University of Glasgow | Institute of Molecular, Cell & Systems Biology

Glasgow, 11 December 2019

Evaluation of the PhD Thesis of I Kaurov
'Biology of the mitochondrial contact site ad cristae organizing system in trypanosomatids'

This PhD thesis focuses on the identification, isolation and functional characterization of the MICOS complex in Trypanosoma Brucei. The work presented is organized in three main parts that basically follow three publications that the student co-authored.

Overall assessment:

The thesis presents a significant body of work that is original and very interesting. The quality if the work is generally very high and published in peer-reviewed journals. **I think the student should be awarded the PhD** and I will be happy to discuss a couple of specific points during the viva. In my opinion the work meets all the required international standards for the award of the PhD in terms of originality, provision of experimental support to the conclusions drawn and a clear explanation of the novel findings. This work will undoubtedly serve as a basis for further studies and develops further our existing knowledge on the MICOS system in T brucei. It is important to study this system in non-opisthokont and plant species both in terms of evolutionary considerations but also in terms of elucidation of the basic mechanistic principles that underpin this system.

Specific comments:

The thesis starts with an Introduction that is very succinct but clearly written. After a very general discussion on mitochondria structure, the student quickly devotes the rest of the introduction to a discussion on the two main themes of the thesis namely the MICOS complex and the MIA import pathway. These topics are both presented with sufficiently in-depth coverage of the literature and set well the ground for the actual experiments in this thesis.

In the first results part, the student presents the identification of the TbMICOS complex and a preliminary characterization of its salient features and differences as compared to the more extensively characterized yeast and human systems. This part of the work was published in Current Biology.

In the second results section, the analysis of the MICOS complex shifts to the organisation in two distinct sub-complexes that are apparently linked to distinct functions. The membrane integrated part is mostly relevant for the cristae formation, while the peripheral part is more relevant for a protein import function. Here, the role of the individual subunits in ablation experiments was investigated as a means to explain the highly divergent origin of the MICOS subunits in Tb. This work is published in a paper in Molecular Microbiology.

Finally, the last chapter deals specifically with TbMic20 and its role in protein import into the intermembrane space. Emphasis is placed on the CIPC motif which is proposed to function as the equivalent CPC motif in Mia40, providing some preliminary analysis of the relevant cysteine mutants. Here, a comparison of the effects of ablation of TbMic20 and TbErv1 is given. This shows an overlap of the proteins that are affected, which is in turn used

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as a main argument for supporting a role of TbMic20 in IMS protein import via the MIA pathway. In an interesting complementation experimentation in bacteria, it was found that TbMic20 can complement DsbA, which is the key thioredoxin-like oxidoreductase in the bacterial periplasmic oxidative folding system.

I would like to discuss with the student the following points:

1. It is clear that TbMic20 is a Thioredoxin-like protein and the assumption is that this Trx domain is important for its import function. On the other hand, it is proposed that TbMic20 'replaces' Mia40 in the Tb system, and that the CIPC motif is the equivalent of the Mia40 CPC motif. However, Mia40 structure is completely unrelated to the Trx structure and this is one of the unique features of the IMS pathway for oxidative folding. In what is the Trx folding domain relevant for the function of the TbMic20? Does the student think that the CIPC motif can really sustain the enzymatic activity of TbMic20 as a disulfide donor?
2. In other system like the QSOX enzymes in human cells, the Erv domain is fused top one or in some cases two Trx domains within the same molecule. Does the student think there is an electron transfer reaction involving the Trx domain in TbMic20 and interacting with TbErv1? Another interesting observation is that even in the cellular location where there is a protein with just one Erv domain (eg cytosol of human cells or ER lumen) there are Trx-like proteins that interact with this domain despite the fact they are not part of the same polypeptide as in the QSOX enzymes. How is this relevant in the Tb system?
3. Although the DsbA complementation experiment is an interesting one, the basis of it is likely that the TbMic20 has a proper Trx domain like DsbA. So this is in a way not very surprising. However, if TbMic20 was used in a system like yeast mitochondria where Mia40 bears no homology to Trx, how could TbMic20 if at all? Equally, could human Mia40 or yeast Mia40 (the soluble part) which both have a CPC motif complement an ablation of TbMic20?
4. When one compares the Tb system to the yeast or human or plant MIA system, it is intriguing that the supposedly equivalent in Tb of Mia40 (ie TbMic20) is part of the MICOS complex. What might be a rational explanation and is this telling us something about a different segregation of the systems within the IM and the cristae in the different organisms? There are reports that the Mia40 may be in physical proximity to the TOM channel. How could this work so differently in the Tb system?
5. What is the basis of the essentiality if the peripheral subcomplex as opposed to the non-essentiality of the membrane subcomplex? Is the function of the membrane subcomplex partially overlapping with other components or complexes in trypanosoma?
6. The student attempts to provide an explanation for the role of TbMic20 in import by arguing for the presence of an equivalent hydrophobic cleft similar to the one found in yeast or human Mia40. However, this is only based on modelling of the structures and in my view would require hard biochemical evidence. Could it not be that the hydrophobic binding is in fact ensured by another protein acting upstream of TbMic20 in the MIA pathway in trypanosoma?

I would be happy to discuss these points with the student. I think he should clearly be awarded the PhD as the work done is very detailed, original and very interesting.

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07.12.2019

Evaluation of the PhD thesis
"Biology of the Mitochondrial Contact Site and Cristae Organizing System in Trypanosomatids"

by
Iosif Kaurov

Mitochondria are ubiquitous organelles of eukaryotic cells. The fulfil a variety of important physiological functions, like oxidative phosphorylation and ATP synthesize, iron-sulphur-cluster biogenesis, β-oxidation or lipid biosynthesize, many of which are vital for most higher organisms. According to the widely accepted endosymbiotic theory, the ancestor of mitochondria was an α-proteobacterium. Over the course of evolution host cell and α-proteobacterium became dependent on each other. For example, most of the α-proteobacterial / mitochondrial genome was transferred into the host cells nuclear DNA and modern mitochondria depend on the import of the vast majority of mitochondrial proteins. At the same time mitochondria evolved to optimize for example ATP production for the cell.
Due to the endosymbiotic event mitochondria are surrounded by two membranes and can be subdivided into four compartments: I) outer membrane (OM), II) intermembrane space (IMS), III) inner membrane (IM) and IV) matrix. Whereas the OM is believed to be a mechanical barrier against the rest of the cell, the IM is intimately connected to many mitochondrial processes. It also has a very different morphology than the OM. It is highly folded and as such as a larger membrane surface. As a result of folding, the IM can be further subdivided into different morphological and functional sub-compartments: the inner boundary membrane (IBM), which runs alongside the OM and invaginations towards the matrix, the cristae. IBM and cristae are connected by cristae junctions (CJs). These are short tubular membrane segments with an inner diameter of 15 – 30 nm. The formation and maintenance of CJs is a lively debated topic of current cell biology, as this structure seems to be key for the organization of the IM. A recently identified multi-subunit complex was found to be enriched at cristae junctions as well as to be important for forming and maintaining these membrane structures and was hence termed mitochondrial contact site and cristae organizing system (MICOS). As the name implies the complex also connects inner and outer membranes.

While MICOS assembly and function is at least to some extend understood in yeast and mammals almost nothing is known about the composition and function in unicellular parasitic protist *Trypanosoma brucei (Tb)*. In the presented PhD thesis Iosif Kaurov set out to characterize the protist complex and analyse its composition and physiological function.
Iosif gives an extensive and well-written introduction into mitochondria and how they evolved. This part of the thesis touches on many different aspects of mitochondrial research like evolution, physiological importance of the mitochondrial metabolism, inner membrane function with a focus on morphology, biogenesis and the MICOS complex, protein import pathways and mitochondrial biogenesis in Trypanosomatids. Even though each of these points is very complex in nature, it is very easy to follow the introduction. Iosif shows a broad knowledge of mitochondrial biology and is able to articulate and explain important and complex networks.

The PhD thesis further includes two published papers in international renowned and peer-reviewed journals and, in chapter 3, promising results that could easily be the basis for another manuscript. Iosif Kaurov is the lead author of the publication '*The Diverged Trypanosome MICOS Complex as a Hub for Mitochondrial Cristae Shaping and Protein Import*' which was published in the prestigious journal ***Current Biology***. He is a co-author on the second publication termed '*The highly diverged trypanosomal MICOS complex is organized in a nonessential integral membrane and an essential peripheral module*' published in ***Molecular Microbiology***.
In the third chapter of his thesis Iosif shows so far unpublished results in which he analyses a newly identified subunit of the Trypanosomas MICOS subunit TbMic20. In his Current Biology paper Iosif found TbMic20 to be a thioredoxin-like protein involved in cristae formation, and to have a CIPC reaction center, similar to the CPC motif of human Mia40. TbMic20 promotes intermembrane space protein import and is now characterized for its role in the potential *Tb* disulphide relay system. Iosif constructed mutations of TbMic20 to exchange specific cysteine residues. In Mia40 such residues were shown to be essential for substrate recognition and disulphide bond formation and therefore oxidative folding of IMS proteins. Performing a quantitative analysis of the effects of these mutants on imported *Tb* mitochondrial proteins of the IMS and the matrix, respectively, revealed a specific defect in IMS protein import as expected. Though these results are not straight forward to interpret, as the mutant proteins showed changed expression levels, in a complementary approach an activity in oxidative protein folding could be shown. For that purpose TbMic20 WT was successfully tested for its ability to rescue the knock-out of bacterial DsbA, a protein important for oxidative folding in the bacterial periplasm.

The thesis triggers a variaty of interesting questions that are worthwhile considering. For example, a number of MICOS subunits with no apparent homologues in yeast or mammals were identified. These proteins were found by interacting with TbMic10. Other eukaryotic MICOS complexes were found to form extensive networks with mitochondrial proteins and protein complexes in the various sub-compartments. Are those interacting partners of TbMic10 part of the *Tb* MICOS or could they also be subunits of interacting complexes of maybe unknown function? Is the membrane lipid composition of *Tb* mitochondrial inner membranes similar to other eukaryotes and do specific lipids have an effect on cellular health of Trypanosomas and the stability of TbMICOS? As *Tb* at different

stages of its life cycle shows changing dependency on mitochondria based metabolic pathways, is the TbMICOS differentially expressed accordingly?

Given the well-presented and excellent published novel findings on the MICOS complex of Trypanosomatids and the overall well-written thesis, I gladly support that the candidate is awarded a PhD.

Göttingen, 07.12.2019

(Prof. Dr. Michael Meinecke)