1. Keywords and Terms

Apicoplast (plastid), “Delayed-death”, episome, endosymbiosis, mitochondrion, bioinformatics, bipartite signal sequence, transgenic parasite

2. Review Questions

1. Name the two Apicomplexan organelles that were derived from endosymbiotic events.

2. What metabolic pathways reside either partially or wholly in the apicoplast.

3. What curious experimental observation led to the eventual discovery of the apicomplexan plastid, or “apicoplast”? 
4. Describe the bipartite signal sequence that allows transport of proteins to the apicoplast.

5. Unfortunately, the apicoplast genome tells us little about its function. Where are the other genes required for apicoplast function located? Is this surprising?

6. Why has the apicoplast been more challenging to study than the chloroplast in plants? What approach has proved most productive in elucidating the function of the apicoplast?

7. Describe the “delayed-death” phenotype.

3. Answers to Review Questions

1. Mitochondrion and plastid

2. Fatty acid synthesis and isoprenoid biosynthesis

3. Surprisingly, the initial observations that led to the discovery of the apicoplast were not cell biological studies, rather they were experiments addressing mechanisms of drug action. It was observed that several well-known antibiotics could kill Plasmodium and Toxoplasma. This was a particularly interesting result because apicomplexa are eukaryotes, their protein machinery (the target of many antibiotics) are not “bacterial-like”.

4. Nuclear-encoded apicoplast proteins must transit to the apicoplast to perform their function. This is achieved by a bipartite signal sequence attached to the amino-terminus of newly synthesized proteins destined for the apicoplast. First, at the most extreme amino-terminal end, a hydrophobic secretory signal directs the secretion of the protein. Fusion of this short sequence to an irrelevant gene is sufficient to target it for secretion into the parasitophorous vacuole space. Second, adjacent to the secretory signal sequence is a longer plastid targeting sequence that dictates transit specifically to the apicoplast. Molecular “cut-and-paste” experiments revealed that the exact sequence necessary for this transit is complex and redundant.

5. They are nuclear-encoded and, no, this is not surprising. As is the case with the genomes of most organelles derived from endosymbiotic events, many genes have
been transferred to the nucleus. This means that the gene product (a protein) must be transported back to the plastid to perform a function.

6. Well, it’s simple (and cheap!) to grow lots of plants, allowing you to collect huge numbers of chloroplasts for biochemical studies. The same is not true for apicomplexan parasites. At only a few millionths of a meter in size, even large-scale experiment in which billions of parasites are cultured will only yield minuscule amounts of purified plastid material – far too little for most biochemical experiments. For these reasons, the most productive approach to studying the function of the apicoplast has been a bioinformatics approach.

7. Unlike bacteria, Apicomplexan parasites do not die rapidly upon exposure to antibiotics. Instead, parasites treated with antibiotics (even at 10,000 times the lethal dose) are still able to grow for 48hrs (6-8 cell cycles), escape from their host cells and invade a new host cell. In this new host cell they will replicate very slowly (a rate that is determined by the dose of drug they saw in their original cell) and will eventually die.

4. Discussion Questions

1. Why is the plastid surrounded by four membranes, while the mitrochondrion is only surrounded by two?

2. As a graduate student you have identified a new gene whose protein product you believe is transported to the apicoplast. Describe at least two methods you might use to test this hypothesis.

3. When during the parasite cell cycle does apicoplast protein transport occur? How was this experimentally determined?

4. Outline the basic workflow that was used to computationally determine which nuclear-encoded apicomplexan genes might target to the plastid.
5. Answers to Discussion Questions

1. This question really comes down the evolutionary origin of these two organelles. The mitochondrion was acquired by a primary endosymbiotic event in which a common ancestor of all eukaryotic organisms was invaded by an alpha-proteobacterium. In contrast, the apicoplast was obtained by way of a secondary endosymbiotic event, 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).

2. A conventional approach to this common dilemma involves creating a fusion of your gene of interest with a gene encoding a fluorescent protein tag. When expressed in parasites, your protein of interest, and its location within the parasite, will be visible in living parasites illuminated under a fluorescent microscope. This allows you to essentially watch the trafficking of your protein as the parasite grows inside the host cell. Unfortunately, fusing a fluorescent tag to your protein of interest can often disturb the synthesis, trafficking or function of a protein. A second and more traditional cell biological approach to this question involves the use of immuno-gold electron microscopy. In this case, antibodies are made to your protein of interest and are labeled with tiny (nanometer scale) gold particles. Thin sections of parasites are incubated with the antibodies, which bind, or stick, very strongly to your protein of interest. Sections are examined by ultra-high resolution electron microscopy, in which the gold particles can be seen as dark spots, indicating the exact location of your protein.

3. Protein targeting to the apicoplast occurs just prior to parasite cell division. This was determined using a technique called “photobleaching”. First, transgenic parasites were made in which a known apicoplast targeted protein was tagged with yellow fluorescent protein (YFP) so that its location in the parasite could be visualized by fluorescent microscopy. In these parasites, the apicoplast will glow bright yellow. An intense light source is used to bleach out a small portion of the yellow fluorescence in the apicoplast, and the parasite is monitored over time under the fluorescent microscope. When this was done just prior to parasite division, new fluorescent protein filled in the photobleached area, indicating that protein was being actively transported to the apicoplast. If, however, the bleaching was done just after parasite division, the photobleached area remained dark, as no YFP-tagged protein was being transported to the apicoplast.
4. Get together all the available genome sequences for apicomplexan organisms. Use computational methods to search the apicomplexan genomes for any plastid genes, based on simple, logical ideas of what these genes might look like. For example:

   a. They should look like plant or algal sequences.
   b. They will probably bear some resemblance to known plastid genes from other organisms.
   c. They should have a unique bipartite signal sequence that we discussed.
   d. Include genes for which there is already experimental data that suggests targeting to the plastid.
   e. This identified a candidate list of over 500 nuclear-encoded genes that might be associated with the apicoplast.
   f. Many of these could be tested experimentally by gene fusions to YFP to see if they co-localize with known apicoplast markers
   g. Finally, after finding a set of genes that target to the apicoplast, these could be used as a training set to further inform computational analyses, thereby constantly improving the predicted dataset.

6. Explain or Teach These Concepts to a Friend

   1. Using the popular (and now quite old) video game “Pac-man” as an analogy, explain the endosymbiotic events that led to the origin of the apicoplast.

   2. Describe how photobleaching can be used to study protein dynamics in a cell.

   3. Explain how a protein encoded by a nuclear gene gets to the apicoplast.

7. Research the Literature on Your Own

   1. It turns out there is one apicomplexan parasite that does NOT have a plastid! What organism is this, why might it lack a plastid, and what are the implications of this difference, both in terms of parasite biology and potential drug targets?

   2. What drugs are currently on the market or in development for treating diseases caused by apicomplexan parasites (e.g. malaria, toxoplasmosis, etc)? Which ones target the apicoplast and how do they work?
3. What is “phylogenomics” and how was it used to help identify the origin of the plastid?

4. There are many instances of endosymbiosis in nature. A particularly interesting one is found in certain parasitic worms and involves a bacterium called Wolbachia. What is this endosymbiotic relationship? Compare and contrast it with what you now know about the apicoplast.

5. How do you suppose proteins that traffic to the apicoplast are able to get across not one, not two, not three, but four membranes, in order to be delivered to the lumen of the organelle?