Project Title: A comparative approach to the 3-dimensional structure of the plantmicrobe interface using FIB/SEM tomography

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Several recent studies dealing with the interaction of plants with microorganisms (beneficial or pathogenic; Parniske, 2000) highlighted the importance and the role of the so-called plant-microbe interface (PMI), e.g. the haustorium, a structure that is produced by plant pathogenic oomycetes and fungi (Bozkurt and Kamoun, 2020). It contains membranes (Limpens, 2019), receptors and other important players. It could also be indicated that a 3-dimensional visualization of the PMI could have a very high potential for a better understanding of the underlying mechanisms (Ivanov et al., 2019; Roth et al., 2019). With the access to a multitude of mutualistic and parasitic symbiosis of plants and microbes, we want to illustrate common features and significant differences.

After chemical (Cerri et al., 2017; Liang et al., 2019) or cryo-fixation (high-pressure freezing (HPF); Rachel et al., 2010) of the respective sample material, we will perform conventional transmission electron microscopy (TEM) but the mahor focus will be on focused ion beam scanning electron microscopy (FIB/SEM) tomography (e.g. Luckner and Wanner, 2018a, b) and serial block-face scanning electron microscopy (SBFSEM). This will be followed by image analysis, segmentation and the generation of 3Dmodels using the AMIRA software package. In our study, we are going to use wild type hosts and mutualistic and parasitic symbiosis with microbes to investigate the following host-microbe interaction scenarios: arbuscular mycorrhiza (AM) in tomato, *Phytophtora* in tomato, nitrogen fixing root nodules by actinobacteria *Frankia* (actinorhizal symbiosis), rhizobia in legumes, downy mildew (*Hyaloperonospora arabidopsidis*) in *Arabidopsis thaliana*, white rust (*Albugo laibachii*) in *A. thaliana* and powdery mildew infection of barley and *Arabidopsis thaliana*.
To facilitate the recognition of the region of interest (ROI), all those approaches will be supported by correlative light and electron microscopy (CALM).

Methods

- High-pressure freezing
- 3D electron microscopy
- FIB/SEM-tomography
- SBF-SEM

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Project Title: Enhancing photosynthesis by synthetic biology and adaptive laboratory evolution

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In this project, parts of the light reactions of photosynthesis from very different species will be combined in a model cyanobacterium by genetic engineering. The goal is to enhance photosynthesis with respect to its potential to use light from different wavelengths. In a complementary approach we use adaptive laboratory evolution to make photosynthetic organisms more tolerant against different stresses like for instance high light or high temperature stress. Corresponding mutations will be identified by whole-genome sequencing, characterized for their molecular effects, and tested for their potential to enhance stress tolerance in several species.

Project Title: Acclimation to fluctuating light: cyclic electron flow

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Light intensities fluctuate under natural conditions. Thus, proper regulation of photosynthesis is pivotal for effective plant performance under fluctuating light (FL). Cyclic electron flow (CEF) involves the two thylakoid membrane proteins PGR5 and PGRL1, both of which are crucial for plant development under FL. Multiple lines of evidence indicate that PGR5 and PGRL1 form a complex in the thylakoid membrane. However, the precise mechanism of their action, the regulation of corresponding activities and whether this process has the potential for enhancing acclimation to FL are elusive. In preparatory work,

we showed that PGR5 and PGRL1 can rebuild CEF in the cyanobacterium Synechocystis sp. PCC 6803, making it now possible to study PGR5-dependent CEF in a prokaryote employing superior genetic tools in relatively short time spans. Moreover, we found that the pgrl2 mutation suppresses the pgrl1 mutation but not the pgr5 mutation - or in other words: PGR5 can function without PGRL1. This result significantly revised our view on PGR5-dependent CEF with PGR5 being the central component that is regulated by PGRL1 and PGRL2.
In this project, we will characterise suppressor mutations of prg5 and pgrl1 at the genetic, physiological and protein level. In addition to the model plant Arabidopsis thaliana, we will employ our cyanobacterial test system with reconstructed PGR5-dependent CEF to rapidly perform molecular and mechanistic studies.

Project title: Tissue-specific regulation of lipid polyester synthesis genes controlling oxygen permeation into *Lotus japonicus* nodules

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Symbiotic nitrogen fixation reduces the dependency on costly and environmentally hazardous synthetic nitrogen fertilizers. The rhizobia nitrogenase that catalyses the reduction of atmospheric nitrogen into ammonia is oxygen sensitive. Legumes protect the nitrogenase by creating a microaerophilic environment inside nodules. A long-standing model posits that oxygen diffusion into nodules is limited by a barrier in the nodule periphery. By exploring the natural diversity of *Lotus japonicus* accessions using comparative transcriptomics, we identified genes involved in the deposition of lipid polyesters on cell walls that are specifically expressed in the nodule periphery. Spatiotemporal analysis of promoter activity controlling the expression of two *Fatty acyl-CoA reductases* (*FARs*) genes showed distinct activation in the root and nodule endodermis. Mutant lines in one of these genes, showed an increase in nodule permeability, higher oxygen concentrations inside nodules, impaired nitrogenase activity, and reduced shoot growth. This supports a model in which nodule-specific lipid polyester synthesis genes mediate the formation of a permeation barrier in the nodule periphery. In this project we will investigate the function and regulation of these genes, as they are promising molecular markers for the establishment of this nodule barrier. To this end we will create mutant lines by CRISPR/Cpf1 gene editing and phenotypically characterize them, we will generate reporter lines to determine the ontogeny of the nodule barrier, and will investigate the regulation of *FAR* genes at a molecular level. This work will advance our understanding of how the nodule barrier is formed, a key adaptation enabling nitrogen fixation in legumes.

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