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Review report on Ph. D. thesis from Mrg. Klara Jirakova

Dear Prof. Zima

In the following you will find my review report on the Ph. D. thesis from Mrg. Klara Jirakova performed at the First Faculty of Medicine, Charles University in Prague.

Mrs. Jirakova's thesis consists of four papers that cover several aspects of the cell cycle and cell differentiation (conversion of trophozoites to cysts and *vice versa*) of the intestinal protozoan parasite *Giardia intestinalis*. *G. intestinalis* is a phylogenetically early diverging ("primitive") eukaryote and thus most cellular pathways in this organism are highly simplified. Therefore, *Giardia* is considered a valuable, if not unique, cellular model for gaining novel insights into basic principles of cell division or differentiation. *Giardia* is unusual in that it contains two morphologically similar nuclei in the vegetative trophozoite stage and four nuclei in differentiated cysts.

In *Giardia*, cell cycle analysis relies on synchronised growth of trophozoites that can be achieved by treatment of *in vitro* cultures with aphidicolin, a mycotoxin reversibly inhibiting replicative DNA polymerase. Synchronisation can also be achieved by treatment with albendazole, a microtubule inhibitor known to block assembly of mitotic spindles in *Giardia* trophozoites. Mrs. Jirakova investigated the influence of these two synchronisation drugs on both cytokinesis and karyogenesis of *Giardia* trophozoites. Further investigations specifically analysed the behavior of the nuclei during conversion of *Giardia* from the bi-nucleated trophozoites to the tetra-nucleated cysts and *vice versa*.

In particular, analyses of cytokinesis and karyogenesis in albendazole pre-treated, mitotic

Giardia trophozoites revealed that the two nuclei are most likely not identical and do not replicate synchronously. Furthermore, data from a second investigation also based on the use of albendazole as synchronising agent suggested that giardial cytokinesis is unique in that it does not involve a conventional spindle assembly checkpoint controlling cellular division. Another study demonstrated that the cytostatic effect of aphidicolin in *Giardia* involves inhibition of DNA synthesis and probably also damage of DNA during transition from G1 to S1 of the cell cycle. In line of these experiments it also became evident that *Giardia* must have functional DNA damage reparatory mechanisms operating prior to the mitotic phase. Finally, the most important study of the present thesis provided novel insights into the events related to nuclear division during the different stages of the life cycle. Here for example, Mrs. Jirakova and co-workers found that after mitosis and at the beginning of encystation, the nuclei form two interconnected pairs, which - in disagreement with findings recently published by Poxleitner et al. in 'Science' - remain associated in cysts and even during excystation.

In my opinion, all these studies were well designed and all experiments were performed with great care. Some of the data presented in this thesis confirm findings from earlier studies, but many others are completely novel and will substantially improve our knowledge on the cell biology of *G. intestinalis*. I expect that part of these results will contribute to the ongoing controversial discussion about the biological significance of the bi-nucleated status in *Giardia* trophozoites. Among the four studies presented the investigation on nuclear organisation during giardial stage conversion probably will have the highest scientific impact and thus may be regarded as the key work of Mrs. Jirakova's thesis.

This thesis has been well written and structured: it contains (i) a short and informative abstract, (ii) a precise formulation of the aims of the thesis (iii) an introduction that includes a comprehensive description of the biology of *G. intestinalis* and, more specifically, provides detailed information on the cell division and differentiation of the parasite, and (iv) a concise overview on the results achieved during her studies.

In conclusion, the document presented by Mrs. Jirakova, without any doubt, meets the international quality standard of a Ph. D. thesis in the field of Biomedical Science. Having learned more about her excellent scientific work now, I conclude that Mrs. Jirakova has gained all technolgical and intellectual skills to continue her professional carrier as an independent scientist in biological research. Accordingly, I am in the fortunate situation to recommend Mrs. Jirakova's thesis for the award of a Ph.D. degree.

Questions to be addressed during the Ph. D. thesis defence:

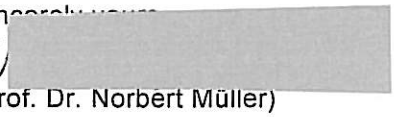
Although Mrs. Jirakova was able to present her research and the respective scientific background in a clear manner I have a few questions that she may address during her Ph. D. thesis defence:

1.) In the article published by Tumova et al. (2007) the authors concluded that "odd chromosome numbers indicate aneuploidy of *Giardia* nuclei, and their stable occurence is suggestive of a long-term asexuality". Although this point has been extensively discussed in the paper the argumentation still remains unclear and should be explained in more detail.

2.) As stated in the article published by Hofstetrova et al. (2010) "a minority of aphidicolin-released trophozoites is able to undergo mitosis even with damaged DNA". From studies on anti-giardial chemotherapy it is known that *G. intestinalis* cultures are phenotypically heterogenous in that individual trophozoites exhibit a differential degree of susceptibility (or resistance) to anti-giardial drugs. Is it possible that the pre-treated trophozoites capable of entering the G2 phase escaped aphodicolin-mediated DNA damage because they represented a relatively aphodicolin-resistant subpopulation of the culture? Did Mrs. Jirakova, or her colleagues, try to select for aphodicolin-resistant trophozoites by periodically increasing the concentration of this drug in a culture? Can Mrs. Jirakova make any comments on these questions?

3.) The study described in the paper of Jirakova et al. (2011) investigated e.g. the behavior of nuclei during, and upon, excystation. For this experiment, the authors used cysts that had been isolated from ovine an human faecal samples. I wonder why the authors did not use *in vitro* generated cysts in order to achieve standardised experimental conditions. Such an experimental set-up would have allowed the authors to assess the behavior of nuclei in excyzoites upon albendazole treatment (e.g. performed during either the encystation or the excystation process). Can Mrs. Jirakova make any comments on both the practicability and reasonability of such an experiment?

Sincerely yours



(Prof. Dr. Norbert Müller)