Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

STATEMENT OF THE DIPLOMA THESIS REVIEWER

Name of the student: Alexander Ch. Heindrich

Thesis title: Late steps in the cytosolic iron-sulfur cluster assembly in Trypanosoma brucei

Supervisor: Priscila Pena Diaz

Reviewer: Ondřej Gahura
Reviewer` affiliation: Institute of Parasitology, Biology Centre CAS, Ceske Budejovice

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced extents of the thesis divisions** (recommended extent of the theoretical part is max. 1/3 of the total extent), **logical structure of the thesis**	0-3	2,5
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	2,5
Adequacy and clarity of the results and conclusions	0-3	2,5
Quality of the annotation	0-3	0,5
Language and stylistics, complying with the valid terminology	0-3	2,5
Accuracy and completeness of figures/tables legends (clarity even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	2,5
Formal requirements – points in total		19
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	2,5
Discussion quality – interpretation of results and their discussion with the literature	0-3	2
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	3
Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2,5

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	3
Formal requirements – points in total		25
POINTS IN TOTAL (MAX/AWARDED)	51	44

My overall impression of the thesis is largely positive. Above all I appreciate the diversity of techniques learned during the work and the effort to discuss the data and put them into the context, in spite of the fact the some observations are somewhat ambiguous.

Suggestions and questions, to which the student has to answer during the defense:

Which constituents of the Fe-S cluster assembly pathway (both in mitochondria and cytosol) are Fe-S cluster proteins themselves? How do you explain that a pathway responsible for adding a cofactor involves the cofactor containing components?

Based on the Table 1 and its footnote – how many Cia2/FAM96 genes had the common ancestor of trypanosomes and humans? Could you describe potential evolution of the Cia2 gene(s)?

Have you considered using two different tags to simultaneously label two proteins and look at their colocalization?

The weakest part of the experimental section is the confocal microscopy of NP-40 treated cells. I have several questions and comments:

1) The principle and rationale of the experiment is not described sufficiently. The technique is not widely used and should be explained. Why do you think Cia2B could interact with DNA?
2) What is the expected effect of the NP-40 treatment on the cells? Individual cells seem to be differentially affected, as revealed by DIC microscopy (Fig. 12).
3) Why did you use anti-TbLa Ab? Is TbLa DNA binding protein or not? This information is missing while it is essential to interpret the data. In addition, TbLa seems to be detected also outside the nucleus (Fig. 11, lower two rows).
4) In Fig. 11 there is hardly any TbCia2B (or any other v5-tagged protein) observed in nucleus, which is in disagreement with Fig. 10. Therefore, the statement that "the signal in the nucleus is weakened" upon NP-40 treatment is wrong.
5) Can you comment on the speckled signal released from the cells after NP-40 treatment? Has this been observed with any other protein?

Is there any other marker apart from the aconitase that is used to assay the functioning of the Fe-S assembly pathway in other models? Are the Fe-S apoproteins stable or not? You mention that KD of TbCfd1 or TbNbp35 results in accumulation of non-thiolated tRNA in cytosol (1.2.4). Why? Could this be used as an indication of impaired CIA pathway?

One of the aims of the thesis was to identify interacting partners and substrates of late CIA components. Even though the MS data were probably not available at the time of submission, the MS analysis (method of detection etc.) and proposed data analysis workflow should have been described to show this part of the project was properly designed.

You mentioned that some bands on the SDS-PAGE from the coIP experiments are non-specific and the signal will be filtered from the MS data. How will you do that?

Did you have chance to do the coIP more than once? If yes, was the band pattern on the Silver-stain gel reproducible?

Giardia intestinalis, an excavate has reduced mitochondrial enzymatic apparatus and therefore needs less mitochondrial Fe-S proteins, but still depends on mitosomal Fe-S pathway. Is there any knowledge on CIA pathway in this (or related) organisms, and are there any target proteins known? Is it reasonable to assume that such proteins are potentially target candidates in trypanosomes?

Are the scaffold proteins, the first recipients of Fe-S cofactors, in the cytosolic CIA and mitochondrial/bacterial ISC pathways homologs? Could any of the scaffold proteins be considered as the ancestral "first" Fe-S protein?

What do you conclude from the fact, that N-terminal KRKR motif in Cia2B is not present in closely related species?

You suggest potential existence of a parallel cytosolic pathway for aconitase maturation. What are the main observations that lead to this hypothesis?

Eventual mistakes, which the students should avoid in the future:

- In several cases the verb "suggest" should be used instead of "confirm" to avoid over-interpretation of some experiments. Be careful about too strong conclusions.
- List of abbreviations should either contain all species names or none (not only *T. brucei*, but also *S. cerevisiae* etc.). Specifically, I miss the full name of bacteria *A. vinelandii* mentioned in the section 1.2.1.
- Writing of scientific name should be consistent. Latin scientific names should be capitalized from the higher taxa to the level of genus. Genus and species names are italicized. In contrast, Anglicized names should be written in the regular lower case font (for example "Kinetoplastida" vs. kinetoplastids, "Excavata" vs excavates, "*Trypanosoma brucei*" vs "trypanosome" etc.)

Eventual additional comments of the reviewer on the student and the thesis:

Conclusion:
In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade 1.

In **České Budějovice** date 14.1.2016

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signature

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University of South Bohemia in České Budějovice

STATEMENT OF THE DIPLOMA* THESIS REVIEWER

Name of the student: Haindrich Alexander Christoph

Thesis title: Late Steps in the cytosolic Iron-Sulfur Cluster Assembly in *Trypanosoma brucei*

Supervisor: Priscila Peña Diaz, PhD.

Reviewer: RNDr. Zdeněk Verner, Ph.D.

Reviewer`s affiliation: Dept. Parasitology, Faculty of Science, Charles University in Prague

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	2
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	3
Formal requirements – points in total		20
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	3
Discussion quality – interpretation of results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	3
Logic in the course of the experimental work	0-3	3
Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2

* Choose one

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Quality of experimental data presentation	0-3	3
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		23
POINTS IN TOTAL (MAX/AWARDED)	48	43

Suggestions and questions, to which the student has to answer during the defense:
See Supplementary material S1.

Eventual mistakes, which the students should avoid in the future:
See Supplementary material S1.

Eventual additional comments of the reviewer on the student and the thesis:
See Supplementary material S1.

Conclusion:

In conclusion, I

recommend

the thesis for the defense and I suggest the grade A .[2]

In **Prague** date **15. 1. 2016**

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signature

[2] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted.

Supplementary Material S1

RNDr. Zdeněk Verner, Ph.D.

Review of master thesis „Late Steps in the cytosolic Iron-Sulfur Cluster Assembly in *Trypanosoma brucei*" by Alexander Christoph Haindrich

The master thesis by Alex Haindrich is by far the best piece of work I have had a privilege to asses so far. This is a result of both Alex's careful attitude towards laboratory work; and also his supervisor's demands where less than best result is not found as satisfactory.

I very much appreciated the introductory note that summarizes all proteins that were described in the thesis showing trypanosomal, yeast as well as human homologues. This is especially helpful as human and yeast biologists tend to name the respective homologous proteins differently w/o thinking about people out of their respective fields. I recommend such table to every student dealing with more than one organism.

Formally, the thesis is very well written with only several minor typos. From time to time I had to re-read a sentence as they occasionally had a different structure than I am used to; this is further stressed by usage of punctuation that differs from both the Czech and English one – as far as I can asses it. I believe this stems from the native language of the author and will be polished during his follow-up career.

From the scientific point of view, the thesis presents and carefully interprets data of high quality. The author familiarized himself with methods ranging from a simple cultivation through molecular cloning and fluorescent microscopy to advanced protein isolation. In the section Material and Methods I finally found the first remark that I could start nagging him. I would hesitate to call the second soluble fraction in crude cellular fractionation (3.4) mitochondrial. Yes, the fraction contains mitochondria; however, other organelles are most likely present as well, e. g. nuclei or glycosomes, hence I would called it granular or organellar instead of mitochondrial.

As I mentioned above, the thesis is very good and I believe the author deserves the title MSc. Regarding the grade, I would like to decide upon author's answers to following questions and comments; so LET MORTAL COMBAT BEGINS!

In the introductory section, pg. 4, you mention "...Additionally eukaryotes obtain a third Fe-S cluster assembly machinery, which is ..." The verb "to obtain" implies that a proto-eukaryotic organism did not posses CIA pathway. Is that what author meant to say? What are possible scenarios of a general Fe-S cluster assembly evolution given the facts mentioned on pg. 2, in brief that Fe-S clusters are given credit for evolution of RNA world? Also later in the text, pg. 7, you state that CIA pathway was most likely present in LECA.

Figure 4, pg. 10, shows different sub-complexes of the late acting CIA components. MMS19 is shown to be involved in maturation of dihydropyrimidine dehydrogenase, ABCE1, XPD and POLD1. There is no interaction with IRP1, yet three pages later you mention that downregulation of trypanosomal homologue of MMS19 shows no influence on c-aconitase activity. What is your interpretation of this result? What would it have meant if you have seen an effect on c-aconitase? Also, could you propose experiments to test effect on at least some of the proteins mentioned on Fig. 4, e. g. POLD1?

Next, in result section, pg. 26, you correctly summarize levels of expression of the tagged proteins. What I miss here is a comparison with available SILAC and other proteomic data. If it is possible, could you please compare your results with data obtained by Butter et al. 2013, Gunasekera et al. 2012 or Urbaniak et al. 2012?

On pg. 32 you present a digitonin permeabilization of procyclic *T. brucei* with markers of cytosol, nucleus, mitochondrial intermembrane space and mitochondrial matrix. Apart from missing a marker for other organelles, could you please compare a sterol composition of nuclear envelope with that of plasma membrane, mitochondrial outer and inner membranes? Has digitonin permeabilization been used for nuclear localization before?

On the following page, you state that "...The crude cellular fractionation could be optimized to separate cytosolic from the other fractions including nucleus, ..." Now if it **could be done**, why was it not performed? Or why an existing protocol for isolation of nuclei was not used?

Next, on the same page you discuss a possibility that KRKR motif could be trypanosomal signal for nuclear import. Could you propose an experiment to test this?

On pg. 36 you speculate that one of the reason as why you did not observe any effect on c-aconitase in the double knock-downs is lower efficiency of RNAi. An apparent question here is: was this evaluated using qRT-PCR or Norther blot?

On the following page, you speculate that *T. brucei* might posses another machinery for Fe-S cluster export from mitochondrion that activates as a result of downregulation of TbCia2B. Do you think a comparative proteomic analysis of parental and RNAi-induced strains could provide hints in identification of this putative machinery?

At the very end of discussion, you hesitate to depict a model on how downregulation of TbCia2A and B could result in upregulation of mitochondrial aconitase activity. Given all your data, could you please propose a model here?

Thank you for your cooperation.

In Prague 15/01/16