H_2O_2 signaling from mitochondria and chloroplast to the nucleus in the green microalga *Chlamydomonas*: *in vivo* and *in vitro* approaches

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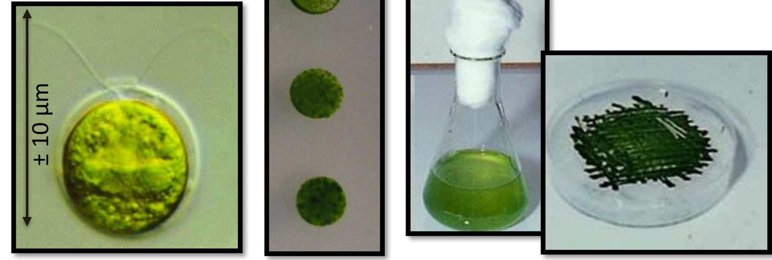
Anna Caccamo[1,2,3,4], Ho Thuy Dung Nguyen[1,2,3,4], Didier Vertommen[5], Sébastien Pyr dit Ruys[5], Steven Fanara[6], Patrick Motte[6], Véronique Larosa[1], Joris Messens[2,3,4], Claire Remacle[1]

[1]Genetics and Physiology of Microalgae, InBios/Phytosystem, University of Liège, Belgium
[2]VIB-VUB Center for Structural Biology, 1050 Brussels, Belgium;
[3]Brussels Center for Redox Biology, 1050 Brussels, Belgium;
[4]Structural Biology Brussels, Vrije Universiteit Brussel, 1050 Brussels, Belgium
[5]UCLouvain and de Duve Institute Avenue Hippocrate 74 box, Université Catholique de Louvain, 1200 Brussels, Belgium
[6]Functional Genomics and Plant Molecular Imaging, InBios/Phytosystem, University of Liège, Belgium

Reactive oxygen species (ROS) are mainly produced in the mitochondrial and in the photosynthetic electron transport chains. Historically, ROS were only considered as toxic molecules for cells, leading to oxidation of proteins, lipids and DNA. Nowadays, the ROS-molecule H_2O_2 is increasingly being recognized as a signaling molecule due to the fact that it is relatively stable compared to the other ROS-molecules and H_2O_2 can potentially travel across membranes. H_2O_2 signals via rapid reactions with protein cysteine sulfurs, which results in an altered protein structure and function. Such cysteine modifications are known as S-sulfenylations (-SOH). So far, hundreds of sulfenylated proteins have been identified in the model plant *Arabidopsis thaliana*. In this project we want to (i) identify *C. reinhardtii* crucial redox enzymes which effect the phenotype under H_2O_2-stress inducing conditions; (ii) trap and identify sulfenylated proteins involved in the redox signaling, using dimedone-based carbon nucleophiles and mass spectrometry; (iii) in vitro characterize the oxidation kinetics and the oxidation induced structural changes on one of the identified redox-sensing proteins.

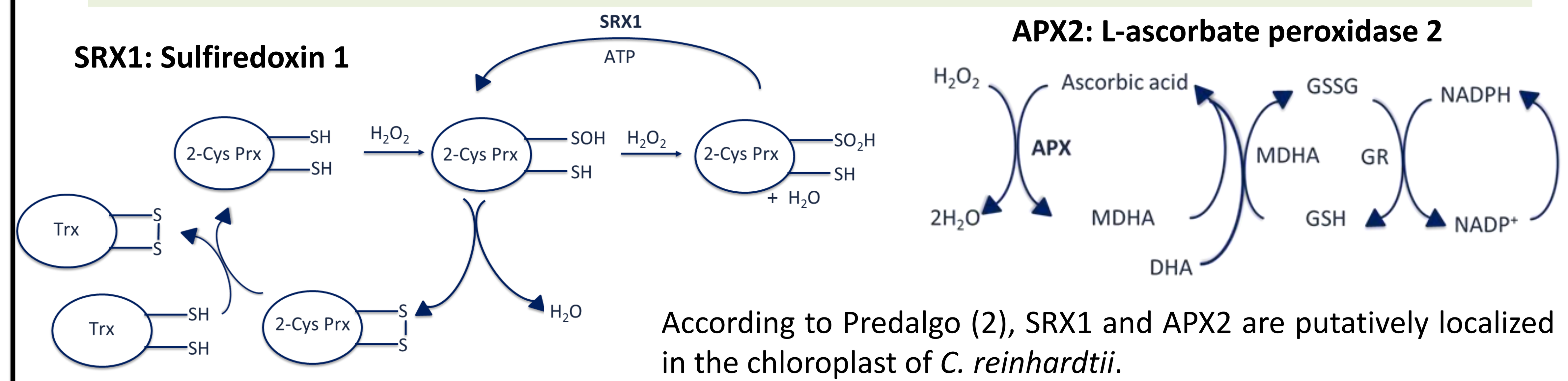
Chlamydomonas reinhardtii, a model of choice

- Unicellular green microalga
- Easy cultivation and genetic manipulation
- Fully sequenced genomes
- Mutant library available [Clip library, (1)]
- Photoautotrophic, heterotrophic, and mixotrophic growth



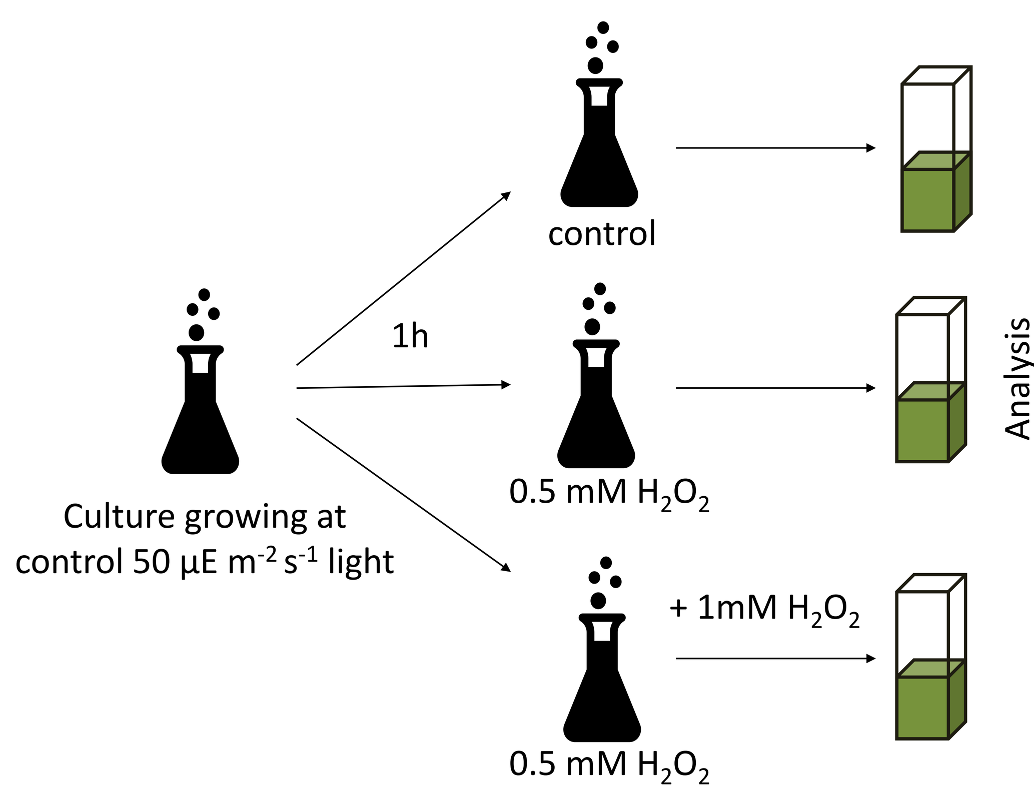
Identification of H_2O_2 scavenging nodes in Chlamydomonas

On 17 Clip mutants analyzed, srx1 and apx2 mutants show the most interesting phenotype in this process so far.



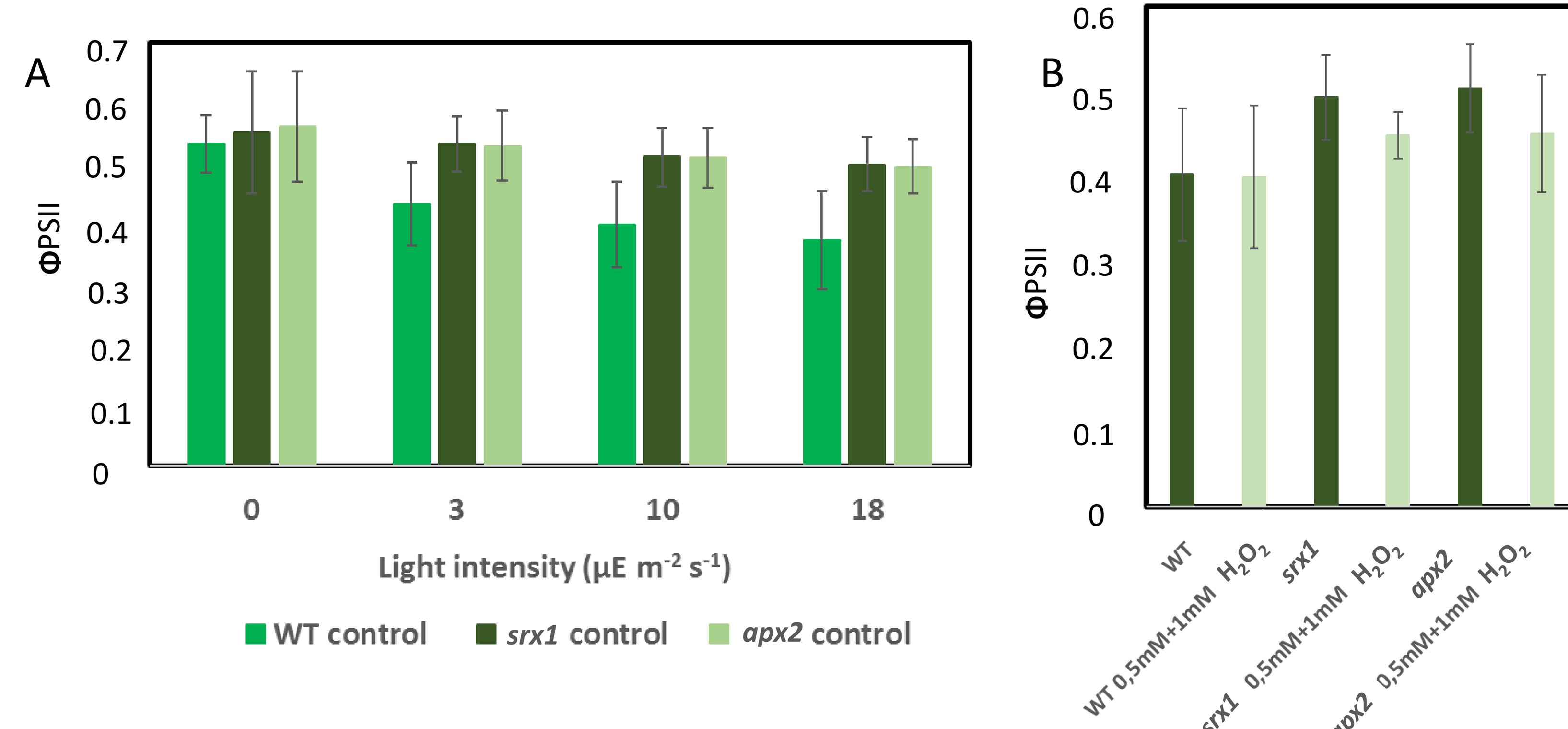
According to Predalgo (2), SRX1 and APX2 are putatively localized in the chloroplast of *C. reinhardtii*.

Characterization of *srx1* and *apx2* Clip mutants



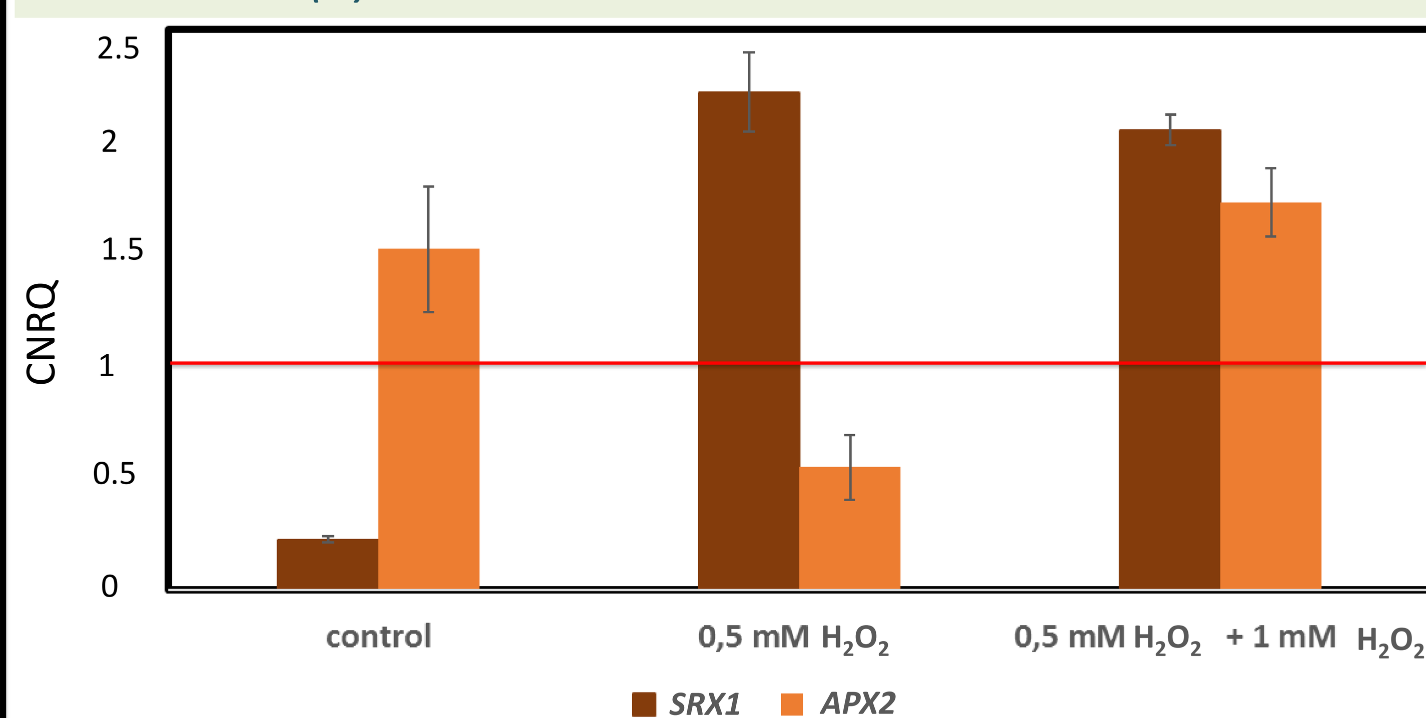
Experimental protocol [adapted from (3)] applied in order to induce the response to oxidative stress in *C. reinhardtii* liquid cultures

Light saturation curves reveal that in control conditions PSII efficiency of srx1 and apx2 mutants does not show the usual reduction when exposed to increasing light intensities such as observed in WT (A) and that mutants show a higher sensitivity than WT when an external H_2O_2 stress is applied (B).



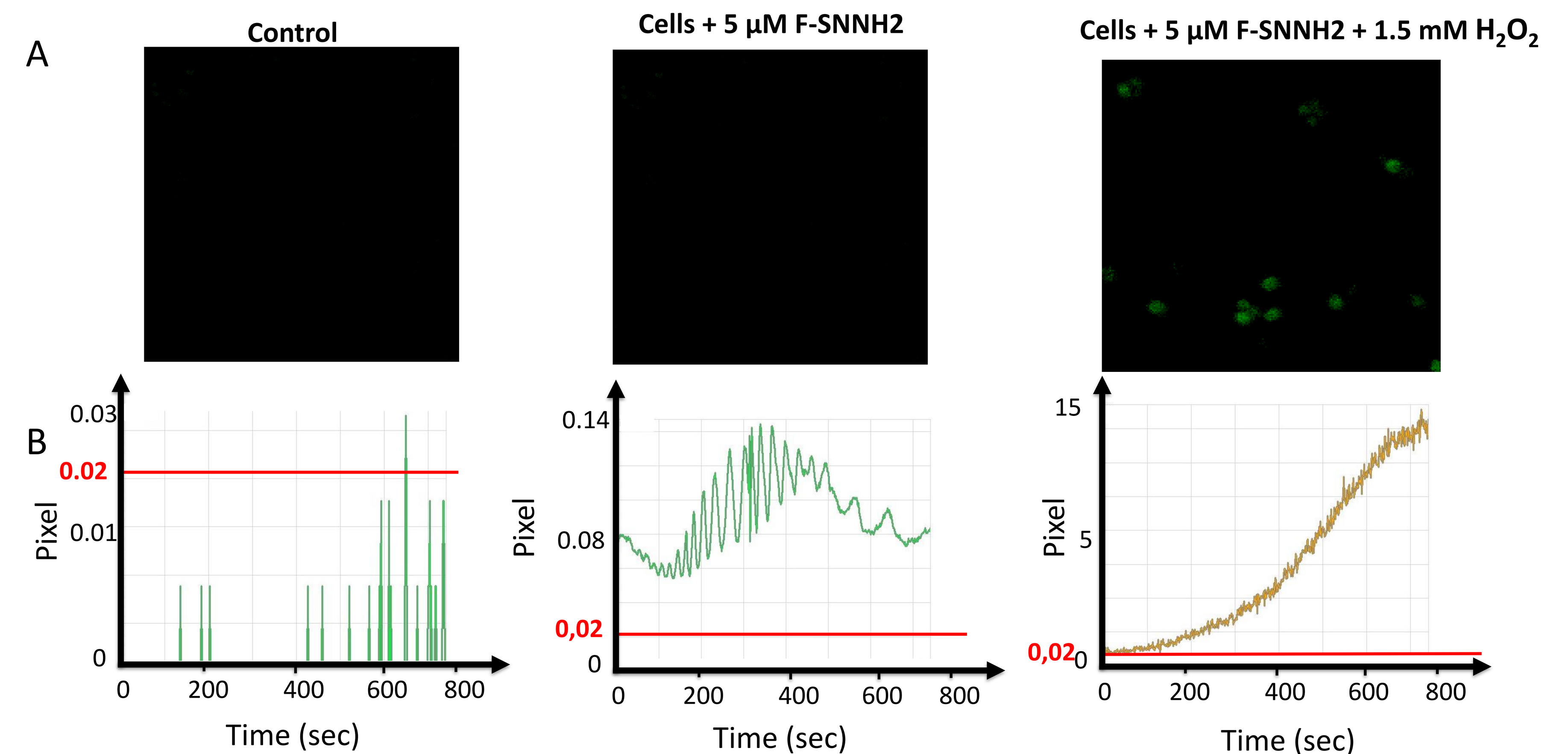
WT and mutants were cultivated and harvested as shown on the left scheme, PSII photochemical activity (**ΦPSII**) was measured with a Joliot-Type-Spectrophotometer in liquid cultures where cells were dark-adapted for 20 min and in (A) fluorescence was measured at increasing light intensities, while in (B) only the fluorescence results concerning the light intensity of 18 µE m^{-2} s^{-1} are shown in control and stress conditions respectively for WT, *srx1* and *apx2* mutants.

RT-qPCR analysis reveals that in WT strain, SRX1 transcripts are highly increased after H_2O_2 addition while APX2 transcripts are already strongly expressed in control conditions, and present a variable expression pattern after H_2O_2 addition. This expression pattern is similar to the one described in (4).



RNA from WT cells, under control and stress (see experimental scheme) conditions, were extracted and qRT-PCR was performed in order to visualize the expression profile of *SRX1* and *APX2*. The red bar indicates the threshold of the reference genes used (CBLP, β subunit-like polypeptide and RPL ribosomal protein L). (Calibrated Normalized Relative Quantity: CNRQ)

Live cell imaging analysis using the new fluorescence probe F-SNNH2 (R. Ferreira and K. Carroll, Scripps Institute, unpublished data) interacting with Cys-SOH could help to decipher if mutants and WT present different sulfenylation patterns. Our preliminary results on WT cells suggest that the probe enters the cells, and fluorescence increases after the addition of H_2O_2.



WT cells, WT cells with the addition of 5 µM of probe and WT cells with the addition of 5 µM of probe and an external H_2O_2 stress (1.5 mM) were analyzed by live cell imaging using confocal microscopy to visualize the fluorescence of the F-SNNH2 probe (Ex: 488 nm, Em: 525 – 625 nm). In (A) the imaged cells at 700 sec are shown to visualize fluorescence stained cells while in (B) the entire kinetics of the live imaging experiment is shown.

Conclusions and perspectives

(1) srx1 and apx2 mutants respond differently to increasing light intensities and to the addition of H_2O_2 while their respective transcripts present different kinetics of expression, but the role of SRX1 and APX2 in H_2O_2 scavenging has still to be clarified.

(2) The fluorescence probe F-SNNH2 is promising for analyzing in vivo sulfenylation patterns of different cell lines. Now, fluorescent kinetics has to be performed in mutants and sulfenylated proteins involved in this kinetic have to be extracted using BTD-based chemical probes.

References

(1) Li *et al.*, (2019). A genome-wide algal mutant library and functional screen identifies genes required for eukaryotic photosynthesis. *Nature Genetics*, 51(4), 627–635. **(2)** Tardif *et al.*, (2012) PredAlgo: a new subcellular localization prediction tool dedicated to green algae. *Mol Biol Evol* 29:3625-39 **(3)** Murik *et al.*, (2014) Dehydroascorbate: a possible surveillance molecule of oxidative stress and programmed cell death in the green alga *Chlamydomonas reinhardtii*. *New Phytol* 202(2):471-84 **(4)** Blaby *et al.*, (2015) Genome-wide analysis on *Chlamydomonas reinhardtii* reveals the impact of hydrogen peroxide on protein stress responses and overlap with other stress transcriptomes. *Plant J* 84(5):974-988

Acknowledgement

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