University of South Bohemia
Faculty of Science
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Ph.D. Thesis

Iron-Sulfur Cluster Assembly in *Trypanosoma brucei*

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Annotation:
This work addresses various aspects of iron-sulfur cluster assembly in *Trypanosoma brucei*, a causative agents of African sleeping sickness. RNAi-mediated reverse genetics and heterologous expression of proteins were used extensively throughout this work. Frataxin appears not to be an iron storage protein in this early branching protist, but it functions primarily in iron-sulfur cluster assembly and protection from reactive oxygen species. Frataxin has to be confined to the mitochondrion for its function. Moreover, *T. brucei* exhibited remarkable conservation in the mechanisms of frataxin import and processing, since its deficiency was rescued by human frataxin, as well as by orthologues from other non-related eukaryotes. Finally, Isa1 and Isa2 were shown to function only for a subset of mitochondrial Fe-S cluster proteins in the procyclic stage, while these proteins are dispensable for the bloodstream stage of *T. brucei*.

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I hereby declare that I did all work summarized in this thesis, on my own or in collaboration with the co-anthours of the presented papers and manuscripts, and only using the cited literature.

Ceske Budejovice, November 20, 2009 Shaojun Long

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Contents

Part 3. Conclusions and perspectives

Abbreviations

ISC	Iron-Sulfur cluster
CIA	Cytosolic iron-sulfur protein assembly
IRP	iron regulatory protein
IRE	iron-responsive element
GSH	glutathione
SAM	S-adenosyl-methionine
XPD	Xeroderma pigmentosum group D protein
Complex I	NADH dehydrogenase
Complex II	Succinate dehydrogenase
Complex III	cytochrome bc_1 complex
Complex IV	cytochrom c oxidase
dsRNA	double stranded RNA
RNAi	RNA interference
MPP	mitochondrial processing peptidase
DAPI	4',6-diamidino-2-phenylindole
TMRE	tetramethylrodamine ethyl ester
ROS	reactive oxygen species
HA	hemagglutinin
NIF	nitrogen fixation
SUF	sulfur assimilatin
DIC	differential interference contrast
TAO	trypanosome alternative oxidase
VSG	variant surface glycoprotein
MT	mitotracker
EGFP	enhanced green fluorence protein
kDNA	kinetoplastid DNA
MRP	mitochondrial RNA binding protein
tet	tetracycline

Wohlgamuth-Benedum et al., J. Biol. Chem. 2009

Due to a complete lack of tRNA genes in the mitochondrial genome of kinetoplastid flagellates, tRNAs are imported into the mitochondrion from the cytoplasm. A single tryptophanyl tRNA ($tRNA^{Trp}$) is encoded in the nucleus of these organisms and contains a CCA anticodon that can decode the UGG codons used in cytoplasmic protein synthesis, but cannot decode the mitochondrial UGA codons. To circumvent this problem, *Trypanosoma brucei* specifically edits the CCA anticodon of $tRNA^{Trp}$ to generate a UCA anticodon that can now decode the predominant mitochondrial UGA tryptophan codons. This tRNA also undergoes an unusual thiolation at position 33 of the anticodon loop, the only known modification at U_{33} in tRNAs in any system. This observation raised questions about the possible involvement of thiolation as a determinant for editing of the neighboring position (C_{34}). In other organisms, tRNA thiolation is mediated by the cysteine desulfurase, Nfs1 (IscS). However, *T. brucei* encodes two Nfs homologues, one localized to the cytosol and the other to the mitochondrion. We show by a combination of RNA interference and Northern analyses that the mitochondria-targeted TbNfs2 is essential for thiolation of both cytosolic and mitochondrial tRNAs. We further show that thiolation specifically affects the stability of thiolated tRNAs in the cells and more surprisingly acts as a negative determinant for C to U editing in *T. brucei*. Taken together our findings indicate that thiolation serves an important role in tRNA processing in these organisms and provides a first line of evidence about the regulation of mitochondrial tRNA editing in any system.

Long et al., Mol. Biochem. Parasitol. 2008

Frataxin is a conserved mitochondrial protein, almost universally present in prokaryotes and eukaryotes, where it is implicated in Fe-S cluster assembly and several other processes. Here we show that frataxins from the diatom *Thalassiosira pseudonana* and the plant *Arabidopsis thaliana* are efficiently targeted and processed in the mitochondrion of the evolutionary distant excavate kinetoplastid flagellate *Trypanosoma brucei*. Moreover, both heterologous frataxins are able to rescue a lethal deficiency for *T. brucei* frataxin.

Long et al., Proc. Natl. Acad. Sci. USA 2008

Trypanosoma brucei is the most genetically tractable representative of the domain Excavata and also the causative agent of human sleeping sickness and ruminant nagana. It has a single mitochondrion, for which a number of unique features is characteristic. We have attempted to rescue the *T. brucei* cells with

down-regulated frataxin using the human frataxin, the defects of which result in Friedriech's ataxia. Despite the evolutionary distance between humans and trypanosomes, human frataxin was not only efficiently imported into the *T. brucei* mitochondrion via its long genuine import signal, but also successfully rescued the phenotype. The rescue was, however, fully dependent on mitochondrial localization of human frataxin. Upon import into the trypanosome organelle, human frataxin was processed by mitochondrial processing peptidase and this processing could have been blocked by mutations, in exactly the same manner as in human cells. However, while in human cells frataxin has to be processed in order to execute its functions, the same protein in the *T. brucei* mitochondrion is functional even in the absence of processing.

Long et al., Mol. Microbiol. 2008

Frataxin is a small conserved mitochondrial protein; in humans, mutations affecting frataxin expression or function result in Friedreich's ataxia. Much of the current understanding of frataxin function comes from informative studies with yeast models, but considerable debates remains with regard to the primary functions of this ubiquitous protein. We exploit the tractable reverse genetics of *Trypanosoma brucei* in order to specifically consider the importance of frataxin in an early-branching lineage. Using inducible RNAi, we show that frataxin is essential in *T. brucei* and that its loss results in reduced activity of the marker Fe-S cluster-containing enzyme aconitase in both the mitochondrion and cytosol. Activities of mitochondrial succinate dehydrogenase and fumarase also decreased, but the concentration of reactive oxygen species increased. Trypanosomes lacking frataxin also exhibited a low mitochondrial membrane potential and reduced oxygen consumption. Crucially, however, iron did not accumulate in frataxin-depleted mitochondria, and since *T. brucei* frataxin does not form large complexes, it suggests that it plays no role in iron storage. Interestingly, RNAi phenotypes were ameliorated by expression of frataxin homologues from hydrogenosomes of another divergent protist *Trichomonas vaginalis*. Collectively, the data suggest trypanosome frataxin functions primarily only in Fe-S cluster biogenesis and protection from reactive oxygen species.