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David Roos' Lecture Part 1:
Toxoplasma and other Apicomplexan Parasites

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1. Keywords and Terms

Protozoa, eukaryotic evolution, subcellular organelles, parasite life cycles, Apicomplexa, *Plasmodium, Toxoplasma,* apical complex, rhoptry, microneme, plastid, inner membrane complex, parasitophorous vacuole, conoid, cytokinesis, schizogony, endodyogeny

2. Lecture Notes

Introduction

While animals and plants are more visible to the naked eye, protozoa (unicellular organisms) constitute the majority of Eukaryotic life. Studying the biology of a model

parasitic protozoan can therefore provide insight into the basic biology of eukaryotes (whose cells contain a nucleus, as distinct from bacteria and archaebacteria). Of course, studying these organisms also provides insight into host-parasite interactions and mechanisms of pathogenesis.

The Phylum Apicomplexa encompasses over 5,000 species of unicellular parasitic protozoa, many of which cause serious disease in humans. For example, *Plasmodium* parasites are transmitted by mosquitoes, infect red blood cells and cause malaria, a disease with devastating impact on children in sub-Saharan Africa, Southeast Asia and South America. Other Apicomplexan parasites of medical importance include *Cryptosporidium* and *Toxoplasma,* which infect a large percentage of the population worldwide, and can cause debilitating and potentially fatal illness in immunocompromised individuals. *Toxoplasma* is a leading cause of congenital neurological birth defects, transmitted by consumption of undercooked meat or ingestion of material contaminated with cat feces (this is why pregnant women are advised not to empty the kitty litter box!)

Using Toxoplasma as a model to understand what is shared with other eukaryotes, and what is unique

Although they cause very different diseases, all apicomplexan parasites originated from a common ancestor and consequently share many characteristics:

- All are obligate intracellular parasites ... they must invade and replicate within host cells in order to survive.
- They traverse a complex life cycle, differentiating (developing) through various forms, often in different host species/tissues in the course infection. In particular, sexual and asexual reproduction often occur in different species (such as mosquitoes and humans for *Plasmodium*).
- From a cell biological perspective, apicomplexan parasites contain all of the organelles one might expect in a eukaryote, including the nucleus, endoplasmic reticulum, Golgi apparatus, and mitochondrion.

Apicomplexan parasites also possess distinctive subcellular organelles, including:

- Specialized secretory organelles, termed rhoptries and micronemes, that deploy their cargo in a coordinated fashion during parasite attachment to

the host cell, invasion, establishment of the intracellular "parasitophorous vacuole" within which they reside and replicate, and modulation of the host cell.
- A plastid organelle, known as the "apicoplast" (apicomplexan plastid), acquired through "secondary endosymbiosis", in which an ancestral parasite ate a eukaryotic alga, and retained the algal plastid (see lecture 2).

Despite the medical importance, and inherent biological interest, of these parasites, the feasibility of laboratory investigation depends on accessibility to experimental manipulation. Several key features make *Toxoplasma* experimentally tractable as a model organism:

- Easily cultivated in the laboratory.
- Mouse infection and disease provides a good model for human toxoplasmosis.
- Genetic crosses can be carried out in cats (without harming the cat).
- Amenable to modern molecular techniques (parasite genes can be removed, replaced, or foreign genes can be inserted).
- Complete genome sequence is available, along with various tools and reagents for functional genomics (e.g. microarrays) and bioinformatic analysis (see lecture 3)
- Outstanding ultrastructural resolution for cell biological studies.

How Toxoplama (and other apicomplexan parasites) replicate.

Once the parasite has invaded a host cell, its survival is critically linked to replication. The replicative process occurs entirely within a specialized intracellular vacuole termed the parasitophorous vacuole. As the parasite divides, the host cell swells and eventually bursts (a lytic infection), causing disease through direct tissue damage. Similarly, in malaria, *Plasmodium* parasites lyse infected red blood cells, causing anemia (other factors may also exascerbate anemia in severe disease). Toxoplasma damages multiple organs systems, but is particularly noted for its ability to quickly destroy brain and fetal tissues.

Apicomplexan parasites replicate via an unusual process in which daughter parasites are assembled within a single mother cell. In the asexual "tachyzoite" stages of *Toxoplasma* infection, two daughter cells emerge from each mother (endodyogeny). In *Plasmodium* "merozoites", up to 16 or more daughters form within a single mother cell

("schizogony"). Assembly of daughter cells within the mother offers several advantages, such as the ability to eliminate indigestible waste products (such as the toxic heme left over after digestion of hemoglobin by *Plasmodium*) by simply leaving them behind. These parasites don't need lysosomes! Assembly of the cytoskeleton from the top (apical end) down, also ensures that polarity is preserved, as required for invasion of the host cell. Replication by endodyogeny or schizogony also poses challenges: a complex process is required to ensure that each daughter inherits a complete set of organelles. Note that unlike division in the typical 'textbook' eukaryotic cell, "M" phase (including organellar replication) is a lengthy process, completely encompassing the DNA replication hallmark of "S" phase. This suggests that unique checkpoints are likely to operate in the apicomplexan cell cycle.

3. Recommended Reading

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4. Review Questions

1. Name (at least) three key features that make *Toxoplasma* a model experimental parasitic organism.

2. Name (at least) two components of the *Toxoplasma* apical complex.

3. Superficially, *Toxoplasma* and *Plasmodium* appear to divide by quite different processes: *Toxoplasma* produces two daughters, while *Plasmodium* produces ~16 (in the acutely lytic stages of human infection). Why might one consider the replication these parasites more notable for their similarities than their differences?

4. What is "endosymbiosis"? What is "secondary endosymbiosis"? Which toxoplasma organelles derived from endosymbiotic events?

5. What are some key events that take place during the process of endodyogeny?

6. There is no evidence that classical lysosomes exist in apicomplexan parasites. If this is true, what happens to waste material generated in parasites?

5. Answers to Review Questions

1.

a. Easily cultivated in the laboratory.
b. Mouse infection and disease provides a good model for human toxoplasmosis.
c. Genetic crosses can be carried out in cats.
d. Amenable to molecular genetic manipulation (transgene expression, homologous and nonhomologous recombination, regulatable expression, etc).
e. Outstanding ultrastructural resolution for cell biological studies.
f. Complete genome sequence, functional genomics tools (e.g. microarrays), and genome databases are available.

2.

a. Micronemes (specialized secretory orgenelles thought to play a critical role in host cell attachment and invasion)
b. Rhoptries (larger, club-shaped secretory organelles, though to be critical for establishment of the intracellular parasitophorous vacuole)
c. Conoid (a distinctive coil of tubulin-based filaments at the exteme apical end of the parasite ... function unknown)

3.

a. Both processes involve the assembly of daughter cells within the mother cell, rather than division of the mother by binary fission (or budding).
b. Both cells uncouple nuclear division from cytokinesis, and employ a complex process to segregate organelles between the developing daughters.
c. Both build daughter parasites from the top down, ensuring polarization (with the microneme and rhoptry organelles needed for invasion at the top).

4.

a. The mitochondria of these parasites was acquired when an alpha-proteobacterium invaded into an ancestor of all eukaryotic cells (endosymbiosis).
b. The apicoplast was acquired by secondary endosymbiosis, when an ancestor of all apicomplexan parasites 'ate' a eukaryotic alga, which had previously engulfed a cyanobacterium (the ancestor of all plastids, including the chloroplasts of green plants).

5.

   a. Centriolar division: centrioles migrate from the apical end of the nucleus to the basal end, where they divide. They then return to the apical end of the nucleus where they associate with other organelles (Golgi, apicoplast, etc).
   b. The Inner Membrance Complex forms in the apical end of the parasite, elongating to form the scaffolding on into which daughter cell organelles are segregated (nucleus, ER, etc).
   c. The mitochondrion is the last organelle to divide, late during the process of endodyogeny.
   d. New micronemes and rhoptries are formed de novo, within each daughter parasite.

6. Essentially parasites leave waste material behind as daughter cells are formed. This is called the residual body.

6. Discussion Questions

1. Why do you think the *Plasmodium* replicative cycle is so much longer than the *Toxoplasma* cell cycle (~48 hr vs ~8 hr)?

2. Parasitologists like to understand the way in which parasites regulate host cell gene expression in the infected cell (and there are some fascinating examples of this). Why might this rationale NOT apply when thinking about *Plasmodium falciparum*?

3. Why do you think apicomplexan parasites have a polarized cellular architecture?

4. You have discovered a new intracellular parasite of birds, and you suspect that it may be a member of the phylum Apicomplexa. How could you confirm this? What would you look for and why?

5. Knowing what you now know about the cell biology of apicomplexa, which organelle would make the best target to develop drugs against, and why?

7. Answers to Discussion Questions

1. The cell cycle of Plasmodium is longer because many daughters must be assembled inside the original mother before segregation. In contrast, Toxoplasma replication occurs much more quickly, as one mother produces only two daughters before cytokinesis.

2. Hint: it has to do with the type of cell this parasite infects. Plasmodium falciparum infects red blood cells, which lack all organelles, including a nucleus. Therefore, there are no host genes there to regulate!

3. This "layout" is likely key to the ability of these parasites to invade cells. As the parasite contacts a host cell, it will briefly glide across the surface before turning the apical end down and invading the cell with a cork-screw motion. As this occurs, the specialized and apically-oriented secretory organelles will deploy their cargo, coordinating essential events of invasion and establishment of the parasitophorous vacuole.

4. You would want to look for any number of "signature" features of the apicomplexa, such as a plastid, conoid, or specialized secretory organelles. These features would all be evident be examining the cellular ultrastructure of the organism, but could also be examined using antibody labeling of fixed speciments. In addition, a molecular approach could be taken in which genetic material could be analyzed for regions of similarity to known and highly conserved apicomplexan genes.

5. The plastid is a likely drug target because it contains essential metabolic enzymes that are of plant origin. Since these bear little, if any, resemblance to components of our own metabolic pathways, they can be targeted without the concern that our own cells and tissues will be damaged.

8. Explain or Teach These Concepts to a Friend

1. Describe the similarities and differences between endodyogeny and schizogony.

2. Explain how the timing of events in the apicomplexan cell cycle differ from those the typical eukaryotic cell cycle.

3. Describe the body-plan, or layout, of an apicomplexan parasite?

9. Research the Literature on Your Own

1. Consider the life-cycle of Toxoplasma -- How might the needs of the parasite change when it differentiates from the rapidly growing tachyzoite form, into slow growing, cyst-forming bradyzoite? How might these changes impact aspects of the parasites cell biology?
2. A recent forward genetic screen was carried out in toxoplasma to identify regulators of the parasite cell cycle (see references; Gubbels, PLOS Pathogens, 2008). How was this screen designed and what was learned from it?
3. What is the global health impact of malaria? Why don't we hear more about this disease in the news? Which apicomplexan parasite has the greatest impact on animal health?
4. Which apicomplexan parasites are zoonotic?