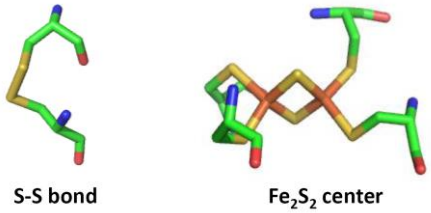
METOX



To bind or not to bind metals, that is the question

Unravelling new metal- or redox-dependent proteins and processes in plants

Principle investigator: Nicolas Rouhier

Collaboration: Pr Roland Lill (Univ. of Marburg) and Pr Johannes Herrmann (Univ. of Kaiserslautern)

Context — Major cellular functions are controlled at several steps through oxidoreduction reactions including electron transfer reactions catalyzed by metalloproteins, notably iron and copper-containing proteins, and by thiol-containing proteins in the case of dithiol-disulfide exchanges. By controlling several developmental stages, the photosynthetic rates and several aspects of the stress responses, these redox mechanisms are particularly crucial in plants. Cysteinyl residues are central to these reactions serving as catalytic or redox centers, as metal ligands or as regulatory switches through their capacity to adopt several oxidation states in proteins in response to different redox signals.

Objectives — While there is no unique protein signature, the CXXC motifs are particularly suited for disulfide bond formation and for the coordination of metals as exemplified by the Fe_2S_2 centers found in ferredoxins. Thus, by identifying and characterizing proteins of unknown function and possessing one or several conserved CXXC motifs, the global objective of the MetOx proposal is to identify new redox-regulated or metal-binding proteins.

Approach — From the *in silico* analyses of several plant genomes, we will select about 50 candidate proteins and perform a thorough biochemical and structural characterization of the corresponding recombinant proteins, before deciphering the biological roles of the most promising candidates using genetic approaches.

Key results —

- Publication of a methodological article describing experimental protocols dedicated to the study of oxidative modifications and redox properties of proteins. Zannini et al., Methods Mol. Biol. 2017.
- Despite the presence of a WCKHC signature, poplar PDI-A does not possess the classic oxidoreductase activity of members of the protein disulfide isomerase family but is capable of binding in vitro a [Fe2S2] center within a dimer. This is the first example of this type for a protein in this family, suggesting that it could have novel roles. Selles et al., Plos one 2017.
- By analyzing the biochemical and functional properties of plant ERV1 and MIA40 proteins, in particular by *in vitro* activity measurements and the complementation of yeast *erv1* and *mia40* mutants, we observed that the ERV1 protein of *Arabidopsis thaliana* can promote protein import and oxidative folding of proteins in the mitochondrial intermembrane space independently of MIA40. This is in sharp contrast with the situation in yeast and animals, where both proteins are essential. Overall, consistent with the absence of Mia40 in many protists, our study suggests that this protein oxidative folding system gradually evolved from a system where Erv1 would act alone to a system where Mia40 would have been added, possibly to improve the specificity of the substrate. Peleh et al., BMC Biology 2017.
- We observed that two mitochondrial thioredoxins, with a typical signature of the thioredoxin family (WCGHC), have the ability to incorporate a [Fe2S2] center *in vitro*. Although the physiological

significance remains unknown, this is the first demonstration that a thioredoxin with this signature possesses this property. Understanding the structural determinants would be particularly interesting. Article in preparation for a special issue in Antioxidants.

Future perspective — To continue the functional analysis of all these proteins:

- Determine the importance of the Fe-S centers for PDI-A and mitochondrial thioredoxins.
- Study the substrate specificity of plant and yeast ERV1 and MIA40 *in vitro*.

Valorisation —

Publications

Selles B, Zannini F, Couturier J, Jacquot JP, Rouhier N. Atypical protein disulfide isomerases (PDI): Comparison of the molecular and catalytic properties of poplar PDI-A and PDI-M with PDI-L1A. PLoS One. 2017, 12:e0174753.

Zannini F, Couturier J, Keech O, Rouhier N. In vitro alkylation methods for assessing the protein redox state. Methods Mol. Biol. 2017, 1653:51-64.

Peleh V, Zannini F, Backes S, Rouhier N*, Herrmann JM*. Erv1 of Arabidopsis thaliana can directly oxidize mitochondrial intermembrane space proteins in the absence of redox-active Mia40. BMC Biology. 2017, 15(1):106. doi: 10.1186/s12915-017-0445-8. *Corresponding authors

Conferences

EMBO Conference on Redox Biology. Moscow (Russia): 17-23 July 2017. Herrmann JM. The mitochondrial disulfide relay.

Posters:

Thiol oxidation in toxicity and signalling. Sant Feliu de Guixols, Spain, 17 – 21 September 2017. Mia40 is not just a “poor man’s thioredoxin”. Backes S, Peleh V, Rouhier N, Herrmann JM

Thiol oxidation in toxicity and signalling. Sant Feliu de Guixols, Spain, 17 – 21 September 2017. Comparison of the redox properties of plant and yeast MIA40/ERV1 couples. Zannini F, Peleh V, Herrmann JM, Rouhier N