Comparison with proteome data

Eukaryotic proteome analyses

In eukaryotes, a number of studies have been performed on thioredoxin, yet all of them aim at the identification of interacting proteins through proteomics, and do not evaluate the effects of thioredoxin levels on transcription. Yet, a closer analysis of the proteomics data could help to shed light on some of the transcriptome results from this study.

Wong and colleagues identified thioredoxin linked metabolic processes in cereal starchy endosperm (5). In corroboration of our results, they identified for instance aldolase, which in both the \textit{ItrxA 25} and the \textit{ItrxA 100} is strongly up-regulated, and malate dehydrogenase. The latter has a close homologue, \textit{yjmC}, in \textit{B. subtilis} that is 39-fold up-regulated upon thioredoxin depletion. In addition they identified a fructose phosphotransferase system as a potential target, which is represented in our results by the \textit{levE} and \textit{levG} genes (down-regulated in \textit{ItrxA 25}). Interestingly, an alpha-amylase/subtilisin inhibitor, were also identified in the study of Wong and colleagues. The genes encoding alpha-amylase and subtilisin are up-regulated in the \textit{ItrxA 25}, and both strains respectively.

Balmer and colleagues have identified thioredoxin interacting proteins plant chloroplasts (1) and mitochondria (2) through affinity chromatography. In chloroplasts, the authors link thioredoxin to terrapyrrole biosynthesis. In our list protoporphyrinogen IX and coproporphyrinogen III oxidase (encoded by \textit{hemY}) and two proteins with similarity to uroporphyrin-III \textit{C-methyltransferase} (encoded by \textit{ylnDF}) show an up-regulation in especially the \textit{ItrxA 25} mutant. Also an enzyme from the thiamin biosynthetic route is present (\textit{thiA}, down-regulated in \textit{ItrxA 25}). In chloroplast also an ATP dependent clp-protease was found; in our study \textit{clpY} shows up-regulation in both \textit{ItrxA} mutants. Furthermore, thioredoxin was found of importance for starch degradation through interaction with beta-amylase. As mentioned already, \textit{amyE} transcription was increased in the \textit{ItrxA 25}. With respect to DNA replication and transcription, the proteomic analysis of thioredoxin interacting proteins in chloroplast yielded a single target: an ATP-dependent helicase. In our results, we identified \textit{yrvN}, a gene with similarity to an ATPase related to the helicase subunit of the Holliday junction resolvase (MGS1/COG2256). Interestingly, cysteine synthase also was found to interact with thioredoxin. In \textit{B. subtilis} the gene encoding this enzyme is \textit{cysK}, and is part of the induction of the sulfur uptake and utilization routes described in the Results section. In plant mitochondria, a substantial number of thioredoxin interacting proteins were identified by Balmer and colleagues. Some of these are in common with chloroplasts, such as the malate dehydrogenase, and cysteine synthase, but the similarity with our dataset goes much further. In addition to the proteins mentioned, dihydrolipoamide acetyltransferase and – dehydrogenase, and pyruvate dehydrogenase were identified in the class the citric acid cycle-associated proteins in their study. In \textit{B. subtilis} these enzymes are encoded by the genes \textit{acoC}, putatively \textit{yqiV} and \textit{pdhB}, respectively, all of which are up-regulated in the \textit{ItrxA 25} strain. Furthermore, alcohol dehydrogenase, katalase and a glutaredoxin like protein were found. In our study, \textit{yhxD} and \textit{yogA} (down and up-regulated, respectively) have an annotated function as alcohol dehydrogenase, \textit{katB} and \textit{katX} encode catalases (down-regulated), and \textit{ytmI} (up-regulated) shows strong similarity to glutaredoxin-like proteins. Finally, thioredoxin was implied in electron transport through interactions with NADH-dependent oxidoreductases. Two genes in the list of genes up-regulated in \textit{ItrxA 25}, \textit{yqiM} and \textit{yqiG}, have been annotated as such on the basis of homology. In both plant organelles, Balmer and colleagues found a chaperonin, HSP70, to be associated with thioredoxin. In \textit{B. subtilis} constitutive expression of thioredoxin seems to lead to an increase of transcription of \textit{groEL}, a class I heat shock gene encoding a chaperonin, which is significant in the \textit{ItrxA 100} strain. The association of a chaperonin with a thioredoxin is consistent with a function for the latter in the reversal of illegal disulfide bond formation.
Lindahl and colleagues have reported that thioredoxin targets in cyanobacteria are different from those identified in chloroplast, based on a proteome analysis in *Synechocystis* (4). Yet, upon examination of their list of thioredoxin-linked processes, again striking similarity is observed with the transcriptome results from this study. Most notably, of the cytosolic proteins, GroEL was found to be associated with thioredoxin, confirming the findings in plant organelles, and the up-regulation of *groEL* in *Bacillus*. Furthermore, both ferredoxin and NADH-dependent glutamate synthase (GOGAT) were identified in the study of Lindahl and colleagues. We found *gltB*, encoding this enzyme, to be up-regulated upon thioredoxin depletion. Furthermore, argininosuccinate synthase (*B. subtilis* argG), was in common between the two datasets. Interestingly, the genes involved in arginine synthesis, seem only to be affected in the *ItrxA* 100 strain. This also counts for *yjaV*, a gene putatively encoding UDP-glucose-4-epimerase, although with a p-value of and a 2.7-fold up-regulation in the *ItrxA* 25 it is possible that this gene has only just been missed due to the stringent cut-off settings imposed in our experiments. Sugar-nucleotide epimerase was also identified in *Synechocystis*. Of the peripheral membrane proteins linked to thioredoxin, the identification of sulfate adenylyltransferase confirmed the strong up-regulation of the genes that encode this enzyme in the *ItrxA* 25 strain, *ylnB* and *yitA*. In addition, Lindahl and colleagues identified ferredoxin sulfite reductase, which also acts in the sulfur metabolic pathways. As pointed out in the Results section, the genes encoding the enzyme responsible for this activity in *B. subtilis*, *cysJl* (*yvgQR*), do not show a change in transcription upon thioredoxin depletion. There is a homologue however, *ykuP*, that does show up-regulation. In *Chlamydomonas reinhardtii*, affinity chromatography led to the identification of new thioredoxin targets, in addition to the confirmation of many of the results from the other studies in plants and cyanobacteria (3). A single protein uniquely identified in this study, has not been discussed yet. Threonine synthase of *B. subtilis* is encoded by the *thrC* gene, and shows a 3-fold up-regulation in the *ItrxA* 25 strain.

To conclude the comparison with proteomics studies in eukaryotes, Yamazaki and colleagues have reported target proteins of cytosolic thioredoxin from *Arabidopsis thaliana* (6). Although a few new putative targets for thioredoxin were identified, the overlap with the transcriptome analysis of the *ItrxA* strain is limited to some of the genes discussed above.

**Prokaryotic proteome analyses**

To our knowledge, only two studies on thioredoxin-linked proteins have been performed in bacteria. Through tandem affinity purification and mass spectroscopy, Kumar and colleagues identified a total of 80 proteins associated with thioredoxin in *E. coli* (38). To our surprise, the overlap between our study and the results of Kumar and colleagues was relatively small. The majority of the genes in common with the *E. coli* study corroborates findings from the eukaryotic proteome studies (such as Clp-protease, chaperonins) or confirms well-established interactions (such as ribonucleotide diphoshate reductase). One of the most striking similarities was association of the transcriptional regulator Fur with thioredoxin. This regulator is involved in iron homeostasis, and is highly conserved between Gram-positive and Gram-negative organisms. In our list of genes with altered transcription in the *ItrxA* strain *fur* itself is not present, but we find almost the entire Fur-regulon, suggesting that also in *B. subtilis* an interaction occurs between Fur and thioredoxin (4). The majority of the Fur-repressed genes are class I genes (*yclP, dhhAC, ydbN, ykuNOP, yuiI, yclN* and *fhuB*), indicating an inactivation of the regulator upon thioredoxin depletion, but the directionality of the response is not strictly conserved. The down-regulated genes belonging to the Fur-regulon include *ywcD, ykvW* (affected in the *IrrxA* 25), *ypbR* and *yfkM* (affected in the *IrrxA* 100). Further experiments will have to establish whether these genes are subject to additional regulation, or that Fur (directly or indirectly) positively regulates the expression of these genes.
Recently, Leichert and Jakob used a novel approach to identify the *in vivo* disulfide bonding state of *E. coli* proteins (40). Interestingly, these authors were able to specifically detect proteins whose redox state was modulated by thioredoxin A by looking at changes in redox state in a thioredoxin A mutant. The results from this study show a substantial overlap with our transcriptome analysis, and point to targets that were not identified in any of the other proteome studies. Most notably, these include oligopeptide permease (*opp*), phosphate transport proteins (*pstA* and *BA*), and ribosomal proteins (*rplA, yhzA, yitA*). In addition, 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (*aroG*), and citrate synthase (*citA*) were in common between the two studies. From the comparison it also seems that thioredoxin A is involved in purine metabolism (*purK* is affected in *ItrxA* 25). Interestingly, *E. coli* YaeC (a probably methionine-binding lipoprotein) was affected in its redox state by thioredoxin A. The homologue of this gene in *B. subtilis* is *yusA*. In the *ItrxA* array study, the genes downstream of *yusA* are transcriptionally affected, and *yusA* itself falls just outside of the criteria for significance in our experiment, indicating that this interaction may be conserved between *E. coli* and *B. subtilis*. 