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# Plant Vacuoles

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## Keywords

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## Abstract

Plant vacuoles are multifunctional organelles. On the one hand, most vegetative tissues develop lytic vacuoles that have a role in degradation. On the other hand, seed cells have two types of storage vacuoles: protein storage vacuoles (PSVs) in endosperm and embryonic cells and metabolite storage vacuoles in seed coats. Vacuolar proteins and metabolites are synthesized on the endoplasmic reticulum and then transported to the vacuoles via Golgi-dependent and Golgi-independent pathways. Proprotein precursors delivered to the vacuoles are converted into their respective mature forms by vacuolar processing enzyme, which also regulates various kinds of programmed cell death in plants. We summarize two types of vacuolar membrane dynamics that occur during defense responses: vacuolar membrane collapse to attack viral pathogens and fusion of vacuolar and plasma membranes to attack bacterial pathogens. We also describe the chemical defense against herbivores brought about by the presence of PSVs in the idioblast myrosin cell.

## Contents



## 1. INTRODUCTION

Vacuoles are the largest compartments in plant cells. Plant vacuoles are separated into two different compartments: storage and lytic (**Figure 1**). A typical storage compartment is the protein storage vacuole (PSV) found in seeds. During seed maturation, large amounts of seed storage proteins are actively synthesized on the endoplasmic reticulum (ER) and efficiently delivered into vacuoles, where they remain in PSVs until they are mobilized during germination. Thus, developing seeds have been used to study the mechanism underlying intracellular transport of vacuolar proteins. These studies have resulted in several important discoveries, including the Golgi-independent transport pathway, vacuolar sorting receptor (VSR), and vacuolar processing enzyme (VPE) responsible for vacuolar protein maturation and activation. After seed imbibition, PSVs are converted into lytic vacuoles.

Lytic vacuoles occur in vegetative tissues and are also called vegetative vacuoles. Lytic enzymes, such as proteinases and nucleases, and defense proteins called pathogenesis-related proteins accumulate in lytic vacuoles. Lytic enzymes and defense proteins defend plants from infections by viruses and avirulent bacteria, respectively. Vacuolar defenses are mediated by vacuolar membrane dynamics; viruses are attacked via vacuolar membrane collapse, and bacteria are attacked via fusion between the vacuolar and plasma membranes. Disruption of the vacuolar membrane releases the vacuolar lytic enzymes and induces hypersensitive cell death. Lytic vacuoles mediate programmed cell death (PCD) in plants. This differs from PCD in animals, which is mediated by macrophages.

Plants also develop specialized vacuoles containing large amounts of specific materials in selected tissues. Vacuoles in the seed coat inner integuments accumulate flavonoids, which protect the embryo from harmful ultraviolet light. In Brassicaceae, the vacuoles in myrosin cells along leaf veins accumulate myrosinases (β-thioglucoside glucohydrolases), which are used for chemical defense against herbivores. In this review, we discuss the molecular machinery that transports soluble

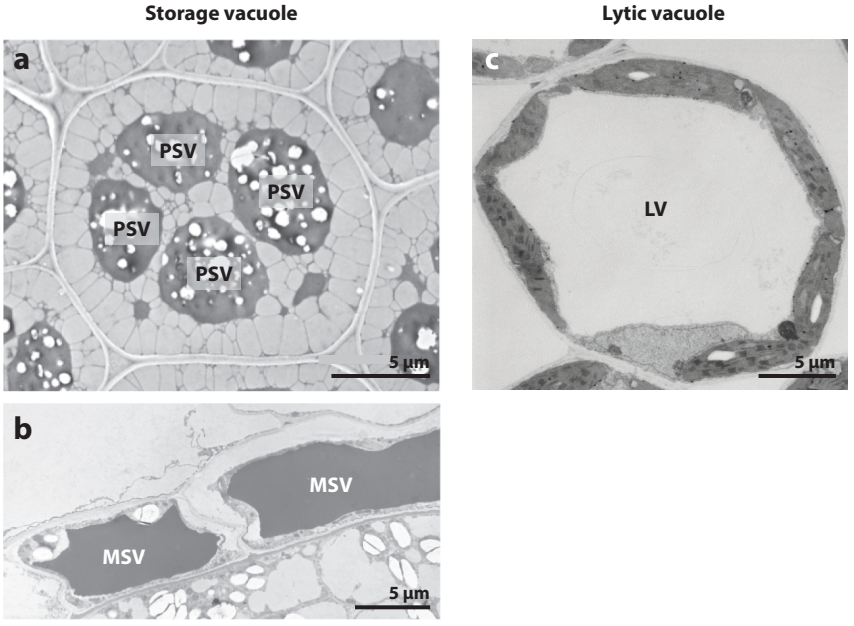


**Figure 1**

Ultrastructures of three types of plant vacuoles. (*a*) Protein storage vacuoles (PSVs) in cotyledon cells of *Arabidopsis thaliana* seed. They abundantly accumulate storage proteins 12S globulin and 2S albumin. (*b*) Metabolite storage vacuoles (MSVs) in the inner integument of *A. thaliana* seed coats. They store flavonoids to protect the embryo from harmful ultraviolet light. (*c*) Lytic vacuole (LV) in pumpkin cotyledon cells.

proteins and metabolites into the vacuoles, the vacuolar processing system that converts vacuolar protein precursors into their mature forms, the vacuolar membrane dynamics that mediate defense responses, and the development of myrosin cells containing specialized vacuoles.

## 2. TRANSPORT OF SOLUBLE PROTEINS AND METABOLITES INTO THE VACUOLES

### 2.1. Golgi-Independent Transport Pathway

Vacuolar proteins initially target the ER through their hydrophobic signal or transmembrane sequence. Some soluble proteins are transported to vacuoles through the Golgi apparatus, whereas others are not. In developing pumpkin cotyledons, storage protein precursors synthesized on the ER are transported to the vacuole via the high-density compartments (44), which were later called precursor-accumulating vesicles (45). Precursor-accumulating vesicles are involved in Golgi-independent aggregation sorting, which is a novel type of vacuolar sorting (**Figure 2**). In aggregation sorting, vacuolar storage proteins form protein aggregates within the ER to produce precursor-accumulating vesicles. Similar protein aggregates were observed in the ER (designated with different names) in several plants. On the other hand, Golgi-dependent aggregation sorting of storage proteins via dense vesicles was observed in developing pea cotyledon (54). The formation of protein aggregates appears to be influenced by the quality and quantity of cargo proteins. Luminal environmental parameters, such as ionic strength, may affect cargo protein solubility in organelles. These parameters appear to determine when and where the protein aggregates form.

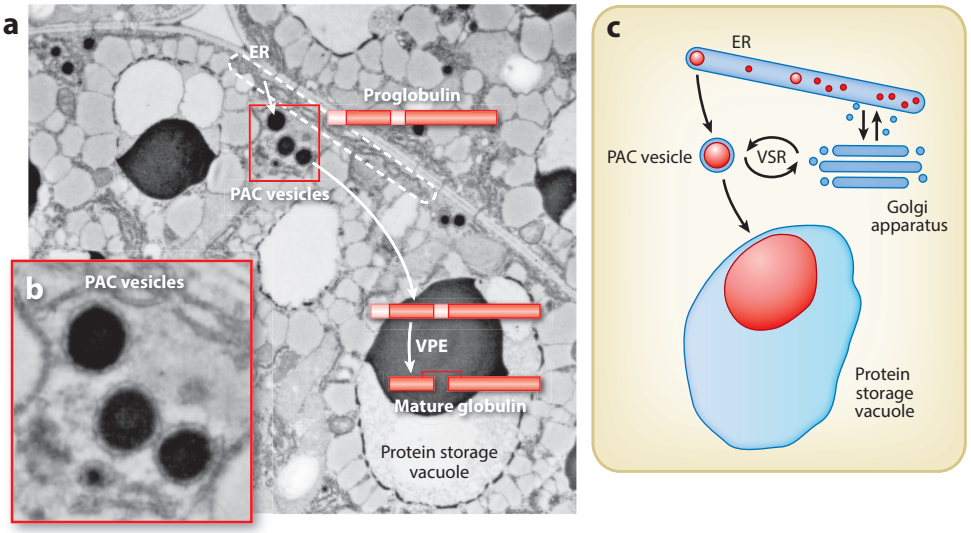


**Figure 2**

Golgi-independent pathway into protein storage vacuoles. (*a*) An electron micrograph of developing pumpkin seed cells. Many precursor-accumulating (PAC) vesicles are shown. A precursor form of the storage protein (proglobulin) synthesized on the endoplasmic reticulum (ER) is delivered into the vacuoles via PAC vesicles and then is converted into the mature form by vacuole processing enzyme (VPE). (*b*) An enlarged micrograph of PAC vesicles. (*c*) Golgi-independent pathway from the ER into the vacuoles via PAC vesicles. Actively synthesized precursors form aggregates in the ER and are packed in the PAC vesicles. They are transported into the vacuoles in a Golgi-independent manner. Abbreviation: VSR, vacuolar sorting receptor.

Protein aggregates in the ER have been observed in developing pumpkin (45), castor bean (45), soybean (91), wheat (79), and rice (128). Aggregate formation in the ER of developing soybean cotyledons depended on protein composition and developmental stage. Soybean seeds express two kinds of globulins, glycinin (11S globulin) and β-conglycinin (7S globulin). Protein aggregates in the ER [designated protein bodies (PBs) in this article] accumulated to high levels in mutants lacking β-conglycinins, whereas they were rarely observed in mutants lacking glycinins (91). Glycinins are preferentially localized in PBs in wild-type seeds. These observations indicate a direct involvement of glycinins in PB formation. PBs are abundant in wild-type soybean at later stages of seed development, but were not observed in mature dry seeds, suggesting that ER aggregates are transported into PSVs.

The mechanism of protein aggregate transport from the ER to PSVs has not been elucidated, but it has been predicted to bypass the Golgi apparatus in wheat (79) and pumpkin (45). Recent studies demonstrated Golgi-independent ER-to-vacuole transport in tobacco leaf cells expressing the cardoon vacuolar aspartic proteinase cardosin A (97) or the human α-mannosidase MAN2B1 (21). These observations suggest that plant cells can deliver ER contents to the vacuoles in a pathway that bypasses the Golgi apparatus. A recent study proposed that ER aggregates are delivered to the PSVs by an autophagy-like process (89). The turnover of ER membrane and its contents in response to ER stress is mediated by an autophagy pathway that is mediated by AUTOPHAGY-RELATED 8 (ATG8)-positive bodies in *Arabidopsis* roots (83). Similar ER-to-vacuole transport is induced by carbon starvation in *Arabidopsis* seedlings (55). Plant-specific ATG8-interacting proteins 1 and 2 are involved in this transport pathway. These combined results suggest that plants have evolved a novel type of autophagy pathway that may mediate the delivery of ER protein

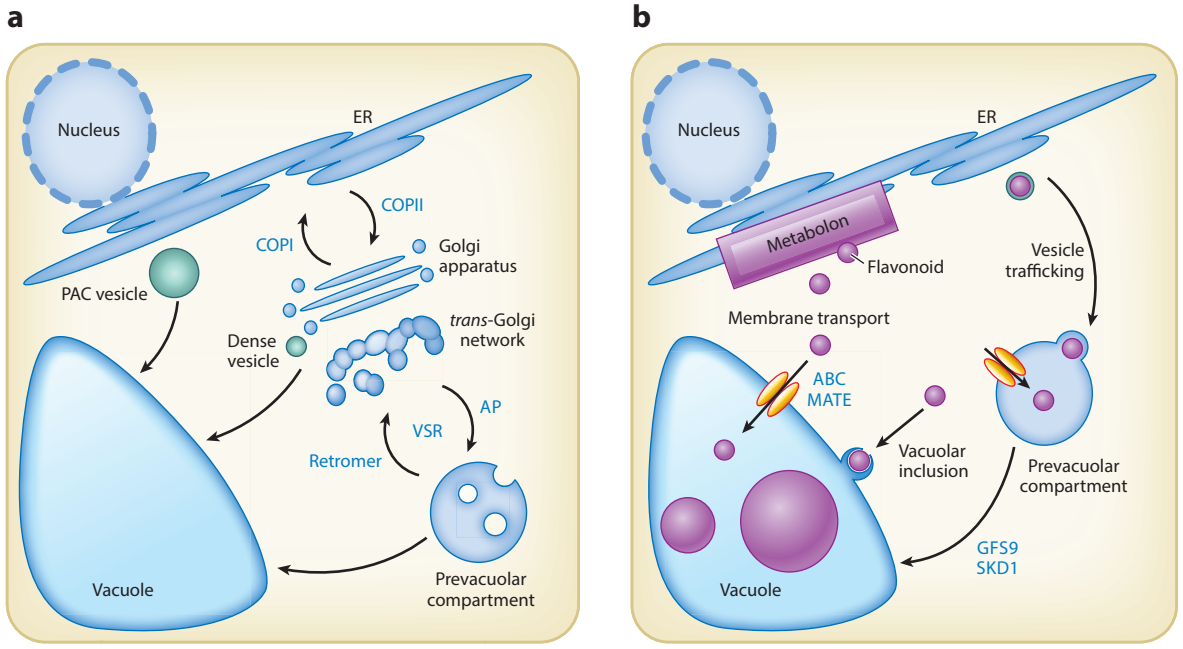


**Figure 3**

Membrane traffic from the ER to vacuoles. (*a*) Transport of soluble proteins into the vacuoles. Vacuolar proteins are synthesized at the ER and then are transported to vacuoles via membrane trafficking. Seed storage proteins are transported to vacuoles by PAC vesicles and dense vesicles. (*b*) Transport of flavonoids into the vacuoles. Flavonoids (*purple circles*) are synthesized by the multi-enzyme complex, flavonoid metabolon, which is located on the ER membrane. Cooperation among membrane transport, vesicle trafficking, and vacuolar development is required for flavonoid accumulation in the vacuole. It has also been proposed that the vacuolar membrane surrounds flavonoids to take up into the vacuole. Abbreviations: ABC, ATP-binding cassette protein; AP, adaptor protein; COPI, coat protein complex I; COPII, coat protein complex II; ER, endoplasmic reticulum; GFS9, GREEN FLUORESCENT SEED 9; MATE, multidrug and toxic compound extrusion-type transporter; PAC, precursor-accumulating; SKD1, SUPPRESSOR OF $K^+$ TRANSPORT GROWTH DEFECT1; VSR, vacuolar sorting receptor.

aggregates to PSVs. Although autophagy is the major pathway for macromolecule degradation in vacuoles, plants might use autophagy for delivery of storage macromolecules to vacuoles in developing seeds.

## 2.2. Membrane Traffic Between the Endoplasmic Reticulum and the Golgi Apparatus

Transport of proteins and lipids between the ER and the Golgi apparatus is facilitated by the membrane trafficking system. Anterograde ER-to-Golgi transport of vacuolar proteins starts with protein export at the ER exit sites, which is mediated by coat protein complex II (COPII) machinery (**Figure 3*a***). By contrast, retrograde Golgi-to-ER transport is mediated by coat protein complex I (COPI) vesicles (**Figure 3*a***).

**2.2.1. COPII vesicles are involved in anterograde transport.** COPII coat assembly is initiated by the small GTPase Sar1, which is activated by Sec12, an ER-localized guanine exchange factor (GEF) (6). Activated Sar1 interacts with Sec23 to recruit the Sec23-Sec24 inner-coat complex (10) and subsequently recruits the Sec13-Sec31 outer-coat complex (11). Sec24 participates in

cargo recognition (90). Another key factor for COPII formation is Sec16, which regulates COPII coat assembly but is excluded from the COPII coat (145). Tethering of COPII vesicles to the Golgi apparatus is mediated by the transport protein particle I (TRAPPI) complex, p115, and the small GTPase Rab1, whereas fusion of the vesicles to the Golgi apparatus is mediated by SNARE proteins (Sed5, Bosf1, and Bet1 on the Golgi apparatus and Sec22 on the vesicles) (85). The TRAPPI complex functions as a Rab1 GEF and recruits activated Rab1 on COPII vesicles (15). p115 is localized on *cis*-Golgi and recognizes activated Rab1 (3). p115 also interacts with Sec22 and Bet1 and facilitates SNARE complex formation (136), which leads to the fusion of COPII vesicles with the Golgi apparatus.

COPII components are conserved among plants. The *Arabidopsis* genome encodes five SAR1, seven SEC23, three SEC24, two SEC13, two SEC31, and two SEC16 proteins (106). Overexpression of a dominant-negative form of SAR1, which inhibits the normal function of SAR1, blocks the vacuolar protein sporamin from exiting the ER (130), suggesting that the transport of some vacuolar proteins from the ER to the vacuole via the Golgi apparatus depends on COPII machinery. This was further supported by a genetic analysis of seed storage protein transport in *Arabidopsis* (114). One of the isolated mutants had a mutation in *SEC16A/MAG5* and exhibited defective precursor storage protein exit from the ER, which led to the formation of abnormal precursor protein aggregations within the ER (127). *Arabidopsis* SEC16A displays low sequence similarity to human and yeast Sec16 proteins, although other COPII components display high similarity to human and yeast proteins. However, SEC16A participates in regulating COPII formation in ways similar to the SEC16 proteins of other eukaryotes. Recent studies report that plant COPII components have diverse functions. Despite high sequence identity between SAR1A and SAR1C, SAR1A interacts with SEC23A but not with SEC23B, whereas SAR1C interacts with SEC23B but not with SEC23A. The dominant-negative form of SAR1C inhibits ER export of the vacuolar protein aleurain, whereas that of SAR1A does not affect aleurain transport but is involved in the ER stress response (148). Plant p115/MAG4 is involved in tethering COPII vesicles at the *cis*-Golgi (64, 129). It remains to be determined whether the TRAPPI complex is involved in ER–Golgi transport. *Arabidopsis* encodes two groups of RAB1 orthologs (one RABD1 and three RABD2). Dominant-negative forms of RABD1 and RABD2A inhibit ER export of vacuolar cargo. However, RABD1 and RABD2 might have distinct functions in ER–Golgi transport (98).

**2.2.2. COPI vesicles are involved in retrograde transport.** Retrograde transport is necessary for recycling the cargo receptors involved in anterograde transport and retrieving ER resident proteins that leak from the ER (6). COPI vesicle formation is initiated by the small GTPase Arf1, which is activated by GBF1 containing a Sec7 domain (14). Activated Arf1 recruits each member of a heptameric (α, β, β′, γ, δ, ε, ζ) coat protein complex, in which α-, β′-, γ-, and δ-COP participate in cargo recognition (126). COPI vesicle tethering is mediated by the Dsl1 complex, which consists of Dsl1, Tip20, and Sec39. The Dsl1 complex recruits COPI vesicles onto the ER membrane (154) and facilitates the formation of SNARE complexes (Ufe1, Sec20, and Use1 on the ER membrane and Sec22 on the vesicles) (104).

COPI machinery is conserved in plants: two α-COP, two β-COP, three β′-COP, one γ-COP, one δ-COP, two ε-COP, three ζ-COP, and several ARF1 (106). GTP-locked ARF1 inhibited the recycling of p24 cargo receptors back to the ER, confirming that retrograde transport to the ER is mediated by COPI machinery in plants (77). GTP-locked ARF1 also inhibited the ER export of sporamin (131). The *Arabidopsis* GBF1-type ARF1-GEF mutant *gnom-like1* (*gnl1*) also displayed defects in vacuolar trafficking of aleurain (133), suggesting that anterograde transport depends on retrograde transport. The tethering complex and SNAREs are also conserved in plants. The ER exit of seed storage proteins is affected in seeds of *mag2*, a mutant of plant Tip20 (81). MAG2

localizes at the ER and interacts with the SNARE proteins SYP81/UFE1 and SEC20 (81). Three MAG2-interacting proteins (MIPs) have been identified: MIP1 and MIP2 are plant homologs of Dsl1 and Sec39, respectively, whereas MIP3 is a novel Dsl1 complex component (80). MIP3 contains a Sec1 domain that is conserved in Sec1/Munc18 proteins, which facilitates SNARE complex formation (105). MIP3 seems to be conserved among animals and plants but not in yeasts (80).

**2.2.3. Endoplasmic reticulum exit site and rapidly moving Golgi apparatus.** In plant cells, the Golgi apparatus is dispersed throughout the cell and undergoes rapid movement via actin- and myosin-based transport (13). Three models have been formulated to understand how proteins are efficiently and accurately transported to rapidly moving Golgi: kiss-and-run, secretory unit, and hybrid. These models focus on the relationship between Golgi and ER exit sites, the ER subdomain where COPII coat assembly occurs (**Figure 3*a***). The kiss-and-run model considers that moving Golgi apparatus pause and transiently associate with stable ER exit sites dispersed over the ER; then, cargo is transported from the ER to the Golgi apparatus during this transient association (94). This model is supported by evidence that SEC13-GFP-labeled ER exit sites outnumber the Golgi apparatus, and some ER exit sites are associated with the Golgi apparatus whereas others are not (144). The secretory unit model considers that moving Golgi apparatus continuously associate with ER exit sites, which facilitates ER export even to moving Golgi stacks (20). This model is based on the following two observations: SAR1-YFP moves together with its associated Golgi apparatus (20), and FRAP analysis shows that protein transport to Golgi apparatus occurs while both organelles are moving (20). The hybrid model integrates the kiss-and-run and secretory unit models and considers that some ER exit sites are associated and move together with Golgi apparatus, whereas others are independent of the Golgi apparatus (62). However, the relationships between Golgi-associated and Golgi-independent ER exit sites remain to be clarified in this model. One possibility is that there are two fundamentally different types of ER exit sites. For example, most SAR1A is closely associated with the Golgi apparatus, whereas most SAR1C is not (148). Other possibilities have been proposed, such as that ER exit sites are Golgi independent during early stages before the formation of the Golgi apparatus (12) or before ER exit sites encounter and become associated with the Golgi apparatus (61). A recent study in yeast reports that the Golgi apparatus approaches ER exit sites to facilitate the ER exit transport of cargo proteins (74). In plants, most COPII vesicles are observed near the Golgi apparatus (64), suggesting that ER exit site association with the Golgi apparatus may be important for anterograde trafficking.

## 2.3. Vacuolar Sorting Receptors

Soluble protein delivery to vacuoles requires the presence of specific signals and their receptors (65). Soluble vacuolar proteins carry vacuolar-sorting signals that enable precise targeting to vacuoles. There are three types of vacuolar-sorting signals: sequence-specific vacuolar-sorting signal (ssVSS), C-terminal vacuolar-sorting signal (ctVSS), and physical-structure vacuolar-sorting signal (psVSS). The ssVSS contains conserved sequences such as the NPIR motif found in barley thiol protease aleurain and sweet potato sporamin. The ctVSS has been identified in C-terminal regions of barley lectin and tobacco chitinase and is often enriched in hydrophobic amino acids. The psVSS has been identified in some storage proteins, such as legumin, an 11S globulin from field bean, but the common molecular feature in the psVSS amino acid sequence has not been determined.

Several VSRs that bind to vacuolar-sorting signals have been identified, including pea BP-80 (96), pumpkin PV72 (115), and *Arabidopsis* VSR1/AtELP (2). Pea BP-80 was the first VSR identified; it binds in vitro to barley aleurain containing an NPIR motif. BP-80 also binds to the ctVSS of Brazil nut 2S albumin. Similarly, pumpkin PV72 binds to the NPIR motif of aleurain

and the C-terminal peptide of pumpkin 2S albumin (138). These results suggest that VSRs have broad specificity for ligands. The VSR-type receptors are found in Viridiplantae (land plants and green algae) but not in rhodophytes (red algae) or glaucophytes (9). The *Arabidopsis* genome encodes seven members of the VSR family. A loss-of-function mutant of *VSR1* missorts vacuolar storage proteins by secreting them from cells in *Arabidopsis* seeds (113). VSRs of land plants can be classified into three subfamilies (22), which do not appear to be functionally equivalent (155). VSR-like genes also have been identified in stramenopiles (brown algae, diatoms, and oomycetes) and alveolates (ciliates), which might have been acquired through horizontal or endocytotic gene transfer from green algae (9). These VSR-like proteins display significant sequence similarities to Viridiplantae VSRs but lack epidermal growth factor (EGF) repeats that are conserved in the luminal domain of Viridiplantae VSRs.

VSRs are type I integral membrane proteins containing a large luminal domain (approximately 550 amino acids) followed by a single transmembrane domain and a short cytoplasmic tail (approximately 40 amino acids). The transmembrane domain and cytosolic tail are required for the homomeric interaction of VSR1, which is crucial for vacuolar protein sorting (67). The VSR luminal domain can be divided into three subdomains: a protease-associated domain at the N terminus, a central domain in the middle, and three EGF repeats at the C terminus. Vacuolar-sorting signals bind the protease-associated and central domains (16, 86, 137). *N*-glycosylation of the VSR luminal domain affects the binding affinity for vacuolar-sorting signals (112). The EGF repeats might regulate ligand protein-receptor dissociation (16). The ligand-receptor interaction depends on calcium (116). The third EGF-like motif has a consensus sequence for calcium binding; therefore, it may modulate a calcium-dependent conformational change of the VSR structure to form a functional pocket for cargo binding.

The VSR cytosolic tail contains multiple signals that are necessary for receptor trafficking. The YMPL sequence is highly conserved among VSR isoforms (22). This tyrosine-based motif is believed to function as an anterograde trafficking signal by interacting with a medium (μ) subunit of the adaptor protein (AP) complex. Yeast two-hybrid analysis revealed a specific interaction between the tyrosine-based motif and μ subunits of AP-1 and AP-4 (31, 33). A Y-to-A substitution in the YMPL sequence affects both intracellular receptor localization and vacuolar protein sorting (19, 27). Mutations in the AP-4 subunit affect VSR1-mediated vacuolar storage protein sorting in *Arabidopsis* seeds (31). The VSR cytosolic tail also contains retrograde trafficking sequence motifs for receptor recycling. The best-characterized retrograde trafficking motif is the conserved Ile-Met dipeptide located immediately upstream of the tyrosine-based motif. Several lines of evidence suggest that the retromer complex has a central role in VSR retrograde trafficking (66, 100, 114, 143).

VSRs are believed to bind their ligands at the *trans*-Golgi network (TGN) and deliver them to the prevacuolar compartment (PVC). In this scenario, AP-1 and/or AP-4 regulates anterograde transport of the ligand-receptor complex at the TGN to the PVC, whereas the retromer complex regulates retrograde transport of ligand-free receptor to the TGN. Künzl et al. (73) recently used nanobody fusion proteins to show that VSRs bind ligands at the ER and Golgi but not at the TGN. These observations suggest that VSRs are required for ligand transport from the ER and/or Golgi to the TGN, whereas post-TGN ligand trafficking toward the vacuole is suggested to occur by default and independently of VSRs (107, 108). Further studies are needed to elucidate the exact actions of VSRs in vacuolar protein trafficking.

## 2.4. Flavonoid Transport into Seed Coat Vacuoles

Plant vacuoles accumulate a variety of secondary metabolites. Flavonoids are the largest group of secondary metabolites and are widely distributed in land plants. Flavonoids have multiple roles

in plants. Anthocyanins and flavonols determine flower and fruit color, which attract pollinators and seed dispersers (36). Flavonoids also function as developmental cues and defense compounds against environmental stresses (47, 132).

Flavonoids accumulate in seed coats to protect embryos and endosperms from external stresses such as UV radiation and pathogen infection. A large amount of proanthocyanidins, a polymer type of flavonoid, accumulates in the vacuoles of the first cell layer of the inner integument (which is the innermost cell layer of the seed coat), resulting in the dark brown color of *Arabidopsis* seeds (78). Genetic screens have isolated a series of *Arabidopsis thaliana* mutants with pale tan-colored seeds, which have been named as *transparent testa* (*tt*) or *tannin-deficient seed* (*tds*), according to their phenotype (1, 70). Characterization of these mutants has revealed the mechanisms of flavonoid biosynthesis (78).

Flavonoid biosynthesis starts at the cytoplasmic face of the ER membrane. Most enzymes involved in flavonoid biosynthesis form a molecular complex called the flavonoid metabolon (78, 139). The synthesized flavonoids are transported into the vacuole, where flavonoids undergo glycosylation, acylation, and polymerization into final products. The molecular mechanisms mediating flavonoid transport from the ER to the vacuole are under investigation (**Figure 3*b***).

**2.4.1. Membrane transporter–mediated flavonoid transport.** Two types of transporters mediate flavonoid transport into the vacuole: ATP-binding cassette (ABC) protein and multidrug and toxic compound extrusion (MATE)-type transporter. The ABC protein transports its substrate coupled to ATP hydrolysis. The Class C ABC proteins MRP3 in *Zea mays* and ABCC1 in *Vitis vinifera* (grapevine) are involved in transporting anthocyanin into the vacuole (29, 35).

The *Arabidopsis* MATE-type transporter TT12 contributes to seed coat pigmentation (23). TT12 localizes on the vacuolar membrane and transports epicatechin 3′-*O*-glucoside, which is a proanthocyanidin precursor (88, 151). *Medicago truncatula* MATE1, an ortholog of TT12, also transports the precursor across the vacuolar membrane (151).

These MATE transporters are proton-coupled flavonoid/$H^+$ antiporters. Vacuolar acidification driven by proton pumps should have a crucial role in flavonoid uptake into the vacuole. The *Arabidopsis* P-type $H^+$-ATPase TT13/AUTOINHIBITED $H^+$-ATPase isoform 10 (AHA10) localizes on the vacuolar membrane and affects proanthocyanidin accumulation, possibly because TT13/AHA10 generates a proton gradient across the vacuolar membrane that is required for TT12-mediated transport (5, 8). A similar mechanism in *Petunia hybrida* is observed; the P-type $H^+$-ATPases PH1 and PH5 regulate vacuolar pH, and lack of either PH1 or PH5 alters the petal color (26, 135).

**2.4.2. Flavonoid transport via vesicle trafficking.** The presence of membrane compartments containing flavonoids stimulates vesicle trafficking of flavonoids. In the tapetum cell of *Brassica napus* and *Arabidopsis*, flavonoids are sequestered into ER-derived vesicle tapetosomes, which are delivered to the pollen surface (57). Various-sized membrane vesicles containing anthocyanin are observed in grapevine: 0.2–0.5 μm–diameter vesicles and 1 μm–diameter membrane compartments (34). Experiments using vesicle trafficking inhibitors suggest that anthocyanin trafficking shares a route with protein trafficking from ER to PVC (101, 109). Microscope observations confirmed that cytoplasmic vesicles and PVC containing anthocyanins or proanthocyanidins can fuse with the central vacuole (1, 149). Recent work proposed that the autophagy machinery may have a role in anthocyanin transport (72, 99). Similarly, the vacuolar membrane surrounds anthocyanins and sequesters them into the vacuole (18). However, the molecular details of flavonoid trafficking remain to be elucidated.

Several lines of evidence provide a link between flavonoid accumulation and vacuolar biogenesis. Four *Arabidopsis tt* mutants display aberrant vacuolar morphologies in their seed coat cells: *tt12*, *tt13/aha10*, the leucoanthocyanidin dioxygenase mutant *tt18/tds4*, and the glutathione-*S*-transferase mutant *tt19* (152). A chemical genetics analysis indicates that the chalcone synthase mutant *tt4* exhibits a vacuolar morphological change in hypocotyl cells (109). Although vacuole morphology might be susceptible to defective flavonoid accumulation (109), the reason why flavonoid metabolism affects vacuolar morphology remains to be determined.

Vacuolar protein sorting mutants in *Arabidopsis* were identified by secretion of vacuole-targeted green fluorescent protein in their seeds and were designated *green fluorescent seed* (*gfs*) (30). *gfs9* is allelic to a previously unmapped locus *tt9* (59). GFS9 functions in proanthocyanidin accumulation in seeds and membrane trafficking events, including protein trafficking to the vacuole and vacuolar development (59). Ectopic expression of a dominant-negative version of *SUPPRESSOR OF* $K^+$ *TRANSPORT GROWTH DEFECT1* (*SKD1*), which encodes a subunit of the ESCRT machinery involved in vacuolar protein trafficking and vacuolar biogenesis, also produces pale, tan-colored seeds and phenocopies the *tt* mutants (110, 111). Therefore, seed coat pigmentation might be dependent on either vacuolar development or vacuolar trafficking.

The collaborative roles among membrane transport, vesicle trafficking, and vacuolar development lead to a model that explains flavonoid transport from the ER to the vacuole (**Figure 3*b***). First, membrane transporters take the synthesized flavonoids up into a membrane compartment such as the ER, vesicle, or PVC. MATE-type transporters TT12, MtMATE1, and VvMATE2 localize on vesicles or Golgi apparatus in addition to vacuolar membranes (150). TT12 and TT19 are involved in flavonoid uptake into the ER lumen for loading to tapetosomes (57). Then, flavonoid-containing vesicles are delivered to the vacuoles or PVC using a similar route as protein trafficking. Finally, the flavonoid-accumulating vacuole and PVC fuse to each other to develop a mature central vacuole. Fusion of the flavonoid-containing vesicle/PVC with the vacuole and development to an enlarged vacuole might be mediated by GFS9 and SKD1. The proposed model is consistent with previous reports that membrane transport and vesicle trafficking function cooperatively and nonexclusively in flavonoid accumulation (34, 101).

## 3. VACUOLAR PROCESSING ENZYME

### 3.1. Vacuolar Protein Maturation and Activation

VPE is a cysteine proteinase originally identified as the processing enzyme responsible for maturation of seed storage proteins (42, 43, 46). VPE converts storage protein precursors into their respective mature forms (46, 117) and processes the single precursor PV100 to produce multiple seed proteins, including two Cys-rich trypsin inhibitors, three Arg/Glu-rich cytotoxic peptides, and the vicilin-like protein (141). VPE is synthesized as an inactive precursor and then activated by self-catalytic removal of an autoinhibitory domain of the C-terminal propeptide (75), indicating that VPE is a key enzyme for the maturation and activation of vacuolar proteins. The enzymatic properties of VPE have been reviewed elsewhere (39).

VPE homologs occur in animals and are referred to as asparagine endopeptidase and legumain. The mammalian VPE homolog (designated asparagine endopeptidase) has a critical role in endosomal/lysosomal degradation (87, 118), tissue homeostasis (146, 147), extracellular matrix remodeling through the degradation of fibronectin (92), and neuronal cell death (84). Deficiency of the mammalian VPE homolog leads to disorders resembling hemophagocytic syndrome (17). These results suggest that a similar VPE-dependent mechanism may function in cell death and tissue degeneration in plants and animals.

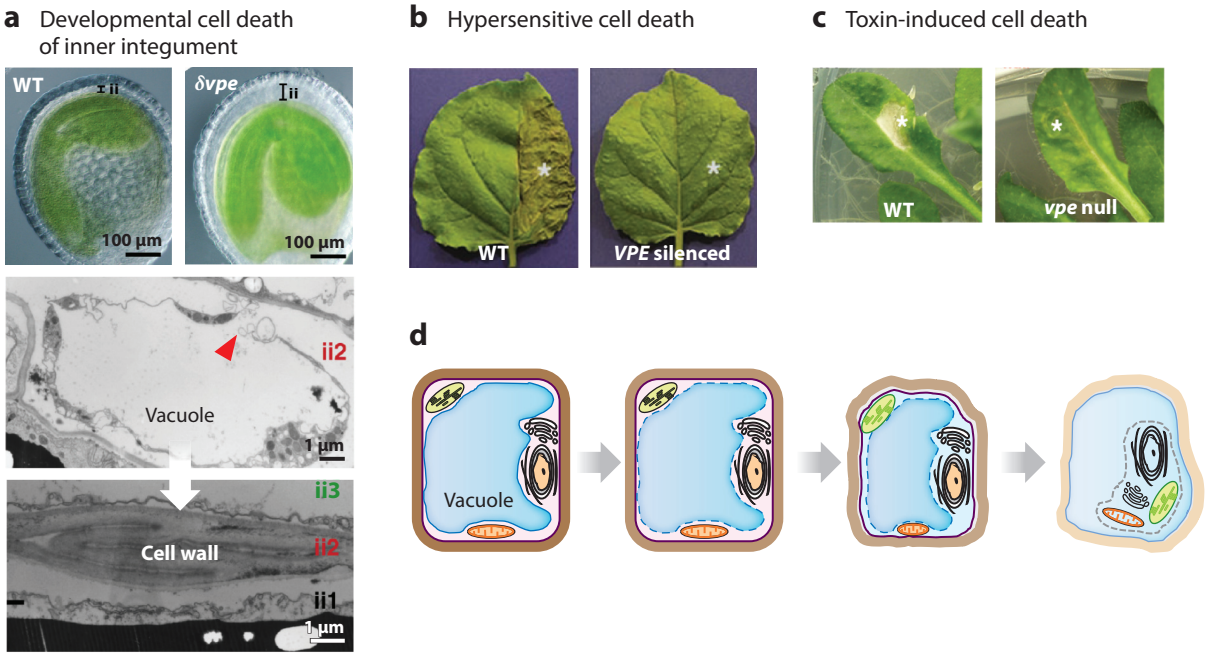


**Figure 4**

Programmed cell death mediated by vacuolar processing enzyme (VPE) responsible for maturation and activation of de novo synthesized vacuolar proteins. (*a*) δVPE-dependent developmental cell death in the inner integument (ii2), the purpose of which is to form a hard seed coat. Unlike the inner integument of the wild type (WT), the inner integument of the *δvpe* mutant remains thick. Electron micrographs show vacuolar collapse. (*b*) VPE-dependent hypersensitive cell death in *Nicotiana benthamiana* leaves that were infected by tobacco mosaic virus. (*c*) VPE-dependent susceptible cell death in the toxin-treated leaves of *Arabidopsis thaliana*. (See Reference 76 for original research.) (*d*) VPE regulates programmed cell death by inducing vacuolar membrane collapse. Image in panel *a* adapted with permission from Reference 93, copyright © 2005 American Society of Plant Biologists. Image in panel *b* adapted with permission from Reference 51, copyright © 2004 *Science*. Image in panel *c* adapted with permission from Reference 76, copyright © 2005 The American Society for Biochemistry and Molecular Biology.

## 3.2. VPE-Dependent Programmed Cell Death

VPE exhibits enzymatic properties similar to those of an executer of PCD in animals (caspase 1), despite the limited sequence identity between these two proteinases. The *Arabidopsis* genome contains four VPE homologs: *αVPE*, *βVPE*, *γVPE*, and *δVPE*. *αVPE* and *γVPE* function in the lytic vacuoles of vegetative tissues during senescence and under various stressed conditions, whereas *βVPE* functions in the PSVs (68). *δVPE* is specifically and transiently expressed in two seed coat cell layers (inner integuments 2 and 3) during early seed development (93). Vacuolar membrane collapse occurs in the inner integuments (**Figure 4*a***). δVPE is involved in PCD of limited cell layers, which leads to the formation of a hard seed coat (93). The vegetative-type VPE is required for hypersensitive cell death (51) and susceptible cell death (76) (**Figure 4*b*,*c***). The VPE-dependent PCD system is involved in the development of several tissues and in the responses to plant immunity and several stress inducers (53).

VPE activity promotes vacuolar membrane collapse, thereby initiating the proteolytic cascade leading to PCD (**Figure 4*d***). Vacuolar collapse also triggers degradation of cytoplasmic structures, leading to cell death (32). VPE-dependent vacuolar cell death, which degrades dying plant cells, is crucial because plant cells are surrounded by rigid cell walls and must degrade materials internally.

## 4. VACUOLAR MEMBRANE DYNAMICS INVOLVED IN DEFENSE RESPONSES

Because plants lack immune cells, each plant cell must defend itself against invading pathogens. Essentially, all vegetative cells have lytic vacuoles containing hydrolytic enzymes and defense proteins, raising the possibility that vacuoles have a role in plant defense. Plants use vacuoles and vacuolar contents to mount defense responses in two different systems: the vacuolar membrane-collapse system and the vacuole-plasma membrane-fusion system. Both systems are associated with vacuolar membrane dynamics. Intriguingly, both strategies rely on enzymes with caspase-like activities. The vacuolar membrane-collapse system requires VPE, which has caspase-1-like activity, and the membrane-fusion system requires a proteasome subunit that has caspase-3-like activity. Thus, plants may have evolved a cellular immune system that involves vacuolar membrane collapse to prevent the systemic spread of viral pathogens and vacuole–plasma membrane fusion to inhibit the proliferation of bacterial pathogens (40, 48).

### 4.1. Vacuolar Membrane Collapse Defends Against Viruses

Vacuolar membrane collapse is initiated by VPE in response to viral infection, which leads to the release of vacuolar contents, including nucleases, into the cytoplasm (41, 50, 51). This system effectively digests viruses that are proliferating in the cytosol (**Figure 5*a***). *VPE* gene silencing completely suppresses lesion formation, vacuolar collapse, and DNA fragmentation in tobacco mosaic virus–infected leaves. Although VPE deficiency blocks these typical immune-mediated responses, it does not interfere with the production of defense proteins (51). This means that the process of vacuolar cell death is independent of defense-protein production. VPE accumulates rapidly at the start of the tobacco mosaic virus–induced hypersensitive response and prevents the formation of visible lesions (51) (**Figure 4*b***). These combined results indicate that VPE is essential for the defense response during the early stage of virus-induced vacuolar cell death.

### 4.2. Vacuole–Plasma Membrane Fusion Defends Against Bacteria

VPE-dependent vacuolar membrane collapse effectively eliminates viruses within the cell. However, it does not prevent bacteria from proliferating outside cells. Plants have evolved a cell-autonomous immune system based on vacuole–plasma membrane fusion to inhibit the proliferation of bacterial pathogens (49).

*A. thaliana* plants are resistant to *Pseudomonas syringae* pv. *tomato* that has avirulence genes. Leaf cells infected with avirulent *Pst* DC3000/*avrRpm1* exhibit cell shrinkage and cytoplasmic aggregation after ~12 h, which are characteristic changes during hypersensitive cell death. Ultrastructural analysis of infected cells reveals that the membrane of the large central vacuole often becomes uniformly fused with the plasma membrane 3 h after infection (**Figure 5*b***). Membrane fusion occurs approximately simultaneously in most cells 6 h after infection (49). Fusion of the vacuolar and plasma membranes leads to the interconnection of vacuoles and extracellular spaces in leaf cells, which enables the discharge of vacuolar defense proteins into the extracellular space where bacteria proliferate. The extracellular fluid surrounding cells in infected leaves had both antibacterial and cell death–inducing activity.

The membrane-fusion system requires proteasome subunit PBA1 with caspase-3-like activity (49) and AP-4 (52). Hence, membrane fusion that is normally suppressed is triggered in a proteasome-dependent manner in response to avirulent bacterial infection. Thus, this defense strategy through proteasome-regulating fusion of the vacuolar and plasma membranes provides plants with a mechanism for attacking intercellular bacterial pathogens.

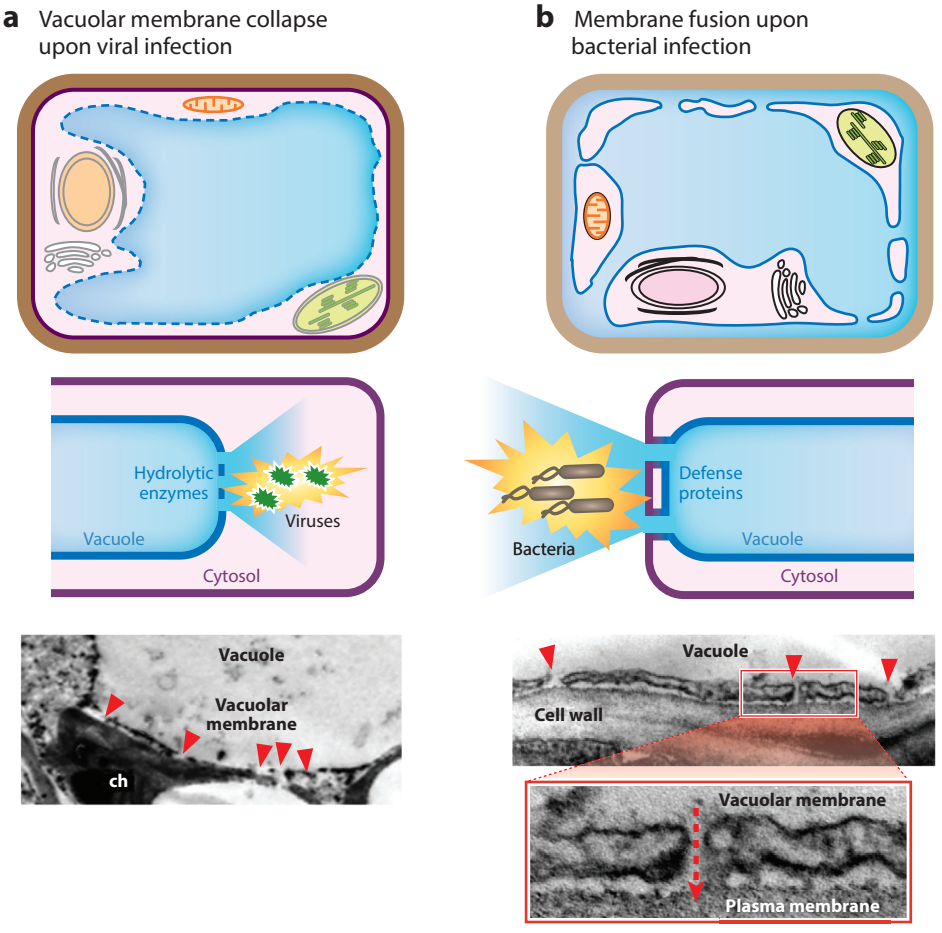


**Figure 5**

Vacuolar membrane dynamics upon pathogen infection. (*a*) Vacuolar membrane-collapse system against viral infection. Red arrowheads indicate vacuolar membrane collapse. (*b*) Membrane-fusion system between the vacuolar and plasma membranes against bacterial infection. Both vacuole-dependent systems cause hypersensitive cell death for defense. Red arrowheads indicate membrane fusion between the vacuolar and plasma membranes. The dashed red arrow shows the discharge of vacuolar defense proteins to the outside of the cells, where bacteria proliferate. Micrograph in panel *a* adapted with permission from Reference 49, copyright © 2009 Cold Spring Harbor Laboratory Press. Micrograph in panel *b* adapted with permission from Reference 51, copyright © 2004 *Science*. Abbreviation: ch, chloroplast.

## 5. MYROSIN CELLS CONTAIN SPECIALIZED VACUOLES FOR CHEMICAL DEFENSE AGAINST HERBIVORES

Vacuoles in seeds accumulate large amounts of storage proteins, whereas vacuoles of aboveground plant organs are generally involved in protein degradation. However, specialized cells of aerial tissues have vacuoles that accumulate large amounts of proteins, such as idioblast myrosin cells that accumulate large amounts of myrosinases [thioglucoside glucohydrolase 1 (TGG1) and TGG2] in their vacuoles. Myrosin cells are characteristic of Brassicales, as TGGs are Brassicales-specific β-glucosidases. When herbivores attack plant tissues, myrosinases are released from the collapsed

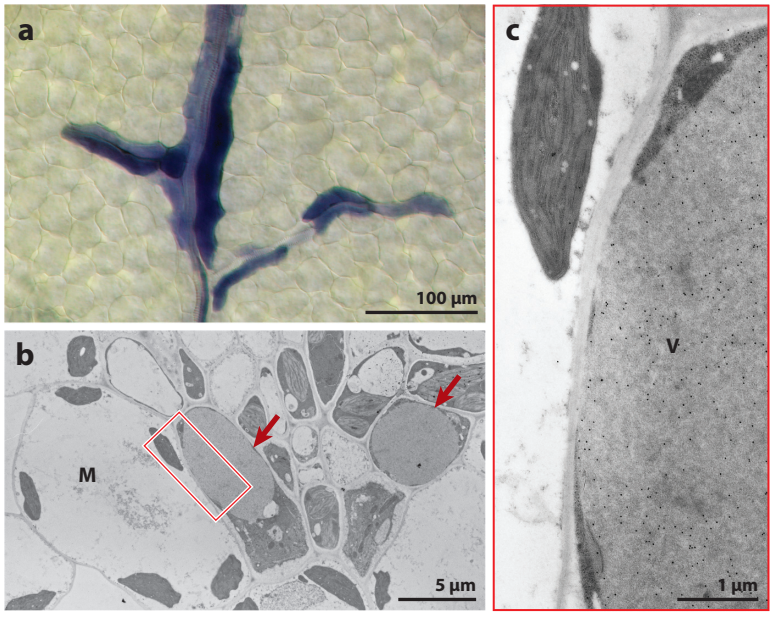


**Figure 6**

Myrosin cells with specialized vacuoles for chemical defense against herbivores. (*a*) Coomassie Brilliant Blue staining of a mature leaf of *Cardamine schinziana*. Myrosin cells are stained by purple color and distributed along leaf veins. Shapes of myrosin cells are not uniform, but they are elongated along leaf veins. (*b*) A section of a caulin leaf of *Arabidopsis thaliana*. Two myrosin cells (*arrows*) are located along a leaf vein. Vacuoles of myrosin cells are filled with smooth materials that have higher electron densities than adjacent mesophyll cells (M). (*c*) An enlarged image of the boxed area in panel *b*. Dots of the anti-TGG2 immunogold label are observed in the vacuole of myrosin cells (V).

vacuoles of myrosin cells to hydrolyze their substrate glucosinolates and produce isothiocyanates, which are toxic for herbivores and bacteria. This chemical defense system is the myrosinase-glucosinolate system, which is also called the mustard oil bomb (37, 38, 56, 69, 102, 140, 156). Myrosin cells containing myrosinases are specifically distributed along veins in a broad range of Brassicales plants, including the model plant *A. thaliana* (**Figure 6*a***) (4, 58, 119, 122, 156), whereas glucosinolates are stored in cells along the primary veins and the leaf periphery (71, 125). This partitioning of substrate and enzyme in different cell types prevents the production of toxic compounds unless attacked by herbivores and effectively protects the veins, which are crucial for water and nutrient transport in plants.

The expression and localization of myrosinase have been analyzed using specific reporter lines and antibodies. *TGG* expression patterns have been determined using β-glucuronidase (GUS) and fluorescent reporters in *Arabidopsis*. *ProTGG1:GUS* is expressed in both myrosin cells and guard cells (7, 58). The *TGG2* expression pattern has not been completely elucidated. *ProTGG2:GUS* is specifically expressed in myrosin cells (7), whereas *ProTGG2:VENUS-2sc* fluorescence is observed in both myrosin and guard cells (121, 122). A comparison of expression patterns of *ProTGG2:VENUS-2sc* and provascular cell–specific reporters reveals that myrosin cells are not differentiated from provascular cells, although myrosin cells develop along the veins (121). The patterns of TGG protein accumulation have been analyzed in both cell types. Immunoelectron microscopy has detected TGGs in myrosin cell vacuoles (134) (**Figure 6*b*,*c***). Proteomic data of purified guard cells indicate that TGGs accumulate in guard cells (153). These combined results

indicate that TGGs accumulate in both cell types. However, the levels of TGG accumulation are much higher in myrosin cells than in guard cells because the guard-cell-defective mutants *speechless* and *mute* accumulate slightly lower levels of TGGs than the wild type does (120).

Physiological functions of TGGs have been examined using T-DNA mutants. Generalist herbivores prefer leaves of *tgg1 tgg2* double mutants over those of the wild type, suggesting that TGGs function in plant defenses (7). Some specialist herbivores and bacteria overcome this defense strategy by having evolutionarily acquired special enzymes that inhibit the production of toxic compounds (25, 103), in what can be described as an arms race between plants and pathogens (24). TGGs in guard cells also may be involved in stomatal opening and closing (60, 153). However, TGG substrates have not been identified in guard cells.

The molecular mechanisms underlying myrosin cell development were largely unknown until 2014. Two groups simultaneously reported that the basic helix-loop-helix transcription factor FAMA is a master regulator of myrosin cell development (82, 120). FAMA expression starts in a subpopulation of stem cells in inner tissue layers, which differentiate into myrosin cells. Loss of *FAMA* leads to the complete absence of myrosin cells and TGG accumulation (82, 120). Conversely, ectopic overexpression of *FAMA* enhances TGG accumulation levels more than 30-fold and confers myrosin cell identity, including expression of the myrosin cell–specific markers *ProTGG2:GUS* and *MYR001:GUS*, in several cell types, including hypocotyl and root cells, both of which normally lack myrosin cells (120). These results indicate that FAMA is essential and sufficient for myrosin cell development. FAMA-interacting factors, such as ICE1/SCREAM (SCRM) and SCRM2, are redundantly essential for myrosin cell development (120). FAMA and SCRM/2 are also required for guard cell differentiation (63, 95), indicating that FAMA-SCRM/2 heterodimers are common master regulators for development of both myrosin cells and guard cells.

FAMA is expressed in cells that differentiate into myrosin cells. We discovered how these cells are selected, in that the vacuolar trafficking pathway regulates myrosin cell fate determination within the pool of stem cells (122). The number of myrosin cells increases in specific mutants of vacuolar trafficking components, including a vacuolar SNARE, SYNTAXIN OF PLANTS 22 (SYP22), and a TGN-localized membrane protein CONTINUOUS VASCULAR RING (COV1) (119, 123, 134). Analysis of *syp22* mutants revealed that more stem cells in inner tissue layers express *FAMA* in leaf primordia in the mutants than in the wild type, resulting in the production of more mature myrosin cells. The *syp22* phenotype is rescued by auxin treatment. SYP22 suppresses the number of *FAMA*-expressing cells by regulating endocytosis/polar localization of the auxin efflux carrier PIN1 (122, 124). Consistent with this, mutants of the endocytic factors VACUOLAR PROTEIN SORTING 9A (VPS9A) and AP-2 exhibit similar phenotypes as *syp22* (122, 142). Despite the vacuolar trafficking defects in *syp22* mutants, TGGs accumulate normally in myrosin cell vacuoles (134). The *GUS* gene in the myrosin cell–specific marker line *MYR001:GUS* is expressed under the control of a 2-kb promoter of *VSR1* (119). The expression of *MYR001:GUS* (i.e., *VSR1*) is dependent on FAMA, the master regulator of myrosin cell development (120). These combined results suggest that vacuolar trafficking of TGGs may be mediated by VSR1.

The key components determining myrosin cell development, FAMA and SYP22, have been identified. However, it is still unclear why myrosin cell vacuoles accumulate large amounts of proteins. Other idioblasts also accumulate specialized proteins and metabolites (28), and myrosin cells could be used as a model idioblast. Analyses of downstream components of the master regulator *FAMA* could shed light on the molecular mechanisms regulating myrosin cell vacuoles. For example, sorting receptors and vacuolar trafficking machinery for TGGs could be identified among FAMA targets. TGG modifications such as glycosylation may be important for TGG stability in vacuoles (134). The myrosin cell system can be used to examine whether exogenous

proteins accumulate in the idioblast vacuoles. Future work could utilize this knowledge to develop strategies for accumulating a large amount of useful proteins in artificially modified vacuoles of important plant cell types.

## 6. CONCLUSION AND PERSPECTIVE

This review summarizes current knowledge of lytic and storage vacuoles in plants. Despite differences in their shapes and contents, these vacuoles are closely related to each other. For example, PSVs are converted into lytic vacuoles during seed germination. During the conversion process, vacuolar proteins are replaced by a balance of degradation and biosynthesis. However, it remains unknown how large vacuolar compartments are formed. Ultrastructural analyses of maturing seeds indicate that vacuoles are directly formed from the ER. Alternatively, they also show that repeated fusion of ER-derived compartments and vesicles produces large vacuoles. These observations suggest that multiple mechanisms are involved in vacuole biogenesis.

This review also summarizes two different means of vacuolar defense against pathogens and discusses how plants use vacuolar cell death to attack invading pathogens. Vacuolar cell death is a useful strategy for plants because they lack immune cells; thus, each cell mounts its own defense system against pathogens. Plants have evolved two systems of vacuolar membrane dynamics depending on the type of pathogenic organism involved. Membrane fusion between the vacuolar membrane and plasma membrane is triggered in a proteasome-dependent manner to attack invading bacterial pathogens. The membrane fusion should be suppressed in an intact cell. Some suppressors might be degraded in response to bacterial infection to trigger membrane fusion. However, the membrane-fusion system does not work on viruses in the cytosol. The vacuolar membrane-collapse system attacks viruses. Vacuolar cell death in plants constitutes a cell-autonomous immune system.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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## LITERATURE CITED

1. Abrahams S, Tanner GJ, Larkin PJ, Ashton AR. 2002. Identification and biochemical characterization of mutants in the proanthocyanidin pathway in Arabidopsis. *Plant Physiol*. 130:561–76
2. Ahmed SU, Bar-Peled M, Raikhel NV. 1997. Cloning and subcellular location of an Arabidopsis receptor-like protein that shares common features with protein-sorting receptors of eukaryotic cells. *Plant Physiol*. 114:325–36
3. An Y, Chen CY, Moyer B, Rotkiewicz P, Elsliger MA, et al. 2009. Structural and functional analysis of the globular head domain of p115 provides insight into membrane tethering. *J. Mol. Biol.* 391:26–41
4. Andreasson E, Bolt Jorgensen L, Hoglund AS, Rask L, Meijer J. 2001. Different myrosinase and idioblast distribution in Arabidopsis and *Brassica napus*. *Plant Physiol*. 127:1750–63

5. Appelhagen I, Nordholt N, Seidel T, Spelt K, Koes R, et al. 2015. TRANSPARENT TESTA 13 is a tonoplast $P_{3A}$-ATPase required for vacuolar deposition of proanthocyanidins in *Arabidopsis thaliana* seeds. *Plant J.* 82:840–49
6. Barlowe CK, Miller EA. 2013. Secretory protein biogenesis and traffic in the early secretory pathway. *Genetics* 193:383–410
7. Barth C, Jander G. 2006. Arabidopsis myrosinases TGG1 and TGG2 have redundant function in glucosinolate breakdown and insect defense. *Plant J.* 46:549–62
8. Baxter IR, Young JC, Armstrong G, Foster N, Bogenschutz N, et al. 2005. A plasma membrane $H^+$-ATPase is required for the formation of proanthocyanidins in the seed coat endothelium of *Arabidopsis thaliana. PNAS* 102:2649–54
9. Becher B, Hoef-Emden K. 2009. Evolution of vacuolar targeting in algae. *Bot. Mar.* 52:117–28
10. Bi X, Corpina RA, Goldberg J. 2002. Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat. *Nature* 419:271–77
11. Bi X, Mancias JD, Goldberg J. 2007. Insights into COPII coat nucleation from the structure of Sec23.Sar1 complexed with the active fragment of Sec31. *Dev. Cell* 13:635–45
12. Brandizzi F. 2017. Transport from the endoplasmic reticulum to the Golgi in plants: Where are we now? *Semin. Cell Dev. Biol.* In press
13. Brandizzi F, Snapp EL, Roberts AG, Lippincott-Schwartz J, Hawes C. 2002. Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching. *Plant Cell* 14:1293–309
14. Bui QT, Golinelli-Cohen MP, Jackson CL. 2009. Large Arf1 guanine nucleotide exchange factors: evolution, domain structure, and roles in membrane trafficking and human disease. *Mol. Genet. Genom.* 282:329–50
15. Cai H, Yu S, Menon S, Cai Y, Lazarova D, et al. 2007. TRAPPI tethers COPII vesicles by binding the coat subunit Sec23. *Nature* 445:941–44
16. Cao X, Rogers SW, Butler J, Beevers L, Rogers JC. 2000. Structural requirements for ligand binding by a probable plant vacuolar sorting receptor. *Plant Cell* 12:493–506
17. Chan CB, Abe M, Hashimoto N, Hao C, Williams IR, et al. 2009. Mice lacking asparaginyl endopeptidase develop disorders resembling hemophagocytic syndrome. *PNAS* 106:468–73
18. Chanoca A, Kovinich N, Burkel B, Stecha S, Bohorquez-Restrepo A, et al. 2015. Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *Plant Cell* 27:2545–59
19. daSilva LL, Foresti O, Denecke J. 2006. Targeting of the plant vacuolar sorting receptor BP80 is dependent on multiple sorting signals in the cytosolic tail. *Plant Cell* 18:1477–97
20. 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. *Plant Cell* 16:1753–71
21. De Marchis F, Bellucci M, Pompa A. 2013. Traffic of human $\alpha$-mannosidase in plant cells suggests the presence of a new endoplasmic reticulum-to-vacuole pathway without involving the Golgi complex. *Plant Physiol.* 161:1769–82
22. De Marcos Lousa C, Gershlick DC, Denecke J. 2012. Mechanisms and concepts paving the way towards a complete transport cycle of plant vacuolar sorting receptors. *Plant Cell* 24:1714–32
23. Debeaujon I, Peeters AJ, Leon-Kloosterziel KM, Koornneef M. 2001. The *TRANSPARENT TESTA12* gene of Arabidopsis encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. *Plant Cell* 13:853–71
24. Edger PP, Heidel-Fischer HM, Bekaert M, Rota J, Glockner G, et al. 2015. The butterfly plant arms-race escalated by gene and genome duplications. *PNAS* 112:8362–66
25. Fan J, Crooks C, Creissen G, Hill L, Fairhurst S, et al. 2011. *Pseudomonas sax* genes overcome aliphatic isothiocyanate-mediated non-host resistance in *Arabidopsis. Science* 331:1185–88
26. Faraco M, Spelt C, Bliek M, Verweij W, Hoshino A, et al. 2014. Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. *Cell Rep.* 6:32–43
27. Foresti O, Gershlick DC, Bottanelli F, Hummel E, Hawes C, Denecke J. 2010. A recycling-defective vacuolar sorting receptor reveals an intermediate compartment situated between prevacuoles and vacuoles in tobacco. *Plant Cell* 22:3992–4008

28. Foster AS. 1956. Plant idioblasts: remarkable examples of cell specialization. *Protoplasma* 46:184–93
29. Francisco RM, Regalado A, Ageorges A, Burla BJ, Bassin B, et al. 2013. ABCC1, an ATP binding cassette protein from grape berry, transports anthocyanidin 3-*O*-glucosides. *Plant Cell* 25:1840–54
30. Fuji K, Shimada T, Takahashi H, Tamura K, Koumoto Y, et al. 2007. *Arabidopsis* vacuolar sorting mutants (*green fluorescent seed*) can be identified efficiently by secretion of vacuole-targeted green fluorescent protein in their seeds. *Plant Cell* 19:597–609
31. Fuji K, Shirakawa M, Shimono Y, Kunieda T, Fukao Y, et al. 2016. The adaptor complex AP-4 regulates vacuolar protein sorting at the *trans*-Golgi network by interacting with VACUOLAR SORTING RECEPTOR1. *Plant Physiol.* 170:211–19
32. Fukuda H. 1996. Xylogenesis: initiation, progression, and cell death. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47:299–325
33. Gershlick DC, de Marcos Lousa C, Foresti O, Lee AJ, Pereira EA, et al. 2014. Golgi-dependent transport of vacuolar sorting receptors is regulated by COPII, AP1, and AP4 protein complexes in tobacco. *Plant Cell* 26:1308–29
34. Gomez C, Conejero G, Torregrosa L, Cheynier V, Terrier N, Ageorges A. 2011. *In vivo* grapevine anthocyanin transport involves vesicle-mediated trafficking and the contribution of anthoMATE transporters and GST. *Plant J.* 67:960–70
35. Goodman CD, Casati P, Walbot V. 2004. A multidrug resistance-associated protein involved in anthocyanin transport in *Zea mays. Plant Cell* 16:1812–26
36. Grotewold E. 2006. The genetics and biochemistry of floral pigments. *Annu. Rev. Plant Biol.* 57:761–80
37. Grubb CD, Abel S. 2006. Glucosinolate metabolism and its control. *Trends Plant Sci.* 11:89–100
38. Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* 57:303–33
39. Hara-Nishimura I. 2012. Plant legumain, asparaginyl endopeptidase, vacuolar processing enzyme. In *Handbook of Proteolytic Enzymes*, ed. AJ Barrett, ND Rawlings, JF Woessner, pp. 2314–20. Cambridge, MA: Academic. 3rd ed.
40. Hara-Nishimura I, Hatsugai N. 2011. The role of vacuole in plant cell death. *Cell Death Differ.* 18:1298–304
41. Hara-Nishimura I, Hatsugai N, Kuroyanagi M, Nakaune S, Nishimura M. 2005. Vacuolar processing enzyme: an executor of plant cell death. *Curr. Opin. Plant Biol.* 8:404–8
42. Hara-Nishimura I, Inoue K, Nishimura M. 1991. A unique vacuolar processing enzyme responsible for conversion of several proprotein precursors into the mature forms. *FEBS Lett.* 294:89–93
43. Hara-Nishimura I, Nishimura M. 1987. Proglobulin processing enzyme in vacuoles isolated from developing pumpkin cotyledons. *Plant Physiol.* 85:440–45
44. Hara-Nishimura I, Nishimura M, Akazawa T. 1985. Biosynthesis and intracellular transport of 11S globulin in developing pumpkin cotyledons. *Plant Physiol.* 77:747–52
45. 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. *Plant Cell* 10:825–36
46. Hara-Nishimura I, Takeuchi Y, Nishimura M. 1993. Molecular characterization of a vacuolar processing enzyme related to a putative cysteine proteinase of *Schistosoma mansoni*. *Plant Cell* 5:1651–59
47. Harborne JB, Williams CA. 2000. Advances in flavonoid research since 1992. *Phytochemistry* 55:481–504
48. Hatsugai N, Hara-Nishimura I. 2010. Two vacuole-mediated defense strategies in plants. *Plant Signal. Behav.* 5:1568–70
49. Hatsugai N, Iwasaki S, Tamura K, Kondo M, Fuji K, et al. 2009. A novel membrane-fusion-mediated plant immunity against bacterial pathogens. *Gene. Dev.* 23:2496–506
50. Hatsugai N, Kuroyanagi M, Nishimura M, Hara-Nishimura I. 2006. A cellular suicide strategy of plants: vacuole-mediated cell death. *Apoptosis* 11:905–11
51. Hatsugai N, Kuroyanagi M, Yamada K, Meshi T, Tsuda S, et al. 2004. A plant vacuolar protease, VPE, mediates virus-induced hypersensitive cell death. *Science* 305:855–58
52. Hatsugai N, Nakatsuji A, Unten O, Ogasawara K, Kondo M, et al. 2017. Involvement of Adapter Protein Complex 4 in hypersensitive cell death induced by avirulent bacteria. *Plant Physiol.* 176:1824–34
53. Hatsugai N, Yamada K, Goto-Yamada S, Hara-Nishimura I. 2015. Vacuolar processing enzyme in plant programmed cell death. *Front. Plant Sci.* 6:234

54. Hohl I, Robinson DG, Chrispeels MJ, Hinz G. 1996. Transport of storage proteins to the vacuole is mediated by vesicles without a clathrin coat. *J. Cell Sci.* 109:2539–50
55. Honig A, Avin-Wittenberg T, Ufaz S, Galili G. 2012. A new type of compartment, defined by plant-specific Atg8-interacting proteins, is induced upon exposure of *Arabidopsis* plants to carbon starvation. *Plant Cell* 24:288–303
56. Hopkins RJ, van Dam NM, van Loon JJ. 2009. Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annu. Rev. Entomol.* 54:57–83
57. Hsieh K, Huang AH. 2007. Tapetosomes in *Brassica* tapetum accumulate endoplasmic reticulum-derived flavonoids and alkanes for delivery to the pollen surface. *Plant Cell* 19:582–96
58. Husebye H, Chadchawan S, Winge P, Thangstad OP, Bones AM. 2002. Guard cell- and phloem idioblast-specific expression of thioglucoside glucohydrolase 1 (myrosinase) in Arabidopsis. *Plant Physiol.* 128:1180–88
59. Ichino T, Fuji K, Ueda H, Takahashi H, Koumoto Y, et al. 2014. GFS9/TT9 contributes to intracellular membrane trafficking and flavonoid accumulation in *Arabidopsis thaliana*. *Plant J.* 80:410–23
60. Islam MM, Tani C, Watanabe-Sugimoto M, Uraji M, Jahan MS, et al. 2009. Myrosinases, TGG1 and TGG2, redundantly function in ABA and MeJA signaling in Arabidopsis guard cells. *Plant Cell Physiol.* 50:1171–75
61. Ito Y, Uemura T, Nakano A. 2014. Formation and maintenance of the Golgi apparatus in plant cells. *Int. Rev. Cell Mol. Biol.* 310:221–87
62. Ito Y, Uemura T, Shoda K, Fujimoto M, Ueda T, Nakano A. 2012. *cis*-Golgi proteins accumulate near the ER exit sites and act as the scaffold for Golgi regeneration after brefeldin A treatment in tobacco BY-2 cells. *Mol. Biol. Cell* 23:3203–14
63. Kanaoka MM, Pillitteri LJ, Fujii H, Yoshida Y, Bogenschutz NL, et al. 2008. SCREAM/ICE1 and SCREAM2 specify three cell-state transitional steps leading to Arabidopsis stomatal differentiation. *Plant Cell* 20:1775–85
64. Kang BH, Staehelin LA. 2008. ER-to-Golgi transport by COPII vesicles in *Arabidopsis* involves a ribosome-excluding scaffold that is transferred with the vesicles to the Golgi matrix. *Protoplasma* 234:51–64
65. Kang H, Hwang I. 2014. Vacuolar sorting receptor-mediated trafficking of soluble vacuolar proteins in plant cells. *Plants* 3:392–408
66. Kang H, Kim SY, Song K, Sohn EJ, Lee Y, et al. 2012. Trafficking of vacuolar proteins: the crucial role of *Arabidopsis* vacuolar protein sorting 29 in recycling vacuolar sorting receptor. *Plant Cell* 24:5058–73
67. Kim H, Kang H, Jang M, Chang JH, Miao Y, et al. 2010. Homomeric interaction of AtVSR1 is essential for its function as a vacuolar sorting receptor. *Plant Physiol.* 154:134–48
68. Kinoshita T, Yamada K, Hiraiwa N, Kondo M, Nishimura M, Hara-Nishimura I. 1999. Vacuolar processing enzyme is up-regulated in the lytic vacuoles of vegetative tissues during senescence and under various stressed conditions. *Plant J.* 19:43–53
69. Kissen R, Rossiter JT, Bones AM. 2009. The 'mustard oil bomb': not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochem. Rev.* 8:69–86
70. Koornneef M. 1990. Mutations affecting the testa colour in Arabidopsis. *Arabidopsis Inf. Serv.* 27:1–4
71. Koroleva OA, Davies A, Deeken R, Thorpe MR, Tomos AD, Hedrich R. 2000. Identification of a new glucosinolate-rich cell type in Arabidopsis flower stalk. *Plant Physiol.* 124:599–608
72. Kulich I, Pecenkova T, Sekeres J, Smetana O, Fendrych M, et al. 2013. Arabidopsis exocyst subcomplex containing subunit EXO70B1 is involved in autophagy-related transport to the vacuole. *Traffic* 14:1155–65
73. Künzl F, Früholz S, Fäßler F, Li B, Pimpl P. 2016. Receptor-mediated sorting of soluble vacuolar proteins ends at the *trans*-Golgi network/early endosome. *Nat. Plants* 2:16017
74. Kurokawa K, Okamoto M, Nakano A. 2014. Contact of *cis*-Golgi with ER exit sites executes cargo capture and delivery from the ER. *Nat. Commun.* 5:3653
75. Kuroyanagi M, Nishimura M, Hara-Nishimura I. 2002. Activation of Arabidopsis vacuolar processing enzyme by self-catalytic removal of an auto-inhibitory domain of the C-terminal propeptide. *Plant Cell Physiol.* 43:143–51

76. Kuroyanagi M, Yamada K, Hatsugai N, Kondo M, Nishimura M, Hara-Nishimura I. 2005. Vacuolar processing enzyme is essential for mycotoxin-induced cell death in *Arabidopsis thaliana*. *J. Biol. Chem.* 280:32914–20
77. Langhans M, Marcote MJ, Pimpl P, Virgili-Lopez G, Robinson DG, Aniento F. 2008. *In vivo* trafficking and localization of p24 proteins in plant cells. *Traffic* 9:770–85
78. Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, et al. 2006. Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol.* 57:405–30
79. Levanony H, Rubin R, Altschuler Y, Galili G. 1992. Evidence for a novel route of wheat storage proteins to vacuoles. *J. Cell Biol.* 119:1117–28
80. Li L, Shimada T, Takahashi H, Koumoto Y, Shirakawa M, et al. 2013. MAG2 and three MAG2-INTERACTING PROTEINs form an ER-localized complex to facilitate storage protein transport in *Arabidopsis thaliana*. *Plant J.* 76:781–91
81. Li L, Shimada T, Takahashi H, Ueda H, Fukao Y, et al. 2006. MAIGO2 is involved in exit of seed storage proteins from the endoplasmic reticulum in *Arabidopsis thaliana*. *Plant Cell* 18:3535–47
82. Li M, Sack FD. 2014. Myrosin idioblast cell fate and development are regulated by the Arabidopsis transcription factor FAMA, the auxin pathway, and vesicular trafficking. *Plant Cell* 26:4053–66
83. Liu Y, Burgos JS, Deng Y, Srivastava R, Howell SH, Bassham DC. 2012. Degradation of the endoplasmic reticulum by autophagy during endoplasmic reticulum stress in *Arabidopsis*. *Plant Cell* 24:4635–51
84. Liu Z, Jang S-W, Liu X, Cheng D, Peng J, et al. 2008. Neuroprotective actions of PIKE-L by inhibition of SET proteolytic degradation by asparagine endopeptidase. *Mol. Cell* 29:665–78
85. Lord C, Ferro-Novick S, Miller EA. 2013. The highly conserved COPII coat complex sorts cargo from the endoplasmic reticulum and targets it to the Golgi. *Cold Spring Harb. Perspect. Biol.* 5:a013367
86. 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. *Plant Cell* 26:3693–708
87. Maehr R, Hang HC, Mintern JD, Kim Y-M, Culvillier A, et al. 2005. Asparagine endopeptidase is not essential for class II MHC antigen presentation but is required for processing of cathepsin L in murine primary antigen presenting cells. *J. Immunol.* 174:7066–74
88. Marinova K, Pourcel L, Weder B, Schwarz M, Barron D, et al. 2007. The *Arabidopsis* MATE transporter TT12 acts as a vacuolar flavonoid/$H^+$-antiporter active in proanthocyanidin-accumulating cells of the seed coat. *Plant Cell* 19:2023–38
89. Michaeli S, Avin-Wittenberg T, Galili G. 2014. Involvement of autophagy in the direct ER to vacuole protein trafficking route in plants. *Front. Plant Sci.* 5:134
90. Miller EA, Beilharz TH, Malkus PN, Lee MC, Hamamoto S, et al. 2003. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* 114:497–509
91. Mori T, Maruyama N, Nishizawa K, Higasa T, Yagasaki K, et al. 2004. The composition of newly synthesized proteins in the endoplasmic reticulum determines the transport pathways of soybean seed storage proteins. *Plant J.* 40:238–49
92. Morita Y, Araki H, Sugimoto T, Takeuchi K, Yamane T, et al. 2007. Legumain/asparaginyl endopeptidase controls extracellular matrix remodeling through the degradation of fibronectin in mouse renal proximal tubular cells. *FEBS Lett.* 581:1417–24
93. Nakaune S, Yamada K, Kondo M, Kato T, Tabata S, et al. 2005. A vacuolar processing enzyme, δVPE, is involved in seed coat formation at the early stage of seed development. *Plant Cell* 17:876–87
94. Nebenfuhr A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, et al. 1999. Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. *Plant Physiol.* 121:1127–42
95. Ohashi-Ito K, Bergmann DC. 2006. Arabidopsis FAMA controls the final proliferation/differentiation switch during stomatal development. *Plant Cell* 18:2493–505
96. Paris N, Rogers SW, Jiang L, Kirsch T, Beevers L, et al. 1997. Molecular cloning and further characterization of a probable plant vacuolar sorting receptor. *Plant Physiol.* 115:29–39
97. 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. *Plant J.* 76:87–100

98. Pinheiro H, Samalova M, Geldner N, Chory J, Martinez A, Moore I. 2009. Genetic evidence that the higher plant Rab-D1 and Rab-D2 GTPases exhibit distinct but overlapping interactions in the early secretory pathway. *J. Cell Sci.* 122:3749–58
99. Pourcel L, Irani NG, Lu Y, Riedl K, Schwartz S, Grotewold E. 2010. The formation of anthocyanic vacuolar inclusions in *Arabidopsis thaliana* and implications for the sequestration of anthocyanin pigments. *Mol. Plant* 3:78–90
100. Pourcher M, Santambrogio M, Thazar N, Thierry AM, Fobis-Loisy I, et al. 2010. Analyses of sorting nexins reveal distinct retromer-subcomplex functions in development and protein sorting in *Arabidopsis thaliana. Plant Cell* 22:3980–91
101. Poustka F, Irani NG, Feller A, Lu Y, Pourcel L, et al. 2007. A trafficking pathway for anthocyanins overlaps with the endoplasmic reticulum-to-vacuole protein-sorting route in Arabidopsis and contributes to the formation of vacuolar inclusions. *Plant Physiol.* 145:1323–35
102. Rask L, Andreasson E, Ekbom B, Eriksson S, Pontoppidan B, Meijer J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. *Plant Mol. Biol.* 42:93–113
103. Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J. 2002. Disarming the mustard oil bomb. *PNAS* 99:11223–28
104. Ren Y, Yip CK, Tripathi A, Huie D, Jeffrey PD, et al. 2009. A structure-based mechanism for vesicle capture by the multisubunit tethering complex Dsl1. *Cell* 139:1119–29
105. Rizo J, Sudhof TC. 2012. The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices—guilty as charged? *Annu. Rev. Cell Dev. Biol.* 28:279–308
106. Robinson DG, Herranz MC, Bubeck J, Pepperkok R, Ritzenthaler C. 2007. Membrane dynamics in the early secretory pathway. *Crit. Rev. Plant Sci.* 26:199–225
107. Robinson DG, Neuhaus JM. 2016. Receptor-mediated sorting of soluble vacuolar proteins: myths, facts, and a new model. *J. Exp. Bot.* 67:4435–49
108. Robinson DG, Pimpl P. 2014. Receptor-mediated transport of vacuolar proteins: a critical analysis and a new model. *Protoplasma* 251:247–64
109. Rosado A, Hicks GR, Norambuena L, Rogachev I, Meir S, et al. 2011. Sortin1-hypersensitive mutants link vacuolar-trafficking defects and flavonoid metabolism in *Arabidopsis* vegetative tissues. *Chem. Biol.* 18:187–97
110. Shahriari M, Hulskamp M, Schellmann S. 2010. Seeds of Arabidopsis plants expressing dominant-negative AtSKD1 under control of the *GL2* promoter show a *transparent testa* phenotype and a mucilage defect. *Plant Signal. Behav.* 5:1308–10
111. Shahriari M, Keshavaiah C, Scheuring D, Sabovljevic A, Pimpl P, et al. 2010. The AAA-type ATPase AtSKD1 contributes to vacuolar maintenance of *Arabidopsis thaliana. Plant J.* 64:71–85
112. Shen J, Ding Y, Gao C, Rojo E, Jiang L. 2014. *N*-linked glycosylation of AtVSR1 is important for vacuolar protein sorting in Arabidopsis. *Plant J.* 80:977–92
113. Shimada T, Fuji K, Tamura K, Kondo M, Nishimura M, Hara-Nishimura I. 2003. Vacuolar sorting receptor for seed storage proteins in *Arabidopsis thaliana*. *PNAS* 100:16095–100
114. Shimada T, Koumoto Y, Li L, Yamazaki M, Kondo M, et al. 2006. AtVPS29, a putative component of a retromer complex, is required for the efficient sorting of seed storage proteins. *Plant Cell Physiol.* 47:1187–94
115. Shimada T, Kuroyanagi M, Nishimura M, Hara-Nishimura I. 1997. A pumpkin 72-kDa membrane protein of precursor-accumulating vesicles has characteristics of a vacuolar sorting receptor. *Plant Cell Physiol.* 38:1414–20
116. 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 Physiol.* 43:1086–95
117. Shimada T, Yamada K, Kataoka M, Nakaune S, Koumoto Y, et al. 2003. Vacuolar processing enzymes are essential for proper processing of seed storage proteins in *Arabidopsis thaliana*. *J. Biol. Chem.* 278:32292–99
118. Shirahama-Noda K, Yamamoto A, Sugihara K, Hashimoto N, Asano M, et al. 2003. Biosynthetic processing of cathepsins and lysosomal degradation are abolished in asparaginyl endopeptidase-deficient mice. *J. Biol. Chem.* 278:33194–99

119. Shirakawa M, Ueda H, Koumoto Y, Fuji K, Nishiyama C, et al. 2014. CONTINUOUS VASCULAR RING (COV1) is a *trans*-Golgi network-localized membrane protein required for Golgi morphology and vacuolar protein sorting. *Plant Cell Physiol.* 55:764–72
120. Shirakawa M, Ueda H, Nagano AJ, Shimada T, Kohchi T, Hara-Nishimura I. 2014. FAMA is an essential component for the differentiation of two distinct cell types, myrosin cells and guard cells, in Arabidopsis. *Plant Cell* 26:4039–52
121. Shirakawa M, Ueda H, Shimada T, Hara-Nishimura I. 2016. Myrosin cells are differentiated directly from ground meristem cells and are developmentally independent of the vasculature in Arabidopsis leaves. *Plant Signal. Behav.* 11:e1150403
122. Shirakawa M, Ueda H, Shimada T, Kohchi T, Hara-Nishimura I. 2014. Myrosin cell development is regulated by endocytosis machinery and PIN1 polarity in leaf primordia of *Arabidopsis thaliana. Plant Cell* 26:4448–61
123. Shirakawa M, Ueda H, Shimada T, Koumoto Y, Shimada TL, et al. 2010. Arabidopsis Qa-SNARE SYP2 proteins localized to different subcellular regions function redundantly in vacuolar protein sorting and plant development. *Plant J.* 64:924–35
124. Shirakawa M, Ueda H, Shimada T, Nishiyama C, Hara-Nishimura I. 2009. Vacuolar SNAREs function in the formation of the leaf vascular network by regulating auxin distribution. *Plant Cell Physiol.* 50:1319–28
125. Shroff R, Vergara F, Muck A, Svatos A, Gershenzon J. 2008. Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *PNAS* 105:6196–201
126. Szul T, Sztul E. 2011. COPII and COPI traffic at the ER-Golgi interface. *Physiology* 26:348–64
127. Takagi J, Renna L, Takahashi H, Koumoto Y, Tamura K, et al. 2013. MAIGO5 functions in protein export from Golgi-associated endoplasmic reticulum exit sites in Arabidopsis. *Plant Cell* 25:4658–75
128. 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 Physiol.* 46:245–49
129. 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 Physiol.* 51:1777–87
130. Takeuchi M, Ueda T, Sato K, Abe H, Nagata T, Nakano A. 2000. A dominant negative mutant of Sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. *Plant J.* 23:517–25
131. Takeuchi M, Ueda T, Yahara N, Nakano A. 2002. Arf1 GTPase plays roles in the protein traffic between the endoplasmic reticulum and the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. *Plant J.* 31:499–515
132. Taylor LP, Grotewold E. 2005. Flavonoids as developmental regulators. *Curr. Opin. Plant Biol.* 8:317–23
133. Teh OK, Moore I. 2007. An ARF-GEF acting at the Golgi and in selective endocytosis in polarized plant cells. *Nature* 448:493–96
134. Ueda H, Nishiyama C, Shimada T, Koumoto Y, Hayashi Y, et al. 2006. AtVAM3 is required for normal specification of idioblasts, myrosin cells. *Plant Cell Physiol.* 47:164–75
135. Verweij W, Spelt C, Di Sansebastiano GP, Vermeer J, Reale L, et al. 2008. An $H^+$ P-ATPase on the tonoplast determines vacuolar pH and flower colour. *Nat. Cell Biol.* 10:1456–62
136. Wang T, Grabski R, Sztul E, Hay JC. 2015. p115-SNARE interactions: a dynamic cycle of p115 binding monomeric SNARE motifs and releasing assembled bundles. *Traffic* 16:148–71
137. Watanabe E, Shimada T, Kuroyanagi M, Nishimura M, Hara-Nishimura I. 2002. Calcium-mediated association of a putative vacuolar sorting receptor PV72 with a propeptide of 2S albumin. *J. Biol. Chem.* 277:8708–15
138. Watanabe E, Shimada T, Tamura K, Matsushima R, Koumoto Y, et al. 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 Physiol.* 45:9–17
139. Winkel BS. 2004. Metabolic channeling in plants. *Annu. Rev. Plant Biol.* 55:85–107
140. Wittstock U, Halkier BA. 2002. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* 7:263–70

141. Yamada K, Shimada T, Kondo M, Nishimura M, Hara-Nishimura I. 1999. Multiple functional proteins are produced by cleaving Asn-Gln bonds of a single precursor by vacuolar processing enzyme. *J. Biol. Chem.* 274:2563–70
142. Yamaoka S, Shimono Y, Shirakawa M, Fukao Y, Kawase T, et al. 2013. Identification and dynamics of *Arabidopsis* adaptor protein-2 complex and its involvement in floral organ development. *Plant Cell* 25:2958–69
143. Yamazaki M, Shimada T, Takahashi H, Tamura K, Kondo M, et al. 2008. *Arabidopsis* VPS35, a retromer component, is required for vacuolar protein sorting and involved in plant growth and leaf senescence. *Plant Cell Physiol.* 49:142–56
144. Yang YD, Elamawi R, Bubeck J, Pepperkok R, Ritzenthaler C, Robinson DG. 2005. Dynamics of COPII vesicles and the Golgi apparatus in cultured *Nicotiana tabacum* BY-2 cells provides evidence for transient association of Golgi stacks with endoplasmic reticulum exit sites. *Plant Cell* 17:1513–31
145. Yorimitsu T, Sato K. 2012. Insights into structural and regulatory roles of Sec16 in COPII vesicle formation at ER exit sites. *Mol. Biol. Cell* 23:2930–42
146. Zeeuwen PL, van Vlijmen-Willems IM, Cheng T, Rodijk-Olthuis D, Hitomi K, et al. 2010. The cystatin M/E-cathepsin L balance is essential for tissue homeostasis in epidermis, hair follicles and cornea. *FASEB J.* 24:3744–55
147. Zeeuwen PLJM, van Vlijmen-Willems IMJJ, Olthuis D, Johansen HT, Hitomi K, et al. 2004. Identification of legumain and cystatin M/E as a functional dyad in skin: Evidence for a role in stratum corneum and skin barrier formation. *Hum. Mol. Genet.* 13:1069–79
148. Zeng Y, Chung KP, Li B, Lai CM, Lam SK, et al. 2015. Unique COPII component AtSar1a/AtSec23a pair is required for the distinct function of protein ER export in *Arabidopsis thaliana*. *PNAS* 112:14360–65
149. Zhang H, Wang L, Deroles S, Bennett R, Davies K. 2006. New insight into the structures and formation of anthocyanic vacuolar inclusions in flower petals. *BMC Plant Biol.* 6:29
150. Zhao J. 2015. Flavonoid transport mechanisms: how to go, and with whom. *Trends Plant Sci.* 20:576–85
151. Zhao J, Dixon RA. 2009. MATE transporters facilitate vacuolar uptake of epicatechin 3′-*O*-glucoside for proanthocyanidin biosynthesis in *Medicago truncatula* and *Arabidopsis*. *Plant Cell* 21:2323–40
152. Zhao J, Dixon RA. 2010. The 'ins' and 'outs' of flavonoid transport. *Trends Plant Sci.* 15:72–80
153. Zhao Z, Zhang W, Stanley BA, Assmann SM. 2008. Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. *Plant Cell* 20:3210–26
154. Zink S, Wenzel D, Wurm CA, Schmitt HD. 2009. A link between ER tethering and COP-I vesicle uncoating. *Dev. Cell* 17:403–16
155. 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. *Plant J.* 64:577–88
156. Shirakawa M, Ueda H, Shimada T, Hara-Nishimura I. 2016. FAMA: a molecular link between stomata and myrosin cells. *Trends Plant Sci.* 21:861–71

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# Contents

**Errata**

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