Evolution of Mitochondria and anaerobic lifestyle

Martin Kolisko

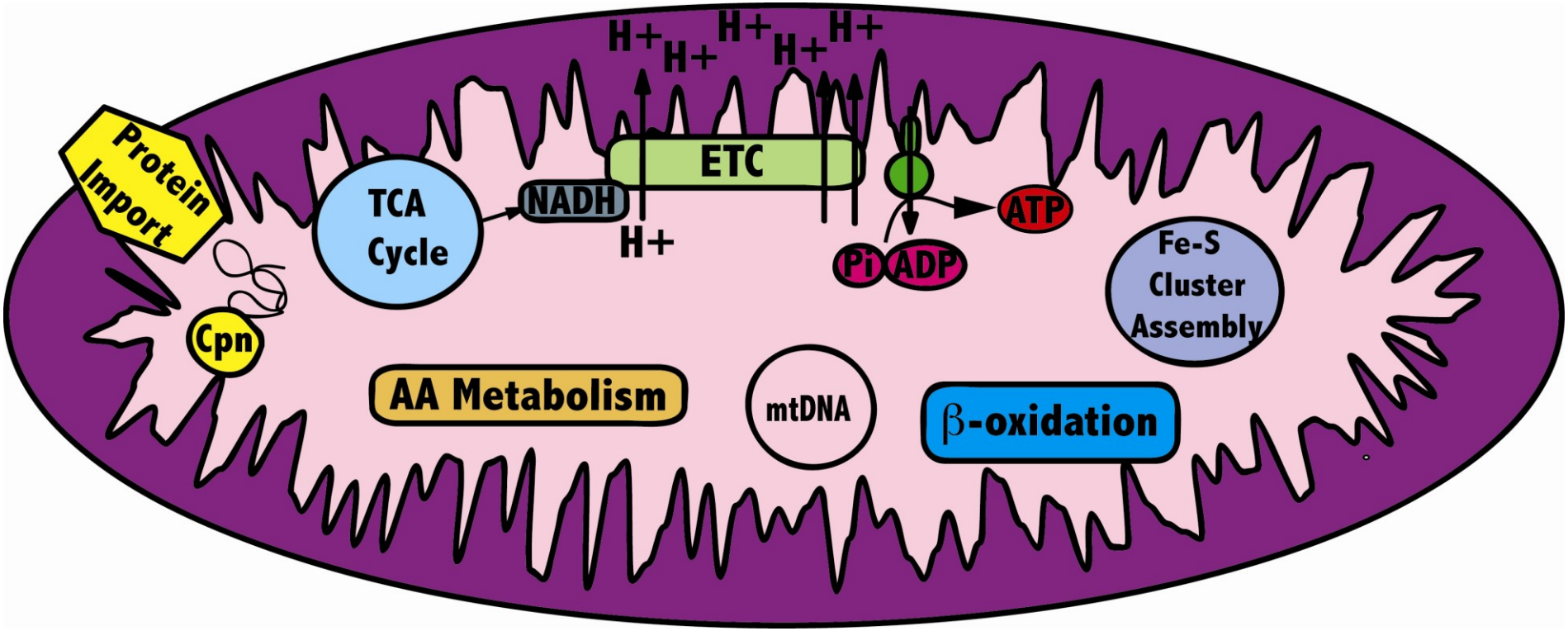
Mitochondria

- Often Considered “The Powerhouse of the Cell”
- Contains its own genome – “semi-autonomous organelle”
- Double membrane bound
- Cristae – formed by the folding of the inner membrane
- One of signature characteristics of Eukaryotic cell

Mitochondria – functions

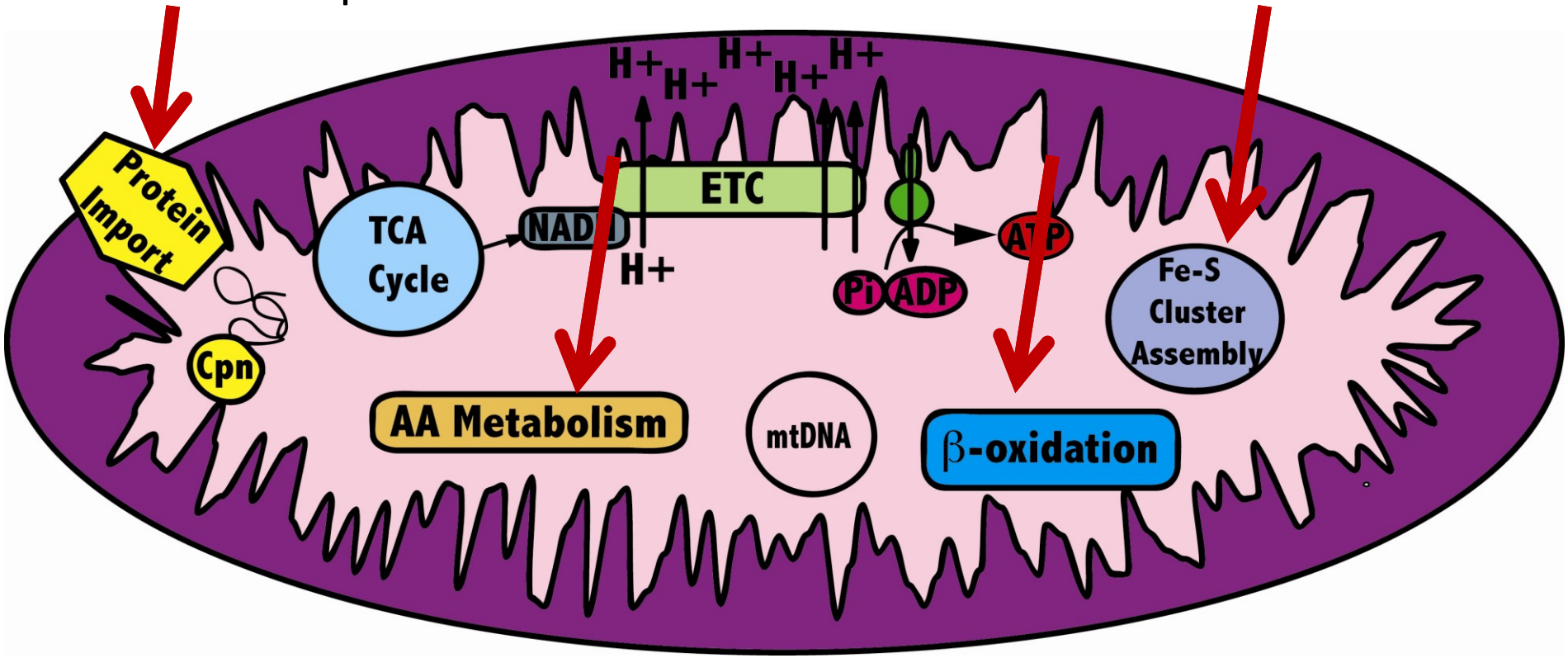
- Oxidative phosphorylation ➔ Pyruvate – Acetyl-CoA – Proton Gradient – ATP synthesis

- Also called cellular respiration



Mitochondria – more functions….

- Energy generation
- Fe-S cluster assembly
- Fatty acid metabolism
- Amino acid metabolism
- Protein import



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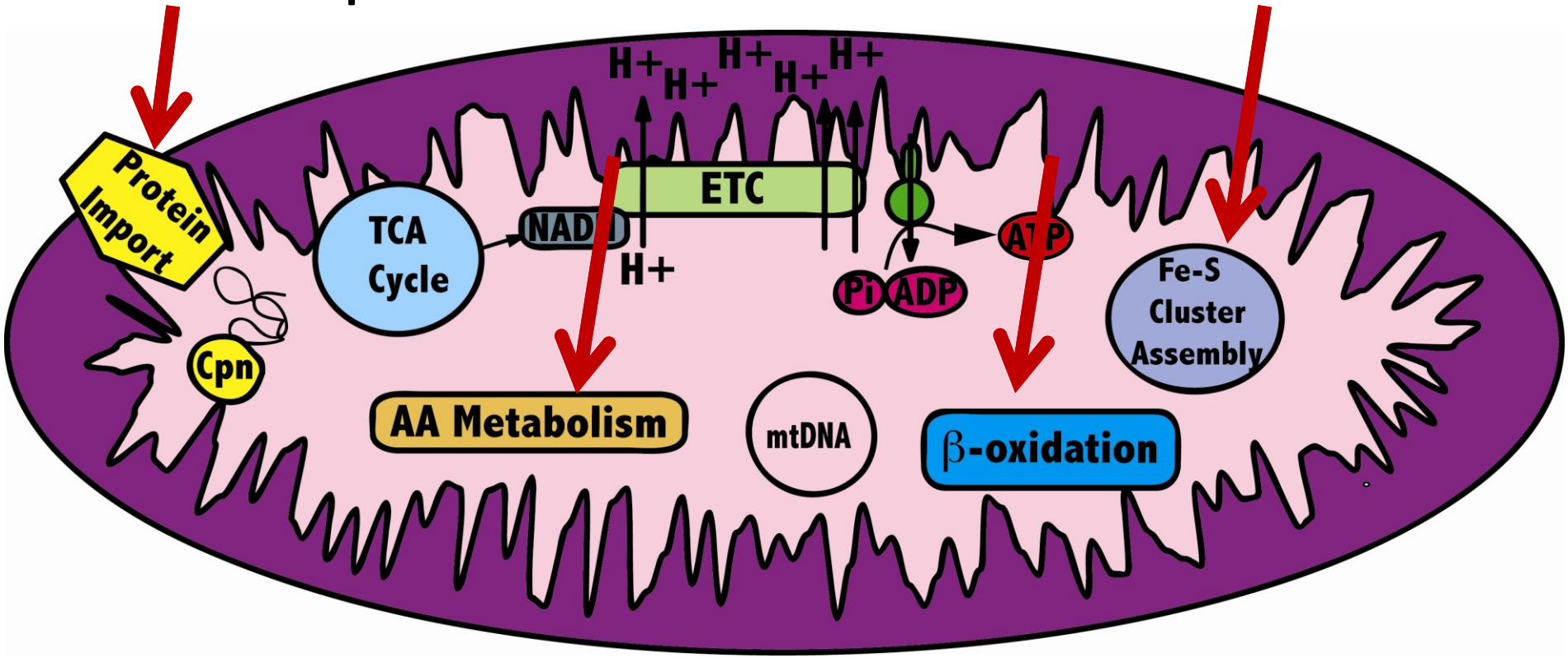
- **Protein import**



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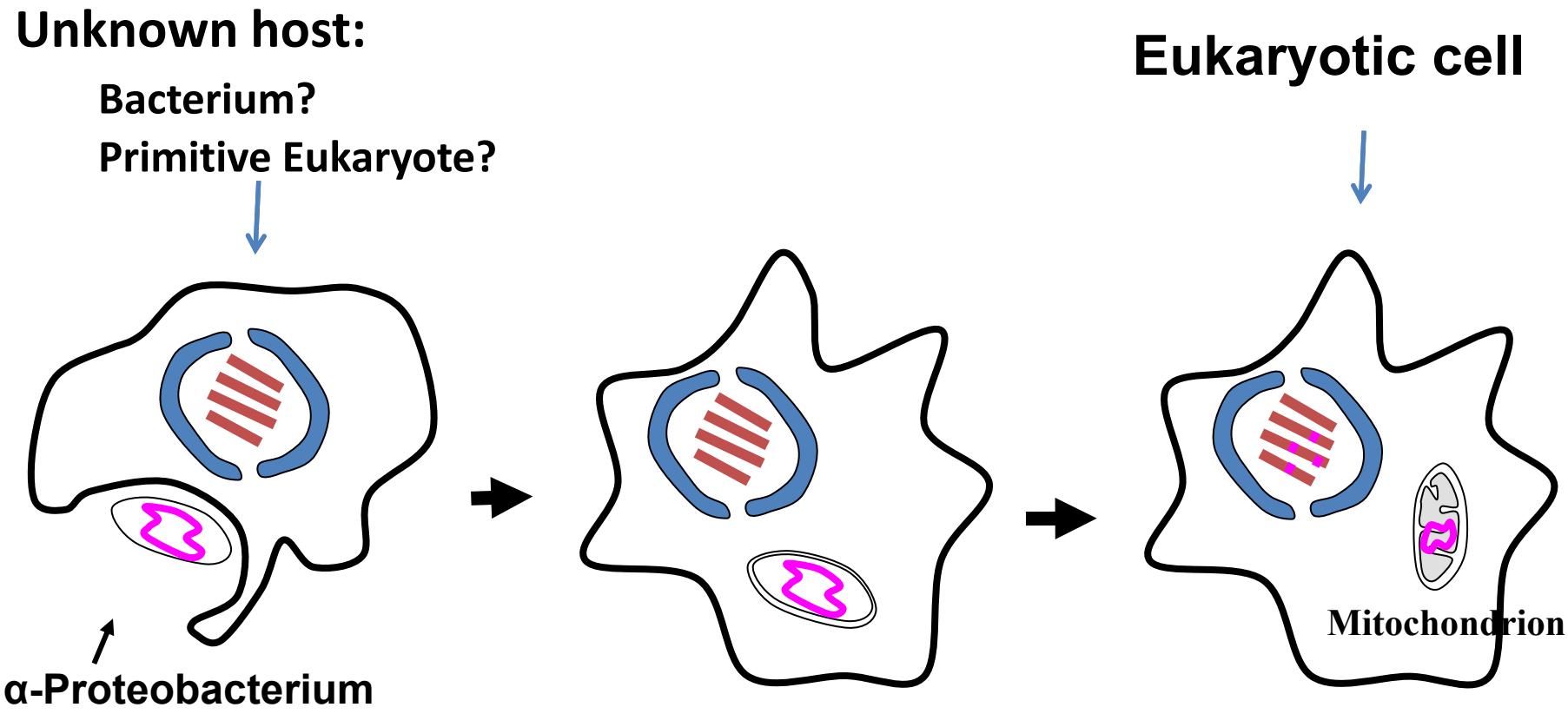
Mitochondria – evolutionary origins

Endosymbiosis



Endosymbiosis

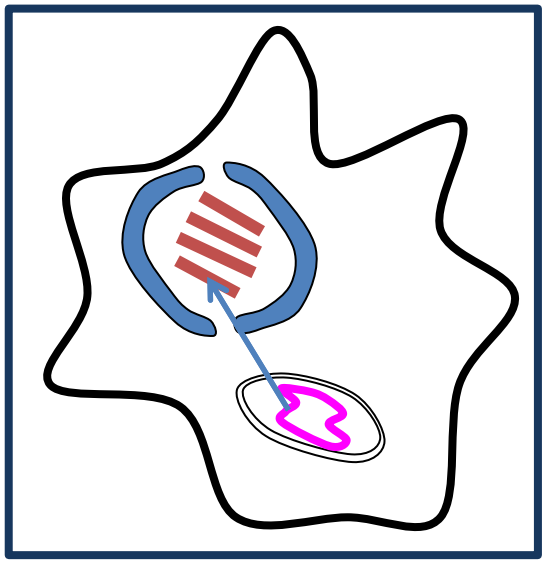
- Internalization of one single cell organism by another single-cell organism
- The internalized organisms is eventually transformed into an organelle (e.g. mitochondrion/ chloroplast)
- Organellar genome is slowly reduced and genes are transferred to the host nucleus

EVIDENCE for endosymbiotic origin of mitochondria?

Mitochondria – evolutionary origins

- Circular genome – bacterial-like
- Different mitochondrial genome reduction. Different organisms possess different number of genes and vary in sizes:
  - genes: **5** genes in *Plasmodium* vs. **94** genes in *Reclinomonas*
  - size: ~**5**Kb in *Plasmodium* vs. ~**100**Kb in *Jakoba*
- Translational (mRNA into protein) machinery similar to bacteria
- Some Bacterial-like biochemical pathways and functions
- Phylogenetic evidence

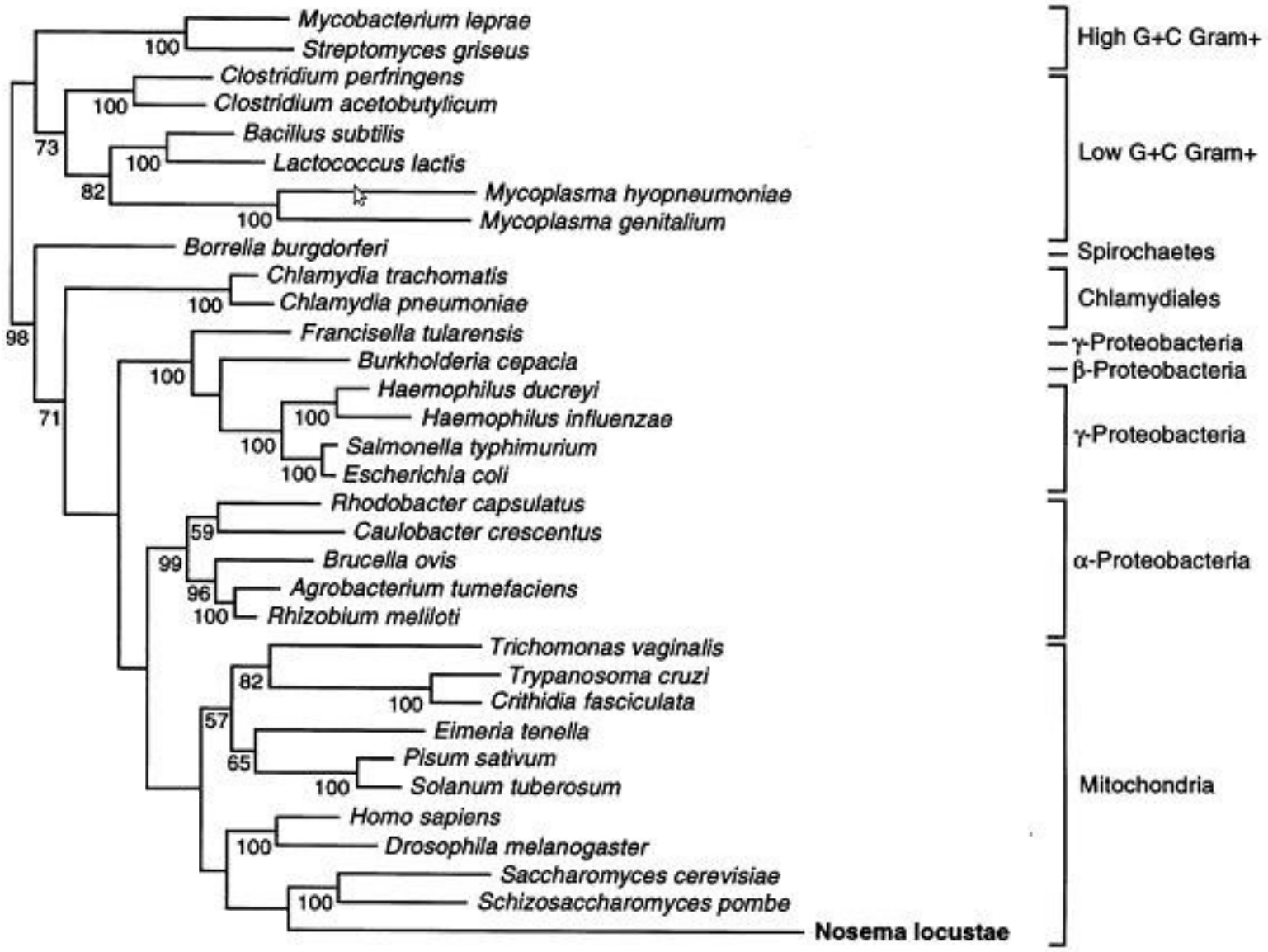
Endosymbiosis



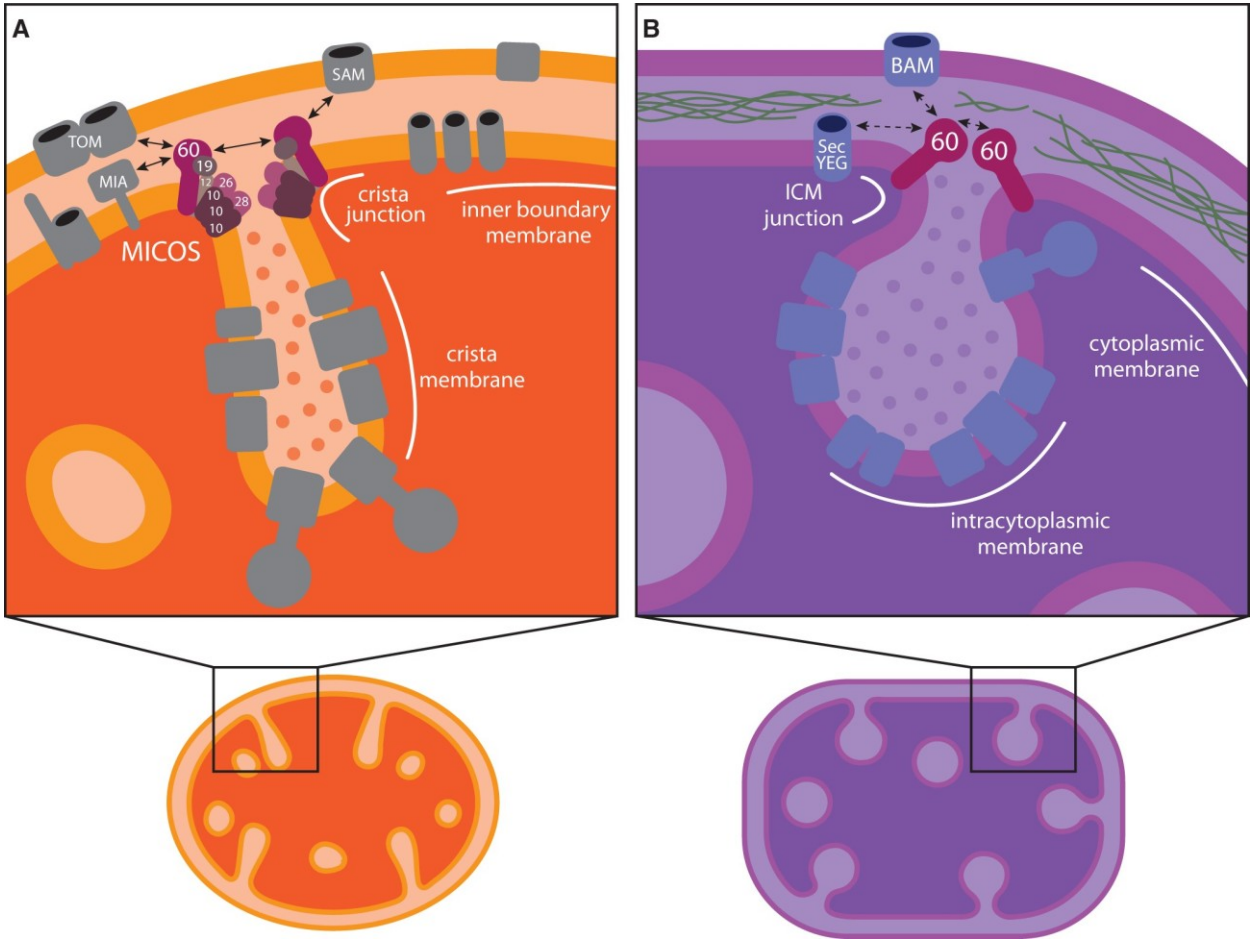
Gene transfer – This means that there are genes in the eukaryotic genomes that originated from the mitochondria.

Making phylogenies based on these genes may help understand the origin of mitochondria

α-proteobacterial origin



Cristae origins



Mol Biol Evol, Volume 34, Issue 4, April 2017, Pages 943–956, https://doi.org/10.1093/molbev/msw298

Targeting of proteins into Mitochondria

Targeting of proteins into the Mitochondria

- Mitochondrial genome codes for only a fraction of proteins that function in mitochondrion (1 – 2%).

- Most of the mitochondrial proteins are imported into the mitochondrion.

- Targeting of the proteins into the mitochondrion is therefore crucial for the function.

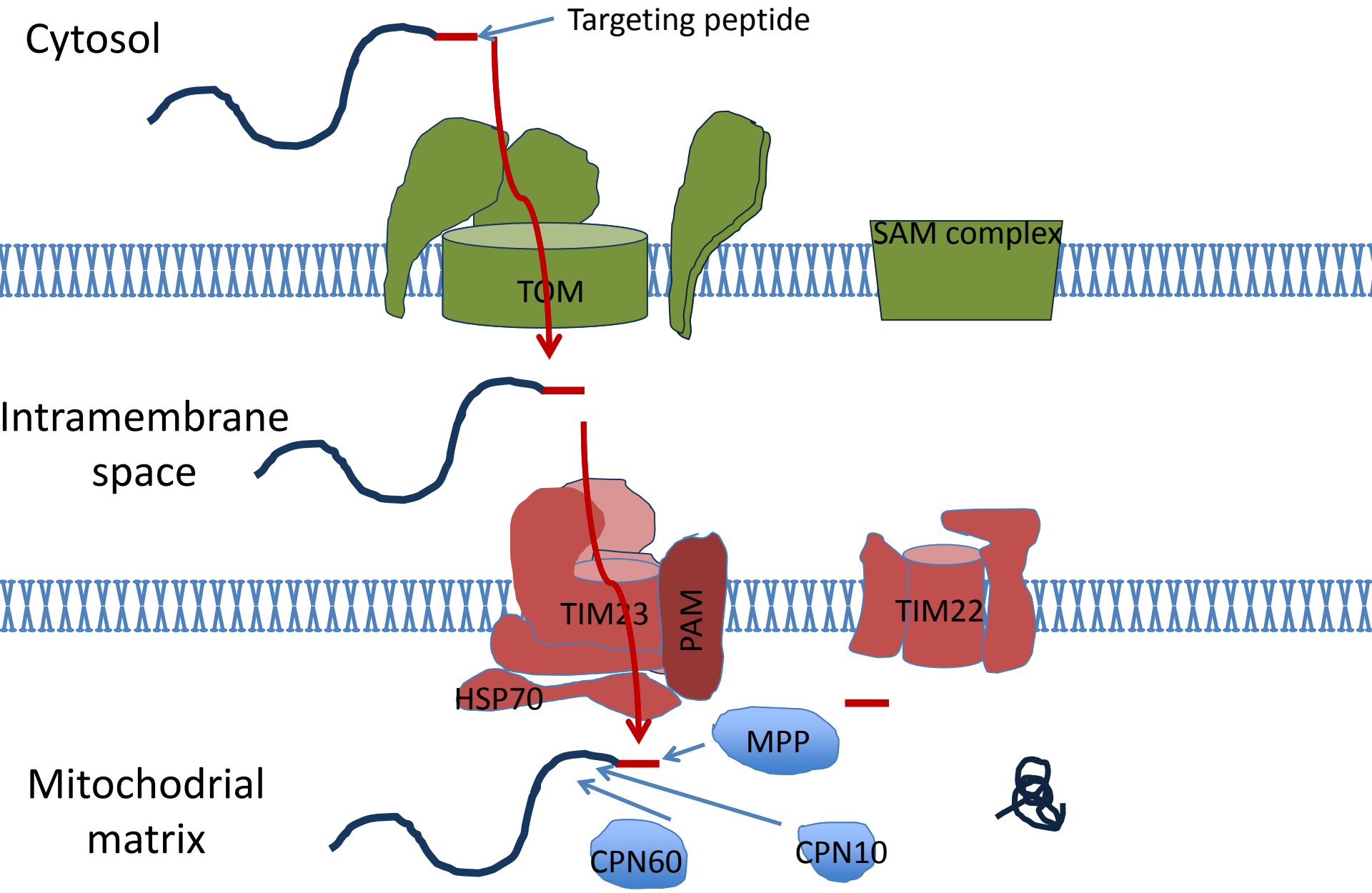
Targeting peptide

- Proteins are mostly imported to mitochondrion through targeting peptide

Targeting peptide:

- usually 10-80 amino acids long
- rich in positively charged residues
- forms amphipathic helix - all charged residues on one site – this is recognized by the translocating proteins

Targeting of proteins into the Mitochondria



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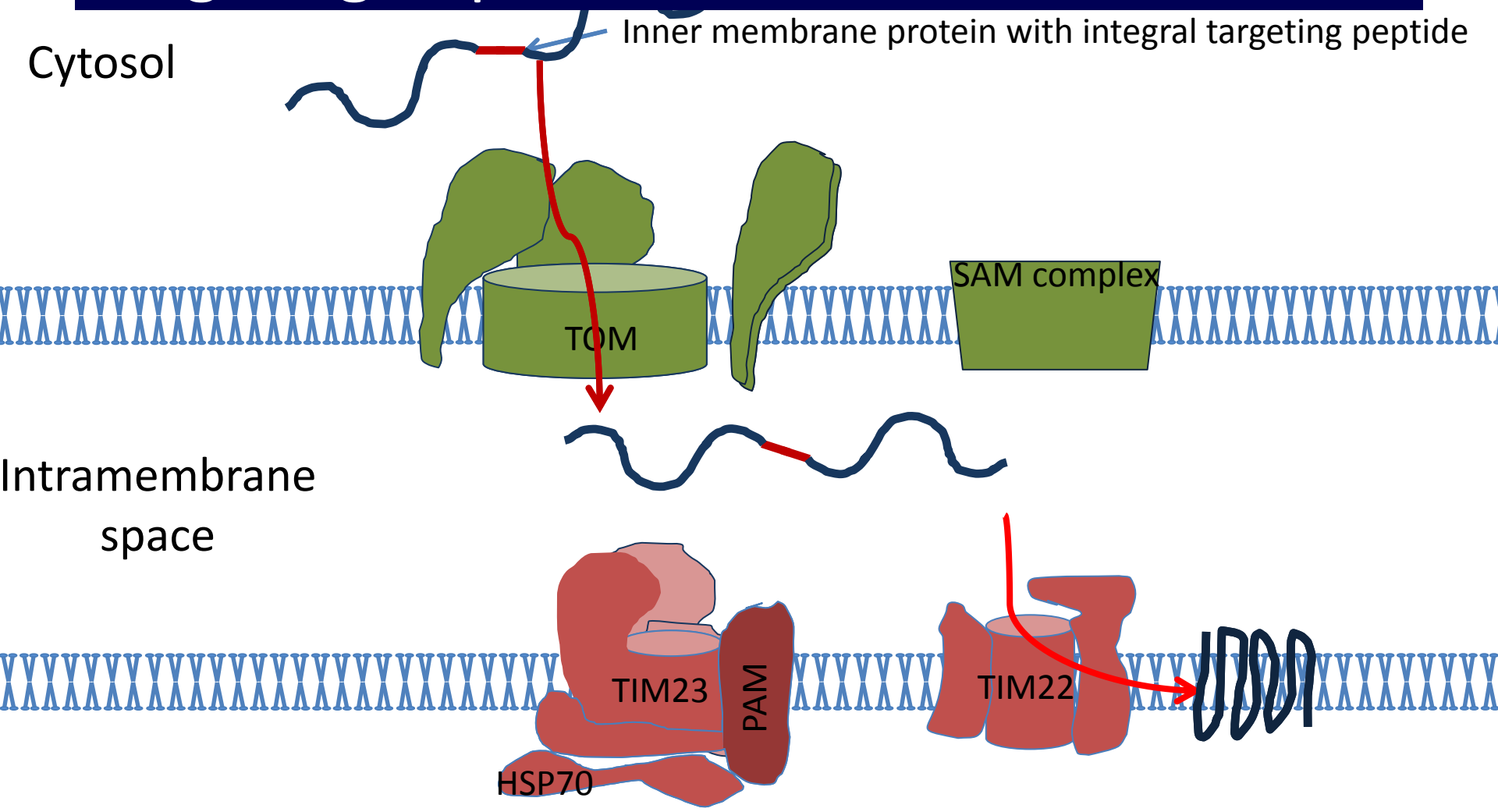

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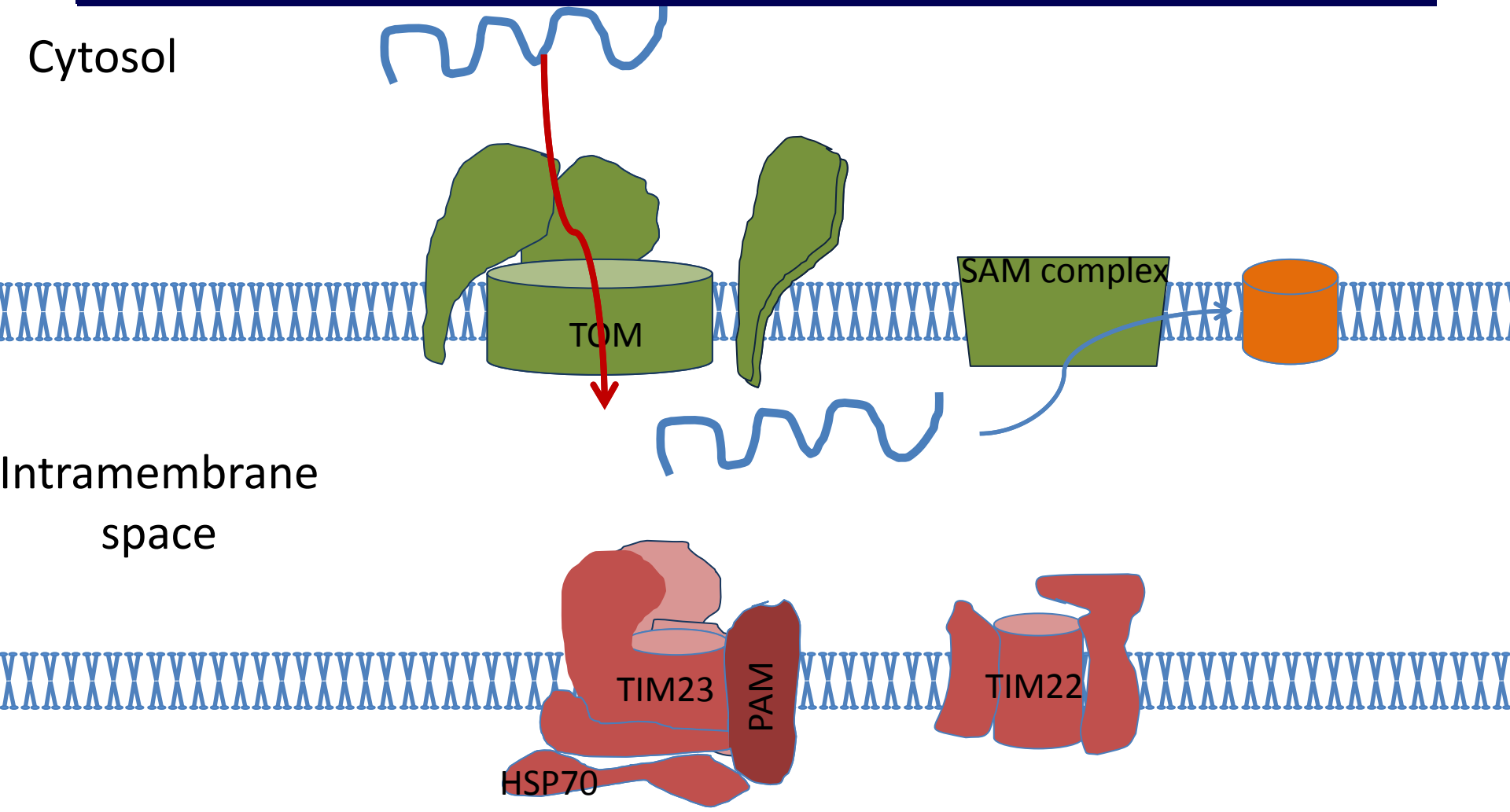

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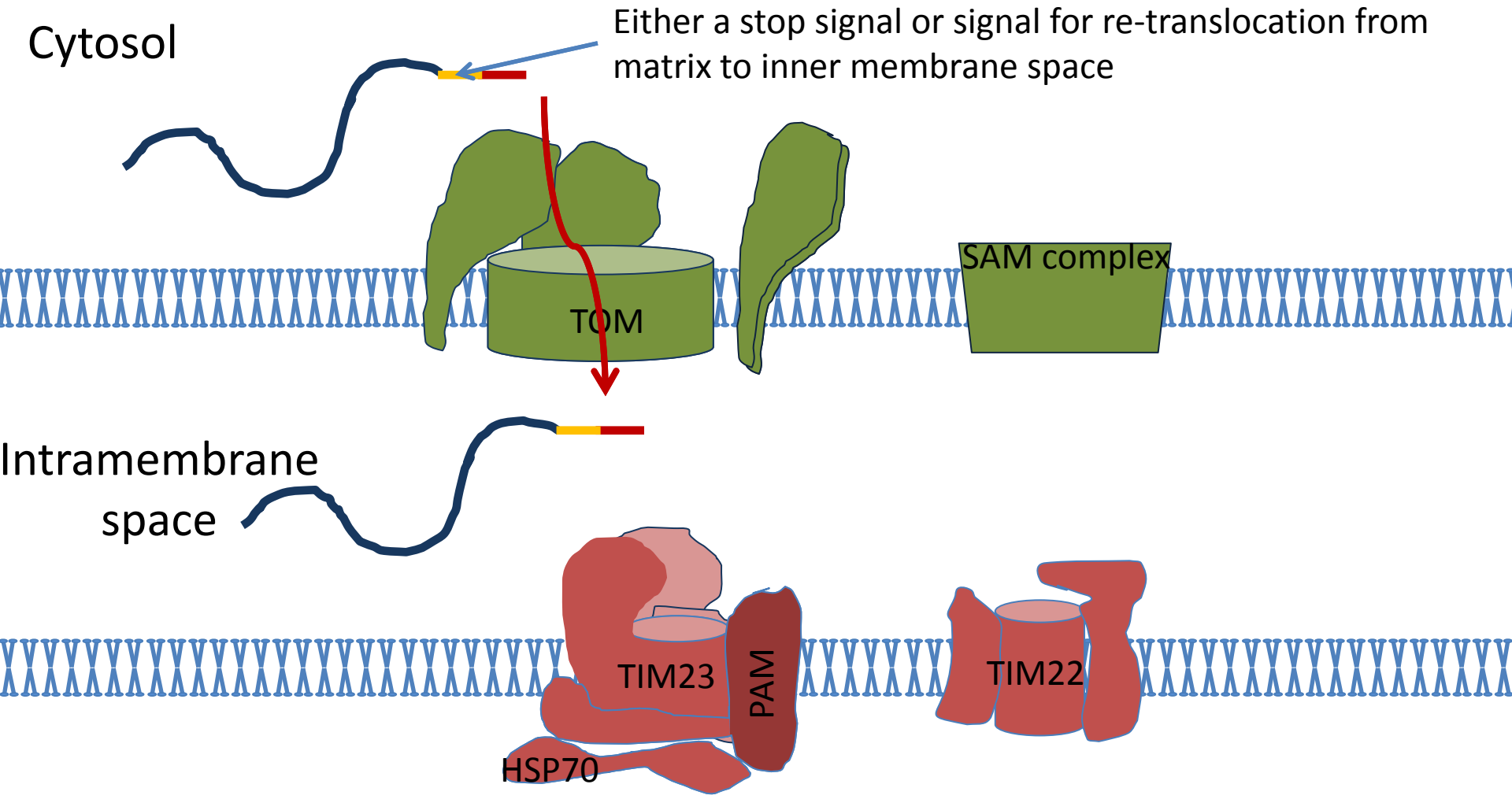
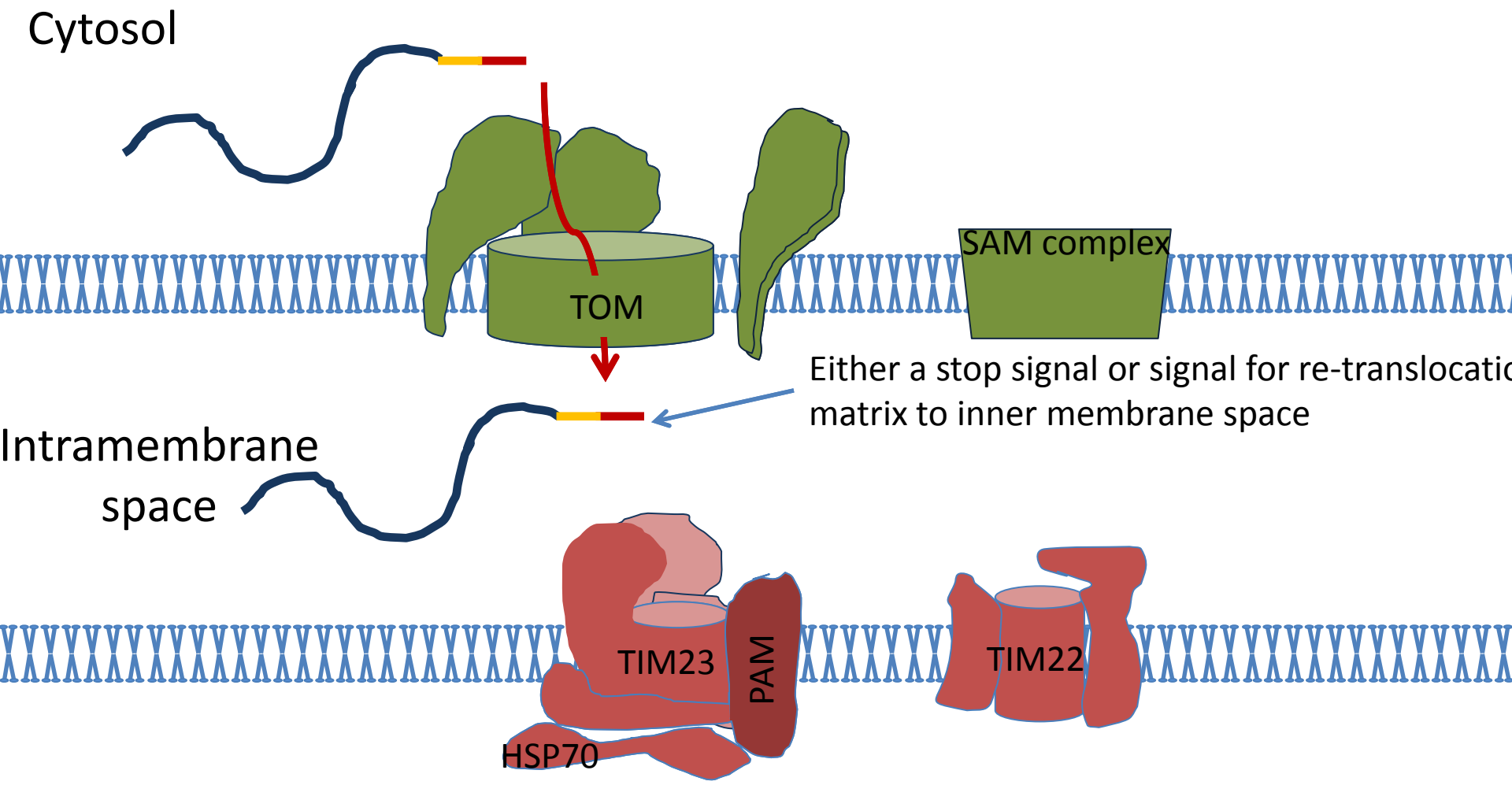
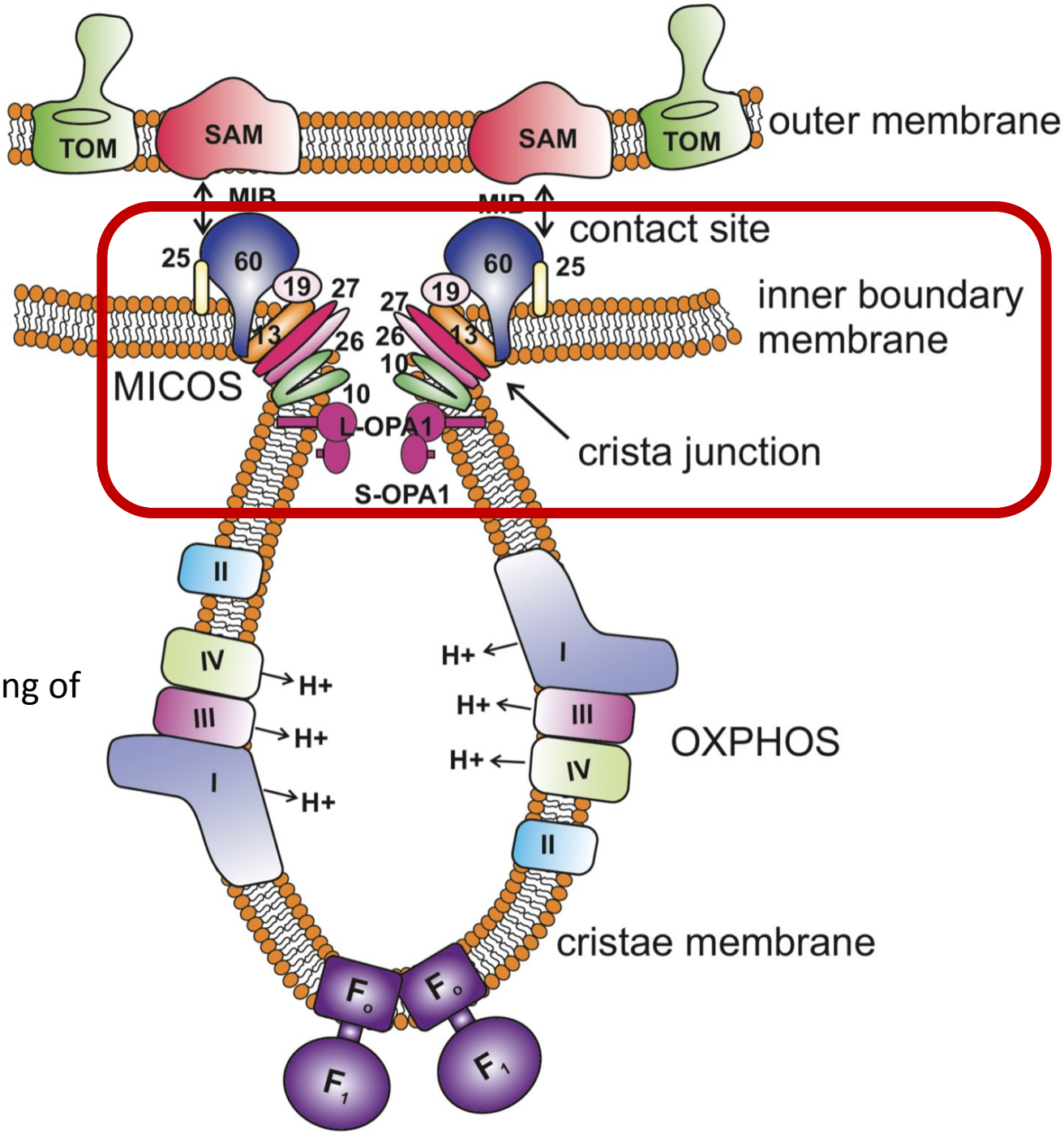


Targeting of proteins into the Mitochondria



Micos



Mitochondrila Cristae Organization:

MIC proteins, that ficiliate flding of the inner membrane.

Targeting of proteins into the Mitochondria

Quick repeat:

Targeting peptide – rich in charged residues

TOM complex transports across outer membrane – cytosolic HSP70 are used to deliver the protein to the TOM complex

TIM complex transports across inner membrane – **mtHSP70** facilitates the transport

Tim22 complex – transports inner membrane proteins

Tim23 complex – transports into the matrix

Mitochondrion – Iron-Sulfur cluster assembly

Iron-Sulfur clusters are part of Iron-Sulfur cluster proteins

There are vital part of …

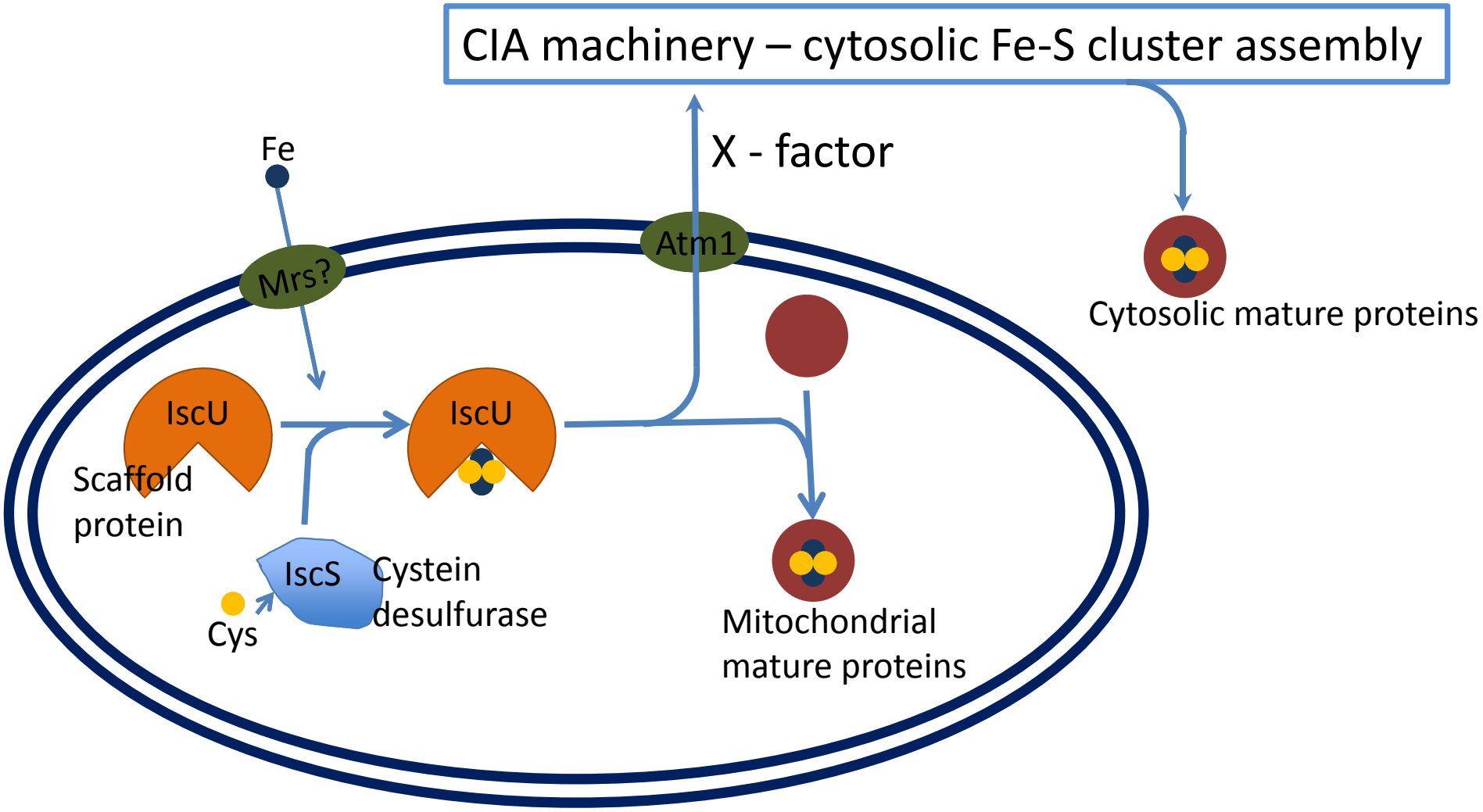
For example:

- Ferredoxins
- aconitase

Several Fe-S assembly systems

Fe-S cluster assembly system	Cytosolic	Mitochondrial	Plastid	Prokaryotes
Cytosolic Iron Sulfur Cluster Assembly (CIA)	+			
Iron sulfur cluster (ISC)		+		+
Sulfur Mobilization (SUF)			+	+
Nitrogen fixation (NIF)				+

Mitochondrion – Iron-Sulfur cluster assembly



Quick rep.

- Mitochondria originated through endosymbiosis
- Most of mitochondrial genes were transferred to nucleus and are imported to mitochondria post-translationaly
- Proteins are imported into mitochondria through TOM and TIM complexes
- Iron-Sulfur assembly is an obligatory function of mitochondria and is done through bacterial type ISC system

Anaerobic lifestyle

Anoxic mud

Deep sea

Gut

Bacterial mat

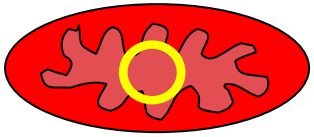
Ocean

marshes

Anaerobic life-style

- Few terms:
    - **Facultative anaerobes:** tolerate oxygen
    - **Strict anaerobes:** oxygen is toxic
    - **Microaerophiles:** live in an environment with low-level of oxygen

What happens to mitochondria?



Anaerobic mitochondria

- Electron transport/oxidative phosphorylation
- use alternate electron acceptor, not O_2

- However many organisms seemed not to contain any mitochondrion
- In past they were considered primarily amitochondriate
- However,…

Amitochondriate organisms

- Organisms that do not possess mitochondria

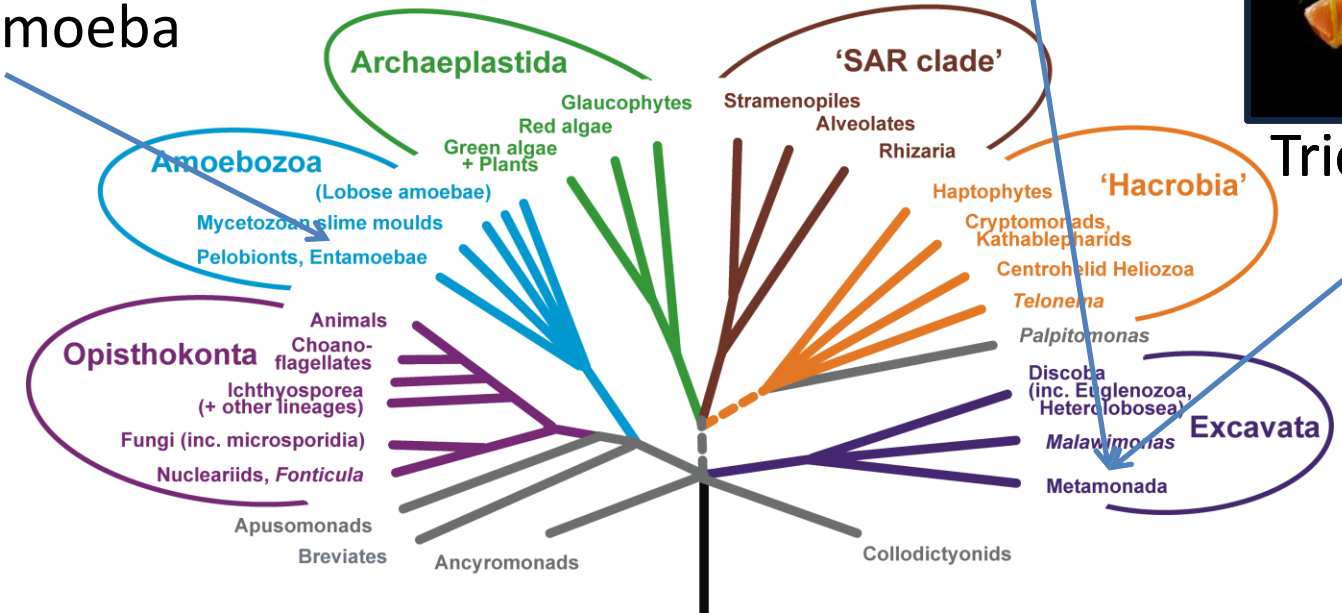
- Primarily amitochondirate – originated before the acquisition of mitochondria

Amitochondriate organisms

Entamoeba

Giardia

Trichomonas

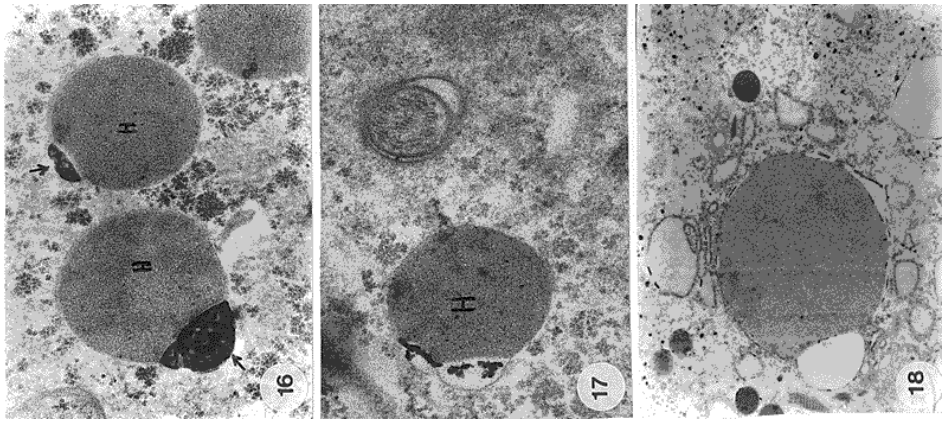


How are they generating energy?

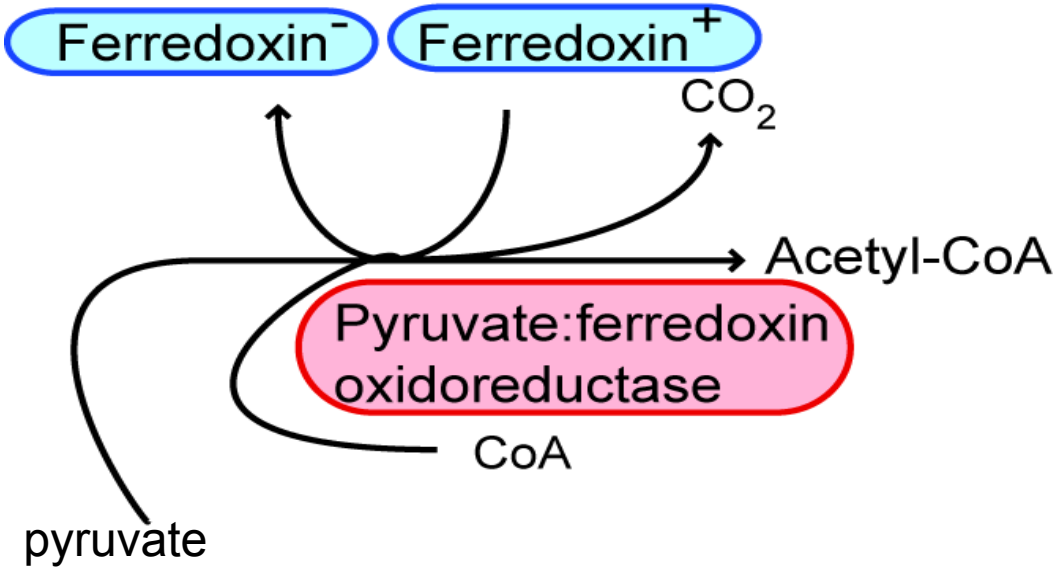
Trichomonas vaginalis

- Parasite of men – STD trichomoniasis

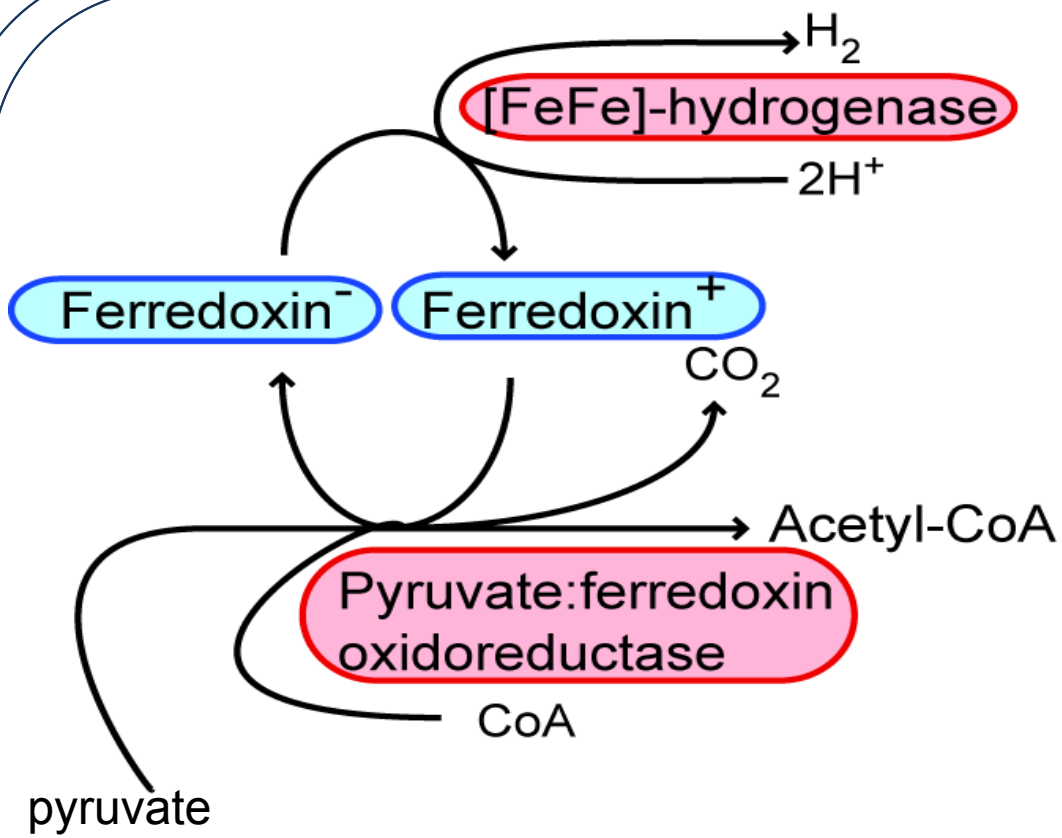
- Posses Hydrogenosomes:
  - Double membrane bound organelle
  - no DNA
  - no cristae



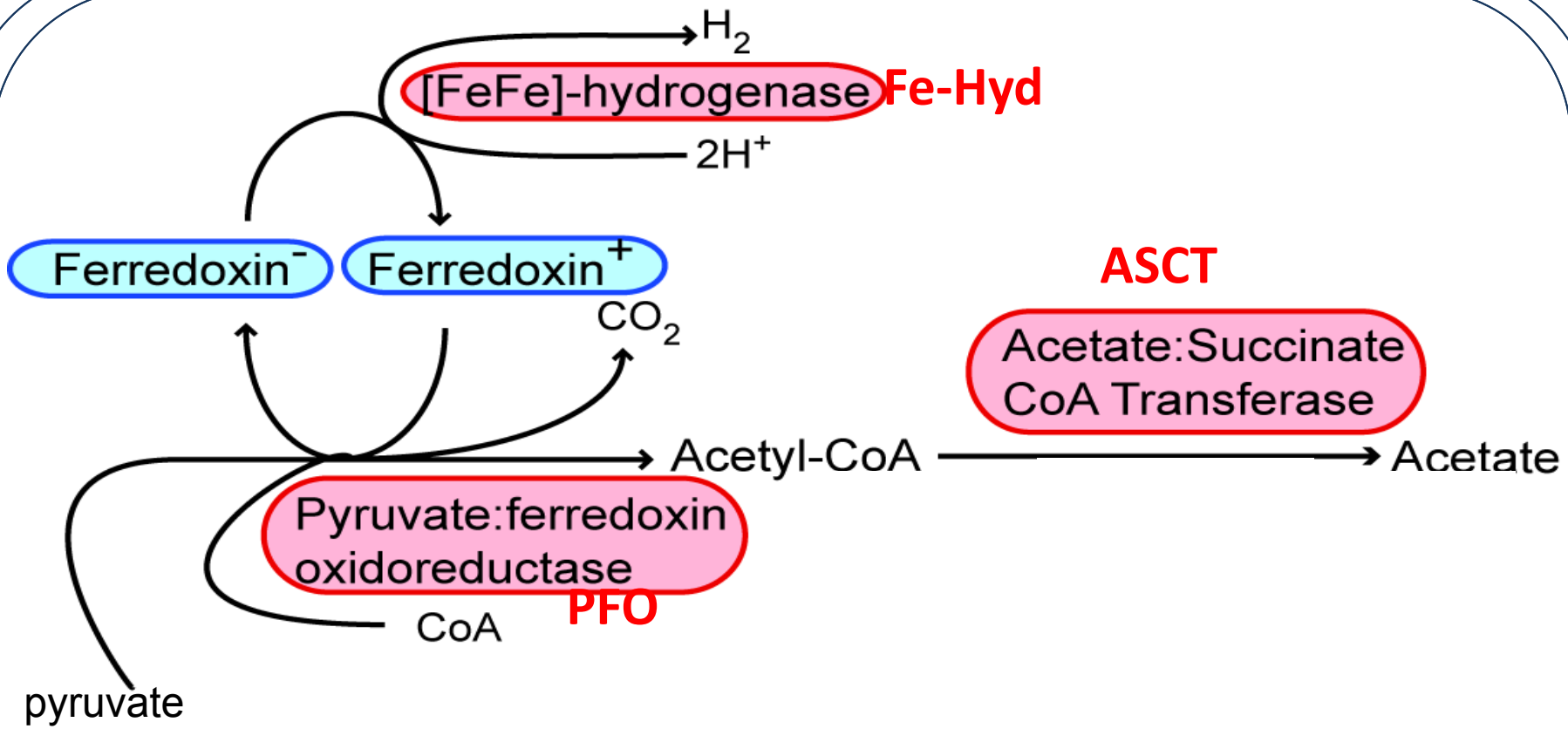
Hydrogenosomes



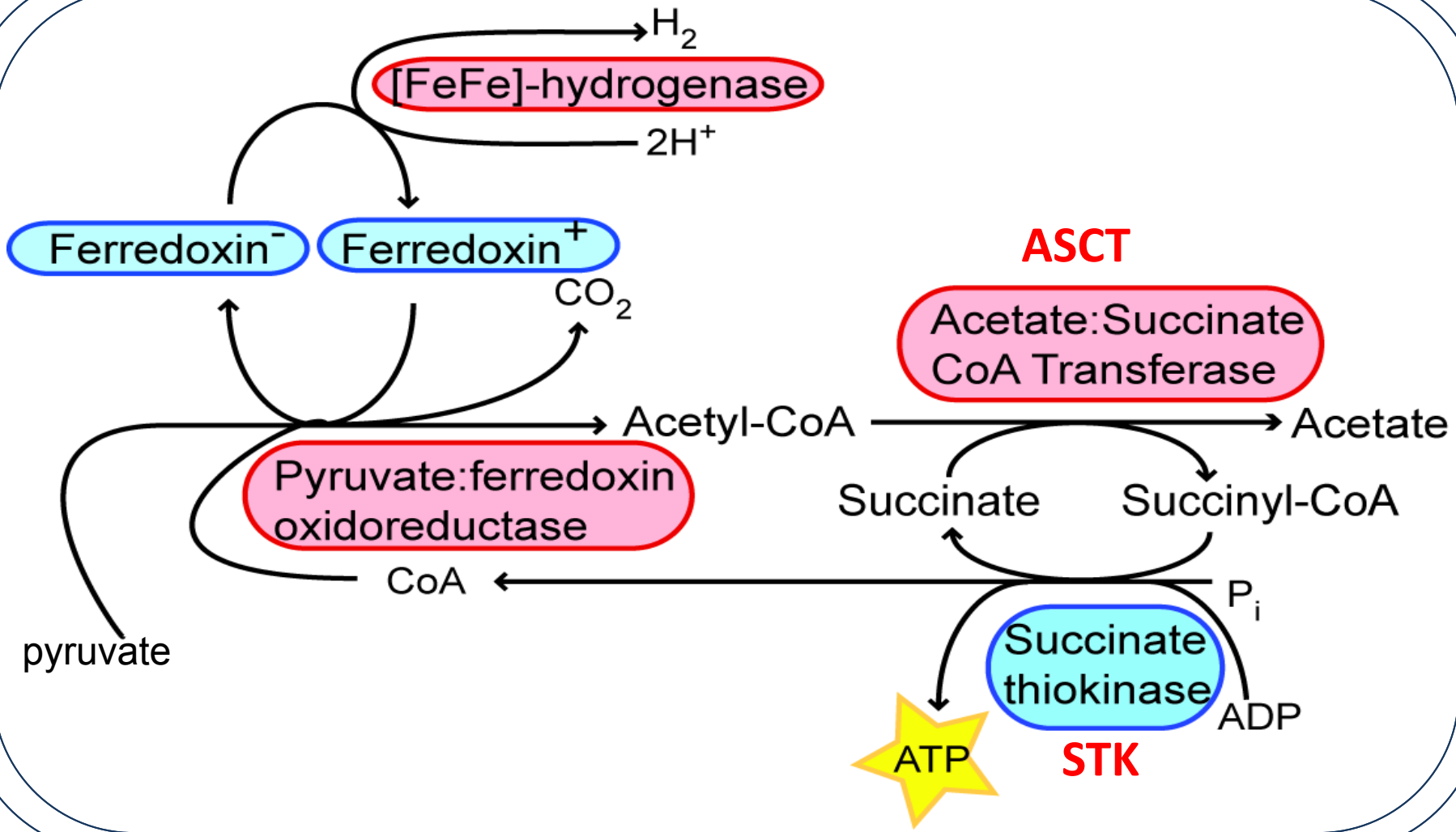
Hydrogenosomes



Hydrogenosomes



Hydrogenosomes

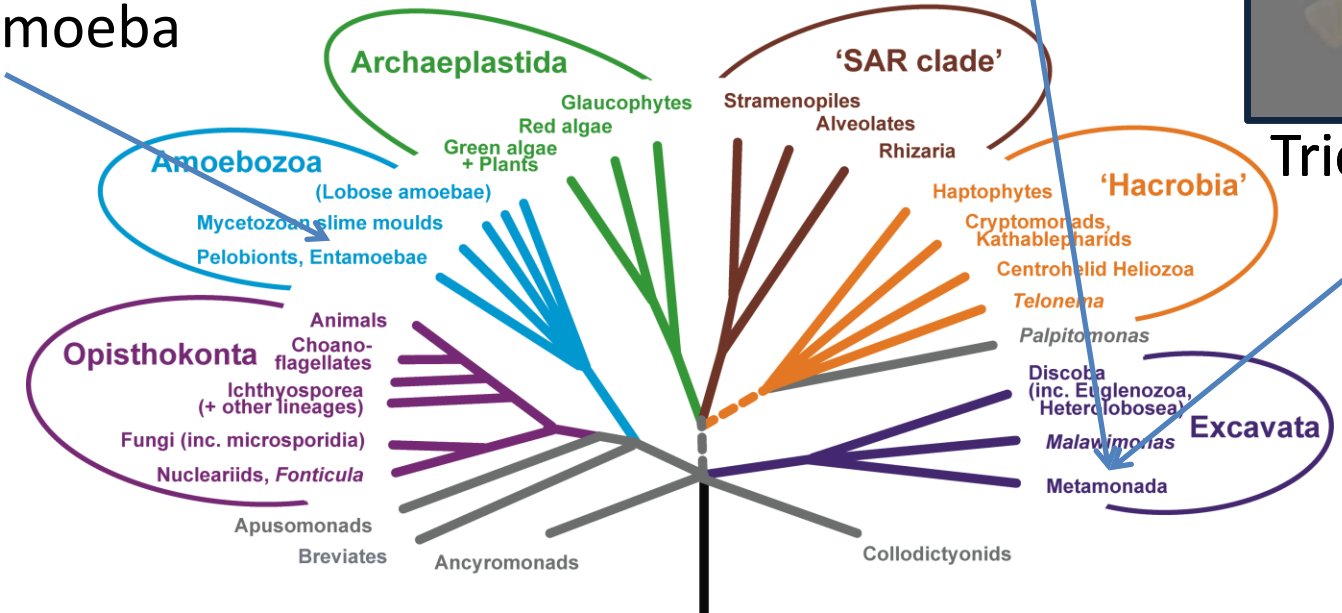


Amitochondriate organisms

Entamoeba

Giardia

Trichomonas



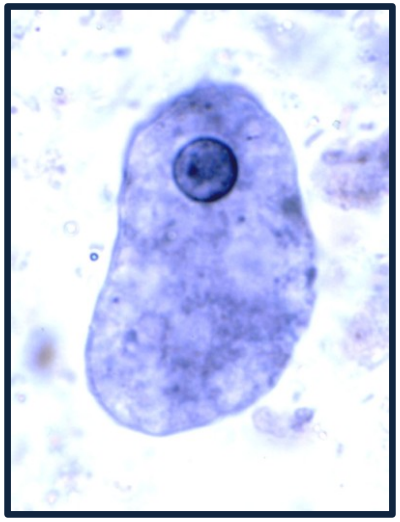
Amitochondriate organisms

Giardia

Intestinal parasite

Beaver fever

No organelle



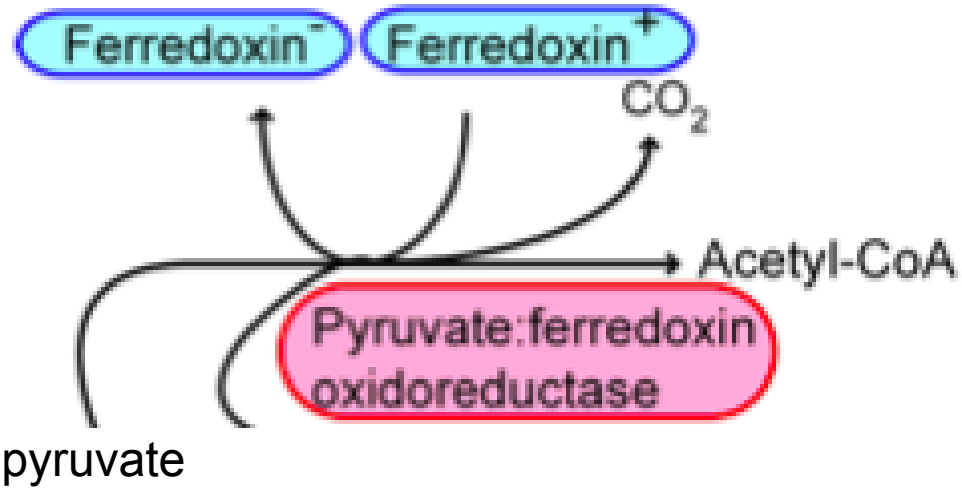
Entamoeba

Intestinal parasite

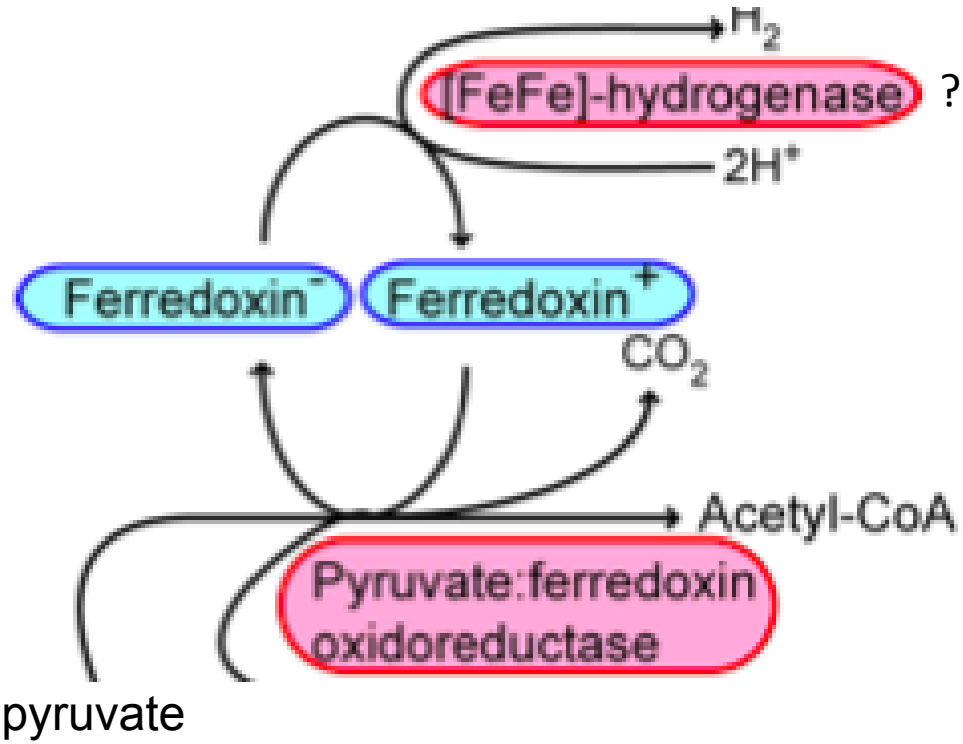
dysentery

No organelle

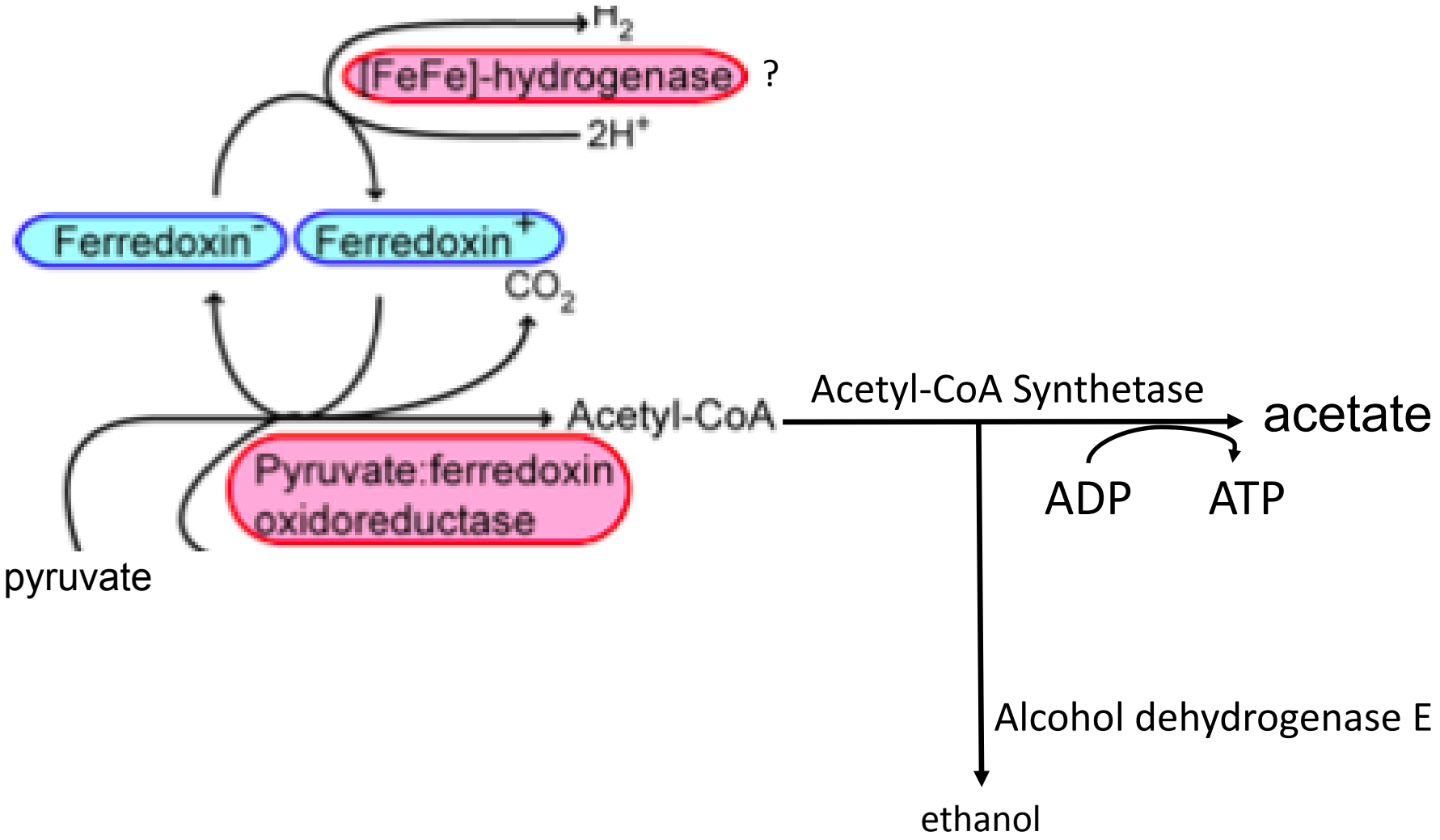
In cytosol


Ferredoxin-
Ferredoxin+
CO2
Acetyl-CoA
Pyruvate:ferredoxin
oxidoreductase
pyruvate

In cytosol

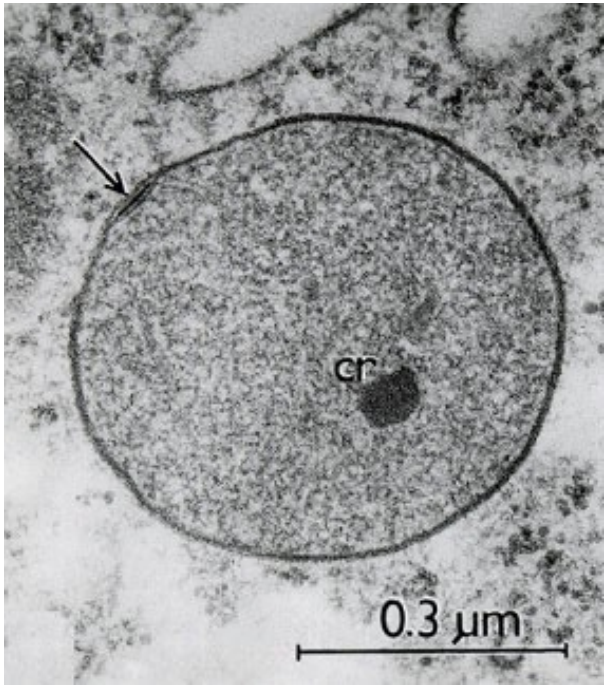

H_2
[FeFe]-hydrogenase
?
$2H^+$
Ferredoxin$^-$
Ferredoxin$^+$
CO_2
Acetyl-CoA
Pyruvate:ferredoxin oxidoreductase
pyruvate

In cytosol


H2
[FeFe]-hydrogenase
?
2H+
Ferredoxin-
Ferredoxin+
CO2
Acetyl-CoA
Acetyl-CoA Synthetase
acetate
ADP
ATP
Pyruvate:ferredoxin oxidoreductase
pyruvate
Alcohol dehydrogenase E
ethanol

Amitochondriate organisms

At this point of the lecture we still think they are truly amitochondriate…



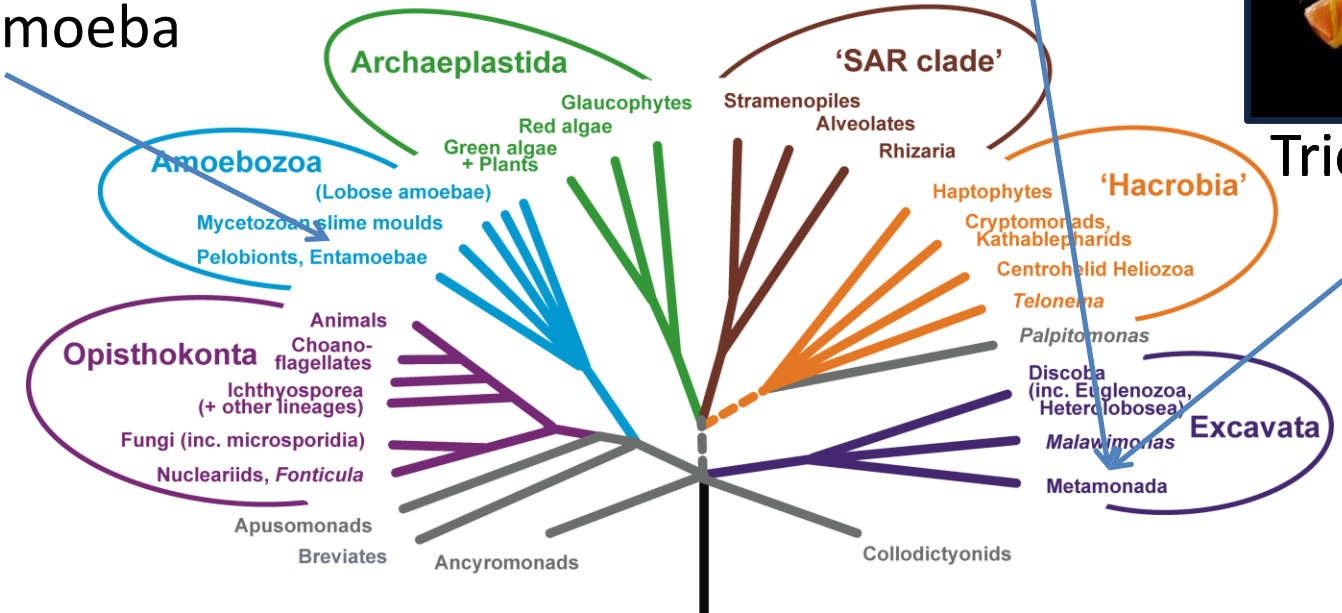
Do we?

Genes of mitochondrial origin

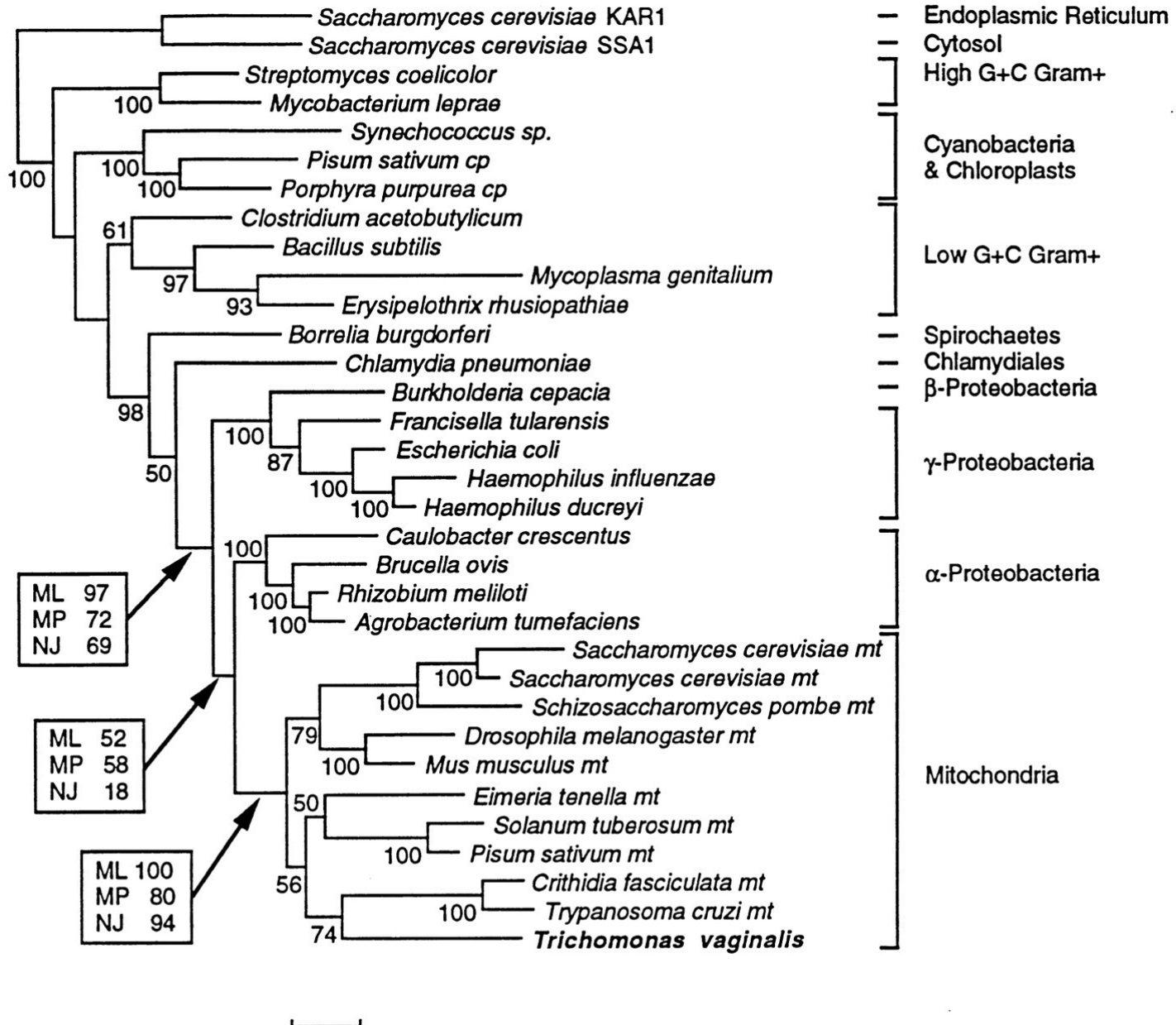
Entamoeba

Giardia

Trichomonas

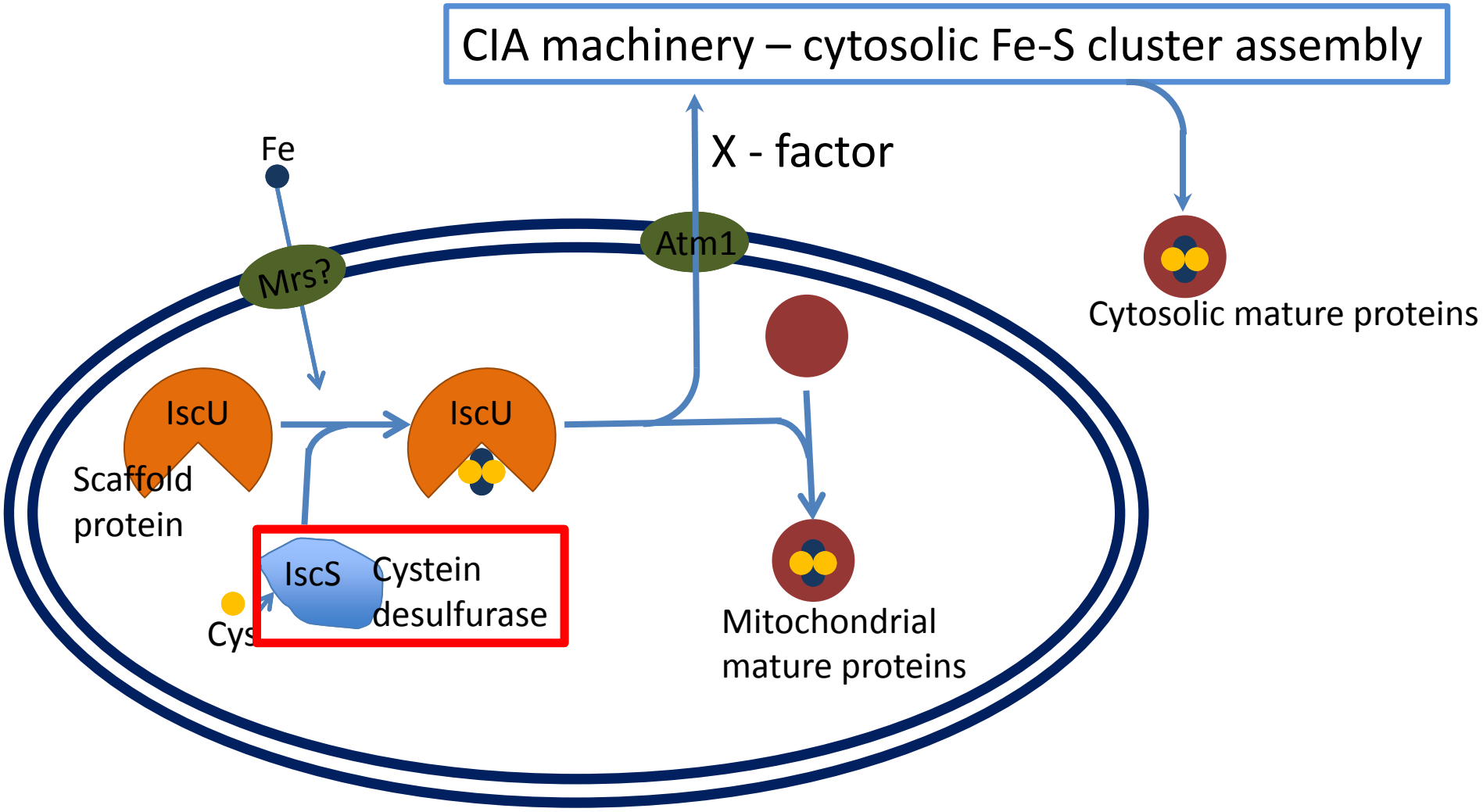


Genes of mitochondrial origin

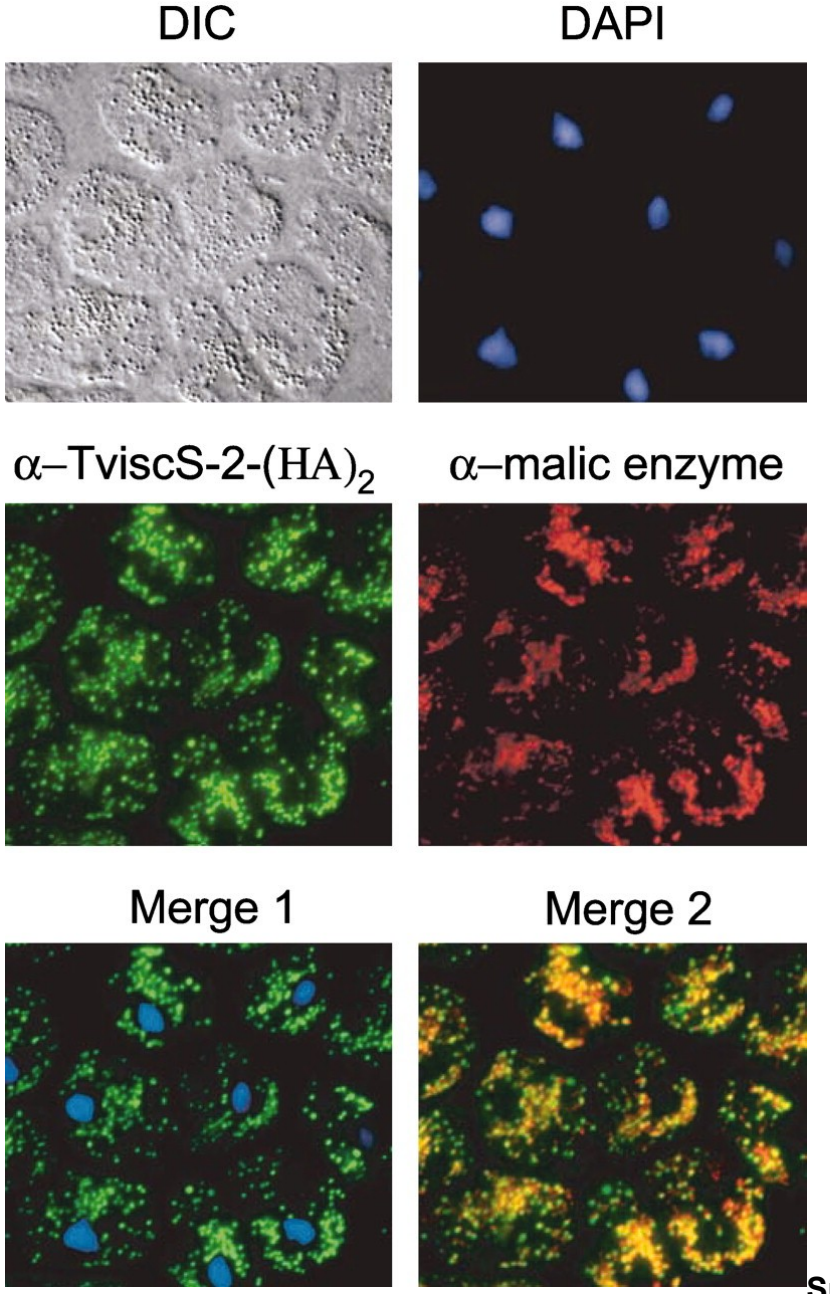


Germot A et al. PNAS 1996;93:14614-14617

Hydrogenosome = mitochondrion



Hydrogenosome = mitochondrion



Other evidence:

- Other proteins of mitochondrial origin targeted to hydrogenosome
- Similar targeting machinery

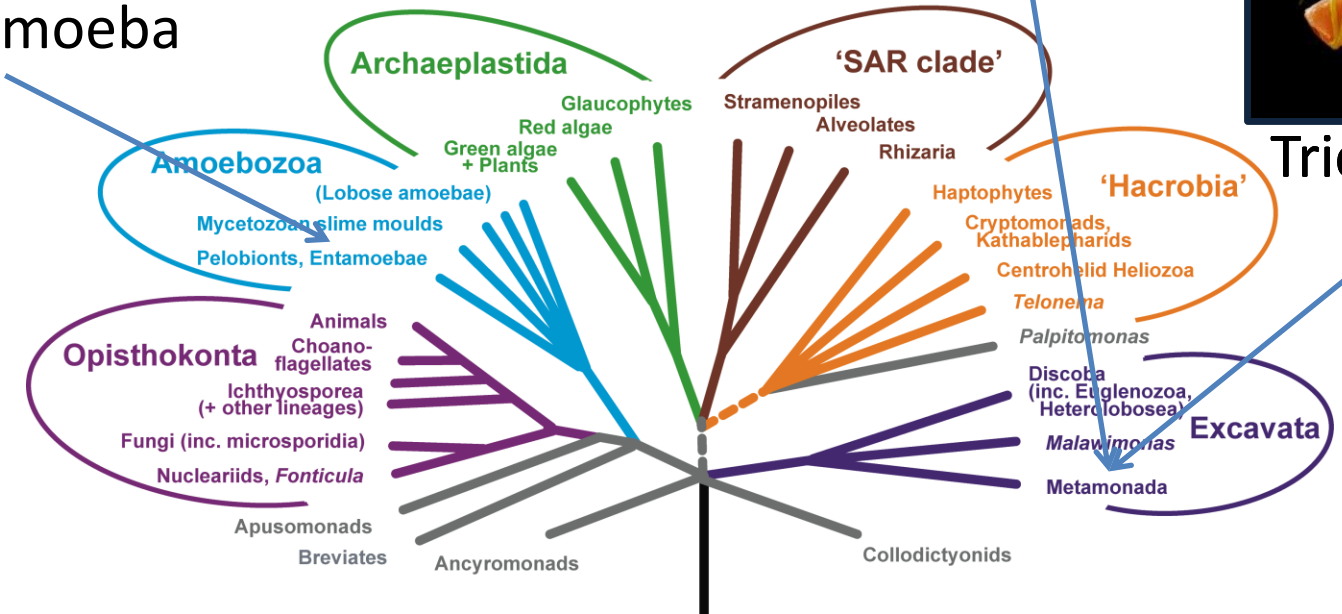
Sutak R et al. PNAS 2004;101:10368-10373

How about *Giardia* and *Entamoeba*

Entamoeba

Giardia

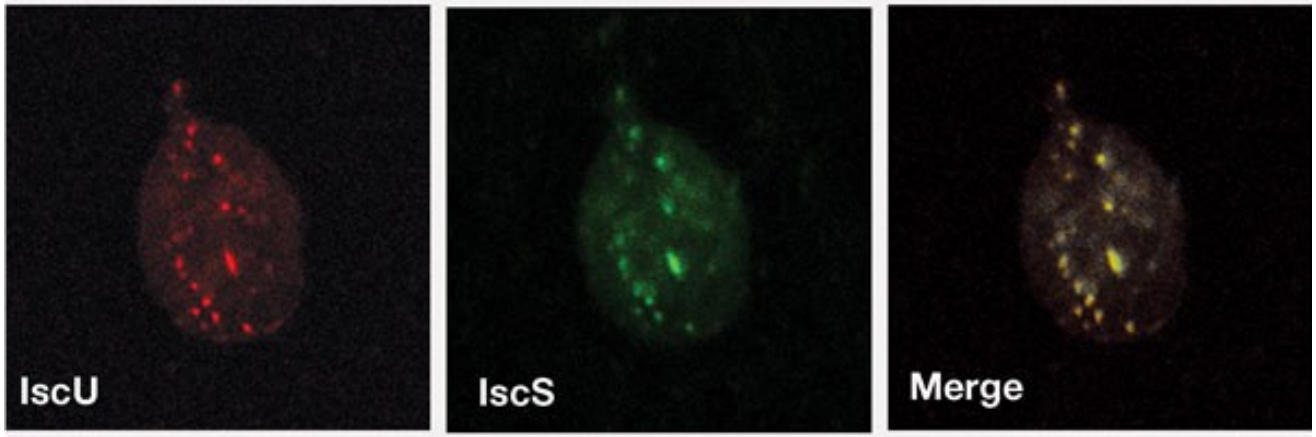
Trichomonas

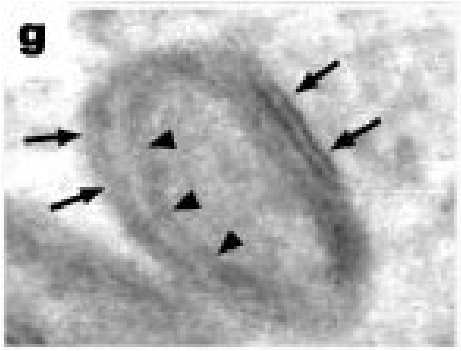


Giardia intestinalis

1. CPN60 gene discovered in *Giardia* genome

2. Subsequently Isc system (Fe-S cluster assembly system) was discovered and localized in Giardia





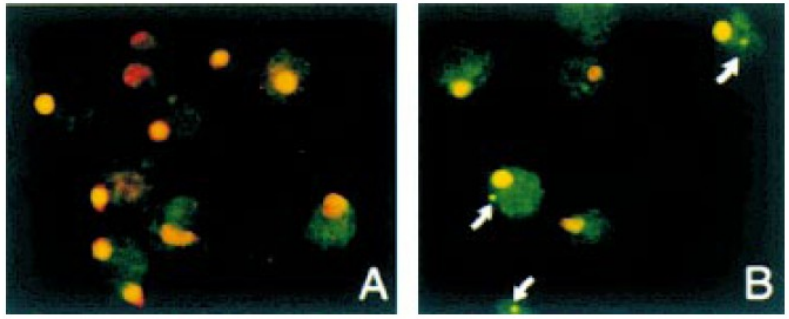
This organelle was called **Mitosome**

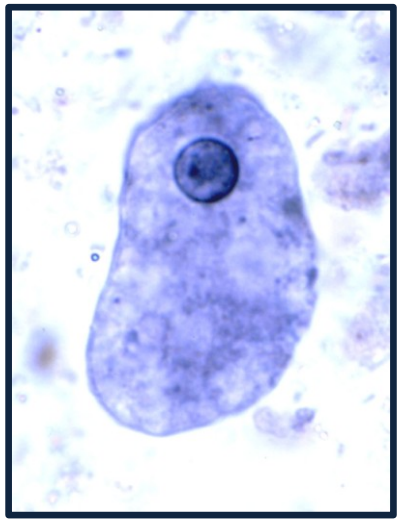
Giardia mitosomes

- Shown to have common targeting system with Hydrogenosomes and Mitochondria
- TOM complex proteins have been identified
- However, TIM pore complex proteins have not yet been identified
- The only known function is Fe-S cluster assembly
- ATP is being imported into mitosome rather than made

Entamoeba mitosomes

CPN60 gene of mitochondrial origin



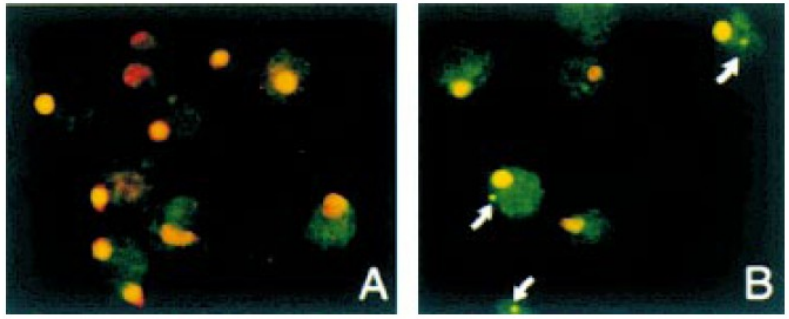


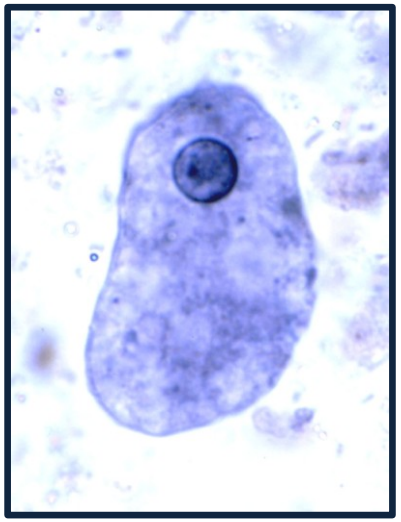
Iron-Sulfur cluster assembly

- Does not have **Ics** system but **Nif** system instead
- Does not localize into the organelle

Entamoeba mitosomes

CPN60 gene of mitochondrial origin





- **Does not have Ics system but Suf system instead**
- **Also functions in cyst formation – again crucial function**

To summarize…

Three basic categories of organelles:

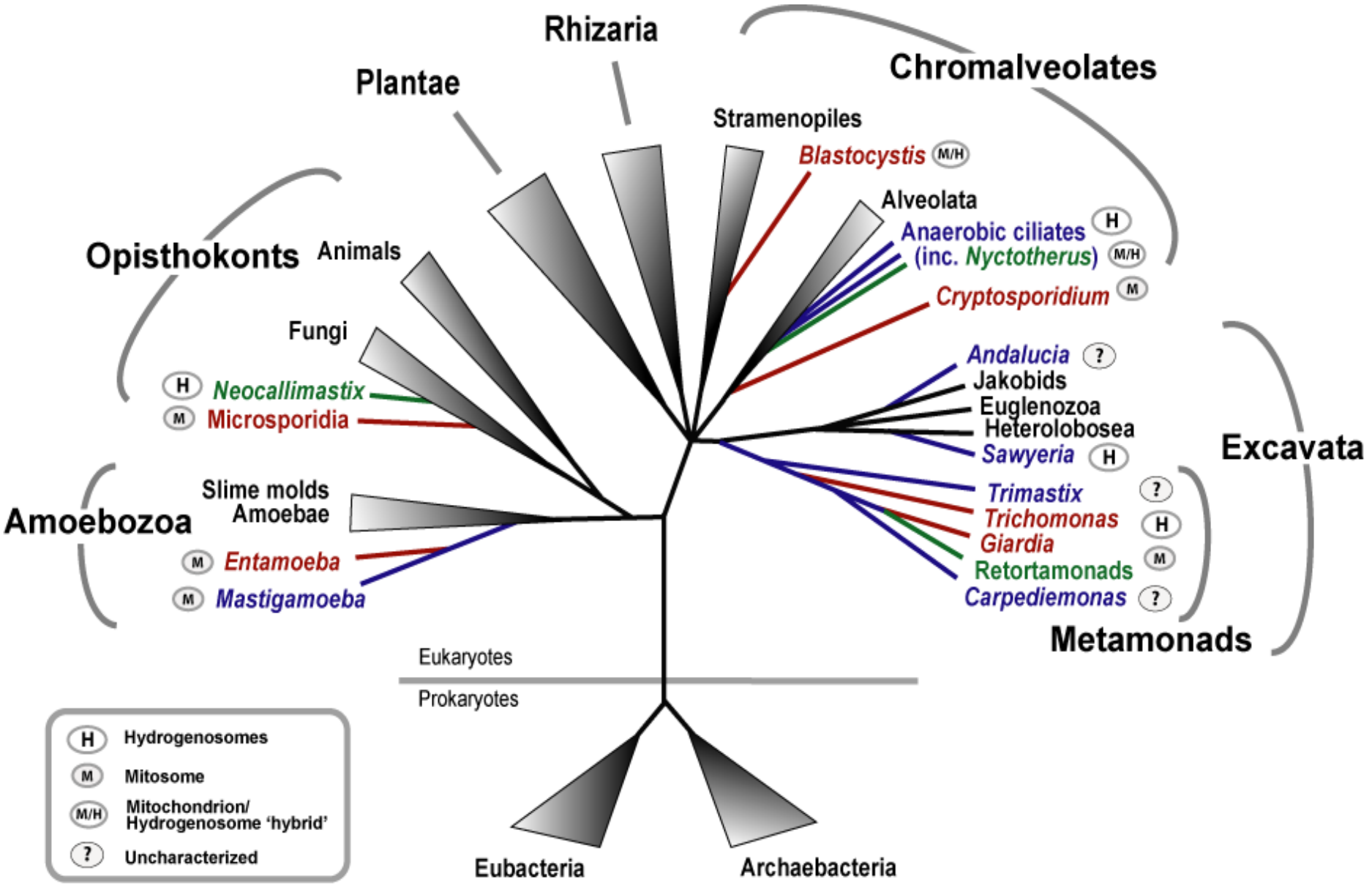
Mitochondrion – genome, electron transport chain, Import machinery, Fe-S cluster synthesis

Hydrogenosome – no genome, PFO/Hydrogenase – genrates energy, Import machinery, Fe-S cluster synthesis

Mitosomes – tiny, no genome, does not generate energy, highly reduced import machinery, Fe-S cluster synthesis (usually)

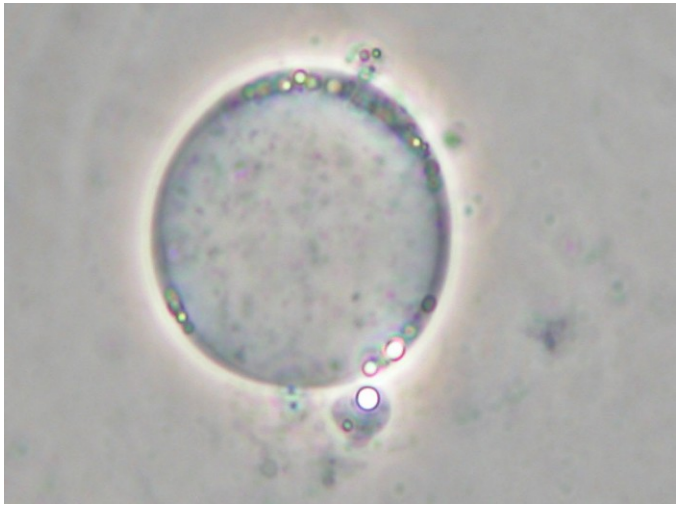
5 minutes break….

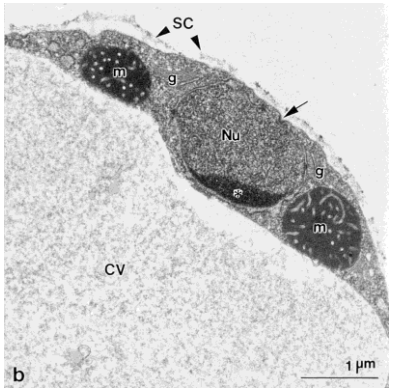
To make it more complicated



Blastocystis

- **Infects gastro-intestinal tract of humans and animals.**
- **9 species of *Blastocystis* that infect humans.**
- **Strict anaerobe**
- **Stramenopile**



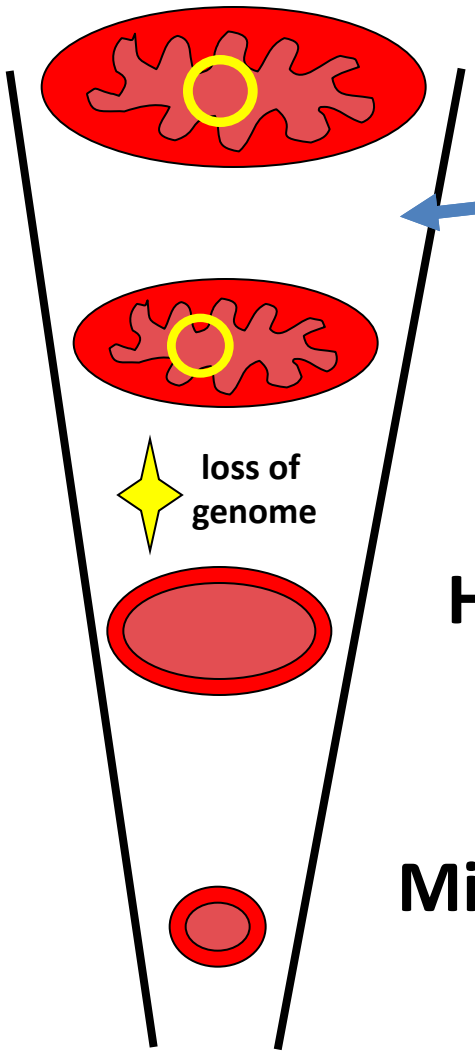


Blastocystis organelle

- **Possess PFO and Fe-Hydrogenase → Therefore it is Hydrogenosome**
- **But also has genome**
- **And partial TCA cycle, and classical mitochondrial pyruvate dehydrogenase**
- **So it is Hydrogenosome/Mitochondria?**
- **There is several organisms whose organelle are bluring the bouindaries**
- **Extremely dynamic system**

What happens to mitochondria?

Continuum



Classical mitochondria

Anaerobic mitochondria

- Electron transport/oxidative phosphorylation
- use alternate electron acceptor, not O_2

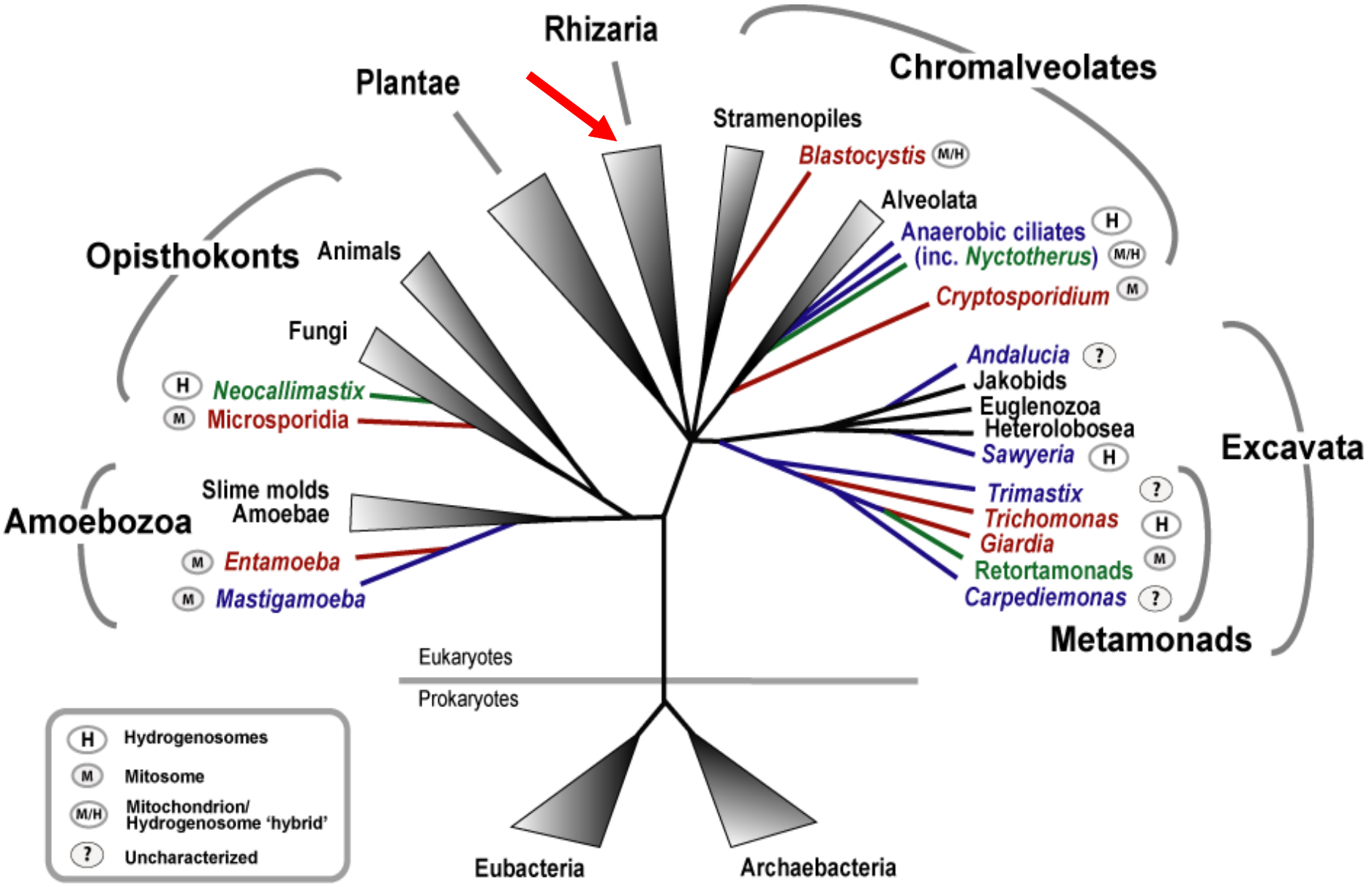
Hydrogenosomes

- No oxidative phosphorylation (usually)
- Anaerobic ATP generation producing H_2 gas

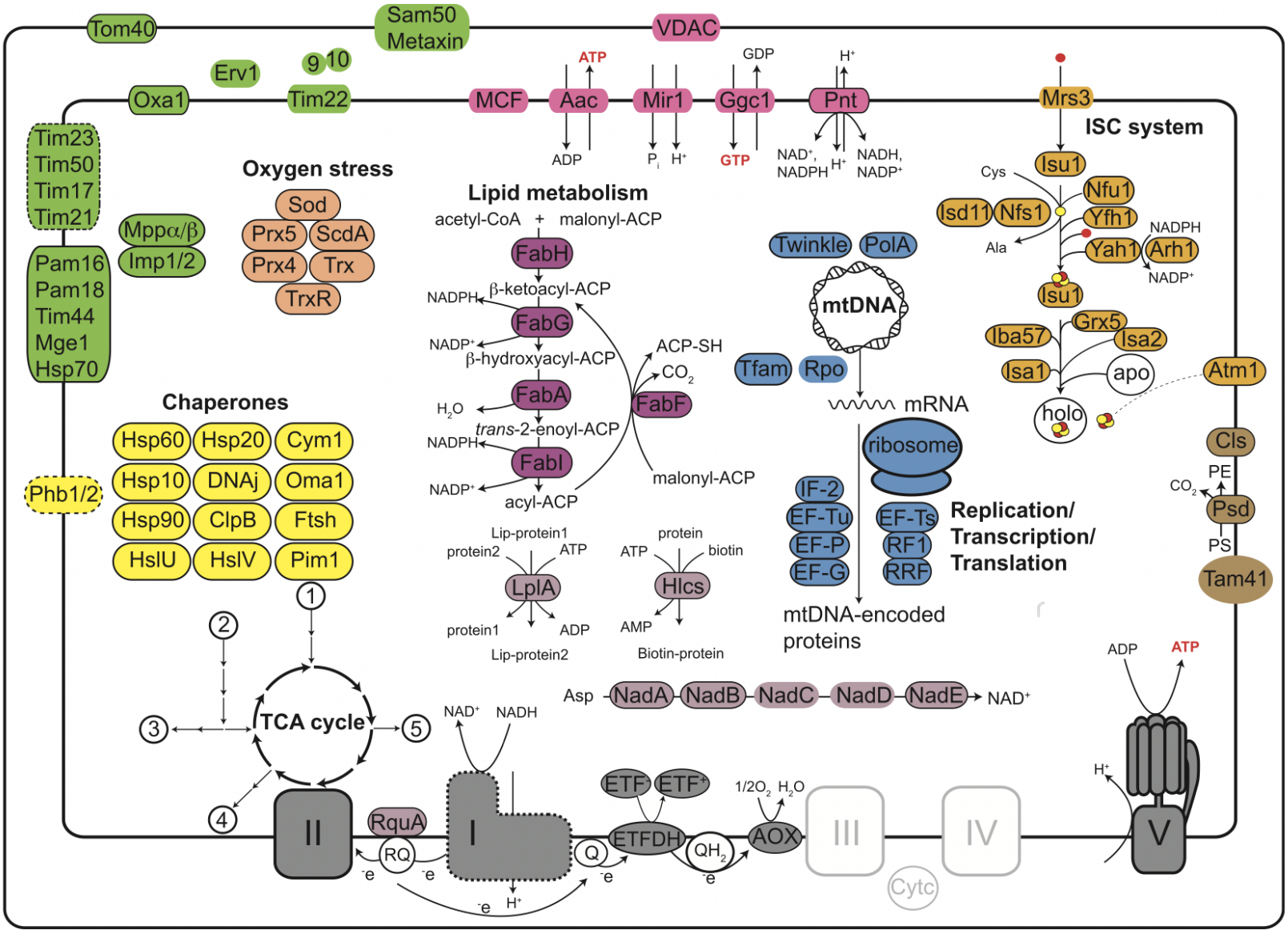
Mitosomes

- No electron transport, oxidative phosphorylation
- Some mitochondrial-derived proteins
- Protein import apparatus and Fe-S cluster biogenesis
- Unknown functions

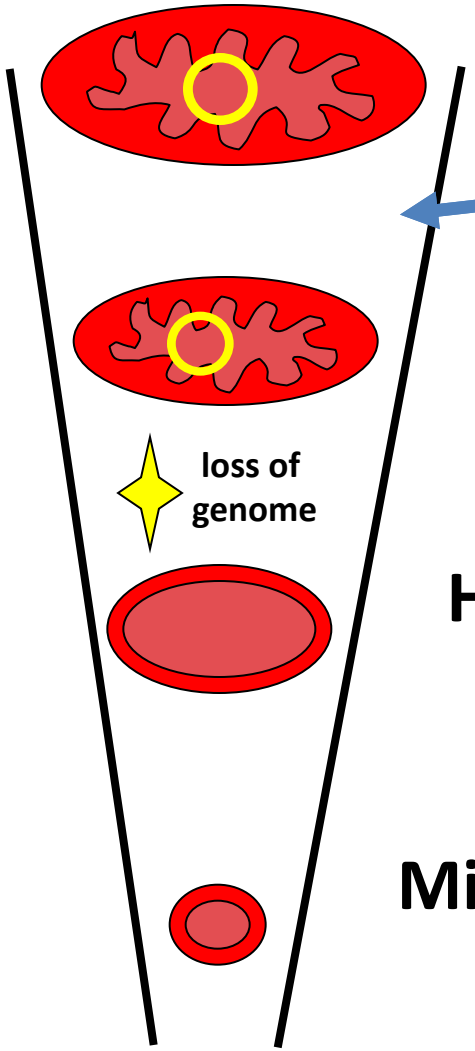
To make it more complicated



Brevimastigomonas motovehiculus



What happens to mitochondria?



Classical mitochondria

Anaerobic mitochondria

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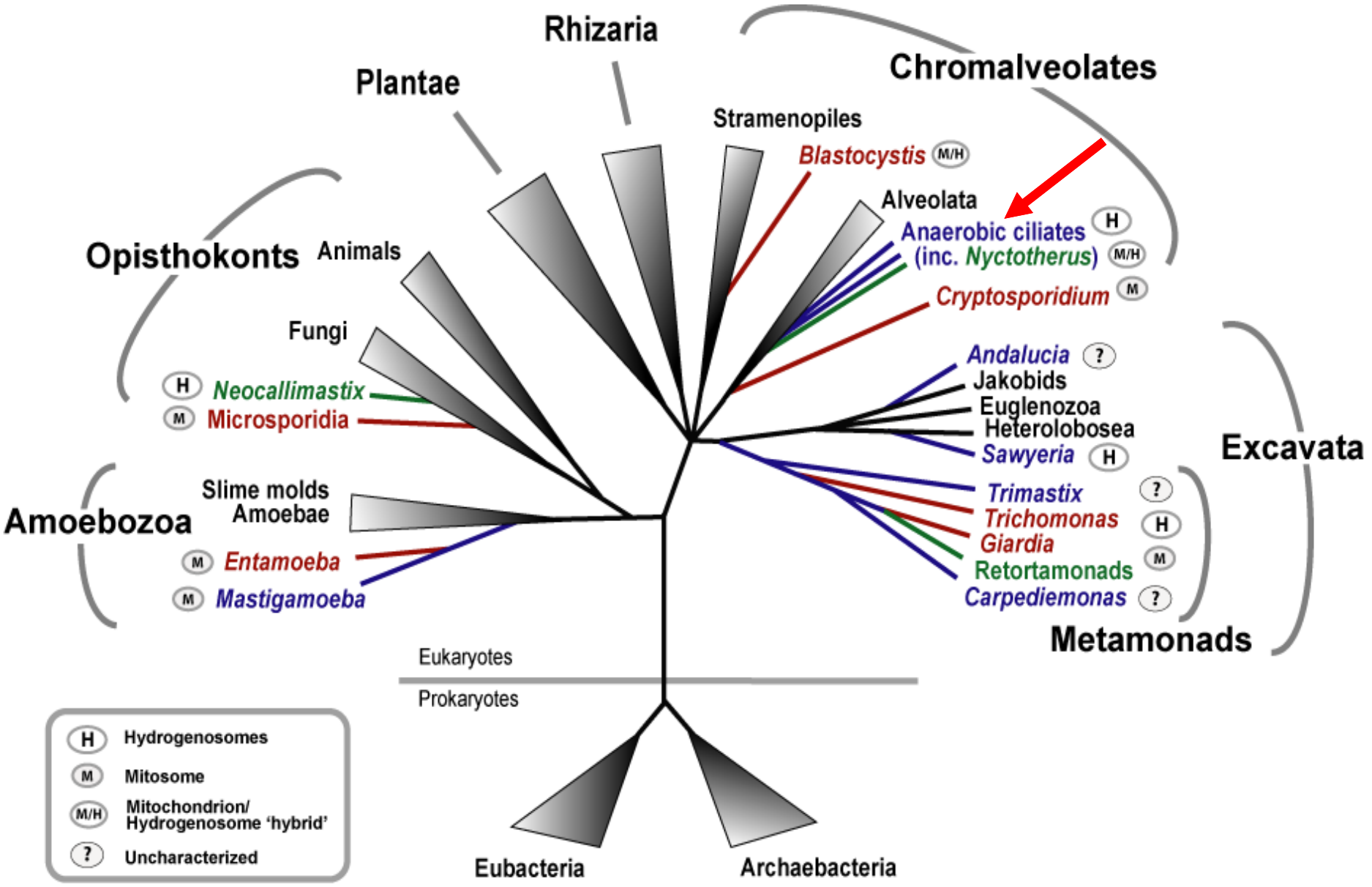
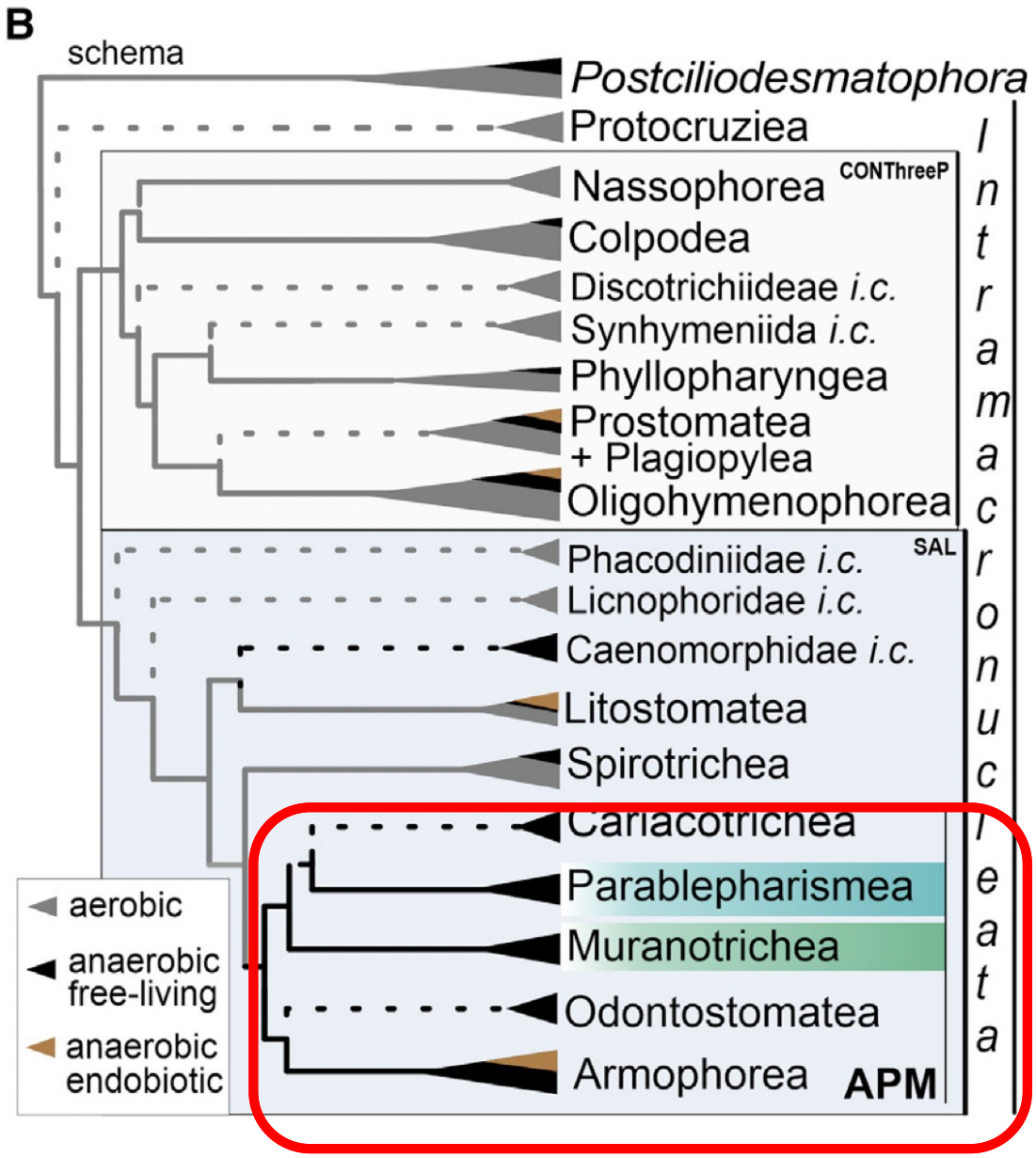
Metopid Ciliates



Metopid Ciliates



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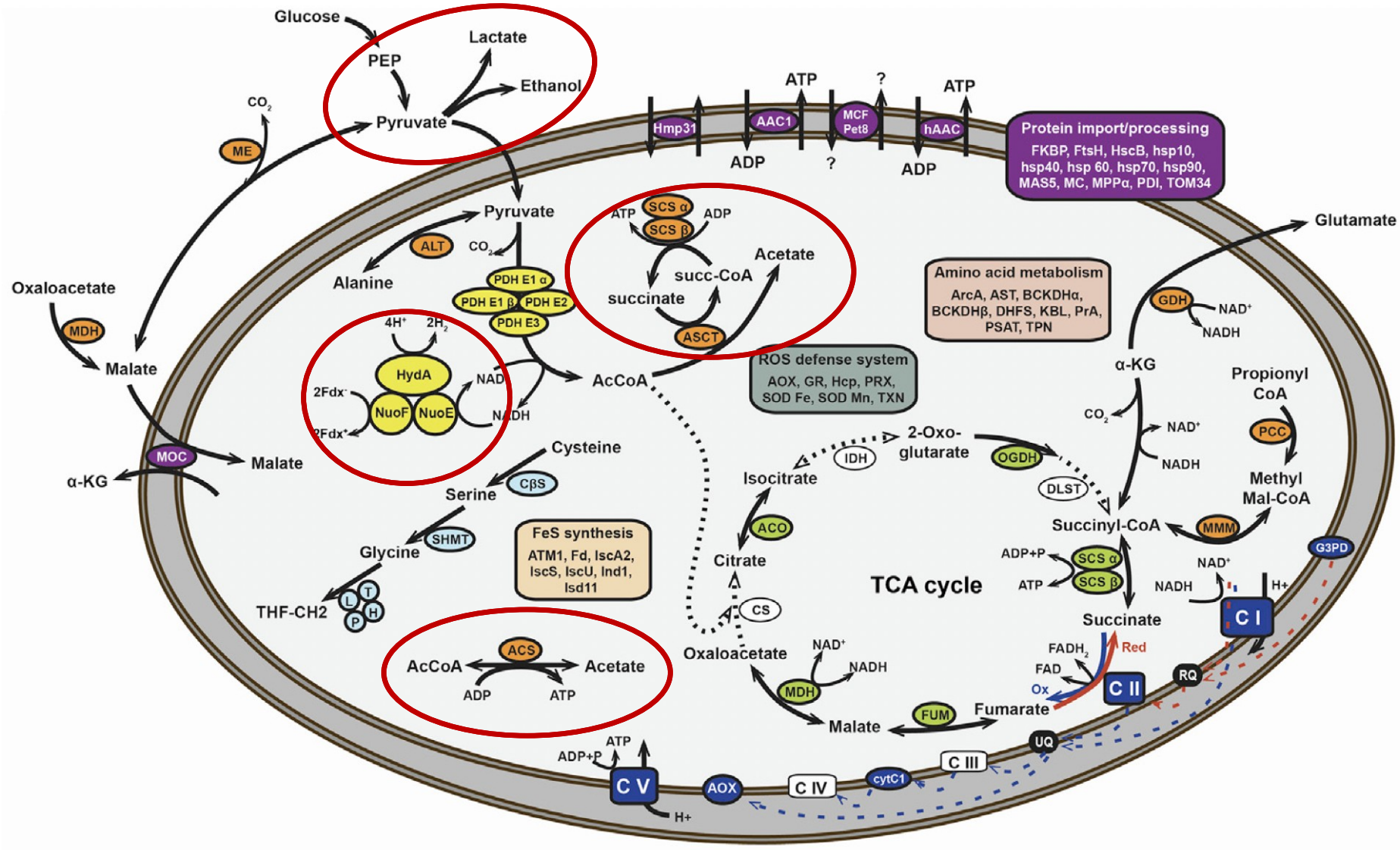

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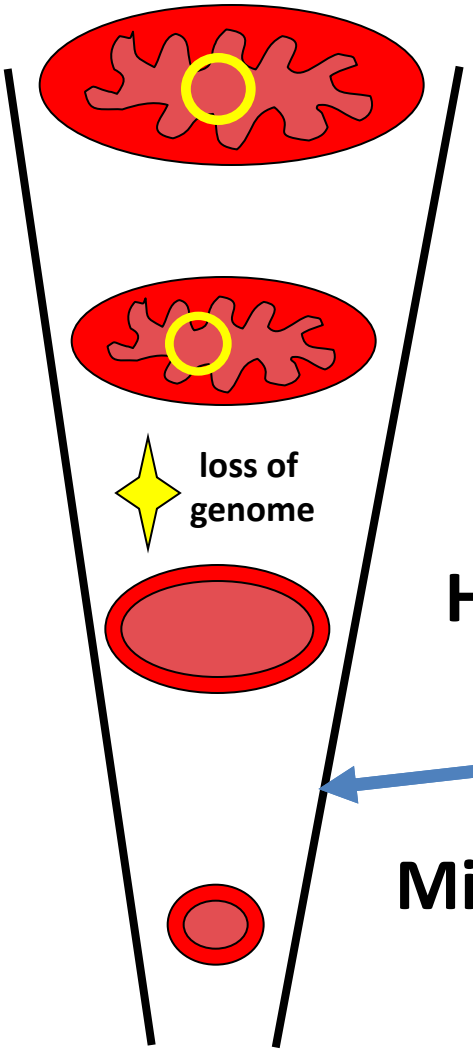

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Metamonada

Trimastix + Oxymonads

Trichomonads

Carpediemonas-like organisms

Diplomonads

MROs of metamonada

Trimastix PCT

Monocercomonoides sp.

Trimastix pyriformis

Tritrichomonas foetus

Pentatrichomonas hominis

Trichomonas vaginalis

Carpediemonas membranifera

Ergobibamus cyprinoides

CLO NY0171

Chilomastix cuspidata

Chilomastix caulleri

Kipferlia bialata

Dysnectes brevis

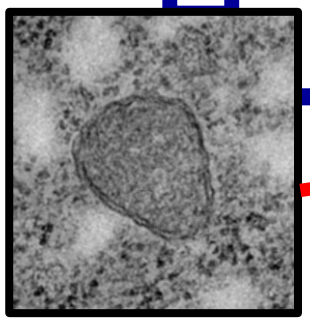
Giardia intestinalis

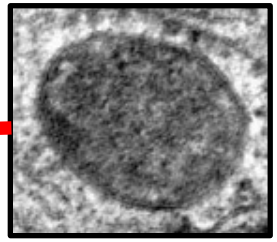
Spironucleus barkhanus

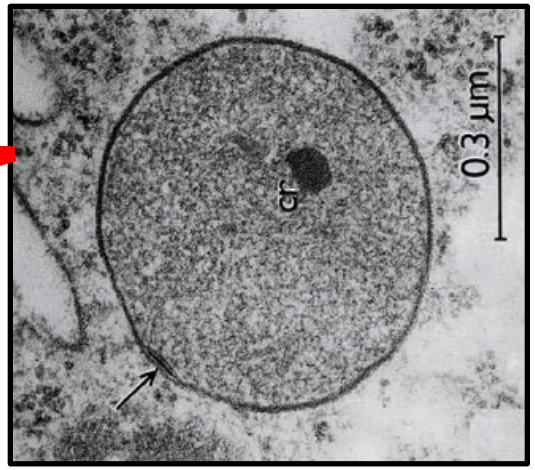
Spironucleus salmonicida

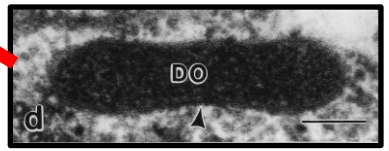
Spironucleus vortens

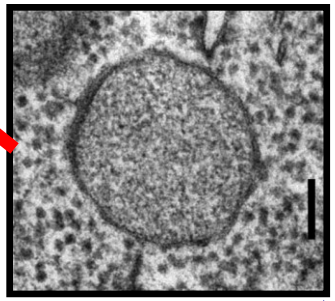
Trepomonas PC1

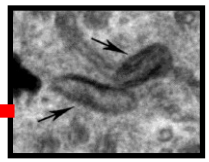






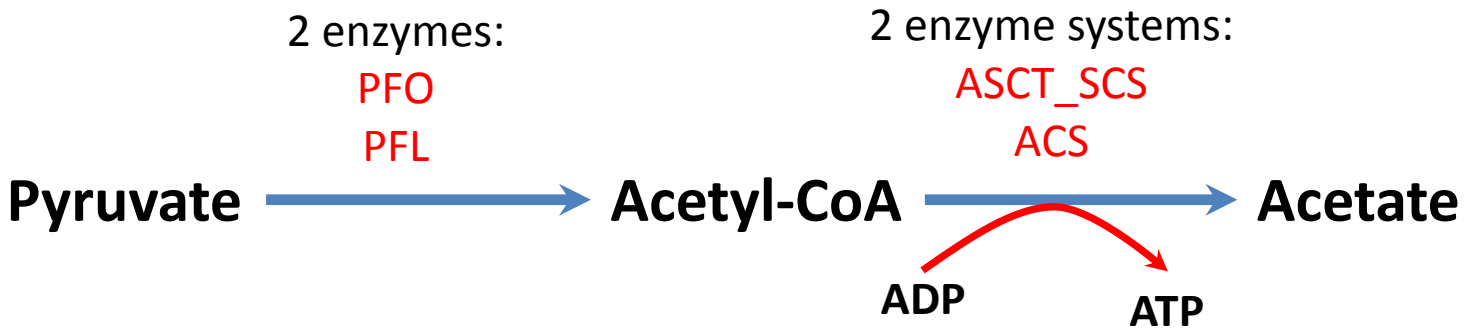




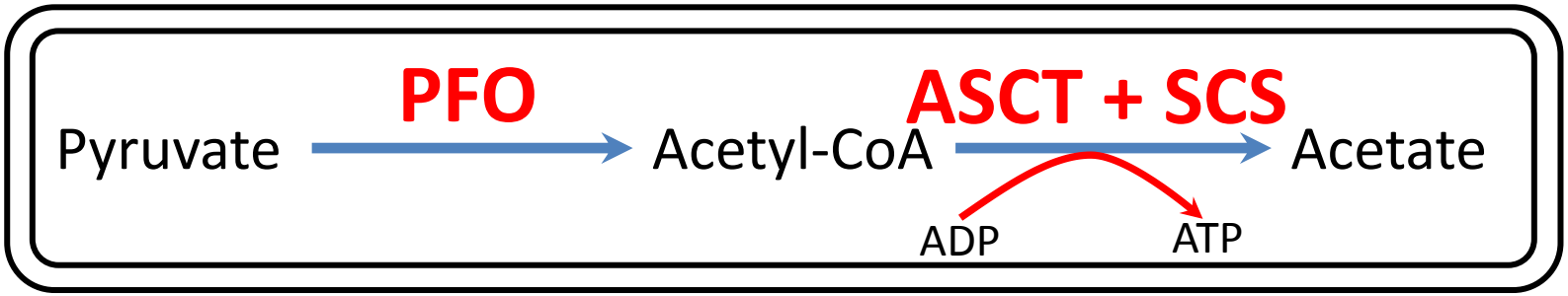


Reductive evolution?

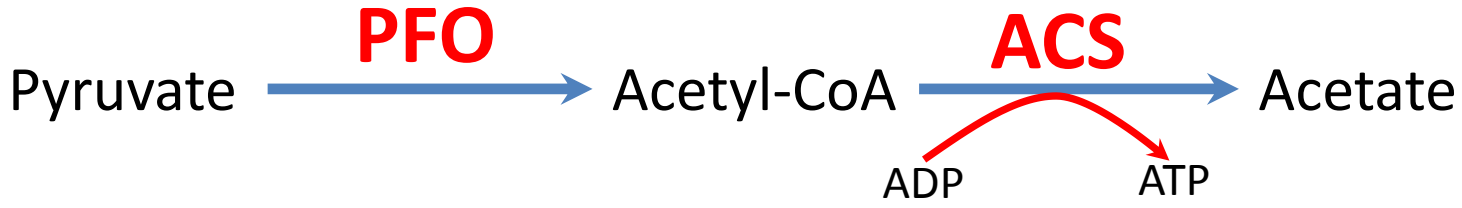
ATP generation

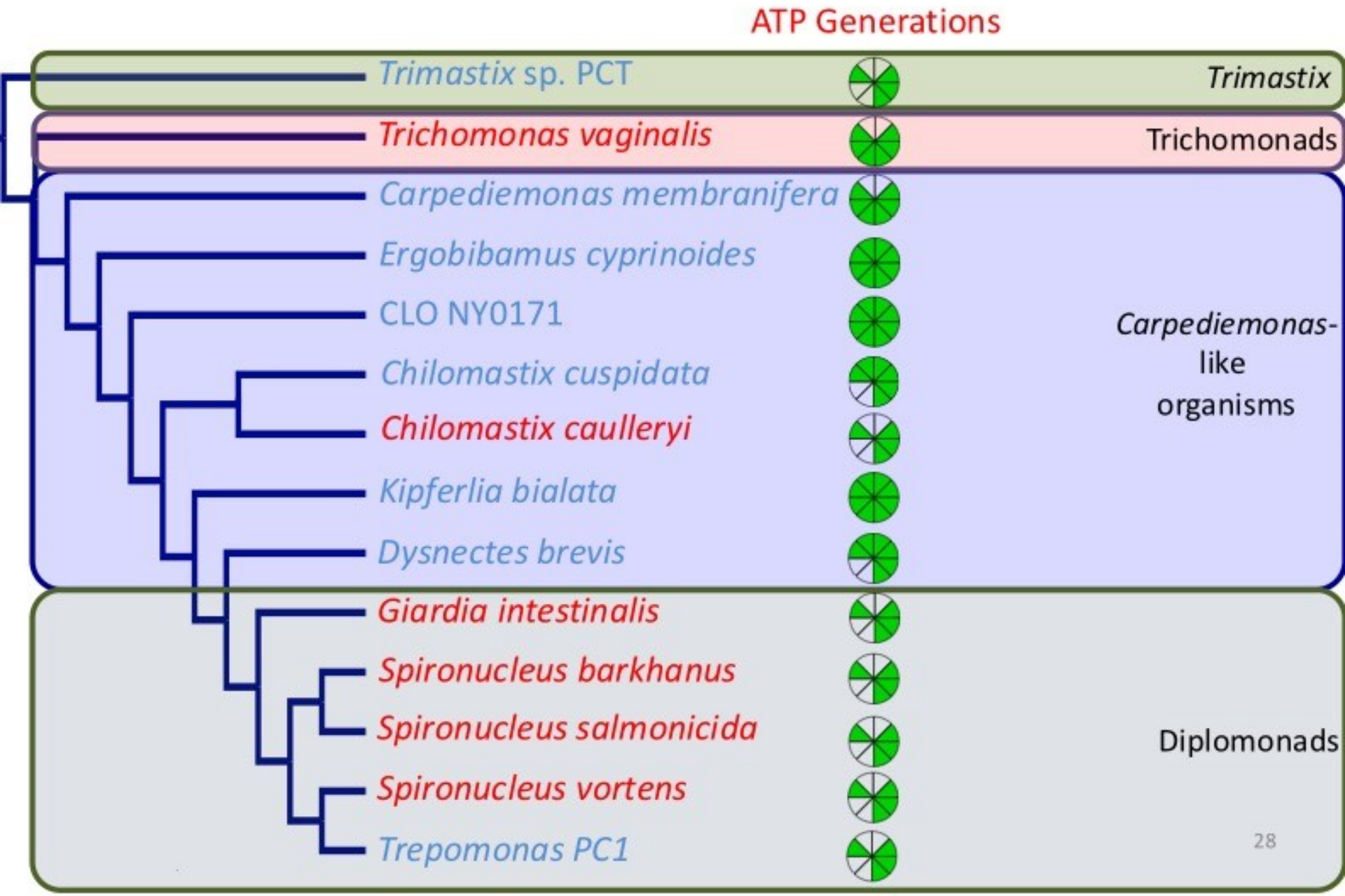


Trichomonas



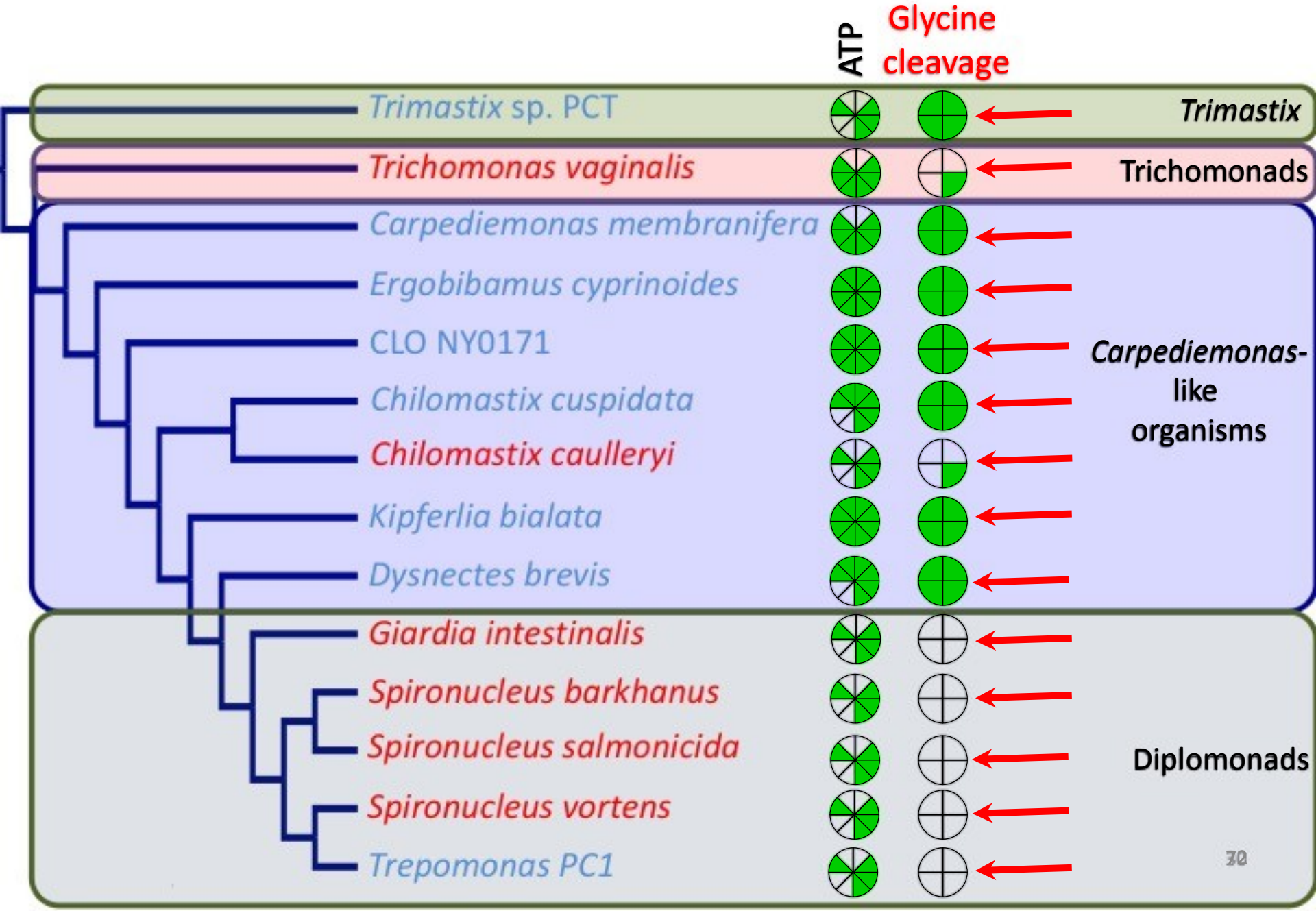
Giardia




ATP Generations
Trimastix sp. PCT
Trichomonas vaginalis
Carpediemonas membranifera
Ergobibamus cyprinoides
CLO NY0171
Chilomastix cuspidata
Chilomastix caulleryi
Kipferlia bialata
Dysnectes brevis
Giardia intestinalis
Spironucleus barkhanus
Spironucleus salmonicida
Spironucleus vortens
Trepomonas PC1
Trimastix
Trichomonads
Carpediemonas-like organisms
Diplomonads

Glycine Cleavage system

- Important part of amino acid metabolism
- Synthesize or degrades glycine
- In eukaryotes present in mitochondria
- 4 component complex:
  - T_protein
  - P_protein
  - H_protein
  - L_protein


ATP
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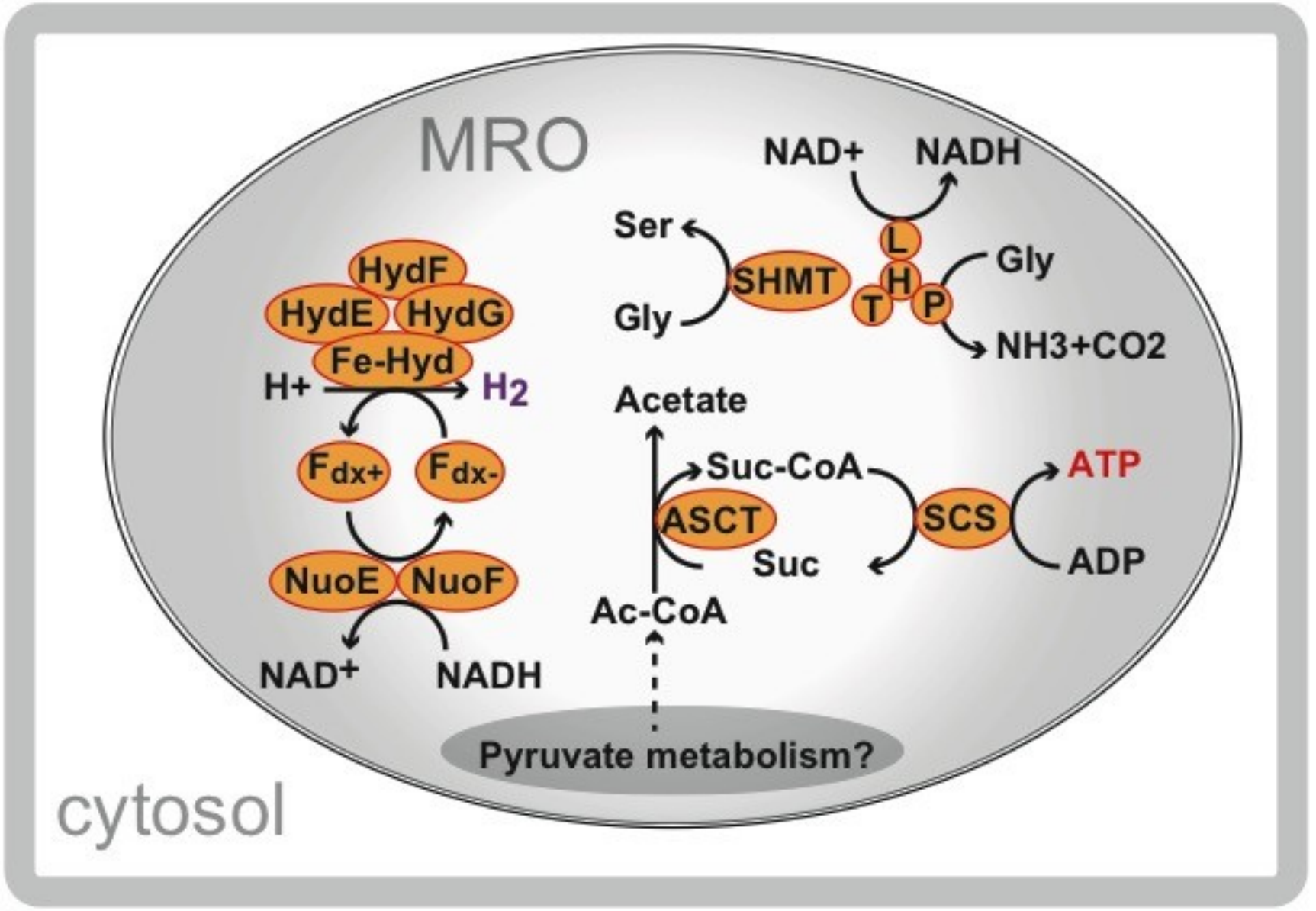
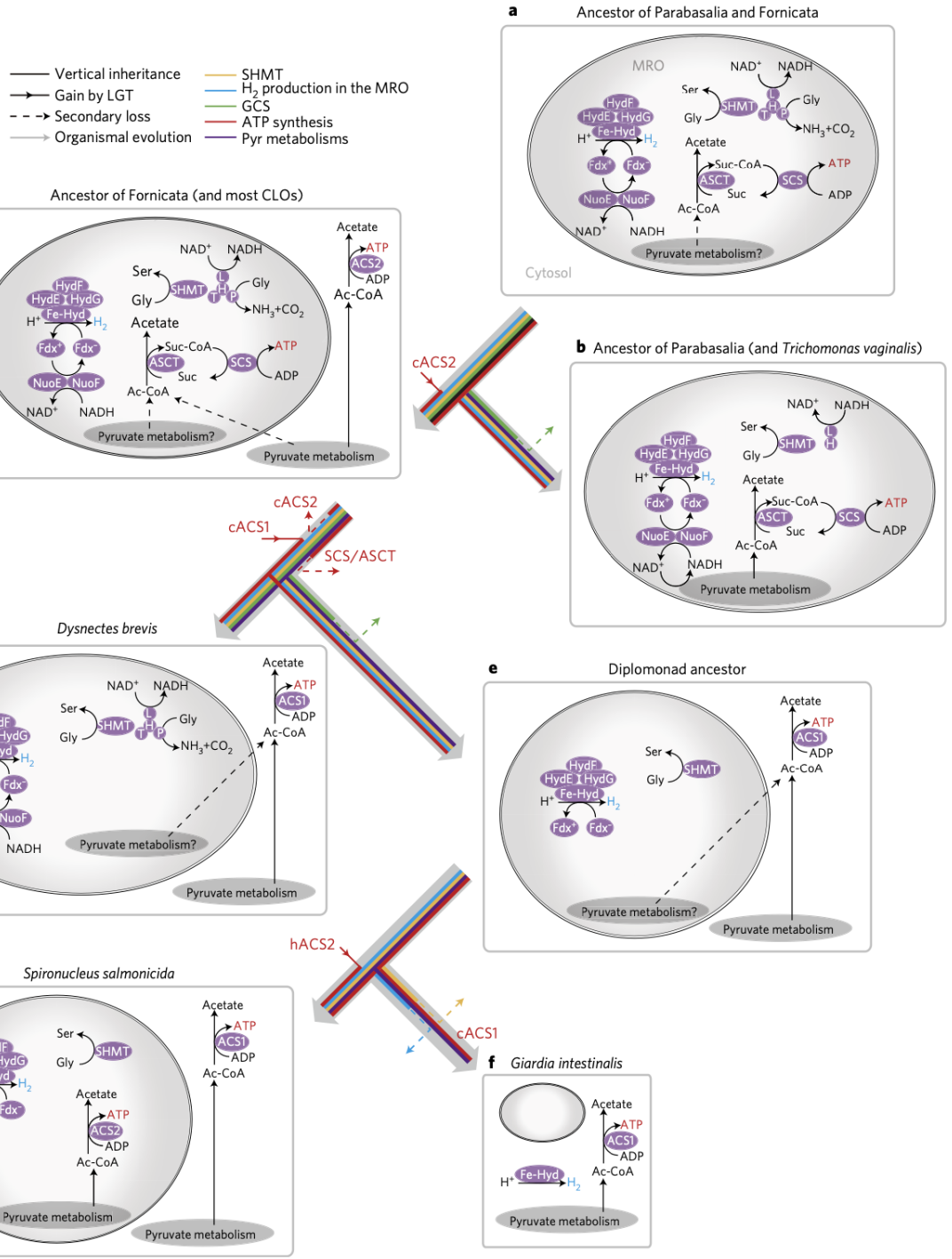
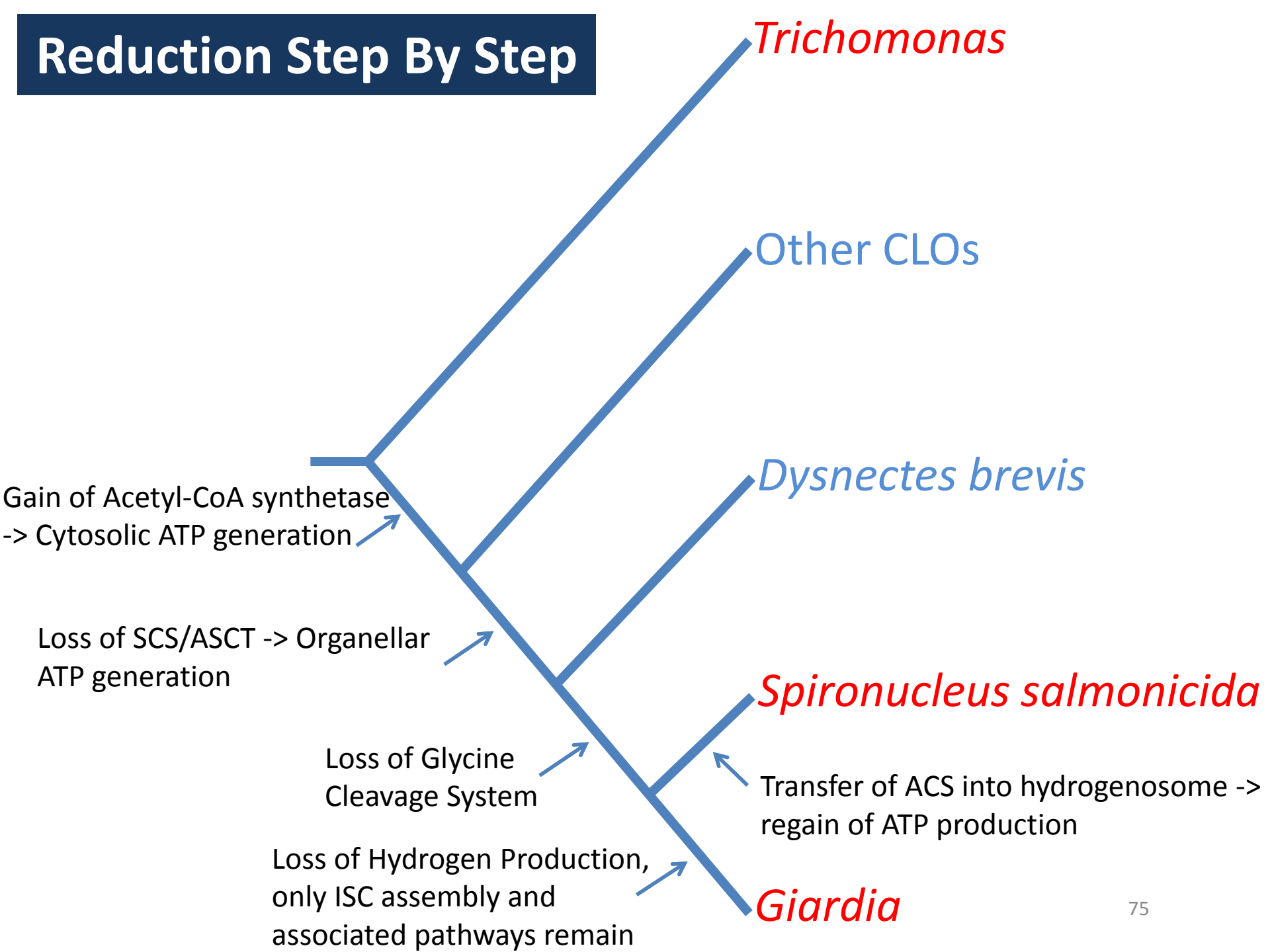

MRO
HydF
HydE
HydG
Fe-Hyd
H+
H2
Fdx+
Fdx-
NuoE
NuoF
NAD+
NADH
NAD+
NADH
Ser
Gly
SHMT
L
H
T
P
Gly
NH3+CO2
Acetate
Suc-CoA
ASCT
Suc
SCS
ATP
ADP
Ac-CoA
Pyruvate metabolism?
cytosol

Metamonada


Vertical inheritance
Gain by LGT
Secondary loss
Organismal evolution
SHMT
H2 production in the MRO
GCS
ATP synthesis
Pyr metabolisms
a Ancestor of Parabasalia and Fornicata
MRO
Cytosol
Pyruvate metabolism?
b Ancestor of Parabasalia (and Trichomonas vaginalis)
Pyruvate metabolism
c Ancestor of Fornicata (and most CLOs)
Pyruvate metabolism? Pyruvate metabolism
cACS2
cACS1
SCS/ASCT
d Dysnectes brevis
Pyruvate metabolism? Pyruvate metabolism
e Diplomonad ancestor
Pyruvate metabolism? Pyruvate metabolism
hACS2
cACS1
g Spironucleus salmonicida
Pyruvate metabolism Pyruvate metabolism
f Giardia intestinalis
Pyruvate metabolism

Reduction Step By Step



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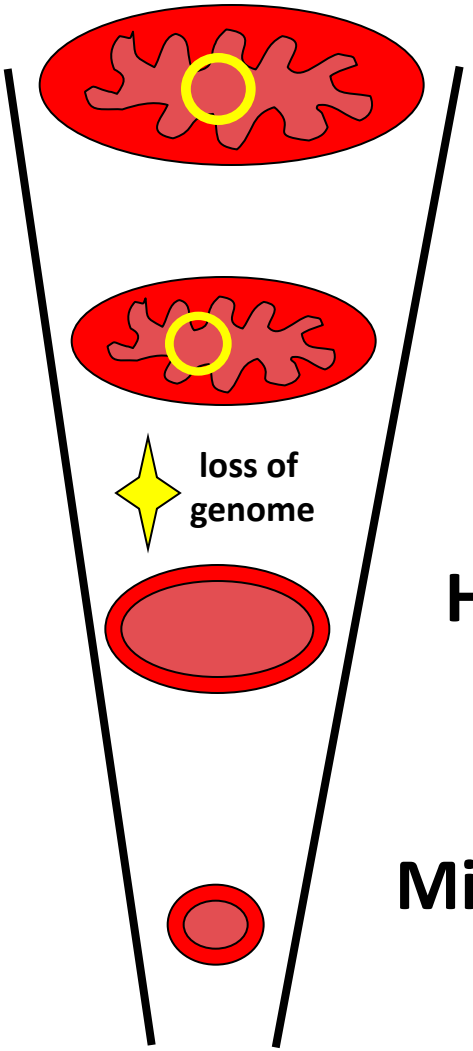

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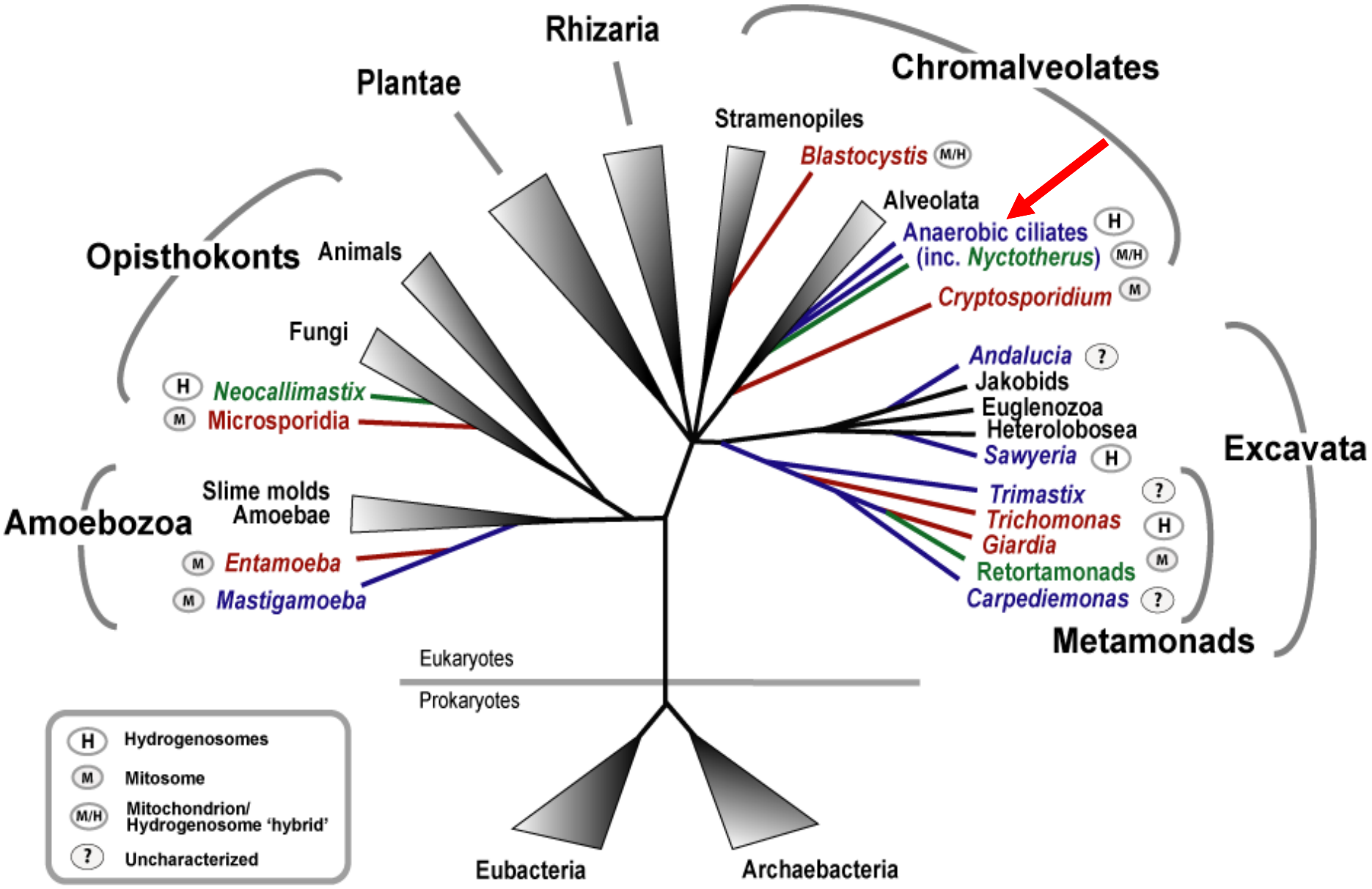
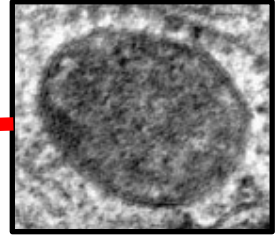


Monocercomonoides



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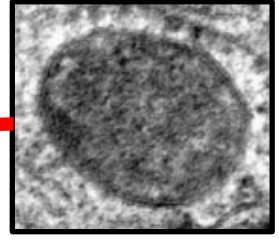

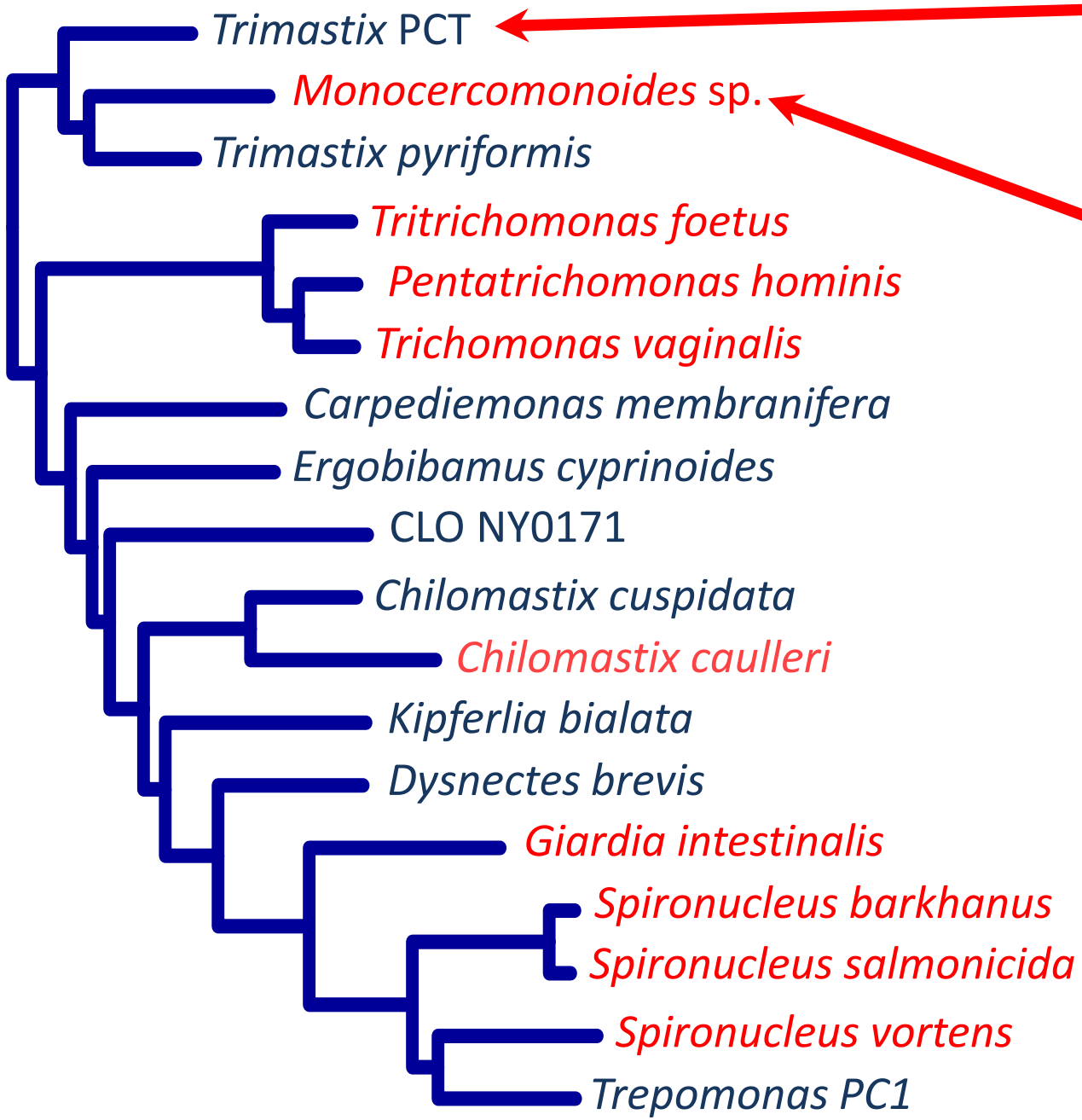

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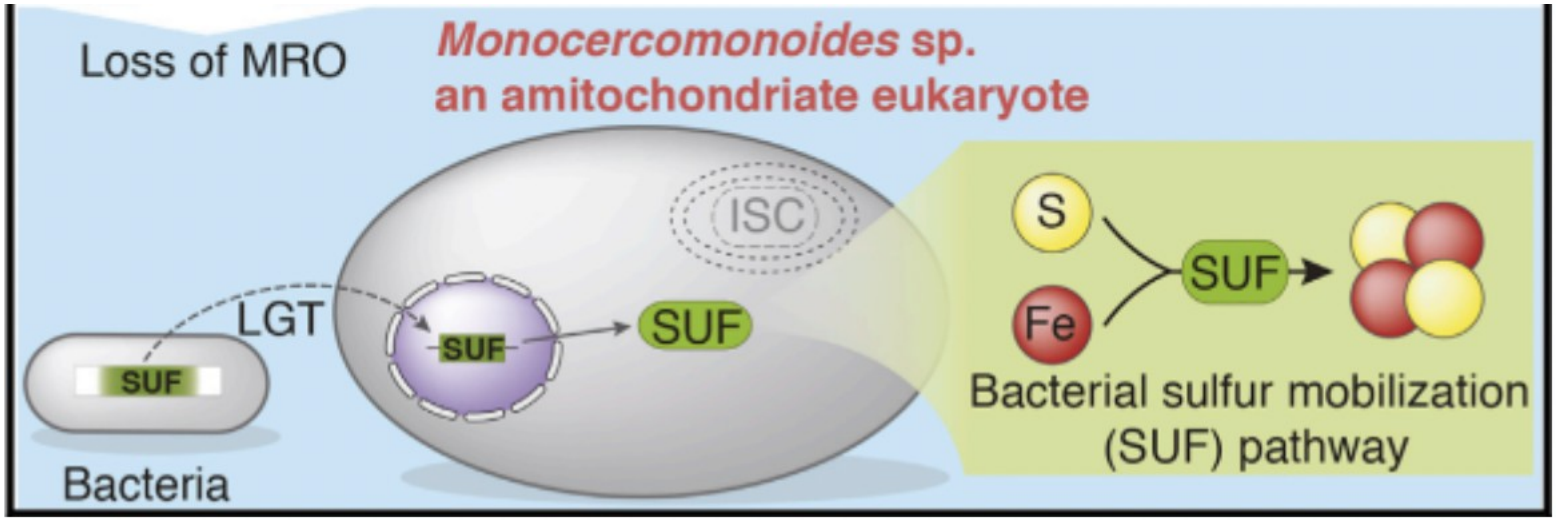

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