

Posudok bakalárskej práce Tomáša Knoblocha

In his BSc. thesis, Tomáš Knobloch studies the localisation of the Tb927.11.1290 protein in the procyclic form of *Trypanosoma brucei*, and attempts to shed more light on its function.

The thesis is written in English, the overall level is good with only minor mistakes (using decimal comma instead of decimal point, missing definite and indefinite articles, very few typos and missing words and some not so elegant expressions). Tomáš references 16 relevant original manuscripts, 1 thesis and 12 web databases and other websites. The thesis satisfies the formal criteria. It contains the chapters it should contain, information is correctly referenced, including the figures. Figures are generally well designed with clear and easy-to-understand legends.

The first half of the introduction nicely describes *Trypanosoma brucei* and its medical and economical impact, and the available bioinformatics databases. Author clearly describes the problem with N-tagging and mitochondrial localisation in the TrypTag database, however, it is not clear whether they decided to study it just as a technical problem (the effect of the tag on mitochondrial localisation signal), or whether it has some broader biological relevance (for example for *Trypanosoma* biology). In the second half of the introduction author writes about the methods used in his project, either describing the method itself or providing details specific for his experiments, such as the percentage of SDS-PAGE gel) – this in my opinion belongs rather to the methods or results section, and author could for example describe and discuss mitochondrial localisation signal, different ways of tagging proteins for their visualisation, or provide more details about the N-tagging vs mitochondrial localisation phenomenon instead.

The aims are clear and fulfilled by the presented results. The methods section is clear and well written, and the results are mostly well presented and described, with appropriate figures. Author showed he gained expertise in performing a number of laboratory techniques, including gene cloning, immunofluorescence staining and microscopy, crude cellular fractionation, western blot, PCR, gel electrophoresis and RNAi – more than sufficient for Bachelor's thesis. However, author should probably put more effort into the discussion section – he discusses the results to some extent, but some key results are not discussed – for example, why there is a difference in the localisation of protein of interest between immunofluorescence staining, crude cellular fractionation, and the method used by the TrypTag database. Author also suggests it is possible that the protein of interest is mitochondrial, but does not discuss how it is possible that the mitochondrial localisation signal is not disturbed by the N-tagging in the TrypTag database. In addition, the last paragraph only very briefly mentions how these results could be followed up and improved.

I have few comments regarding the formal side of the thesis pointing out issues that could be improved:

1. Latin names of organisms are not always written in italics
2. In the thesis aims, it is written „The aim is to localise gene XXX..“ – but it was the protein that was localised, not gene.
3. Methods section 3.2.2 says „the molecular weight of DNA fragments was verified by gel electrophoresis“ – author probably meant the length of the fragments, not the molecular weights

4. In results section 4.2, it is written that the program Phobius predicted there is a probability of a transmembrane domain. It does not say how big the probability is.
5. Figure 9 does not have a description or units for x axis.
6. Results section 4.4 (before the start of 4.4.1) does not contain any text, just a figure (number 15) with a legend. The figure 15 is not referenced anywhere in the text of the thesis. It would be good to have this figure introduced in the main text.

Questions for the author regarding the information that should be in the thesis, but it is not there:

1. Why you aimed to use smaller tag than the one used in the TrypTag database? Is that one big? How smaller is the V5 tag you used compared to the tag in TrypTag database? How does V5 tag look like? Why did you choose V5 tag and not some other tag? (for example HA tag).
2. Is anything published about N-terminal tagging of mitochondrial proteins, did it work or not work for someone in the past?
3. Did you test the localisation of your protein of interest just as a technical problem, to observe the effect of the tag on hypothetical mitochondrial localisation signal and localisation itself, or because of some broader biological relevance (for example for *Trypanosoma* biology) for which you wanted to determine the precise localisation within the cell?
4. Why did you choose protein Tb927.11.1290? Was it a random choice from all the proteins with suggested mitochondrial localisation?
5. What was the reasoning behind the bioinformatic analyses (results section 4.2)? Did you have a hypothesis that the protein of the interest might have a transmembrane domain, which would help to explain the discrepancy between mitochondrial localisation and N-tagging?
6. Do you have an explanation or hypothesis why the results of the localisation differed between immunofluorescence and crude cellular fractionation, and between the TrypTag database results?
7. Can the protein of interest be localised to some other organelles instead of mitochondria, considering the fact it was found to be within the „organellar fraction“ after crude cellular fractionation?
8. Can the protein of interest be mitochondrial or not, in your opinion? Can the N-terminal tagging work for mitochondrial proteins?
9. Why do you conclude that that the protein of interest might be essential for the parasite based on the result that RNAi of C-terminally tagged protein failed to reduce the amount of the protein?
10. Do you have any suggestions how to continue with the project except the overexpression of the protein?

Other questions:

1. Is it possible to look for any other phenotype apart from the growth curve after RNAi?

I recommend the thesis for defence with grade 2.

In České Budějovice, 9.1.2019

A handwritten signature in blue ink, consisting of a stylized 'G' followed by a horizontal line.

Mgr. Lenka Gahurová, Ph.D.