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Vacuolar trafficking and biogenesis: a maturation in the field

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The vacuole is a prominent organelle that is essential for plant viability. The vacuole size, and its role in ion homeostasis, protein degradation and storage, place significant demands for trafficking of vacuolar cargo along the endomembrane system. Recent studies indicate that sorting of vacuolar cargo initiates at the ER and Golgi, but not the trans-Golgi network/early endosome, as previously thought. Furthermore, maturation of the trans-Golgi network into pre-vacuolar compartments seems to contribute to a major route for plant vacuolar traffic that works by bulk flow and ends with membrane fusion between the pre-vacuolar compartment and the tonoplast. Here we summarize recent evidence that indicates conserved and plant-specific mechanisms involved in sorting and trafficking of proteins to this major organelle.

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Introduction

Vacuoles are essential organelles and important for protein storage, detoxification, recycling and defense [1]. Recent discoveries are revealing both conserved and distinctive mechanisms for trafficking to the plant vacuole when compared to animal cells and yeast. With the role of the plant trans-Golgi network (TGN) as an early endosome, cargo sorting of endocytic and anterograde traffic to the vacuole as well as traffic to the plasma membrane must occur at or before this important hub [2,3]. Recent findings underscore earlier sorting events in the plant endomembrane system when compared to yeast and animals, and an increased reliance on organelle maturation, rather than vesicles, for vacuolar traffic. This includes the interaction between vacuolar sorting receptors and their cargo at the Golgi and their dissociation at the TGN [4••], the biogenesis of the pre-vacuolar compartment (PVC) from maturation of the TGN, and initiation of intraluminal vesicle budding as early as the TGN [5,6]. At the end of this major maturation pathway is the pre-vacuolar compartment (PVC) which can only reach the vacuole via membrane fusion mediated by highly conserved complexes including SNAREs (Soluble NSF Attachment protein Receptors) and HOPS (**ho**motypic fusion and vacuole **p**rotein **s**orting) [7,8]. We discuss here the latest developments on the vacuolar trafficking pathway and highlight future research that may lead to a better understanding of the mechanisms for vacuolar traffic.

Post-Golgi traffic via maturation of endomembrane organelles

The plant endomembrane system comprises a series of organelles that traffic cargo to the vacuole or the plasma membrane, and include the ER, the Golgi apparatus, the TGN, the PVC and the vacuole [3,7,8]. PVCs, also called multi-vesicular bodies (MVBs), are formed by maturation of specific domains of the TGN, and transport of vacuolar cargo between TGN and PVC is thought to occur by bulk flow rather than vesicle traffic [5,6]. This maturation into the PVC involves the formation of intraluminal vesicles (ILVs), which carry plasma membrane proteins destined for degradation at the vacuole. The endosomal sorting complex required for transport (ESCRT) machinery is responsible for ILV formation and this process is important for both vacuole trafficking and autophagy [5,9]. Localization of VPS28, a member of the ESCRT-I complex, suggested that formation of intraluminal vesicles starts as early as the TGN [6]. A plant unique ESCRT component, FYVE1/FREE1, was recently identified for having a critical role in formation of ILVs, vacuole traffic and biogenesis, degradation of membrane-associated ubiquitylated proteins, and seedling viability [9,10•,11•]. FYVE1/FREE1 binds to vps23A and Snf7A, members of the ESCRT-I and ESCRT-III complexes, respectively, and therefore, may function in ESCRT-I-III bridging [12•]. The current model for PVC biogenesis from TGN maturation supports the hypothesis of an organelle continuum in the endomembrane system were bulk flow of luminal cargo could result in vacuolar proteins reaching the PVC in the absence of vesicle budding and fusion between these two organelles [4••,13••]. Alternative pathways from this major route could arise from budding of specific cargo into vesicles or the differentiation of such maturing organelles into distinct subdomains of the TGN or the PVC [7].

Rab GTPases from the RAB5 and RAB7 families regulate vacuolar trafficking at the PVC. An exchange of RAB5 for RAB7, which is mediated by the CCZ1-SAND complex, is important for the trafficking of aleurain, phaseolin and 12S globulin [7]. Yet a RAB5-dependent RAB7-independent pathway has been reported for trafficking of SYP22 [14], and VHA-a3 [15]. Similarly, another pathway that is independent of both RAB5 and RAB7, but is dependent on AP-3, is utilized by VAMP713 [7]. How different proteins are sorted into these different pathways and which other regulatory elements are involved, is still unknown. Recently, a connection between RAB5 and phosphoinositides was uncovered using a yeast-two-hybrid approach to identify RAB5 effectors. This work identified EREX, a phox homology (PX) domain containing protein that binds exclusively to the GTP active form of RAB5 proteins ARA7 and RHA1, but not to ARA6, the plant-specific RAB5. EREX localization to endosomes is dependent on ARA7-GTP and phosphatidylinositol 3-phosphate. Both EREX and EREX-like 1 (EREL1) work together to mediate vacuole traffic of 12S globulins. Future research that identifies other protein interactors of EREX is needed to define the role of this protein in vacuole trafficking [16••].

pH and regulatory lipids are important for PVC and vacuole traffic and homeostasis [17,18]. Two tonoplast proton pumps, the V-type H^+-ATPase (V-ATPase) and H^+-pyrophosphatase (V-PPase), are required for vacuole acidification, but proton pumps at the TGN may also contribute to this function [18,19]. Moreover, NHX-type Na+/H+ exchangers (NHXs) are also important for regulating the pH of cellular organelles, and their activity is important for vacuolar traffic [18]. For example, NHX5 and NHX6 are important for trafficking of 2S albumin and 12S globulin [20], and the localization of two vacuolar SNARE proteins, SYP22 and VAMP727 [21]. Given the synergistic effects of the triple *nhx5 nhx6 syp22* mutant, it is possible that NXH transport activity is important for SNARE-mediated fusion events. However, since the direct interaction between SNAREs and NHX proteins has not been detected, the mechanism of this regulation is still unknown [21].

Membrane lipids are also important for vacuole traffic, biogenesis and dynamics [22]. Phosphoinositides have important roles in pollen development [23,24], homotypic vacuole fusion [25], endosome maturation [26], and vacuole morphology [27]. Sterols and sphingolipids are also important for vacuole traffic because genetic or pharmacological disruption of their biosynthesis results in abnormal trafficking of vacuolar cargo and developmental defects [28•]. Biosynthesis of these lipids is induced by auxin and is regulated by the ribosomal protein RPL4. In the absence of abundance information of sterols and sphingolipids along the endomembrane system, it is not yet clear how lipid composition regulates protein trafficking to the vacuole [29]. These data indicate that more studies are required to understand how phosphoinositides and other lipids regulate vacuole biogenesis and dynamics.

SNARE-mediate fusion with the vacuole

Fusion between the PVC with the vacuole is the last step of vacuolar traffic [7,9,30]. Mechanisms of membrane fusion are highly conserved and include the function of SNAREs, RAB GTPases and the HOPS tethering complex. SNAREs belong to a highly conserved family of proteins that mediate membrane fusion in eukaryotes. According to the SNARE hypothesis, two fusing membranes contribute either one R-SNARE or three Q-SNAREs to form of a four-helical bundle called a trans-SNARE complex. Zippering of the trans-SNARE complex brings the two apposing membranes together to promote fusion [31,32] fusion. Paralogous expansion in the SNARE complement in seed plants suggests diversification in SNARE functions [33]. An important vacuolar SNARE complex in Arabidopsis is composed of SYP22, VTI11, SYP51 and VAMP727 [34]. Mutant phenotypes indicate redundancy for SYP22/SYP21 [35], while two more R-SNAREs, VAMP713 and VAMP711, can replace VAMP727 and participate in the SNARE complex [7,36,37]. The VTI11 paralogue VTI13 has been detected at the tonoplast [38], but its association in the complex has not been demonstrated. Intriguingly, SNAREs may function beyond membrane fusion because VTI11 is involved in auxin-induced changes in vacuole morphology [39•] by an unknown mechanism that involves the actin cytoskeleton [40]. A conserved HOPS tethering complex is required for fusion of vacuoles in yeast and lysosomes in mammalian cells [41,42], and a similar complex is likely to exist in plants [43]. The HOPS subunit Vps33 belongs to the family of Sec1/Munc18 (SM) proteins, and has been implicated in assembly and regulation of the SNARE complex [44,45]. The predicted plant HOPS complex is composed of six proteins, VPS16/VACUOLESS1 (VCL1), VPS18, VPS11, VPS33, VPS39 and VPS41, which are all encoded by a single gene and are essential in Arabidopsis [43,46,47•]. VCL1 is important for vacuole development and pollen fertility, and it interacts with the SNARE SYP22, and HOPS subunits VPS33 and VPS11 [48–50]. VPS41 localizes to PVC and the tonoplast, and is necessary for vacuole fusion in pollen tubes [47•]. HOPS gene expression appears to correlate with changes in vacuole morphology in symbiotic cells of *Medicago truncatula* [51]. Together these results strongly suggest a conserved role of HOPS in modulating membrane fusion in plants, but it is not yet clear if it interacts with SNAREs to mediate membrane fusion at the vacuole.

Vacuolar cargo sorting

Vacuolar proteins contain one or more vacuolar sorting determinants (VSDs) that are recognized by sorting receptors [30,52]. Sorting receptors belong to the Vacuole