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REVIEW PAPER

The trafficking machinery of lytic and protein storage vacuoles: how much is shared and how much is distinct?

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Abstract

Plant cells contain two types of vacuoles, the lytic vacuole (LV) and protein storage vacuole (PSV). LVs are present in vegetative cells, whereas PSVs are found in seed cells. The physiological functions of the two types of vacuole differ. Newly synthesized proteins must be transported to these vacuoles via protein trafficking through the endomembrane system for them to function. Recently, significant advances have been made in elucidating the molecular mechanisms of protein trafficking to these organelles. Despite these advances, the relationship between the trafficking mechanisms to the LV and PSV remains unclear. Some aspects of the trafficking mechanisms are common to both types of vacuole, but certain aspects are specific to trafficking to either the LV or PSV. In this review, we summarize recent findings on the components involved in protein trafficking to both the LV and PSV and compare them to examine the extent of overlap in the trafficking mechanisms. In addition, we discuss the interconnection between the LV and PSV provided by the protein trafficking machinery and the implications for the identity of these organelles.

Keywords: Lytic vacuole, molecular machinery, protein storage vacuole, trafficking pathway.

Introduction

Plant cells contain two types of vacuoles, the lytic vacuole (LV), which occurs mainly in vegetative cells, and the protein storage vacuole (PSV), found mainly in seed cells. PSVs can also be found in vegetative tissues (Müntz, 1998; Park *et al.*, 2004; Sohn *et al.*, 2007; Zouhar *et al.*, 2010) or may coexist with the LV in the same cell (Paris *et al.*, 1996; Park *et al.*, 2004; Olbrich *et al.*, 2007). LVs and PSVs can transform into each other, depending on the physiological conditions (Herman *et al.*, 1999; Feeney *et al.*, 2013). They can be distinguished by the presence of different tonoplast intrinsic proteins (TIPs): α-TIP and δ-TIP in PSVs, and γ-TIP in LVs (Maeshima, 1992). Both LVs and PSVs are part of endomembrane systems. The LV is functionally equivalent to the lysosome and the vacuole in animal and yeast cells, respectively (Jiang and Rogers, 2003), and is found in all plant species including *Marchantia*, an ancestor of plants. In contrast, PSVs are found only in seed plants. Thus, the PSVs in plants must have evolved after the LV. It is possible that the

evolution of PSVs was a crucial event for the evolution of seeds in plants. However, how PSVs evolved in seeds remains elusive.

The LV plays a key role in cellular homeostasis through its hydrolytic activities toward various cellular components, whereas PSVs mainly serve as a location for the storage of large quantities of seed storage proteins used as nitrogen or carbon sources during germination, and for toxic proteins such as lectins, protease inhibitors, and ribosome-inactivating proteins that function in defense responses against predators (Jiang *et al.*, 2001; Cândido *et al.*, 2011; Jørgensen *et al.*, 2011; Lord *et al.*, 2011; Xiang *et al.*, 2013). The acidic pH in the lumen of the LV is critical for the activity of various hydrolytic enzymes. In contrast, PSVs have neutral pH (Isayenkov, 2014). Indeed, the neutral pH in PSVs is a key mechanism to inhibit the hydrolytic activity of proteinases such as C1 protease in soybean, thereby enabling both storage proteins and proteases to be stored in the PSVs (He *et al.*, 2007). However, another study showed that in Arabidopsis, the pH of the PSV lumen changes from neutral at the torpedo stage to acidic at the mature stage during embryogenesis (Otegui *et al.*, 2006).

For the LV and PSVs to function properly, newly synthesized LV and PSV proteins must be delivered to them accurately. Many studies have been carried out to elucidate the molecular mechanisms of protein trafficking to these organelles (Fig. 1). In this review, we compare the mechanisms of protein trafficking to the LV and PSVs. We focus on the molecular machinery involved in the biogenesis of vesicles in the Golgi apparatus and/or the *trans*-Golgi network (TGN) that function in trafficking to these two organelles, and in vesicle fusion to the LV or PSVs. In addition, we discuss the relationship between the molecular machinery of the trafficking pathways to the LV and PSVs and scenarios for how the PSV trafficking pathway might have arisen during the evolution of seed plants.

Generation of vesicles specific to the LV and PSVs

Cargo receptors: VSRs and RMRs

In the trafficking of newly synthesized proteins to the LV and PSVs in plant cells, the sorting of cargoes depending on their destination is pivotal. The sorting of vacuolar proteins is carried out by vacuolar sorting receptors (VSRs) that specifically recognize LV cargoes and PSV proteins (Miao *et al.*, 2006; Kim *et al.*, 2010). VSRs bind their cargoes at the TGN, traffic to the prevacuolar compartment (PVC) together with the cargoes, and then recycle back to the TGN for the next round sorting of vacuolar proteins (Kirsch *et al.*, 1994; Tse *et al.*, 2004). However, recently it has also been shown that VSRs bind vacuole-targeted proteins in the endoplasmic reticulum (Watanabe *et al.*, 2004; daSilva *et al.*, 2005; Niemes *et al.*, 2010) and *cis*-Golgi (Gershlick *et al.*, 2014), thereby facilitating their transport to the LV or PSVs (Künzl *et al.*, 2016), and then recycle from the TGN/early endosome back to the *cis*-Golgi to reload ligands (Fruholz *et al.*, 2018). For sorting cargoes, VSRs recognize a specific signal of the cargo proteins. The vacuolar sorting signals (VSSs) of soluble proteins have been divided into three categories: sequence-specific VSSs (ssVSSs), C-terminal VSSs (ctVSSs), and physical structure VSSs (psVSSs) (Neuhaus and Rogers, 1998). Proteins that are targeted to the LV, such as barley aleurain, sweet potato sporamin, and cysteine

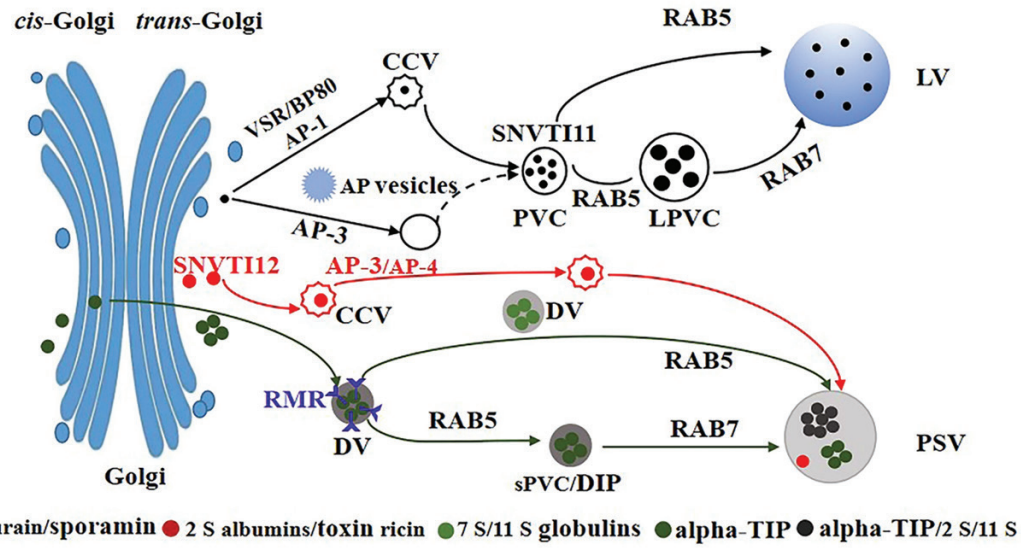

Fig. 1. Multiple LV and PSV trafficking pathways. Multiple pathways have been demonstrated to participate in the transport of lytic vacuolar proteins and storage proteins from the Golgi apparatus to the LV and PSVs, respectively.

protease, have a sequence-specific motif such as the NPIR-like motif (Nakamura and Matsuoka, 1993).

VSRs were first found in a purified clathrin-coated vesicle (CCV) fraction from developing pea (Kirsch *et al.*, 1994). Subsequently, the function of VSRs has been extensively studied in Arabidopsis, which contains seven AtVSR isoforms. The roles of AtVSRs in LV trafficking have been confirmed by examination of the effects of dominant-negative mutant forms (daSilva *et al.*, 2005; Foresti *et al.*, 2010; Kim *et al.*, 2010). In *atvsr1 atvsr4* double-mutant plants, a small amount of Arabidopsis aleurain-like protein (AALP), a lytic vacuole protease, is secreted into the apoplast in leaf tissues (Zouhar *et al.*, 2010). However, AALP is not secreted into apoplasts in *atvsr1* or *atvsr4* single mutants (Zouhar *et al.*, 2010). In *atvsr1 atvsr3* double-mutant protoplasts, AALP and carboxypeptidase Y (CPY) accumulate as precursors in protoplasts and are secreted into the incubation medium (Lee *et al.*, 2013). The defective protoplast trafficking in *atvsr1 atvsr3* and *atvsr1 atvsr4* double-mutant plants can be complemented by transient expression of *AtVSR1* or *AtVSR4* (Lee *et al.*, 2013). Although AtVSR1, AtVSR3, and AtVSR4 are involved in protoplast trafficking in vegetative cells, single and double mutants of *atvrs1*, *atvsr3*, and *atvsr4* show no obvious abnormal phenotype in their vegetative tissues.

In addition to the role of VSRs in trafficking to the LV, they have also been shown to be involved in trafficking to PSVs. In the case of barley lectin, its C-terminal propeptide functions as a sorting determinant (Bednarek and Raikhel, 1991). However, the C-terminal sorting signals of PSV proteins do not have any consensus sequence (Matsuoka *et al.*, 1999). AtVSR binds the C-terminal peptides of 12S globulin, 2S albumin, and 7S β-conglycinin *in vitro* (Shimada *et al.*, 2003; Fuji *et al.*, 2007). Pea BP-80 and pumpkin PV72, which belong to the VSR family, also bind the C-terminal peptide of Brazil nut 2S albumin (Watanabe *et al.*, 2004). In the *atvsr1* loss-of-function mutant, 12S globulin and 2S albumin precursors accumulate in PSVs and a portion is secreted into the extracellular matrix (Shimada *et al.*, 2003). There also exists major portions of mature 12S globulin and 2S albumin in PSVs, which is likely due to the functional redundancy among AtVSR family members (Shimada *et al.*, 2003). In addition, the members of the AtVSR family exhibit cargo preference: *atvsr1 atvsr3* and *atvsr1 atvsr4* double-mutant plants exhibit a small increase in 12S globulin precursor accumulation but a large increase in 2S albumin precursor accumulation (Zouhar *et al.*, 2010). Moreover, seed germination is completely inhibited in *atvsr1 atvsr2* double-mutant plants, but just strongly affected in *atvsr3 atvsr4* double-mutant plants (Nguemelieu, 2006); this difference might be due to the differential reduction in PSV proteins related to the different mutations.

Besides VSRs, receptor homology region-transmembrane domain-RING-H2 (RMR) proteins have been identified to be involved in trafficking to PSVs. Unlike VSRs, RMRs serve as sorting receptors without recycling back to the Golgi. However, RMR proteins display a more variable localization. AtRMR1, AtRMR2, and OsRMR1 localize to the endoplasmic reticulum, Golgi, post-Golgi compartments such as the TGN, PSVs of embryos, and dense vesicles (DVs) (Park et al., 2005, 2007; Hinz *et al.*, 2007; Shen *et al.*, 2011; Occhialini *et al.*, 2018). The fact that the luminal domain of RMR1 and RMR2 bind specifically to the C-terminal vacuolar sorting signal of PSV proteins such as bean phaseolin, tobacco chitinase, and barley lectin (Park et al., 2005, 2007) suggests that AtRMRs may function as cargo receptors in the PSV trafficking pathway. In support of this idea, the co-expression of AtRMR1 deletion mutants strongly inhibits the trafficking of phaseolin to the PSVs and causes the secretion of phaseolin into the medium (Park *et al.*, 2005). However, neither *atrmr* single- nor double-mutant plants show the accumulation of 12S globulin or 2S albumin precursors (Zouhar *et al.*, 2010). In addition, the PSVs in these mutant plants exhibit no differences in size or morphology from those in wild-type plants. One possible explanation for these observations is that AtVSRs may compensate for the loss of function of AtRMRs in the PSV trafficking pathway. Another possibility is that AtRMRs are involved in the trafficking of certain storage proteins, such as phaseolin and chitinase, but not 12S globulin or 2S albumin.

Vesicles for the LV and PSVs

The cargoes sorted by sorting receptors need to be packaged into vesicles for trafficking to the next step in the pathway. CCVs have been shown to localize to the TGN and to contain LV cargoes (Kim *et al.*, 2010; Park *et al.*, 2013). The CCVs play a crucial role in trafficking in all eukaryotic cells (Ghosh *et al.*, 2003). Earlier studies showed that CCVs bud from the TGN and fuse with plant PVCs/multivesicular bodies (MVBs) (Hinz *et al.*, 1999; Tse *et al.*, 2004), suggesting that CCVs are involved in trafficking to the LV. However, other lines of evidence suggest that the TGN can mature into PVCs/MVBs that are involved in LV trafficking (Pedrazzini *et al.*, 2013; Viotti *et al.*, 2013). Thus, it is possible that LV cargoes are transported from the TGN to PVCs via multiple mechanisms, one employing vesicles such as CCVs and another involving the maturation of the TGN into PVCs/MVBs.

Vesicles involved in PSV trafficking in the Golgi or TGN appear to differ from those that participate in LV trafficking. DVs that are unique to plants and that carry several major PSV proteins have been identified (Chrispeels, 1983; Hohl *et al.*, 1996; Wenzel *et al.*, 2005). DVs that are slightly bigger than CCVs (Dhonukshe *et al.*, 2007) are characterized by high-density electron-opaque osmiophilic contents (Vitale and Hinz, 2005; Hinz *et al.*, 2007). In contrast to CCVs, which are generated in the TGN, the formation of DVs is initiated from the *cis*-Golgi, and they are released from the TGN. DVs either fuse directly with PSVs, or fuse first with PVCs/MVBs and then with PSVs (Hohl *et al.*, 1996; Jiang *et al.*, 2001; Tse *et al.*, 2004). PVCs characterized by the presence of RMR proteins

are called storage PVCs (Hillmer *et al.*, 2001). Additionally, aleurain, which predominantly shows up in CCVs, was also detected in DVs (Hinz *et al.*, 2007).

Adaptors for the LV and PSVs

One crucial factor in the sorting of cargo proteins into vesicles is adaptor proteins, which can interact with both cargoes/cargo receptors and vesicle coat proteins. Multiple types of adaptors have been identified to play roles in endosomal trafficking pathways. Of these adaptor proteins, heterotetrameric adaptor protein complexes (APs) play a key role in LV trafficking (Kim *et al.*, 2010; Park *et al.*, 2013). Arabidopsis contains five different APs, designated AP-1 to AP-5 (Happel *et al.*, 2004; Hirst *et al.*, 2011). The role of AP-1 in LV trafficking has been demonstrated using *ap1m2* plants, which have a mutation in *AP1M2*, one of two medium-sized adaptins. AP-1 co-localizes closely with clathrin to the TGN (Park *et al.*, 2013). In *ap1m2* plants, LV cargoes are secreted, indicating the role of AP-1 in LV trafficking (Park *et al.*, 2013). AP-3 also plays a role in LV trafficking (Lee *et al.*, 2007; Sanmartín *et al.*, 2007; Niihama *et al.*, 2009). *zip4*, a loss-of-function mutant of AP-3 μ adaptin, was identified as a suppressor of the phenotype of *zig1* plants. *zig1* is a mutant in *AtVTI11* involved in vesicle trafficking from the TGN to the PVC in LV trafficking (Surpin *et al.*, 2003; Sanmartín *et al.*, 2007; Niihama *et al.*, 2009). In addition, loss-of-function mutants of AP-3 β and δ adaptins also exhibit a suppressive effect on the *zig1* phenotype (Niihama *et al.*, 2009). Consistent with this finding, *protein affected trafficking* (*pat2*) plants, which have a mutation in AP-3 β adaptin, exhibit defects in LV function and in the transition of PSVs into LVs (Feraru *et al.*, 2010). Moreover, plants carrying the *protein affected trafficking 4* (*pat4*) mutation in AP-3 σ adaptin have a defect in LV protein sorting (Zwiewka *et al.*, 2011).

By contrast, AP-4 is implicated in trafficking to PSVs. *Green fluorescent seeds 4* (*gfs4*), *gfs5*, and *gfs6* mutant plants, identified from screening of seeds expressing a green fluorescent protein (GFP)-CT24 fusion, showed the secretion of GFP-CT24 to the apoplast (Fuji *et al.*, 2007, 2016; Ichino *et al.*, 2014; Teh *et al.*, 2015). GFP-CT24 consists of GFP fused to a signal peptide consisting of 24 C-terminal amino acid residues of the β-conglycinin α subunit, and serves as a marker of PSVs (Fuji *et al.*, 2007). The mutations in the *gfs4*, *gfs5*, and *gfs6* plants reside in the genes encoding AP-4 β, μ, and σ adaptins, respectively. Consistent with these phenotypes, a T-DNA insertion mutant of AP-4 ε adaptin shows the accumulation of 12S globulin precursors (Fuji *et al.*, 2016). Interestingly, AP-3 mutant *pat4* seeds exhibit correct processing of 12S globulin and 2S albumin, but contain abnormally sized PSVs (Zwiewka *et al.*, 2011), implying that AP-3 has additional roles in PSV trafficking.

Monomeric adaptors have also been shown to play roles in LV trafficking. Epsins are monomeric adaptors that play roles in vacuolar trafficking at various locations in animal and yeast cells (Mills *et al.*, 2003). Of these epsin homologs, EpsinR1 and EpsinR2 localize to the TGN (Song *et al.*, 2006; Lee *et al.*, 2007). EpsinR1 interacts with the subunit of AP-1 and AtVTI11, suggesting a role in LV trafficking. In contrast, EpsinR2 interacts with the δ subunit of AP-3 and AtVTI12 (Lee *et al.*, 2007; Sanmartín *et al.*, 2007). AtVTI12 is thought to play a role in protein trafficking to PSVs. Thus, EpsinR2 may be involved in PSV trafficking.

Molecular machinery involved in vesicle fusion associated with trafficking to the LV or PSVs

RAB GTPases

RAB GTPases, members of the family of small GTPases, play crucial roles in vesicle trafficking. In particular, they are involved in determining the specificity of vesicle fusion to target organelles (Saito and Ueda, 2009). Arabidopsis contains 57 RAB GTPases that are classified into eight subgroups (Woollard and Moore, 2008). RAB GTPases belonging to subgroups RAB5, RAB6, RAB7, RAB8, and RAB11 function in post-Golgi trafficking pathways (Rutherford and Moore, 2002; Sohn *et al.*, 2003). Recent studies showed that RAB5, RAB7, and RAB11, acting at different steps of the pathway, interfere with the trafficking of LV and PSV cargoes indiscriminately (Bottanelli et al., 2011, 2012). *rab11* plants show a defect in the arrival of cargo at prevacuoles, indicating the role of RAB11 at an early transport step, whereas *rab7* plants show a defect in the final delivery to the vacuole, with an increase in levels of cargo to the PVC (Bottanelli et al., 2011, 2012). The RAB5 subgroup (also called RABF) includes three members: the plant-unique ARA6, also known as RABF1, and the canonical RAB5 members ARA7 and RHA1, also called RABF2b and RABF2a, respectively (Saito and Ueda, 2009). Canonical RAB5s and ARA6 localize to distinct but overlapping populations of multivesicular endosomes, and are thought to be involved in the post-Golgi vacuolar trafficking pathway (Bolte *et al.*, 2000; Sohn *et al.*, 2003; Ueda *et al.*, 2004; Ebine *et al.*, 2011). RHA1 plays a critical role in trafficking from the PVC to the LV (Sohn *et al.*, 2003). The soluble vacuolar cargo proteins Spo:GFP and AALP:GFP were not delivered to the central vacuole, but instead accumulated primarily in the PVC and tonoplast or were secreted into the medium when a Rha1 dominant-negative mutant was co-expressed (Sohn *et al.*, 2003).

The RAB5 subgroup also plays a role in the PSV trafficking pathway. *rha1 syp22-1* double-mutant plants accumulate 12S globulin precursors and secrete GFP-CT24 (Ebine *et al.*, 2011). In the knockout mutant of *VPS9a*, an activator of RAB5, glutelin precursors accumulate and localize to the apoplast in the form of DVs (Fukuda *et al.*, 2013). Furthermore, OsVPS9A interacts with OsRAB5A to regulate DV-mediated post-Golgi

pro-glutelin trafficking to the PSVs in rice (Liu *et al.*, 2013). ENDOSOMAL RAB EFFECTOR WITH PX-DOMAIN (EREX) and EREX-LIKE1 (EREL1) are RAB5 effectors (Sakurai *et al.*, 2016). Seeds of *erex erel1* double-mutant plants accumulate 12S globulin precursor but not 2S albumin precursor (Goh *et al.*, 2007; Sakurai *et al.*, 2016), indicating that EREX and EREL1 are involved in trafficking to the PSVs. Consistent with this notion, both *vps9a-2* and *erex erel1* embryos show secretion of GFP-CT24 to the apoplast but no changes in the morphology of PSVs (Sakurai *et al.*, 2016). In rice, the Rab5a effector GAP5a, which is homologous to Arabidopsis EREX, mediates tethering and membrane fusion of DVs with PSVs in endosperm (Ren *et al.*, 2020). *erex erel1* double-mutant plants also exhibit severe dwarfism, indicating additional functions of RAB5 in vegetative tissues. The expression of a dominant-negative Rab7 mutant (RABG3f^{T22N}) causes enlargement of the PVC and deformation of the vacuolar morphology (Cui *et al.*, 2014). Consistent with the abnormal morphology of these organelles, in the presence of RABG3f^{T22N}, soluble vacuolar cargoes are mistargeted to the apoplast, and vacuolar storage proteins are not properly degraded during the germination process (Cui *et al.*, 2014). In addition, Rab7 (RABG3f) plays a crucial role in the biogenesis of PSVs; *rab7* plants showed fragmented PSVs in mature embryos, accumulation of unprocessed 12S globulin, and mis-secretion of GFP-CT24 into the extracellular space (Ebine *et al.*, 2014). Sextuple-mutant *rabG3a-f* plants that have mutations in the members of the *RAB7* subgroup exhibited semi-dwarfism at the early developmental stages (Ebine *et al.*, 2014) and severe growth defects in seedlings (Cui *et al.*, 2014). Mutations in *MON1* or *CCZ1*, which encode RAB7 guanine nucleotide exchange factors (GEFs) in Arabidopsis, cause the secretion of GFP-CT24 and 12S globulin to the apoplast as well as a defect in the morphology of PSVs (Cui *et al.*, 2014; Ebine *et al.*, 2014), supporting the role of RAB7 in PSV trafficking. The RAB5- and RAB7-related double mutants *erex erel1* and *ccz1a ccz1b* also show severe defects in plant growth, consistent with the role of RAB5 and RAB7 in LV trafficking (Cui *et al.*, 2014; Ebine *et al.*, 2014). Thus, RAB5 and RAB7 act in both LV trafficking in vegetative cells and PSV trafficking in seed cells.

SNARE proteins

SNARE proteins, which play central roles in the vesicle fusion process, are another important factor in specificity determination (Wang *et al.*, 2017; Yoon and Munson, 2018). Many SNAREs have been shown to localize to endosomes (Carter *et al.*, 2004). Two discrete SNARE complexes, SNARE-VTI11 (SNVTI11) and SNARE-VTI12 (SNVTI12), have been studied to determine their roles in vacuolar trafficking. Some earlier studies showed that SNVTI11 and SNVYI12 are involved in trafficking to the LV and PSVs, respectively (Sanmartín *et al.*, 2007). The SNVTI11 complex, which consists of AtVTI11 and AtSYP22 (or its paralog AtSYP21), AtSYP51, and AtVAMP727, resides in the PVC and is critical for PVC-to-LV trafficking. *atvti11* plants exhibit abnormal morphology and biogenesis of the LV together with a defect in gravitropism (Sanderfoot *et al.*, 2001; Hashiguchi *et al.*, 2010). Two SYP2 family members, SYP21 and SYP22, found in the SNVTI11 complex may perform functions that are distinct from each other. Overexpression of SYP21 causes homotypic fusion of the PVC and traps vacuolar cargoes in the PVC in tobacco cells, whereas overexpression of SYP22 does not lead to such effects (Foresti *et al.*, 2006). In contrast, Arabidopsis *syp21* plants do not exhibit any abnormal phenotype, while *syp22* plants show semi-dwarfism, late flowering, and a poorly developed vascular network in leaves (Shirakawa *et al.*, 2010; Uemura *et al.*, 2010). These results suggest that the SNVTI11 complex plays a role in LV trafficking and/or processes involved in vegetative growth. The other SNVTI12 complex, consisting of VTI12, SYP41 (SYP42), and SYP61, resides in the TGN (Sanmartín *et al.*, 2007; Ebine *et al.*, 2008; Wu *et al.*, 2016). Subsequent studies showed that the SNVTI11 complex is also involved in trafficking to PSVs. Various double mutants of the components of the SNVTI11 complex (*vamp727 syp22*, *syp21 syp22*, and *syp22 syp23*) accumulate precursors of both 12S globulin and 2S albumin, accompanied by abnormal morphology of the PSVs, and cause the secretion of PSV proteins to the extracellular space (Ebine *et al.*, 2008; Shirakawa *et al.*, 2010). *atvti11 atvti12* double mutants are lethal, suggesting the functional redundancy of VTI11 and VTI12 (Sanmartín *et al.*, 2007). A functional difference has also been found between the two members (SYP51 and SYP52) of the SYP5 family. Both SYP51 and SYP52 localize to the vacuolar membrane and the TGN when expressed in Arabidopsis leaf cell protoplasts. SYP51 plays a role in post-Golgi transport of GFP-chitinase, whereas SYP52 plays a role in the transport of aleurain-GFP to the LV (De Benedictis *et al.*, 2013). It is possible that SYP51 functions in trafficking to the PSVs in seed cells and SYP52 functions in trafficking to the LV in leaf cells.

Conclusions and perspective

It is generally believed that the LV and PSVs are two different types of organelle. In fact, they show a great deal of difference in terms of their morphology, biochemical composition, and functions. They are also mostly present in different cell types (Xiang *et al.*, 2013). However, as summarized here, there is a great deal of interconnection between the LV and PSVs in terms of their trafficking machinery. Thus, one fundamental question is that of what defines the LV and PSVs. Additionally, at certain time points of plant growth, such as during germination, the lytic vacuolar pathways are used to deliver to the PSVs proteins that are required for the degradation of protein

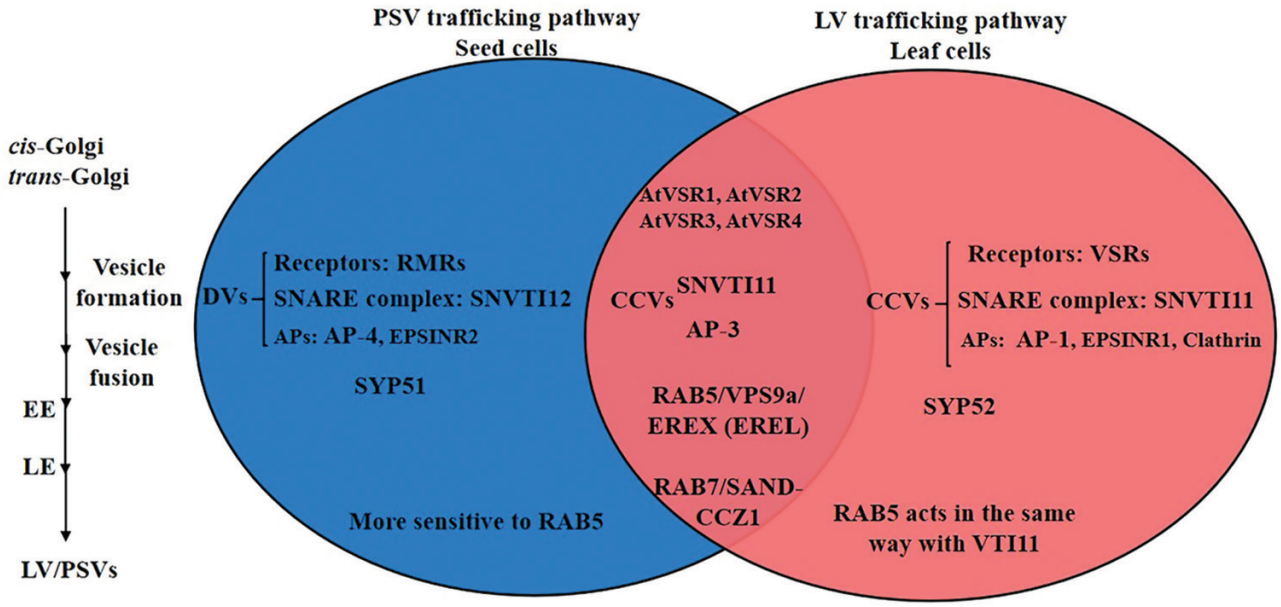

Fig. 2. Components of the trafficking machinery of the LV and PSV trafficking pathways can be categorized into three groups. LVs and PSVs appear to be different types of organelle. However, many components of the trafficking machinery are shared by the trafficking pathways to both organelles. In addition, certain components are specific to either the LV or the PSV pathway.

storage proteins and acidification of the PSVs. Close examination of the trafficking factors reveals that they can be divided into two groups: one comprising factors shared by both the LV and PSV trafficking pathways, and the other comprising factors that are specific or preferential to one or other of the trafficking pathways (Fig. 2). So, one interesting and fundamental question would be that of how the trafficking pathways of the LV and PSVs have ended up having such a relationship. This question may be related to the origin of vacuoles. Thus, one direction for future study would be to elucidate their evolution in plants. In the evolution of these organelles, the most crucial process should be the establishment of the sorting signal and the trafficking machinery. Thus, the trafficking machinery may contain certain clues toward answering the question. Another direction for future research would be to elucidate the process of PSV biogenesis at the molecular and cellular levels. Recently, Feeney *et al.* (2018) showed that PSVs are derived from the embryonic vacuole via reprogramming during seed maturation. These future studies may provide an answer to the fundamental question of whether the LV and PSV are two truly different organelles, or the same organelle that performs different functions depending on the type of cell in which it resides.

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Author contributions

IH and HLu conceived the concept of the work; XZ collected the references and wrote most of the manuscript under the guidance of HLi.

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