Abstract:

In the search for the mitochondrion of oxymonads, DNA of *Monocercomonoides exilis* – an oxymonad isolated from the gut of *Chinchilla,* was isolated and its genome was sequenced. Sequencing resulted in a fairly complete genome which was extensively searched or genes for mitochondrion related proteins, but no reliable candidate for such gene was identified. Even genes for the ISC pathway, which is responsible for Fe-S cluster assembly and considered to be the only essential function of reduced mitochondrion-like organelles (MROs), were absent. Instead, we were able to detect the presence of a SUF pathway which functionally replaced the ISC pathway.

Closer examination of the SUF pathway based on heterologous localisation revealed that this pathway localised in the cytosol. *In silico* analysis showed that SUF genes are highly conserved at the level of secondary and tertiary structure and most catalytic residues and motifs are present in their sequences. The functionality of these proteins was further indirectly confirmed by complementation experiments in *Escherichia coli* where SUF proteins of *M. exilis* were able to restore at least partially Fe-S cluster assembly of strains deficient in the SUF and ISC pathways. We also proved by bacterial adenylate cyclase two-hybrid system that SufB and SufC can form complex.

SUF genes were also found in transcriptomes and genomes of nine other Preaxostyla species (a group of anaerobic protists that belong to Metamonada), while the ISC pathway was consistently absent. Interestingly, in most Preaxostyla, we were able to identify at least partial fusions of SufD, S, and U, which is a unique situation. In a phylogenetic analysis of concatenated genes SufB, C, D, and S, Preaxostyla formed a well-supported clade nested inside of bacteria. The resulting clade is clearly distinct from that of the SUF pathways known from plastids and previously described SufBC systems of *Blastocystis hominis, Pygsuia biforma*, and related protists.

These results strongly suggest that the SUF pathway was acquired from bacteria by the last common ancestor of Preaxostyla, where it functionally replaced the mitochondrial ISC pathway. Based on these results it was proposed that *M. exilis* has completely lost its MRO and therefore it is the first described amitochondriate organism. The loss of MRO in the case of *M. exilis* was probably allowed by a combination of an anaerobic and endobiotic lifestyle of this organism, together with the replacement of the mitochondrial ISC pathway by cytosolically - localised SUF pathway which rendered the MRO expendable.