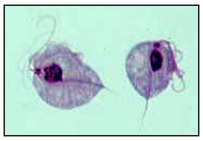
One more amitochondriate

Trichomonads

Trichomonads

- Class Trichomonada
  - All parasitic
- Family Trichomonadidae



Trichomonas tenax

Human - mouth

Trichomonas vaginalis

Human - urogenital

Tritrichomonas foetus

Bovine - urogenital

Trichomonas vaginalis

- **Worldwide distribution (cosmopolitan)**

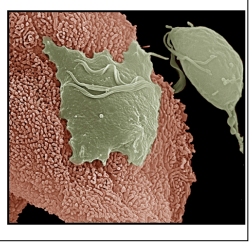
  7.4 million cases/yr in US (2007)
  200 milion cases/yr worldwide

- **Trichomoniasis - vaginitis**

- **Variable symptoms**

  Females - only ~ 15 are asymptomatic
  Asymptomatic, to mild or moderate infections, to extreme vaginitis
  - **50-75% abnormal discharge (frothy yellowish or greenish)**
  - **50% experience painful intercourse**
  - **vaginal erythema - (2%)**
    'strawberry cervix'

  Males - 50-90% are asymptomatic
  - **minor urethral discharge**



Trichomonas and HIV Transmission

T. vaginalis amplifies HIV transmission

- The pathology caused by *Trichomonas* infection includes:
  - punctate hemorrhages
  - infiltration of white blood cells (including lymphocytes and macrophages)

Trichomonas in an HIV Negative Person

Trichomonas in an HIV Positive Person

- *Trichomonas* makes an HIV + person more infectious
- Hemorrhages allow leakage of free virus
- Infiltration of white blood cells includes HIV infected leucocytes
- This results in higher concentration of HIV in the infected area

1: Sex Transm Dis. 2009 Jan;36(1):11-6.

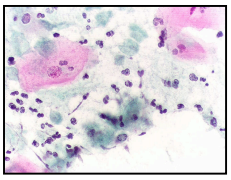
Trichomonas vaginalis treatment reduces vaginal HIV-1 shedding.

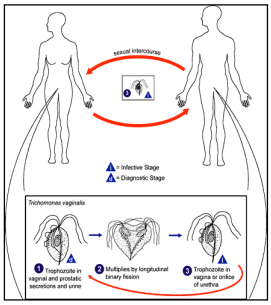
Kissinger P, Amedee A, Clark RA, Dumestre J, Theall KP, Myers L, Hagensee ME, Farley TA, Martin DH.

Tulane University Health Sciences Center - School of Public Health and Tropical Medicine, New Orleans, Louisiana 70012, USA. kissing@tulane.edu

Trichomonad Life Cycle - Direct

- One form - trophozoite
  - Binary fission
- Direct Transmission
- Direct contact with the parsite
- Classified as a STD





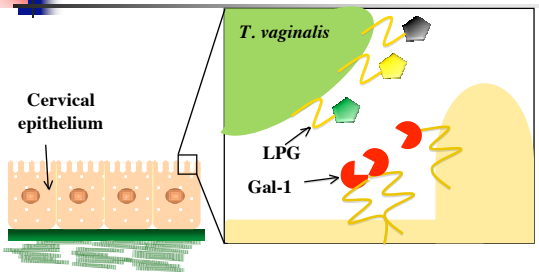
Cellular Microbiology (2008)

Galectin-1 on cervical epithelial cells is a receptor for the sexually transmitted human parasite *Trichomonas vaginalis*

Cheryl Y. Okumura, Linda G. Baum and Patricia J. Johnson

Department of Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA 90095, USA.

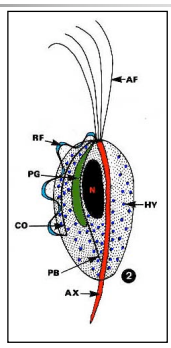
Galectin-1 binding



- Gal-1 in cervical epithelia is involved in parasite cross-linking
- Gal-1 is the first identified receptor in cervical epithelium that binds *T. vaginalis*
- Monosacharides in LPG interact with gal-1 homodimer

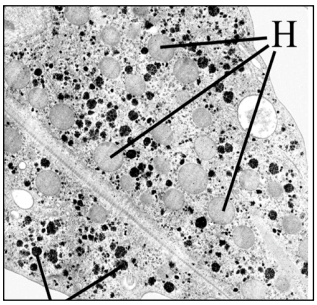
Trichomonad structure

- Anterior nucleus
- Anterior flagella
  - *T. vaginalis* - 4
  - *Tritrichomonas foetus* - 3
- Recurrent flagellum
- Costa
- Axostyle



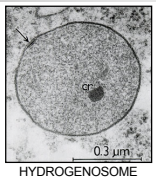
Amitochondriate??

- Contain a typical golgi
- Lack peroxisomes
- Lack mitochondria
- Granules associated with axostyle and costa
- Hydrogenosomes
  - Abundant double membrane organelles
  - Not a microbody!

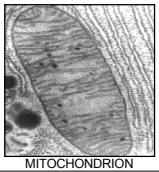


The Hydrogenosome

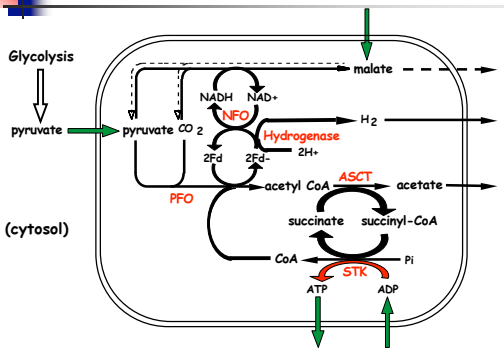
- Hydrogenosome
  - Anaerobic equivalent to mito
  - Pyruvate fermentation
    - ATP, CO_2, Acetate, H_2
- Differences with mitochondria
  - Morphology - no cristae
  - No oxidative phosphorylation
  - No DNA
- Evolution of mitochondria
- Drug target!



HYDROGENOSOME


MITOCHONDRION

Hydrogenosomal Metabolism

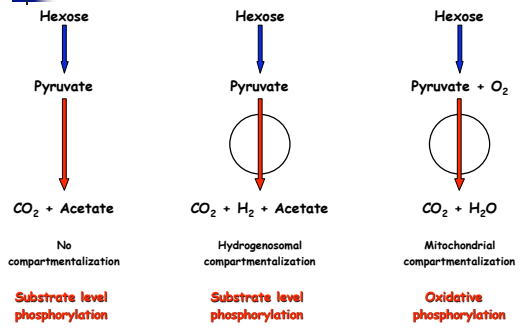


Hydrogenosomal/Mitochondrial Comparison

Only Mitochondrial	Shared	Only Hydrogenosomal
PDC complex	Malic enzyme	PFO
TCA cycle	Ferrodoxins	Hydrogenase
Cytochromes	Adenylate kinase	
Cytochrome oxidase	ATP/ADP exchanger	
DNA	Superoxide dismutase	
Cardiolipin		
F_0F_1 ATPase		

Oxidative phosphorylation vs. substrate level phosphorylation

Carbohydrate Metabolism is Eukaryotes



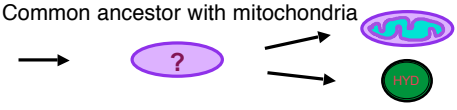
Synthesis of ATP

- Oxidative phosphorylation - coupling of ATP formation to the respiratory chain (electron transport, membrane associated, O_2 as final e- acceptor). As electrons move through complexes, a proton gradient is generated which drives ATP formation. **Chemiosmotic theory - P. Mitchell, 1978.**
- Substrate level phosphorylation - direct phosphorylation of ADP via the transfer from a high-energy intermediate.

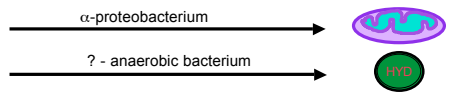
Origin of Hydrogenosomes

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

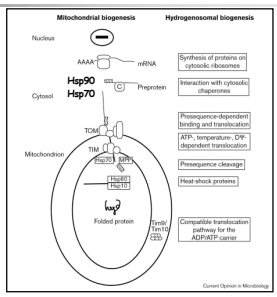
(2) Examine protein targeting to the organelle
- matrix protein targeting
- membrane protein targeting

(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 you are 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

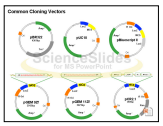
• Nuclear targeting
Basic, internal sequence (not cleaved)
Often bipartite

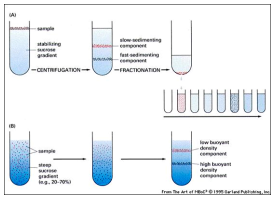
• Peroxisomal targeting
C-terminal peptide (not cleaved)
3 aa (Ser Lys Leu; SKL)
Other signals used also

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

Methodology to study processes

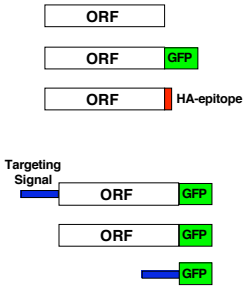
- Molecular Biology Tools
  - Manipulate organism
  - Cloning methodologies
- Cell Biology Tools
  - Observe the organism
  - Specific localization via tags, antibodies
- Biochemistry Tools
  - Cell fractionation
  - Proteomics





Molecular Engineering

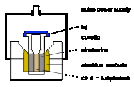
- Can changes the properties of any ORF that one wants to study
  - Visualization
    - introduce a tag
  - Mutations
- Overexpression vs. endogenous expression levels



Necessary and Sufficient

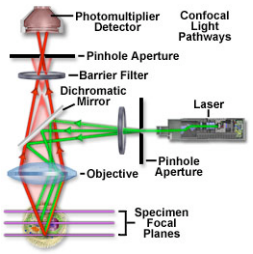
Transfection of DNA

- 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



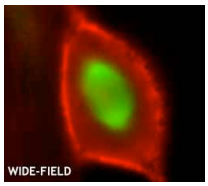
Confocal Microscopy

- 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

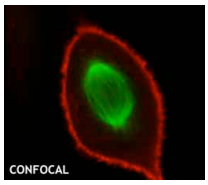


Comparison of Images

Background fluorescence



Sharper fluorescent images

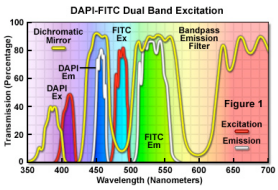


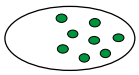
Single section

Optical sectioning

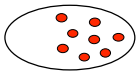
Co-localization studies

- Importance of appropriate markers
- Different colored fluorophores are used

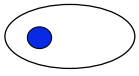




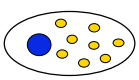
IscS



Mitotracker

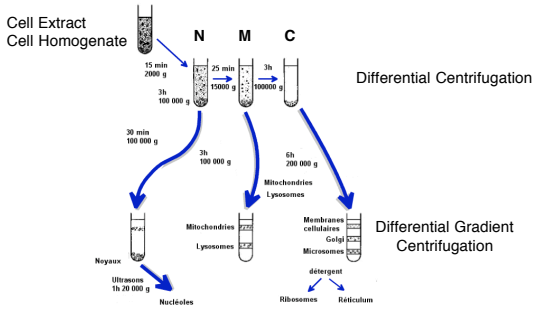


DAPI



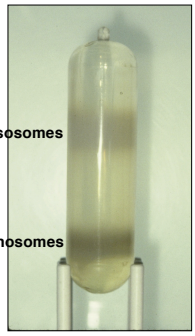
Merged Images

Cell Separation Techniques



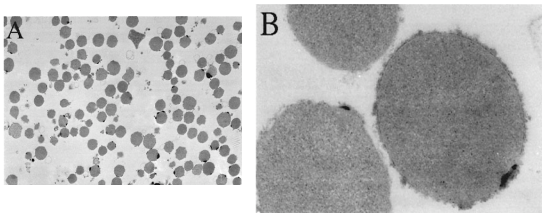
Density Gradient Centrifugation

- Different buoyant densities of organelles
- Linear gradient of viscous solution
- Purification of organelle of interest from other similar sized particles



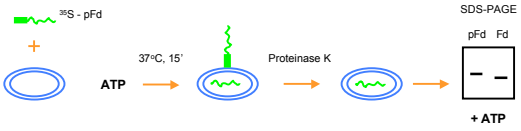
Discovered by Lindmark and Muller, 1973
Rockefeller University
Worked down the hall from C. de Duve!

Purified Hydrogenosomes

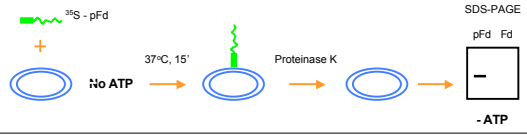


Protein Import Studies

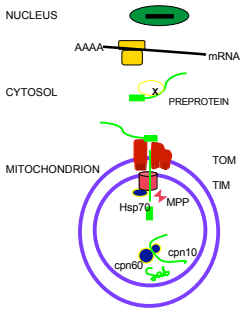
in organello translocation of pFd into isolated hydrogenosomes



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

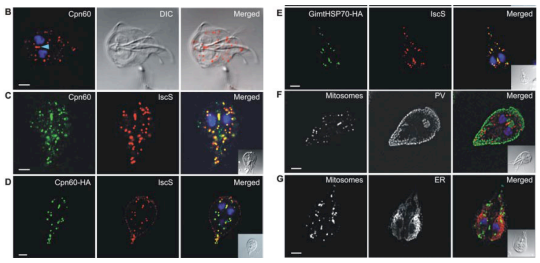
Presequence-dependent translocation
ATP, temperature, ΔΨ-dependence

Presequence cleavage (MPP)
Similar presequences

Mt-type Hsp 70
Chaperonins, cpn60/cpn10

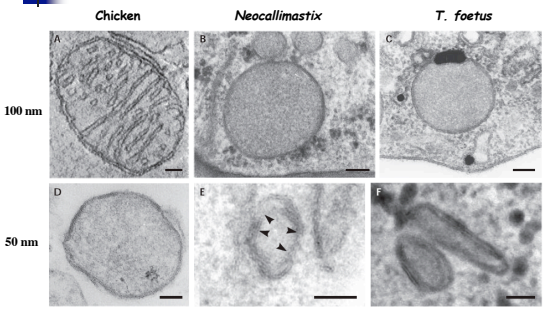
Common membrane carriers

Giardia Mitosome Localization



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

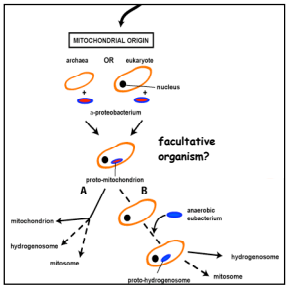
Mitochondrial Diversity



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

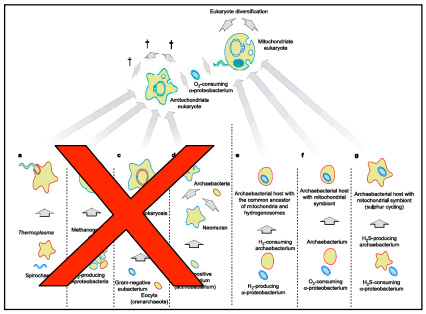
Current dogma - mitochondria and related organelles arose just once in evolution



Reconstructing Evolution

- Mitochondrial evolution
  - well established endosymbiotic theory
  - α-proteobacterium - *Rickettsia prowazekii*
- Hydrogenosomal evolution
  - No DNA (NOW 1 example 2005, *Nyctotherus!*)
  - Several proteins similar to mitochondria
- Mitosome evolution
  - No DNA
  - Few proteins identified similar to mitochondria

Reconstructing Evolution



Embley and Martin (2006) Nature 440:623