al.*, 2013), whereas VSP35b and VPS35c are required for SSP transport in developing embryos (Yamazaki *et al.*, 2008). Furthermore, the *snx1 snx2a snx2b* triple mutant is partially impaired in the processing of 12S globulin but not the 2S albumin precursors (Pourcher *et al.*, 2010), whereas the processing of both proteins is abnormal in the *vps29* mutant (Shimada *et al.*, 2006).

A conserved role for the retromer in SSP trafficking of cereal crops has yet to be demonstrated. Positional cloning of a maize quantitative trait locus (QTL) affecting kernel morphology revealed that *ZmVPS29* plays an important role in kernel shape and development. Overexpression of *ZmVPS29* resulted in a slender kernel morphology, probably by regulating kernel auxin accumulation (Chen *et al.*, 2019). However, whether SSP deposition is affected by ZmVPS29 overexpression has not been determined.

Adaptor proteins mediate receptor trafficking

Once VSRs bind cargo proteins via their luminal domain, the receptor–ligand complex is sorted by an adaptor protein (AP) complex, which recognizes the cytosolic domain of VSRs. The AP complexes are evolutionarily conserved machineries that are responsible for the formation of transport vesicles and cargo sorting in all eukaryotes (Robinson, 2015; Mettlen *et al.*, 2018). Thus far, five types of AP complexes have been identified in eukaryotic cells, AP-1 to AP-5 (Hirst *et al.*, 2011). Each AP complex consists of two large subunits (β1–β5 and one each of γ/α/δ/ε/ζ, respectively), one medium subunit (μ1–μ5), and one small subunit (σ1–σ5). Although they all seem to be involved in cargo sorting, these AP complexes operate at distinct subcellular localizations or even in different subdomains of the same organelle. For example, both AP-1 and AP-4 localize to the TGN, but AP-1 strongly interacts with clathrin (Hirst *et al.*, 2012; Park *et al.*, 2013) and co-localizes with the SNARE protein VAMP (vesicle-associated membrane protein)-721, while AP-4 only interacts with clathrin weakly and co-localizes with VAMP727 (Shimizu *et al.*, 2021). Experimental evidence supports that whereas AP-1/VAMP721 are involved in clathrin-dependent exocytosis/secretion (Zhang *et al.*, 2011; Park *et al.*, 2013; Shimada *et al.*, 2018a), AP-4/VAMP727 participates in vacuolar trafficking, including transport of SSPs. Gershlick *et al.* (2014) reported that the μ subunits of AP-1 and AP-4 complexes are able to interact with the cytosolic tail of AtVSR1 in a yeast two-hybrid assay. Moreover, Fuji *et al.* (2016) uncovered that mutations in genes coding for three subunits of the AP-4 complex (AP4β, μ, and σ) led to accumulation of VSR1 and abnormal trafficking of 12S globulin precursors, but not 2S albumin precursors, indicating once again that the 2S and 12S SSPs may be targeted to the PSV via different sorting mechanisms.

Dense vesicles

The molecular identity of the vesicles that carry storage proteins from the Golgi/TGN to the MVEs for vacuolar delivery is still unclear. Dense vesicles containing an electron-dense core and no discernible coat were first discovered by TEM in common bean (*Phaseolus vulgaris*) (Chrispeels, 1983), and have subsequently been identified in rice (Krishnan *et al.*, 1986), wheat (Kim *et al.*, 1988), faba bean (*Vicia faba* L.) (Hohl *et al.*, 1996), pea (*Pisum sativum*) (Hinz *et al.*, 1999), and Arabidopsis (Otegui *et al.*, 2006; Hinz *et al.*, 2007). In the pea and Arabidopsis cotyledon cells, dense vesicle buds were identified in peripheral bulges of the *cis*-Golgi using immunogold electron microscopy (Hinz et al., 1999, 2007; Otegui *et al.*, 2006). The SSP precursors accumulate within these buds and progressively form electron-dense protein aggregates. As Golgi cisternae mature, dense vesicles ultimately reach the TGN where they bud off (Robinson and Hinz, 2007). Both dense vesicles and PAC vesicles share some common structural characteristics: both have an electron-dense core consisting of SSPs and no discernible coats; however, dense vesicles are slightly smaller than PAC vesicles (150–200 nm in diameter) and bud from the TGN instead of the ER (Shimada *et al.*, 2018b).

There are many open questions regarding the formation, sorting, budding, and fate of dense vesicles. For example, although VSRs have been identified in dense vesicles, their exact function and nature have been controversial. They might facilitate protein aggregation or recover 'escaped' protein for delivery to the PSVs. In addition, some reports indicated that BP-80/VSRs were enriched in clathrin-coated vesicles and excluded from dense vesicles, which instead contain RMRs (Hillmer *et al.*, 2001; Hinz et al., 1999, 2007).

A study by Otegui *et al.* (2006), however, found that dense vesicles in Arabidopsis embryos carry both SSP and VSRs, which is consistent with the later reports on AP-4 acting on clathrin-free domains of the TGN (Shimizu *et al.*, 2021), and interacting with the VSR for the vacuolar delivery of SSPs. However, how dense vesicles are connected with VSR and AP-4 sorting is presently unclear. Finally, in Arabidopsis, dense vesicles deliver their SSP-enriched cores to MVEs by fusion (Otegui *et al.*, 2006), whereas in rice, dense vesicles carrying glutelins seem to fuse directly with the PSVs (Ren *et al.*, 2020).

Factors involved in targeting, tethering, and fusion of trafficking vesicles to protein storage vacuoles

Rab GTPases

Post-TGN trafficking of SSP-carrying vesicles involves a complex set of proteins to regulate targeting, docking, and fusion with acceptor compartments (MVEs or PSVs, see Fig. 2F, G). RAB GTPases are key regulators of membrane trafficking that act as a molecular switch cycling between active (GTP-bound) and inactive states (GDP-bound). RAB5 and RAB7 are two evolutionarily conserved subgroups of Rab GTPase that act in early and late endosomal trafficking in mammalian cells, respectively (Langemeyer *et al.*, 2018). The plant counterparts of RAB5 and RAB7 localize to late/multivesicular endosomes and vacuoles, respectively, and are involved in SSP trafficking in Arabidopsis and rice (Minamino and Ueda, 2019). There are three RAB5 GTPases in Arabidopsis, two canonical-type ones (ARA7 and RHA1), and a plant-specific isoform, ARA6 (Ebine *et al.*, 2011). Although the gametophytic lethality of the double mutant, *ara7 rha1*, has precluded any direct study on the role of RAB5 in SSP trafficking, the characterization of mutants for either the RAB5 activator VPS9a or RAB5 effectors indicates a role for RAB5 in SSP transport (Goh *et al.*, 2007; Sakurai *et al.*, 2016). As a guanine nucleotide exchange factor (GEF), VPS9a controls the exchange from GDP to GTP in the Arabidopsis RAB5 GTPases. The *vps9-2* mutant seeds abnormally accumulate 12S and 2S precursors (Ebine *et al.*, 2011; Sakurai *et al.*, 2016), whereas double mutant plants for two redundant Rab5 effectors, Endosomal Rab Effector with PX-domain (EREX) and EREX-like 1 (EREL1), fail to process 12S globulin but not 2S albumin precursors (Sakurai *et al.*, 2016). In addition, green fluorescent protein (GFP)–CT24, an artificial seed protein generated by fusing GFP to the vacuolar targeting signal CT24 of soybean β-conglycinin, was found to be mis-sorted to the apoplast of embryo cells in both *vps9a-2* and *erex erel1* mutants (Sakurai *et al.*, 2016).

Likewise, genetic analyses of mutants for rice Rab5 GTPases, their activator, and effectors have revealed the importance of Rab5 in dense vesicle-mediated traffic in rice. The rice *gpa1/rab5a* (also known as *glup4*) mutant overaccumulates proglutenin precursors and partially mistargets dense vesicles to the apoplasts, resulting in a reduction in PSV/PB-II size (Wang *et al.*, 2010; Fukuda *et al.*, 2011). Similar SSP mis-sorting defects were also found in several mutants for the Rab5 activator and effectors, including *gpa2/glup6/Osvps9a*, *gpa3*, and *gpa5/Oserex* mutants (Fukuda *et al.*, 2013; Liu *et al.*, 2013; Ren *et al.*, 2020). GPA3 encodes a plant-specific kelch-repeat-containing protein and can interact directly with VPS9a to form a regulatory complex for OsRab5 (see Fig. 2G/ Ren *et al.*, 2014).

The RAB7 subfamily has also been implicated in PSV trafficking. The Arabidopsis genome encodes eight putative RAB7 homologs (RABG1, G2, and G3a-f) and RABG3a-f share a common GEF complex consisting of MON1 (MONENSIN SENSITIVITY1) and CCZ1 (CALCIUM CAFFEINE ZINC SENSITIVITY1) (Cui *et al.*, 2014). Studies on *rabg3* quintuple mutants, as well as *mon1*, and *ccz1a ccz1b* mutants showed that Rab7 activity is essential for correct vacuolar targeting of 12S globulins and GFP–CT24, and for normal PSV morphology (Cui *et al.*, 2014; Ebine *et al.*, 2014; Singh *et al.*, 2014). Rice contains five Rab7-encoding genes, which play multiple roles in plant growth, tolerance to heat, drought and salt stresses, and regulation of leaf senescence and grain yield (Langemeyer *et al.*, 2018; El-Esawi and Alayafi, 2019). A recent study by Pan *et al.* (2021) showed that mutation in rice CCZ1/GPA7 leads to abnormal accumulation of storage PVC-like structures and mistargeting glutelins to the apoplast, indicating a conserved role for the Rab7 complex in SSP trafficking in both Arabidopsis and rice.

SNARE proteins

SNARE proteins are another key component of the protein machinery that mediates fusion between vesicles and target membranes. Our understanding of the role of SNAREs in SSP trafficking has mostly come from studies in Arabidopsis. Two distinct SNARE complexes are known to be involved in vacuolar trafficking in Arabidopsis: one complex is composed of SYP (Syntaxin)-22, SYP5, VTI (Vesicle transport v-SNARE)-11, and VAMP727, and the other involves VAMP71 instead of VAMP727. The first complex works with canonical RAB5 GTPases and the tethering complex CORVET (class C core vacuole/endosome tethering) to facilitate the fusion between endosomes and vacuoles, whereas the latter probably works with RAB7 and the HOPS (homotypic fusion and vacuole protein sorting) tethering complex to mediate homotypic vacuolar fusion (Takemoto *et al.*, 2018). However, the VAMP71-containing SNARE complex and HOPS also control membrane fusion between the MVEs and the PSV in embryo cells during the transport of 12S globulin and GFP–CT24 (Ebine *et al.*, 2014; Singh *et al.*, 2014; Takemoto *et al.*, 2018; Minamino and Ueda, 2019). In contrast, the rice RAB5 effector GPA5/OSEREX was shown to associate with CORVET and the VAMP727-containing SNARE complexes

in developing endosperm cells to mediate the fusion between dense vesicles and PSVs (Fig. 2G; Ren *et al.*, 2020).

The SNARE VTI11 and its paralog VTI12 also participate in PSV trafficking. A report by Sanmartin *et al.* (2007) indicated that VTI11 and VTI12 have distinct roles in trafficking to lytic and storage vacuoles, respectively. However, more recent studies showed that VTI11 is required for the homotypic fusion of not only lytic vacuoles but also PSVs (Zheng *et al.*, 2014), suggesting the function of the two SNARES overlap more than previously thought.

Endosomal sorting complexes required for transport

SSPs and their processing proteases have been detected inside MVEs in Arabidopsis embryo cells (Otegui *et al.*, 2006). MVEs do not act just like passive carriers to the vacuole as the proteolytic processing of at least the 2S albumin precursors start within endosomes (Otegui *et al.*, 2006). The characteristic feature of these organelles in the transmission electron microscope is the presence of intralumenal vesicles involved in the sorting of membrane proteins for degradation in the vacuole (Paez Valencia *et al.*, 2016). This sorting and vesiculation event at endosomes required the ESCRT (endosomal sorting complex required for transport) machinery. The Arabidopsis ESCRT mutant *chmp1a chmp1b*, which is drastically impaired in the endosomal sorting and vacuolar degradation of plasma membrane proteins, is able to transport SSPs to PSVs in embryo cells (Spitzer *et al.*, 2009), suggesting that the capability of sorting membrane proteins into intralumenal vesicles is independent from endosomal transport of soluble cargo. However, the plant-unique ESCRT protein FREE1 (known as FYVE domain protein required for endosomal sorting 1), which acts upstream of CHMP1, is required for processing and PSV targeting of 2S and 12S storage proteins as well as the sorting of the soluble vacuolar cargo GFP–CT24 (Gao *et al.*, 2014).

In cereal crops, ESCRT complexes have been implicated in drought, heat, and salt stress response (see review by Ibl, 2019) as well as in seed development (Shen *et al.*, 2003; Zhang *et al.*, 2013). A proteomic study by Roustan *et al.* (2020) in developing barley endosperm suggested a potential role for ESCRT proteins in SSP trafficking, but experimental evidence is still lacking.

Other endomembrane proteins involved in SSP trafficking

During the screening of mutants mis-sorting the artificial soluble vacuolar cargo GFP–CT24, a protein named GFS9 (GREEN FLUORESCENT SEED 9) was identified as a factor critical for vacuolar targeting of SSPs (Fuji *et al.*, 2007). GFS9 is a peripheral protein associated with the Golgi membranes, but its molecular function is unknown. Besides mis-sorting vacuolar cargo, the *gfs9* mutant also has smaller vacuoles, including PSVs, which supports a role for GSF9 in vacuolar biogenesis and homeostasis (Ichino *et al.*, 2014).

The ionic balance within the endomembrane system is critical for cargo transport. For example, the sodium (Na^+)–proton (H^+) antiporters NHX5 and NHX6 localize to the TGN and MVEs, and are required for SSP targeting to the PSV. Mutations in *NHX5* and *NHX6* cause the overaccumulation of the 2S and 12S SSP precursors through their mistargeting to the apoplast (Ashnest *et al.*, 2015; Reguera *et al.*, 2015; Wu *et al.*, 2016). In the *nhx5 nhx6* double mutant, the binding between VSR and 12S globulins is reduced and the lumen of the TGN and MVEs is more acidic, highlighting the importance of the ionic environment of the endomembrane system in the regulation of VSR–ligand association and SSP sorting (Reguera *et al.*, 2015). Similarly, disruption of rice NHX5, which also localizes to the TGN and MVEs, results in the abnormal accumulation of proglutelins and their mis-sorting to the apoplast in rice endosperm (Zhu *et al.*, 2019). Another example emphasizing the importance of ionic balance within the endomembrane system came from the study of the rice *gpa8* mutant, which shows disruption of the function of subunit E isoform 1 of vacuolar H^+-ATPase (OsVHA-E1) (Zhu *et al.*, 2021). In the *gpa8* mutant, the lumenal pH of the TGN and vacuole is significantly increased and TGN-derived dense vesicles loaded with proglutelins are misdirected to the apoplast.

Conclusions and future perspectives

The coordinated synthesis, trafficking, and accumulation of SSPs is a complex and dynamic process that requires an extremely flexible and efficient endomembrane system. As summarized here, it has become clear that the cellular mechanisms that regulate SSP trafficking are strikingly different depending on species, tissues, and even developmental stages. For example, prolamins in starchy endosperm cells of cereals such as rice and maize directly aggregate inside the ER lumen to develop into PBs. Arabidopsis SSPs and rice glutelins are initially synthesized at the ER, transported through the Golgi, and deposited into the PSVs, whereas in pumpkin and castor bean, some SSPs traffick directly from the ER to the PSVs. However, the regulation of these trafficking mechanisms and how different proteins are sorted into these different pathways are still largely unknown. It has become clear, though, that the intrinsic properties of SSPs, their interaction with each other, and the cell type where they are expressed all affect their trafficking. Thus, the same prolamins that accumulate inside ER PBs in starchy endosperm cells of maize are transported to PSVs in aleurone cells.

There is also much to be learned about the molecular identify of SSP-containing vesicles as they have been mostly characterized by their morphological features as seen in the transmission electron microscope. For example, are the VSR-positive dense vesicles that carry SSPs from the TGN to

MVEs in Arabidopsis embryo cells equivalent to the glutelin-containing dense vesicles that fuse directly with the PSVs in rice endosperm (Fig. 2)? Interestingly, these trafficking routes share some conserved components, such as COPII, Rab5 cascade, CORVET, SNARE, and NHX, for targeting, tethering, and fusion to the PSVs, indicating that they may have evolved from canonical pathways in response to the unique challenges posed by the massive synthesis and transport of SSPs in seed tissues.

An additional avenue for future study involves the proteomic analysis of SSP trafficking vesicles and PSVs. G. Wang *et al.* (2016) identified multiple non-zein PB-associated proteins through a proteomic analysis of maize PBs. A similar approach can also be applied to study the PSV and other SSP-containing vesicles. A thorough proteomic investigation would not only allow the identification of PSV- or vesicle-resident proteins and sorting signals for targeting these proteins, but also help to clarify the substructures of these complex organelles and their changes in response to environmental/physiological cues. In summary, these future studies will enhance our knowledge of the molecular mechanisms of SSP trafficking in Arabidopsis and cereal crops.

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Conflict of interest

The authors have declared no conflicts of interest.

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References

Adachi M, Kanamori J, Masuda T, Yagasaki K, Kitamura K, Mikami B, Utsumi S. 2003. Crystal structure of soybean 11S globulin:glycinin A3B4 homohexamer. Proceedings of the National Academy of Sciences, USA **100**, 7395–7400.

Ashnest JR, Gendall AR. 2018. Trafficking to the seed protein storage vacuole. Functional Plant Biology **45**, 895–910.

Ashnest JR, Huynh DL, Dragwidge JM, Ford BA, Gendall AR. 2015. Arabidopsis intracellular NHX-type sodium–proton antiporters are required for seed storage protein processing. Plant & Cell Physiology **56**, 2220–2233.

Backhaus RA, Walsh S. 1983. The ontogeny of rubber formation in guayule, *Parthenium argentatum* Gray. Botanical Gazette **144**, 391–400.

Barlowe C, Orci L, Yeung T, Hosobuchi M, Hamamoto S, Salama N, Rexach MF, Ravazzola M, Amherdt M, Schekman R. 1994. COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell **77**, 895–907.

Bednarek SY, Raikhel NV. 1991. The barley lectin carboxyl-terminal propeptide is a vacuolar protein sorting determinant in plants. The Plant Cell **3**, 1195–1206.

Brandizzi F. 2018. Transport from the endoplasmic reticulum to the Golgi in plants: where are we now? Seminars in Cell and Developmental Biology **80**, 94–105.

Brown JC, Jolliffe NA, Frigerio L, Roberts LM. 2003. Sequence-specific, Golgi-dependent vacuolar targeting of castor bean 2S albumin. The Plant Journal **36**, 711–719.

Cao X, Rogers SW, Butler J, Beevers L, Rogers JC. 2000. Structural requirements for ligand binding by a probable plant vacuolar sorting receptor. The Plant Cell **12**, 493–506.

Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, Ueda T, Eliceiri KW, Grotewold E, Otegui MS. 2015. Anthocyanin vacuolar inclusions form by a microautophagy mechanism. The Plant Cell **27**, 2545–2559.

Chen L, Li YX, Li C, Shi Y, Song Y, Zhang D, Wang H, Li Y, Wang T. 2019. The retromer protein ZmVPS29 regulates maize kernel morphology likely through an auxin-dependent process(es). Plant Biotechnology Journal **18**, 1004–1014.

Choi SB, Wang C, Muench DG, Ozawa K, Franceschi VR, Wu Y, Okita TW. 2000. Messenger RNA targeting of rice seed storage proteins to specific ER subdomains. Nature **407**, 765–767.

Chrispeels MJ. 1983. The Golgi apparatus mediates the transport of phytohemagglutinin to the protein bodies in bean cotyledons. Planta **158**, 140–151.

Chrispeels MJ, Higgins TJ, Spencer D. 1982. Assembly of storage protein oligomers in the endoplasmic reticulum and processing of the polypeptides in the protein bodies of developing pea cotyledons. Journal of Cell Biology **93**, 306–313.

Coleman CE, Herman EM, Takasaki K, Larkins BA. 1996. The maize gamma-zein sequesters alpha-zein and stabilizes its accumulation in protein bodies of transgenic tobacco endosperm. The Plant Cell **8**, 2335–2345.

Cui Y, Zhao Q, Gao C, Ding Y, Zeng Y, Ueda T, Nakano A, Jiang L. 2014. Activation of the Rab7 GTPase by the MON1–CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in *Arabidopsis*. The Plant Cell **26**, 2080–2097.

daSilva LL, Snapp EL, Denecke J, Lippincott-Schwartz J, Hawes C, Brandizzi F. 2004. Endoplasmic reticulum export sites and Golgi bodies behave as single mobile secretory units in plant cells. The Plant Cell **16**, 1753–1771.

Delgadillo MO, Ruano G, Zouhar J, *et al*. 2020. MTV proteins unveil ER- and microtubule-associated compartments in the plant vacuolar trafficking pathway. Proceedings of the National Academy of Sciences, USA **117**, 9884–9895.

Di Berardino J, Marmagne A, Berger A, Yoshimoto K, Cueff G, Chardon F, Masclaux-Daubresse C, Reisdorf-Cren M. 2018. Autophagy controls resource allocations and protein storage accumulation in Arabidopsis seeds. Journal of Experimental Botany **69**, 1403–1414.

Durand TC, Cueff G, Godin B, Valot B, Clément G, Gaude T, Rajjou L. 2019. Combined proteomic and metabolomic profiling of the *Arabidopsis thaliana vps29* mutant reveals pleiotropic functions of the retromer in seed development. International Journal of Molecular Sciences **20**, ijms20020362.

Ebine K, Fujimoto M, Okatani Y, *et al*. 2011. A membrane trafficking pathway regulated by the plant-specific RAB GTPase ARA6. Nature Cell Biology **13**, 853–859.

Ebine K, Inoue T, Ito J, Ito E, Uemura T, Goh T, Abe H, Sato K, Nakano A, Ueda T. 2014. Plant vacuolar trafficking occurs through distinctly regulated pathways. Current Biology **24**, 1375–1382.

El-Esawi MA, Alayafi AA. 2019. Overexpression of rice Rab7 gene improves drought and heat tolerance and increases grain yield in rice (*Oryza sativa* L.). Genes **10**, 56.

Feeney M, Kittelmann M, Menassa R, Hawes C, Frigerio L. 2018. Protein storage vacuoles originate from remodeled preexisting vacuoles in *Arabidopsis thaliana*. Plant Physiology **177**, 241–254.

Frigerio L, Jolliffe NA, Di Cola A, Felipe DH, Paris N, Neuhaus JM, Lord JM, Ceriotti A, Roberts LM. 2001. The internal propeptide of the ricin precursor carries a sequence-specific determinant for vacuolar sorting. Plant Physiology **126**, 167–175.

Früholz S, Fäßler F, Kolukisaogl Ü, Pimpl P. 2018. Nanobody-triggered lockdown of VSRs reveals ligand reloading in the Golgi. Nature Communications **9**, 643.

Fuji K, Shimada T, Takahashi H, Tamura K, Koumoto Y, Utsumi S, Nishizawa K, Maruyama N, Hara-Nishimura I. 2007. *Arabidopsis* vacuolar sorting mutants (green fluorescent seed) can be identified efficiently by secretion of vacuole-targeted green fluorescent protein in their seeds. The Plant Cell **19**, 597–609.

Fuji K, Shirakawa M, Shimono Y, Kunieda T, Fukao Y, Koumoto Y, Takahashi H, Hara-Nishimura I, Shimada T. 2016. The Adaptor complex AP-4 regulates vacuolar protein sorting at the trans-Golgi network by interacting with VACUOLAR SORTING RECEPTOR1. Plant Physiology **170**, 211–219.

Fukuda M, Kawagoe Y, Murakami T, Washida H, Sugino A, Nagamine A, Okita TW, Ogawa M, Kumamaru T. 2016. The dual roles of the Golgi Transport 1 (GOT1B): RNA localization to the cortical endoplasmic reticulum and the export of proglutelin and α-globulin from the cortical ER to the Golgi. Plant & Cell Physiology **57**, 2380–2391.

Fukuda M, Satoh-Cruz M, Wen L, *et al*. 2011. The small GTPase Rab5a is essential for intracellular transport of proglutelin from the Golgi apparatus to the protein storage vacuole and endosomal membrane organization in developing rice endosperm. Plant Physiology **157**, 632–644.

Fukuda M, Wen L, Satoh-Cruz M, *et al*. 2013. A guanine nucleotide exchange factor for Rab5 proteins is essential for intracellular transport of the proglutelin from the Golgi apparatus to the protein storage vacuole in rice endosperm. Plant Physiology **162**, 663–674.

Gao C, Luo M, Zhao Q, Yang R, Cui Y, Zeng Y, Xia J, Jiang L. 2014. A unique plant ESCRT component, FREE1, regulates multivesicular body protein sorting and plant growth. Current Biology **24**, 2556–2563.

Gershlick DC, Lousa Cde M, Foresti O, Lee AJ, Pereira EA, daSilva LL, Bottanelli F, Denecke J. 2014. Golgi-dependent transport of vacuolar sorting receptors is regulated by COPII, AP1, and AP4 protein complexes in tobacco. The Plant Cell **26**, 1308–1329.

Goh T, Uchida W, Arakawa S, Ito E, Dainobu T, Ebine K, Takeuchi M, Sato K, Ueda T, Nakano A. 2007. VPS9a, the common activator for two distinct types of Rab5 GTPases, is essential for the development of *Arabidopsis thaliana*. The Plant Cell **19**, 3504–3515.

Gruis D, Schulze J, Jung R. 2004. Storage protein accumulation in the absence of the vacuolar processing enzyme family of cysteine proteases. The Plant Cell **16**, 270–290.

Gruis DF, Selinger DA, Curran JM, Jung R. 2002. Redundant proteolytic mechanisms process seed storage proteins in the absence of seed-type members of the vacuolar processing enzyme family of cysteine proteases. The Plant Cell **14**, 2863–2882.

Hara-Nishimura I, Shimada T, Hatano K, Takeuchi Y, Nishimura M. 1998. Transport of storage proteins to protein storage vacuoles is mediated by large precursor-accumulating vesicles. The Plant Cell **10**, 825–836.

Heath JD, Weldon R, Monnot C, Meinke DW. 1986. Analysis of storage proteins in normal and aborted seeds from embryo-lethal mutants of *Arabidopsis thaliana*. Planta **169**, 304–312.

Hegedus DD, Coutu C, Harrington M, Hope B, Gerbrandt K, Nikolov I. 2015. Multiple internal sorting determinants can contribute to the trafficking of cruciferin to protein storage vacuoles. Plant Molecular Biology **88**, 3–20.

Heucken N, Ivanov R. 2018. The retromer, sorting nexins and the plant endomembrane protein trafficking. Journal of Cell Science **131**, jcs203695.

Hillmer S, Movafeghi A, Robinson DG, Hinz G. 2001. Vacuolar storage proteins are sorted in the cis-cisternae of the pea cotyledon Golgi apparatus. Journal of Cell Biology **152**, 41–50.

Hinz G, Colanesi S, Hillmer S, Rogers JC, Robinson DG. 2007. Localization of vacuolar transport receptors and cargo proteins in the Golgi apparatus of developing *Arabidopsis* embryos. Traffic **8**, 1452–1464.

Hinz G, Hillmer S, Baumer M, Hohl II. 1999. Vacuolar storage proteins and the putative vacuolar sorting receptor BP-80 exit the Golgi apparatus of developing pea cotyledons in different transport vesicles. The Plant Cell **11**, 1509–1524.

Hirst J, Barlow LD, Francisco GC, Sahlender DA, Seaman MN, Dacks JB, Robinson MS. 2011. The fifth adaptor protein complex. PLoS Biology **9**, e1001170.

Hirst J, Borner GH, Antrobus R, Peden AA, Hodson NA, Sahlender DA, Robinson MS. 2012. Distinct and overlapping roles for AP-1 and GGAs revealed by the 'knocksideways' system. Current Biology **22**, 1711–1716.

Hohl I, Robinson DG, Chrispeels MJ, Hinz G. 1996. Transport of storage proteins to the vacuole is mediated by vesicles without a clathrin coat. Journal of Cell Science **109**, 2539–2550.

Holding DR. 2014. Recent advances in the study of prolamin storage protein organization and function. Frontiers in Plant Science **5**, 276.

Holwerda BC, Padgett HS, Rogers JC. 1992. Proaleurain vacuolar targeting is mediated by short contiguous peptide interactions. The Plant Cell **4**, 307–318.

Ibl V. 2019. ESCRTing in cereals: still a long way to go. Science China Life Sciences **62**, 1144–1152.

Ichino T, Fuji K, Ueda H, *et al*. 2014. GFS9/TT9 contributes to intracellular membrane trafficking and flavonoid accumulation in *Arabidopsis thaliana*. The Plant Journal **80**, 410–423.

Jaillais Y, Fobis-Loisy I, Miège C, Rollin C, Gaude T. 2006. AtSNX1 defines an endosome for auxin-carrier trafficking in *Arabidopsis*. Nature **443**, 106–109.

Kang H, Kim SY, Song K, Sohn EJ, Lee Y, Lee DW, Hara-Nishimura I, Hwang I. 2012. Trafficking of vacuolar proteins: the crucial role of *Arabidopsis* vacuolar protein sorting 29 in recycling vacuolar sorting receptor. The Plant Cell **24**, 5058–5073.

Kawakatsu T, Hirose S, Yasuda H, Takaiwa F. 2010. Reducing rice seed storage protein accumulation leads to changes in nutrient quality and storage organelle formation. Plant Physiology **154**, 1842–1854.

Kim WT, Franceschi VR, Krishnan HB, Okita TW. 1988. Formation of wheat protein bodies: involvement of the Golgi apparatus in gliadin transport. Planta **176**, 173–182.

Kirsch T, Paris N, Butler JM, Beevers L, Rogers JC. 1994. Purification and initial characterization of a potential plant vacuolar targeting receptor. Proceedings of the National Academy of Sciences, USA **91**, 3403–3407.

Koide Y, Hirano H, Matsuoka K, Nakamura K. 1997. The N-terminal propeptide of the precursor to sporamin acts as a vacuole-targeting signal even at the C terminus of the mature part in tobacco cells. Plant Physiology **114**, 863–870.

Krishnan HB, Franceschi VR, Okita TW. 1986. Immunochemical studies on the role of the Golgi complex in protein-body formation in rice seeds. Planta **169**, 471–480.

Krishnan HB, White JA, Pueppke SG. 1992. Characterization and location of rice (*Oryza sativa* L.) seed globulins. Plant Science **81**, 1–11.

Langemeyer L, Fröhlich F, Ungermann C. 2018. Rab GTPase function in endosome and lysosome biogenesis. Trends in Cell Biology **28**, 957–970.

Larkins BA, Hurkman WJ. 1978. Synthesis and deposition of zein in protein bodies of maize endosperm. Plant Physiology **62**, 256–263.

Larkins BA, Wu RY, Song RT, Messing J. 2017. Maize seed storage proteins. In: Larkins BA, ed. Maize kernel development. Wallingford, UK: CABI Publishing, 175–189.

Levanony H, Rubin R, Altschuler Y, Galili G. 1992. Evidence for a novel route of wheat storage proteins to vacuoles. Journal of Cell Biology **119**, 1117–1128.

Li F, Chung T, Pennington JG, Federico ML, Kaeppler HF, Kaeppler SM, Otegui MS, Vierstra RD. 2015. Autophagic recycling plays a central role in maize nitrogen remobilization. The Plant Cell **27**, 1389–1408.

Li L, Shimada T, Takahashi H, Ueda H, Fukao Y, Kondo M, Nishimura M, Hara-Nishimura I. 2006. MAIGO2 is involved in exit of seed storage proteins from the endoplasmic reticulum in *Arabidopsis thaliana*. The Plant Cell **18**, 3535–3547.

Li L, Shimada T, Takahashi H, *et al*. 2013. MAG2 and three MAG2-INTERACTING PROTEINs form an ER-localized complex to facilitate storage protein transport in *Arabidopsis thaliana*. The Plant Journal **76**, 781–791.

Li W, Xie T, Qiu Q, Ning T, Yang D. 2013. A short peptide in rice glutelin directs trafficking of protein into the protein storage vacuoles of the endosperm cells. Plant Molecular Biology Reporter **31**, 1492–1505.

Li X, Franceschi VR, Okita TW. 1993. Segregation of storage protein mRNAs on the rough endoplasmic reticulum membranes of rice endosperm cells. Cell **72**, 869–879.

Liu F, Ren Y, Wang Y, *et al*. 2013. OsVPS9A functions cooperatively with OsRAB5A to regulate post-Golgi dense vesicle-mediated storage protein trafficking to the protein storage vacuole in rice endosperm cells. Molecular Plant **6**, 1918–1932.

Luo F, Fong YH, Zeng Y, Shen J, Jiang L, Wong KB. 2014. How vacuolar sorting receptor proteins interact with their cargo proteins: crystal structures of apo and cargo-bound forms of the protease-associated domain from an *Arabidopsis* vacuolar sorting receptor. The Plant Cell **26**, 3693–3708.

Marshall RS, Vierstra RD. 2018. Autophagy: the master of bulk and selective recycling. Annual Review of Plant Biology **69**, 173–208.

Masclaux-Daubresse C, Chen Q, Havé M. 2017. Regulation of nutrient recycling via autophagy. Current Opinion in Plant Biology **39**, 8–17.

Mettlen M, Chen PH, Srinivasan S, Danuser G, Schmid SL. 2018. Regulation of clathrin-mediated endocytosis. Annual Review of Biochemistry **87**, 871–896.

Minamino N, Ueda T. 2019. RAB GTPases and their effectors in plant endosomal transport. Current Opinion in Plant Biology **52**, 61–68.

Mori T, Maruyama N, Nishizawa K, Higasa T, Yagasaki K, Ishimoto M, Utsumi S. 2004. The composition of newly synthesized proteins in the endoplasmic reticulum determines the transport pathways of soybean seed storage proteins. The Plant Journal **40**, 238–249.

Müntz K. 1998. Deposition of storage proteins. Plant Molecular Biology **38**, 77–99.

Mylne JS, Hara-Nishimura I, Rosengren KJ. 2014. Seed storage albumins: biosynthesis, trafficking and structures. Functional Plant Biology **41**, 671–677.

Nakamura T, Liu Y, Hirata D, Namba H, Harada S, Hirokawa T, Miyakawa T. 1993. Protein phosphatase type 2B (calcineurin)-mediated, FK506-sensitive regulation of intracellular ions in yeast is an important determinant for adaptation to high salt stress conditions. The EMBO Journal **12**, 4063–4071.

Neuhaus JM, Sticher L, Meins F Jr, Boller T. 1991. A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. Proceedings of the National Academy of Sciences, USA **88**, 10362-10366.

Niemes S, Labs M, Scheuring D, Krueger F, Langhans M, Jesenofsky B, Robinson DG, Pimpl P. 2010a. Sorting of plant vacuolar proteins is initiated in the ER. The Plant Journal **62**, 601–614.

Niemes S, Langhans M, Viotti C, Scheuring D, San Wan Yan M, Jiang L, Hillmer S, Robinson DG, Pimpl P. 2010b. Retromer recycles vacuolar sorting receptors from the trans-Golgi network. The Plant Journal **61**, 107-121.

Nishizawa K, Maruyama N, Satoh R, Fuchikami Y, Higasa T, Utsumi S. 2003. A C-terminal sequence of soybean β-conglycinin α' subunit acts as a vacuolar sorting determinant in seed cells. The Plant Journal **34**, 647–659.

Nodzynski T, Feraru MI, Hirsch S, De Rycke R, Niculaes C, Boerjan W, Van Leene J, De Jaeger G, Vanneste S, Friml J. 2013. Retromer subunits VPS35A and VPS29 mediate prevacuolar compartment (PVC) function in *Arabidopsis*. Molecular Plant **6**, 1849–1862.

Oliviusson P, Heinzerling O, Hillmer S, Hinz G, Tse YC, Jiang L, Robinson DG. 2006. Plant retromer, localized to the prevacuolar compartment and microvesicles in *Arabidopsis*, may interact with vacuolar sorting receptors. The Plant Cell **18**, 1239–1252.

Osborne TB. 1924. The vegetable proteins. Nature **114**, 822–822.

Otegui MS, Herder R, Schulze J, Jung R, Staehelin LA. 2006. The proteolytic processing of seed storage proteins in *Arabidopsis* embryo cells starts in the multivesicular bodies. The Plant Cell **18**, 2567–2581.

Paez Valencia J, Goodman K, Otegui MS. 2016. Endocytosis and endosomal trafficking in plants. Annual Review of Plant Biology **67**, 309–335.

Pan T, Wang Y, Jing R, *et al*. 2021. Post-Golgi trafficking of rice storage proteins requires the small GTPase Rab7 activation complex MON1–CCZ1. Plant Physiology **187**, 2174–2191.

Park JH, Oufattole M, Rogers JC. 2007. Golgi-mediated vacuolar sorting in plant cells: RMR proteins are sorting receptors for the protein aggregation/membrane internalization pathway. Plant Science **172**, 728–745.

Park M, Lee D, Lee GJ, Hwang I. 2005. AtRMR1 functions as a cargo receptor for protein trafficking to the protein storage vacuole. Journal of Cell Biology **170**, 757–767.

Park M, Song K, Reichardt I, Kim H, Mayer U, Stierhof YD, Hwang I, Jürgens G. 2013. *Arabidopsis* μ-adaptin subunit AP1M of adaptor protein complex 1 mediates late secretory and vacuolar traffic and is required for growth. Proceedings of the National Academy of Sciences, USA **110**, 10318–10323.

Pereira C, Pereira S, Satiat-Jeunemaitre B, Pissarra J. 2013. Cardosin A contains two vacuolar sorting signals using different vacuolar routes in tobacco epidermal cells. The Plant Journal **76**, 87–100.

Pourcher M, Santambrogio M, Thazar N, Thierry AM, Fobis-Loisy I, Miege C, Jaillais Y, Gaude T. 2010. Analyses of sorting nexins reveal distinct retromer-subcomplex functions in development and protein sorting in *Arabidopsis thaliana*. The Plant Cell **22**, 3980–3991.

Reguera M, Bassil E, Tajima H, Wimmer M, Chanoca A, Otegui MS, Paris N, Blumwald E. 2015. pH regulation by NHX-type antiporters is required for receptor-mediated protein trafficking to the vacuole in *Arabidopsis*. The Plant Cell **27**, 1200–1217.

Ren Y, Wang Y, Liu F, *et al*. 2014. *GLUTELIN PRECURSOR ACCUMULATION3* encodes a regulator of post-Golgi vesicular traffic essential for vacuolar protein sorting in rice endosperm. The Plant Cell **26**, 410–425.

Ren Y, Wang Y, Pan T, *et al*. 2020. GPA5 encodes a Rab5a effector required for post-Golgi trafficking of rice storage proteins. The Plant Cell **32**, 758–777.

Reyes FC, Chung T, Holding D, Jung R, Vierstra R, Otegui MS. 2011. Delivery of prolamins to the protein storage vacuole in maize aleurone cells. The Plant Cell **23**, 769–784.

Robinson MS. 2015. Forty years of clathrin-coated vesicles. Traffic **16**, 1210–1238.

Robinson DG, Hinz G. 2007. Golgi-mediated transport of seed storage proteins. Seed Science Research **9**, 267–283.

Robinson DG, Neuhaus JM. 2016. Receptor-mediated sorting of soluble vacuolar proteins: myths, facts, and a new model. Journal of Experimental Botany **67**, 4435–4449.

Robinson DG, Oliviusson P, Hinz G. 2005. Protein sorting to the storage vacuoles of plants: a critical appraisal. Traffic **6**, 615–625.

Roustan V, Hilscher J, Weidinger M, *et al*. 2020. Protein sorting into protein bodies during barley endosperm development is putatively regulated by cytoskeleton members, MVBs and the HvSNF7s. Scientific Reports **10**, 1864.

Saalbach G, Jung R, Kunze G, Saalbach I, Adler K, Muntz K. 1991. Different legumin protein domains act as vacuolar targeting signals. The Plant Cell **3**, 695–708.

Saito Y, Shigemitsu T, Yamasaki R, *et al*. 2012. Formation mechanism of the internal structure of type I protein bodies in rice endosperm: relationship between the localization of prolamin species and the expression of individual genes. The Plant Journal **70**, 1043–1055.

Sakurai HT, Inoue T, Nakano A, Ueda T. 2016. ENDOSOMAL RAB EFFECTOR WITH PX-DOMAIN, an interacting partner of RAB5 GTPases, regulates membrane trafficking to protein storage vacuoles in *Arabidopsis*. The Plant Cell **28**, 1490–1503.

Sanmartin M, Ordonez A, Sohn EJ, Robert S, Sanchez-Serrano JJ, Surpin MA, Raikhel NV, Rojo E. 2007. Divergent functions of VTI12 and VTI11 in trafficking to storage and lytic vacuoles in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **104**, 3645–3650.

Shen B, Li C, Min Z, Meeley RB, Tarczynski MC, Olsen OA. 2003. *sal1* determines the number of aleurone cell layers in maize endosperm and encodes a class E vacuolar sorting protein. Proceedings of the National Academy of Sciences, USA **100**, 6552–6557.

Shen Y, Wang J, Ding Y, Lo SW, Gouzerh G, Neuhaus JM, Jiang L. 2011. The rice RMR1 associates with a distinct prevacuolar compartment for the protein storage vacuole pathway. Molecular Plant **4**, 854–868.

Shewry PR, Napier JA, Tatham AS. 1995. Seed storage proteins: structures and biosynthesis. The Plant Cell **7**, 945–956.

Shimada T, Fuji K, Tamura K, Kondo M, Nishimura M, Hara-Nishimura I. 2003. Vacuolar sorting receptor for seed storage proteins in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **100**, 16095–16100.

Shimada T, Koumoto Y, Li L, Yamazaki M, Kondo M, Nishimura M, Hara-Nishimura I. 2006. AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. Plant & Cell Physiology **47**, 1187–1194.

Shimada T, Kunieda T, Sumi S, Koumoto Y, Tamura K, Hatano K, Ueda H, Hara-Nishimura I. 2018a. The AP-1 complex is required for proper mucilage formation in Arabidopsis seeds. Plant & Cell Physiology **59**, 2331–2338.

Shimada T, Takagi J, Ichino T, Shirakawa M, Hara-Nishimura I. 2018b. Plant vacuoles. Annual Review of Plant Biology **69**, 123–145.

Shimada T, Watanabe E, Tamura K, Hayashi Y, Nishimura M, Hara-Nishimura I. 2002. A vacuolar sorting receptor PV72 on the membrane of vesicles that accumulate precursors of seed storage proteins (PAC vesicles). Plant & Cell Physiology **43**, 1086–1095.

Shimizu Y, Takagi J, Ito E, *et al*. 2021. Cargo sorting zones in the trans-Golgi network visualized by super-resolution confocal live imaging microscopy in plants. Nature Communications **12**, 1901.

Shy G, Ehler L, Herman E, Galili G. 2001. Expression patterns of genes encoding endomembrane proteins support a reduced function of the Golgi in wheat endosperm during the onset of storage protein deposition. Journal of Experimental Botany **52**, 2387–2388.

Singh MK, Krüger F, Beckmann H, *et al*. 2014. Protein delivery to vacuole requires SAND protein-dependent Rab GTPase conversion for MVB–vacuole fusion. Current Biology **24**, 1383–1389.

Spitzer C, Reyes FC, Buono R, Sliwinski MK, Haas TJ, Otegui MS. 2009. The ESCRT-related CHMP1A and B proteins mediate multivesicular body sorting of auxin carriers in *Arabidopsis* and are required for plant development. The Plant Cell **21**, 749–766.

Takagi J, Renna L, Takahashi H, *et al*. 2013. MAIGO5 functions in protein export from Golgi-associated endoplasmic reticulum exit sites in *Arabidopsis*. The Plant Cell **25**, 4658–4675.

Takahashi H, Saito Y, Kitagawa T, Morita S, Masumura T, Tanaka K. 2005. A novel vesicle derived directly from endoplasmic reticulum is involved in the transport of vacuolar storage proteins in rice endosperm. Plant & Cell Physiology **46**, 245–249.

Takahashi H, Tamura K, Takagi J, Koumoto Y, Hara-Nishimura I, Shimada T. 2010. MAG4/Atp115 is a Golgi-localized tethering factor that mediates efficient anterograde transport in *Arabidopsis*. Plant & Cell Physiology **51**, 1777–1787.

Takemoto K, Ebine K, Askani JC, Kruger F, Gonzalez ZA, Ito E, Goh T, Schumacher K, Nakano A, Ueda T. 2018. Distinct sets of tethering complexes, SNARE complexes, and Rab GTPases mediate membrane fusion at the vacuole in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **115**, E2457–E2466.

Templeman TS, Demaggio AE, Stetler DA. 1987. Biochemistry of fern spore germination: globulin storage proteins in *Matteuccia struthiopteris* L. Plant Physiology **85**, 343–349.

Tian L, Dai LL, Yin ZJ, Fukuda M, Kumamaru T, Dong XB, Xu XP, Qu LQ. 2013. Small GTPase Sar1 is crucial for proglutelin and alpha-globulin export from the endoplasmic reticulum in rice endosperm. Journal of Experimental Botany **64**, 2831–2845.

Tosi P, Parker M, Gritsch CS, Carzaniga R, Martin B, Shewry PR. 2009. Trafficking of storage proteins in developing grain of wheat. Journal of Experimental Botany **60**, 979–991.

van Doorn WG, Papini A. 2013. Ultrastructure of autophagy in plant cells: a review. Autophagy **9**, 1922–1936.

Vitale A, Hinz G. 2005. Sorting of proteins to storage vacuoles: how many mechanisms? Trends in Plant Science **10**, 316–323.

Wang G, Wang G, Wang J, Du Y, Yao D, Shuai B, Han L, Tang Y, Song R. 2016. Comprehensive proteomic analysis of developing protein bodies in maize (*Zea mays*) endosperm provides novel insights into its biogenesis. Journal of Experimental Botany **67**, 6323–6335.

Wang H, Rogers JC, Jiang L. 2011. Plant RMR proteins: unique vacuolar sorting receptors that couple ligand sorting with membrane internalization. The FEBS Journal **278**, 59–68.

Wang Y, Liu F, Ren Y, *et al*. 2016. GOLGI TRANSPORT 1B regulates protein export from the endoplasmic reticulum in rice endosperm cells. The Plant Cell **28**, 2850–2865.

Wang Y, Ren Y, Liu X, *et al*. 2010. OsRab5a regulates endomembrane organization and storage protein trafficking in rice endosperm cells. The Plant Journal **64**, 812–824.

Wang Y, Zhu S, Liu S, *et al*. 2009. The vacuolar processing enzyme OsVPE1 is required for efficient glutelin processing in rice. The Plant Journal **58**, 606–617.

Watanabe E, Shimada T, Tamura K, Matsushima R, Koumoto Y, Nishimura M, Hara-Nishimura I. 2004. An ER-localized form of PV72, a seed-specific vacuolar sorting receptor, interferes the transport of an NPIR-containing proteinase in *Arabidopsis* leaves. Plant & Cell Physiology **45**, 9–17.

Withana-Gamage TS, Hegedus DD, Qiu X, Yu PQ, May T, Lydiate D, Wanasundara JPD. 2013. Characterization of *Arabidopsis thaliana* lines with altered seed storage protein profiles using synchrotron-powered FT-IR spectromicroscopy. Journal of Agricultural and Food Chemistry **61**, 901–912.

Wu X, Ebine K, Ueda T, Qiu QS. 2016. AtNHX5 and AtNHX6 are required for the subcellular localization of the SNARE complex that mediates the trafficking of seed storage proteins in *Arabidopsis*. PLoS One **11**, e0151658.

Xiang L, Etxeberria E, Van den Ende W. 2013. Vacuolar protein sorting mechanisms in plants. The FEBS Journal **280**, 979–993.

Yamagata H, Sugimoto T, Tanaka K, Kasai Z. 1982. Biosynthesis of storage proteins in developing rice seeds. Plant Physiology **70**, 1094–1100.

Yamagata H, Tamura K, Tanaka K, Kasai Z. 1986. Cell-free synthesis of rice prolamin. Plant & Cell Physiology **27**, 1419–1422.

Yamazaki M, Shimada T, Takahashi H, Tamura K, Kondo M, Nishimura M, Hara-Nishimura I. 2008. *Arabidopsis* VPS35, a retromer component, is required for vacuolar protein sorting and involved in plant growth and leaf senescence. Plant & Cell Physiology **49**, 142–156.

Yang X, Bassham DC. 2015. New insight into the mechanism and function of autophagy in plant cells. International Review of Cell and Molecular Biology **320**, 1–40.

Zelazny E, Santambrogio M, Pourcher M, Chambrier P, Berne-Dedieu A, Fobis-Loisy I, Miege C, Jaillais Y, Gaude T. 2013. Mechanisms governing the endosomal membrane recruitment of the core retromer in *Arabidopsis*. Journal of Biological Chemistry **288**, 8815–8825.

Zhang L, Zhang H, Liu P, Hao H, Jin JB, Lin J. 2011. *Arabidopsis* R-SNARE proteins VAMP721 and VAMP722 are required for cell plate formation. PLoS One **6**, e26129.

Zhang XQ, Hou P, Zhu HT, Li GD, Liu XG, Xie XM. 2013. Knockout of the VPS22 component of the ESCRT-II complex in rice (*Oryza sativa* L.)

causes chalky endosperm and early seedling lethality. Molecular Biology Reports **40**, 3475–3481.

Zheng J, Han SW, Rodriguez-Welsh MF, Rojas-Pierce M. 2014. Homotypic vacuole fusion requires VTI11 and is regulated by phosphoinositides. Molecular Plant **7**, 1026–1040.

Zhu J, Ren Y, Wang Y, *et al*. 2019. OsNHX5-mediated pH homeostasis is required for post-Golgi trafficking of seed storage proteins in rice endosperm cells. BMC Plant Biology **19**, 295.

Zhu J, Ren Y, Zhang Y, *et al*. 2021. Subunit E isoform 1 of vacuolar H^+-ATPase OsVHA enables post-Golgi trafficking of rice seed storage proteins. Plant Physiology **187**, 2192–2208.

Zouhar J, Munoz A, Rojo E. 2010. Functional specialization within the vacuolar sorting receptor family: VSR1, VSR3 and VSR4 sort vacuolar storage cargo in seeds and vegetative tissues. The Plant Journal **64**, 577–588.