Chapter 11

General Apicomplexan Biology

The apicomplexa are an extremely large and diverse group (>5000 named species). Seven genera infect humans (Table 11.1) and several other apicomplexan species are important pathogens of livestock. Most notable among the apicomplexan parasites that are important in terms of veterinary medicine and agriculture are Babesia and Theileria in cattle and Eimeria in poultry. Plasmodium species, as the causative agent of malaria, probably have the greatest impact on human health of any protozoan pathogen. Most of the medically important Apicomplexa are transmitted via an oral route and some cause intestinal disease. Many of these parasites, most notably Cryptosporidium and Toxoplasma, generally cause benign self-limiting diseases if the host has an intact immune system, but can cause serious and even life-threatening disease in immunocompromised patients. The AIDS epidemic has resulted in many of these organisms, which were previously considered to be rare and exotic infections, becoming prominent opportunistic infections of humans.

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<tr>
<th>Genera</th>
<th>Ch.*</th>
<th>Transmission</th>
<th>Disease</th>
</tr>
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<tbody>
<tr>
<td>Plasmodium</td>
<td>15</td>
<td>mosquito</td>
<td>malaria</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>12</td>
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<td>watery diarrhea</td>
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<td>13</td>
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<td>13</td>
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<tr>
<td>Toxoplasma</td>
<td>14</td>
<td>felines are definitive host</td>
<td>neurological manifestations</td>
</tr>
<tr>
<td>Sarcocystis</td>
<td>14</td>
<td>predator-prey</td>
<td>extremely rare infection</td>
</tr>
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<td>Babesia</td>
<td>16</td>
<td>tick</td>
<td>rare zoonotic disease</td>
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Apicomplexa, along with ciliates and dinoflagellates, form a higher order group known as Alveolata. The apicomplexa probably evolved from predatory flagellates and like many dinoflagellates have retained a chloroplast remnant called the apicoplast (Box 11.1). A major defining characteristic of the alveolata are the cortical alveoli, which are membrane bound vesicles that are found just underneath the plasma membrane. In the apicomplexa these vesicles are flattened and give the appearance of a tri-layered pellicle and the alveolar membranes are often called the **inner membrane complex**. The Apicomplexa are a monophyletic group composed almost entirely of parasitic species. At some point during their life cycle, members of the Apicomplexa either invade or attach to host cells. This interaction between the parasite and host cell is mediated by unique organelles localized to one end of the parasite. These ‘apical organelles’ are the defining characteristic of the Apicomplexa. The apical organelles are only evident by electron microscopy, and thus this group was not defined until after the application of electron microscopy to the study of protozoa during the 1960’s. Formerly the apicomplexa were part of a group called sporozoa and this name is sometimes still used.
General Apicomplexan Structure and Life Cycle

The apicomplexa generally exhibit complex life cycles involving specialized invasive stages with apical organelles and other ultrastructural features (Figure 11.1). At the most anterior end of the invasive stages are rings of microtubules known as the polar ring. In some species, a hollow cone-shaped structure, called a conoid, is part of the polar ring. The polar ring is a microtubule organizing center and microtubules which run the length of the organism emanate from the polar ring. These longitudinal microtubules are also associated with the inner membrane complex. Three distinct types of secretory organelles are also found at the apical ends of the invasive stages: rhoptries, micronemes, and dense granules.

**Figure 11.1. Schematic representation of generalized apicomplexan.**
Invasive and motile forms of apicomplexa exhibit distinctive apical organelles and ultrastructural features in addition to the typical eukaryotic organelles.

*Rhoptries* are described as being tear-drop or club shaped membrane bound organelles due to a duct connecting with the anterior end of the organism. *Micronemes* are small elliptical shaped vesicles found in close proximity of polar ring. *Dense granules* are secretory vesicles found throughout the organism. However, some of the dense granules are concentrated at the apical end and appear to play a role in invasion.

**Life cycle phases.** The apicomplexa have complex life cycles that are characterized by three distinct processes: *sporogony*, *merogony* and *gametogony* (Figure 11.2). Although most apicomplexa exhibit this overall general life cycle the details vary between species. Furthermore, the terminology used to describe these various life cycle stages vary between the species. The life cycle consists of both asexually reproducing forms and sexual stages. In monoxenous species all three of these processes will be carried out in a single host and often in a single cell type or tissue. Whereas, in
heteroxenous species the various processes will be carried out in different hosts and generally involve different tissues.

Sporogony occurs immediately after a sexual phase and consists of an asexual reproduction that culminates in the production of sporozoites. Sporozoites are an invasive form that will invade cells and develop into forms that undergo another asexual replication known as merogony. Merogony and the resulting merozoites are known by different names depending of the species. In contrast to sporogony, in which there is generally only one round of replication, quite often there are multiple rounds of merogony. In other words, the merozoites, which are also invasive forms, can reinvade cells and initiate another round of merogony. Sometimes these multiple rounds of merogony will involve a switch in the host organism or a switch in the type of cell invaded by the parasite resulting in distinct stages of merogony. As an alternative to asexual replication merozoites can develop into gametes through a process variously called gametogony, gamogony or gametogenesis. As in other types of sexual reproduction, the gametes fuse to form a zygote which will undergo sporogony.

Many apicomplexa exhibit unique modes of cell division. Apicomplexans generally do not exhibit a typical binary fission as is often associated with the replication of most other protozoa. This is in part due to replication in apicomplexans generally results in the formation of specialized invasive forms, or zoites. Furthermore, cellular replication in several of the apicomplexa is a process by which the organism undergoes numerous rounds of nuclear division without cytoplasmic division resulting in a multinucleated form. During merogony these multinucleated forms are generally called either schizonts or meronts. The progeny are then formed by a budding process in which a single nucleus will associate with the nascent apical organelles resulting in the formation of the invasive stages. Microtubule organizing centers (i.e., polar rings) are established at several positions on the plasma membrane of the meront. The nascent
apical organelles and nuclei associated with these microtubule organizing centers and the formation of the budding zoites is driven by the emergence of the subpellicular microtubules from the forming apical complex. The inner membrane complex is also formed along the subpellicular microtubules during the budding process. The mitochondria and apicoplasts also divide and are incorporated into the newly formed zoite.

Some life cycle stages of *Toxoplasma* exhibit a unique form of binary fission called **endodyogeny** (Figure 11.3). During endodyogeny the nascent apical organelles and inner pellicular membranes of the daughter cells start to form within the cytoplasm of the cell instead of at the plasma membrane. Other organelles (i.e., nuclei, mitochondria, and apicoplasts) divide and also associate with the newly forming daughter cells within the mother cell. The inner pellicular membranes of the mother cell disappear and are replaced by the inner pellicular membranes of the daughter cells. The outer plasma membrane of the mother cell is reused to form the outer plasma membrane of the daughter cells. Other stages of *Toxoplasma* and related parasites replicate by **endopolygeny**. This is similar to both schizogony and endodyogeny in that the organism undergoes multiple rounds of nuclear division without cytoplasmic division and formation of the progeny is initiated within the cytoplasm of the parent cell instead of at the plasma membrane.

![Figure 11.3. Schematic representation of endodyogeny. The zoites of Toxoplasma are formed by an internal budding of the daughter cells.](image)

**Mechanism of Host Cell Invasion**

The vast majority of the apicomplexa exhibit an intracellular stage during part of their life cycle. The exceptions to this are some gregarines and *Cryptosporidium*. In these cases the parasite attaches to the host cell and derives its nutrients from the host cell by
myelocytosis. The attachment and invasion process are related in that the apical organelles are involved in both processes. The mechanism of invasion is best characterized in the more medically important genera of *Plasmodium* and *Toxoplasma* and has been especially well characterized in erythrocyte invasion by *Plasmodium* merozoites. Although the details of invasion differ between species and life cycle stages the overall mechanism is presumably conserved throughout the Apicomplexa. During the invasion process the invasive forms (e.g., merozoites and sporozoites) of most apicomplexan species orientate themselves so that the apical end is juxtaposed to the host cell. The contents of the rhoptries, micronemes and dense granules are expelled as the parasite invades. Experiments in *Toxoplasma gondii* indicate that the micronemes are expelled first and the expulsion occurs with initial contact between the parasite and host. The rhoptries are discharged immediately after the micronemes and dense granule contents are released last. Many dense granules are extruded after the parasite has completed its entry, and thus probably play a role in modifying the host cell.

![Figure 11.4. Schematic representation of micronemal adhesins.](image)

**Figure 11.4. Schematic representation of micronemal adhesins.** (upper) Diagram of TRAP showing signal sequence (SS); von Willebrand A-domain; thrombospondin type 1 repeat (TSR); transmembrane domain (TM); and cytoplasmic domain (CD). (lower) Comparison of the gene structures of *Plasmodium* erythrocyte binding proteins.

Adhesion proteins are found in the micronemes. Many proteins found within the micronemes are characterized by having domains that are homologous to known adhesive domains. For example, the thrombospondin-related anonymous protein (TRAP) family of micronemal proteins is characterized by having multiple copies of thrombospondin type-1 domains and von Willebrand A domains (Figure 11.4). Thrombospondin is a multifunctional adhesive protein that binds to a wide range of cellular and extracellular matrix molecules. Other adhesive domains found in other micronemal proteins include epidermal growth factor (EGF)-like domains, apple domains and lectin domains. These ‘adhesins’ play a role in the adherence of the motile and invasive zoites to either host cells or the substratum. In this sense, the micronemal adhesins can be viewed as ligands that bind to host cell receptors. Some micronemal proteins are specific and only bind to particular molecules on the host, whereas as others exhibit a wider range of binding.
The specificity of the adhesins will reflect the host and cell specificity exhibit by the various species and stages of apicomplexan zoites.

The Duffy binding protein of *P. vivax* and erythrocyte binding antigen (EBA)-175 of *P. falciparum* are two rather well characterized proteins of *Plasmodium* merozoites which are not related to the TRAP family and other micronemal adhesins. The Duffy binding protein binds the Duffy antigen on the surface of the erythrocyte, whereas EBA-175 binds to sialic acid moieties on erythrocyte glycophorins. Thus, *P. vivax* and *P. falciparum* utilize different receptors on the host erythrocyte. Persons lacking the Duffy antigen on their erythrocytes are refractory to *P. vivax* infection. In particular, *P. vivax* is not very prevalent in western Africa where a large portion of the population is Duffy negative. This recognition of specific receptors on the host cell partly accounts for the host species specificity exhibited by *Plasmodium* species as well as the specificity *Plasmodium* merozoites have for erythrocytes.

Despite the different binding specificities the Duffy binding protein and erythrocyte binding antigen exhibit similar structures (Figure 11.4) and are members of a gene family of erythrocyte binding proteins. In particular, the receptor-binding activity has been mapped to a domain in which the cysteine and aromatic amino acid residues are conserved between species. This sequence motif is referred as a Duffy-binding domain and is found in other adhesive proteins expressed by the malaria parasite (see Chapter 15). The Duffy-binding domain is duplicated within the EBA-175 protein. Signal sequences and transmembrane domains are found on the N- and C-termini respectively. The topography of the transmembrane domain is consistent with the parasite ligands being integral membrane proteins with the receptor-binding domain exposed within the lumen of the microneme. This is also true for members of the TRAP family and other micronemal proteins. Thus, the fusion of the microneme membrane with the plasma membrane during microneme discharge will result in the exposure of the receptor-binding domain on the merozoite surface at the apical end.

Coincident with the release of the microneme contents is the formation of an electron dense junction between the host and parasite (Figure 11.5, left). This and the receptor-ligand interactions imply that the contents of the micronemal adhesins play a role in the formation of this junction and the attachment of the parasite to the host cell. This junction converts from its initial disk shape to a ring, or band, surrounding the zoite and is pulled back along the length of the invading zoite (Figure 11.5, right). The translocation of this ‘moving junction’ towards the back of the parasite results in a forward movement of the parasite into the host cell. Coincident with the movement of the zoite into the host cell is the formation of a parasitophorous vacuole. The parasitophorous vacuolar membrane (PVM) is likely derived from both the host membrane and parasite components and expands as the parasite enters the erythrocyte. Formation of the parasitophorous vacuole is coincident with the release of the rhotropies and the contents of the rhotropies are believed to be important in the formation of the parasitophorous vacuolar membrane. The parasitophorous vacuole continues to expand as the zoite enters the host cell and when the moving junction reaches the posterior end of the parasite there is a closure resulting in a separation of the PVM and erythrocyte membrane and the enclosure of the parasite within the parasitophorous vacuole.
Invasion and zoite motility are powered by a molecular motor complex. Apicomplexan parasites actively invade host cells and entry is not due to uptake or phagocytosis by the host cell. In addition, the zoites are often motile forms that that crawl along the substratum by a type of motility referred to as ‘gliding motility’. Gliding motility, like invasion, also involves the release of micronemal adhesins, attachment to the substratum, and a capping of the adhesins at the posterior end of the zoite. One difference between gliding motility and invasion is that the micronemes must be continuously released as the organism is moving. Thus, gliding motility does not involve
this relatively small moving junction, but a continuous formation of new junctions between the zoite and the substratum. In addition, the adhesins are cleaved from the surface of the zoite as the adhesions reach the posterior of the zoite and a trail of the adhesive molecules are left behind the moving zoite on the substratum [include figure?]. However, the overall mechanism of motility and invasion are quite similar and thus, during invasion the parasite literally crawls into the host cell through the moving junction. In addition, some apicomplexans use this type of motility to escape from cells and can traverse biological barriers, such as epithelial cell layers, by entering and exiting cells.

![Current model of the motor protein complex driving gliding motility.](image)


Cytochalasins inhibit zoite entry and this inhibition suggests that the force required for parasite invasion is based upon actin-myosin cytoskeletal elements. The ability of myosin to generate movement and force is well characterized (e.g., muscle contraction). A myosin unique to the Apicomplexa has been identified and localized to the inner membrane complex which lies just beneath the plasma membrane of the zoites. This myosin is part of a motor complex which is linked to the adhesins (Figure 11.6). Members of the TRAP family and other adhesins have a conserved cytoplasmic domain. This cytoplasmic domain is linked to short actin filaments via aldolase. The actin filaments and myosin are oriented in the space between the inner membrane complex and plasma membrane so that the myosin propels the actin filaments toward the posterior of the zoite. The myosin is anchored into the inner membrane complex and does not move.
Therefore, the transmembrane adhesins are pulled through the fluid plasma membrane due to their association with the actin filaments. Thus the complex of adhesins and actin filaments is transported towards the posterior of the cell. Since the adhesins are either complexed with receptors on the host cell or bound to the substratum, the net result is a forward motion of the zoite. When the adhesins reach the posterior end of the parasite they are proteolytically cleaved and shed from the zoite surface.

A trophic period characterized by the parasite taking up nutrients from the host and increasing in size generally follows the completion of parasite entry. This is then followed by a replicative stage which leads to the production of more invasive forms. In some Apicomplexa the mechanism of zoite egress involves the apical organelles and motility in a fashion similar to invasion. In other cases the egress of the invasive forms involves a destruction of the host cell and the release of the zoites.

**Summary and Key Concepts**

- The Apicomplexa are a monophyletic group of almost exclusively parasitic protozoa characterized by specialized invasive stages.

- The invasive stages exhibit a polarity and contain unique organelles and subcellular structures at the apical end (i.e., apical complex).

- During part of their life cycles most apicomplexans invade and replicate within the host cells.

- Micronemes and rhoptries are specialized membrane bound apical organelles that play a major role in interactions with and invasion of host cells.

- Micronemes contain adhesive proteins that are important in attaching to host cells or the substratum.

- The force needed for zoite motility and invasion is provided by a motor protein complex including a myosin unique to apicomplexans.

**Further Reading**


**Box 11.1. Algal Origins of the Apicomplexa**

Historically the apicomplexa have been described as a group with only parasitic forms. This and their unique apical organelles bring up questions in regards to the origin of the group. Phylogenetic analysis indicates that members of the genus *Copodella* form a sister group with the apicomplexa (1). The colpodellids are predatory flagellates that feed on unicellular algae by a process called myzocytosis. Myzocytosis involves the predator (or parasite) attaching to the prey (or host) and literally sucking out the cytoplasm of the prey cell via specialized structures. In these predatory apicomplexans, this attachment and interaction with the prey cell is mediated by organelles similar to those that are utilized by the parasitic apicomplexans for attachment to or invasion of host cells. Thus the evolution of the apicomplexa likely evolved from this myzocytic predation to myzocytic parasitism, as exhibited by gregarines and *Cryptosporidium* (see Box 12.1), to intracellular parasitism.

Other myzocytic organisms with apicomplexa like apical organelles include *Perkinsus*, parasites of oysters and clams, and *Parvilucifera*, a predator of dinoflagellates. These perkinsids, however, form a sister group with the dinoflagellates and not the apicomplexa (Figure 11A). This suggests that the progenitor of dinoflagellate and apicomplexan clades may have been a predatory flagellate and that the apical organelles were retained in the apicomplexan clade, but lost in most members of the dinoflagellate clade.

![Figure 11A. Cladogram showing relationships between apicomplexans and other alveolata. Node A is the Alveolata branch and node B is the Apicomplexa branch.](image-url)
The other connection between algae and the apicomplexa is a chloroplast remnant, called the apicoplast, found in most apicomplexans (2). The apicoplast is likely the result of a secondary endosymbiosis of a red algae and is likely the same endosymbiotic event giving rise to the plastids of dinoflagellates. The apicoplast is nonphotosynthetic but exhibits activities associated with type II fatty acid biosynthesis, isoprenoid biosynthesis, and possibly heme synthesis. These pathways are essentially prokaryotic and represent excellent drug targets. A photosynthetic alveolate, *Chromera velia*, that appears to be the earliest branching apicomplexan has also been identified (3).

