Exploring How a Mutant Form of Yeast Thioredoxin Reductase Trr1 Causes Growth Arrest

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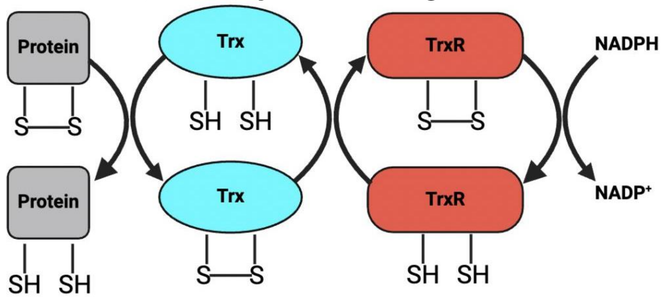
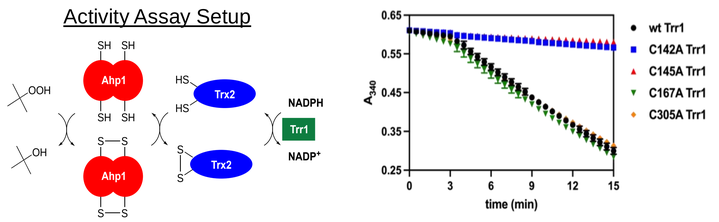
Abstract

Thioredoxin reductase (Trr1) partners with thioredoxin (Trx) to protect against reactive oxygen species (ROS) and support deoxyribonucleotide biosynthesis. In yeast, Trr1 has two critical cysteines, C145 (catalytic) and C142 (resolving), which facilitate Trx reduction. Yeast lacking Trr1 exhibit growth arrest when overexpressing a C145A mutant of Trr1. This growth sensitivity is specific to strains lacking Trr1, as shown by testing different genetic backgrounds (wild-type, trr1Δ, trx1Δ trx2Δ, and trr1Δ trx1Δ trx2Δ) using a galactose-inducible system. Non-reducing SDS-PAGE analysis revealed high molecular weight (~70 kDa) bands in trr1-deficient yeast expressing either wild-type or C145A Trr1, suggesting the formation of disulfide-linked Trr1 homodimers. These findings imply that other conserved cysteines in Trr1, such as C142 or a semi-conserved C-terminal cysteine, may form non-productive complexes with unknown proteins. This raises the possibility of Trx-independent roles for Trr1 and related reductases. Further studies aim to identify these interactions and elucidate the mechanism behind the observed growth arrest.

Thioredoxin Reductase Uses NADPH to Reduce Thioredoxin, Thereby Influencing Redox Homeostasis

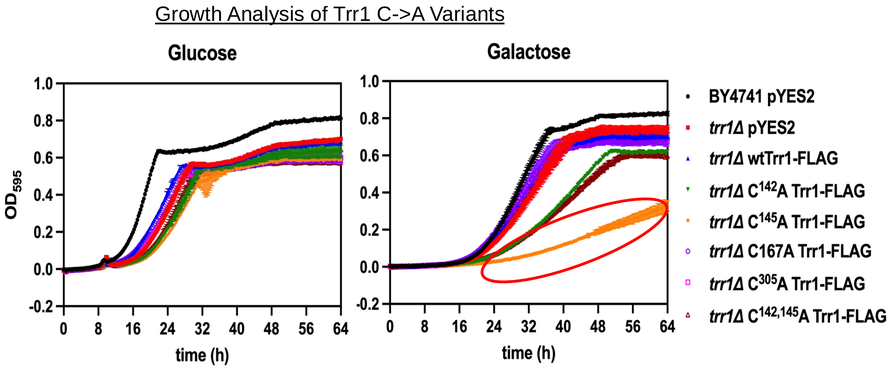


C142 and C145 Are Critical for Trr1 Activity in a Coupled Activity Assay

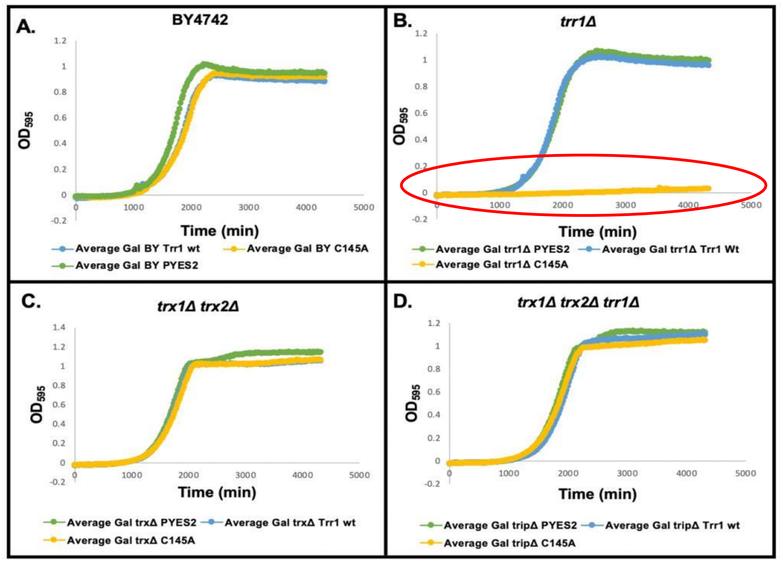


•C145 is predicted to be the principal nucleophilic cysteine in Trr1's active site, from studies of the *E. coli* TrxR.

The C145A Trr1 Variant Slows Growth Considerably When Overexpressed in *trr1*Δ Cells

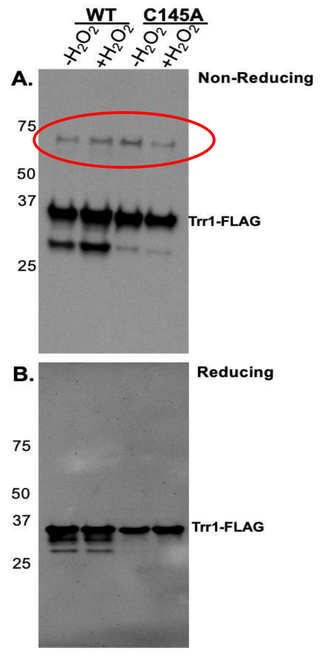


Genetic Background Influences Growth Arrest Caused by C145A Trr1 Following Induction in Galactose



•C145A overexpression only causes growth arrest in the *trr1Δ* deletion strain.

Trr1-FLAG WT and C145A Show Possible Higher Molecular Weight Complexes Under Non-Reducing Conditions.

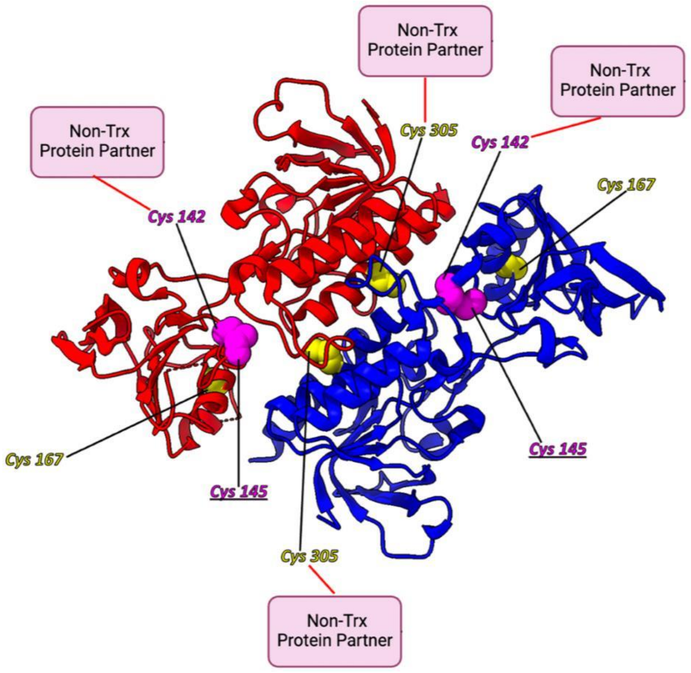


- 70 kDa bands can be observed in the Non-Reducing SDS Page, potential homodimer formation
- 30 kDa bands indicate possible protein degradation

Summary of Key Findings

- C145A Trr1 in *trr1*-deficient yeast when placed in Galactose causes the growth arrest phenotype
- Non-Reducing SDS PAGE shows the presence of 70 kDa bands in both WT and C145A Trr1 variants, possible Trr1 homodimer formation as Trr1 is found at 35 kDa, the presence of peroxide had no effect on either variant

How Might C145A Trr1 Cause Growth Arrest?



•Since C142 and C305 are in close proximity to C145, either of them may form 'unresolvable' disulfides with novel redox partners that are essential for growth with the C145A Trr1 variant. Neither cysteine has been reported to have a redox role, other than C142 as a resolving cysteine in reducing Trx.

Future Work

- Preform another round of Western blotting using DVSF
- Identify whether C142 or C305 is involved in this proposed interaction.
- Isolate the protein(s) that are trapped in a non-productive complex with C145A Trr1 using immunoprecipitation and identify using mass spectrometry-based proteomic approaches.

Acknowledgments

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