The protein interactome of *Escherichia coli* Glutaredoxin 3 Expands its Possible Cellular Functions

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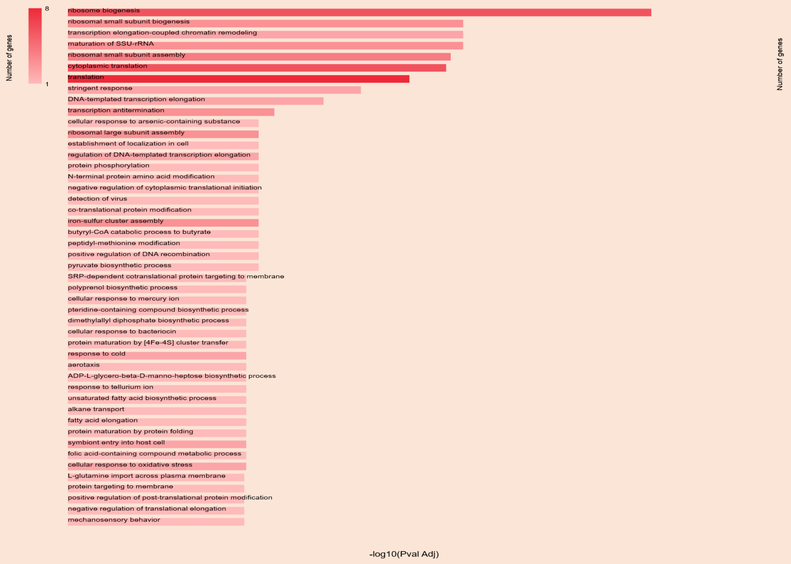
Introduction

Glutaredoxin 3 (Grx3) catalyzes thiol-disulfide exchange reactions between protein substrates and glutathione (GSH)[1,2] but its only biologically relevant activity is the inefficient in vitro reduction of ribonucleotide reductase 1a3. To investigate the greater role of Grx3 for the cell, we used affinity columns with immobilized monothiol mutant Grx3, through which cell lysates from Escherichia coli were chromatographed. These columns are expected to trap dithiol substrates of the wild-type Grx3. Further analysis of the interactions between Grxs and identified protein partners was performed by protein docking using the Prism webserver.

Methodology

The *E.coli* Grx3 C14S C65Y (monothiol) mutant was overexpressed, purified and immobilized on Affi-Gel 15 resin. Approximately 6 mg of Grx3 were immobilized per mL of gel beads. *E. coli* cells were grown in LB-medium and harvested at the exponential and stationary phases of growth. Affinity chromatography of selected cell lysates started with increasing salt (KCl) concentrations to be followed by acidic (CH_3COOH/HCOOH) and finally reducing (DTT) conditions. All experiments were performed thrice. Eluants were analysed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) followed by bioinformatics and gene ontology evaluations. In addition, whole proteome comparisons were performed between the wild-type and the *grxC* null mutant for the exponential and stationary phases of growth.

Results



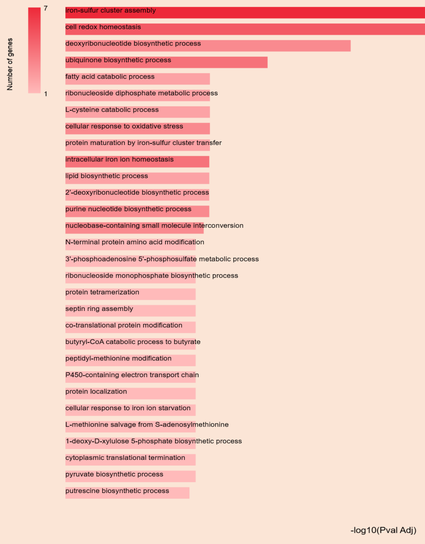


Figure 1. Gene Ontology annotation of the statistically significant proteins in exponential phase, derived from affinity chromatography, performed in GeneCodis4. Ontologies were retrieved from the databases Biological process and Molecular function. Acidic elution (left) and DTT elution (right).

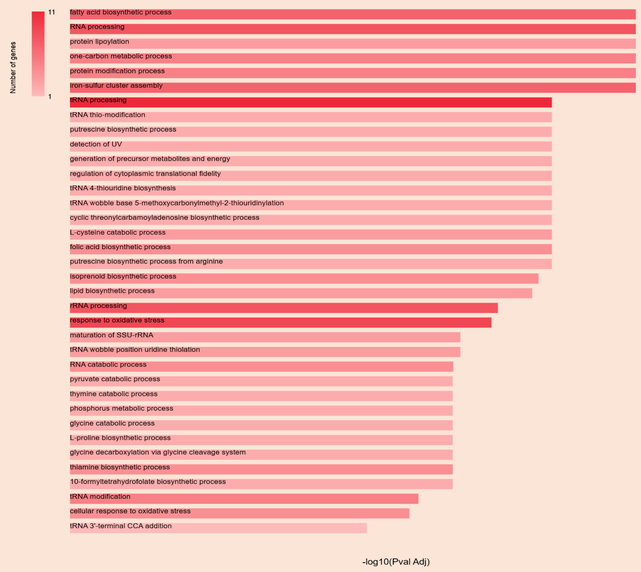
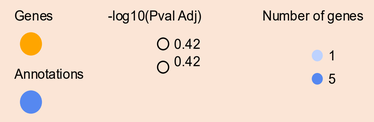
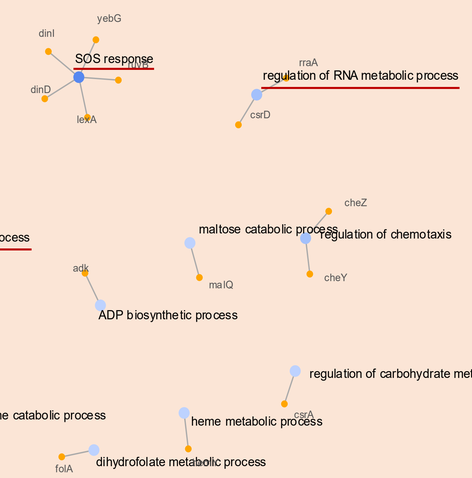


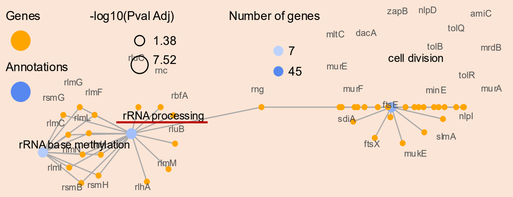
Figure 2. Gene Ontology annotation of the statistically significant proteins in stationary phase, derived from affinity chromatography, performed in GeneCodis4. Ontologies were retrieved from the databases Biological process and Molecular function. Acidic elution (left) and DTT elution (right).

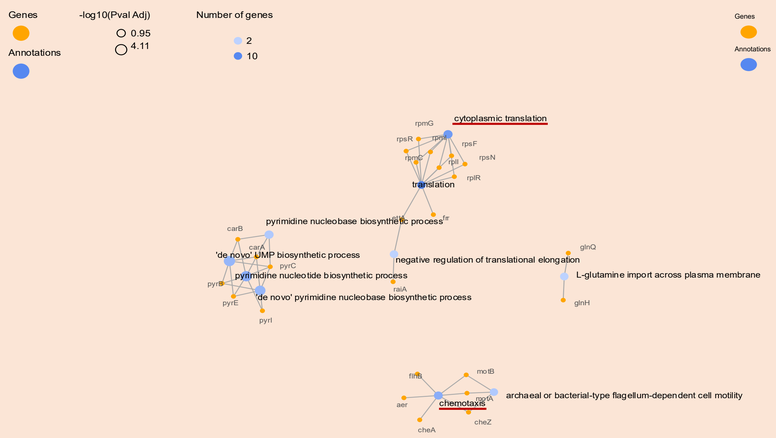
Table 1. Percentage of successful *in silico* docking by Prism[4]. Top 100 potential interactors of each growth phase, in acidic and DTT elutions, were selected for docking experiments. In the exponential phase 42% agreement was detected between docking and affinity chromatography results. In the stationary phase 48% agreement was detected.

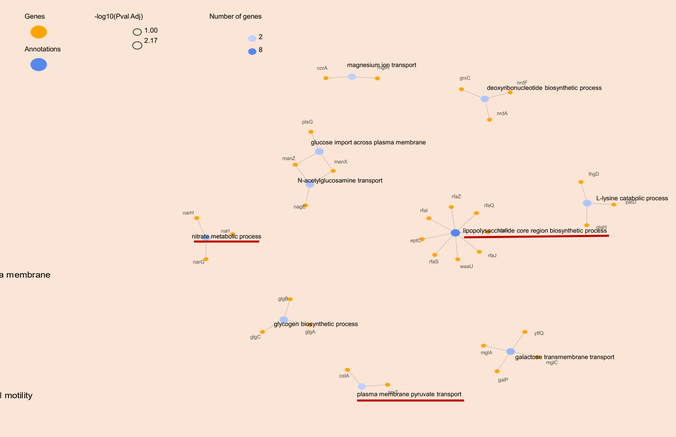
Docking experiments		
	Exponential Phase	Stationary Phase
Acidic elution	54% agreement (24 complexes)	58% agreement (29 complexes)
DTT elution	36% agreement (18 complexes)	38% agreement (19 complexes)











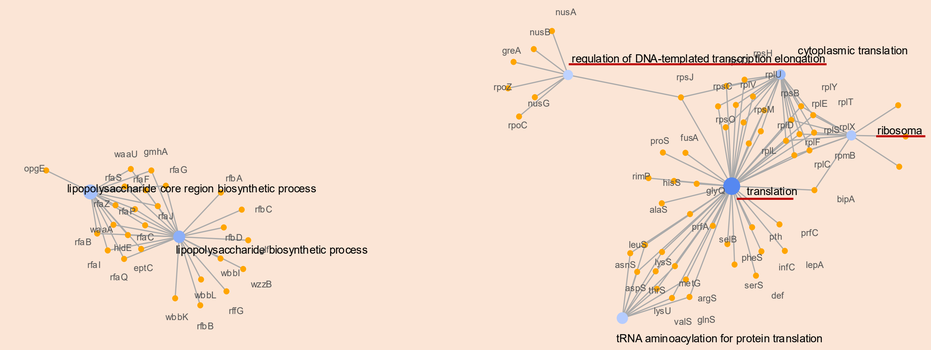


Figure 4. Gene Ontology annotation of the statistically significant proteins, derived from *grxC* null mutant whole proteom analysis, performed in GeneCodis 4. Ontologies were retrieved from the databases Biological process and Molecular function. Up- regulated (left) and Down- regulated (right) proteins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

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.

Conclusions

1. The mean docking score was 45%.
2. The non-covalent interactions (acid elutions) of Grx3 implicated the molecule to ribosome biogenesis, ribosomal small subunit biogenesis, transcription elongation-couple chromatin remodelling (exponential phase), fatty acid biosynthesis, RNA processing and protein lipolysation (stationary phase).
3. The covalent interactions (reducing) of Grx3 corresponding to the iron sulphur cluster assembly, cell redox homeostasis (exponential phase), cytoplasmic translation, translation and ribosomal large subunit assembly (stationary phase).
4. The information derived from whole proteome analysis agrees with the results obtained by affinity chromatography (underlined in red, Figure 3 and Figure 4).

References

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Acknowledgements

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