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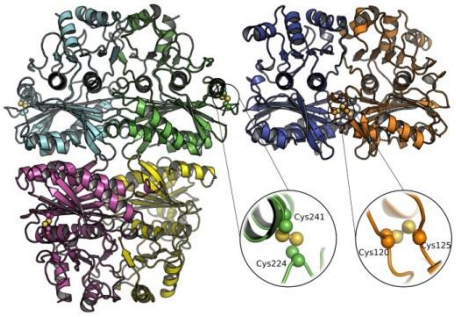

Use of *Physcomitrella patens* to generate Mutants of the Redox Systems

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Collaboration: Plant Biotechnology AG Reski Freiburg Universität)

Context — Study of the redox regulation of CO_2 fixation in photosynthesis. This regulation conditions the rate of photosynthesis and hence plant growth/ agronomical yields. The regulation system is called the ferredoxin-thioredoxin system it depends on the photosystems and includes a series of stroma-soluble electron and proton transporters allowing the reduction of disulfide bonds on selected target enzymes.

Objectives —

- Creation of mutants of the ferredoxin-thioredoxin reductase (FTR) in *Physcomitrella patens*
- Comparative biochemical and structural study of two target enzymes of the Calvin-Benson cycle, the phosphatases FBPase and SBPase.

Approach — Mutation and replacement of the ferredoxin-thioredoxin reductase (FTR) genes in *P. patens* by homologous recombination . Cloning of the cDNA sequences of FBPase and SBPase and expression and purification of the recombinant proteins from *E. coli*. Biochemical and structural studies of FBPase and SBPase.

Key results —

- Construction of point mutations for FTR cysteines. Isolation of simple and double mutants.
- Determination of the 3D structures of FBPase et SBPase.
- Differential spatial positioning of the regulatory sequences in the phosphatases.
- Phylogenetic origin of FBPase et SBPase.
- Structural and phylogenetic properties of redox target enzymes.

Main conclusions including key points of discussion — The point mutants of the FTR genes are viable. Experiments aiming at deleting completely the genes are under way to understand this behavior FBpase and SBPase have distinct phylogenetic origins, deriving from alpha proteobacteria and archaea, primitive non photosynthetic prokaryotes. Different spatial positions have been found by evolution to introduce regulatory sequences in proteins with similar architecture 3D.

Future perspectives — Complete deletion of the FTR genes. Structure/ function studies of *P. patens* thioredoxins f, the proximal reductants of the phosphatases.

Valorisation —

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