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Genomics of Preaxostyla flagellates

Genomika bičíkovců skupiny Preaxostyla

Summary of the PhD thesis

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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.

1. Introduction

Protistological research of the past 3 decades has uncovered a great diversity of types of mitochondria adapted to anaerobic life. Almost all eukaryotic lineages previously considered amitochondriate have been shown to possess a more or less reduced remnants of mitochondria with varying metabolic capabilities. Some mitochondria-related organelles (MROs) are known to produce ATP in a process of extended glycolysis, which leaves molecular hydrogen as a waste product. Parts of the amino acid metabolism can also be localized in these organelles. Other MROs do not play any role in energy or amino acid metabolism at all and retain very limited physiological roles.

Biologists have categorized MROs into discrete classes like hydrogenosomes and mitosomes, but it is increasingly apparent, that each lineage of anaerobic protists has unique MROs with a specific set of physiological functions and these organelles form a continuous spectrum, rather than distinct types (Roger, Muñoz-Gómez, and Kamikawa 2017). One function seems to unite a great majority of MROs: the synthesis of iron-sulfur (FeS) clusters, ubiquitous and vital cofactors in a number of (mostly) electron transfer-related proteins. The synthesis of FeS clusters by either the mitochondrial ISC system, or other pathways, has been suggested to represent the most crucial and indispensable function of MROs, which prevents total loss of MROs even in organisms, where all the other roles are unnecessary or localized elsewhere (Williams et al. 2002).

The largest eukaryotic taxon containing only anaerobic organisms with MROs and no aerobes with canonical mitochondria is Metamonada. The hydrogenosomes of *Trichomonas vaginalis* (Parabasalia) and mitosomes of *Giardia intestinalis* (Metamonada, Fornicata), as well as other cellular systems of these human parasites, have been extensively studied and characterized using methods of biochemistry and cell biology. Recent progress in genomic and transcriptomic sequencing has allowed study of other metamonads of lesser practical importance and new MROs with unique characteristics have been identified all across the metamonad tree of life (Leger et al. 2017; Hampl et al. 2008).

However, one lineage of metamonads, the exclusively endobiotic Oxymonadida, has never been shown to have any relictual mitochondria at all. Oxymonadida are very likely not primarily amitochondriate, because they are closely related to free-living lineages with known MROs: Trimastigidae and Paratrimastigidae, with which they are grouped in the phylum Preaxostyla (Dacks et al. 2001; Simpson 2003; Zhang et al. 2015).

We obtained a good quality genome assembly of a representative of Oxymonadida, chinchilla gut symbiont *Monocercomonoides exilis*, and shown convincingly that this organism indeed lacks any traces of mitochondria (Karnkowska et al. 2016). This discovery, first among eukaryotes, has transformed Preaxostyla from an unimportant and understudied taxon into an attractive model system for the study of reductive evolution of mitochondria.

We followed this breakthrough with more detailed investigations of various cellular systems in Preaxostyla. The genome of *M. exilis* was subjected to a thorough functional annotation with the goal of understanding how other features of its cell either contributed to – or reacted to – the loss of mitochondria (Karnkowska et al. 2019).

Distribution of proteins involved in the FeS cluster synthesis pathways was mapped in Preaxostyla, which uncovered an ancient lateral gene transfer of the bacterial SUF system into an ancestor of Preaxostyla. This newly gained pathway likely later allowed the loss of mitochondria, as the mitochondrial ISC system was no longer indispensable (Vacek et al. 2018).

The ATP producing arginine deiminase (ADI) pathway has been found in *M. exilis*, which motivated us to search for it in a broad sample of eukaryotes and to try to uncover its evolutionary history. Our findings show that the ADI pathway is more widespread than previously thought and is likely an ancestral feature of Metamonada if not all eukaryotes (Novák et al. 2016).

A comprehensive comparative genomic project on 5 members of Preaxostyla is currently underway. Genomic and transcriptomic data from *Trimastix marina* (Trimastigidae), *Paratrimastix pyriformis* (Paratrimastigidae), *Monocercomonoides exilis*, *Blattamonas nauphoetae*, and *Streblomastix strix* (Oxymonadida) are being functionally annotated in parallel in order to map changes in their metabolic and other capabilities, as they happened during the evolutionary history of Preaxostyla. We hope to gain many new insights into the evolution of mitochondria reduction and loss, energy metabolism, biosynthetic capabilities, plasma membrane transporters, cytoskeleton, endomembrane system, and many other systems thanks to this project. Selected preliminary results of *P. pyriformis* genome functional annotation are presented in supplement of this thesis.

2. Aims

- To generate the genomic assembly of oxymonad *Monocercomonoides exilis*, perform automatic and manual prediction and annotation of protein-coding genes. To evaluate the completeness and quality of the genomic assembly by annotating various cellular systems. To thoroughly search for genes of mitochondrial origin.
- To search for genes coding for enzymes of the arginine deiminase (ADI) pathway in a broad sample of eukaryotes *in silico*. To explore the evolutionary history of the pathway in eukaryotes by molecular-phylogenetic methods.
- To search for genes coding for components of iron-sulfur cluster assembly systems in a broad sample of Preaxostyla. To explore the evolutionary history of these systems in Preaxostyla by molecular-phylogenetic methods.
- To annotate various cellular systems of *Monocercomonoides exilis* based on the previously obtained genomic assembly. To explore how these systems might have contributed to the loss of mitochondria, or how they responded to it, by comparing the results to other studied anaerobic protists.

3. Materials and methods

Multiple next generation sequencing technologies were used to produce genomic and transcriptomic assemblies. Proteomes predicted from the genomic assemblies were functionally annotated based on homology searches and presence of particular sequence motives. Hypothetical maps of cellular systems were assembled based on functional knowledge from other organisms. Evolutionary history of selected genes and metabolic pathways was investigated using maximum likelihood phylogenetic methods and statistical topology tests. Methods are described in greater detail in the individual publications and in the supplement.

4. Results and discussion

We have obtained a good quality genomic assembly of *M. exilis*. An *in silico* predicted proteome of *M. exilis* was thoroughly searched for genes of mitochondrion origin and no were conclusively found. To investigate whether this reflects a real lack of the mitochondrial genes, or an artefact caused by imperfect sequencing and assembly, we performed an analogous search for genes associated with the Golgi apparatus, another organelle which was never observed in an oxymonad cell. We have identified numerous Golgi-associated genes, which suggests that the Golgi functions are performed by *M. exilis* cell, only in other, less conspicuous, arrangement than the typical stacked Golgi apparatus. This, together with multiple independent evaluations of the genome assembly completeness, strongly indicates, that the lack of genes of mitochondrial origin indeed reflects a complete lack of the mitochondrial compartment in *M. exilis* (Karnkowska et al. 2016).

Energy metabolism in *M. exilis* was shown to be similar to mitosome-bearing protists in its reliance on glycolysis extended by enzymes typical for anaerobic protists: pyruvate:ferredoxin oxidoreductase (PFO) and acetyl-CoA synthetase (ACS). The mitochondrial FeS cluster assembly system ISC is completely missing, although the downstream cytosolic system CIA is retained. FeS cluster biosynthesis is likely performed by the SUF system of bacterial origin, which was found in a complete state in *M. exilis* (Karnkowska et al. 2016).

A great number of various cellular systems was later annotated based on the *M. exilis* genome assembly in order to understand eventual evolutionary changes connected to the loss of mitochondria (Karnkowska et al. 2019). These systems include genome maintenance, cytoskeleton, endomembrane system, autophagy, oxidative stress response, amino acid metabolism, fatty acid metabolism, purines and pyrimidines scavenging, and other. None of these systems was shown to be substantially altered in *M. exilis* when compared to other

metamonad species with MROs. The only features clearly connected to the loss of mitochondria are 1) the replacement of FeS cluster assembly system (which likely facilitated the loss of mitochondria), 2) lack of glycine cleavage system (which is always localized in the mitochondrion), and 3) lack of mitophagy machinery (which is unnecessary in the absence of mitochondria). The apparent unreduced complexity of *M. exilis* cell in absence of mitochondria may inform further thinking about the origin of eukaryotes and the role of mitochondria in that process (Hampl, Čepička, and Eliáš 2019).

The complete arginine deiminase (ADI) pathway, which catabolizes arginine while producing ATP, and which was previously known among eukaryotes only from members of Parabasalia and Fornicata, was discovered also in *M. exilis*. We searched for sequences coding for the 3 enzymes composing the ADI pathway in multiple publicly available gene databases and in unpublished data from our laboratory, as well as our collaborators, in order to map their distribution in eukaryotes. Individual enzymes were discovered in a broad diversity of eukaryotes and the complete set of 3 was discovered in representatives of Metamonada, Heterolobosea, Breviatea, Amoebozoa, and Viridiplantae. Results of phylogenetic analyses of the sequences suggest that the ADI pathway was present already in the common ancestor of Metamonada, and do not contradict its presence already in the last eukaryotic common ancestor (LECA). Evolution of the ADI pathway in eukaryotes involved frequent gene losses and likely also lateral gene transfers (Novák et al. 2016).

To understand evolutionary history of the FeS cluster synthesis systems in Preaxostyla, we searched available genomic and transcriptomic data for components of all 4 systems known from eukaryotes: CIA, ISC, SUF, and NIF. We identified components of both CIA and SUF systems, and no components of the ISC and NIF systems in all studied Preaxostyla species. Phylogenetic analysis of the SUF system shows a common origin of all Preaxostyla sequences, which was however different from any other eukaryotic SUF known to date. This implies a lateral gene transfer of the entire SUF system into an ancestor of all currently living Preaxostyla. The source lineage is unclear. However, the phylogenetic analysis points towards Bacteria and the composition of the Preaxostyla SUF system, as well as presence of a unique fusion of SufD, SufS, and SufU proteins, suggests that the source lineage might have been in Firmicutes, Thermotogae, Spirochaetes, Proteobacteria, or Chloroflexi (Vacek et al. 2018).

Preliminary functional annotation of *P. pyriformis* genome (not published, see supplement I) resulted in a reconstructed putative proteome of MRO, set of putative plasma membrane transporters, and hypothetical metabolic maps of the energy and amino acid metabolism. The

MRO of *P. pyriformis* is suggested to have no role in energy metabolism, although it contains [FeFe] hydrogenases and hydrogenase maturases. Role of these proteins is hypothesized to be an electron sink for reoxidizing NADH, required by the glycine cleavage system, which was previously shown to be localized in the organelle (Zubáčová et al. 2013). The fully cytosolic energy metabolism is predicted to be very similar to *M. exilis*, consisting of glycolysis using both canonical ATP-dependent, and alternative pyrophosphate-dependent enzymes (Stechmann et al. 2006; Liapounova et al. 2006), and the glycolysis extension by PFO and ACS. The hypothetical amino acid metabolism is more complex and biosynthetically capable compared to not only *M. exilis*, but even *T. vaginalis*, which has the most complex amino acid metabolism known in metamonads so far (Carlton et al. 2007). Among notable features of the *P. pyriformis* amino acid metabolism are ability to synthesize selenocysteine (previously known only from *Spironucleus salmonicida* among metamonads; Xu et al. 2014), presence of the glycine cleavage system together with connected folate and methionine cycles, and absence of the ADI pathway. Complement of the plasma membrane transporters responsible for nutrient scavenging and keeping cellular homeostasis is similarly diverse as in *T. vaginalis*, but less numerous, which likely reflects differences between the parasitic and free-living lifestyles.

5. Conclusions

We have obtained and thoroughly annotated the genome assembly of *M. exilis*, the first known completely amitochondriate eukaryote. The unreduced cellular complexity shown by the genome informs research on eukaryogenesis by demonstrating that presence of mitochondria is not necessary for existence of a complex eukaryotic cell. The complete loss of mitochondria in *M. exilis* was likely facilitated by a lateral gene transfer of a bacterial SUF system for FeS clusters assembly, which further underlines the essential role of FeS clusters for mitochondria retention in eukaryotes. The lateral transfer of the SUF system occurred before the split of currently living Preaxostyla lineages. The ADI pathway, previously demonstrated to be important for ATP production in *T. vaginalis* and *G. intestinalis*, is more widespread among eukaryotes than previously known and was likely present already in the last common ancestor of Metamonada. *P. pyriformis* has a unique type of MRO and likely a completely cytosolic energy metabolism. Amino acid metabolism in *P. pyriformis* is most complex of all metamonads reported so far.

6. References

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7. Curriculum Vitae

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Education & Related Experience:

- 2013 – Present: PhD study of Parasitology, Faculty of Science, Charles University.
    - 2019: Whole Transcriptome Data Analysis Course, EMBL Heidelberg.
    - 2019: Workshop on single DNA molecule sequencing, Vestec.
    - Since 2018: Supervision of a BSc student on the cell biology of *Paratrimastix pyriformis*.
    - 2017 – 2018, 2018 – 2019: Teaching – Molecular phylogenetics and systematics, practical course.
    - 2017: Invited seminar talk "Genomics and cell biology of *Paratrimastix pyriformis*", University of Warsaw.
    - 2017: Co-supervision of a visiting MSc student on the cell biology of Preaxostyla.
    - 2017: Workshop on Genomics, Český Krumlov.
    - 2016: Opponent for a BSc thesis "Biogenesis and function of peroxisomes, particularly in parasitic protists".
    - 2014: Research stay at the Dalhousie University in Halifax, NS, Canada.
- 2011 – 2013: MSc study of Parasitology, Faculty of Science, Charles University. Thesis: Mitochondrion of *Trimastix pyriformis*.
    - 2013: Course of Methods of Functional Genomics, České Budějovice.
- 2008 – 2011: BSc study of Biology, Faculty of Science, Charles University. Thesis: Lateral gene transfer and its utilization in the phylogeny of eukaryotes.

Funding & Awards:

- 2019: Živa Magazine Award for the best popular science article by authors 26 – 30 years old (Losers Finders: Life without Semiautonomous Organelles), Živa, Czech Academy of Sciences.
- 2017: Bedřich Hrozný Award for creative work (A Eukaryote without a Mitochondrial Organelle), Charles University.
- 2014, 2015: Holz-Conner Travel Award, International Society of Protistologists.
- 2013 – 2017: STARS – Supporting Talented PhD Research Students, Charles University.

Membership:

- Since 2018: International Society for Evolutionary Protistology**.**
  - 2018 – Present: Newsletter editor.
- Since 2014: International Society of Protistologists**.**
  - 2016 – Present: Community manager.
  - 2016 – 2017: Nominating committee.
- Since 2011: Czech Parasitological Society**.**

Publications:

- Karnkowska A et al.: The oxymonad genome displays canonical eukaryotic complexity in the absence of a mitochondrion. Molecular Biology and Evolution 2019, msz147.
- Vacek V, Novák LVF, Treitli SC, Táborský P, Čepička I, Kolísko M, Keeling PJ, Hampl V: Fe-S Cluster Assembly in Oxymonads and Related Protists. Molecular Biology and Evolution 2018, msy168.
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Selected conferences:

- 5th joint meeting of the Phycological Society of America & International Society of Protistologists, Vancouver, BC, Canada, 29/7–2/8/2018. Talk: How to lose a mitochondrion: Comparative genomics of Preaxostyla.
- XXII meeting of the International Society for Evolutionary Protistology, Droushia, Cyprus, 18–27/5/2018. Talk: A comparative genomic study on metabolism and cell biology of Preaxostyla flagellates.
- 15th International Congress of Protistology, Prague, 30/7–4/8/2017. Talk: Genomics and cell biology of the free living preaxostylan flagellate *Paratrimastix pyriformis*. Co-organizer of social events.
- VII European Congress of Protistology (ECOP-ISOP), Seville, Spain, 5-10/9 2015. Talk: Evolutionary History of the Arginine Deiminase Pathway among Eukaryotes.
- Protist 2014, Banff, Alberta, Canada, 3–8/08, 2014. Talk: Mitochondrial organelle of *Trimastix pyriformis*.

8. Selected publications

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