Trends in Plant Science

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Spotlight

VPS34 Complexes in Plants: Untangled Enough?

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Phosphatidylinositol-3-phosphate (PI3P) is essential for endocytosis and autophagy. VPS38 (endocytosis) and ATG14 (autophagy) are required for localized biosynthesis of PI3P. Liu *et al*. have shown that mutant arabidopsis (*Arabidopsis thaliana*) lacking both proteins are viable and synthesize PI3P, suggesting that the enzymatic complex VPS34 can function in absence of these regulatory subunits.

The pathways of endocytosis and autophagy (in particular macroautophagy) allow the eukaryotic cell to respond to internal and external stimuli by recycling and/or degrading defined components. In plant cells, these two pathways lead to the lytic vacuole as a common site of degradation of vesicles and their contents. The molecular machinery involved in these two pathways are very different, as are the membrane compartments that orchestrate these intracellular vesicular transports: endosomes and prevacuolar/multivesicular compartments (PVC/MVB) for endocytosis, phagophores, and autophagosomes for autophagy. Despite these differences, there is crosstalk between these two pathways [1] and their specific membrane compartments share the particularity of being enriched in phosphatidylinositol-3 phosphate (PI3P). The presence of this signaling lipid acts like a molecular fingerprint for these membrane compartments and facilitates the recruitment of various effector proteins based on their specific interaction with PI3P [2]. The transformation of phosphatidylinositol (PI) into PI3P is catalyzed by the lipid kinase of class III phosphatidylinositol-3 kinase (PI3K) vacuolar protein sorting 34 (VPS34), initially described in yeast. VPS34 is part of a heteromeric complex (VPS34 complex) with the kinase VPS15 and the protein VPS30 or Beclin1/ATG6 (core complex). In addition, the ATG6 protein in the core complex can recruit ATG14 protein or VPS38 protein to form VPS34 complex I or complex II, respectively. Complex I acts in the pathway of autophagy, whereas complex II is involved in endocytosis [3] (Figure 1). Although VPS38 had already been characterized in plants [4], no ATG14 homolog had been found. This void has just been filled by pioneering work in the laboratory of Richard Vierstra [5], which identified and characterized the only two paralogs, ATG14a and ATG14b, of arabidopsis (*Arabidopsis thaliana*). Among other intriguing observations, the authors showed that the homozygous triple mutant *atg14a-1 atg14b-4 vps38-1* is not only viable but also fertile, although its growth and development are substantially affected. This observation suggests that in plants, the VPS34 complexes can function without either of the regulatory factors, VPS38 or ATG14, and questions the ability of the remaining core complex to localize correctly in the plant cell in the absence of these activating and positioning factors.

Structure and Function of VPS38/UVRAG in Endocytosis and Beyond

The plant VPS38 is the homolog of yeast Vps38p and UV radiation resistance-associated gene (UVRAG) in animal cells. Arabidopsis *vps38-1* plants are almost not affected in their levels of PI3P, but at the cellular level, the mutants show an enlargement of the endosomes and deficient endocytic trafficking to the vacuole, leading to accumulation of ubiquitylated proteins that affect the physiology of the cell [4]. The vacuolar degradation of an autophagy marker (GFP-ATG8) is little affected in these mutants, except in conditions of nitrogen deficiency. Loss of VPS38 function seems not to perturb significantly the autophagic pathway in plants [4,5], suggesting that VPS38 is not required for autophagy in the plant cell. However, the *vps38* mutant exhibits crinkled leaves and defects in vascular tissue development, gravitropism, pollen germination, and seed development. These are all manifestations likely related to defective endocytic trafficking [5].

Structurally, angiosperm VPS38 proteins consist of essentially two functional domains, namely a coiled-coil (CC) domain followed by a related β-α repeated autophagy-specific (BARA2) domain. This structure is similar to that of yeast Vps38p, which possess an additional calcium-dependent lipid-binding (C2) domain. The BARA domain, also present at the C terminal end of Beclin-1/ATG6, contributes to anchoring the complex to the lipid bilayer [6]. Comparatively, metazoan UVRAG has a more complex structure, containing the following domains: a proline-rich (PR) domain, C2 domain, CC, BARA domain, and a C terminal part capable of associating with different additional effectors. The PR domain allows the interaction of UVRAG with other factors such as the endophilin Bif-1 in its phosphorylated form and this interaction promotes the formation of the autophagosome [7]. The presence of the UVRAG protein in the VPS34 complex can also lead to the recruitment of Run domain Beclin-1 interacting and cysteine-rich containing protein (RUBICON), which inhibits the maturation of the autophagosome and its fusion with the lysosome [8]. VPS34 complexes containing UVRAG therefore play a role in both the endocytic pathway and the autophagy pathway, depending on the additional protein partners of UVRAG [7,8].

Unlike plant VPS38 and yeast Vps38p, UVRAG in the VPS34 complex allows the formation of larger complexes involved in the formation of the autophagosome, its maturation, and that of the endosome, but also is involved in other functions

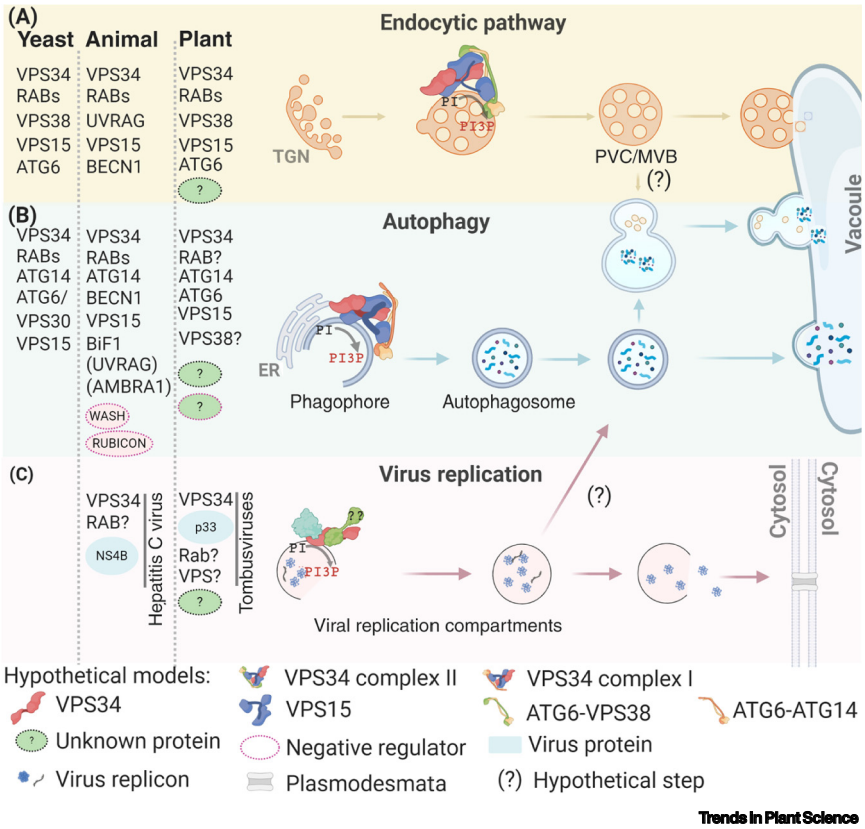

Figure 1. Model for Plant Vacuolar Protein Sorting 34 (VPS34) Complexes in Various Intracellular Processes. (A) Plant VPS34 complex II components (VPS34 as catalytic subunit, the kinase VPS15, Beclin-1/ATG6, VPS38) and additional described factors such as Rab-GTPases (RABs), as compared with other eukaryotes (yeast and animal cells, left) regulating endocytic vesicular trafficking. The functional VPS34 complex II catalyzes the enrichment of the endosomal compartment [prevacuolar compartment or multivesicular body (PVC/MVB)] membrane with phosphatidylinositol-3 phosphate (PI3P) from phosphatidylinositol (PI). Ultimately, the endosomal compartment fuses with the vacuole when its content is destined for degradation.(B) During autophagy induction in the plant, the phagophore can grow from the initiation membrane as an omega-shaped structure at an endoplasmic reticulum (ER) subdomain. VPS34 complex I (VPS34 as catalytic subunit, the kinase VPS15, Beclin-1/ATG6, ATG14) is recruited early on to the initiation membrane to enrich the phagophore membranes with PI3P, which is used as a platform for the recruitment of PI3P-binding effectors. Up-to-date plant VPS34 complex I composition is compared with the known VPS34 complex I composition from other eukaryotic cells (left), including additional factors regulating the activity of the complex, such as Rab-GTPases, in yeast and animal cells, or for animal cells, specifically, the endophilin B1 (Bif-1), UV radiation resistance-associated gene protein (UVRAG) acts as a substitute for ATG14-like (ATG14) and binds the autophagosome maturation and fusion inhibitor Run domain Beclin-1-interacting and cysteine-rich domain-containing protein (RUBICON). Additionally, possible regulatory components of the animal VPS34 complex I are Wiskott–Aldrich syndrome protein and SCAR homolog (WASH), which can suppress Beclin-1 (BECN1) ubiquitination to inhibit VPS34 activity, and activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1). Related factors are not yet described in plants. *En route* to the vacuole, the plant autophagosome could fuse with an endocytic membrane compartment before delivering its content to the vacuole. (C) Animal and plant VPS34 complexes may also be involved in virus particle trafficking in the cell through the generation of virus replication compartments, the membrane of which is also enriched in PI3P. Specific virus proteins, such as hepatitis C virus nonstructural protein 4B (NS4B) and plant *Tombusvirus* tomato bushy stunt virus (TBSV) viral protein-p33, may be part of this VPS34 complex; the full composition and the structure of the targeted membrane compartment is not yet clear. Hypothetically (denoted by a question mark), the viral replication compartment could fuse with the autophagosome for its degradation in the vacuole, or release the virus particles in the cytoplasm for their cell-to-cell movement through the plasmodesmata. This figure was created using BioRender (https://biorender.com/). Abbreviations: ER, endoplasmic reticulum; TGN, *trans*-Golgi network.

independent of VPS34 such as apoptosis, centrosome stability, DNA repair, and genomic stability. It remains to be seen whether the plant VPS38 protein could functionally interact with Bif1-related BAR domain proteins such as SH3-domain-containing protein 1 or 2 (SH3P1 or SH3P2). RUBICON-related proteins are also present in plants; whether they play a role in this context remains to be determined.

Structure and Function of ATG14/Atg14L/Barkor in Autophagy

Two paralogs of ATG14, ATG14a and ATG14b, are present in arabidopsis and at least one is expressed in all major tissue. Only the double mutant *atg14a-1/atg14b-4* exhibits a cellular phenotype affecting essentially the autophagy pathway and manifested by a strong decrease in the vacuolar degradation of the autophagy marker GFP-ATG8, during nitrogen or carbon deficiency [5]. Under normal physiological conditions, *atg14a/atg14b* mutant plants are indistinguishable from wild-type plants. Plant ATG14 proteins may therefore be essential for the autophagy pathway. Nevertheless, their absence in the VPS34 complex I does not seem to adversely affect the biosynthesis of PI3P and the physiology of the plant. These observations suggest that the role of ATG14 proteins during autophagy may not be limited to the activation of the VPS34 complex.

Plant ATG14 does not complement the absence of Atg14p in yeast. The conservation rate between the two proteins is very low, although they are organized in similar functional domains, namely an N terminal cysteine repeat followed by a CC domain that interacts specifically with Beclin1/ATG6. Their animal counterpart, ATG14L or Beclin 1-associated autophagy-related regulator (Barkor), additionally contains a Barkor autophagosome targeting sequence (BATS) domain at its C terminus, which allows interaction with $PI(4,5)P_2$ and its corresponding biosynthetic kinase

[9,10]. ATG14L, without the VPS34 core complex, promotes the tethering and fusion of autophagosomes with endolysosomes by physically and physiologically interacting with cognate SNAREs [11]. The membrane tethering activity of ATG14L requires its BATS domain.

Plant VPS34 Core Complex Activity without VPS38 and/or ATG14?

Liu *et al.* [5] achieved the genetic *tour de force* of generating viable triple mutants deficient in VPS38/ATG14a/ATG14b activities. Although the phenotype of these plants is marked by an accentuation of the phenotype of the *vps38-1* mutant plants, it was surprising to find that these triple mutants still synthesize about 50% of PI3P found in the wild-type plant. This observation suggests that VPS38 and ATG14 are not essential for the metabolism of PI3P, even if their loss of activity has substantial physiological consequences. It is possible that the absence of VPS38 and ATG14 compromises the activity of the corresponding complexes much less than their spatiotemporal positioning in the cell and access to their substrate. Of the various VPS34 complexes, it seems that only the loss of the VPS34 catalytic subunit is vital.

Concluding Remarks and Future Directions

The VPS34 complexes I and II play an important role in the autophagy and endocytosis pathways, respectively. The pioneering work of Liu *et al.* [5] in the elucidation of the molecular components of these complexes has generated novel tools to study their structure–function and their regulation by both internal and external stimuli. It cannot be excluded that the composition of these complexes in plants involves multiple other factors, such as RAB proteins, which also define the membrane identity or territories, and may contribute, directly or indirectly, to the selective intracellular positioning of a VPS34 complex. It also remains to be seen whether the complex role of VPS34 in the cell could go beyond autophagy and endocytosis, such as also facilitating the trafficking of viral particles [12].

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Declaration of Interests

No interests are declared.

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