Evaluation of the MSc thesis of Piay Chungmai entitled "Functional analysis of Iron-Sulfur cluster assembly protein Isd11 in procyclic and bloodstream *Trypanosoma brucei*"

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General comments

The first part of MSc thesis of Piay Changmai consists of 8 pages comprising Introduction, Conclusions and Literature. Further, the manuscript called "Isd11 but not Mtu1 is essential for tRNA thiolation in the excavate protist *Trypanosoma brucei*" is attached (21 pages).

The Introduction part is very well written and introduces reader into the topic. The author in details describes the pathway of Fe-S clusters biogenesis both in the yeast *S. cerevisiae* and humans. Three figures are included the introduction, all are (only) pasted from various publications. I am missing detail legends under these pictures. I would also appreciate a list of Abbreviations, which could help reader to orientate in the text.

The Conclusion part summarizes author's results, which are mostly described and discussed in the attached manuscript. The first part of thesis ends with a detailed list of literature used in the Introduction.

However, the whole work contains an unbelievable number of mistakes in all sections, including the manuscript. The author has a problem with "spaces" between letters in the individual words. I do not understand how this could happen, so maybe Piay has some explanation.

The usual consensus of MSc thesis at our faculty is to divide the text into Introduction, Methods, Results, Discussion, and Literature. Obviously, Methods and Results are not present in this thesis. Both, in the Introduction and in the Manuscript, respectively, author say, that many "data are not shown". I think that they should be shown, e.g. in the Results. In the Abstract, the last sentence says that Piay got "interesting data", but these are again not shown. May it be possible to see some of these data at least during the defense presentation?

The second part of Piya´s work comprises manuscript "Isd11 but not Mtu1 is essential for tRNA thiolation in the excavate protist *Trypanosoma brucei*" by Paris, Changmai, Rubio, Zikova, Alfonzo and Lukes. Although the manuscript is still not ready for submission and some experiments still need to be finished I can say, that it is a nice piece of work, the text is understandable, experiments are well designed and done, documented and finally discussed. Nevertheless, there are also many typing mistakes and the literature summary is also not unified into one style.

Pia Changmai claims in the Paper's preface that he contributed substantially to the results. He measured the growth curve of procyclic and bloodstream forms after Isc11 KD (although the curve for the bloodstream form is again "not shown" in the work), measured mitochondrial potential before and after KD, prepared and electroporated RNAi constructs into *T. brucei* (not described and results not shown). He also "determined the level of iron sulfur cluster proteins (which are these??) using Western blot (this I did not find in the work; could you explain it?). Finally, Piya prepared publication-quality pictures.

I can summarize that MSc work of Piya Changmai is a work of big scientific interest and shed some light on the function of Isc11, ISC assembly and tRNA thiolation in trypanosomes. Nevertheless, the work contains many typing mistakes and does not include all data that should be shown. It is definitely not usual for MSc students to have almost ready

manuscript attached in their thesis so I think that this is something exceptional and I really appreciate it. It is obvious that if the author spent some more time over the thesis it might became to be something really exceptional.

Finally, I can recommend this thesis for MSc defense.

Questions to the author

1) How the author explains the observation that bloodstream forms of trypanosomes do not need Isd11? Does it mean that they don't need FE-S cluster proteins or that Ids11-3 might be involved?

2) Could be the decrease in Nfs2 and IscU levels explained by their decreases in the gene expressions after Isd11 KD (Northern blot of these genes after Isc11 KD)? Is the KD really only Isd11 specific?

3) Fig. 4A - how could you exactly measure the percentage level of -S^2U decrease?

4) Is it correct to name Isd11 KD non-growing culture of procyclic *T. brucei* as a lethal phenotype?

5) Manuscript, page 1 - Are you sure that really all prokaryotic organisms contain and need FE-S cluster proteins?

In Ceske Budejovice
May, 27, 2009

RNDr. Ondrej Hajdusek, Ph.D.

In Prague 26th of May, 2009

The review of the Master thesis of Bsc. Piyi Changmai: „ Functional Analysis of the Fe-S cluster assembly protein Isd11 in procyclic and bloodstream *Trypanosoma brucei.*"

The Master thesis of Mr. Piya Changmai has two separate parts: First, a review written by Mr. Changmai on the current state of knowledge on the formation of iron-sulfur (Fe/S) protein biogenesis in eukaryotes. Second, an unpublished manuscript of new experimental work by six co-workers with Mr. Changmai as a second author. These two parts are evaluated separately and the summarizing report on the thesis follows.

The review is brief and well written and contains all essential information, which should be mentioned in such text. The writing is read nicely and it is obvious that Mr. Changmai understands the topic well. This is especially important as the subject of Fe/S cluster formation includes combination of inorganic chemistry, biochemistry and cell biology. Moreover, the author nicely keeps the balance between going into exhausting details and too much of simplification. Several negative features however appeared in this otherwise nice piece. The protein nomenclature is confusing in some places as the yeast, bacterial and mammalian names of the proteins seem to be mixed (IscU x Isu1, IscS x Nfs, Yfh1 x frataxin). This comes mainly from the fact that experimental data come from these different cellular systems but should be unified for the sake of this review. After the Introduction section of the review, Conclusions part begins (page 6). This is a summary of the following manuscript and as such it may appear bit confusing to the reader. I would welcome a short section on the experimental aims of the work shown later, which would put the trypanosome biology in the context of the general information. This would help the reader to jump from the introduction into the specific conclusions. References should also be ordered in better way, alphabetically or numbered.

The manuscript by Paris et al. describes function of two proteins Isd11 and Mtu1 involved in the sulfur metabolism in *Trypanosoma brucei.* While Isd11 is known as a partner of IscS, which is dominant cystein desulfurase providing sulfur either during Fe/S cluster biosynthesis or for thiolation of some tRNAs, Mtu1 is specialized mitochondrial thiourydilase itself. Based on the experiments presented, authors of the manuscript formulate a hypothesis that also in *T. brucei* Isd11 mediates both functions when in complex with IscS. However, Mtu1 homologue doesnt seem to participate in tRNA thiolation. One line of the experiments is very clear, Isd11 is localized to mitochondrial fraction of the cell lysate and pull down of the TAP-tagged protein co-precipitates anticipated protein partners, IscS and IscU. Moreover, silencing of Isd11 has a profound effect on cell viability and is responsible for downregulation of the

interacting partners, IscS and IscU. Such phenotype is demonstrated as decrease in Fe/S cluster biosynthesis as determined by the activity of Fe/S proteins and also as reduced thiolation of two tRNAs. On the other hand, the silencing of Mtu1 is without a detectable phenotype and the protein of unknown localization due to the lack of specific antibody.

In the foreword to the thesis Mr. Changmai specifies his contribution to the manuscript production. It is a substantial part and requires great understanding in molecular and cell biology. The manuscript is clear and well written. Few parts need some clarifications or removal of repetitive statements (indications of all corrections will be sent to Mr. Changmai by mail). Figures are clear and well organized only with few typos. As a whole, the manuscript represent a piece of work of high scientific quality and should be published sooner or later in a good journal. I, however, feel (see bellow question number 3) that additional data will have to be shown in order to demonstrate direct role of Isd11 both sulfur-metabolizing processes in *T. brucei.*

To conclude, I believe that Mr. Changmai has proven here that he is a smart young man with great experimental skills and his thesis should be marked with the best mark. The questions for the discussion follow:

Specific questions:

1. Given that most eukaryotes contain just single Hsp70 in their mitochondria, what are the functions of the three distinct Hsp70 orthologues present in *S. cerevisiae* mitochondria? Can they individually substitute (for instance when overexressed) the other Hsp70s?
2. Is there a functional explanation for tight interaction between Isd11, IscS and IscU with no frataxin present in the complex? Why do you think frataxin levels do not follow the decrease in Isd11?
3. Concerning the essential role of IscS plus the crucial character of the interaction of Isd11 and IscS, how can you distinguish between direct or indirect effect of Isd11 on both Fe/S cluster formationa and tRNA thiolation? Could it be just the decrease in IscS (and IscU), which is responsible for all phenotypes?
4. Can you distinguish whether tRNAs in your assay get thiolated in the cytosol or in the mitochondria?

Pavel Doležal, PhD.