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Review – Michaela Veselikova- Master thesis

Michaela Veselikova in her Master thesis performed an extensive analysis of a putative MTase with the focus on its fundamental properties in *Trypanosoma brucei* which are not determined yet. In order to uncover the MT420 characteristic she made two different complicated constructs. To specify the intracellular localization she analysed a signal sequence using different software tools which shows a high probability that MT420 might be targeted to the mitochondrion. This result was confirmed with other approaches as by an immunofluorescence assay and sub-cellular fractionation made by digitonin. Furthermore, she studied by the tandem affinity purification and glycerol gradient purification following by LC-MS/MS analysis if *T. brucei* MT420 interacts with any other proteins. In addition, she showed that MT420 is probably not essential for the *T. brucei* growth. However, she was not able to purify the native protein for further *in- vitro* experiments.

Master thesis of Michaela Veselikova is very well written with very good english and large amount of data. She shows excellent abilities and skills in many molecular biology methods and a deep literary review. It seems the science is challenging her. I fully recommend the thesis to be accepted. Despite of a high quality I have a few comments and questions to her. Concerning comments , there are some missing references (page 3, 4, figure 5 is not explained in text at all), worse quality of some pictures and non-uniform names of constructs, which could be confusing.

Questions:

1. Did you try a longer measurement of the cell growth in RNAi silenced cells. There are some cases in literature which show the depletion after 7 days.

2. Did you test the growth of wild-type cells in the presence of tetracycline as a control?

3. Did you consider to ask some company produce the specific antibody for MT420?

4. Did you make a picture of wild-type 29-13 visualised by fluorescence microscopy using anti-c-myc antibody to see the background?

5. How you can explain that according to the annotation MT420 might be around 47kDa (table 4.) and in Fig. 17 the size of endogenous protein is more than 55 kDa?

6. You said on pade 33 that in the band 2 you did not reveal any significant match, not even your Tb10.6k5.0440 which might be present in all. How to explain?

7. Are you planning to do some of these experiments with bloodstream forms?

Eva Chocholova

Comments on 'Characterization of a putative methylttransferase MT420 in *Trypanosoma brucei*'

1. The mitochondrial localization of the MT420-TAP fusion protein by Immunofluorescence looks very nice. Was MT420 found in the mitochondrial proteome by Panigrahi *et al.*, 2009? If not, how could the absence be explained? How would one demonstrate that the putative amino-terminal leader sequence on MT420 is necessary and sufficient for mitochondrial import? Does the yeast homolog Mtq1 have a predicted import-signal leader? If so, can the alignment (Fig 26) be improved at NH terminus?

2. Can the putative substrate release factor Mrf1 be identified in *T. brucei* by bioinformatics? Assume that you have developed an *in vitro* assay for methylation of Mfr1 by MT420, how would you determine theoretically the site of methylation in Mtrf1?

3. On page 9, it is stated that VP39 is the first cap MTase to be studied. In the interest of accuracy it should be stated that VP39 is the first "2'-*O*-ribose methyltransferase". This distinguishes it from the m^7G "base" MTase.