The Three Kingdoms of Life

New Eukaryotic Phylogeny
Alveolates - diversity

Phylum Apicomplexa (sporozoa)

- Large and diverse group (>5000 species)
- All members of this phylum are parasitic
- No cilia or flagella (except for some microgametes)
- Movement by gliding motility
- All members possess an apical complex
- Complex life cycles
  - Spore-like forms - cysts
  - Sexual and asexual stages
  - Intracellular stages
- Class Perkinsasidea
- Class Conoidasida - Coccidia
- Class Aconoidasida - Haemosporidia
Apicomplexans

- **Gregarines**
  - parasites of invertebrates, some quite big (used as early research models)

- **Coccidians**
  - tissue parasites of vertebrates and invertebrates (can have single (e.g. *Eimeria*) or two host (e.g. *Toxoplasma*). Many parasites of medical and veterinary importance. Sex produces a sporelike oocyst

- **Haemosporidians**
  - (*Plasmodium*) and Piroplasms (*Babesia & Theileria*): small parasites of blood cells which are transmitted by arthropods

Important human and animal parasites

- *Plasmodium* - Malaria
- *Toxoplasma* - Toxoplasmosis
- *Cryptosporidium* - Cryptosporidiosis
- *Eimeria* - Coccidiosis
- *Sarcocystis* - Sarcocystosis
- *Cyclospora* - Cyclosporosis
- *Isospora* – Isosporiasis - rare
- *Babesia* – Babesiosis - rare
Morphological diversity

- Basic biology and life cycle
- Host cell invasion
- Apicomplexan cell division
- Newly discovered organelles
- Modification of the host cell
- Pathogenesis of disease
- Mechanisms of drug action and resistance
- Why don’t we have a malaria vaccine?

- Will use mainly *Plasmodium*, *Toxoplasma* and *Eimeria* as examples.
General Apicomplexan Life Cycle

- Sporogony - 1 zygote gives rise to many sporozoites
- Gamogony - gamont gives rise to many gametes
- Merogony - process that increase the number of infective cells

Apicomplexan General Features

- Apicomplexans are haplonts and meiosis directly follows fertilization
- All replication occurs inside of host cells (with the exception of the conclusion of meiosis in certain species)
- There are several invasive zoite stages
Apicomplexa can separate nuclear division from cytokinesis

- Growth
- Multiple rounds of nuclear division (B)
- Segregation (C)
- Cytokinesis (D)
- Many progeny from 1 cell (E)

3 modes of intracellular replication

- Endodyogeny
  - *Toxoplasma*

- Schizogony
  - *Plasmodium*

- Endopylogeny
  - *Sarcocystis*
**Apical complex**

- Ultrastructural complexity at the anterior end
- Electron dense structures
- Concentration of organelles

**Apicomplexan Ultrastructure**

- Apical complex plays a role in invasion
  - Rhoptries and Micronemes - modified secretory organelles
- Apicoplast

Ultrastructure of a *Toxoplasma gondii* tachyzoite
Specialized Secretory Organelles

- microneme
- rhoptry
- dense granule

Apicomplexan host cell invasion
Invasion depends on parasite actin
NOT host cell actin

- Cytochalasin treatment does not appear to inhibit attachment (bar graph in c shows number of parasites bound to cells at different drug doses)
- CytD inhibits the movement of the parasite into the host cell.
- A parasitophorous vacuole (PV) is still set up, however the parasite can not move in, and the moving junction remains at the apical tip of the parasite.


Secretion during invasion is ordered

Not via phagocytosis!
Gliding Motility

- Substrate-dependent motion that requires an actin-myosin motor
  - Cytochalasins inhibit (actin destabilizer)
  - Gliding is coupled to translocation of cell surface adhesins (deposit on surface)
- Differs from amoeboid movement
  - Also actin based, cell deformation
  - Apicomplexan gliding - no deformation
- Assists in 3 vital functions
  - Migration
  - Invasion
  - Egress
- Movement includes:
  - Circular, upright twirling, helical

Toxoplasma Motility

Hakansson et al 1999
Mol Biol Cell 10: 3539

Circular Gliding by Toxoplasma

Figure 1
Hakansson et al., 1999

Stationary Twirling by Toxoplasma

Figure 2
Hakansson et al., 1999

Antibody staining of a surface antigen
The gliding machinery is anchored in the inner membrane complex.

**Gliding Translocation Model**

MIC2/M2AP - hexameric complex essential role

Migration

Essential role

Translocation (capping direction)
The Moving Conveyor Belt

The Glideosome

The Molecular Machinery
Responsible for
Gliding Motility, Host Cell Invasion and Egress in Apicomplexan Parasites

The conveyor-belt model

- Motility depends of parasite actin/myosin (MyoA)
  The MyoA is parasite specific - different from host myosin
- Myosin is anchored into the outer IMC membrane
  Complex of proteins (GAP45/50, MyoA, MLC)
- Short actin filaments form and are moved towards the posterior end of the parasite by the myosin power stroke
- The short actin filaments are linked to microneme proteins by an adaptor Aldolase - moonlighting protein
- Movement of actin filaments results in movement of microneme proteins
- Microneme proteins are shed at the back end (rhomboid proteases are the best candidates for this activity)
- The parasite glides over the substrate
The parasitophorous vacuole IS NOT fusing with lysosomes

- Macrophage cells were incubated with live (A/B) or heat killed (C/D) parasites.
- Note that only vacuoles containing heat killed parasites show staining for a lysosomal marker protein.
- Dead parasites go in by phagocytosis, living parasites enter differently.

Joiner et al. 1990, Science 249:641-6

The Parasitophorous Vacuole

- After invasion parasites reside within a new compartment the PV.
- The PV is derived from host cell membrane but behaves different from a phagosome.
- The PV membrane is derived from the host cell plasma membrane.
- The PV is provided by the parasite (e.g. by secretion).
- Both contribute to the PV.
The PV is highly modified to suite the parasite’s needs

- Tubular network increases surface (dense granule)
- Sieving pores give access to small nutrient molecules in the host cell cytoplasm (probably dense granule)
- Specific host cell organelles are recruited close to the PV membrane (rhoptry)

Dense granules are involved in establishing the intravacuolar network
**Intestinal Coccidian Species**

- **Apicomplexa**
  - Coccidia
    - Piroplasmida
      - Theileriidae
        - Babesiidae
    - Eimeriida
      - Cryptosporidiidae
        - Cryptosporidium
      - Sarcocystidae
    - Haemosporida
      - Theileriidae
      - Babesiidae
      - Haemosporida
        - Plasmodium

**Similarities**

- Direct life cycles - no intermediate hosts
  - Homoxenous
- Oral-fecal transmission
- Infective stage - oocyst
- Oocysts in contaminated feces are not immediately infective
- Usually contaminated food or water
- Human infections
- Direct Human-Human infection is unlikely
- Oocysts must “mature”
- Of significance as opportunistic infections in immunocompromised people
**Eimeria coccidiosis**

- Disease of chickens
  - other animals as well! (2500 species)
- Can cause high mortality
  - Young birds
- Serious disease causing bloody diarrhea, death
- Parasite replication causes bleeding, and massive swelling in gut
- Once infection is established there is no effective chemotherapy
- In US alone, cost of disease is about $80 million/year including coccidiostats (in the feed).

**Eimeria Life Cycle**

[Diagram showing the life cycle of Eimeria]
**Sporogony**

- Unsporulated cysts are non-infective
  - UV (sunlight) and dessication sensitive
- Sporulation requirements
  - Oxygen
  - Moisture
  - Lowered temperature (exp: 20-23°C)
  - Sporulation can be fast - 24 hr
- 1st nuclear division is meiosis
- Subsequent divisions are mitotic
- Sporulated cyst
  - 4 sporocysts - each with 2 sporozoites
  - Resistant form - environmental factors
  - Storage in 2% potassium dichromate

**Merogony - Programmed Amplification**

- Excystation - release of sporozoites
  - Mechanical, enzymatic, acid, bile
- Asexual reproduction
  - 1 merozoite produces 2-10,000
- Each *Eimeria* species has a set number of merogonous generations
Gamogony

- Sexual reproduction
- Majority of gamonts produced are macrogamonts
  - Macrogamont (F) - unicellular
    - Large number of granules - destined to be oocyst cell wall
  - Microgamont (M)- multiplication, release biflagellated microgametes

Interesting factoids

- A single oocyst of *Eimeria tenella* will produce 1 million more
- 1 gram of chicken litter (waste) can contain between 100,000 and 200,000 oocysts
- Birds (animals) that are in constant contact with small numbers of oocysts develop immunity to that specific oocyst species
- An ounce of prevention….
### Coccidiosis in Chickens

<table>
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<th>E. acervulina</th>
<th>E. tenella</th>
<th>E. brunetti</th>
<th>E. necatrix</th>
<th>E. mitis</th>
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