Possible Redox Roles of Non-Active Site Cysteines in Yeast Mitochondrial Thioredoxin 3

Shashank R. Pandey, Jude A. Cerniglia, and James D. West

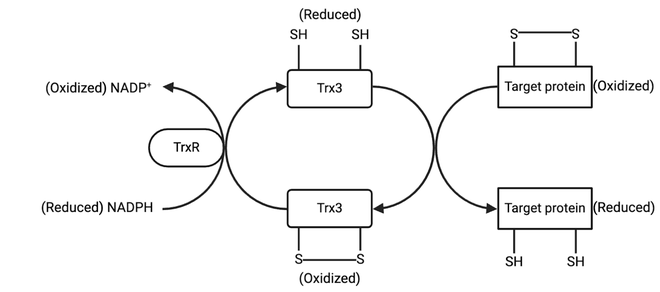
Biochemistry & Molecular Biology Program; Departments of Biology and Chemistry; The College of Wooster; Wooster, OH

THE COLLEGE OF WOOSTER

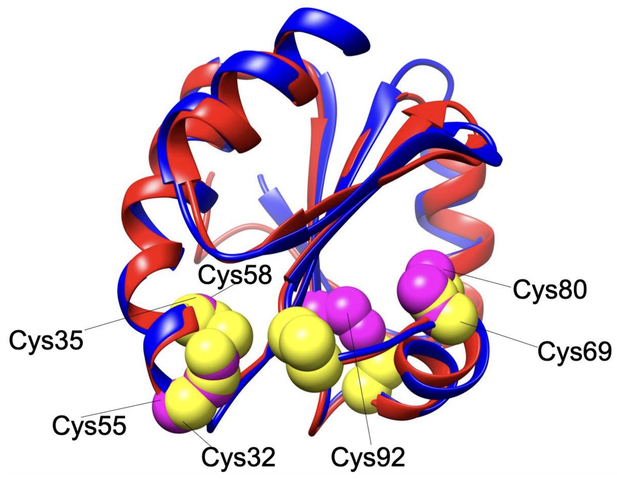
Abstract

Thioredoxins (Trxs) are highly conserved oxidoreductase enzymes that maintain cellular redox balance by reducing disulfide bonds in oxidized proteins, thus protecting cells from oxidative damage. While the active-site cysteines of mitochondrial thioredoxin (Trx3) in *Saccharomyces cerevisiae* are well-characterized, the role of non-active site cysteines remains largely unexplored. This study primarily investigates the functional importance of these residues under oxidative stress and, secondarily, Trx3 branched-chain amino acid (BCAA) limitation conditions. Phenotypic analysis demonstrated that yeast strains expressing the Trx3 protein relatively resisted oxidative stress, whereas strains expressing cysteine mutants (C80S, C92S, and C80,92S) exhibited reduced growth under similar stress conditions. Western blotting and purification experiments revealed that mutations in non-active site cysteines result in lower protein expression and stability, suggesting that these cysteines may contribute to maintaining structural integrity rather than directly catalyzing redox reactions. These findings indicate that non-active site cysteines in Trx3 potentially play crucial roles in maintaining protein stability and potentially regulating protein-protein interactions under oxidative stress. Future research should focus on detailed structural and biochemical analyses to further elucidate the specific mechanisms by which these residues influence Trx3 function and stability.

Thioredoxin 3 Functions Alongside Thioredoxin Reductase to Catalyze Disulfide Reduction in Substrate Proteins



Thioredoxin 3 Overlapped with Human Cytosolic Thioredoxin 1 Shows Highly Conserved Cysteine Residues

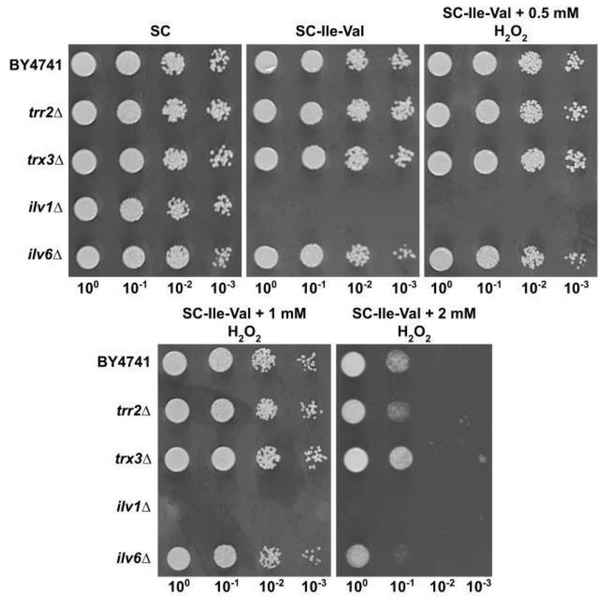


Experimental Goals

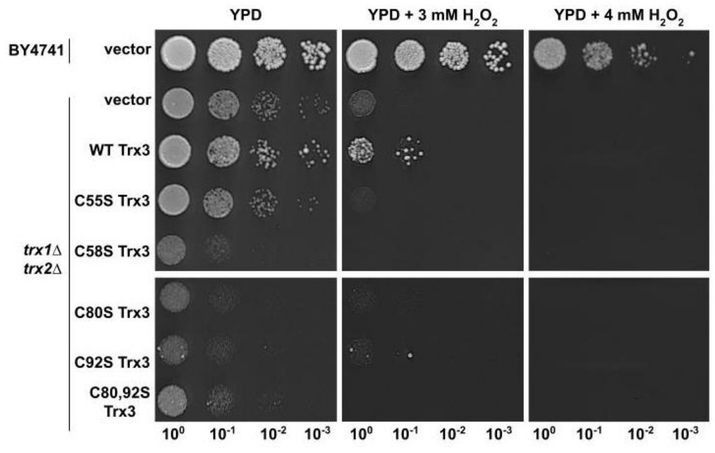
•To explore the potential contribution of non-active site cysteines in the stability and function of yeast mitochondrial thioredoxin 3

•To further elucidate the antioxidant roles of active site cysteines in yeast mitochondrial thioredoxin 3 under oxidative stress conditions

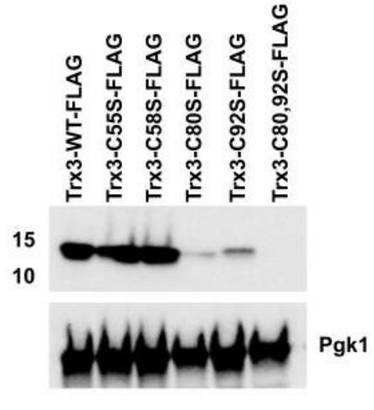
Deletion of Trx3 Does Not Increase Sensitivity to Oxidative Stress or BCAA Limitation



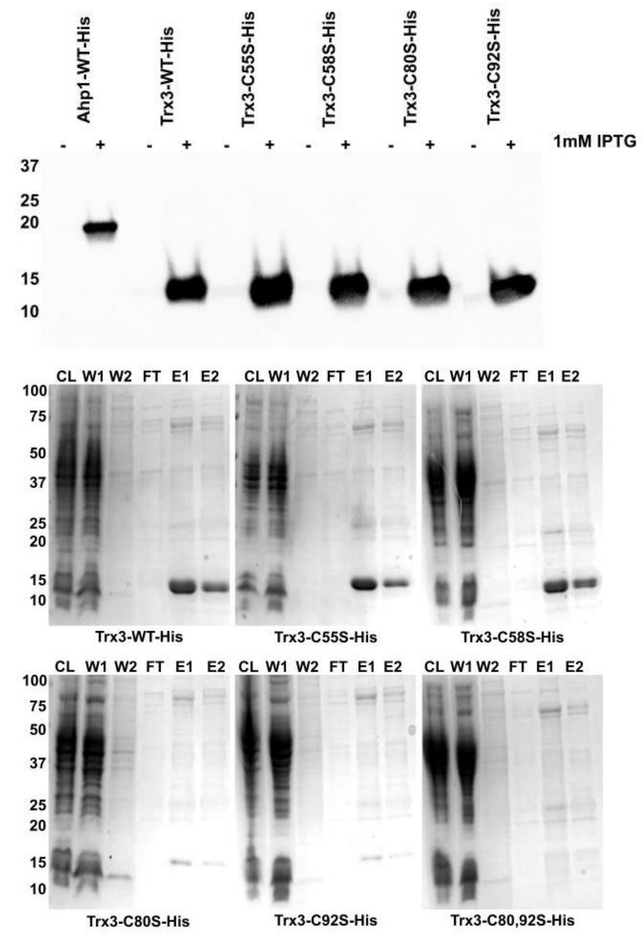
Cytosolic Trx3 Cysteine Mutants Exhibit Differential Sensitivity to Oxidative Stress



Mutation of Non-Active Cysteines in Trx3 Results in Decreased Protein Expression



Low Thioredoxin 3 Protein Yield in *E. coli* When Non-Active Site Cysteines Are Mutated to Serine



•Similar results were observed in previous unpublished work with alanine substitutions at the *C*-terminal cysteines.

Next Steps in Studying the Redox Roles of Non-Active Site Cysteines in Trx3

•Determine if there are other cysteine substitutions that are more stable than C->S substitutions in Trx3.

•Optimize bacterial expression conditions to improve yields of Trx3 mutants, especially for C80S, C92S, and C80,92S variants.

•Employ structural studies (e.g., X-ray crystallography) to understand how non-active site cysteines impact Trx3 stability and conformation.

•Conduct *in vitro* reduction assays to uncover potential redox activity of non-active site cysteines in Trx3

Summary of Findings

•Deletion of Trx3 did not significantly impair yeast growth under BCAA limitation or oxidative stress, suggesting compensatory pathways.

•Mutations at non-active site cysteines (C80S, C92S, C80,92S) led to severe growth defects under oxidative stress, likely due to reduced protein stability.

•Protein purification showed reduced yields of C80S, C92S, and C80,92S mutants, reinforcing their role in structural stability.

Acknowledgments

This work was funded by the Departments of Biology and Chemistry and the Copeland Fund for Independent Study from The College of Wooster.