Evaluation of the Master Thesis by Bc. Michaela Kunzová

Bc. Michaela Kunzová submitted a thesis entitled **"Differential gene expression as a tool to sort individual life stages of a mammalian parasite *Trypanosoma brucei*"**. The thesis is written in clear scientific English, extends 53 pages and follows the common structure required by the Faculty of Science of the University of the South Bohemia in České Budějovice. The introduction is brief but it goes directly to the point and includes all relevant information for the thesis aims and results. The aims of the research are clear and of importance in the field. The methodology is disclosed in sufficient detail and I appreciate also short descriptions of method principles and very nice illustrations (maps) of constructed plasmids. The results and discussion are well-written as far as it concerns both their size and content, and accompanied with relevant figures.

As a reviewer, I was impressed by the way the thesis is written and the level of English. I highly appreciate and respect the courage of the author to pioneer a system of sorting individual life cycle stages of the *T. brucei in vitro* based on regulatable fluorescent protein expression. Although an establishment of such a platform is rather challenging and demanding, the author succeeded in this task. Moreover, the system offers a great research potential and I believe (hope) that the established technique will become a standard method in AZ lab. In my opinion, there is no doubt about Michaela scientific expertise and I would like to encourage her to continue in doctoral study.

In the thesis, I have found just two minor weak points. First, I would appreciate the list of abbreviation, it would highly facilitate the orientation in the text. Second, it was quite difficult to suddenly switch from the generation of fluorescent protein expression system to TbIF1 double knock-out (chapter 4.5). Since it is the additional chapter, I would appreciate more introduction into the problematics and I believe there are incorrect numbers of figures in the text.

Despite these minor comments, I am fully convinced that submitted master thesis by Michaela Kunzová fulfills the criteria required by the Faculty of Science of the University of the South Bohemia in České Budějovice for the Master degree graduation and I recommend it for defense with evaluation 1 (excellent).

České Budějovice, May 15, 2018

Marie Jalovecká

Questions:

1. The author mentioned that *Trypanosoma brucei* can invade the central nervous system. Could the author briefly discuss how the invasion into central nervous system is mediated?

2. Do the media (used for parasites cultivation) contain any supplements like antibiotics or antimycotics which are commonly used for *in vitro* cultivation of parasites or mammalian cells? Does the cultivation of parasite procyclic form require the higher percentage of CO_2 in the atmosphere like it is common for blood forms of these parasites?

3. As author described in the thesis, the RBP6 overexpression was triggered by adding of tetracycline (1-10μg/ml) into the medium. However, recent publication (Moullan et al. 2016, PMC4565776) reported that tetracycline can cause an oxidative stress and lead to changes in nuclear gene expression in many model organisms like worms, flies, mice or plants. What is the author opinion on the possibility of the same phenomenon in the *Trypanosoma* platform of Tet-controlled transcription (by Tet-On and Tet-Off system)?

4. As author claimed in the thesis (and as is apparent from Figures 12 and 13), the fluorescence signal was not stronger in cells with localization of fluorescent protein to nucleus (NLS), although it was expected. Does the author have any explanation for it?

5. From the histogram of PF_NLS_tdTomato_Act (Figure 12) it seems that this cell line does not show any fluorescent signal (no or very minor shift compared to the wt line) but in PF_tdTomato_Act cell line the clear fluorescence signal (shift) can be detected. Based on histogram (Figure 12), I would expect no fluorescence of the PF_NLS_tdTomato_Act line also in microscopy result (Figure 13). However, in the Figure 13 the signal from PF_NLS_tdTomato_Act line appears the same (or even stronger) compared to PF_tdTomato_Act line (although the signal is generally very weak which was presumably caused by fixing solution as the author explained). Could the author explain this discrepancy?

6. The author developed the system to sort individual life cycle stages of *T. brucei in vitro* based on expression of developmentally regulatable fluorescent protein. What is the author opinion on the function of the same platform in *in vivo* model of this parasite? What would be the limitations of this system *in vivo*?

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Würzburg, 18.05.2018

Review of the master thesis of Bc. Michaela Kunzová, "**Differential gene expression as a tool to sort individual life stages of a mammalian parasite *Trypanosoma brucei*"**

Trypanosoma brucei is a unicellular, eukaryotic parasite, which is responsible for African sleeping sickness in humans and *Nagana* in livestock in sub-Saharan Africa. A substantial part of biomedical research about trypanosomes is based on the urgent need to develop new drugs. However, *T. brucei* is also a well-established model system to study fundamental biological processes such as transcriptional regulation, DNA repair, membrane biology, cell cycle regulation and cell differentiation. In the past 10 years the field witnessed an enormous increase of systemic information due to several large genomic, transcriptomic and proteomic studies. However, it needs sophisticated tools to put this information into a cell biological context to fully understand this parasite.
The aim of Ms. Kunzová master thesis was to develop such a tool to better understand developmental biology processes of *T. brucei*. Using information from high-throughput experiments, which described differential gene expression during developmental differentiation of trypanosomes, Ms Kunzová wanted to generate a reporter cell line that expresses GFP under the control of UTRs that mediate stage-specific gene expression. Anticipated expression can be verified *in vitro* with an optimized RBP6-driven inducible differentiation system. This reporter cell line could then be used to isolate specific life cycle stages for further analysis. This is a very elegant approach, which is exactly what is required in a "post-genome" era to translate information generated by high-throughput experiments to cell biology.

The introduction of this thesis, which prepares the reader well to the topic of this work, is well researched and demonstrates that Ms Kunzová gained substantial insights into the theoretical background of the project. The references are well chosen and sufficiently balanced between "classical" milestone literature (eg. Vickerman, 1988 or Crowe, 1983) and very recent publications. However, sometimes references are missing (eg. phylogenetic position of trypanosomes) and the overall extent of the references is rather average for a master thesis (eg. changes in mitochondrion structure and function during development). A few times, scientific terminology is not used correctly. For example, Ms. Kunzová tries to explore her

hypothesis by "tagging a fluorescence protein with 3'UTRs of a stage specific gene..." (page 7). It is not impossible to tag a protein with nucleic acids but I guess the experimental design of this thesis was different. Overall the introduction, which is written in fluent English with very few mistakes, is remarkably concise and fully sufficient for the topic of this thesis.
The methods section is very detailed, precise and well organized. The figures support the information about the methods well and are all annotated correctly. However, there is no information about puffers, media or most of the other materials used in this study.
The results part is also well organized and the graphic layout of this paragraph is very good. It is not entirely clear to me who generated the data and figures 8 – 11. If Ms Kunzová did differentiation, sample preparation and analysis of the data it is missing in the methods section. If someone else did this it should be explained more clearly in the text. The FACS analyses are quite nice and demonstrate that the concept of this project is working. NeonGreen is detectable in the nucleus and regulated by 15610 and RBP10 UTRs as expected from the mass spec data. I assume the 15610-based cell line is supposed to be a negative control. This is a very good outcome of this part of the thesis. The figure numbering doesn't always correspond with the text. Y-axis in figure 14 is not labeled and it would have been nice to have error-bars in this figure. Finally, the reporter cells were subjected to cell sorting with a flow cytometer, which seemed to have work according to the text of the result section. A post-sort analysis to verify the sorting process would have been nice.
In summary, the original idea to establish a reporter cell line for stage-specific enrichment of cells by FACS was executed at least as a proof of principle with one cell line.
All of the open questions of the result section are discussed in details in the last part of this thesis. Adequate experiments to use and to improve the reporter system are suggested and the results are discussed in the context of the current literature.

This work established the basis for a very useful tool to investigate several aspects of trypanosome cell biology and I clearly recommend the thesis for a defense.

Sincerely,

Christian Janzen

Přírodovědecká fakulta
Faculty of Science

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

STATEMENT OF THE BACHELOR/DIPLOMA* THESIS REVIEWER

Name of the student: Michaela Kunzová

Thesis title: Differential gene expression as a tool to sort individual life stages of a mammalian parasite *Trypanosoma brucei*
Supervisor: Alena Zikova

Reviewer: Christian Janzen
Reviewer` affiliation: Biocenter, University Würzburg, Germany

	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	3
quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	2
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
Adequacy and clarity of the results and conclusions	0-3	2
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 even without reading the rest of the text, explanation of the symbols and labeling, indicating the units)	0-3	2
Formal requirements – points in total		20
(2) PRACTICAL REQUIREMENTS		
Clarity of the aims	0-3	3
Fulfillment of the aims	0-3	3
Discussion quality – interpretation of results and their discussion with the literature	0-3	3
Logic in the course of the experimental work	0-3	2
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
Formal requirements – points in total		23
POINTS IN TOTAL (MAX/AWARDED)	**51**	**43**

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

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

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

See attached review

Conclusion:

In conclusion, I

r e c o m m e n d

the thesis for the defense and I suggest the grade

In date **21.05.2018**

.....

signature

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Würzburg, 24.05.2018

Master thesis of Bc. Michaela Kunzová, "**Differential gene expression as a tool to sort individual life stages of a mammalian parasite *Trypanosoma brucei***"

To whom it may concern

With this letter, I confirm that Ms Michaela Kunzová answered all of my questions correctly and in a satisfying way.

Sincerely,

Christian Janzen