Biochemistry, Stefan-Meier-Str. 17, 79104 Freiburg, Germany

Faculty of Science
University of South Bohemia in České Budějovice
Czech Republic

University of Freiburg

Institute of Biochemistry and Molecular Biology

Dr. Heike Rampelt, Group Leader
Stefan-Meier-Straße 17
79104 Freiburg, Germany
Tel. +49-761-203-5245

heike.rampelt@biochemie.uni-freiburg.de

www.biochemie.uni-freiburg.de

January 11, 2022

REVIEW OF MASTER THESIS

Name of the student: Bc. Lawrence Rudy Cadena III

Thesis title: The Mitochondrial Contact Site and Cristae Organization System and F_1F_o-ATP Synthase Crosstalk is a Fundamental Property of Mitochondrial Cristae

Supervisor: Mgr. Hassan Hashimi, Ph.D.

In his master thesis that led to a first author publication, Bc. Lawrence Rudy Cadena employed the model organism *Trypanosoma brucei* to study the crosstalk between the mitochondrial contact site and cristae organizing system (MICOS) and the F_1F_o-ATP synthase. These two high molecular weight protein complexes play crucial roles in shaping the inner mitochondrial membrane and are both required for the native architecture of cristae membranes where oxidative phosphorylation takes place. It was shown previously that in the yeast *S. cerevisiae*, the MICOS core component Mic10 interacts with dimeric ATP synthase via the dimerization factor subunit e (Su e / Atp21) and regulates its higher order assemblies. Trypanosomes are evolutionarily very distant from fungi and metazoa, and both their MICOS and ATP synthase differ substantially from their fungal or human counterparts. Despite the numerous differences, the requirement for native cristae architecture for efficient respiratory growth and the roles of the two machineries in membrane organization are conserved between these disparate eukaryotic supergroups.
L.R. Cadena demonstrated in his master's work that trypanosomal Mic10 interacts with the ATP synthase, and this interaction is specific for the isoform Mic10-1, since Mic10-2 does not interact. Moreover, he identified the protein ATPTb8 as a subunit e ortholog in trypanosomes and showed that it is required for dimerization of the ATP synthase and crosslinks directly to Mic10-1. Thus his results suggest that crosstalk between MICOS and the ATP synthase represents a widely conserved and therefore fundamental regulatory principle that may organize cristae architecture.

This work is an impressive achievement considering the limited time available to a master student. L.R. Cadena successfully investigated specific interactions between membrane proteins and also characterized a novel subunit of the ATP synthase in *Trypanosoma brucei*. To address his research questions, he and his co-authors employed a variety of well-suited

methods including biochemical analyses such as immunoprecipitations and crosslinking, mass spectrometry and electron microscopy of mitochondrial ultrastructure. It would have been helpful to include an overview of experiments and methods performed by the student himself. The premise and aims of his work are well-founded and highly interesting. The results are robust and convincing and represent a valuable contribution to the field, in particular because of the implications regarding evolutionary conservation of the crosstalk. They open many exciting questions about common principles and parasite-specific adaptations in cristae morphogenesis.
The thesis is of excellent quality. The writing is very clear and scientifically precise, and the individual parts of the text are well balanced in their lengths, numbering 53 pages in total. The introduction covers all relevant aspects in a structured, logical and highly accessible manner and effectively highlights critical aspects. The choice of figures is conducive to illustrate central concepts. It would have been even better if some of the figures had been prepared by the student for the thesis rather than adapted from review articles. The text provides adequate references. For future scientific texts, perhaps more original publications, in addition to reviews, can be cited for central statements.

Questions for the student:

- The specificity of the ATP synthase interaction for Mic10-1 is very intriguing. Did you perform mass spectrometry of Mic10-2 interactors, as well? How do they differ from Mic10-1 interactors? Can you comment on the functional diversification of the two Mic10 paralogs, do they differ in other properties such as oligomerization propensity, or in their expression in the procyclic vs. bloodstream form of trypanosomes?
- Do other MICOS subunits interact with the ATP synthase in *Trypanosoma brucei*?
- Does the ATP synthase form dimers or oligomers in the bloodstream form, and is the interaction with Mic10-1 maintained?
- What is known about dimerization subunits in type IV ATP synthase and about their position in dimers or oligomers? Type IV ATP synthase differs in its subunit composition, structure and dimer arrangement to oligomeric rows from fungal and mammalian type I ATP synthase. Based on type IV topology and ATPTb8 position, do you expect that Mic10-1 would be able to interact with mature oligomers or rather with individual dimers? In yeast, Mic10 enhances the stability of ATP synthase oligomers. Are there indications for a similar or distinct function in *Trypanosoma*?
- Regarding the enhanced stability of ATP synthase dimers upon downregulation of Mic60, have you tested whether the Mic10-1 / ATPTb8 interaction is affected in these cells?
- Are there any lipidomics results from trypanosomes that might implicate MICOS or Mic60 in cardiolipin biogenesis or enrichment?

In conclusion, I recommend the thesis for the defense and I suggest the grade 1.

Dr. rer. nat. Heike Rampelt