Iron-Sulfur Proteins and Iron-Sulfur Cluster Assembly in 'Amitochondriate' Eukaryotes

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Ph.D. thesis

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2000- Postgraduate study of Biomedicine (Parasitology), Faculty of Science, Charles University, thesis:
Iron-sulfur proteins and iron-sulfur cluster assembly in 'amitochondriate' eukaryotes.

2003 Visiting student at laboratory of Andrew Dancis, MD. University of Pennsylvania, PA, USA

2001 course on Biology of Parasitism: Modern Approaches
MBL in Woods Hole, MA, USA

- 1994 – 2000: Master degree in Biology, Charles University,
thesis: *Malic enzymes in* Trichomonas vaginalis

Introduction

Mitochondrion is the name reflecting a typical morphology of an organelle that was originally observed as granules in muscle cells (*mitos*=thread+ *chondros*=grain). It is usually characterized as a double membrane-bound organelle that produces ATP by oxidative phosphorylation. The process is dependent on consumption of oxygen as the terminal electron acceptor, which is coupled with the citric acid cycle generating reducing equivalents. Additionally, the mitochondria are involved in a number of metabolic pathways such as fatty acids catabolism by β-oxidation, amino acid biosynthesis, and urea cycle. Although mitochondria are unequivocally present in all metazoa, recent interest in unicellular

eukaryotes (protists) reveal that a number of free-living as well as parasitic protists that inhabit oxygen-poor environments, do not possess mitochondria as we know them. These organisms were then called “amitochondriates”.

More recent studies of evolution and cell biology of “amitochondriate” protists, however, challenged their amitochondriate status, at least in the case of those organisms possessing double-membrane bound organelles such as hydrogen-producing hydrogenosomes and newly characterized organelles, the mitosomes. These organelles were suggested to evolve either from a common pro-mitochondrial endosymbiont, or they represent highly modified or reduced mitochondria. Although the metabolic pathways in mitochondria and hydrogenosomes are rather different, and metabolic functions of mitosomes are virtually unknown, both of these organelles possess homologous machineries required for formation of FeS clusters. This process was recognized as a novel fundamental mitochondrial function, thus providing a useful tool for comparative study of mitochondria, hydrogenosomes and mitosomes including tracing their evolutionary history.

Aims of the thesis

1) Iron-sulfur cluster assembly in trichomonads
    a) To localize and examine mechanism of iron-sulfur cluster biosynthesis in hydrogenosomes
    b) To characterize frataxin homologue in *Trichomonas vaginalis* and assess its function
    c) To investigate iron distribution in *Tritrichomonas foetus*

2) NADH dehydrogenase complex in hydrogenosomes
    a) To purify components of NADH dehydrogenase complex in *Trichomonas vaginalis*
    b) To determine its activity and reconstruct its evolutionary history

3) Protein import in mitosomes of *Giardia intestinalis*
    a) To analyze protein targeting signal of mitosomal proteins
    b) To test the ability of mitosomal proteins to translocate into hydrogenosomes
    c) To characterized components of mitosomal protein targeting pathway

Results

1) **Iron-sulfur cluster assembly in trichomonads**

We showed the hydrogenosomal localization of IscS and demonstrated that hydrogenosomes are the site of FeS cluster biosynthesis in trichomonads. Within hydrogenosomes, frataxin homologue may also participate in FeS cluster biosynthesis as showed by its heterologous expression in *Saccharomyces cerevisiae.* Hydrogenosomal ferredoxin was characterized as the major iron-binding protein. These data suggest that mitochondria and hydrogenosomes possess conserved mechanism for biogenesis of FeS proteins.

2) **NADH dehydrogenase complex in hydrogenosomes**

We purified and cloned two subunits of hydrogenosomal NADH dehydrogenase complex and showed that it can reduce a variety of electron carriers including ubiquinone, but unlike the mitochondrial enzyme it can also reduce ferredoxin. Phylogenetic analyses show that the *T. vaginalis* shares common ancestry with the mitochondrial enzyme.

3) **Protein import in mitosomes of *Giardia intestinalis***

We demonstrated that mitosomal proteins contain N-terminal and/or internal signal which ensure their organellar localization. Upon translocation into the mitosomes the N-terminal targeting sequence is removed by the activity of an organellar processing peptidase, a homologue of which is present in the mitosomes, as well. The targeting signals are inter-recognizable between hydrogenosomes and mitosomes and the importing complex employs the components that also function in mitochondrial protein import.

List of publications:

Dolezal, P., Smid, O., Rada, P., Zubacova, Z., Bursac, D. Sutak, R., Nebesarova, J., Lithgow, T., Tachezy, J. (2005). Giardia mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting. ***Proc Natl Acad Sci USA*** (accepted)

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Sutak, R., Dolezal, P., Fiumera, H.L., Hrdy, I., Dancis, A., Delgadillo-Correa, M.,Johnson, P.J., Miller, M., Tachezy, J. (2004). Mitochondrial-type assembly of FeS centers in the hydrogenosomes of the amitochondriate eukaryote *Trichomonas vaginalis*. ***Proc Natl Acad Sci USA***. 200, 101:10368-73

Dolezal, P., Vanacova, S., Tachezy, J., Hrdy, I.(2004). Malic enzymes of *Trichomonas vaginalis*: two enzyme families, two distinct origins. ***Gene****,* 329:81-92

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Djikeng, A., Ferreira, L., D'Angelo, M., Dolezal, P., Lamb, T., Murta, S., Triggs, V., Albert, S., Villarino, A., Renzi, S., Ullu, E., Tschudi, C. (2001). Characterization of a candidate Trypanosoma brucei U1 small nuclear RNA gene. ***Mol Biochem Parasitol***, 113:109-15

Dolezal,P., Dancis,A., Lesuisse,E., Embley,T.M., Tachezy,J. (2005). Functional frataxin in hydrogenosomes of *Trichomonas vaginalis.* **in preparation**.

List of selected abstracts

Dolezal, P., Smid, O., Rada, P., Zubacova, Z., Tachezy, J. Targeting of *Giardia intestinalis* proteins involved in FeS cluster assembly into mitosomes and trichomonad hydrogenosomes. *Molecular Parasitology Meeting XV,2004 Woods Hole*
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Dolezal, P, Dancis, A., Embley, M., Tachezy. Funtional frataxin homologue in hydrogenosomes of *Trichomonas vaginalis.. Molecular Parasitology Meeting XV,2004 Woods Hole*

Dolezal, P., Smid, O., Rada, P., Zubacova, Z, Sutak, R. Tachezy, J. Iron-sulfur cluster assembly in amitochondrial protists. *4th International Biometals, Symposium 2004, Garmisch, Germany*

Dolezal, P., Dancis, A., Embley, M., Tachezy. Frataxin homologue in *Trichomonad vaginalis.. Bioiron Meeting, NIH, Bethesda 2003*

Dolezal, P. Proost, P., Tachezy, J., Hrdy, I. Malic enzymes of *Trichomonas vaginalis*: two

enzyme families, two distinct origins. *Meeting of the International Society for Evolutionary Protistology XIV, 2002 Vancouver*

Dolezal, P. Tachezy, J., Proost, P., Hrdy, I. Malic enzyme of *Trichomonas vaginalis*: purification, function and phylogeny. *Anaerobic Protozoan Parasites: From Basic Science to Drug Targets, COST-B9 Meeting, 2001, Prague*.

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The thesis will be defended on friday, **22nd July, 2005, 10:00** at the Department of Parasitology, Charles University, Viničná 7, Prague 2, Czech Republic.