Ilon Liu

Dr. med.

In vivo characterization of Px IV, a novel glutathione peroxidase-type enzyme, in African trypanosomes.

Fach: Biochemie

Doktormutter: Prof. Dr. rer. nat. Luise Krauth-Siegel

T. brucei subspecies are the causative agent of human African sleeping sickness and Nagana cattle disease. Based on their specific trypanothione-tryparedoxin system, hydroperoxide detoxification is achieved by 2-Cys-peroxiredoxins (TXNPx) and glutathione peroxidase-type enzymes (Px). So far, the two cytosolic Px I-II and the mitochondrial Px III have been characterized in bloodstream *T. brucei*.

In this thesis, the role of a fourth, distantly related glutathione peroxidase type enzyme, Px IV, was studied.

As specific antibodies against the protein were not available, vectors that allow the inducible expression of N- and C-terminally myc_6-tagged versions of the protein (pRPa^{ix6Mx}_N-myc_6_*px* IV and pRPa^{ix6Mx}_*px* IV_C-myc_6) were cloned and bloodstream *T. b. brucei* were transfected with the constructs. Immunofluorescence analyses of different clones revealed a mitochondrial localization of Px IV. The C-terminally tagged protein clearly co-localized with the mitochondrial marker MitoTracker^{TM}, while the N-terminally tagged version co-localized with the cytosolic markers TR and TXNPx. This contrasts with TcGPXII, an orthologue of Px IV in *T. cruzi*, described to be an ER resident protein. A co-localization with the ER marker BiP was excluded for both the N- and C-terminally myc_6-tagged *T. brucei* Px IV due to the significantly lower Pearson correlation coefficient (R_r) for BiP compared to the ones for the respective mitochondrial or cytosolic markers. Thus, Px IV is likely to be a mitochondrial protein possessing an N-terminal mitochondrial targeting signal.

To elucidate if Px IV is essential, the mRNA of *px* IV was depleted in four different bloodstream RNAi cell lines after respective transfection with constructs derived from the

pRPa vector. The sizes of the mRNA of *px* IV deriving from *trans*-splicing were determined. cDNA analysis using a spliced leader primer and a reverse internal primer as well as a forward internal primer and an Oligo(T) primer, respectively, revealed two possible *px* IV mRNAs of 628 bases and 978 bases. These two forms corresponded to bands in the Northern blot. Both were not downregulated after RNAi, suggesting that the RNAi was not efficient. A high throughput RNAi approach had indicated essentiality of Px IV in bloodstream parasites using RNAi constructs that differed from those used in this work. Overexpression of the myc_6-tagged versions likewise did not reveal a difference in proliferation compared to WT parasites.

Because of the insufficient mRNA depletion, cloning of *px* IV knockout constructs was carried out. As a first step for the generation of (conditional) knockout cell lines, the vectors pHD 1747_*px* IV (puromycin resistance) and pHD 1748_*px* IV (blasticidin resistance) were generated and WT bloodstream *T. b. brucei* cells as well as Px IV overexpressing cells were successively transfected with both vectors.

This thesis adds a new potential candidate to the antioxidant defense mechanisms in the mitochondrion of *T. brucei*, so far consisting of a 2-Cys-peroxiredoxin and Px III, another non-selenium glutathione peroxidase type enzyme. Since both peroxidases proved to be not essential in bloodstream *T. brucei*, the role of Px IV in the mitochondrion of trypanosomatids will be of high interest.