Developing a specific multiplex analytical strategy for redox proteomics

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Introduction

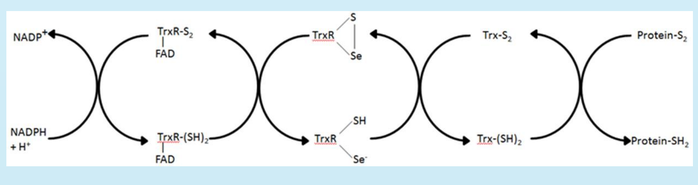
A large number of proteins transit through the endoplasmic reticulum, and most of them necessitate the formation of disulfide bonds for their folding in order to be secreted. The underlying mechanism catalyzing disulfide bond formation is well known. However, the reverse reduction process, probably involving glutathione, remains less understood.

Objectives

We aim at the developing of specific analytical tools dedicated to the study of the redox regulated secretion of proteins. Such an approach appears to be mandatory for the study of redox phenomena in protein chemistry.

Procedure

OcSILAC is a shotgun proteomics approach based on the differential enrichment of thiols in cysteines (Cys), coupled to a SILAC strategy. It has been successfully applied to a thioredoxin reductase-1 knockout yeast model (ΔTrr1).



Schematic diagram of Trr's cellular role

Results

Identification: over 1700 proteins (MaxQuant)

Quantification: Expression profiles of over 1400 proteins were compared in wild type and ΔTrr1 yeast cells (MaxQuant)

Localization of oxidized cysteines: 1920 oxidized Cys in 902 different proteins (In-house software)

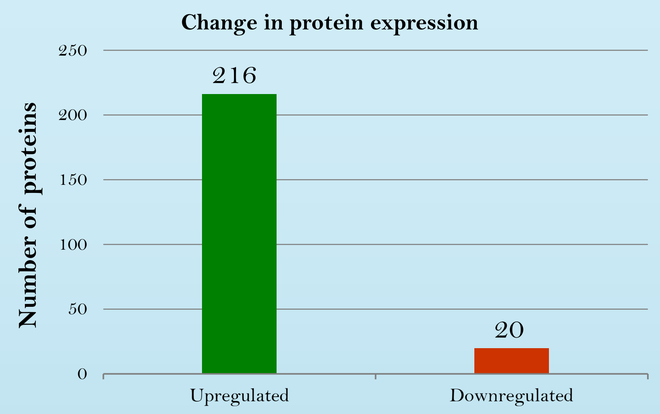
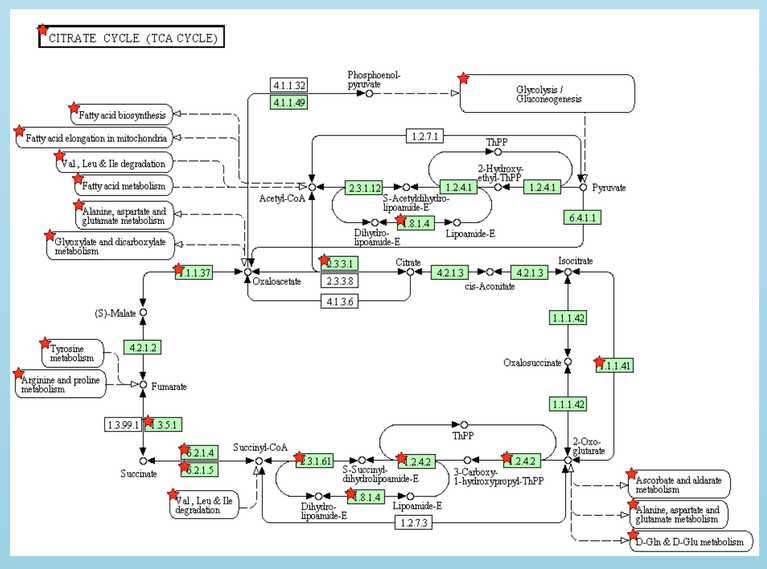


Diagram showing the number of proteins that underwent a significant change in their expression profile

A preliminary ontology study (DAVID bioinformatics) of 80 of the 216 upregulated proteins allowed us to detect a number of affected pathways, such as pathways directly related to the TCA cycle.



Schematic diagram of proteins identified in the TCA cycle as well as other pathways that are directly related

Conclusions & Perspectives

OcSILAC is an adequate strategy to obtain the differential expression profiles of proteins and to monitor the redox index of cysteine residues.

Following this discovery step, a targeted analysis would allow us to finely monitor changes in the redox states and profile expressions of specific proteins that are involved in the affected pathways.

Extending this type of study to a variety of cell lines (different knockouts) would allow us to better understand the mechanisms underlying redox regulation and the response to oxidative stress.

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