Evolutionary history of the mitochondrial outer membrane channels of a predicted beta-barrel topology

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The biogenesis and proper function of mitochondria require import of 90-99% of mitochondrial proteins as well as the metabolite exchange with the cytoplasm. These transport processes are mediated by proteins located in both mitochondrial membranes but initiated by proteins of the mitochondrial outer membrane that display a channel activity, namely VDAC, Tom40 and Sam50/Tob55. VDAC, known also as mitochondrial porin, supports the flux of metabolites. Tom40 and Sam50/Tob55 are crucial components of protein import complexes, namely the TOM complex and the SAM/TOB complex, respectively. The SAM/TOB complex is essential for insertion of beta-barrel integral proteins into the mitochondrial outer membrane as well as for biogenesis of the TOM complex while the TOM complex is regarded as general entry gate of mitochondria as the complex who is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting.

The presented study focused on the amoeba *Acanthamoeba castellanii* and the slime mold *Dictyostelium discoideum* VDAC, Tom40 and Sam50/Tob55. Both microorganisms, currently proposed to be members of *Amoebozoa,* are commonly applied as model organisms in different biological studies, including the research of animal and plant mitochondria. However, till now only Tom40 and VDAC of *D. discoideum* were identified. The aim of this work was to identify Sam50/Tob55 of *D. discoideum* as well as VDAC, Tom40 and Sam50/Tob55 of *A. castellanii*.

Owing to the fact that amoebozoans are considered as a sister group of the fungal/animal lineage and also that many of their proteins are similar to animal orthologs we decided to perform comprehensive analysis of the evolutionary history of mitochondrial outer membrane channels of a predicted beta-barrel topology in order to verify the currently proposed *A. castellanii* and *D. discoideum* membership to the *Amoebozoa* group.

Here we report the discovery of all of the proteins of interest for both *D. discoideum* and *A. castellanii* with the usage of bioinformatic and proteomic approaches. Results of the phylogenetic survey indicate that despite the strong functional and structural conservation, the studied mitochondrial beta-barrels present high sequence divergence between different eukaryotic lineages. This can be explained by the evolution of lineage specific, additional components of the mitochondrial outer membrane import machinery, which partially took over some functions of the very first, simple translocases, e.g. receptor functions. The obtained phylogenetic trees result in clustering of *A. castellanii*, *D. discoideum* and other amoebozoans with representatives of *Chromoalveolata* and *Excavata* in close proximity to *Archaeplastida.* Furthermore, the representatives of *Amoebozoa*, *Chromoalveolata* and *Excavata* usually form homogenous branches that partially supported the new system of classification. Nevertheless, further genomic sequences of these poorly analyzed groups will obviously provide a good opportunity to verify the relevance of the presented study.