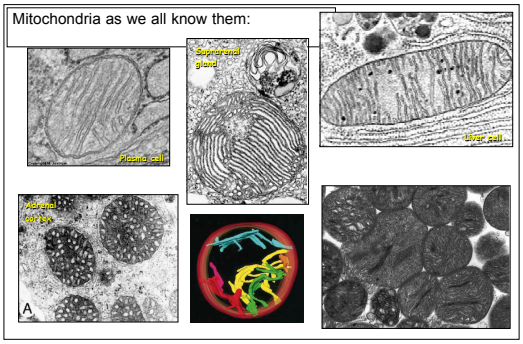
Reconstructing Mitochondrial Evolution??

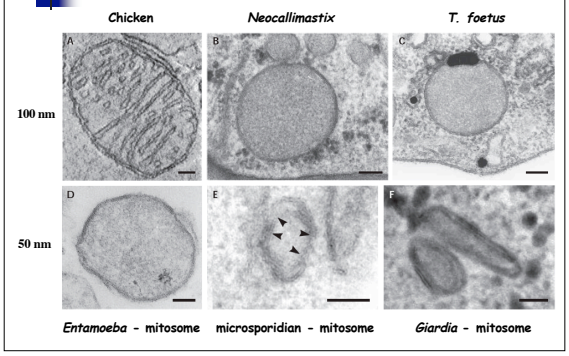
What is your definition of a mitochondrion??

Morphological Diversity

Mitochondria as we all know them:



Mitochondrial Diversity???



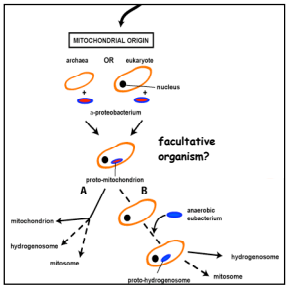
Reconstructing Evolution

- Mitochondrial evolution
  - well established endosymbiotic theory
  - α-proteobacterium - *Rickettsia prowazekii*
- Hydrogenosomal evolution
  - No DNA
  - NOW 2 examples *Nyctotherus and Blastocystis* (MLO)
  - Several proteins similar to mitochondria
- Mitosome evolution
  - No DNA
  - Few proteins identified similar to mitochondria

Origins via Endosymbiosis

- Aerobic α-proteobacterium prokaryote gave rise to present day mitochondria.
- Are hydrogenosomes and mitosomes of anaerobic protists derived from the same proto-mitochondrion?
- **Evidence for:** accumulating evidence for several proteins that are currently found in mitochondria - **Proteins of Fe-S cluster formation.**
- **Scenario A** Common ancestor
- **Scenario B** degenerate mitochondrion invoke lateral gene transfer from anaerobic prokaryotes

Current dogma - mitochondria and related organelles arose just once in evolution



Hypotheses for Mito Acquisition

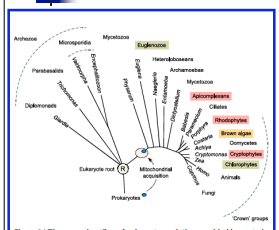
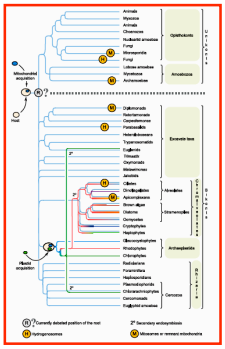


Figure 1 | The general outline of eukaryote evolution provided by rooted rRNA trees. The tree has been redrawn and modified from ref. 92. Until recently, lineages branching near the root were thought to primitively lack mitochondria and were termed Archezoa*. Exactly which archezoans branched first is not clearly resolved by rRNA data*, hence the polytomy (more than two branches from the same node) involving diplomonads, parabasalids and microsporidia at the root. Plastid-bearing lineages are indicated in colours approximating their respective pigmentation. Lineages furthest away from the root, including those with multicellularity, were thought to be the latest-branching forms and were sometimes misleadingly (see ref. 60) called the 'crowns' groups.



Mitochondrion-related Organelles

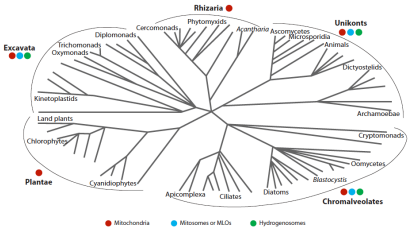


Figure 1

Mitochondrion-related organelles are found in all eukaryotes. Eukaryotes can be divided into several major groups, all of which contain mitochondria (*red*), mitosomes or mitochondrion-like organelles (*blue*), or hydrogenosomes (*green*). Three of these groups have lineages with organisms that contain combinations of these organelles. This tree is adapted from Reference 59 with permission.

2 way to interpret this summary - can you think of both?

Groups with mitochondrial homologues

Table 1. Taxa with mitochondrial homologues for which there are protein or DNA localization data.

species*	higher taxonomic level*	mitochondrial homologue	protein or DNA localized to the organelle
E. histolytica	Amoebozoa, Entamoebida	mitosome	Cpn60 (Mai *et al.* 1999; Tovar *et al.* 1999), Cpn10 (van der Giezen *et al.* 2005), mtHsp70 (Tovar *et al.* 2007), MCF ADP/ATP transporter (Chan *et al.* 2005)
M. balamuthi	Amoebozoa, Mastigamoebidae	mitochondria-like organelle	Cpn60 (Gill *et al.* 2007)
T. hominis	Opisthokonta, Microsporidia	mitosome	mtHsp70 (Williams *et al.* 2002), Nfs (Goldberg *et al.* 2008)
E. cuniculi	Opisthokonta, Microsporidia	mitosome	mtHsp70, NTT3 (Tsaousis *et al.* 2008), Nfs, Isu, frataxin (Goldberg *et al.* 2008), ferredoxin (Williams *et al.* 2008*a*)
Nosocallimastix sp.	Opisthokonta, Fungi	hydrogenosome	mtHsp70, Cpn60 (van der Giezen *et al.* 2003), [Fe]-hydrogenase (Voncken *et al.* 2002), MCF ADP/ATP transporters (van der Giezen *et al.* 2002; Voncken *et al.* 2002), pyruvate formate-lyase (Akhmanova *et al.* 1999)
Piromyces sp.	Opisthokonta, Fungi	hydrogenosome	pyruvate formate-lyase (Akhmanova *et al.* 1999)
C. parvum	Chromalveolata, Apicomplexa	mitosome	Cpn60 (Riordan *et al.* 2003), mtHsp70 (Slapeta & Keithly 2004)
Blastocystis sp.	Chromalveolata, Stramenopiles	mitochondria-like organelle	DNA, [Fe]-hydrogenase (Stechmann *et al.* 2008), DNA, NAD7, β-SCS, PFOR (Wawrzyniak *et al.* 2008), DNA (Perez-Brocal & Clark 2008)
N. ovalis	Chromalveolata, Ciliophora	hydrogenosome	DNA and hydrogenase activity (Boxma *et al.* 2005)
T. vaginalis	Excavata, Parabasalia	hydrogenosome	mtHsp70, mtHsp40, Cpn60, Cpn10, Pam18, (Bui *et al.* 1996; Dyall *et al.* 2003; Dolezal *et al.* 2005), α and βMPP (Brown *et al.* 2007; Šmíd *et al.* 2008), acetate:succinate CoA-transferase (van Grinsven *et al.* 2008), succinyl CoA synthetase α- and β-subunits (Lahti *et al.* 1994), adenylate kinase (Lange *et al.* 1994), malic enzyme (Hrdy & Müller 1995*b*; Dyall *et al.* 2004), ferredoxin, frataxin, Nfs, Isu (Sutak *et al.* 2004; Dolezal *et al.* 2005, 2007), [Fe]-hydrogenase, [Fe]-hydrogenase maturases HydG, HydE, and HydF, PFOR (Hrdy & Müller 1995*a*; Bui & Johnson 1996; Pütz *et al.* 2006), NdhA510Nuo8,Ndh24NuoE (Dyall *et al.* 2004; Hrdy *et al.* 2004), serine hydroxymethyl transferase, glycine cleavage H proteins H1 and H2, dihydrolipoamide dehydrogenase (Mukherjee *et al.* 2006*a*,*b*), SOD, rubrerythrin, thiol peroxidase (Pütz *et al.* 2005), MCF ADP/ATP transporter Hmp31 (Dyall *et al.* 2000), Hmp35 (Dyall *et al.* 2003), TrxR (Mentel *et al.* 2008), flavodiiron protein (Smutna *et al.* 2009)
G. lamblia	Excavata, Diplomonadida	mitosome	Nfs, Isu (Tovar *et al.* 2003), mtHsp70, Cpn60, ferredoxin (Regoes *et al.* 2005), βGFP (Šmíd *et al.* 2008), Tom40 (Dagley *et al.* in press)

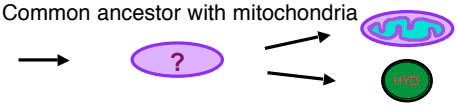
*Species are grouped according to super-groups and first rank or second rank names as defined in Adl *et al.* (2005). SOD, superoxide dismutase. Other abbreviations of protein names are defined in the text.

Hjort et al *Phil. Trans. R. Soc. B* 2010 **365**, 713-727

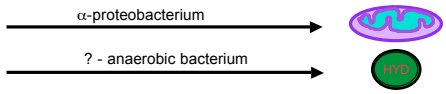
Origin of Hydrogenosomes (Mitosomes?)

1. a) Conversion of Mitochondria

b) Common ancestor with mitochondria



2. Independent Origin



Organelles - origins and biogenesis

Approaches:

(1) Conduct phylogenetic analyses of similar proteins
- Hsp70 Fd
- Hsp60 Isc subunits

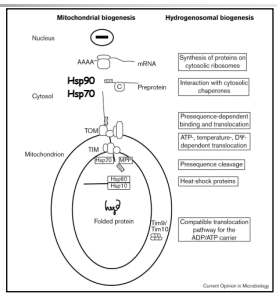
(2) Examine protein targeting to the organelle
- matrix protein targeting
- membrane protein targeting

(3) Characterize membrane/translocation components
- These components could have evolved as the endosymbiont was converted to organelle.
- Reveals evolutionary history.

Comparison of Biogenesis

- Nuclear encoded preproteins synthesized on cytosolic ribosomes.
- Kept in a translocation competent form by cytosolic chaperones
- Related TOM and TIM components.
- Similar energetic requirements
- Matrix chaperones and processing protein.
- Similar targeting complex to the inner membrane.

Review mitochondrial targeting if this is unfamiliar.



Translocase of the outer membrane - TOM
Translocase of the inner membrane - TIM
Mitochondrial processing peptidase - MPP

Protein sorting and targeting signals

Co-translational targeting of secretory proteins via the RER

• ER targeting
N-terminal cleaved peptide
20-50 amino acids (aa)
Basic & hydrophobic enriched

• Lysosomal targeting
Mannose-6-phosphate
(sugar moiety, not removed)

Post-translational targeting of organellar proteins via cytosolic chaperones

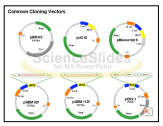
• Nuclear targeting
Basic, internal sequence (not cleaved)
Often bipartite

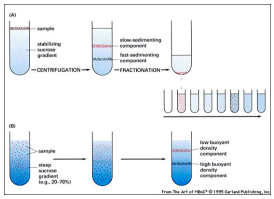
• Peroxisomal targeting
C-terminal peptide (not cleaved)
3 aa (Ser Lys Leu; SKL)
Other signals used also

• Mitochondrial Matrix targeting
N-terminal cleaved peptide
20-80 aa
Rich in Arg, Leu, Ser

Methodology to study processes

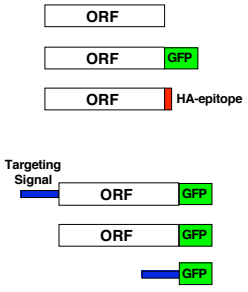
- Molecular Biology Tools
  - Manipulate organism
  - Cloning methodologies
- Cell Biology Tools
  - Observe the organism
  - Specific localization via tags, antibodies
- Biochemistry Tools
  - Cell fractionation
  - Proteomics





Molecular Engineering

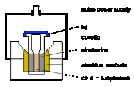
- Can changes the properties of any ORF that one wants to study
  - Visualization
    - introduce a tag
  - Mutations
- Overexpression vs. endogenous expression levels



Necessary and Sufficient

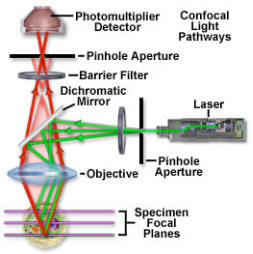
Transfection of DNA

- Electroporation of DNA
  - Electrical discharge
    - Can be used for various cell types
    - Emperically determine conditions for each cell type
  - Reversible destabilization of the cell membrane
  - Transient formation of membrane pores
  - Potentiates uptake of DNA



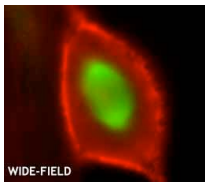
Confocal Microscopy

- Significant advancement
- Single point of light emission that can scan across the specimen
- Spatial filtering techniques to eliminate out-of-focus light
- Digital cameras
- Three-dimensional renderings of images

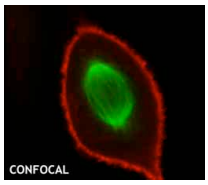


Comparison of Images

Background fluorescence



Sharper fluorescent images

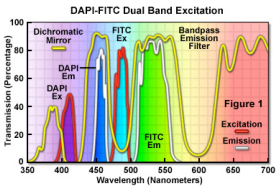


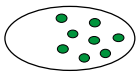
Single section

Optical sectioning

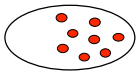
Co-localization studies

- Importance of appropriate markers
- Different colored fluorophores are used

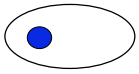




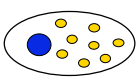
IscS



Mitotracker



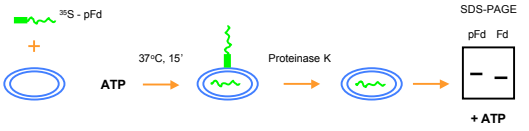
DAPI



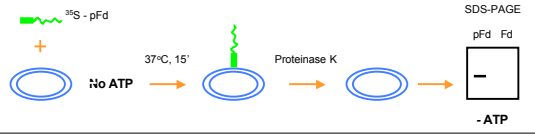
Merged Images

Protein Import Studies

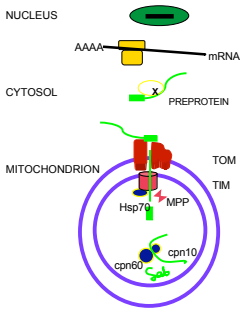
in organello translocation of pFd into isolated hydrogenosomes



Trap precursor using ATP depleted assay



Common features of mitochondrial and hydrogenosomal biogenesis established using protein targeting assays and biochemistry



Synthesis of preproteins on cytosolic ribosomes with mitochondrial-like presequence

Interaction with cytosolic chaperones
NEM-sensitive

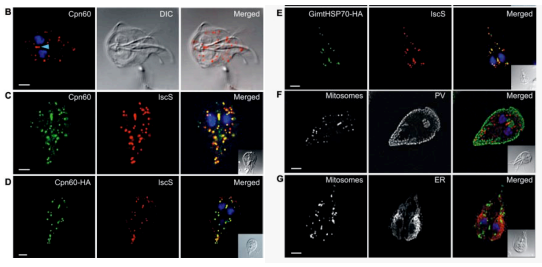
Presequence-dependent translocation
ATP, temperature, ΔΨ-dependence

Presequence cleavage (MPP)
Similar presequences

Mt-type Hsp 70
Chaperonins, cpn60/cpn10

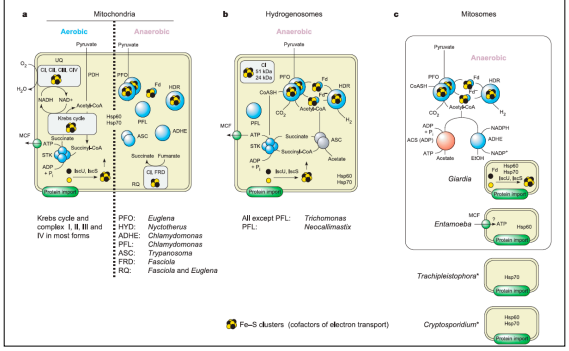
Common membrane carriers

Giardia Mitosome Localization



Key mitochondrial markers localize to the mitosome: Cpn60, Hsp70, IscS, IscU, Fd

Organellar Comparison



Questions that Remain

- **Why have the remnant organelles been retained?**
- **What is the function of the remnant organelle?**
- **How many other organisms have some variation of the classical mitochondrion?**
- **Will proteomic studies reveal the function of the mitosome?**