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Vacuole Biogenesis in Living Soybean Root Tip Cells

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The most prominent organelle in most living plant cells is the large central vacuole. In meristem cells the vacuole is derived *de novo* from other membrane systems. Currently there is not a widely accepted model of vacuole biogenesis in plants [1]. Electron micrographs show that developing vacuoles (provacuoles) in root tip meristem cells comprise complex membrane structures, including large numbers of vesicles and tubular membrane elements at various stages of fusion [2-3]. Using high voltage EM of thick sections stained *en bloc* with ZIO, Marty and coworkers have shown that a complex array of provacuole tubules forms a cage around cytoplasm in root tip meristem cells [2]. These tubules fuse together to form an autophagic vacuole that digests the enclosed cytoplasm. According to this view, the provacuole tubular elements derive from the TGN. Consistent with this view, thin sections show proximity of Golgi stacks and TGN to the provacuolar compartment [3]. Alternatively, other evidence suggests that the provacuolar compartment may derive directly from smooth ER [4]. Clearly, vacuole biogenesis is a highly dynamic process that is difficult to interpret using static electron micrographs. Live cell imaging could provide information on development that is not possible in EM samples, by continuously monitoring the dynamics of vacuole biogenesis in single cells.

We have discovered that genetic transformation of legume roots by *Agrobacterium rhizogenes*, a plant pathogenic bacterium that causes the disease “hairy roots”, induces the production of an autofluorescent compound in the lumen of vacuoles of transformed roots. Based on staining with DPBA (a flavonoid stain) [fig. 1], and similar to other studies of legume roots challenged with bacteria, the compound is likely a dihydroxyflavone [5]. Making use of this autofluorescence, which can be imaged using 790 nm two-photon excitation, we have made 3D images over time (4D movies) of vacuole biogenesis (http://www.danforthcenter.org/imf/4danimation.htm). Use of this system has allowed us to address many of the previously unanswered questions regarding vacuole biogenesis. Provacuoles exhibit remarkable dynamics in this system. At early stages, branched and polygonal networks of tubular provacuoles [fig. 2] undergo constant rearrangements, including sliding of tubules along their intersections with other tubules. Vesicles bud from provacuolar tubules and fuse again with other tubular elements. Nascent vacuole begins to develop at two poles of the meristem cell [fig. 3], by aggregation of vesicles and tubular elements and swelling/fusing of these components. 4D movies of this process show structures consistent with Marty’s model of autophagic tubule cage formation. To analyze derivation of the provacuolar compartment, a gene encoding an ER:GFP construct was included in the bacterium’s root-inducing plasmid to facilitate simulteaneous imaging of the ER. The provacuole signal is distinct from the ER signal in these cells [fig. 4], consistent with Marty’s model of derivation from the TGN. Live cell imaging is the imaging method of choice for understanding the dynamics of vacuole development.

References

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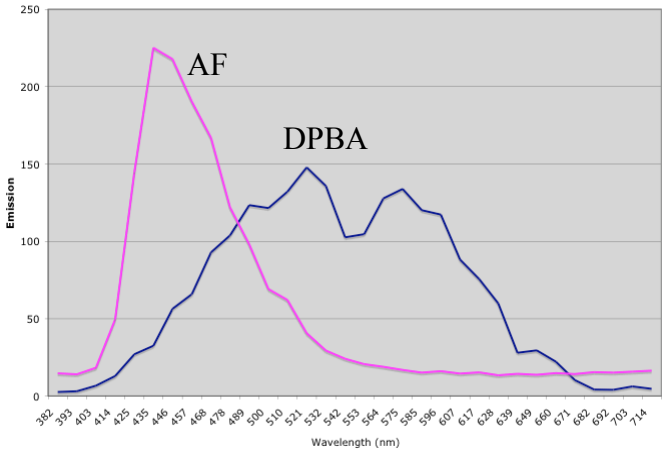

Fig. 1. Signal from Zeiss META spectral image for autofluorescence (AF) and DPBA-stained compound in vacuole lumen

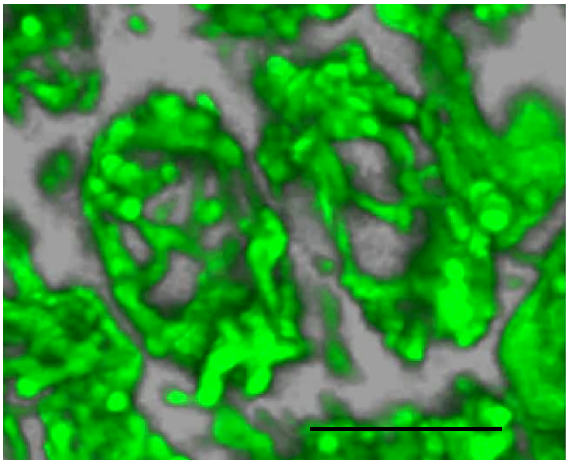

Fig. 2. Early stage of vacuole biogenesis showing arrays of provacuole tubular elements in two meristem cells. Scale bar = 7 µm.

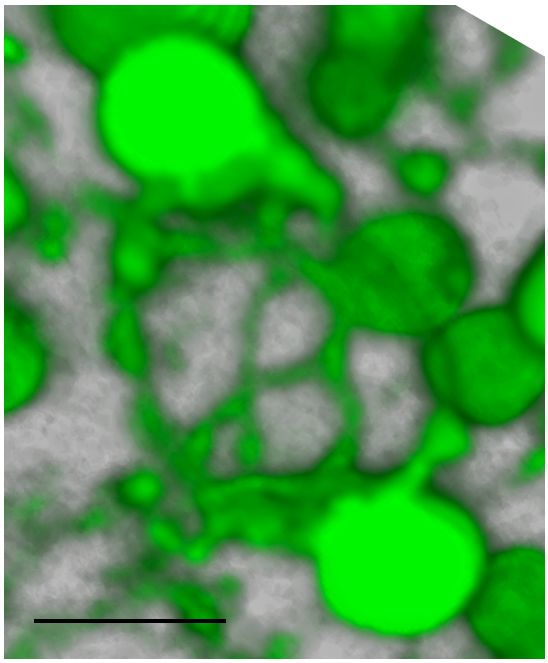

Fig. 3. Nascent vacuole stage, with developing vacuoles at two poles of the meristem cell. Scale bar = 4 µm.

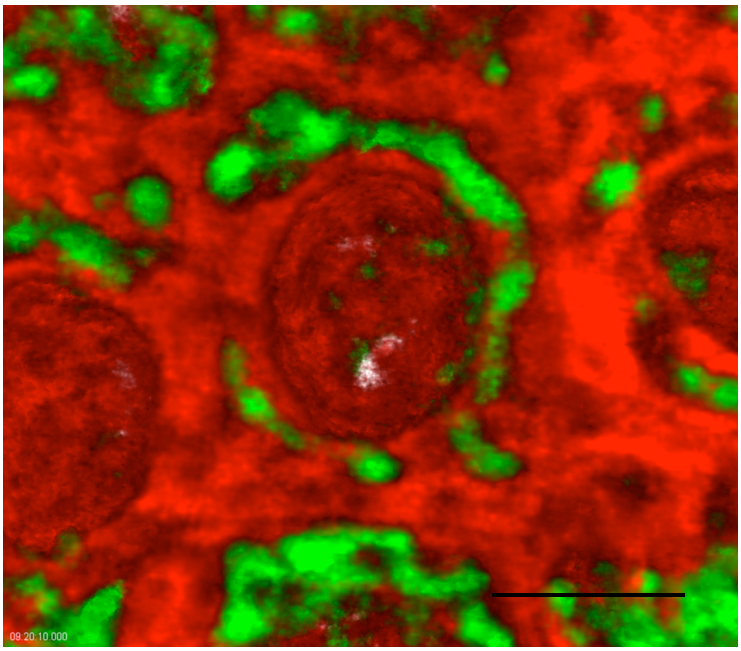

Fig. 4. ER:GFP (red) and provacuole components (green) are not colocalized. Nucleus in cell center is surrounded by ER. Scale bar = 4 µm,

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