The role of kinetoplastid MICOS complex in cristae shaping and intermembrane space import.

Iosif Kaurov[1,2], Marie Vancova[1,2], Lawrence Rudy Cadena[2], Jiři Heller[1], Tomaš Bily[1,2], David Potěšil[3], Zbyněk Zdrahal[3], Julius Lukeš[1,2], Hassan Hashimi[1,2]

1. Institute of Parasitology, Biology Center, Czech Academy of Sciences, Česke Budějovice, Czech Republic

2. Faculty of Science, University of South Bohemia, Česke Budějovice, Czech Republic

3. Central European Institute of Technology, Masaryk Univeristy, Brno, Czech Republic

Cristae, the internal ridges of the mitochondrial inner membrane, contain protein complexes that are responsible for oxidative phosphorylation (OXPHOS) and therefore proper cristae formation is vitally important for aerobic organisms. Cristae junctions (CJs) form the connection between the cristae and mitochondrial inner boundary membrane. The Mitochondrial Contact Site and Cristae Organization System (MICOS) is responsible for the formation of CJs in opisthokonts. Knowledge about MICOS is limited to opisthokont models, especially yeast. Our model species, *Trypanosoma brucei*, has only one bioinformatically recognizable subunit, homolog of the core MICOS protein Mic10. The two paralogs of this subunit in *T. brucei* have redundant functions under our experimental conditions. We have allele tagged both paralogs and have confirmed that they associate with mitochondrial cristae. Immunoprecipitation of both TbMic10 paralogs pulls down the same set of kinetoplastid MICOS subunits. While some subunits share similar domains with opisthokont MICOS subunits, they are all quite diverged. RNAi cell lines targeting these TbMICOS candidates were obtained. Most depletion cell lines developed growth phenotype both in glucose-poor and glucose-rich media. Transmission electron microscopy revealed significant alterations in cristae shape and structure of some depletion cell lines, similar to those observed in yeast after deletion of core MICOS subunits. Moreover, TbMICOS contains a novel subunit that we demonstrate is important for the import of intermembrane space (IMS) proteins, including OXPHOS complex assembly factors. We propose that in addition to cristae junction formation and cristae shaping, which we establish are conserved MICOS functions, kinetoplastid MICOS takes part in IMS protein import.