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The distinct roles of the tryparedoxin peroxidases Px I, Px II and Px III in African trypanosomes

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Trypanosoma brucei species are the causative agents of human sleeping sickness as well as Nagana cattle disease in Africa. Hydroperoxide detoxification in these parasites is mediated by 2-Cys-peroxiredoxins and non-selenium glutathione peroxidase-like enzymes (Px I–III), which both obtain their reducing equivalents from the unique trypanothione/-tryparedoxin system.

In bloodstream *T. brucei* the cytosolic enzymes Px I–II are essential. Deletion of both genes leads to cell death within a few hours. In contrast, the specific lack of the mitochondrial Px III results only in a transient proliferation defect.

Aim of the thesis was to knockout the Px-type enzymes in procyclic *T. brucei* and to characterize the mutant cell lines.

Deletion of Px I-II did not affect the proliferation rate of the parasite under culture conditions. However, when treated with linoleic acid hydroperoxide – but not with H_2O_2 – the Px I-II double-knockout cell lines showed a proliferation defect in comparison to wt cells. An up-regulation of other thiol redox enzymes to compensate for the missing peroxidases could be ruled out by Western blot analysis.

Cells lacking Px III also did not show any proliferation defect unless when they were stressed with linoleic acid hydroperoxide. Interestingly, the growth defect was only pronounced during the first 24 hours. Afterwards the cells regained a nearly normal proliferation rate.

The gene of Px III encodes, in addition to the mitochondrial, also a putative glycosomal targeting sequence. Fractionated lysis of the Px I-II double-knockout cells clearly revealed that the remaining Px III is absent from the cytosol but it could not finally be decided if the protein occurs only in the mitochondrion or also in the glycosomes.

Since a previous RNA interference approach suggested that the Px-type enzymes are also essential in the procyclic insect form of the parasite new RNA interference cell lines were generated. One of them indeed revealed a proliferation defect together with Px-depletion. However, others had an impaired growth but no down-regulation of Px or vice versa displayed normal proliferation despite a depleted Px level. Very recently it was shown that the knock-out of all three *px* genes is lethal for the procyclic parasites. Thus one can assume that RNA interference resulted in a variable degree of down-regulation. Furthermore, there might be an additional off-target effect.

In summary, the results obtained in this thesis clearly show that also in the insect form of *T. brucei*, the Px-type enzymes are involved in the detoxification of lipid-derived hydroperoxides. However, under normal culture conditions, either the cytosolic or the mitochondrial enzyme(s) are/is dispensable. This suggests that the endogenous oxidative stress in procyclic *T. brucei* is much lower compared to that in the mammalian bloodstream form of the parasites.