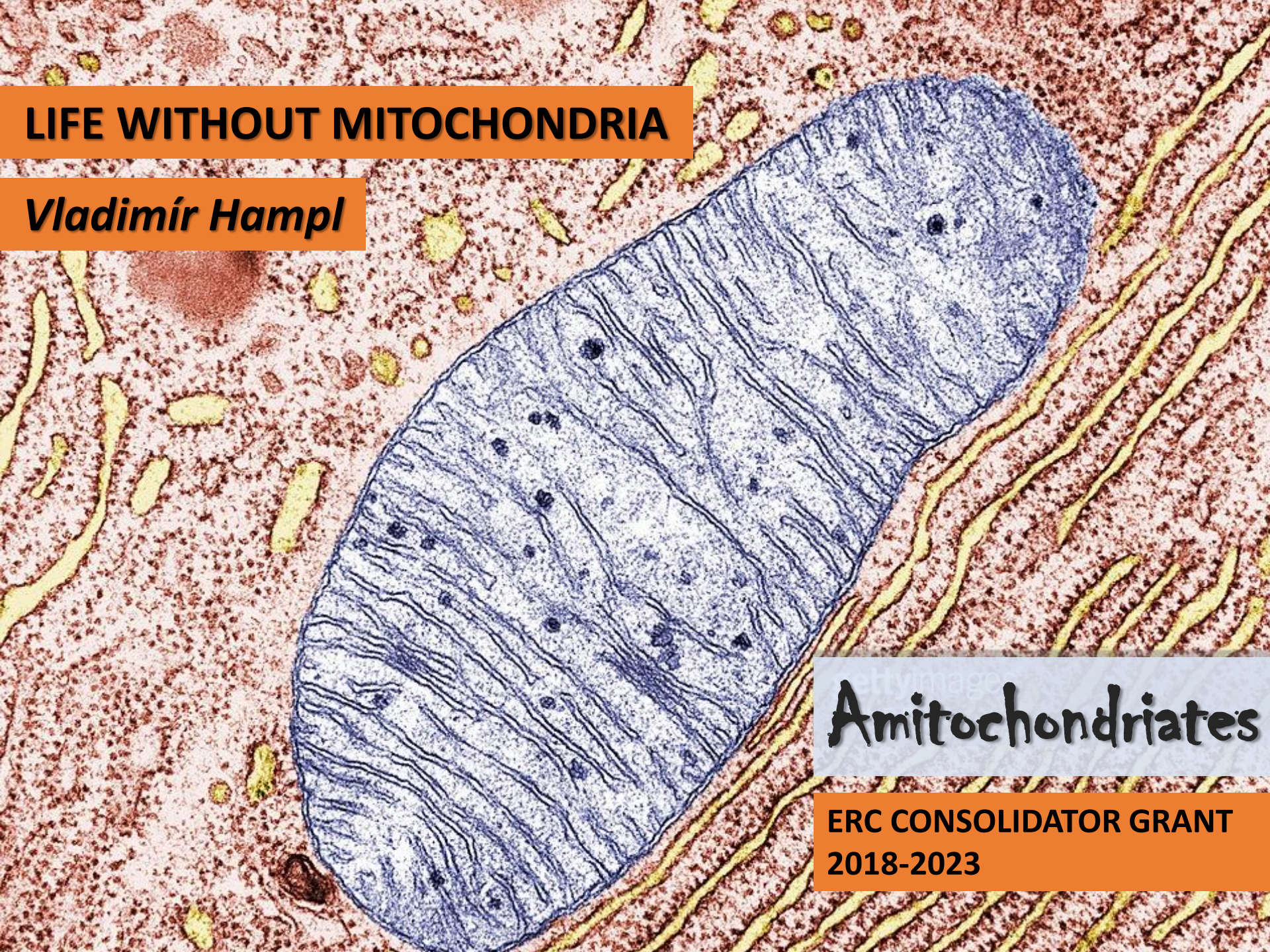
LIFE WITHOUT MITOCHONDRIA

Vladimír Hampl



Amitochondriates

ERC CONSOLIDATOR GRANT
2018-2023

B1 - CURRICULUM VITAE

VLADIMÍR HAMPL, Ph.D.

Head of the **Laboratory of Evolutionary Protistology** (Faculty of Science, Charles University) http://www.protistologie.cz/hampllab

- 1994-1999 – Faculty of Science Charles University in Prague - Biology
- 2001 and 2003 – research stay in laboratory of Dr. Embley at Museum of Natural History London
- 2005 – Ph.D. in Parasitology
- 2006-2007 – Postdoctoral fellow in laboratories of Andrew J Roger and Alastair GB Simpson, Dalhousie University, Halifax, Canada
- Since 2008 – Assistant of Professor
- 2015 – Habilitation
- 2017 – Main organiser of International Congress of Protistology, Prague

B1 – CURRICULUM VITAE

SHOW YOUR IMPACT ON PEOPLE

SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS

Name	Position	Period	Positions after
Zuzana Zubáčová	Postdoc	2012-2013	Postdoc - Institute of Molecular Genetics, Prague
Anna Karnkowska	Postdoc	2013-2015	Postdoc – University of British Columbia (see collaboration with Patrick Keeling)
			Research assoc. – University of Warsaw
Naoji Yubuki	Postdoc	2015-2017	Research assoc. – University of British Columbia
Lucia Hadáriová	Postdoc	Since 2016	
Jana Szabová	Grad. st.	2008-2015	Genomic lab, Faculty hospital Motol, Prague
6 unfinished graduate students, 8 defended master students, 1 unfinished master student.			

I started to build my team in 2008 when I returned from my postdoctoral fellowship. The core of the team consists of PhD students, many of which continue in my team from master studies, but some (Jana Szabová, Sebastian Treitli) come from other laboratories. I am happy that the reputation of the team and the research topics that we deal with are increasingly **attracting students and postdocs from abroad (Slovakia, Romania, Poland, Japan). My current team has 13 members**. My former postdoc **Anna Karnkowska entered from January 2017 research associate position** at the University of Warsaw. Her newly established group **will collaborate on this project**.

B1 - Track record

46 publications
sum of citations=1660
H index of 19.

Present you scientific life like a story

Section c: Ten year' track-record
My major scientific achievements

My impact on the scientific community can be illustrated by two papers, which I consider most substantial. My contribution to both of them was significant and they would not have been published without my involvement. The **first paper is a reconstruction of the eukaryotic tree of life:**

Hampl V, Hug L, Leigh JW, Dacks JB, Lang BF, Simpson AGB and Roger AJ: Phylogenomic Analyses Support the Monophyly of Excavata and Resolve Relationships among Eukaryotic "Supergroups". Proc Natl Acad Sci USA, 2009 Mar 10;106(10):3859-64. Times Cited: 266

This paper was published after my postdoctoral fellowship and at the very end of my career of being a young researcher without my own independent position. We have focused on resolving very difficult nodes in the eukaryotic tree of life, which have largely been avoided by others due to immense methodological difficulties. Thanks to the original approach in handling the data, we were able to recover the existence of a eukaryotic kingdom Excavata.

Since 2008, I have started to build my team and since 2015 it moved to a newly established institute BIOCEV, which is affiliated with our university. **Last year our team published a paper, which I consider our most significant discovery yet. This paper also fulfils a scientific goal which I set 15 years ago, at the beginning of my PhD studies:**

Karnkowska A, Vacek V, Zubáčová Z, Treitli SC, Petrželková R, Eme L, **Novák L,** Žárský V, Barlow LD, Herman EK, **Soukal P,** Hroudová M, Doležal P, Stairs CW, Roger AJ, Eliáš M, Dacks JB, Vlček Č, **Hampl V**. A Eukaryote without a Mitochondrial Organelle. Curr Biol. 2016 May 11. pii: S0960-9822(16)30263-9. Times Cited: 11

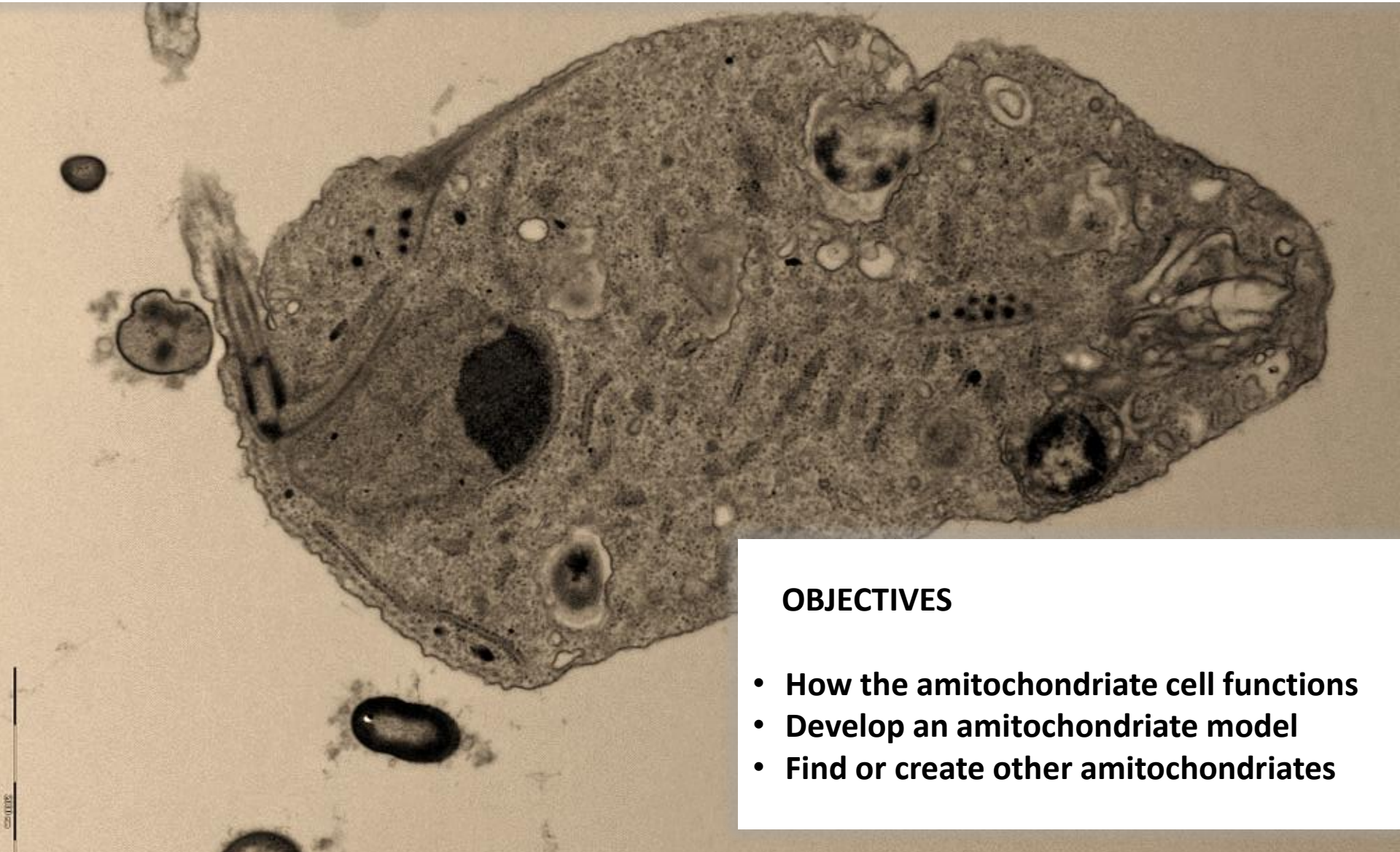
This **ground breaking work demonstrated the absence of mitochondrion in a microbial eukaryote** *Monocercomonoides* (oxymonads) using genomic and cell biological data. The study **refutes the long-held paradigm that amitochondriate eukaryotes do not exist**. It took many years for the methods of genomics and transcriptomic sequencing to become effective and cheap enough to enable our study, in which we show the absence of otherwise canonical organelle. The study included complete genome and transcriptome sequencing and annotation and I am proud that our young team was able to carry out such a demanding task. At the same time the work represents a broad international collaborative project coordinated by our team, from which there are seven authors (given in bold) including the first four. The contribution of our team was by all measures major. I am happy that **the publication received substantial attention in media worldwide (74 news articles)**. According to the Nature index altmetric score, the paper is ranked as 2nd from the Czech Republic in the period September 2015 - August 2016. **The discovery of amitochondriates represents the starting point of this ERC proposal**, in which I plan to develop the research of these interesting organisms up from the genome to the level of cell function.

Organising congress in 2017

SUPERVISORS, ALLOW YOUR PHD STUDENTS PUBLISH WITHOUT YOU!

PROJECT ITSELF

STARTING POINT: The key organism is already discovered. I decided to come up with a mixture of safe (lower gain) and risky (higher gain) objectives for its exploitation.



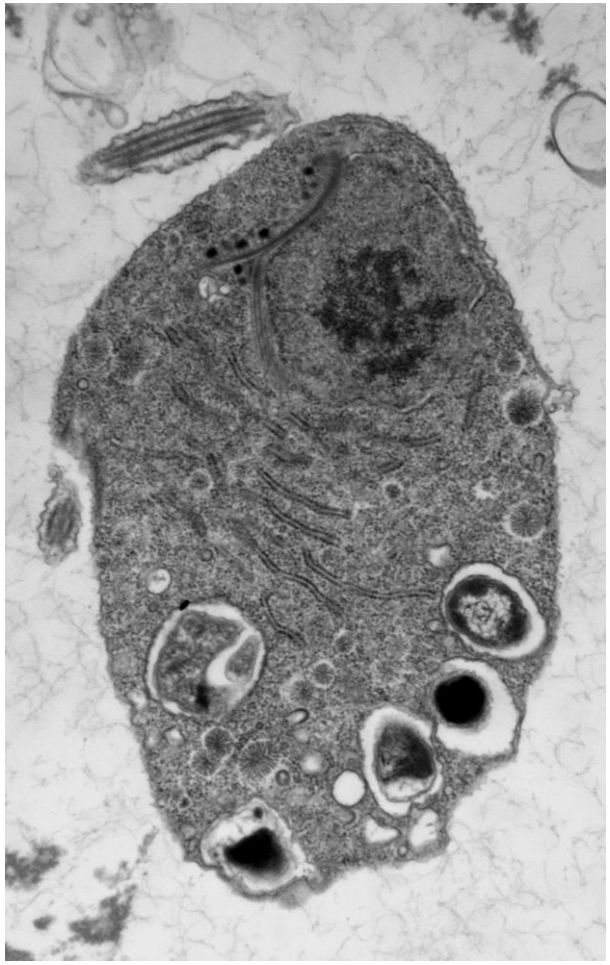
OBJECTIVES

- **How the amitochondriate cell functions**
- **Develop an amitochondriate model**
- **Find or create other amitochondriates**

OBJECTIVE 1: PHYSIOLOGY OF THE AMITOCHONDRIATE CELL

WHY:
To know how the amitochondriate cell functions

HOW:
- Metabolomics
- Functional study on the synthesis of FeS clusters



OBJECTIVE 2: OXYMONADS AS MODEL SYSTEM FOR CELL BIOLOGY

WHAT

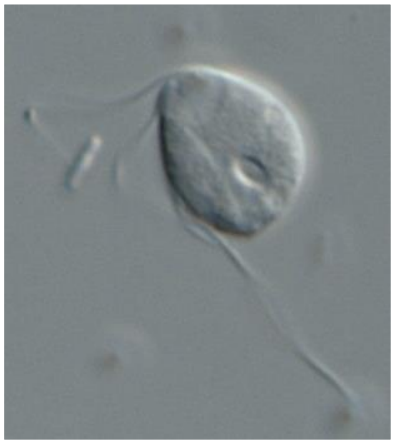
- Axenic cultivation
- Transfection system
- Genetic manipulation

WHY

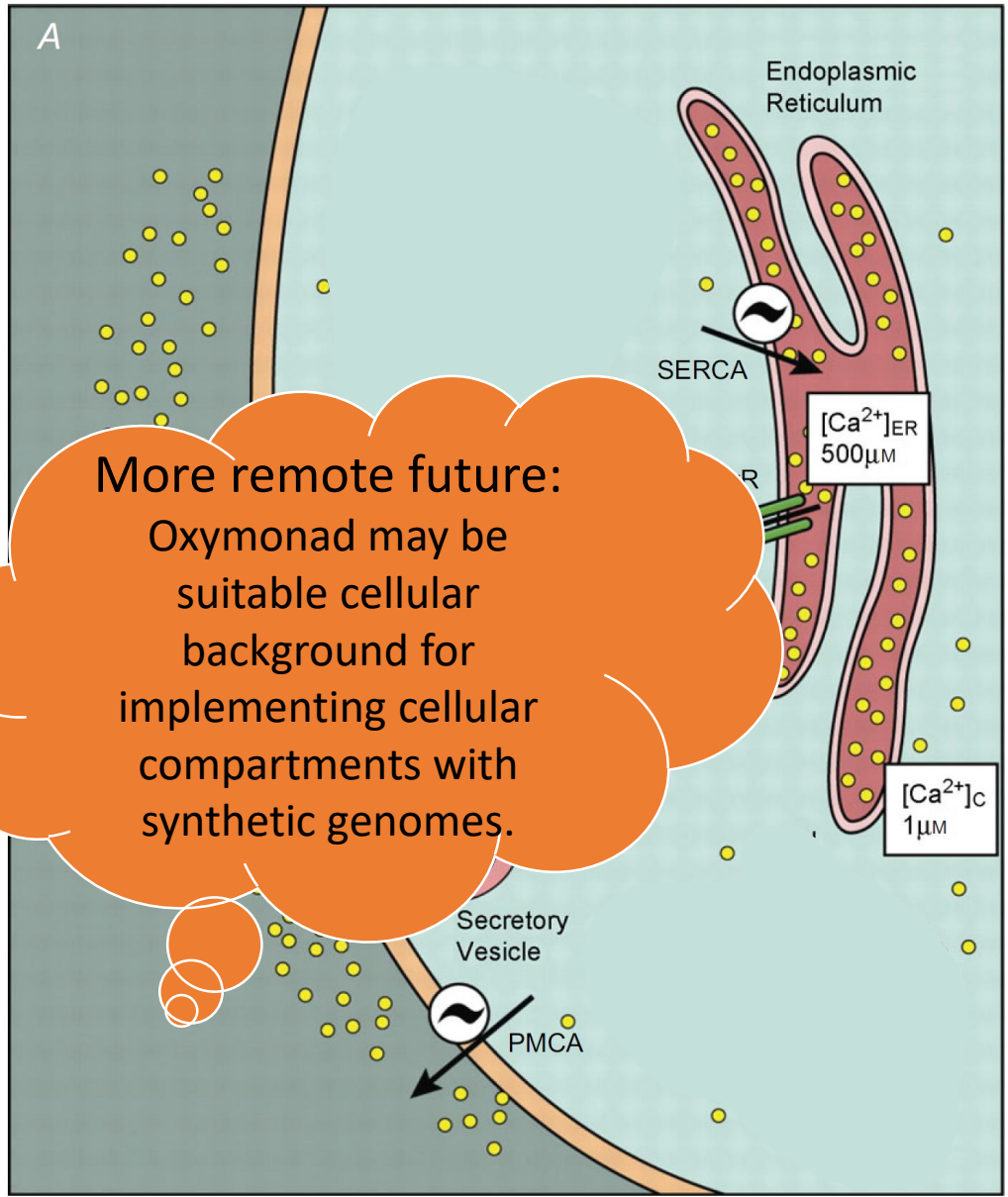
Oxymonad model can be used for experiments in which the influence of mitochondrion needs to be suppressed.

OBJECTIVE 2: OXYMONADS AS MODEL SYSTEM FOR CELL BIOLOGY

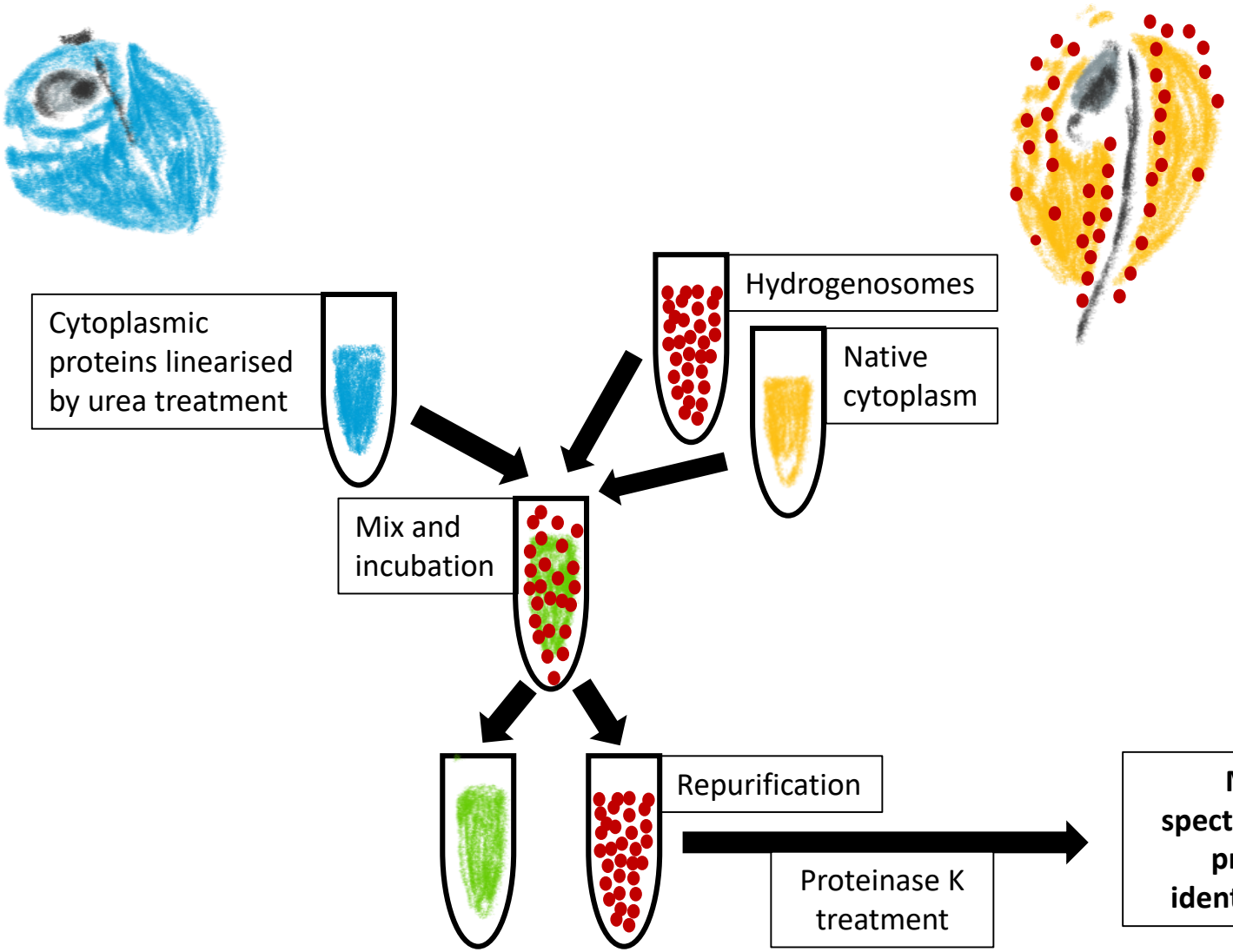
Study of calcium flux in the cell



García-Sancho, 2014



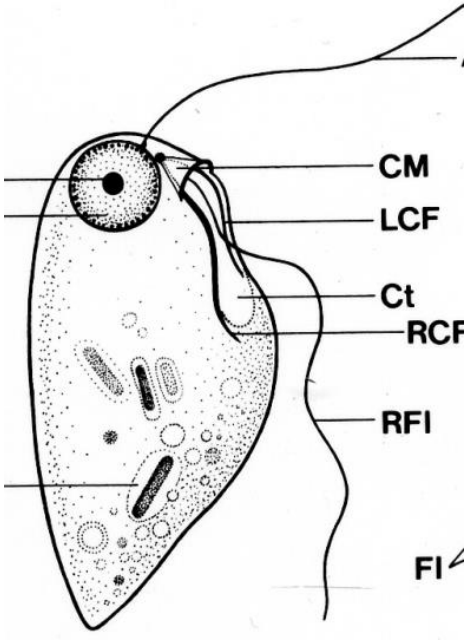
OBJECTIVE 2: EVOLUTION OF MITOCHONDRIAL IMPORT



OBJECTIVE 3: GENOMICS

WHAT:
Investigate more candidates potentially without mitochondria

WHY:
To find more examples of amitochondriates with their specific adaptations

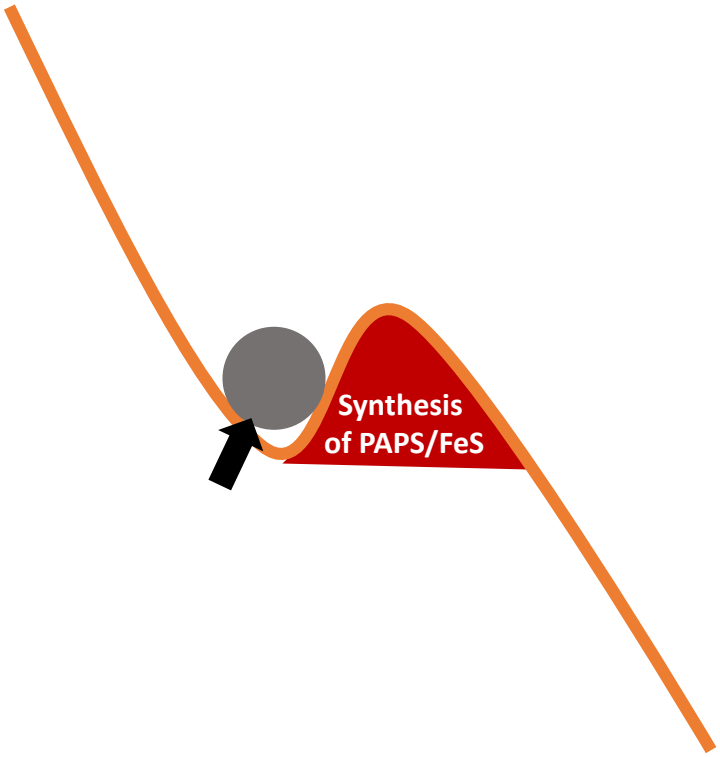


Retortamonas dobelli

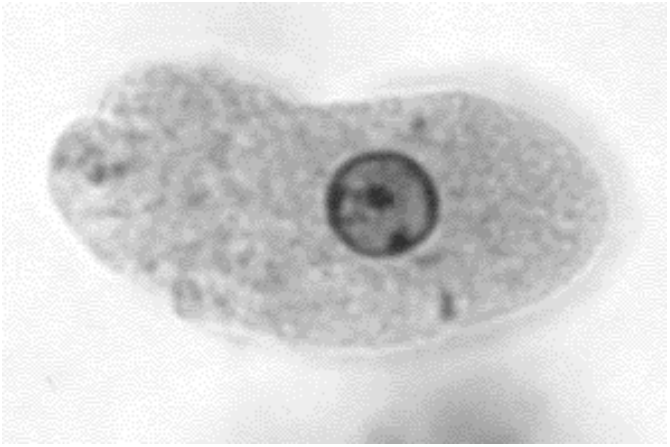
Pelomyxa schiedti

OBJECTIVE 3: EVENT OF MITOCHONDRIAL LOSS

Hypothesis:
Maintenance of higly reduced mitochondria is an evolutionary trap



Model: *Entamoeba histolytica*
(and *Giardia intestinalis*)



B1 – SYNOPSIS

Section a: Extended Synopsis of the scientific proposal (max. 5 pages)

WHAT ARE WE PROPOSING TO STUDY AND WHY?

1. **How the amitochondriate cell functions.**
   WHY: *The cell of* Monocercomonoides *is the first representative of a new cellular type – amitochondriate eukaryotes. As such, its biochemistry and cell biology should be investigated in detail. It will show plasticity of processes connected to mitochondria – namely the synthesis of iron sulphur clusters.*
2. **Simulating the events of mitochondrial origin and loss.**
   WHY: *We would like to understand how difficult it is loose mitochondria and, through experiments, to induce a situation partially similar to the moments when the cell gained and lost this organelle.*
3. **Develop axenic culture and genetic tools for *Monocercomonoides*.**
   WHY: *To create the first amitochondriate laboratory tractable cell culture. This would, firstly, facilitate the research of this unique cell and, secondly, serve as an amitochondriate (blank) for studies of cellular processes, where the role of mitochondrion and other compartments needs to be dissected. An example of a research area where such a simpler model system could find its use is the cellular flux of calcium.*

STATE OF THE ART

Paradigm of mitochondria everywhere

Mitochondria are key organelles of eukaryotic cells. Although they **are often regarded as ATP producing factories, their functions are much broader (Figure 1).** All mitochondria originated in a single endosymbiotic event in which an ancestor of the eukaryote nucleocytoplasmic lineage engulfed an α-proteobacterium[1–4]. This proto-mitochondrion developed into an integrated cellular compartment after it established a specific transport of proteins and intermediates of metabolism between host cell cytosol and itself and after the proto-mitochondrion synchronised its division with the cell cycle[5–7]. This functional integration led to the substantial reduction of mitochondrial genomes, which in some special cases have been lost completely[8–10].

Mitochondria are structurally and functionally very diversified and this is particularly true for the mitochondria of **protists – single cell eukaryotic microbes representing the widest evolutionary diversity of eukaryotes[11]. In several independent lineages of protists living in low oxygen environments, mitochondria underwent functional and structural reductions (Figure 2)[9,12–14].** Outcomes of this reductive process are hydrogenosomes and mitosomes, organelles in which the most typical mitochondrial functions, oxidative phosphorylation and mitochondrial genome expression and maintenance, are absent. Mitosomes are the most reduced mitochondria, and have completely lost the capacity for ATP synthesis. Mitosomes are found only in parasitic protists (e.g. *Giardia* and *Entamoeba*).

Prior to 2016, it was widely thought that despite the existence of very reduced mitochondria in several lineages, the organelle itself had never been lost. This applied not only to naturally occurring organisms but also to laboratory mutants. Of the latter, probably the best known are Rho0 yeast mutants (known also as petite mutants) that had completely lost the mitochondrial genome and oxidative phosphorylation but still retained small mitochondria[15]. Other examples include various tumour cells lines that have shifted their energy metabolism towards glycolysis (Warburg effect), yet still keep mitochondria[16]. It should be noted that mature mammalian red blood cells do not contain mitochondria, but they also lose other essential cellular components such the nucleus, endoplasmic reticulum and Golgi body and so are not viewed as cells but rather as haemoglobin filled bodies. In summary, **until 2016 it was a widely accepted paradigm that all eukaryotic cells in nature contain mitochondria** in some form. The reason for the existence of

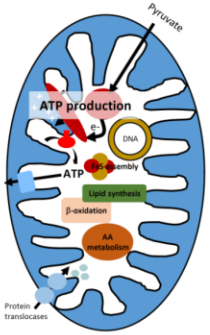


Figure 1: *Mitochondrion has more functions than the production of ATP.*

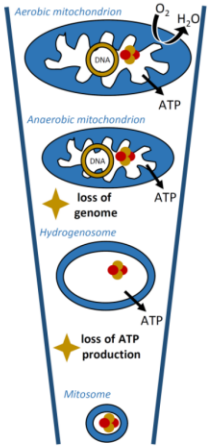


Figure 2: *Mitochondria of anaerobes are functionally reduced. Synthesis of iron sulphur clusters remains.*

the minimalistic mitosomes remains, in some cases, unclear, but mostly they function to synthesize iron-sulphur clusters[17].

Mitochondria and the synthesis of iron-sulphur clusters

Around 100 proteins in the cell (70 in humans) contain **iron-sulphur clusters (Figure 3)** in their active sites[18]. This **inorganic prosthetic group serves to transfer electrons in redox reactions, in catalysis, in regulation of gene expression and as a sulphur donor**[19]. In the form of pyrite minerals, it hypothetically formed part of the most ancient biochemistry at the origin of life[20,21]. Iron-sulphur clusters are part of several indispensable proteins, and so their synthesis is essential for every cell[19]. **The clusters can be formed** and inserted into the target protein spontaneously, but efficiency of this process is low[22]. In living cells, this process is catalysed **by one of four known enzymatic systems** along with some specific helper proteins. In the prokaryotic world, three enzymatic machineries, which are distantly related to each other, are known to perform this task – the nitrogen fixation (NIF), iron-sulphur cluster assembly (ISC) and sulphur mobilisation (SUF) systems[23]. In a nutshell, the process starts by extracting sulphur from a cysteine by the activity of cysteine desulphurase (NifS, IscS or SufS) and coupling the elemental sulphur with elemental iron. This process requires ATP and the reducing power of electrons. The cluster is first built on a dedicated scaffold protein (e.g. NifU, IscU, SufB) and then transferred to the target protein via an intermediate carrier.

A typical eukaryotic cell contains the ISC pathway, which is always localised in its mitochondrion and it was clearly inherited from the prokaryotic mitochondrial ancestor[24,25]. The chloroplasts (or other types of plastids) always contain SUF pathway, analogously inherited from the cyanobacterial ancestor of these organelles[24,25]. Iron-sulphur cluster containing proteins in these organelles are thus formed by the action of the dedicated organellar pathways. The iron-sulphur clusters **in the cytosolic and nuclear proteins are formed by yet another eukaryotic specific machinery called CIA[26].** An important feature of CIA is that it lacks the cysteine desulphurase, and so it cannot synthesise iron-sulphur clusters *de novo*. In all studied cases, the **CIA is dependent on** an unknown intermediate produced by the mitochondrial **ISC pathway (Figure 4)**[24]. When the mitochondrial ISC pathway is repressed, the formation of cytosolic and nuclear clusters also stops[27]. In some cases, like in the minimalistic mitochondrion (mitosome) of *Giardia*, the ISC pathway in the organelle appears to serves only for the assembly of iron-sulphur clusters in the cytosolic and nuclear proteins, because the organelle itself does not contain any pathway in which iron-sulphur cluster containing proteins function[28,29].

Oxymonads – a group rejecting the paradigm

Oxymonads are a group of flagellates distantly related to *Trichomonas* and *Giardia*. This group contains ~140 described species, which live in the intestine of insect and mammals[30]. In termites and cockroaches, they form an important component of the intestinal microflora digesting cellulose[31]. **Many oxymonad species are poorly known and we know close to nothing about the functioning of these cells.** In 2016 the existence of oxymonads helped to reject the paradigm that all eukaryotic cells require mitochondria to survive. **Our team showed that oxymonad cells are a result of a unique series of evolutionary events in which mitochondrion has been lost[32].** This situation is unprecedented. It is clear that during the process of mitochondrial loss the organisms must have solved several life threatening problems and we speculate that **the pre-requisition for this loss was a major rearrangement in the biosynthetic pathway of iron-sulphur clusters[32].**

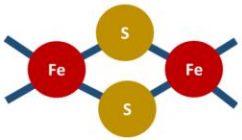


Figure 3: *Iron sulphur clusters are co-factors of redox reactions present in several essential proteins*

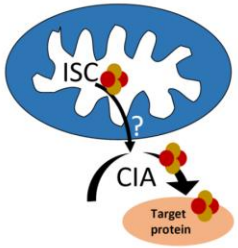


Figure 4: *Synthesis of cytosolic iron sulphur clusters is dependent on the mitochondrial ISC pathway.*

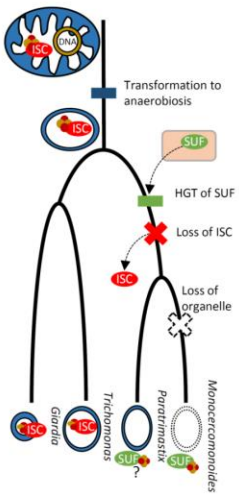


Figure 5: *Pre-requisition for the loss of mitochondrion in* Monocercomonoides *was the acquisition of SUF pathway and loss of mitochondrial ISC pathway*

TIMING OF THE WRITING

- One year – contemplation in the backgroud
- 6 months – serious thinking
- 4 months – writing
  - start early (procrastination is more painful than the writing itself)
  - set deadlines and keep them (better to have sleepless night due to personal deadline one month before submission than just before submission)
  - feed back is absolutely neccesary (Do not be shy to ask for feedback people you know and people you do not know, e.g. me. BUT you must allow them enough time! Week is not enough)

LIFE WITH THE GRANT

- Money source until 2023
- Hired 5 new postdocs (total 20 members of the team)
- Management relatively easy for you – budget for grant manager

OUR TEAM

Amitochondriates

THE END