

Franziska K. Kretschmar¹, Laura Mengel¹, Oliver Valerius², Gerhard Braus², Till Ischebeck¹

¹Georg-August-University Göttingen, Albrecht-von-Haller-Institute for Plant Sciences, Department of Plant Biochemistry, 37077 Göttingen, Germany
²Georg-August-University Göttingen, Dept. Molecular Microbiology and Genetics, Institute of Microbiology and Genetics, 37077 Göttingen, Germany

Introduction

Lipid droplets are energy storage organelles found in most eukaryotes. In animals, lipid droplets are found in hepatocytes and adipocytes; in plants, they are ubiquitous but most prominent in seeds and pollen.

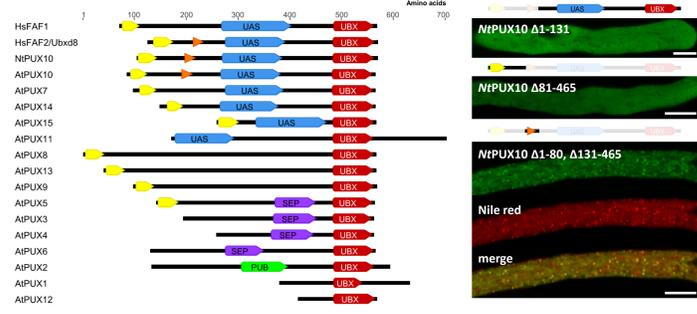
Despite their omnipresence, little is known about lipid droplet formation, degradation and protein composition.

To elucidate the function of lipid droplet as well as their synthesis and degradation, we analyzed the proteome of lipid droplets isolated from tobacco pollen tubes. Using a comparative approach with total and cytosolic extracts, we were able to identify several candidates and to confirm their localization at the lipid droplets in transiently transformed pollen tubes by fluorescence microscopy. One of these proteins, a UBX-domain containing scaffold protein, harbors a unique hydrophobic region not found in most members of the gene family. This short domain alone is able to target a fluorophore to lipid droplets.

The scaffold protein can recruit the AAA-type ATPase CDC48 that is involved in protein degradation. Homologues of CDC48 and UBX domain-containing proteins are involved in the ERAD-pathway both in yeast and mammals. We therefore suspect a similar protein degradation pathway situated at lipid droplets in plants. This pathway might be important especially upon temperature stress during lipid droplet formation.

AtPUX10 is a unique member of a 15 member gene family

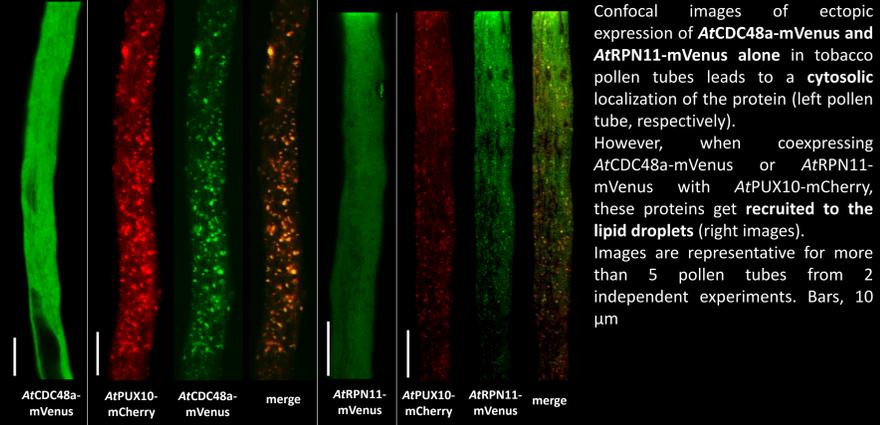
The family of PUX proteins is very diverse but shares the UBX-domain. *NtPUX10* and *AtPUX10* harbor the UBX (ubiquitin regulatory X) domain, a UAS (ubiquitin-associating) and a UBA (ubiquitin-homolog) domain (displayed in yellow) similar to *HsFAF1*, *HsFAF2* and *AtPUX7*, 14



and 15. In addition, they contain a hydrophobic stretch (displayed in orange) not present in any other member of the Arabidopsis gene family. The hydrophobic stretch of *HsFAF2* is not conserved. The plot was created with Geneious.

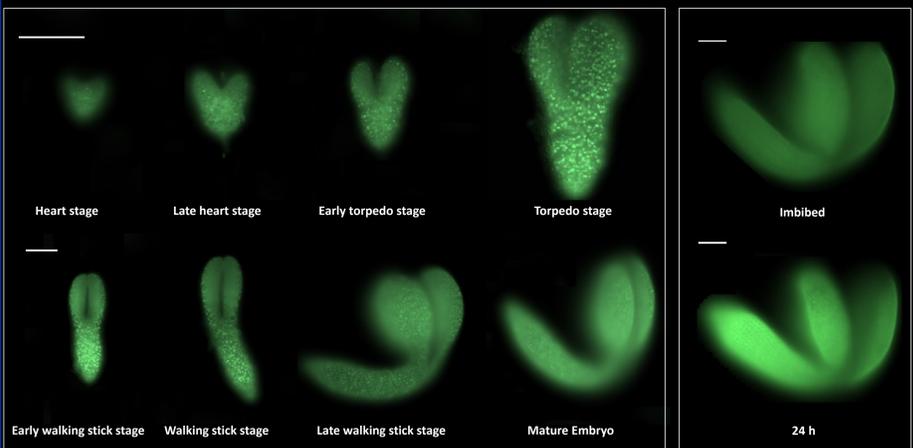
The unique hydrophobic stretch targets *NtPUX10* to the LDs. Truncated variants of *NtPUX10* were transiently expressed in tobacco pollen tubes. Colocalization was confirmed by Nile Red staining. Bars, 10 μm

PUX10 recruits *AtCDC48a* (*Hsp97/VCP* homolog) and proteasomal protein RPN11 to lipid droplets



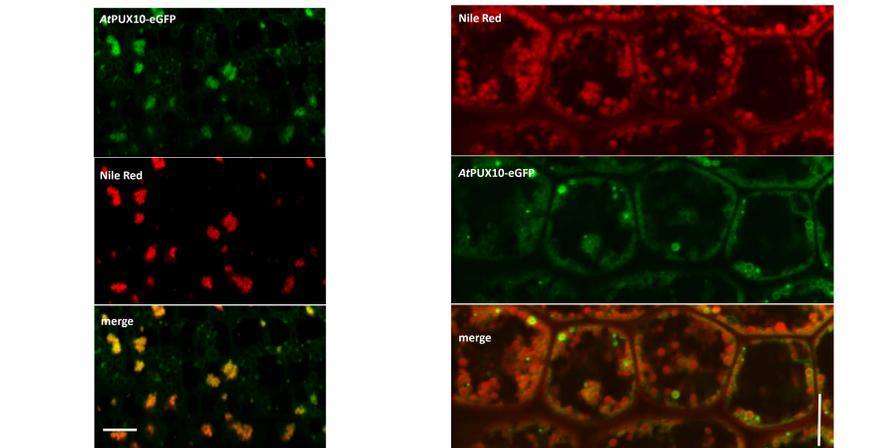
Confocal images of ectopic expression of *AtCDC48a-mVenus* and *AtRPN11-mVenus* alone in tobacco pollen tubes leads to a cytosolic localization of the protein (left pollen tube, respectively). However, when coexpressing *AtCDC48a-mVenus* or *AtRPN11-mVenus* with *AtPUX10-mCherry*, these proteins get recruited to the lipid droplets (right images). Images are representative for more than 5 pollen tubes from 2 independent experiments. Bars, 10 μm

Expression of *AtPUX10* starts during embryogenesis



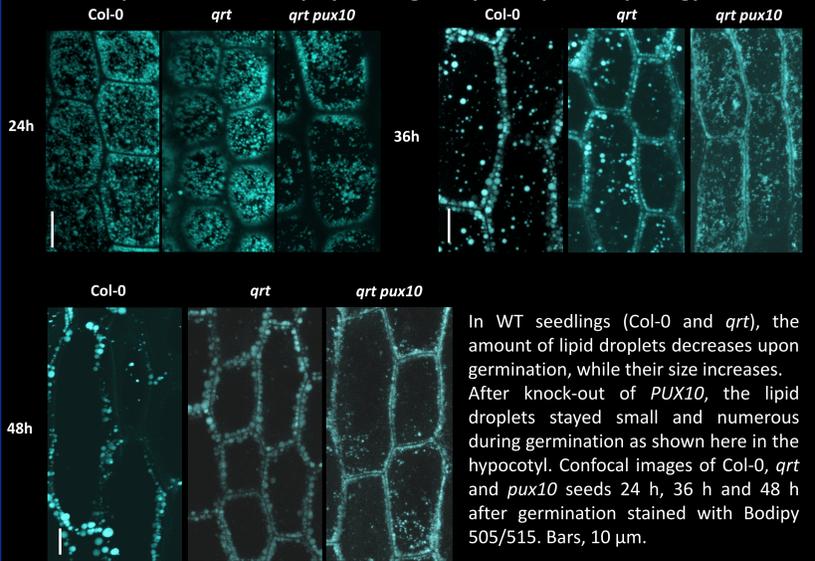
Epifluorescence microscopy of embryos expressing *AtPUX10-eGFP* under its native promoter. LD colocalization and presence during embryogenesis was confirmed for at least 5 embryos per stage of 3 independent transgenic lines. Bars, 100 μm

During embryogenesis and germination, *AtPUX10* localizes to lipid droplets



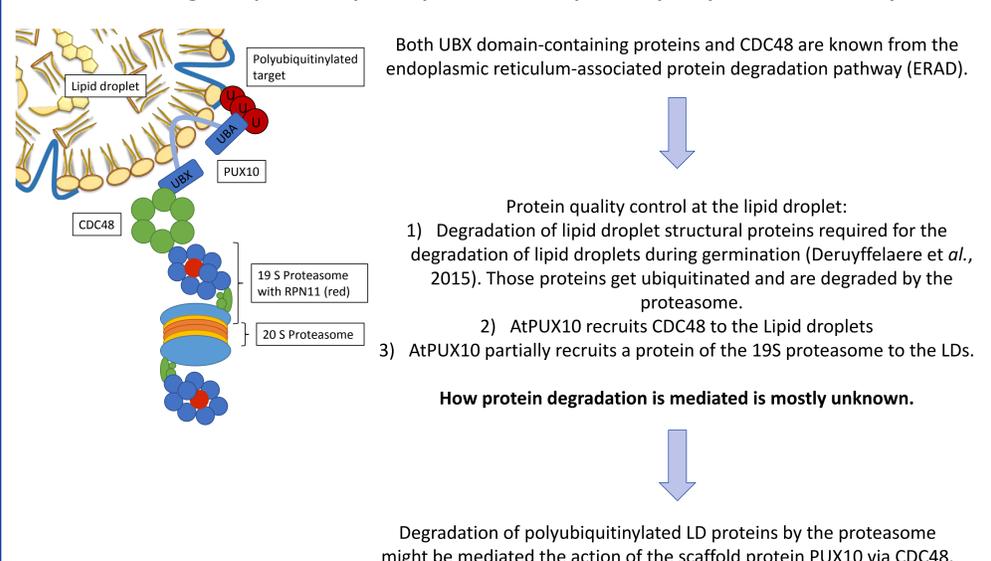
Confocal images of embryonic LDs show that during germination, *AtPUX10-eGFP* localizes to all lipid droplets (stained in Nile Red). Observation was confirmed for at least 5 seedlings for 3 independent lines. Confocal images of hypocotyl cells (24 h after germination) show that during germination, *AtPUX10-eGFP* localizes stronger to specific lipid droplets (stained in Nile Red). Observation was confirmed for at least 5 seedlings for 3 independent lines. Bars, 10 μm.

pux10 mutants display a change in lipid droplet morphology



In WT seedlings (*Col-0* and *qrt*), the amount of lipid droplets decreases upon germination, while their size increases. After knock-out of *PUX10*, the lipid droplets stayed small and numerous during germination as shown here in the hypocotyl. Confocal images of *Col-0*, *qrt* and *pux10* seeds 24 h, 36 h and 48 h after germination stained with Bodipy 505/515. Bars, 10 μm.

PUX10 might be part of a lipid droplet-associated protein quality control machinery



Summary and conclusion

- AtPUX10* is a unique member of a 15 member gene family
- AtPUX10* gets recruited to the lipid droplets by its hydrophobic sequence
- AtPUX10* recruits the AAA ATPase *AtCDC48a* and the proteasomal protein RPN11 to the lipid droplets
- AtPUX10* is expressed both during embryogenesis and germination during which it localizes to specific lipid droplets
- pux10* mutants display a change in lipid droplet morphology
- AtPUX10* might be part of a lipid droplet-associated protein quality control machinery

Outlook

- Determination of potential targets of the lipid droplet-associated protein quality control machinery by tandem mass spectrometry
- Determination of further effectors of lipid droplet-associated protein quality control machinery by tandem mass spectrometry
- Analysis of mutants exposed to different abiotic stress conditions like temperature stress, drought stress or pharmacological stress

References

Deruyffelaere, C., Bouchez, I., Morin, H., Guillot, A., Miquel, M., Froissard, M., Chardot, T., and D'Andrea, S. (2015). Ubiquitin-mediated proteasomal degradation of oleosins is involved in oil body mobilization during post-germinative seedling growth in Arabidopsis. *Plant Cell Physiol.* 56, 1374–1387.

