Investigating Thioredoxin Independent Roles of Trr1 in *Saccharomyces cerevisiae*

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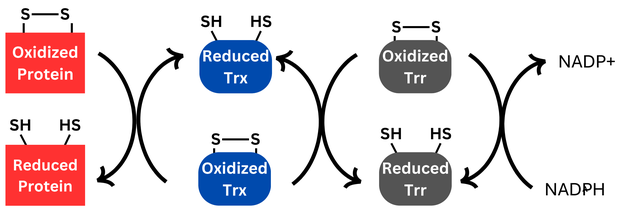
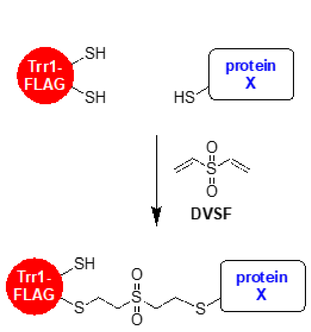
Background and Significance

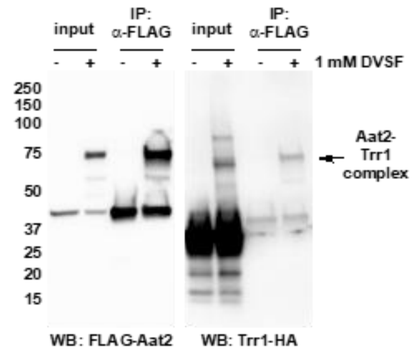
- Thioredoxin systems defend against oxidative stress using disulfide bond chemistry
- Baker's yeast lacking the thioredoxin reductase Trr1 exhibit more severe phenotype changes than yeast lacking the corresponding thioredoxins, highly suggesting that Trr1 has thioredoxin-independent roles
- Six proteins, Ade13, Aat2, Aro8, Asc1, Tsa1 and Grx3, have been found to interact with Trr1



Protein Interaction Partners Show Crosslinks with Trr1 Using Thiol-Reactive Cross-Linker DVSF

Protein	Biological Function
Tsa1	Peroxiredoxin, chaperone with holdase activity
Grx3	Monothiol glutaredoxin involved in Fe-S cluster assembly
Aat2	Aspartate aminotransferase, possible role in translation regulation
Asc1	G-protein beta-subunit
Aro8	Aromatic aminotransferase
Ade13	Adenyl succinate lyase





Hypothesis & Research Objectives

- Characterize phenotypic changes in protein deletion mutants to understand ER stress responses and peroxide sensitivity
- Purify Ade13, Aro8, Aat2, and Trr1 proteins using His-Tag chromatography for activity assays to understand potential redox interactions and biological corollary

Main Conclusions

- Yeast lacking Trr1 have a more severe slow growth phenotype than yeast lacking the corresponding thioredoxins.
- Trr1 is cross-linked to several other proteins besides thioredoxin in yeast treated with the thiol-reactive cross-linker DVSF. These interactions have been validated by co-IP analysis.
- Single deletion mutants of interaction partners were genotyped
- Aro8, Aat2, Ade13, and Trr1 proteins were purified using His-Tag Chromatography

Strategies for Validating Whether Novel Trr1 Interaction Partners Are Also Redox Partners

Biochemical Approach

purify His-tagged partner proteins with known enzymatic activities

↓

oxidize proteins with diamide

↓

determine whether enzymatic activities are altered by oxidation (mostly using spectroscopic analysis)

↓

if protein activity is redox-sensitive, add purified Trr1 + NADPH to determine if activity is recovered

Cellular Approach

make yeast deletion strains that lack both TRR1 and the novel redox partner

↓

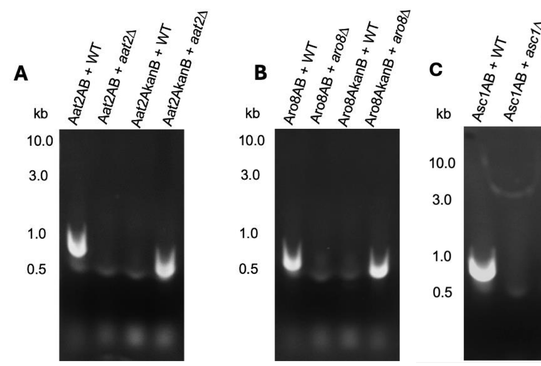
genotype deletion strains using PCR

↓

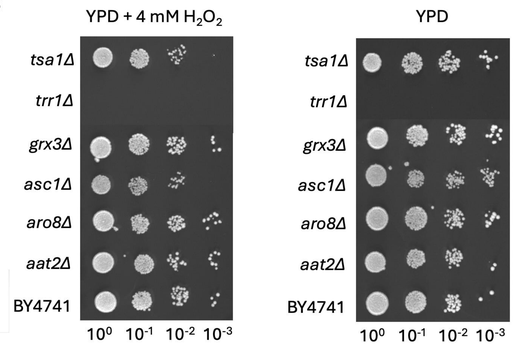
determine whether single and combined deletion of new Trr1 partner proteins impacts phenotypes associated with TRR1 loss

•slow growth
•peroxide sensitivity
•constitutive UPR activation

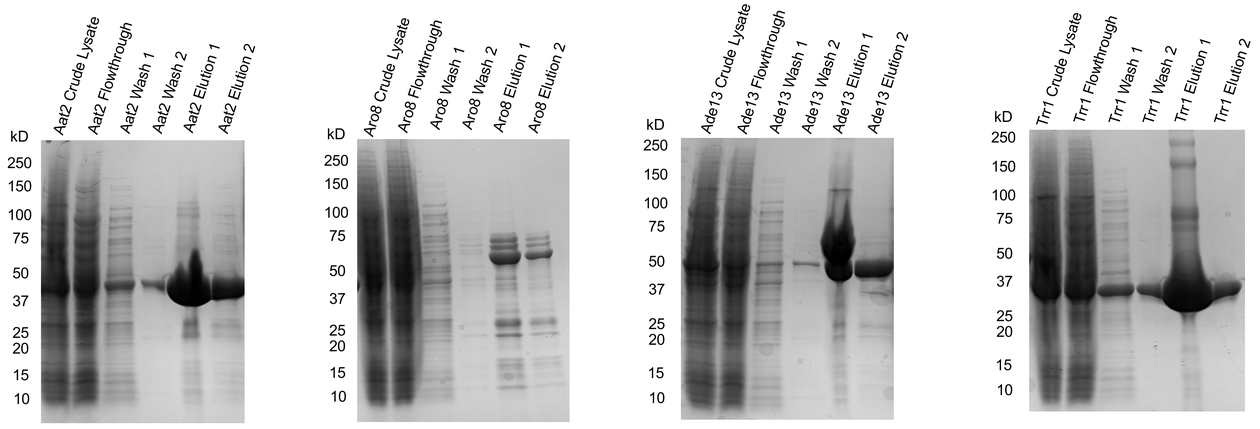
Genotyping *aat2Δ, aro8Δ, asc1Δ,* and *grx3Δ* Deletion Strains For Phenotypic Characterization Experiments



Investigating Slow Growth Using Peroxide Sensitivity Assay



Protein Purification for Activity Assays to Probe Trr1 Redox Capabilities with Interaction Partners



Future Research

- Preparation of Trr1 and interaction partner double deletion strains for phenotypic assays
- Troubleshoot beta-galactosidase and peroxide sensitivity assays
- Construct activity assays to investigate oxidation of interaction partners and Trr1's ability to rescue the protein through reduction

Acknowledgements

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