Max-Planck-Institut für molekulare Physiologie

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Evaluation of the Master thesis entitled "Expression system in Leishmania tarentolae"

Submitted by
of Zdenek Paris, BSc form the Faculty of Biological Sciences
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The thesis work describes a series of experiments on a *Kinetoplastida Leishmania tarentolae*. This organism has been extensively used as a model for studying Leishmania biology due the ease of cultivation and its inability to infect mammalian hosts. Recently, *L.terentolae* was exploited as a biotechnological host for production of recombinant proteins. However, the existing expression prototypes based on *L.terentolae* fell short of the requirements for a generally suitable expression system due to the insufficiently high yields.

The presented thesis deals with two aspects of the protein expression namely mRNA stability and translational initiation. The main part of the work is dedicated to the analysis of *L.terentolae* clones inducibly overexpressing a fusion of a coat protein (VP2) of parvovirus B19 and either bleomycin resistance protein or Enhanced Green fluorescent Protein (EGFP). The goal of the project was to analyze the stability of the VP2 mRNA and to establish a system where the factors controlling mRNA stability in *L.terentolae* could be identified. The author used a combination of Northern and Western blotting to evaluate the expression of fusion genes by analyzing the mRNA and recombinant protein levels. The author reaches the conclusion that in contrary to previous observations the mRNA message of VP2 containing fusion genes is not degraded completely and present in the cells at the detectable levels. However, both qualitative and functional analysis failed to detect the corresponding proteins leading author to propose several possible explanations such as inhibition of translation or/and low stability of resulting fusion proteins.

Thesis is well written and illustrated. The introduction demonstrates that author is familiar with molecular biology of *Leishmania* and can put his experimental plan into perspective. The experimental part of thesis is preformed very well. The experiments are well designed and executed, the results are properly controlled and illustrations are well designed. The author clearly understands what he was doing, why he was doing it, what was the outcome and what needs to

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be done next. The latter two aspects are particularly important since the experimental outcome deviated significantly form the expected results.

There are several minor flaws in the thesis that I would like to point out.

Introduction: page 2 author mentions the public health significance of *Kinetoplasitdae* but gives no details. Since this group contains several most dangerous human parasites the exact nature of medical significance should be discussed.

Page 6-7 While talking about *Leishmania* as an expression system author refers only to publications describing use of *L.tarentolae* as an expression host. I believe that these reports should be put into perspective based on the analysis of the literature dealing with protein expression problem in general. Moreover, several earlier attempts to use kinetoplastidae members for protein expression also should be mentioned.

Materials and methods:

Page 13. DAB is missing form the list of abbreviations.

Page 14. Based on the description of the expression constructs the *E.coli* strain should be BL21(DE3) and not BL21.

Page 15. Figure legend. Stuffer is mentioned – this either has to be removed or explained .

Page 19. Table 2 and text . The VP2BLe strains failed to grow on the medium containing phleomycin. Analysis of phleomycin sensitivity of a strain expressing only bleomycin resistance gene is needed as a positive control.

Page 20. figure legend referees to cultures derived form different « shipments » this has to be either explained or removed .

Page 22. figure legend. "Expression of VP2 mRNA in *E.coli* (strain BL21) was performed as a positive control". Should be " total RNA isolated form *E.coli* strain expressing VP2 gene was used as a positive control".

The panel A shows a Northern blot comparing mRNA of *L.tarentolae* strains expressing VP2-EGFP fusion protein with the strain expressing EGFP alone (lane 17). It appears form this experiment that when EGFP is used as a probe > 20 times reduction in the amount of reactive mRNA observed in the strains expressing VP2-EGFP compared with strains expressing EGFP. This is glossed over and needs to be further discussed.

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Discussion: Page 27 first paragraph. Author supports his conclusion that the sensitivity of the antibodies is not the reason for the failure to detect VP2 fusion proteins with the statement " as the VP2EGFP2 fusion protein is readily detected by relatively high sensitive monoclonal anti-EGFP antibody." This statement contradicts the observations described in the page 21 and figure 12, page 24.

Despite these minor drawbacks the presented work constitutes a nice and relevant study and deserves the highest grade.

With best regards

Yours sincerely
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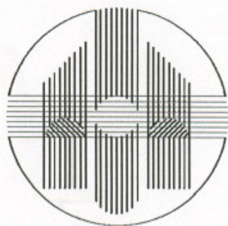

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Review of the Masters thesis of Zdenek Paris

One part of this thesis comprises studies of the effects of pre-ATG nucleotides on translation efficiency in *Leishmania tarentolae*. This is an extensive study of gene expression involving transfection, measurement of reporter gene expression, and the subsequent statistical analysis. I am informed that the student made a very considerable contributionbut this should have been made much clearer in the thesis - the summary here is so short that it is not even possible to tell the number of plasmids for which he was responsible.

In the second part of the work, the candidate expressed fusions of the parvovirus VP2 gene with GFP or bleomycin open reading frames in *L. tarentolae*, with the aim of developing a genetic screen for reduced mRNA degradation. (Webb et al also recently described a screen for deregulated mRNA degradation in trypanosomes which has a very simlar design to this study - why is this not cited?) The mRNAs were detectable in both cases, and the student concludes that the mRNAs were stable. This conclusion is not at all warranted by the data, since tha abundances of the mRNAs were not quantitated and the stabilities were not measured. The downstream selectable marker RNA was also detected, and the student draws conclusions about its structure based on size alone, which is again not legitimate.

The proteins were not detected, but the reason for this was not investigated. The Ble fusion protein was clearly not present in adequate amounts to give drug resistance, which means that the cell line would in principle be suitable for a screen to find higher expression. The student mentions that protein degradation could cause the apparent abscence of the products. but appears to be unaware of the considerable evidence for translational regulation (mediated by the 3'-UTR) in Leishmania and trypanosomes. I would have expected to see here a reference to the paper of Boucher et al on translational regulation in amastigotes, and to a review on translation regulation in eukaryotes.

The student's technical achievements are considerable. However the part which he wrote himself reveals that he still has not learned how to interpret his results critically: there is a lot of over-interpretation here based on little data, and the student

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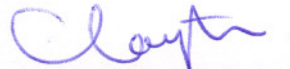
makes no attempt at all to propose methods which could be used to verify the interpretations. Several critical references are also missing. If I were evaluating this thesis in Germany I would therefore assign a grade of 2.0 (scale 1-5, where 1 is best and 5 is fail).



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