ISTITUTO SUPERIORE DI SANITA'

DIPARTIMENTO DI MALATTIE INFETTIVE

REPARTO: Parassitosi Alimentari e Neglette

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Review of the PhD candidate Magdalena Uzlíková

Overall assessment of the thesis

I have read with interest the thesis as well as the associated publications of the Ph.D. candidate Magdalena Uzlíková. I was impressed by the quality of the experimental work performed, the appropriateness of methodological approaches as well as by the rigorous analysis of the results and the critical manner in which the collected data were presented and discussed. This is particularly true for the two works on the characterization of telomeres and telomerase and, the effect of metronidazole on Giardia cell cycle, for which the candidate (being responsible for almost 80% of the performed work) clearly shows a good overall knowledge of the subjects and, the capacity to translate and apply such knowledge to the unusual biological system represented by the protozoan parasite *Giardia*.

Being the Ph. D. thesis work spanning almost a decade (from the firs publication in 2010 to the last one in 2017) the methodologies applied in the different part of the work where appropriate. Moreover, some methods has been adapted to cope with specific issue associated to *Giardia* biology (i.e. chromosome visualization; TRAP assay; etc.).

Collectively the work performed and presented by the PhD candidate has contributed to improve the current knowledge in the scientific community on the mechanisms behind the maintenance, integrity and inheritance of *Giardia* genome. Indeed, the evidence provided have already inspired and oriented publications from other groups. In particular:

- despite its efficacy in synchronize Giardia cells cycle, the observation that aphidicolin induces double-stranded DNA breaks and causes dissociation of the nuclear and cytoplasmic cycles, has prompted to develop new strategies for *Giardia* synchronization (Horlock-Roberts et al., mSphere. 2017; 2: pii: e00384-16);
- observation of phosphorylation of histone H2A in MTZ-treated parasite was a premise for a publication on metronidazole induced DNA homologous recombination response in Giardia by Ordoñez-Quiroz et al., Exp. Paras. 2018; 194: 24-31, as well as for a publication on post-translational modifications in metronidazole-resistant Giardia (Emery et al., 2018 Gigascience; 7: giy024)

Evaluation of the introductory chapter should demonstrate critical thinking and deeper insight by the candidate into the background of material relevant to the thesis.

The Introduction section present in a comprehensive manner the general information associated to Giardia and giardiasis (basic biology, epidemiology, clinical manifestation, treatment) as well as provide background information to contextualize the different topics being the subject of the thesis. Overall the candidate showed to know the subject and be able to focus on the aspect more strictly

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associate with her experimental work. However, some of the arguments presented in the introductive chapter would benefit of the use of graphical schemes to help readers to follow the information provided in the text (e.g. SAC mechanism; chromosome condensation proteins, telomeres maintenance).

Specific critical comments

Being aware that English is not the mother language of the Ph- D. candidate, I should underline that the quality of written English of the thesis is not exceptional and sentences are not always fluent and do not sound grammatically correct. More attention should have been done during text revision.

General questions to the defendant dealing with the subject, methods or interpretations that would allow the candidate to show in the reply how he/she is mastering the discipline. These questions will be asked at the defense.

Question 1: In the part of the work related to evaluation of the effect of aphidicolin on Giardia, isolate HP-1 has been used instead of the broadly and better characterized WBC6 strain. Why such choice? As aphidicolin synchronization is more efficient when incubation last for the number of hours grossly corresponding to cell replication time, is it not clear to me if this parameter was taken into account and HP-1 replication time measured prior to plan the experimental design. Moreover HP-1 is constitutively infected by the GLV (giardiavirus) that can affect parasite growth rate and eventually be a confounder in the right interpretation of the results. Can the candidate comment on this?

Question 2: In the work related to evaluation of the effect of aphidicolin treatment, results suggest that "replacement of histone H2A and reentry into mitosis" after the release from aphidicolin "is a strong indication for the presence of a DNA damage checkpoint in Giardia". Recently some components of the DNA damage repair machinery were characterized in Giardia. In light of this new knowledge, how methodologically and conceptually the candidate would further demonstrate if and how components of a DNA damage checkpoint signaling cascade are activated in Giardia in response to exposure to aphidicolin?

Question 3: In the work related to telomere maintenance, as the goal was to evaluate telomere localization during the cell cycle, could the candidate comment on the reason why FISH was not performed (or additionally performed) on synchronized Giardia cell, despite the protocol was available? This would have strength the provided conclusions.

Question 4: In the work related to telomere maintenance, despite the lack of the T motif in the GlTERT the enzyme is functional. Could the candidate comment on this and provide alternative explanations and how assess experimentally the alternative hypothesis?

Question 5: In the work related to telomere maintenance, the differences in size of the telomeric smears produced after TRF were linked to differences in the restriction sites between the Giardia isolates. Why the candidate did not apply the BAL-31 assay to the other Giardia isolates to verify this statement? More recent approaches have been developed to characterise telomeric ends/sub-telomeric regions using NGS. Is the candidate aware of it? How this could be applied to solve the differences in telomeric smear? Alternatively, the obtained result could be associated to a different processivity or amount of TERT in the isolates: How the candidate would further assess this alternative hypothesis?

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Question 6: In the work related to telomere maintenance, repeat other than TAGGG where found in the cloned TRAP products. It is not clear to me if this is due to *in vitro* condition of the TERT activity assay or there are true alternative repeats occurring within Giardia telomeres, eventually associated to presence of multiple RNA telomerase components or poor accuracy of TERT. Could the candidate comment on this and eventually design a strategy to solve this issue?

Question 7: In the work related to MTZ effect on cell cycle, the IC50 for MTZ treatment of WBC6 was extrapolated from a previous publication (Muller et al., 2006). Other publications report an IC50 between 1.5 to 3.5 μM. This value is then important to define lethal vs sub-lethal concentrations of the drug thus affecting interpretation of the results. Why the candidate did not experimentally establish the IC50 for its parasite isolate, as differences in culture condition are known to affect the drug response?

Question 8: In the work related to MTZ effect on cell cycle, H2A staining in cell treated with 10 μM MTZ could be assessed only for the few cell attached to the coverslip. Why the candidate did not test unattached cell using a different protocol for fixation (i.e. 4% formaldehyde)? Please comment on this.

Question 9: In the work related to MTZ effect on cell cycle, the percentage of cell positive for phosphorylated H2A was higher following treatment with 5μM MTZ compared to 10μM. Is it conceivable that higher concentration of MTZ impairs DNA DSB signalling phosphorylation inhibiting specific protein kinase pathway and preventing H2A phosphorylation? Could the candidate comment on it based on the most recent literature?

Statement of whether the reviewer considers the thesis suitable or not for award of a Ph.D.

In conclusion, for its content and quality, I consider the thesis of Magdalena Uzlíková suitable for award the Ph.D title.

Rome, September 10th, 2019

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