

Abstract

Protists inhabiting oxygen-depleted environments have evolved various adaptation to thrive in their niches, including modified mitochondria to various degrees adapted to anaerobiosis. The most radically altered forms of these organelles (Mitochondria-Related Organelles, MROs) have completely lost their genomes and other defining features of canonical aerobic mitochondria. Anaerobic protists are often found as endobionts (parasites, mutualists, etc.) of larger organisms. The endobiotic lifestyle combined with anaerobiosis poses another source of evolutionary pressure forcing unique adaptations in the endobionts. Here we present new insights into the adaptations of an anaerobic protistan phylum Preaxostyla, especially with regard to the reductive evolution of mitochondria, which, uniquely among all known eukaryotes, led to a complete loss of the organelle in the oxymonad *Monocercomonoides exilis*.

We have obtained *M. exilis* genomic assembly of good quality and completeness, as well as genomic and transcriptomic data of varying quality and completeness from 9 other Preaxostyla species. Based on extensive, thorough gene searches and functional gene annotation on these datasets, as well as phylogenetic analyses and protein localization experiments, we conclude: 1) *M. exilis* has completely lost the mitochondrion. This was likely facilitated by a replacement of the mitochondrial system for iron-sulfur (Fe-S) cluster assembly (ISC) with an unrelated SUF system of bacterial origin, which was employed for function in the cytosol; 2) Despite the loss of mitochondria, *M. exilis* displays no major reduction in genomic or cellular complexity compared to other anaerobic protists endowed with MROs; 3) The SUF system for Fe-S clusters assembly is present in all studied Preaxostyla and was likely gained in a single lateral gene transfer event from bacteria into a common ancestor of extant Preaxostyla. No studied member of Preaxostyla has the mitochondrial ISC system; 4) The ATP-producing arginine deiminase (ADI) pathway is present in most studied Metamonada including Preaxostyla and likely represents an ancestral feature of Metamonada. Distribution and phylogeny of the 3 ADI pathway genes among eukaryotes is consistent with presence of the pathway already in the last eukaryotic common ancestor (LECA) and their evolutionary history was shaped by frequent losses and lateral gene transfers.