Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
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Host laboratory:
LPCV, https://www.lpcv.fr/en

Host group/team:
MetalStress : Plants, Stress & Metals team Web page

Title of the M2 research internship:

Biochemical and structural characterization of Casparian strip membrane domain protein 1 from Arabidopsis thaliana

Project summary:
Roots play a crucial role in the supply of essential ions for plants growth and development (i.e. water and nutrients) but may also absorb toxic substances. To control the molecule uptake from the soil, vascular plants developed a protective sheath surrounding the vascular cylinder called Casparian strips. A genetic analysis revealed the importance of Casparian strip membrane domain proteins (CASPs) in the formation of the Casparian strip (Roppolo et al., 2011). However, what is the exact role of these plasma membrane proteins in this process remains elusive. Intriguingly, based on gene ontology annotation, CASP proteins are predicted to incorporate a [4Fe-4S] cluster as a cofactor (Przybyla-Toscano et al., 2021). In line with this prediction, two cysteines contained in the loop between the third and fourth transmembrane domains are strictly conserved among the CASP representatives in Arabidopsis, and could serve as residue ligands. Therefore, several questions arise. Are CASP proteins representing a novel Fe-S protein family? What is the function of this metallic cofactor? How the [4Fe-4S] cluster is delivered toward transmembrane CASP proteins? To answer some of these questions, the aim of this M2 research internship will be to biochemically and structurally characterize Arabidopsis CASP1 (AtCASP1). For this, AtCASP1 will be produced in an Escherichia coli and/or Lactococcus lactis heterologous system as recombinant protein under aerobic and anaerobic conditions. In order to purify AtCASP1, several protocols involving different detergents will be assayed. This characterization will be completed by spectroscopic analyses of AtCASP1 (i.e. electron paramagnetic resonance, circular dichroism, Mössbauer). This work will be performed in collaboration with Patrice Catty from the LCBM. Our preliminary results demonstrate that AtCASP1 is well expressed in the E. coli system. Ultimately, the determination of the tri-dimensional structure of holo-AtCASP1 will be performed in partnership with the IBS.

Keywords:
casparian strip membrane domain proteins, iron-sulfur cluster

Relevant publications of the team:

Roland, M.; Przybyla-Toscano, J.*; Vignols, F.*; Berger, N.*; Azam, T.; Christ, L.; Santoni, V.; Wu, H.-C.; Dhalleine, T.; Johnson, M. K.; Dubos, C.; Couturier, J.; Rouhier, N. The Plastidial Arabidopsis thaliana NFU1 Protein Binds and Delivers [4Fe-4S] Clusters to Specific Client Proteins. J. Biol. Chem. 2020.

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Azam, T.*; Przybyla-Toscano, J.*; Vignols, F.; Couturier, J.; Rouhier, N.; Johnson, M. K. The Arabidopsis Mitochondrial Glutaredoxin GRXS15 Provides [2Fe-2S] Clusters for ISCA-Mediated [4Fe-4S] Cluster Maturation. Int J Mol Sci 2020.

Przybyla-Toscano, J.; Boussardon, C.; Law, S.; Rouhier, N.; Keech, O. Gene Atlas of Iron-Containing Proteins in Arabidopsis thaliana. Plant J 2021.

Przybyla-Toscano, J.*, Maclean, AE.*, Franceschetti, M., Liebsch, D., Vignols, F., Keech, O., Rouhier, N., Balk J. The Fe-S cluster assembly factors NFU4 and NFU5 are primarily required for protein lipoylation in mitochondria. Plant Physiol. 2021.

Revel B, Catty P, Ravanel S, Bourguignon J, Alban C. High-affinity iron and calcium transport pathways are involved in U(VI) uptake in the budding yeast Saccharomyces cerevisiae. J Hazard Mater 2022.

Sarthou MCM, Devime F, Baggio C, Figuet S, Alban C, Bourguignon J, Ravanel S. Calcium-permeable cation channels are involved in uranium uptake in Arabidopsis thaliana. J Hazard Mater 2022.