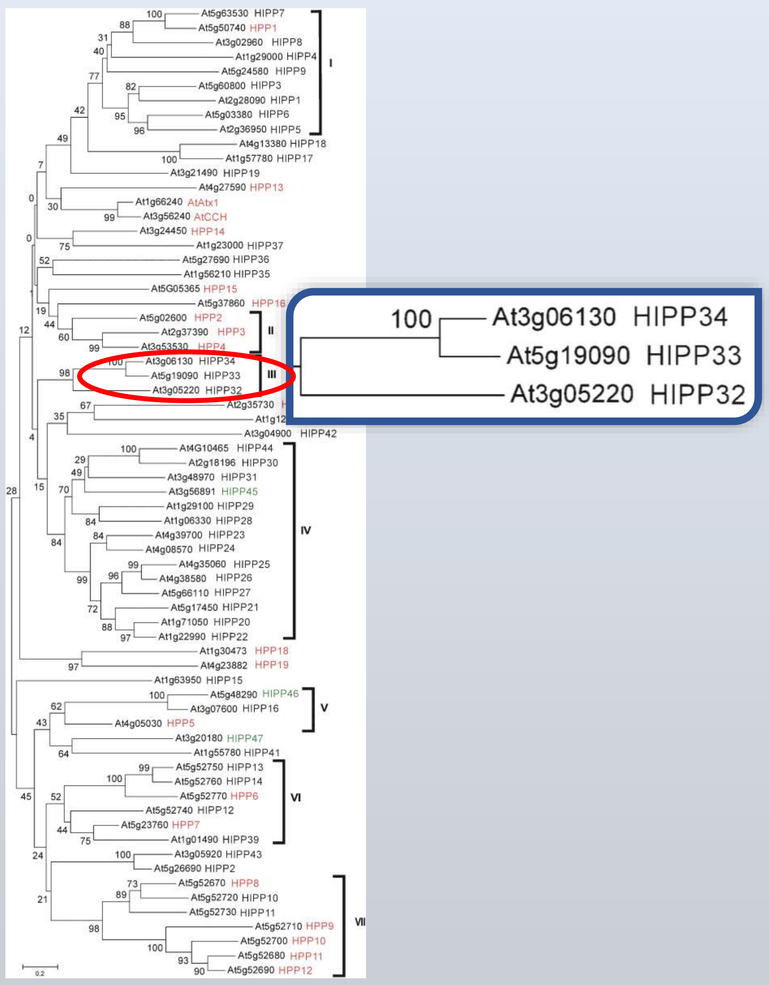
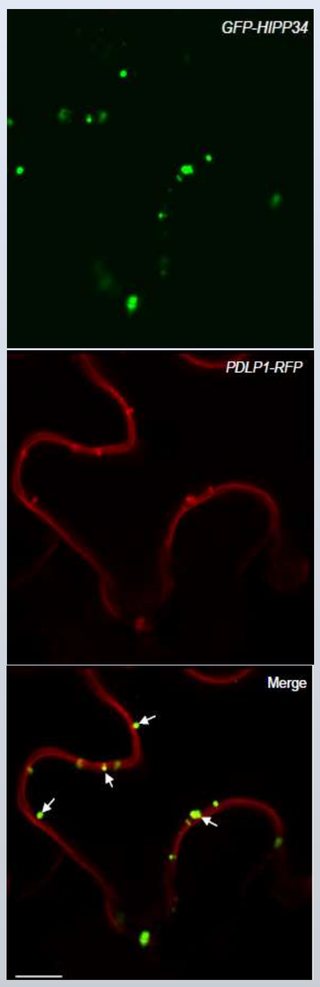
Molecular and functional characterization of heavy metal associated isoprenylated plant proteins localized to plasmodesmata

Paul Tschuden[1], Georgeta Leonte[2], Tomáš Werner[1]
[1]Institute of Biology, University of Graz, Austria
[2]Institute of Biology/Applied Genetics, Freie Universität Berlin, Germany
Email: **paul.tschuden@uni-graz.at**

Cell-to-cell communication is crucial to coordinate plant development. The intercellular transmission of information between adjacent cells is conducted via membrane-lined channels within the rigid plant cell walls – the plasmodesmata (PD). Recently we found a group of heavy metal-associated isoprenylated plant proteins (HIPPs) that specifically localize to PD. The biological function of these vascular plant-specific proteins, however, is entirely unknown. The goal of this work is to molecularly and functionally characterize the phylogenetically closely related HIPP proteins targeted to PD. Confocal microscopy is employed to address expression patterns as well as subcellular localization of different *HIPP*-reporter gene constructs. The function of the *HIPP* genes in plant growth and development will be analyzed using loss- and gain-of-function mutants. Based on our preliminary findings, a specific emphasis is laid on their role in regulatory activities within shoot and root apical meristems. The function of *HIPP* genes in mediating general PD-dependent protein trafficking as well as transport of specifically selected transcriptional regulators in the apical meristems will be explored. In essence, my doctoral project will not only provide first insights into the biological role of *HIPP* genes in controlling growth and development but it will also contribute to the better understanding of PD-associated molecular mechanisms and communication in plants

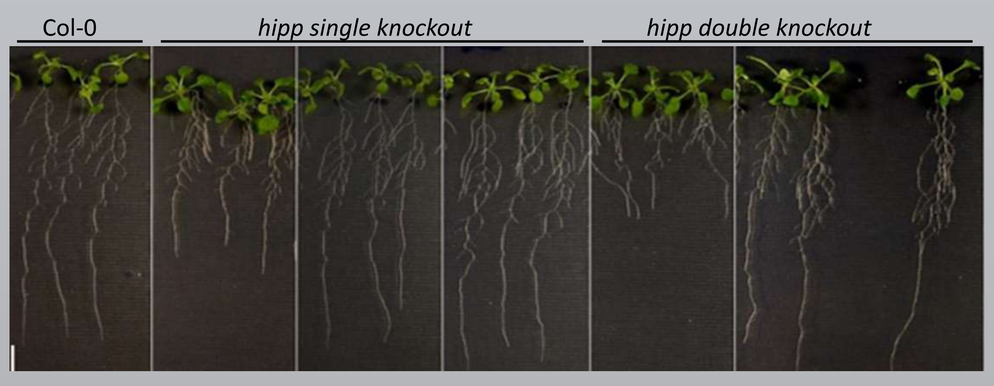
1. HIPP proteins from phylogenetic cluster III are specifically targeted to plasmodesmata





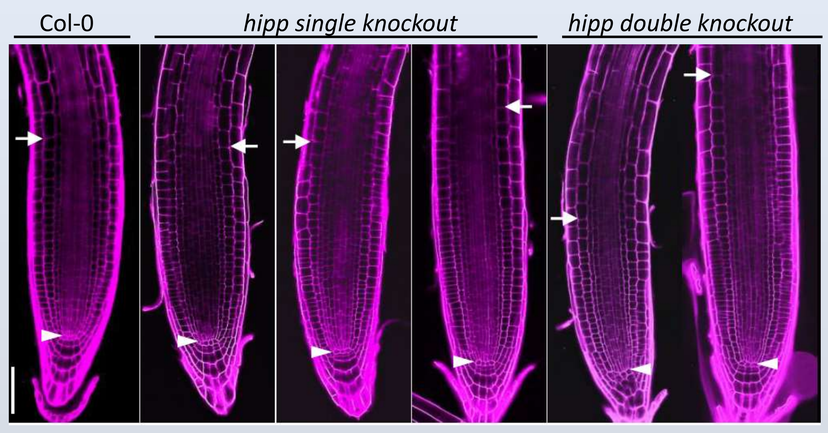
The investigated *HIPPs* share a high level of sequence homology and belong to the distinct phylogenetic cluster III (Tehseen et al., 2010). In transient expression experiments, in *Nicotiana benthamiana* leaves, the investigated GFP-HIPP34 fusion protein localized to plasmodesmata (PD, indicated by arrows). The specific localization was confirmed by colocalization with the established PD marker PDLP1-RFP (Thomas *et al*., 2008). Scale bar = 10 µm.

2. Phenotypic analysis of *hipp* knockout plants



Primary root (PR) elongation in the *hipp* knockout lines is influenced in a gene-specific way. The *hipp* single knockouts show either reduced or weakly enhanced PR elongation in comparison to the wild-type. In the *hipp* double knockout plants, reduction or increase of PR elongation or increase of PR elongation was enhanced in comparison to the respective single knockout lines suggesting that the homologous genes not only act in the same regulatory pathway but also that they have antagonistic roles in the regulation of PR elongation. Plants were analyzed twelve days after germination. Scale bar = 10 mm

3. Loss of *hipp* gene function influences the proximal root apical meristem of Arabidopsis



The number of proximal meristematic root cells is altered in *hipp* knockout lines in comparison to the wild-type. Consistent with the PR elongation, the *hipp* single knockouts had either a slightly increased number of cells in the cortex cell file or a reduced number in this particular cell file in comparison to the wild-type control. The combination of two *hipp* knockout alleles leads to an enhancement of the observed changes in the single knockout lines, suggesting that the cluster III *HIPPs* act in part redundant as positive or negative regulators of root meristem cell divisions. Furthermore, in one mutant line, the usually precise and regular patterning of the QC and the distal RAM is lost, indicating that *HIPPs* act in the root stem cell niche. Plants were analyzed six days after germination. Arrow = transition zone. Arrowhead = QC cells. Scale bar = 75 µm

4. Outlook

- Because *HIPP32* and *HIPP34* are genetically linked, CRISPR/Cas9-mediated knockout of *hipp32* in the *hipp33,34* background will be used to generate the triple knockout.
- Phenotypic analysis of all double knockouts and the triple knockout will be conducted and thoroughly quantified.
- The function of *HIPPs* in root apical meristem formation and patterning will be further investigated.
- Plasmodesmata-localization of cluster III HIPP proteins will be analyzed in stably transformed *Arabidopsis thaliana* lines.
- Reporter gene constructs containing mutated *HIPP* versions will be employed to uncover the nature of their specific plasmodesmata localization.
- The potential influence of HIPPs on plasmodesmata transport rate will be measured in *hipp* loss-of-function lines using photoswitchable fluorescent proteins.
- Expression patterns of individual *HIPP* genes will be mapped in the shoot and root apical meristems.

References:
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