Title
Autophagy Sustains the Arabidopsis Root Meristem during Phosphate Starvation

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Introduction

Autophagy is a conserved eukaryotic catabolic pathway which was shown to participate in many cellular physiological processes including nutrient recycling (Klionsky, 2004). The autophagy pathway involves formation of double membrane compartments, called autophagosomes, which sequester a portion of cytoplasm and deliver to vacuole for further degradation (Bassham et al., 2006). In plants, analyses of several autophagy mutants reveal crucial roles of autophagy during carbon and nitrogen starvation (Bassham et al., 2006). However, the function of autophagy in response to other types of nutrient starvation has never been elucidated. Phosphate (Pi) is an essential plant macronutrient whose availability is very limited in every ecosystem. To survive under this common Pi limitation, plants evolved a number of responses to increase Pi use efficiency and acquisition. One dramatic Pi starvation response involves changing root architecture in which primary root growth is attenuated and lateral root number and growth are increased (Ticconi and Abel, 2002). Here, we demonstrate that autophagy plays a significant role in remodeling root system architecture by sustaining root meristem during Pi starvation.

Result

MDC staining detected numerous autophagosome spots in root meristem under Pi starvation

Monodansylcadavarine (MDC) is an autofluorescent dye widely used to detect autophagosomes in animal and plant cells during nutrient starvation (Contento et al., 2005). 5 d-old wild type seedlings were transferred to media without Pi (-Pi) and stained with MDC at each time point. At about 4 days after transfer, numerous moving spot of autophagosomes were detected in root tip cells (Fig. 1).

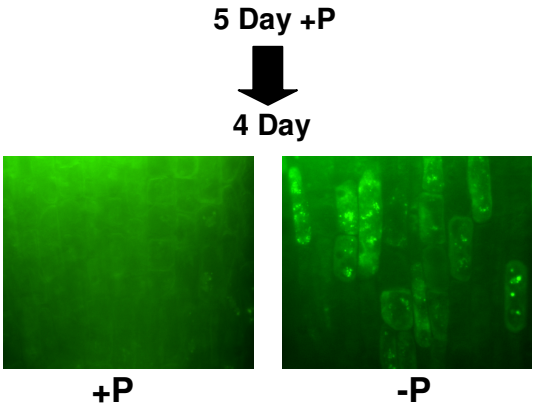

Figure 1. MDC staining of Arabidopsis root. 5 day old wild type seedlings after transfer to complete and –Pi media

atATG8s were upregulated in root tip during Pi starvation

ATG8 encodes an ubiquitin-like protein that undergoes cascade of modifications before conjugation with phosphatidylethanolamine (PE) and incorporation into preautophagosomal membrane where it plays crucial roles in autophagosome formation and expansion (Klionsky, 2004). In *Arabidopsis*, nine homologs of *ATG8* genes, known as *atATG8a-i*, were identified and shown to participate in autophagy process (Yoshimoto et al., 2004). To observe changes in expression of *atATG8* genes, we performed Quantitative RT-PCR at several time points using RNA extracted from Arabidopsis primary root tip after transfer to –Pi media. Consistent with the time course MDC staining experiment, we found that some *atATG8s* were significantly upregulated in wild

type after transfer to –Pi media for 3 to 4 days (Fig. 2). Among upregulated genes, *atATG8a*, *atATG8e* and *atATG8f* were upregulated more than 3 folds.

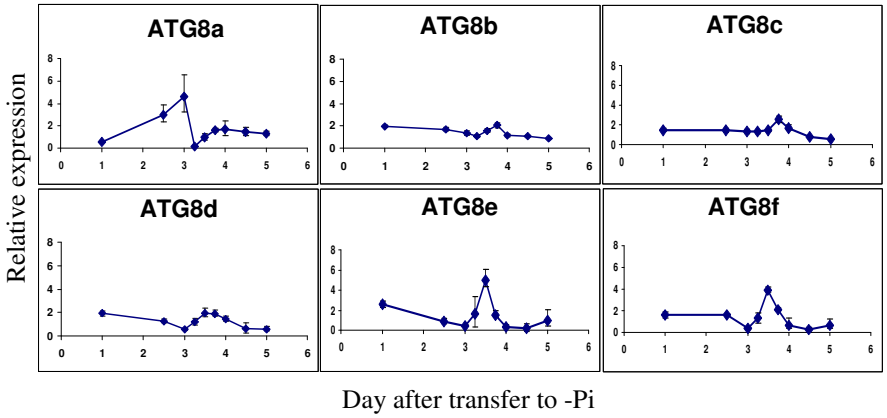

Figure 2. QRT-PCR of *atATG8s*. Seedlings were grown on complete media for 5 days before transfer to complete and –Pi media. 3-4 mm of root tips were collected at each time point and used for RNA extraction. Relative expression values calculated using ΔΔCt method. Samples from complete media at the same time points were used as calibrator. Actin was used as the endogenous control.

Disruption of *atATG5* causes early root meristem consumption in low Pi condition

To understand the role of autophagy during Pi starvation, we isolated *atATG5* T-DNA insertion line, called *atg5-4*, from SALK collection. This *atATG5* insertion line contains T-DNA insertion in the first exon 1 bp upstream of start codon. *atATG5* knockouts have been previously shown to prevent formation of autophagosome and caused sensitivity to nitrogen starvation (Thompson et al., 2005). To test whether *atg5-4* is sensitive to Pi starvation, we performed transfer experiments in which 5 d-old seedlings of wild type and *atg5-4* were transferred to complete and –Pi media. After transfer to –Pi media for 8-12 days, we found that lateral root length of *atg5-4* is dramatically reduced whereas the number of lateral root per seedling is the same when compared to wild type (Fig. 3a and b). To monitor root meristem activity, we introduced a cell division marker CYCB1::GUS into *atg5-4* background. Time course GUS staining indicated early loss of primary and secondary root meristem in *atg5-4* (Fig. 3a and c). Moreover, low primary root recovery rate upon transfer back to complete media in *atg5-4* suggests this loss of root meristem is irreversible (Fig. 3d and e).

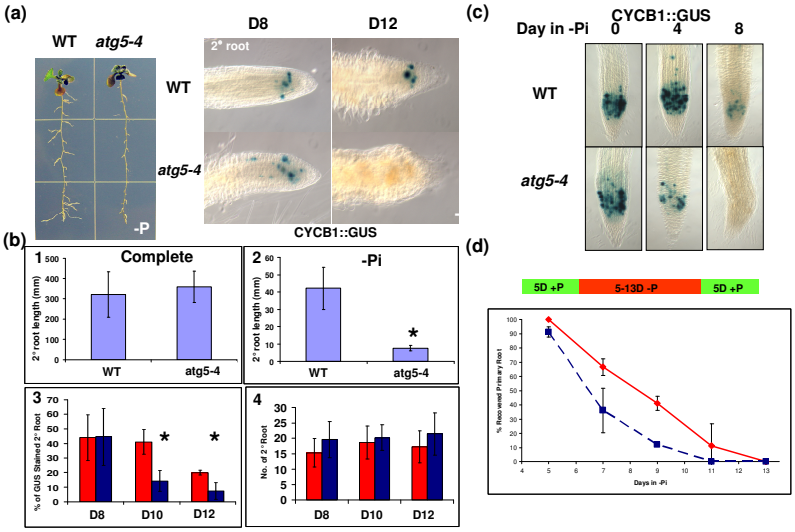

Figure 3. Acceleration of Root Meristem Consumption in *atg5-4*. **(a)** Comparison of WT and *atg5-4* lateral root growth after transfer to –Pi media for 10 days. **(b)** total secondary root length per seedling in wt and *atg5-4* after transfer to (1) complete and (2) –Pi media for 12 days, (3) percentage of secondary roots having GUS signal per seedling and (4) number of secondary roots per seedling after transfer to –Pi media for 8-12 day. Wild type is red and *atg5-4* is blue. **(c)** GUS staining of CYCB1::GUS marker after transfer to –Pi media. **(d)** Percentage of recovered primary root after transfer to –Pi media for indicated days. Red is WT and blue is *atg5-4*.

Summary

We have shown that autophagy is induced during Pi starvation. Moreover, we showed that disruption of the autophagy pathway caused acceleration of root meristem consumption and results in abnormal root system architecture in low Pi condition. Further work is needed in order to demonstrate the mechanism by which autophagy sustains root meristem.

References

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