The Putative Interactome of Escherichia coli Glutaredoxin 2 as revealed by Affinity Chromatography

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Introduction. Glutaredoxin 2 (Grx2) contributes more that 80 % of glutathione (GSH) mediated redox activity in cellular extracts and protects cells from general oxidative damage (formation of carbonyls[1,2]). The specific substrates of Grx2 however, are unknown. To this aim, immobilized monothiol/athiol Grx2 mutants were used in affinity chromatography with cellular lysates from *Escherichia coli* (*E. coli*). The athiol Grx2 (Grx2 C9S C12S) served as a bait for proteins interacting in a non-thiol manner while the monothiol Grx2 C12S was used to trap dithiol substrates of Grx2. In addition, lysates from *E. coli* null mutants for *grxB,* encoding Grx2, were compared to those of the wild type. All proteomic analyses were performed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) followed by bioinformatics and gene ontology evaluations.

Methodology. Overexpression and purification of *E. coli* Grx2 C9S C12S and Grx2 C12S mutants. Each protein (approximately 6 mg) was immobilized on Affi-Gel 10 beads in chromatographic columns. *E. coli* cells were grown in LB-medium and collected during both exponential and stationary phases. Cell lysates were prepared and chromatographed through columns with of the immobilized Grx2 mutants. Empty resin served as control. Bound proteins were eluted with salt (KCl, step gradients), CH3COOH/HCOOH and finally DTT. The eluted proteins were analyzed by LC-MS/MS. All cromatographies were performed in triplicates. Furthermore, *E. coli* wild type strain and the null mutant for *grxB* were grown and harvested in both growth phases. Their proteome was analyzed by LC-MS/MS.

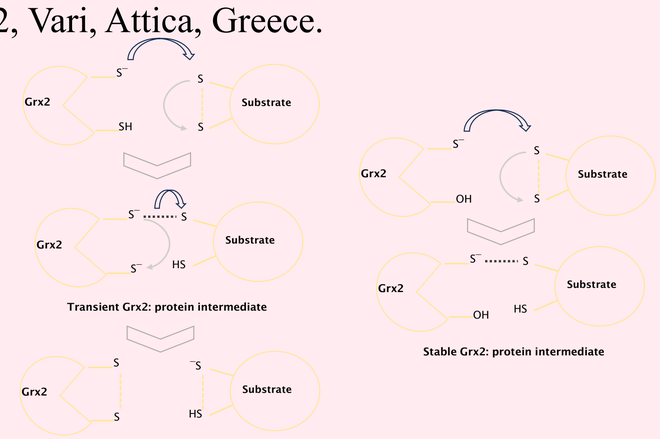

Figure 1. Catalytic mechanism and monothiol Grx substrate trap.

Results

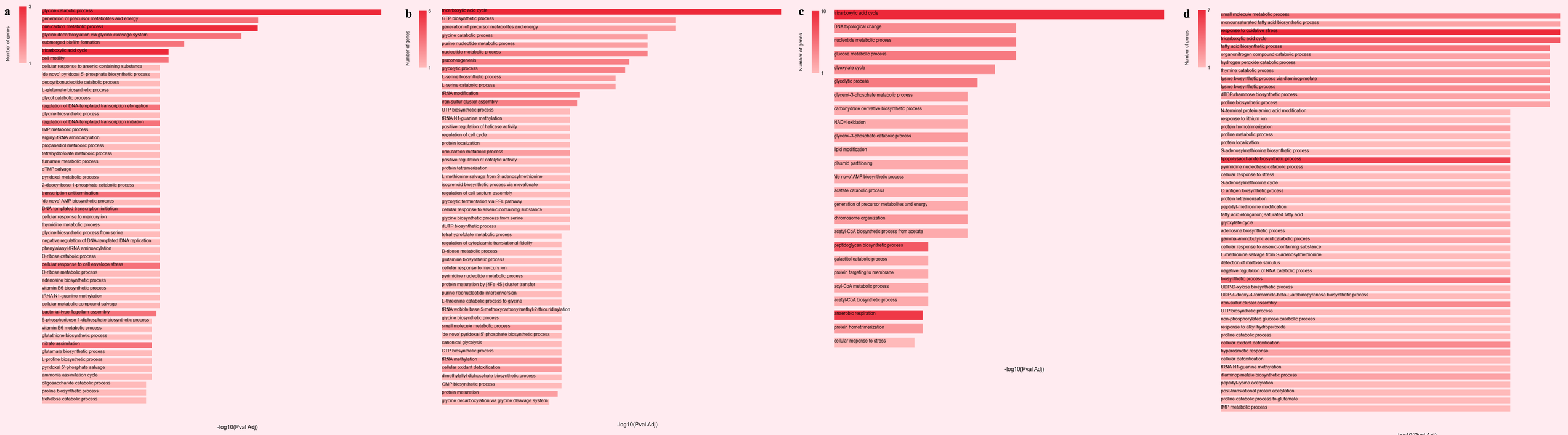

Figure 2 Gene Ontology annotation of the statistically significant proteins, derived from affinity chromatography, performed in GeneCodis4. Ontologies were retrieved from the databases Biological process and Molecular function. Exponential phase acidic elution (a) and DTT elution (b). Stationary phase acidic elution (c) and DTT elution (d).

Table 1. Top 50 potential interactors of each growth phase, in acidic and DTT elutions, were selected for docking experiments.

Docking simulation					
Acidic elution DTT elution	Exponential Phase 52% (13 complexes) 44% (11 complexes)	58% agreement in exponential phase	Stationary Phase 84% (21 complexes) 72% (18 complexes)	**68% agreement in stationary phase**	**Total 63% agreement**

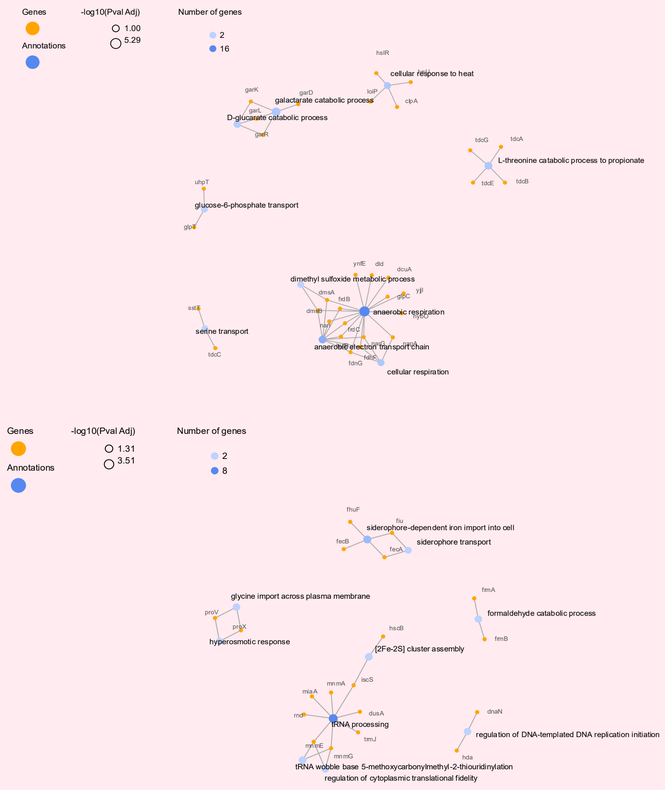

Figure 3. Gene Ontology annotation of the statistically significant proteins, derived from *grxC* null mutant whole proteom analysis, performed in GeneCodis 4. Ontologies were retrieved from the databases Biological process and Molecular function. Up- regulated (up) and Down-regulated (down) proteins compared to wild-type in exponential growth phase.

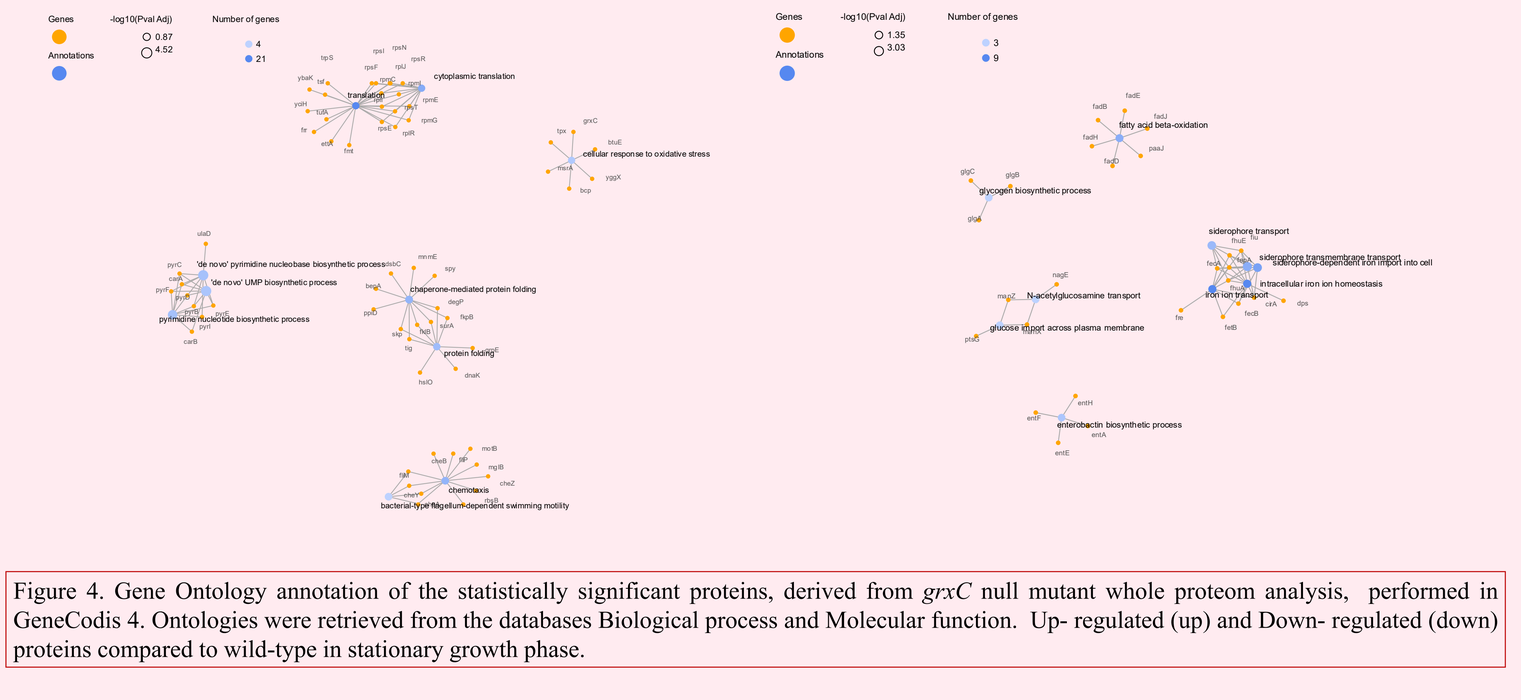

Figure 4. Gene Ontology annotation of the statistically significant proteins, derived from *grxC* null mutant whole proteom analysis, performed in GeneCodis 4. Ontologies were retrieved from the databases Biological process and Molecular function. Up- regulated (up) and Down- regulated (down) proteins compared to wild-type in stationary growth phase.

Conclusions

1. Affinity chromatography experiments revealed that Grx2-interacting proteins may be involved tricarboxylic acid cycle, respose to oxidative stress, peptidoglycane biosynthesis, iron-sulfur cluster assembly and regulation of cell cycle.
2. *grxB*⁻-wild type whole proteome comparisons showed significant changes in the levels of proteins involved in anaerobic respiration, tRNA processing, iron ion homeostasis, protein folding and chemotaxis.
3. Grx2 appears as a multifunctional protein involved in many more biological pathways than its known general antioxidant function.

References

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2. Ogata F. T., Branco V., Vale F. F., Coppo, L. (2021). Glutaredoxin: Discovery, redox defense and much more. *Redox Biology*, 43, 101975.

Acknowledgements

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