One more amitochondriate

Trichomonads

Class Trichomonada
  - All parasitic
Family Trichomonadidae

Trichomonas tenax
  - Human - mouth

Trichomonas vaginalis
  - Human - urogenital

Tritrichomonas foetus
  - Bovine - urogenital

Trichomonads

- Worldwide distribution (cosmopolitan)
  - 7.4 million cases/yr in US (2007)
  - 200 million 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, whitish, 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 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

Trichomonas in an HIV Negative Person
- Hemorrhages allow ready access of HIV
- Infiltration of white blood cells provides cellular targets for HIV

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

Trichomonad Life Cycle - Direct
- One form - trophozoite
- Binary fission
- Direct Transmission
- Direct contact with the parasite
- Classified as a STD

Diagnosis
- Clinical presentation: discharge, strawberry cervix
- Visualization of the motile parasites in vaginal secretions (45-60% sensitivity)
- Culture of parasites in special media. (InPouch).This method will identify up to 95% of infections.
- Antigen detection test from Genzyme Corp. (78.5-98.6% sensitivity)
- Oligonucleotide probe test (80-90% sensitivity)
- PCR-based tests

Trichomonas and HIV Transmission

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OSOM® Trichomonas Rapid Test
A rapid test for the detection of Trichomonas Vaginalis
A rapid test for the detection of Trichomonas Vaginalis
- Easy - to - Run - Dipstick simple, one reagent.
- Accurate - 95% agreement against Composite Reference Standard (culture and wet mount).
- Unaffected by other common infections.
Treatment

- METRONIDAZOLE: 2g orally in a single dose (97% cure rate)
  Brand name: Flagyl.
  Sexual partner should be treated
- TINIDAZOLE: recently licensed for use in the USA. Useful for resistant infections. (Really??)

Sexual education helps prevent the spread of this disease

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Similarities and Differences

<table>
<thead>
<tr>
<th>G. lamblia</th>
<th>E. histolytica</th>
<th>T. vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Flagellate</td>
<td>Flagellate/Amoeba</td>
</tr>
<tr>
<td>Life Stages</td>
<td>Trophozoite Resistant Cyst</td>
<td>Trophozoite Only</td>
</tr>
<tr>
<td>Host</td>
<td>Many Mammals Including Humans</td>
<td>Human Only</td>
</tr>
<tr>
<td>Disease Manifestations</td>
<td>Diarrhea</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Transmission</td>
<td>Ingestion of Cyst</td>
<td>Ingestion of Cyst</td>
</tr>
</tbody>
</table>

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“Amitochondriate” Comparison

- Early Diverging Eukaryote
- Lumen-dwelling
- Extracellular Parasite
- Treatment via Metronidazole
- Microaerophilic
- Pyruvate fermentation
- No “Classical” Mitochondria
Genome sequenced in 2007

- Highest coding capacity among Eukaryotes
- Massively expanded gene families
- One of the most repetitive genomes sequenced
- Large number of lateral gene transfers
- Newly discovered metabolic pathways
- Multiple ferredoxin genes

Genome Sequenced 2007

- Dense glycocalyx composed of LPG
- Genes for LPG biosynthetic pathway
- Monosaccharide rhamnose - absent in the human
- Largest family of membrane proteins - BspA-like
  - Similar to leucine rich adherence proteins in bacteria
- Much more work is required to understand the roles of these surface proteins.

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 (CEC) is involved in parasite cross-linking
- Gal-1 is the first identified receptor in CE that binds T. vaginalis
- Monosaccharides 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, CO₂, Acetate, H₂
- Differences with mitochondria
  - Morphology - no cristae
  - No oxidative phosphorylation
  - No DNA
- Evolution of mitochondria
- Drug target!
- Christian de Duve (relationship?)

Exploring Cells

- Light Microscopy - localization
- Electron Microscopy - localization
- Genetics - Phenotypic changes

Exploring cells with a centrifuge!

Founders of Modern Cell Biology

Keith Porter
National Medal of Science 1976

"If I have seen further it is by standing on the shoulders of giants" - Isaac Newton
Subcellular Fractionation

- Methodologies developed in the ‘50s
- Development of the ultracentrifuge
- Instrumental in the discovery of:
  - Lysosomes - turned 55 in 2011!
  - Peroxisomes
  - Hydrogenosomes - will turn 40 in 2013
- Ability to correlate the sedimented enzymatic activities with similar kinetics; could be attributed to structure(s) observed via EM.

Subcellular Fractionation

- The ultimate reductionist approach
- ‘80s-'90s - popularity of this method declined
  Why?
- 21st century - is this still a valid approach?

Organellar Proteomics!!
Think about Johnson Rev2011 - several proteomic studies

Cell Separation Techniques
Important Concepts/Assumptions

- Different sedimentation coefficients
- Polydispersity of a population
- Looked at the distribution of single enzymes across the fractionation ( Histograms )
- Postulate of biochemical homogeneity
  
  large particles contain same biochemical composition - just more of it
- Postulate of single location
  
  One enzyme - one location

Quality Control

- Balance sheet that contains:
  - Recorded volumes of fractions
  - Distribution of protein among the fractions
  - Distribution of marker enzymes - proteins
  - Latency of those marker enzymes
  - Confirmation via EM studies

Histogram Analysis

- 1st introduced by de Duve

<table>
<thead>
<tr>
<th>Protein %</th>
<th>N</th>
<th>M</th>
<th>P</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative specific activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Histogram Analysis

- 1st used by de Duve

- Cytochrome oxidase enriched in mitochondria

- N-nuclear
- M-mitochondrial
- P-microsomal
- S-supernatant

- % protein

- Relative specific activity

- Original data

- De duve (1955)

- Organelles (structures) are separated by their physical properties.

- Problem: some of these compartments share physical properties.

- Pattern I
- Pattern II
- Pattern III

- Original data

- De duve (1959)

- N-nuclear
- M-heavy mitochondrial
- L-light mitochondrial
- P-microsomal
- S-supernatant

- Possible problems:
  - Breakage of particles
  - Adsorption of soluble parts
  - Leakage from particles

- Much more fundamental:
  - How to separate populations that display polydispersity
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

Hydrogenosomal Metabolism

(cytosol)
**Hydrogenosomal/Mitochondrial Comparison**

<table>
<thead>
<tr>
<th>Only Mitochondrial</th>
<th>Shared</th>
<th>Only Hydrogenosomal</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDC complex</td>
<td>Malic enzyme</td>
<td>PFO</td>
</tr>
<tr>
<td>TCA cycle</td>
<td>Ferredoxins</td>
<td>Hydrogenase</td>
</tr>
<tr>
<td>Cytochromes</td>
<td>Adenylate kinase</td>
<td>ATP/ADP exchanger</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>DNA</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>Cardiolipin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F_{0}F_{1} ATPase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Oxidative phosphorylation vs. substrate level phosphorylation

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**Carbohydrate Metabolism is Eukaryotes**

- Hexose → Pyruvate → CO₂ + Acetate → No compartmentalization
- Hexose → Pyruvate → CO₂ + H₂ + Acetate → Hydrogenosomal compartmentalization
- Hexose → Pyruvate → CO₂ + H₂O → Mitochondrial compartmentalization

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**Synthesis of ATP**

- **Oxidative phosphorylation** - coupling of ATP formation to the respiratory chain (electron transport, membrane associated, O₂ 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.