The essential processes of FeS cluster assembly and mitochondrial protein import in parasitic protists

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Ph.D. Thesis

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Introduction

Mitochondria of most eukaryotic organisms are organelles whose most prominent function in a cell is ATP production by oxidative phosphorylation. Parasitic protists possess organelles that are often deviated structurally and functionally from the canonical mitochondrion. Protists that encounter aerobic enviroment during their lifecycle have mitochondria that are able to produce ATP by oxidative phosphorylation. Many protists however, like *Trichomonas vaginalis*, live in an oxygen-poor enviroment. Their mitochondria lost citric acid cycle and respiratory chain and employ anaerobic metabolic pathways for production of ATP by substrate-level phosphorylation. These mitochondria were named hydrogenosomes after the hallmark metabolic end product, the molecular hydrogen. Mitochondria of other protists, like those of *Giardia intestinalis*, *Entamoeba histolytica, Cryptosporidium parvum* or microsporidia, underwent more radical reductive evolution. They lost ability to produce ATP altogether, being downsized to remnant organelles called mitosomes.

Even though mitochondria are sometimes highly reduced, they are present in all eukaryotic organisms studied to date. It indicates that the organelle harbours fundamental process(es) required for life of a eukaryotic cell. In *Saccharomyces cerevisiae*, the model eukaryotic organism, the mitochondrion is a compartment where the crucial part of the essential iron-sulfur (FeS) cluster biosynthetic pathway is localized. FeS clusters are cofactors of a number of proteins, including those essential for DNA metabolism (Rad3, Pri2) and protein translation initiation (Rli1). The components of the FeS cluster biosynthesis are encoded in the nucleus and translated in the cytosol. Thus, machineries involved in mitochondrial protein import and maturation are also indispensable for *S. cerevisiae*.

Aims of the thesis

1. **FeS cluster assembly in *Trypanosoma brucei***
   a) identification and subcellular localization of IscS and IscU, the key components of FeS cluster assembly, in *T. brucei;*
   b) verification of the involvement of the two proteins in FeS cluster assembly;
   c) assessment of the significance of the FeS cluster assembly machinery for the insect stage of *T. brucei*.

2. **Function of mitosomes in *Giardia intestinalis***
   a) development of a method for subcellular fractionation of *G. intestinalis;*
   b) proteomic analysis of the mitosome-rich fraction;
   c) verification of the mitosomal localization of the identified proteins.

3. **Protein import to mitosomes of *Giardia intestinalis***
   a) overexpression of *G. intestinalis* mitosomal proteins IscS, IscU, and 2Fe2S ferredoxin in *G. intestinalis* and *T. vaginalis;*
   b) comparison of the targeting and translocation of the three proteins into mitosomes and hydrogenosomes;
   c) identification of the components of the mitosomal protein import machinery.

4. **Protein processing in mitosomes of *Giardia intestinalis* and hydrogenosomes of *Trichomonas vaginalis***
   a) identification of hydrogenosomal and mitosomal processing peptidases (HPP and GPP, respectively);
   b) characterization of HPP and GPP processing reactions *in vitro*;
   c) analysis of substrate specifity and protein structure of HPP, GPP and the mitochondrial processing peptidase.

Results of the thesis

1. **FeS cluster assembly in *Trypanosoma brucei***
   We demonstrated that the cysteine desulfurase IscS and scaffold protein IscU are essential for FeS cluster formation and consequently the viability of the procyclic stage of *T.*

brucei. Even though both IscS and IscU were specifically localized to the mitochondrion, their deficient expression affected the maturation of FeS proteins operating not only in the mitochondrion, but also in the cytosol. This indicates that a crucial part of FeS cluster assembly is localized to the mitochondrion of *T. brucei*. One of the major differences between the *T. brucei* of the insect vector and the stage parasitizing mammals is the way they generate energy, in particular the use of the mitochondrion, in the process. Remarkably, the overall metabolic changes observed in the FeS cluster-impaired cells resulted in a phenotype that mimics the interstagial transition of the organelle, most notably by decreased production of ATP and acetate. Based on these results we proposed that the function of FeS cluster assembly machinery is critical for the interstagial changes in the *T. brucei* life cycle.

2. Function of mitosomes in *Giardia intestinalis*

FeS cluster assembly is so far the only known function of *G. intestinalis* mitosomes. To identify other metabolic pathways, we analysed protein content of these organelles. Interestingly, only proteins involved in FeS cluster biogenesis and mitosomal protein import were found by this approach. One of the identified proteins is the monothiol glutaredoxin. We demonstrated that, same as the homologue of *S. cerevisiae* that is involved in FeS cluster transfer to apoproteins, the *G. intestinalis* glutaredoxin binds an FeS cluster and glutathione.

3. Protein import to mitosomes of *Giardia intestinalis*

When IscS, IscU, and 2Fe2S ferredoxin of *G. intestinalis* were overexpressed in *G. intestinalis* or *T. vaginalis*, they were specifically delivered into the mitosomes or into the hydrogenosomes, respectively. The delivery of the proteins into *G. intestinalis* mitosomes was mediated by two different mechanisms requiring either N-terminal targeting sequences (ferredoxin, IscU) or internal targeting sequences (IscS). The N-terminal extensions predicted in IscU and ferredoxin were found to be both necessary and sufficient for targeting to mitosomes. A homologue of mitochondrial protein import motor component was identified in the *G. intestinalis* mitosomes.

4. Protein processing in mitosomes of *Giardia intestinalis* and hydrogenosomes of *Trichomonas vaginalis*

We demonstrated that hydrogenosomal processing peptidase of *T. vaginalis*, HPP, is a heterodimeric metalloprotease composed of subunits homologous to α and β subunits of mitochondrial processing peptidase, MPP. So far uniquely among eukaryotes, mitosomal processing peptidase of *G. intestinalis*, GPP, functions as a βGPP monomer. Our phylogenetic and functional analyses show that GPP is a striking example of reductive evolution from a heterodimeric to a monomeric enzyme. The structure and negative surface charge distribution of βGPP appear to have co-evolved with the properties of mitosomal targeting sequences, which, unlike classic mitochondrial targeting signals, are short and devoid of positively-charged residues except for the arginine of the cleavage motif. The majority of hydrogenosomal presequences resemble those of mitosomes, but longer positively charged mitochondrial-type presequences were also identified, consistent with the retention of the *T. vaginalis* αHPP.

List of publications

Dolezal, P., Smid, O., Rada, P., Zubacova, Z., Bursac, D., Sutak, R., Nebesarova, J., Lithgow, T., and Tachezy, J. (2005). Giardia mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting. ***Proc. Natl. Acad. Sci. U. S. A.*** 102:10924-10929.

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Smid,O., Matuskova, A., Harris, S., Kucera, T., Novotny, M., Horvathova, L., Hrdy, I., Kutejova, E., Hirt, R.P., Embley, T.M., Janata, J., and Tachezy, J. (2008). Reductive evolution of the mitochondrial processing peptidases of unicellular parasites.

Smid, O., Sutak, R., and Tachezy, J. (2008). Monothiol glutaredoxin in the mitosomes of Giardia intestinalis. **In preparation.**

List of selected abstracts

Smid, O., Matuskova, A., Harris, S., Kucera, T., Novotny, M., Horvathova, L., Hrdy, I., Hirt, R., Embley, M., Janata, J., Tachezy, J. Reductive evolution of processing peptidases and targeting presequences in mitosomes and hydrogenosomes. *4th International Conference on Anaerobic Protists, Taoyuan (Taiwan), 2008*

Smid, O., Matuskova, M., Zubacova, Z., Harris, S., Janata, J., Tachezy, J. Characterization of the *Giardia intestinalis* mitosomal processing peptidase. *Molecular Parasitology Meeting XVIII, Woods Hole (USA), 2007*

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Smid, O., Tumova, P., Tachezy, J. The mitosome of Giardia. *Extended COST B-22 Expert Meeting, Prague (Czech Republic), 2006*

Smid, O., Vondruskova, E., Vilimova, V., Sutak, R., Lukes, J., Tachezy, J. Role of IscS in FeS cluster assembly in *Trypanosoma brucei*. *4th International Biometals Symposium, Garmisch-Partenkirchen, 2004*

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