Molecular characterization of the ER oxidoreductases ERO1 and ERO2 from *Arabidopsis thaliana*.

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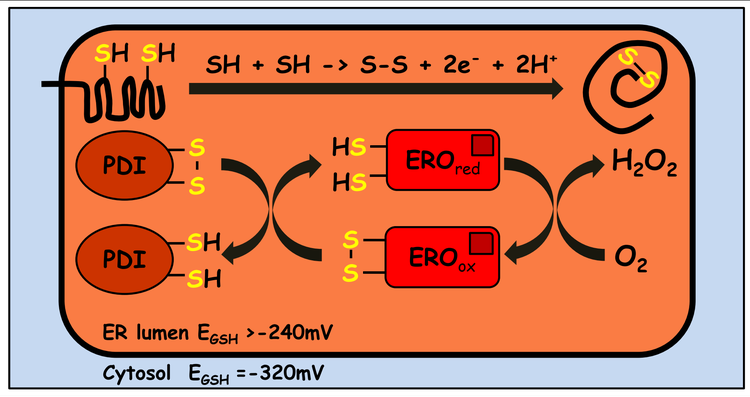
INTRODUCTION

The Endoplasmic Reticulum (ER) is a key compartment for oxidative protein folding. This requires the formation of disulfide bridges, a process in which the ER oxidoreductases (ERO) play a crucial role. The process causes the formation of H_2O_2 which is linked to the balance of the glutathione redox state in the ER. We have developed a novel redox-sensitive GFP (roGFP) with which we seek to understand the influence of *At*ERO1 and *At*ERO2 on the redox homeostasis in the ER of *Arabidopsis thaliana*.

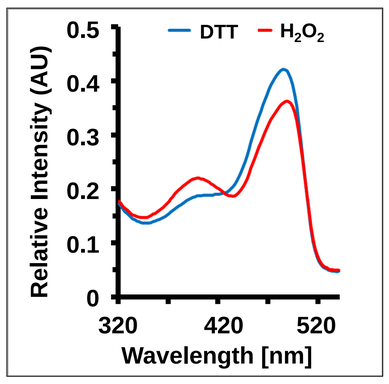


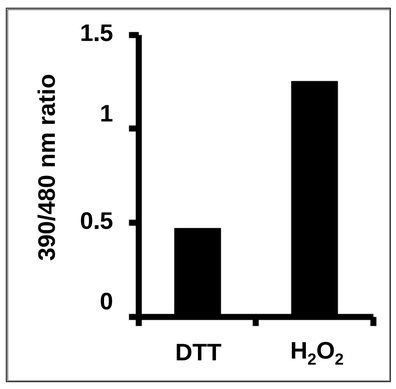
1. Development of a novel roGFP2 based sensor suitable for oxidizing compartments.

	147
roGFP2	NYNC-H
roGFP2iL	NYNCLS
	147

Compared to roGFP2, roGFP2iL has a leucine insertion next to C147 and a H148S substitution leading to a reduced stability of the disulfide C147/ C205 and to changes in the protonation state of the chromophore.

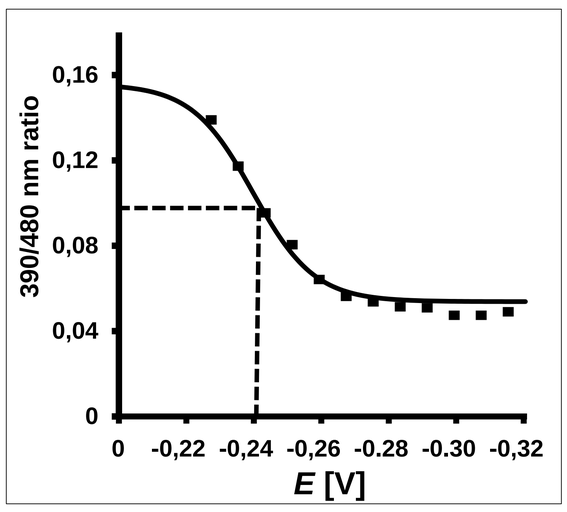
(A) Redox-dependent changes in the absorption spectrum and fluorescence ratio of roGFP2iL.





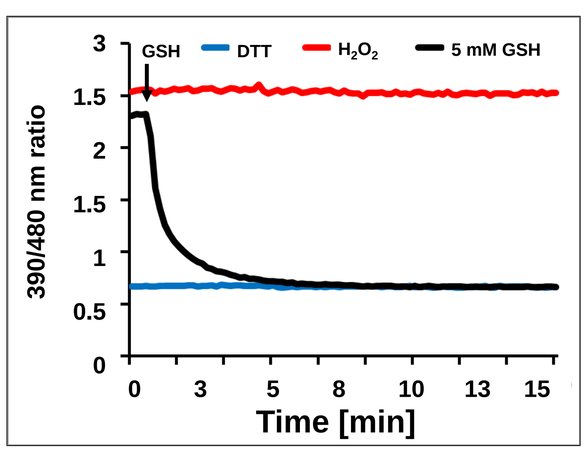
Redox dependent changes in the absorption spectrum of roGFP2iL (left side). After treatment with reducing (10mM DTT) and oxidizing (10mM H_2O_2) agents, the 390/480 nm fluorescence ratio significantly changes compared to untreated roGFP2iL (right side).

(B) The midpoint potential of roGFP2iL is shifted towards -0.24V.



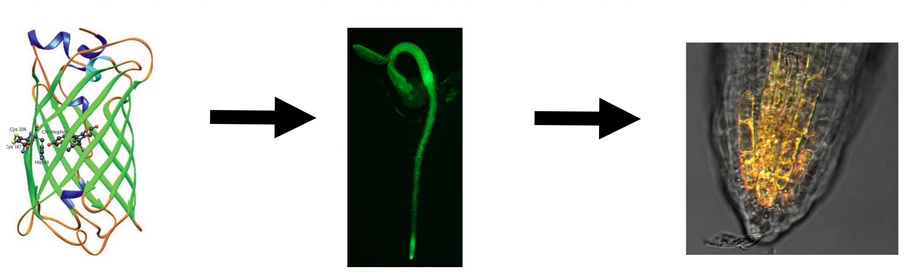
First results support a midpoint potential of -240 mV and indicate roGFP2iL is more suitable for dynamic measurements in oxidized compartments like the ER than roGFP2 with a midpoint potential of -280 mV

(C) GRX-roGFP2iL is rapidly and completly reduced by physiological GSH concentrations.

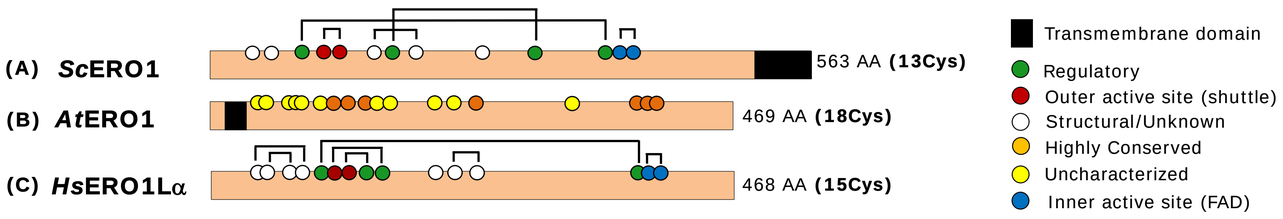


Glutaredoxin catalyzes the transfer of electrons from GSH to roGFP2iL. The injection of 5 mM reduced GSH solution into GRX-roGFP2iL solution leads to a rapid and complete reduction of the sensor within 5 minutes.

(D) Stable transformation of Arabidopsis plants with low *At*ERO1 and *At*ERO2 levels with roGFP2iL in the ER.

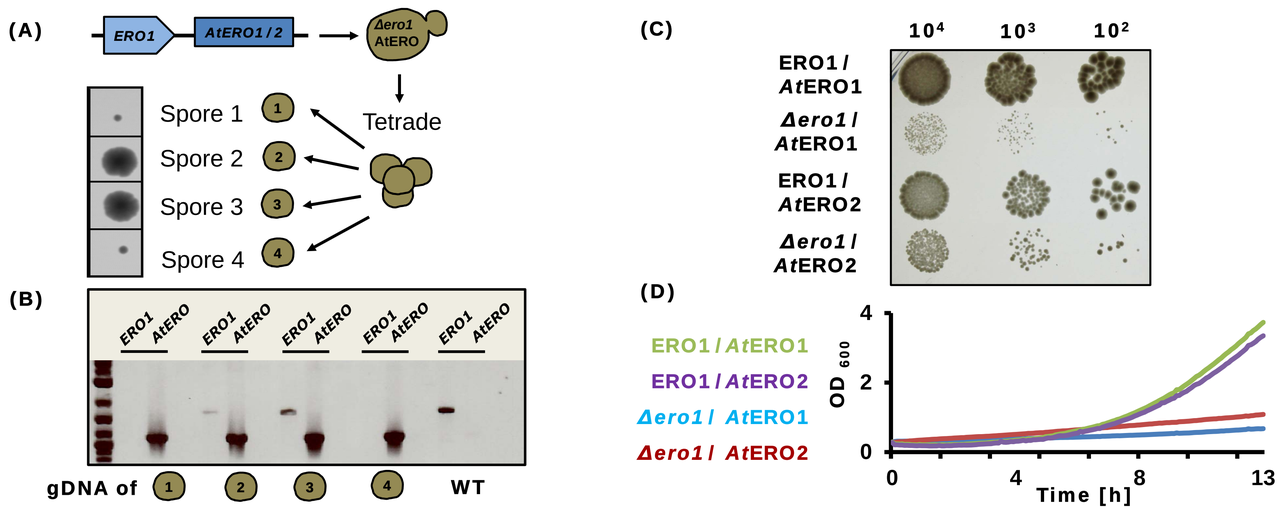


2. Comparison of ERO proteins from different eukaroytic model organisms.



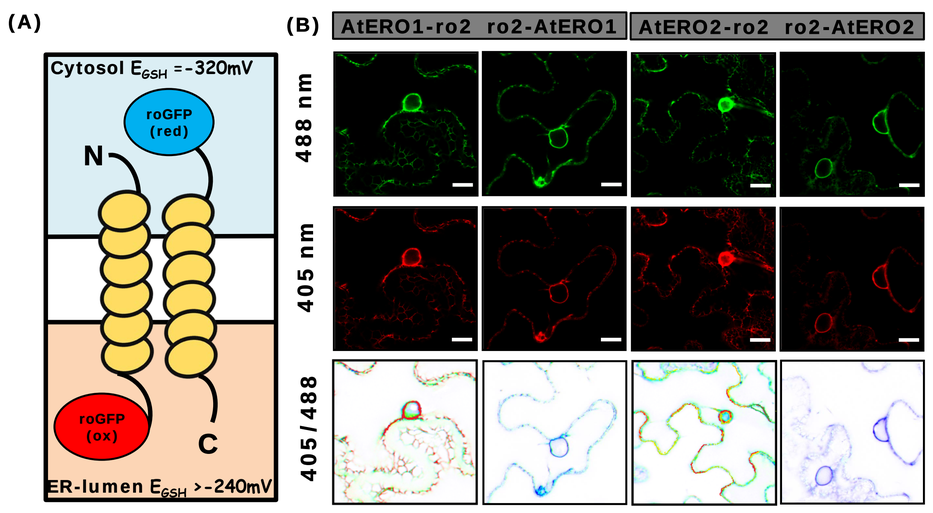
Alignment of the protein sequences of yeast **(A)** ERO1, **(B)** ERO1 from *Arabidopsis thaliana* and **(C)** human ERO1Lα shows a high conservation of regulatory and catalytic cysteins that indicates functional conservation of ERO proteins. Interestingly, the membrane association between the three eukaryotic ERO proteins is different.

3. AtERO1 and AtERO2 from *Arabidopsis thaliana* partially complement an ERO1 deficient yeast strain.



(A) An ERO1 deficient yeast strain was transformed with the CDS of *At*ERO1 and *At*ERO2 from *Arabidopsis thaliana*. Both *At*EROs were able to partially complement yeast ERO1. **(B)** PCR on gDNA isolated from the single spores confirmed the presence of *At*EROs. **(C)** In a yeast spotting assay a slightly higher complementation capacity of *At*ERO2 compared to *At*ERO1 could be observed. **(D)** Growth assays in liquid media support the results from the yeast spotting assay.

4. *At*ERO1 and *At*ERO2 are ER resident, Type II membrane proteins.



(A) Scheme of the *At*ERO proteins N- and C-terminally fused to redox-sensitive GFP2 (roGFP2).

(B) roGFP2-*At*ERO1 and *At*ERO1-roGFP2 (left) or roGFP2-*At*ERO2/and *At*ERO2-roGFP2 (right) in tobacco epidermis cells at 405 (red) and 488 nm (green) excitation. Ratio images (bottom) indicate roGFP2 redox state and therefore its localization on the cytosolic or luminal side of the ER membrane.

PERSPECTIVE: roGFP enables visualization of oxidation processes in the ER lumen in real time.

An Arabidopsis root expressing the redox probe roGFP2 in the ER was perfused with H_2O and DTT, respectively. The response of the roGFP status is displayed as a series of ratiometric images. The impact of *At*ERO proteins on the re-establishement of the oxidized redox poise will be assessed in mutants of *At*ERO1 and *At*ERO2 of *Arabidopsis thaliana*.