Defining H_2O_2 signaling from chloroplast and mitochondria to the nucleus in *Chlamydomonas reinhardtii*

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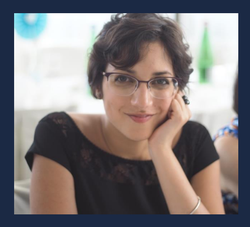

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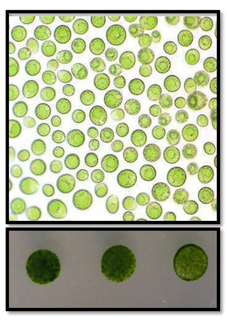
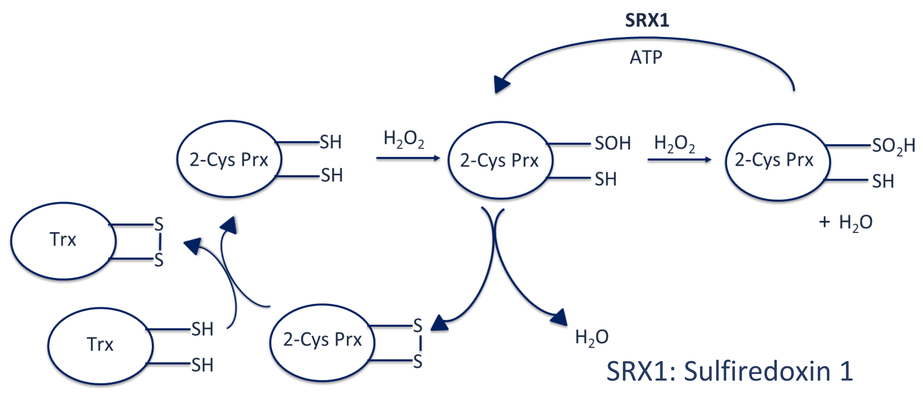
To investigate H_2O_2 signaling in the model organism *Chlamydomonas reinhardtii*, we identified different phenotypes for mutants affected in oxidative stress scavenging.

Chlamydomonas reinhardtii

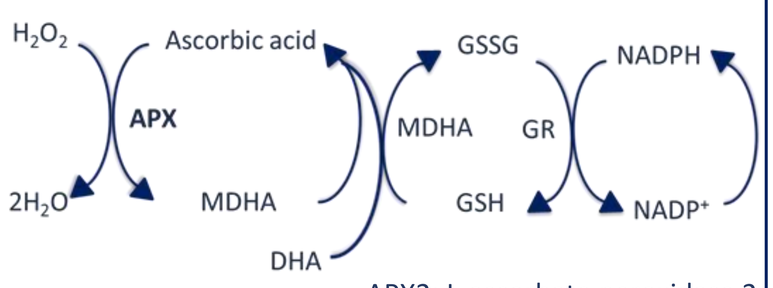
- a unicellular green microalga
- has a single chloroplast.
- relatively easy culture and genetic manipulation
- genome has been sequenced
- grows photoautotrophically, heterotrophically, and mixotrophically



H_2O_2 scavenging: SRX1 and APX2 enzymes involved in the process



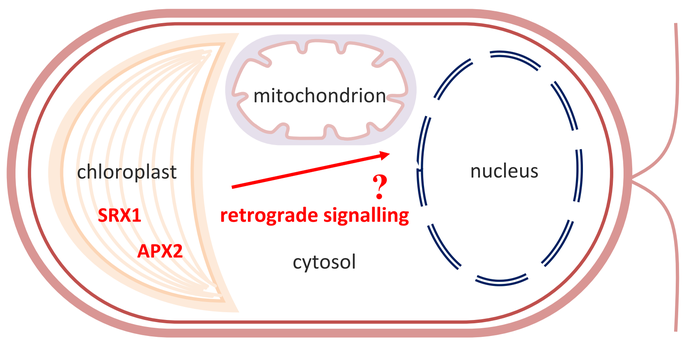
SRX1: Sulfiredoxin 1



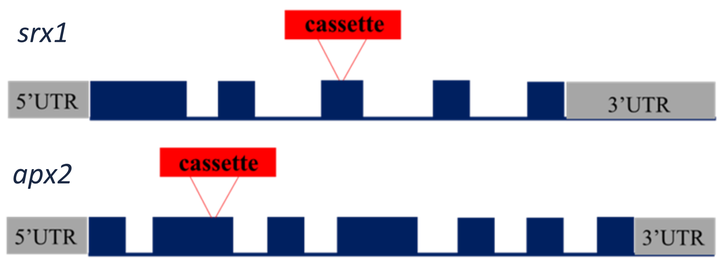
APX2: L-ascorbate peroxidase 2

Mutant characterization

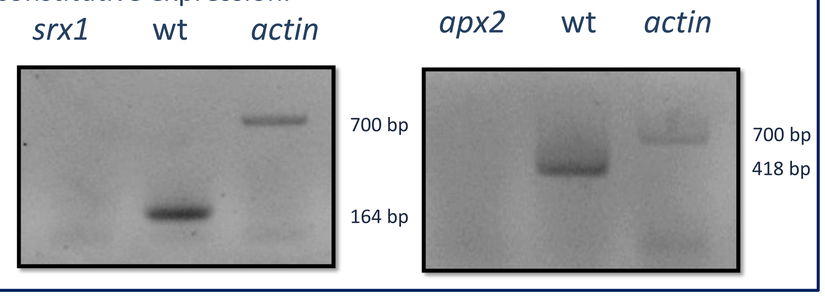
According to Tardif *et al.,* (1) SRX1 and APX2 are putatively localized in the chloroplast.



Corresponding mutants were obtained from CLiP Library (2). The insertion of a paromomycin resistance cassette which disrupts the gene was identified by PCR and sequencing.
srx1 – cassette in the third exon
apx2 – cassette in the second exon

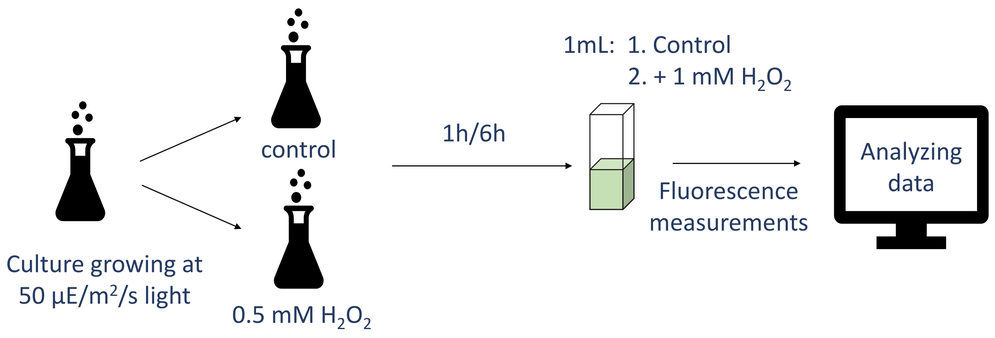


RT-PCR was performed to confirm the absence of the corresponding transcript. The same pair of primers was used for mutant and wt to analyze the presence and the absence of the *SRX1* and *APX2* transcripts. *Actin* was used as control of constitutive expression.

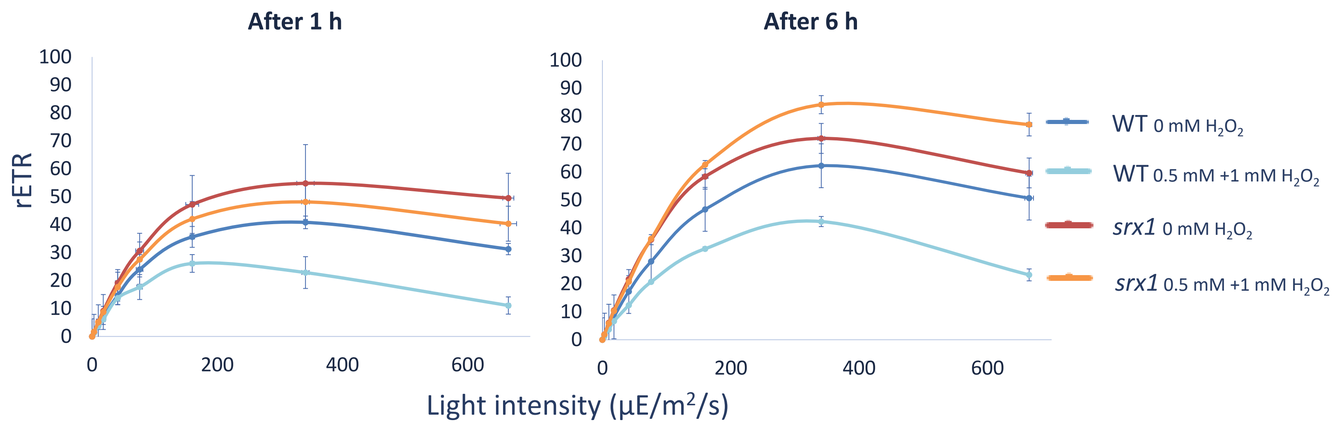


Results

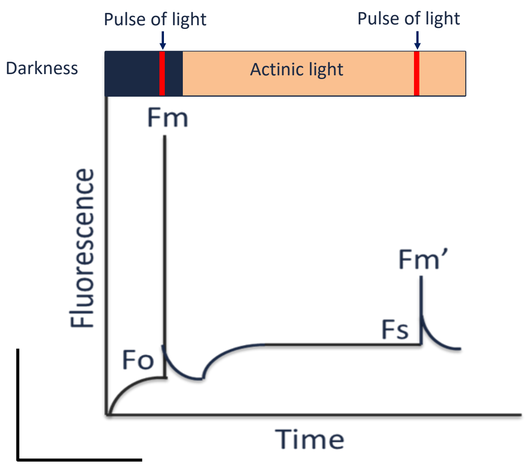
Protocol to induce the response to oxidative stress is performed by treating the cultures with 0.5 mM H_2O_2 during 1 and 6 hours followed by measurements of the relative photosynthetic electron transport rate (rETR)



rETR of *srx1* is higher than WT in all conditions tested.



rETR is based on chlorophyll a fluorescence measurements by using a Joliot Type Spectrophotometer (JTS-10, BeamBio, France) to obtain fluorescence-related parameters (3).



Quantum yield of PSII

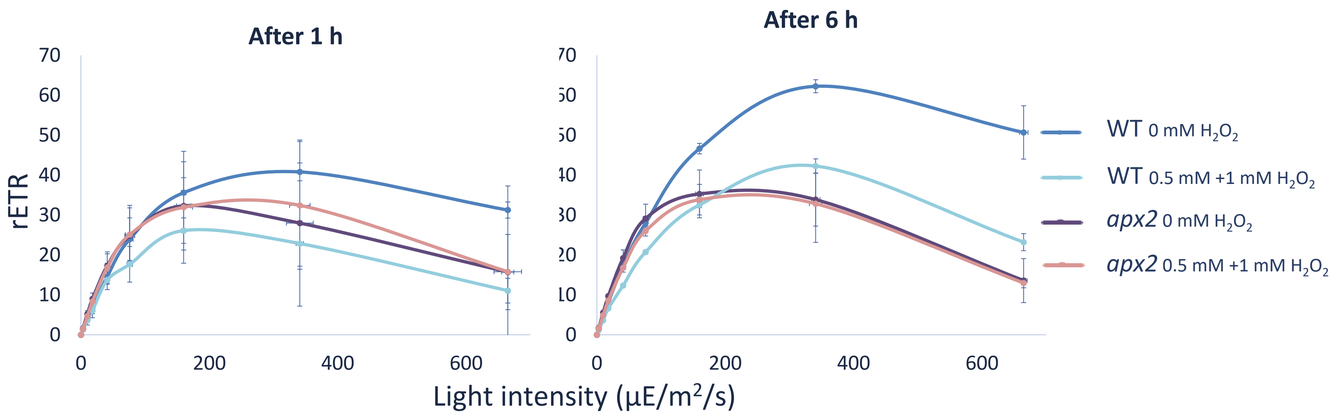
$$\phi PSII = \frac{Fm'-Fs}{Fm'}$$

Relative Electron Transport Rate

$$rETR = \phi PSII * hv$$

hv: actinic light intensity

rETR of *apx2* is insensitive to H_2O_2 treatment.



<u>Conclusion</u>: In the presence of H_2O_2, SRX1 seems to be essential since photosynthetic activity is not repressed in the *srx1* mutant, while APX2 seems to have a different role.

Perspectives

- Clarify the role of SRX1 and APX2 in H_2O_2 scavenging
- Identify sulfenylated proteins involved in redox signaling with chemical probes
- Structural and functional study of one or two redox-sensing proteins

References

1. Tardif M *et al.,* (2012) PredAlgo: a new subcellular localization prediction tool dedicated to green algae. *Mol Biol Evol* 29:3625-39
2. Li *et al.,* (2016) An Indexed, Mapped Mutant Library Enables Reverse Genetics Studies of Biological Processes in *Chlamydomonas reinhardtii. Plant Cell* 28: 367–387
3. Maxwell and Johnson (2000) Chlorophyll fluorescence – a practical guide. *J. Exp. Bot.* 345: 659- 668

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