Identification of the protein interactome of *Escherichia coli* Glutaredoxin 3

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Introduction. Glutaredoxin 3 (Grx3) catalyses thiol-disulfide exchange reactions between protein substrates and glutathione (GSH)[1,2] but its biological role is unknown. Its only biologically relevant activity is the inefficient *in vitro* reduction of ribonucleotide reductase 1a[3]. To investigate its function, affinity chromatography was employed for *Escherichia coli* cell lysates through columns with immobilized monothiol Grx3 mutant. Possible substrates of dithiol Grx3 are expected to interact with the monothiol Grx3 species (Figure 1, right column). In addition, lysates from *E. coli* null mutants of the *grxC* gene encoding Grx3, 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.

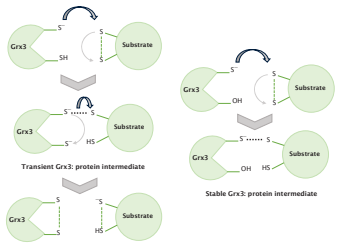

Figure 1. Mechanism of monothiol Grx3 mutant substrate trap.

Methodology. The *E.coli* Grx3 C14S C65Y mutant was overexpressed and purified. Approximately 6 mg of Grx3 were immobilized per mL of Affi-Gel 15 beads and placed in chromatographic columns. *E. coli* cells were grown in LB-medium and harvested at the exponential and stationary growth phases to provide a pool of possible interacting partners. Chromatography of the lysate supernatants followed under increasing salt (KCl), acid (CH_3COOH/HCOOH) and reductive (DTT) conditions. All experiments were performed thrice. Eluants were analyzed with LC-MS/MS. Proteomic comparisons at the exponential phase of growth were also performed between the wild-type and the *grxC* null mutant. All protein fractions were analyzed by LC-MS/MS.

Results

Table 1. The 16 most up- regulated genes whose proteins bound to monothiol Grx3 (affinity chromatography).

Exponential Phase Elutions					
50 mM KCl	100 mM KCl	250 mM KCl	500 mM KCl	Acid	DTT
lptF	*yhdW*	*yhdW*	*gatZ*	*yjdM*	*ydfZ*
yecF	*clsB*	*ptsH*	*gatC*	*yiaD*	*fimH*
pbpG	*acnA*	*amiB*	*lpp*	*gmhB*	*pspE*
rlmE	*grxB*	*fdx*	*grxB*	*fimH*	*ibaG*
amiA	*glrR*	*ybaQ*	*purL*	*yhbY*	*iscA*
yhdW	*lpp*	*clsB*	*kbp*	*iscA*	*nrdE*
yqjC	*emrK*	*birA*	*clsB*	*gnsB*	*hpf*
eamB	*gatZ*	*rsmJ*	*yhdW*	*yibN*	*elaA*
ymdB	*nrdH*	*gpmA*	*ompF*	*fhuA*	*ulaR*
ratA	*ptsH*	*pliG*	*malF*	*ompA*	*slyD*
yfdH	*ridA*	*yjaG*	*ppsA*	*proXp-y*	*yecA*
atpE	*ydjL*	*rfaH*	*acnA*	*raiA*	*iscU*
dcuR	*menC*	*ycjX*	*amiB*	*dolP*	*ychJ*
nuoA	*fre*	*purL*	*tnaB*	*ybcJ*	*yceD*
ybjQ	*yecF*	*lpp*	*mglC*	*iscU*	*fdhD*
yfeY	*rbsB*	*rsmF*	*eptC*	*erpA*	*trxC*

Stationary Phase Elutions					
50 mM KCl	100 mM KCl	250 mM KCl	500 mM KCl	Acid	DTT
rnpA	*yajQ*	*gnsB*	*hslV*	*hisF*	*rraB*
galR	*gcvT*	*ypeA*	*rpoS*	*murF*	*yhhZ*
trxC	*sodB*	*hslV*	*rppH*	*fimH*	*acpP*
fucR	*pckA*	*skp*	*mqsA*	*gcvH*	*ydjL*
sbp	*mioC*	*pliG*	*ybjD*	*fldB*	*msyB*
rfaH	*malM*	*yjbD*	*yibL*	*yjdM*	*yodC*
cysB	*yecA*	*yggU*	*smpB*	*murA*	*rpmA*
ratA	*apt*	*ybaK*	*endA*	*gltA*	*aroL*
rlmA	*nlpD*	*yccX*	*hldD*	*fldA*	*rpsN*
potF	*gltI*	*yhbP*	*dapF*	*rng*	*hdfR*
gmk	*fkpB*	*ptrB*	*yfiF*	*aroL*	*zntR*
rluE	*iscX*	*hupA*	*rplB*	*ydcJ*	*rmf*
artJ	*ptsH*	*flgA*	*rna*	*yoeB*	*aegA*
dacD	*yccJ*	*tadA*	*dicA*	*rimM*	*yjdM*
lrp	*gnsB*	*sra*	*hfq*	*yghA*	*fucK*
rfaF	*amiB*	*rpmF*	*rnr*	*gcvT*	*queG*

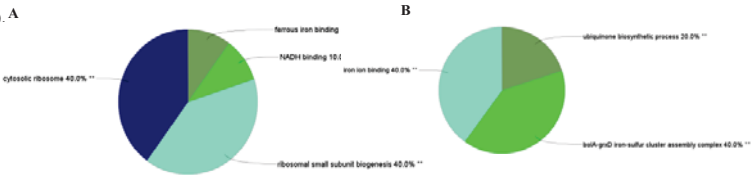

Figure 2. Gene Ontology annotation of the statistically significant proteins in exponential phase, derived from affinity chromatography, performed in Cytoscape. Ontologies were retrieved from the databases Biological process, Molecular function Cellular component and Complexes. A) Acidic elution and B) DTT elution.

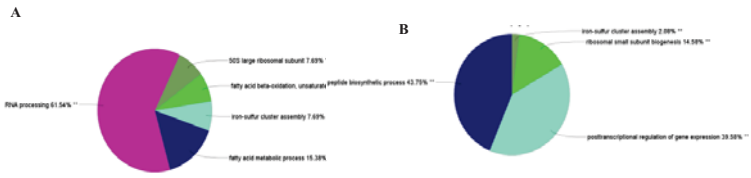

Figure 3. Gene Ontology annotation of the statistically significant proteins in stationary phase, derived from affinity chromatography, performed in Cytoscape. Ontologies were retrieved from the databases Biological process, Molecular function Cellular component and Complexes. A) Acidic elution and B) DTT elution.

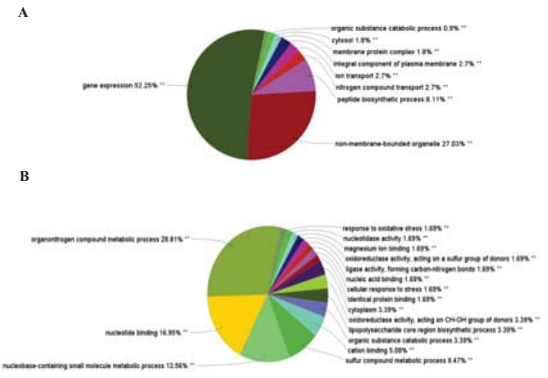

Figure 4. Gene Ontology annotation of the statistically significant proteins, derived from *grxC* null mutant whole proteom analysis, performed in Cytoscape. Ontologies were retrieved from the databases Biological process, Molecular function, Cellular component and Complexes. A) Up- regulated and B) Down- regulated proteins compared to wild-type.

Table 2. Top 10 up- and down- regulated proteins in *grxC*⁻ compared to wild type.

Up-regulated	Protein description	Down-regulated	Protein description
cysU	Sulfate transport system permease protein CysT	*secB*	Protein-export protein SecB
hfq	RNA-binding protein Hfq	*hyfA*	Hydrogenase-4 component A
ydiE	Uncharacterized protein YdiE	*rfaY*	Lipopolysaccharide core heptose(II) kinase RfaY
cirA	Colicin I receptor	*rfaQ*	Lipopolysaccharide core heptosyltransferase RfaQ
fabZ	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	*htrL*	Protein HtrL
ydcH	Uncharacterized protein YdcH	*waaU*	Lipopolysaccharide 1,2-N-acetylglucosaminetransferase
yphA	Inner membrane protein YphA	*torZ*	Trimethylamine-N-oxide reductase 2
rpmE	50S ribosomal protein L31	*rfaB*	Lipopolysaccharide 1,6-galactosyltransferase
racR	Prophage repressor RacR	*rfaI*	Lipopolysaccharide 1,3-galactosyltransferase
ompX	Outer membrane protein X	*rfaS*	Lipopolysaccharide core biosynthesis protein RfaS

Conclusions

1. Affinity chromatography implied involvement of Grx3, in translation, metabolism and iron-sulfur cluster assembly.
2. Comparative analysis of the *grxC*⁻ proteome showed that interacting proteins with Grx3 were involved in metabolism, translation, iron homeostasis, ribosome biogenesis, RNA biosynthesis and transport of bacteriosin .
3. Grx3 correlates with many more functions than the reduction of ribonucleotide reductase.

References

1. Alexios Vlamis-Gardikas . (2008). The multiple functions of the thiol-based electron flow pathways of Escherichia coli: Eternal concepts revisited. *Biochim Biophys Acta* 1780(11):1170-200.
2. Åslund, F., Nordstrand, K., Berndt, K. D., Nikkola, M., Bergman, T., Ponstingl, H., … Holmgren, A. (1996). Glutaredoxin-3 from Escherichia coli. *Journal of Biological Chemistry*, 271(12), 6736–6745.
3. Aslund, F., Ehn, B., Miranda-Vizuete, A., Pueyo, C., & Holmgren, A. (1994). Two additional glutaredoxins exist in Escherichia coli: glutaredoxin 3 is a hydrogen donor for ribonucleotide reductase in a thioredoxin/glutaredoxin 1 double mutant. Proceedings of the National Academy of Sciences, 91(21), 9813–9817.

Acknowledgements

The project is financed by Hellenic Foundation of Research and Innovation (H.F.R.I.) through the program «GluTrxomics», 03352. 2022-2025.