Escherichia coli glutaredoxins 2 and 3 interact with proteins essential for bacterial survival.

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Introduction

Grx2 (encoded by the *grxB* gene) representing up to 1 % of total soluble protein in the stationary phase of growth, contributes to the protection of cells against oxidative stress induced by H_2O_2 [1]. Grx3 (*grxC* gene) with 0.4 % of total soluble protein may reduce ribonucleotide reductase in vitro. Both Grx2 and 3 participate in thiol-disulfide exchange but their biological role remains unknown. Proteins essential for survival of the Gram-negative pathogen *Escherichia coli,* may represent novel targets for multitargeting antibiotics[2]. We have shown that both glutaredoxins (monothiol trapping mechanism[3]) may interact with essential for survival proteins, whose levels were also altered in null mutants for *grxB* and *grxC*. In this work, we examined the interactions of the two glutaredoxins and the essential proteins.

Methodology

The protein ligands of *E. coli* Grx2 and Grx3 were identified by affinity chromatography experiments using as bits monothiol grxs. Cellular lysates corresponded to cells grown to LB medium (exponential and stationary phase). Cell lysates were prepared and chromatographed through columns with of the immobilized Grx2 and Grx3 mutants. Columns with uncoupled resin served as control. Bound proteins were eluted with salt (KCl, step gradients), CH_3COOH/HCOOH pH 2,1 and finally DTT. All experiments were performed in triplicates. Furthermore, the whole proteomes of *E. coli* wild type and the null mutant for *grxB* and *grxC* were compared for cells grown in LB (exponential and stationary growth phase). All proteomic analyses were performed by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) followed by bioinformatics and gene ontology evaluations.

Results

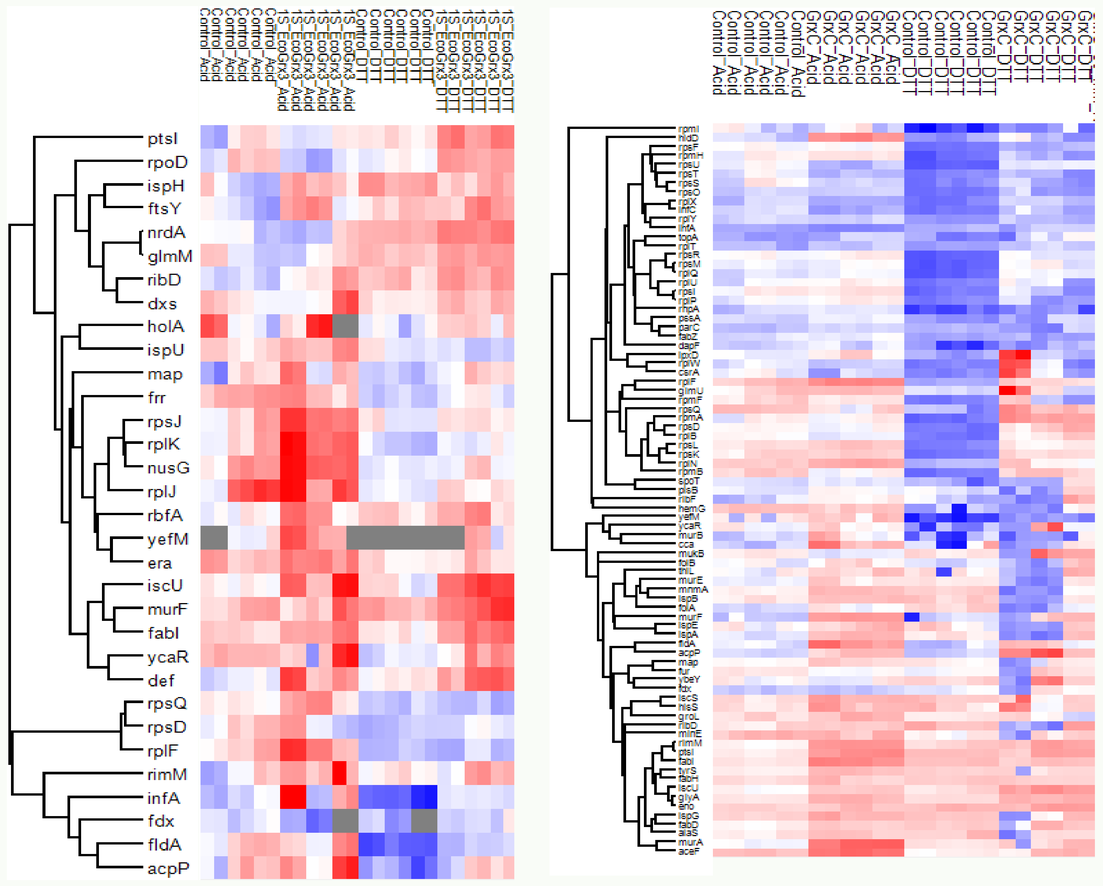

Figure 1. Heatmaps of the statistically significant proteins, derived from affinity chromatography (DTT and acidic elutions), performed in Perseus. Grx3 exponential (left) and stationary (right) phase.

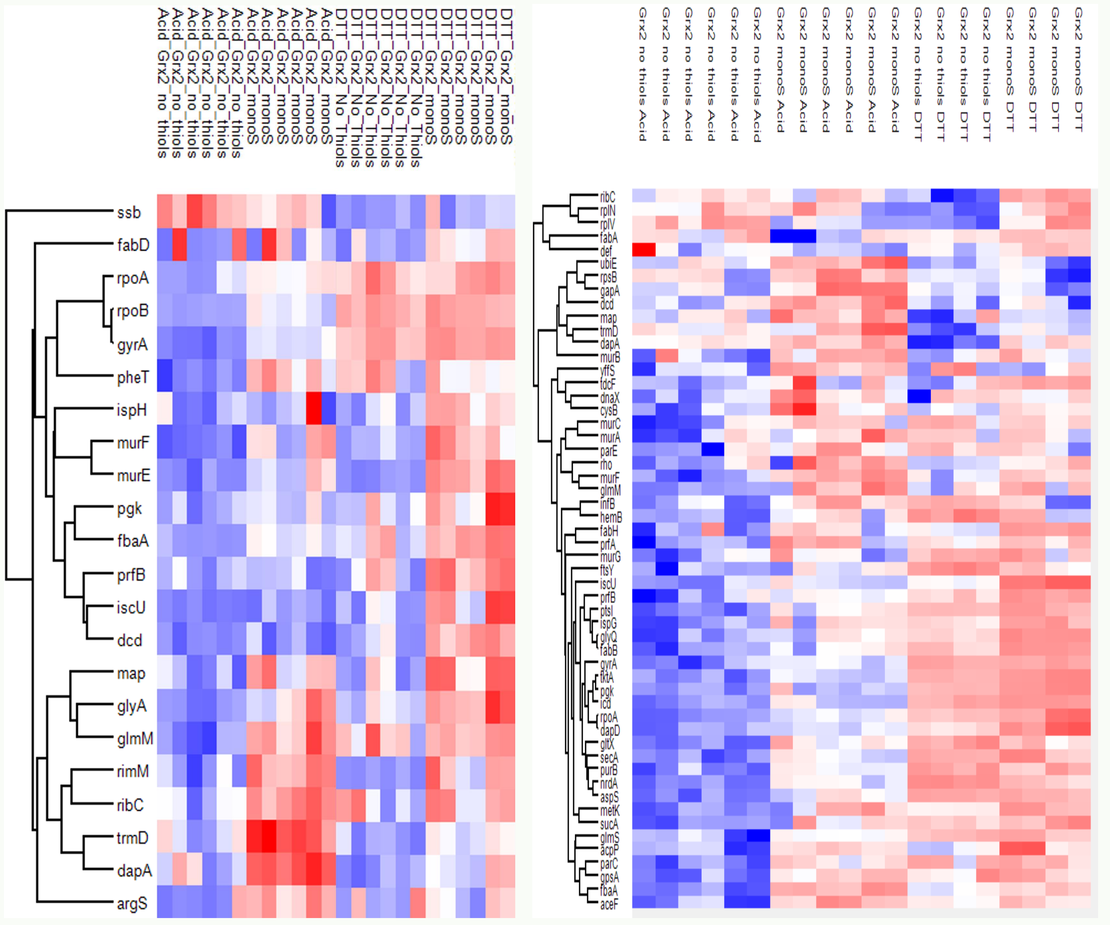

Figure 2. Heatmaps of the statistically significant proteins, derived from affinity chromatography (DTT and acidic elutions), performed in Perseus. Grx2 exponential (left) and stationary (right) phase.

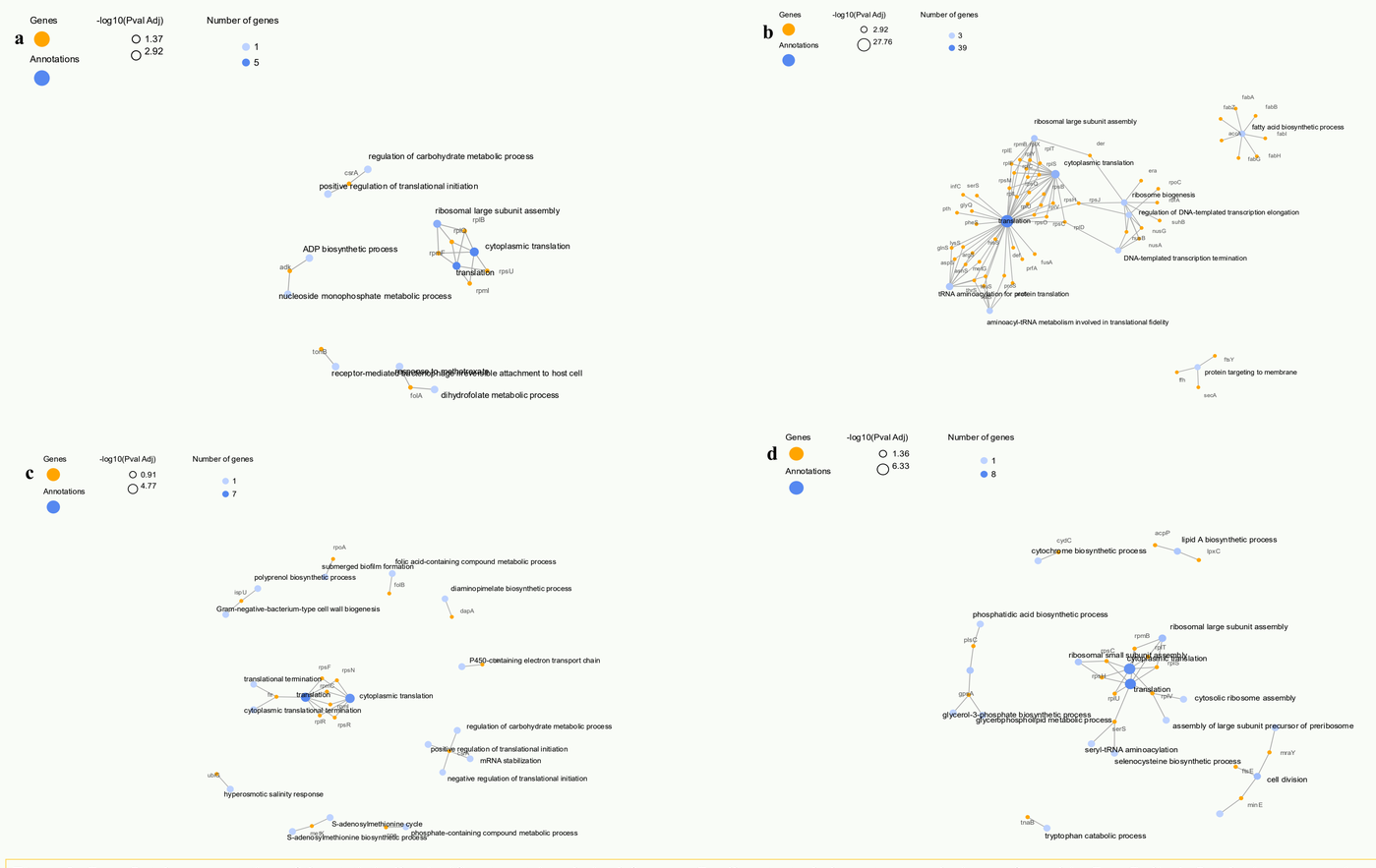

Figure 3. Gene Ontology annotation of the statistically significant proteins, derived from *grxC* null mutant whole proteom analysis, performed in GeneCodis 4. Ontologies were retrieved from the databases Biological process and Molecular function. Up- regulated (a) and Down- regulated (b) proteins compared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

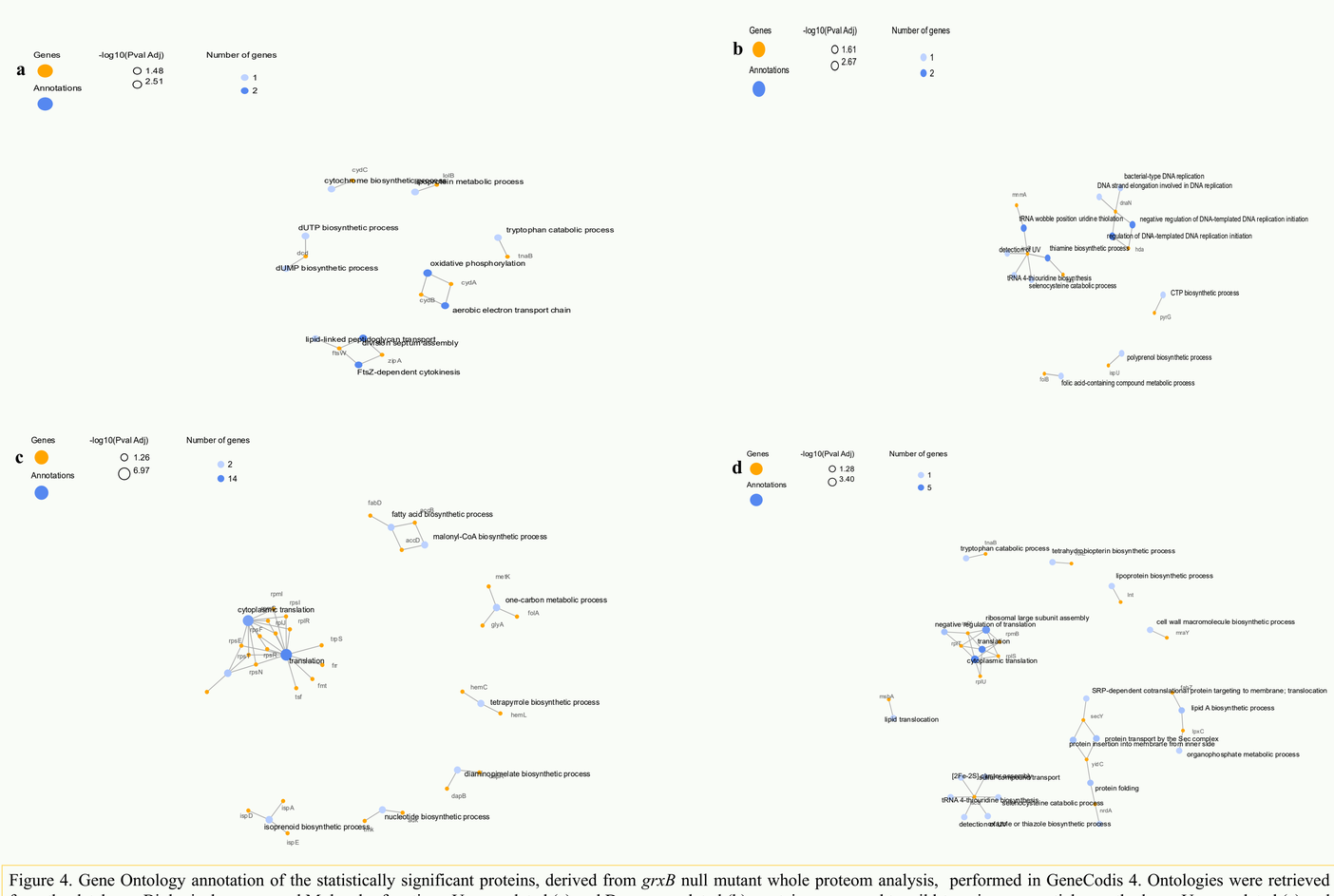

Figure 4. Gene Ontology annotation of the statistically significant proteins, derived from *grxB* null mutant whole proteom analysis, performed in GeneCodis 4. Ontologies were retrieved from the databases Biological process and Molecular function. Up- regulated (a) and Down- regulated (b) proteins compared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

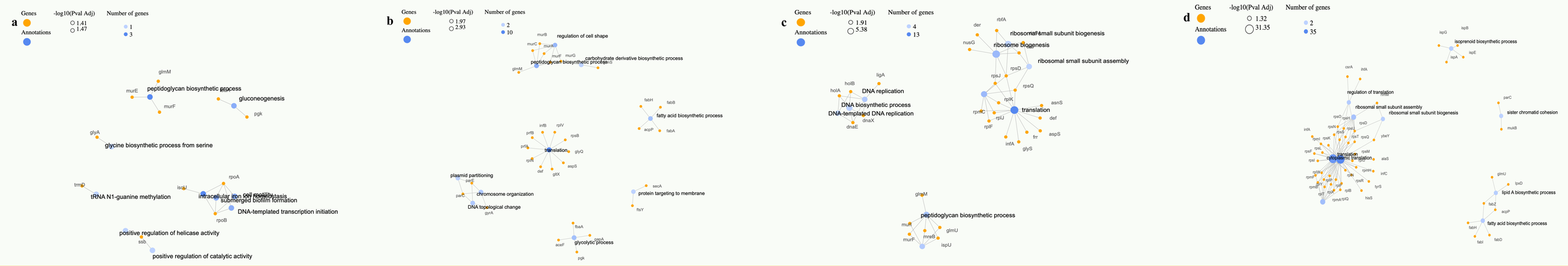

Figure 2. Gene Ontology annotation of the statistically significant proteins, derived from affinity chromatography (DTT and acidic elutions), performed in GeneCodis4. Ontologies were retrieved from the databases Biological process and Molecular function. Grx2 exponential (a) and stationary (b) phase and Grx3 exponential (c) and stationary (d) phase.

Conclusions

- Affinity chromatography experiments revealed that Grx2 interacted with 65 essential for survival proteins involved in biofilm formation, cell motility, peptidoglycan biosynthesis and translation.
- Grx3, in affinity chromatography results, interacted with 125 essential for survival proteins that participate in translation, ribosome biogenesis, DNA replication and regulation of sister chromatin cohesion.
- Whole proteome analysis showed that the levels of 92 essential proteins were altered between the wild type and null mutants for *grxB*¯. Changes in protein levels (down regulation in grxB null mutant) were mostly observed in the stationary phase of growth and involved proteins related to the regulation of DNA replication initiation, detection of UV, lipoprotein biosynthesis, translation and protein folding.
- Whole proteome analysis showed that the levels of 160 essential proteins were altered between the wild type and null mutants for *grxB*¯. Changes in protein levels (down regulation in grxB null mutant) were mostly observed in the exponential phase of growth and involved proteins related to the translation, ribosome assembly, chemotaxis, lipopolysaccharide biosynthesis and metabolic processes.
- These findings highlight the multifunctional roles of Grx2 and Grx3 and provide insights into potential targets for antibacterial strategies.

Acknowledgements

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References

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