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REVIEW OF PH.D. THESIS

Name of the student: Shaghayegh Sheikh, MSc.

Thesis title: Factors involved in *Trypanosoma brucei* mitochondrial morphology

Supervisor: doc. Hassan Hashimi, Ph.D.

In her PhD work, Shaghayegh Sheikh has studied mitochondrial morphology in the model organism *Trypanosoma brucei* using advanced microscopic techniques, as well as investigated the mechanistic basis of cristae architecture and inner membrane dynamics. Her work has focused on a range of protein determinants of cristae morphology, including the mitochondrial contact site and cristae organizing system (MICOS) in trypanosomes and its evolutionarily ancient subunit Mic60 in alphaproteobacteria, as well as dynamin-related proteins. Her results are the subject of two first author publications as well as a publication where she is a co-author, and she has contributed to an additional study that is as yet unpublished. Her thesis documents an astonishing range of methods expertise and subject areas she has investigated with scientific rigour. With these studies she has contributed exciting and highly relevant findings to the field of mitochondrial inner membrane architecture and the evolution of its determinants.

In the study "Ultrastructural changes of the mitochondrion during the life cycle of *Trypanosoma brucei*" published in Journal of Eukaryotic Microbiology, S. Sheikh, who shares the first co-authorship, and coworkers have characterized mitochondrial and cristae architecture in unprecedented detail using focused ion beam-scanning electron microscopy and 3D reconstruction. They provide not only highly informative images, but also quantitative measures of cristae volume and surface as well as of other parameters, to describe morphological differences between the bloodstream form (BSF) and the procyclic form (PCF) of trypanosomes. They find that the BSF, whose metabolism relies on glycolysis and that was thought to have few to no cristae, harbour a significant number of cristae. However, they are clearly distinct from PCF cristae, for instance with respect to their average volume which is much lower and thus reflects the metabolic requirements of the cells.

To the study "Intracytoplasmatic-membrane development in alphaproteobacteria involves the homolog of the mitochondrial crista-developing protein Mic60, published in Current Biology, S. Sheikh contributed electron microscopic analyses as well as growth tests. Alphaproteobacteria harbour intracytoplasmatic membranes (ICM) that can be vesicular or lamellar and that are likely evolutionarily related to eukaryotic mitochondrial cristae. The study finds that knockout or overexpression of the alphaproteobacterial gene encoding Mic60 or of an adjacent gene encoding Orf52 affects ICM morphology and growth of the cells. Strikingly, Mic60 interacts with the alphaproteobacterial β-barrel protein BamA, the ortholog of eukaryotic outer mitochondrial membrane protein Sam50. These findings establish that contact site formation via Mic60, which in eukaryotes is known to interact with Sam50, is highly conserved in evolution.

Shaghayegh Sheikh is the first author of yet another study, "A novel group of dynamin-related proteins shared by eukaryotes and giant viruses is able to remodel mitochondria from within the matrix", which was submitted to Molecular Biology and Evolution. The authors performed a comprehensive phylogenetic analysis of dynamin-related proteins, among which are factors required for mitochondrial fission and fusion. This work discovered two novel groups of this protein superfamily, including the MidX proteins. Excitingly, this group that the authors identified in the genomes of 6 lineages of unicellular eukaryotes, originates from giant viruses. The authors demonstrate that a MidX representative is targeted to the mitochondrial matrix and remodels the inner membrane, indicating that a strategy employed by viruses appears to have become exploited by some protozoa after horizontal gene transfer. This work also finds that the mitochondrial fusion factors OPA1 in animals and Mgm1 in fungi do not share a common ancestor and thus cannot be considered homologs.

Finally, important unpublished data by S. Sheikh on the MICOS complex of *Trypanosoma brucei* has uncovered that two subunits, Mic34 and Mic40, contain mitofilin domains. TbMic60 does not contain the mitofilin domain, a signature domain of Mic60 in alphaproteobacteria, fungi and animals, which posed the question whether this otherwise conserved feature is not required in TbMICOS. S. Sheikh's exciting results reveal that mitofilin domains can contribute their functionality to MICOS in trans, as part of separate MICOS subunits. Knockdown of each of these subunits results in altered mitochondrial morphology as well as cristae architecture. Strikingly, expression of the Mic34 or Mic40 mitofilin domains in *E. coli* causes the formation of intracytoplasmic vesicles, showing that this domain has membrane remodeling activity.

With the wealth of scientific work covered in this PhD thesis, Shaghayegh Sheikh has demonstrated impressive practical skills over a wide range of methods, from biochemical and microbiological techniques to highly advanced electron microscopic approaches. Importantly, she has also proven her ability to analyse, understand and scientifically dissect multiple aspects of mitochondrial biology in a variety of model organisms, demonstrating her aptitude as a rigorous and adaptable researcher. This is underlined by her extraordinarily well written, comprehensive and precise PhD thesis. Her very well structured text summarizes the relevant information, important aspects and arising questions in professional, exact and eminently engaging language. She highlights crucial points while including all pertinent background information, refrains from far-flung speculation or unbased conclusions, and illustrates central concepts and findings with clear, informative models and data figures. Moreover, she contextualizes her findings in the framework of preceding and current research.

Questions for the student:

- What could be the physiological role of tubulovesicular cristae in mitochondria of BSF *T. brucei*? Do these cells still contain respiratory chain complexes at a low level, like for instance yeast cells that are grown under fermentative conditions?
- What is known about the mitochondrial lipid composition in BSF versus PCF forms?
- What could the molecular function of Orf52 be based on its topology, homology with studied proteins and protein-protein interactions?
- Have you tested or can you speculate on whether Mic60 or Orf52 interact with BamA individually in separate complexes or whether they could be part of one complex? Does the interaction of one of them with BamA persist in the absence of the other? Do you think the Orf52-BamA interaction might form Mic60-independent membrane contact sites? How would you test this?
- You mention that OPA1 or Mgm1 are present only in a small subset of eukaryotic lineages. Which ones do or do not have OPA1/Mgm1, and are there general differences in the mitochondrial dynamics or morphology of these two groups that correlate / might be a consequence of this difference?
- The protease protection assay of HypMidX expressed in *T. brucei* shows that a significant proportion of the protein is degraded in absence of detergent, indicating that it might have a dual localization in the matrix and possibly the intermembrane space. Can you exclude this possibility? If not, can you speculate on membrane remodeling mechanisms for a dually localized MidX protein as well as on regulation of protein localization? Since this experiment was done using mitoplasts, is the protein and the prohibitin control fully protease-protected in intact mitochondria?
- What is the basis of membrane binding by Mic34 and Mic40, which is a prerequisite for their membrane remodeling activities? Have you or your colleagues tested this activity *in vitro* using liposomes?

In conclusion, I recommend the thesis for the defense.

Dr. rer. nat. Heike Rampelt