

Cytoskeleton of Apicomplexan Parasites

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THE APICOMPLEXA

Infection by parasitic protozoa of the phylum Apicomplexa causes incalculable morbidity and mortality to humans and agricultural animals (2, 3, 19, 26, 28, 39, 90). Apicomplexan parasites include *Plasmodium* spp., the agents of malaria; *Toxoplasma gondii*, a significant opportunistic pathogen in immunocompromised individuals; *Eimeria* spp., pathogens of chicken and cattle; *Theileria* spp., tick-borne parasites of cattle in Africa; and *Cryptosporidium*, an animal parasite as well as an opportunistic pathogen of humans. The phylum also includes gregarines, parasites of the guts of invertebrates including cockroaches and shrimp. This review is restricted to apicomplexan parasites of medical and agricultural importance since the bulk of the research has been done in this area.

All apicomplexans are obligate intracellular parasites. Most apicomplexan parasites grow and replicate within the parasitophorous vacuole, a nonphagosomal, membrane bound compartment that is segregated from most cellular trafficking pathways (78, 110, 143, 186). Proliferation of these organisms occurs by invasion of a host cell and is followed by parasite growth and cell division until the host cell is lysed by the replicating parasites. Parasites released by host cell lysis do not grow or undergo cell division extracellularly and must rapidly reinvade other host cells in order to survive. Repeated cycles of host cell invasion, parasite replication, host cell lysis, and par-

asite invasion of new cells account for much of the tissue damage associated with apicomplexan infections. Although apicomplexans are haploid for the bulk of their life cycles, they have complex life cycles, involving differentiation to forms that invade distinct tissues and hosts (Fig. 1). Differentiation can generate gametes that undergo fusion to generate a transient diploid zygote. The zygote immediately undergoes meiosis to reestablish haploid organisms. In some cases, differentiation also permits infection of organisms (such as mosquitoes or ticks) that serve as vectors to transmit parasites from host to host (2, 26, 39, 60, 67, 90, 157).

Apicomplexan parasites share a variety of morphological traits that are considered diagnostic for this phylum (Fig. 2). These protists have an elongated shape and a conspicuous specialization of the apical region (2, 3, 26, 157). Many of the distinct characteristics constitute a collection of unique organelles termed the apical complex. These organelles include the rhoptries, the micronemes, the apical polar ring, and the conoid. Rhoptries and micronemes are unique secretory organelles that contain products required for motility, adhesion to host cells, invasion of host cells, and establishment of the parasitophorous vacuole (2, 22, 24, 26, 41, 139). The conoid is a small cone-shaped structure composed of a spiral of unidentified filaments (120, 138). It is thought to play a mechanical role in invasion of host cells and is present in only some apicomplexans. The apical polar ring is a hallmark organelle of all members of the Apicomplexa (120, 134). It serves as one of the three microtubule-organizing centers (MTOCs) in these parasites; spindle pole plaques and centrioles/basal bodies are the other MTOCs (26, 157). In addition to the apical complex,

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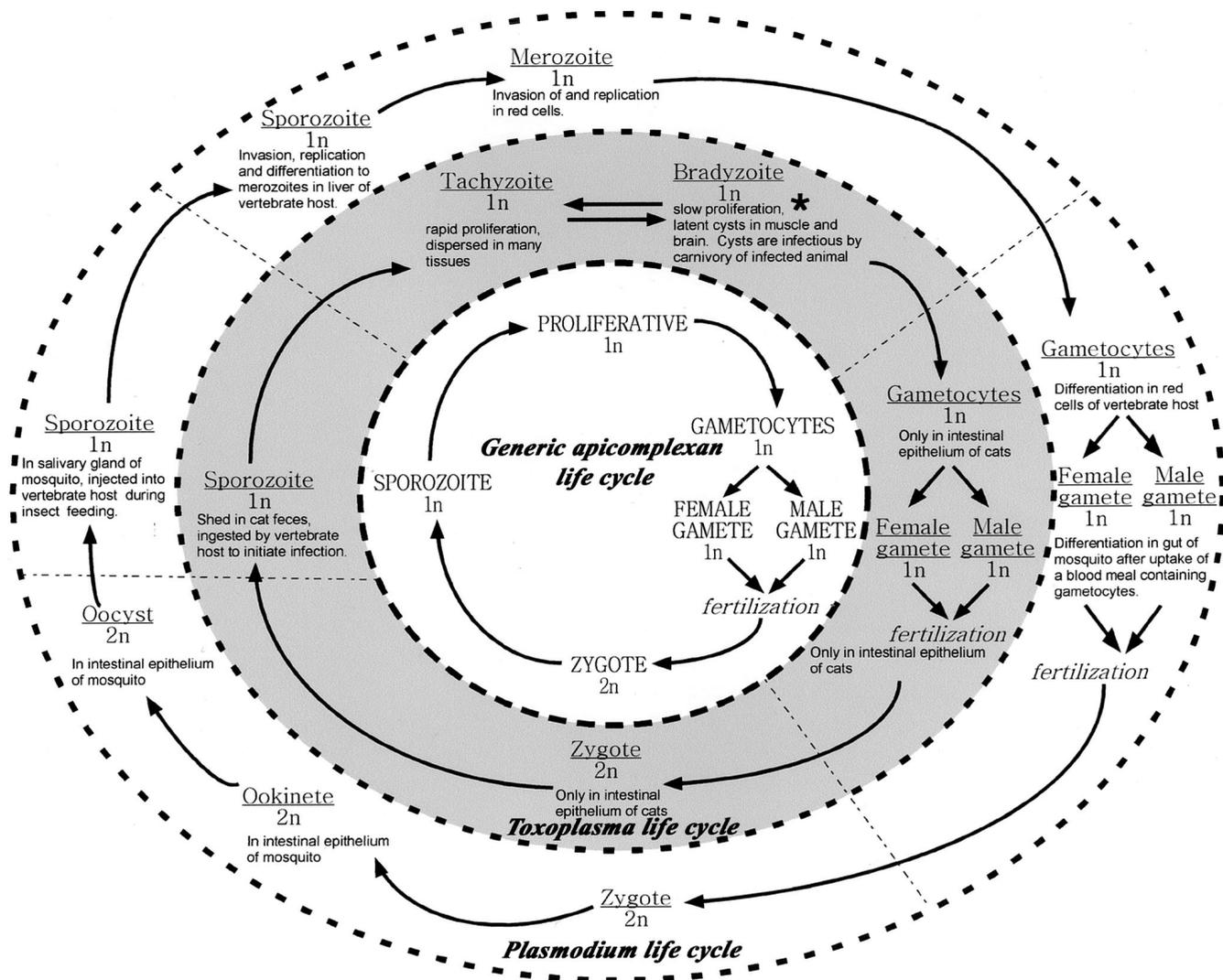


FIG. 1. Life cycle of apicomplexan parasites. For the faint of heart or those not interested in details, the center circle illustrates a generic apicomplexan life cycle. The rapidly proliferating haploid form has the capacity to differentiate into gametes that fuse to produce a diploid zygote. Meiosis reestablishes the haploid state and leads to sporozoites, the form most widely associated with establishment of new infections after inoculation of a naive host by an infected vector. The outer two circles represent the specific life cycles for *T. gondii* and *P. falciparum*. The bradyzoite form of *Toxoplasma* (*) is responsible for reactivation of latent infection and is an obligatory stage between tachyzoites and gametes. *Plasmodium* spp. do not have an analogous stage, although "hypnozoites" (latent sporozoites) are implicated in reactivation by *P. vivax* and *P. ovale*.

the apicomplexans have other unique structural features, such as an essential chloroplast-like organelle called the apicoplast (87, 99, 169, 194). The parasites are bounded by the pellicle, a composite structure consisting of the plasma membrane and the closely apposed inner membrane complex (IMC) (2, 4, 26, 43, 44, 58, 103, 104, 177, 183). The pellicle is intimately associated with a number of cytoskeletal elements, including actin, myosin, microtubules, and a network of intermediate filament-like proteins (Fig. 3).

Apicomplexan protozoa share a number of cytoskeletal elements (microtubules, actin, myosin, and intermediate filament-like proteins) with other "typical" eukaryotic systems used to study the cytoskeleton. Nonetheless, studies of the cytoskeletons of apicomplexans have revealed startling differ-

ences from model organisms that are worth mentioning at the outset. For example, the singlet subpellicular microtubules of apicomplexans are unusually stable and withstand the high pressure, cold, and detergents typically used to isolate them, conditions incompatible with survival for most microtubules (113). In contrast, the microfilaments of apicomplexans are thought to be exceedingly transient. Microfilaments are observed only after treatment with jasplakinolide (a drug that drives actin polymerization), and the bulk of actin (>98%) is sequestered in globular form (12, 36, 151). Apicomplexan myosins are unconventional, constituting a new class of unusually small "neckless" motors (68–70, 73, 123). In summary, apicomplexan protozoa constitute an ancient and diverse phylum with peculiar cell biological traits that make these parasites an in-

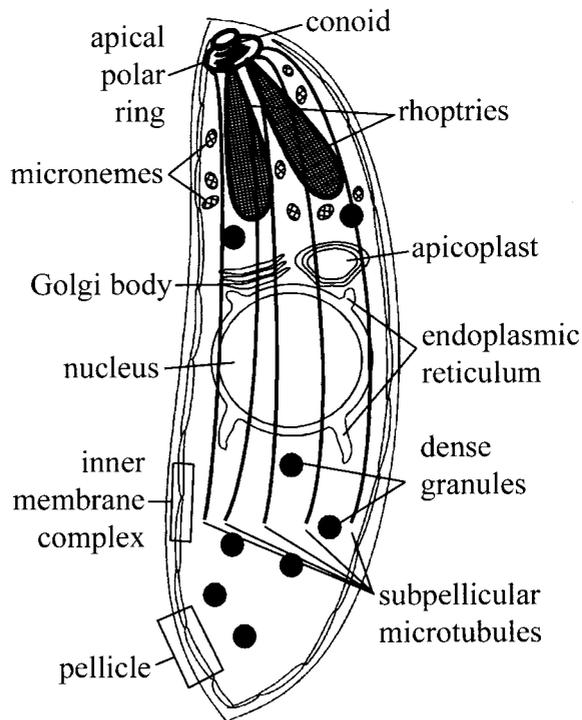


FIG. 2. The morphology of apicomplexan parasites. Apicomplexans are highly polarized cells containing a collection of organelles that are specific to the phylum. Rhoptries, micronemes, and dense granules are secretory organelles that contain products required for motility, invasion, and establishment of the parasitophorous vacuole. The conoid is a small cone-shaped spiral of unidentified filaments. It is thought to play a mechanical role in invasion and can be protruded from or retracted into the apical polar ring. The apical polar ring serves as an MTOC for the subpellicular microtubules. The spirally arranged subpellicular microtubules closely follow the serpentine body shape of apicomplexans. The parasites are bounded by the pellicle, a composite structure consisting of the plasma membrane and the closely apposed IMC. The endoplasmic reticulum surrounds the nucleus, and the Golgi body is immediately above it. The apicoplast is immediately adjacent to the Golgi body.

triguing topic for study. This review will survey our current understanding of the cytoskeleton of apicomplexan parasites.

MICROTUBULES IN THE APICOMPLEXA

Organization

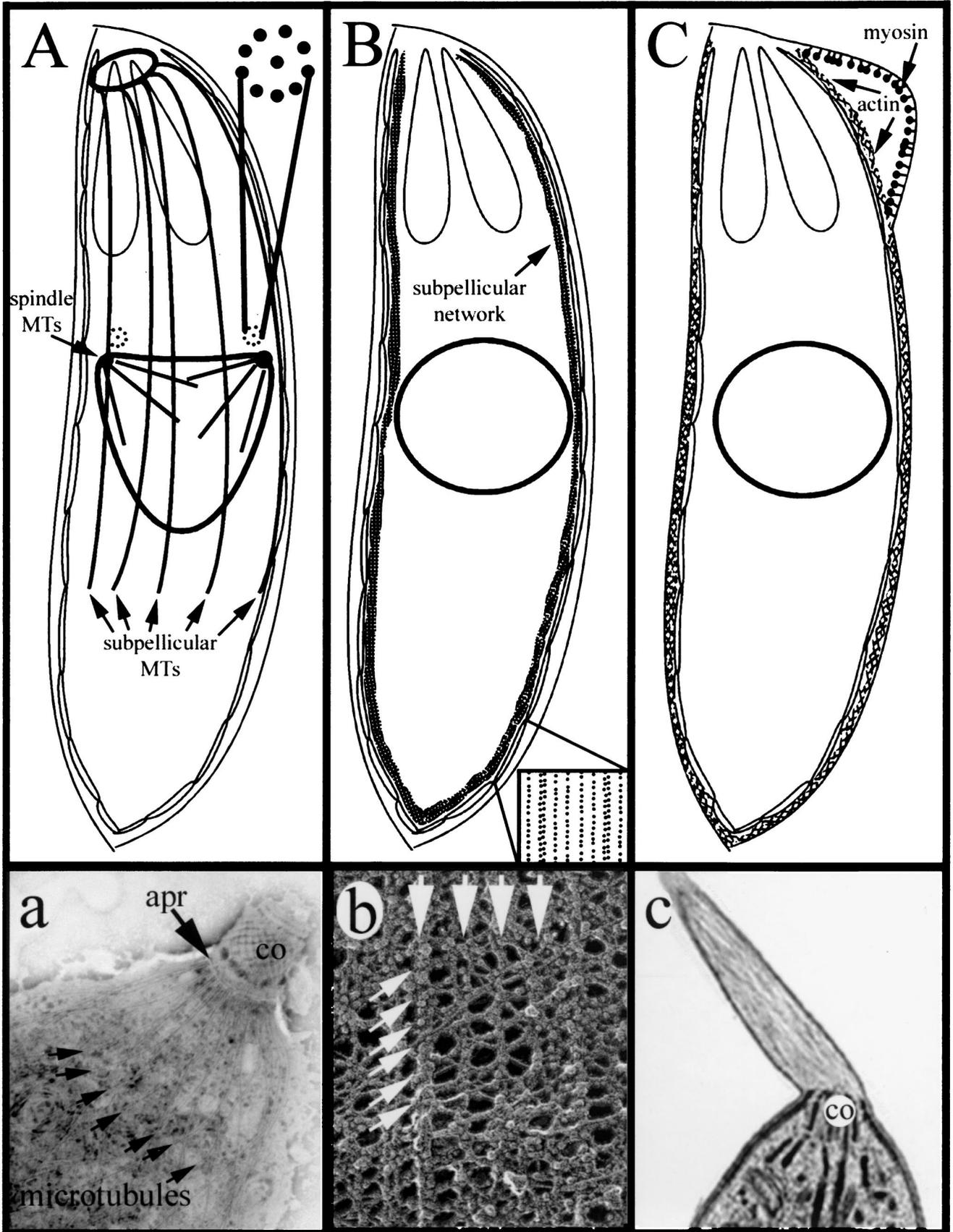
The haploid forms of apicomplexan parasites have two discrete populations of microtubules: the subpellicular microtubules and the spindle microtubules (Fig. 3A and a). The subpellicular microtubules radiate from the apical polar ring and run down the cytosolic face of the pellicle, ending in the region below the nucleus (approximately two-thirds of the length of the parasite [1, 12, 13, 26, 31, 120, 131]). These spirally arranged microtubules closely follow the serpentine body shape of apicomplexans. Subpellicular microtubules confer both elongated shape and apical polarity to apicomplexan parasites. Replicative forms or drug-treated parasites that lack subpellicular microtubules are nonpolar, nonmotile, and noninvasive (72, 168). Subpellicular microtubules are organized by lateral association with the apical polar ring (APR), a circular MTOC

(120, 134). Attachment of subpellicular microtubules to the APR is supported by blunt projections of the APR, which form a cogwheel pattern in transverse views. The plus end of the subpellicular microtubules is distal to this MTOC, and the ends of the subpellicular microtubules do not appear to be physically capped (134).

The arrangement of subpellicular microtubules varies among apicomplexan species, but the number, length, and organization are absolutely stereotyped within the life cycle stage of a species. The number of subpellicular microtubules ranges from a band of 3 or 4 in the tiny *Plasmodium falciparum* merozoites to ~60 in the considerably larger *Plasmodium* ookinetes (2, 3, 12, 26, 136, 156, 157). In coccidian parasites (*Toxoplasma* and *Eimeria*), the subpellicular microtubules are evenly spaced beneath the periphery of the pellicle; however, in *Plasmodium* species, most of the microtubules occupy two-thirds of the circumference and one microtubule is centered within the latter one-third of the pellicle (3, 157, 180). *P. falciparum* merozoites are an exception to this spacing generalization; they have a reduced number (three or four) of subpellicular microtubules termed f-MAST (*falciparum* merozoite-associated assemblage of subpellicular microtubules) that extend down one side of the merozoite membrane from the apex toward the posterior (12, 59). Other *P. falciparum* life cycle stages (sporozoites and ookinetes) have a full complement of subpellicular microtubules, as do merozoites of other *Plasmodium* species. *Theileria* sporozoites also deviate from this pattern. Although the tick-borne kinete stage of *Theileria* contains subpellicular microtubules and an IMC, *Theileria* sporozoites lack subpellicular microtubules and the IMC altogether and enter host cells in a distinct fashion (see below) (49, 50, 147, 148, 152).

Replicating parasites employ spindle microtubules during mitosis. Nuclear division proceeds without nuclear membrane breakdown (12, 26, 59, 79, 130, 161). Spindle microtubules are nucleated from electron-dense, amorphous plaques associated with nuclear invaginations and embedded in the nuclear membrane (12, 26, 82, 109, 140, 146, 157). This spindle-organizing structure has been variously referred to as the centrocone, the centriolar plaque, the spindle pole body, or the centriolar equivalent. We have chosen to characterize this structure as a "spindle pole plaque" to distinguish it from the adjacent centrioles that are sometimes present in members of the Apicomplexa. Centrioles are apparently not required for spindle assembly, since *Plasmodium* merozoites and *Theileria* sporozoites lack these structures and construct spindles by using only the spindle pole plaques (5, 12, 51, 140, 150, 157, 159). However, it is also possible that centrioles exist in these organisms and are obscured by inclusion in the electron-dense spindle pole plaque.

In many apicomplexans, centrioles are located in the cytoplasm close to but separate from the spindle pole plaques (26, 31, 42, 82, 100, 116, 157). Centrioles are highly ordered MTOCs typically consisting of a 9+0 structure of nine triplet microtubule blades organized in a turbine fashion. Apicomplexan centrioles have an unconventional form consisting of a central single microtubule surrounded by nine singlet microtubules, a deviation from the canonical structure (31, 40, 42, 82, 157, 165, 182). It is curious that apicomplexans apparently contain both spindle pole plaque structures and centrioles in



addition to the apical polar ring to organize microtubules. It may be that the centrioles are maintained throughout the asexual life cycle in order to serve as a template for construction of basal bodies that nucleate flagellar axonemes in the male gametes. The centriole is also associated with inheritance of the apicoplast during replication in *Toxoplasma* (169). One intriguing possibility is that the centrioles function as a “super-organizing center” coordinating the apical polar ring MTOC and the spindle pole plaque MTOC.

Although most parasite replication occurs by asexual division, apicomplexans also differentiate to gametes that fuse to form a diploid zygote. The male gamete (microgamete) is flagellated and swims to the female gamete (macrogamete) to carry out fertilization. The anterior end of apicomplexan microgametes is pointed and contains three basal bodies in close proximity to the apical pole (116, 137, 158, 181). These basal bodies nucleate two or three flagella that extend past the nucleus and away from the apical end. Additional microtubules originate in the basal apparatus zone and extend to the posterior end of the microgamete. Two of the flagella are long and are free from association with the gamete body. The third flagellum is shorter and is attached to surface of gamete at its anterior end. In some species this third flagellum is present only in rudimentary form as a band of microtubules that extends along the length of the gamete. In contrast to the atypical centrioles observed in other stages, the basal body of male gametes has a typical triplet microtubule structure with nine-fold symmetry and the flagellar axoneme contains a conventional 9+2 arrangement of doublet microtubules surrounding the central pair of microtubules (137, 158–160, 181). In *Plasmodium*, genesis of the basal bodies involves an intermediate 9+1 singlet form similar to the centrioles in other apicomplexans (157).

Conoid

Some apicomplexan parasites (*Toxoplasma*, *Eimeria*, and *Sarcocystis*) contain an additional cytoskeletal structure, the conoid, not found in other apicomplexans (*Plasmodium* and *Theileria*). It has been suggested that the conoid plays a mechanical role in invasion by parasites that must penetrate the robust barrier of the intestinal epithelium of vertebrates (26, 108, 121, 136, 138). The conoid consists of a set of counterclockwise spiraling filaments that create a pointed or cone-shaped structure at the extreme apex of these parasites (26, 64, 136, 138, 153). Extrusion of the conoid can be stimulated by ionomycin-triggered calcium influx, whereas pretreatment with cytochalasin D inhibits conoid extrusion (108). The filamentous

subunits of the conoid resemble microtubules but are curled into an extremely tight coil, suggesting that if they are microtubules they are unusually constructed or deformed. (Recent work by K. Hu, D. Roos, and J. Murray [submitted for publication] suggests that the *Toxoplasma* conoid is constructed from tubulin organized into a novel polymer form consisting of a comma-shaped sheet of nine protofilaments.) The conoid is approximately 250 nm in diameter and can be extended beyond or retracted into the apical polar ring. There are two ~400-nm-long, closely associated microtubules in the middle of the conoid. These microtubules are tightly bound to each other and are eccentric to the longitudinal axis of the conoid. This arrangement may be due to contact with the conoid and preconoidal rings via lateral projections (120). The course of the conoid subunits parallels the counterclockwise spiral of the subpellicular microtubules.

Role in apicomplexan replication

Apicomplexan parasites replicate by internal budding to create either two daughter cells or multiple progeny. Nuclear divisions within the Apicomplexa are cryptomitoses (the nuclear membrane remains intact throughout), and kariokinesis occurs without chromosomal condensation (26, 79, 130, 157). Replication in *Toxoplasma* and *Neospora* occurs by endodyogony, which creates two daughter parasites (63, 71, 91, 154). Replication by *Plasmodium*, *Theileria*, *Eimeria*, and *Babesia* occurs by schizogony, which can create 64 daughter parasites (3, 12, 72, 79, 150, 157). The processes of endodyogony and schizogony are quite similar, differing mainly in the preservation or loss of maternal cell specialization. In endodyogony, two daughter parasites are formed within an intact, fully polarized mother parasite (Fig. 4A). This preserves the ability of replicating tachyzoites to invade throughout the cell cycle. The internal daughter cells are delimited by an IMC and associated subpellicular microtubules, and each contains (in addition to the nucleus, mitochondrion, Golgi, and plastid) a complete set of apical organelles (100, 154, 183). When the daughter cells are fully mature, the maternal apical complex is disassembled and the daughter parasites bud from the mother, adopting her plasma membrane. In schizogony, after host cell invasion, the parasite subpellicular microtubules and apical complex are disassembled (Fig. 4B). After growth and multiple nuclear divisions, polarized parasites are regenerated when the nuclei move to the schizont periphery and associate with the assembling IMC, subpellicular microtubules, and apical organelles (3, 12, 72, 157). The newly repolarized parasites then bud out of the maternal cell as merozoites. Due to their large

FIG. 3. Cytoskeletal elements in the Apicomplexa. (A) The subpellicular microtubules radiate out of the apical polar ring and run down the cytosolic face of the IMC. The spindle microtubules are nucleated from spindle pole plaques within the nuclear membrane. Centrioles (consisting of nine singlet microtubules surrounding a central singlet microtubule) are adjacent to the spindle pole plaques. (a) Isolated, negatively strained conoid (co), apical polar ring (apr), and subpellicular microtubules from *T. gondii* (113). (B) A network of intermediate filaments, the subpellicular network, underlies the length of the IMC. The lower right inset illustrates the pattern of IMPs revealed by freeze fracture of the IMC. These particles may represent the transmembrane domains of receptors that link the subpellicular network to the cytoplasmic face of the inner membrane complex. (b) Glycerol- and detergent-extracted, freeze-dried replicas of *Toxoplasma* tachyzoites illustrate the regular array of the subpellicular network filaments. (Image of the *Toxoplasma* network kindly provided by J. Heuser.) (C) Actin is localized between the plasma membrane and the IMC. When the plasma membrane is separated from the IMC, actin remains associated with the IMC and myosin is associated with the plasma membrane. (c) Actin filaments protrude beyond the conoid (co) of *Toxoplasma* tachyzoites treated with jasplakinolide, a drug that induces actin polymerization. (Panel c reprinted from reference (151) with the permission of the publisher.)

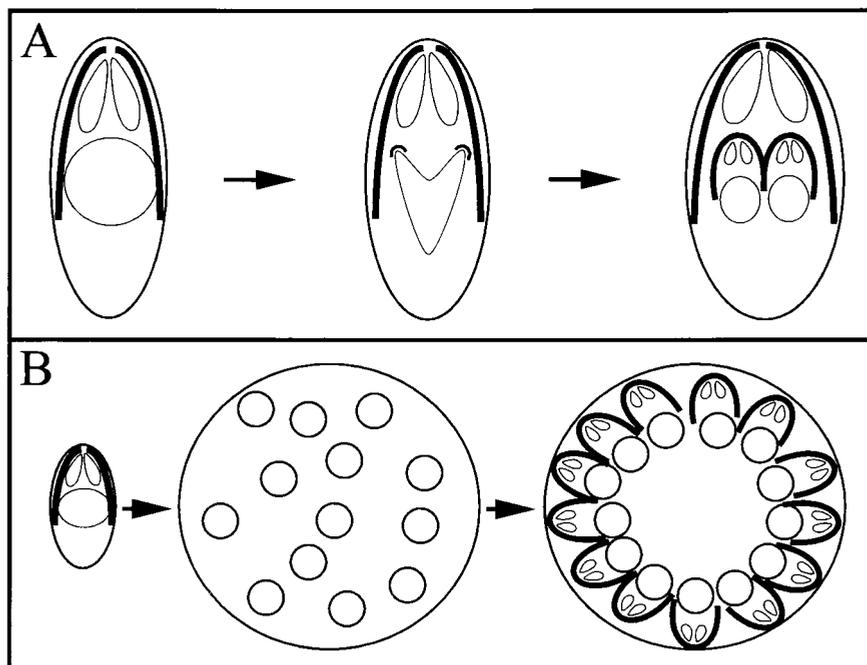


FIG. 4. Replication by the Apicomplexa. (A) Endodyogeny proceeds without loss of maternal cell shape and apical polarity. Formation of a curved nucleus is coupled to construction of two buds composed of nascent daughter IMC and subpellicular microtubules. The daughter cells develop surrounded by their own subpellicular microtubules and IMC in the fully polarized mother cell. Once the daughter cells are mature, they bud out of the remnants of the mother cell. (B) Schizogeny proceeds with loss of apical polarity. Invasion of a polarized elongated parasite is followed by disassembly of the subpellicular microtubules and IMC and by extensive cell growth and nuclear divisions. To reassemble polarized daughter cells, the multiple nuclei align with individual sets of apical organelles, subpellicular microtubules, and IMC at the periphery of the replicating cell.

round shape and lack of apical specialization, replicating parasites are noninvasive during schizogeny.

P. falciparum replication has been studied by observing microtubule structures during replication in red cells (130). Premitotic intracellular parasites have a diffuse distribution of unpolymerized tubulin. The first visible structure is a spindle pole plaque that is localized within the nuclear membrane. This nucleates microtubules to give rise to a hemispindle, which partitions into two. Partitioning of the spindle pole plaque has also been observed by electron microscopy. A band of striated fibers termed the couche fibrillaire spans the separating plaques (42, 140, 161). This structure may represent centrin fibers, since similar structures are observed in algae and yeast (89, 164, 192). The spindle halves segregate in opposite directions by migration within the nuclear membrane to ultimately produce a full spindle. The multiple nuclear divisions of schizogeny are not completely synchronous; spindles in various stages of assembly are often present in a single late-stage schizont. Distinct postmitotic microtubule structures appear in late schizogeny. Each structure is associated with an individual nucleus and consists of extranuclear microtubules arranged in a regular radial array like the spokes on a cartwheel. These microtubules extend beyond the nucleus toward the center of the schizont. Remains of this microtubule organization persist as a rod-like structure in budding merozoites. This remnant may represent f-MAST, the reduced set of three or four subpellicular microtubules that occurs in *P. falciparum* merozoites (12, 59).

The microtubules of extracellular apicomplexans are not

dynamic and are therefore impervious to microtubule-disrupting drugs (135). Assembly of microtubules occurs in the course of replication; therefore, intracellular parasites are susceptible to microtubule-depolymerizing drugs (Table 1) (168). In *Toxoplasma* and *Plasmodium*, the spindle and subpellicular microtubule populations are differentially stable to disruption by oryzalin or colchicine (14, 115). Lower concentrations (0.5 μM oryzalin or 1.0 mM colchicine) shorten microtubules. Under these conditions, *Toxoplasma* and *Plasmodium* continue to undergo nuclear division and budding but lack functional subpellicular microtubules and are incapable of invading new host cells. When removed from 0.5 μM oryzalin, *Toxoplasma* recovers normal morphology and is invasive (115). In contrast, higher concentrations of drug (2.5 μM oryzalin or 5.0 to 10.0 mM colchicine) disrupt both subpellicular and spindle microtubules (149, 168). Parasites under these conditions are incapable of nuclear division or budding, although cell growth, DNA synthesis, and centriole replication continue unchecked (115, 149, 168). When removed from 2.5 μM oryzalin, *Toxoplasma* tachyzoites attempt to bud as crescent-shaped parasites. Since the polyploid nuclear mass cannot be correctly segregated, daughter parasites are made that lack nuclei altogether (115).

Assessment of the activity of microtubule-destabilizing drugs on intracellular parasites is complicated by the activity of these drugs on host cells. In many cases, toxicity to host cells antedates any effects on parasite microtubules. In this situation, parasites fail to replicate, but this is due to adverse effects on the host cell rather than to direct inhibition of parasite func-

TABLE 1. Drug disruption of cytoskeletal elements in the Apicomplexa

Drug and cytoskeletal element (drug mechanism)	Concn	Action	Comments
Actin microfilaments			
Cytochalasin D (disrupter) ^a	0.1–0.2 μ M	Inhibits actin-based gliding motility (extracellular) and invasion	Inhibits gliding motility in extracellular parasites; use resistant host cells to avoid host cell effects.
Latrunculin (disrupter) ^b	10 nM–5 μ M	Inhibits motility and invasion	Will also show host cell actin effects.
Jasplakinolide (stabilizer) ^c	50 nM–2 μ M	Filament polymerization inhibits gliding and invasion	Acts on extracellular parasites; will also act on host cell actin in intracellular settings.
Microtubules			
Oryzalin (disrupter) ^d			Also other dinitroanilines such as trifluralin, ethafluralin
Subpellicular microtubules	0.5 μ M	Parasites lack apical polarity	No effect on extracellular parasites; no effect on host cell microtubules, but intracellular parasites are affected. As for subpellicular microtubules.
Spindle microtubules	2.5 μ M	Parasites are incapable of replication	
Colchicine (disrupter) ^e			
Subpellicular microtubules	10 μ M–1 mM	Parasites lack apical polarity	No drug effect on extracellular parasites; use resistant host cells to avoid host cell disruption (no microtubules in RBCs). As for subpellicular microtubules.
Spindle microtubules	5–10 mM	Parasites are incapable of replication	
Colcemid (disrupter) ^f	50 μ M	Inhibits intracellular growth	No cell biological observations accompany study.
Vinblastine (disrupter) ^g	15–100 nM	Inhibits intracellular growth	No cell biological observations accompany study.
Vincristine (disrupter) ^h	7 nM	Inhibits intracellular growth	No cell biological observations accompany study.
Tubulozole (disrupter) ⁱ	10 μ M	Parasites are killed	May inhibit parasite protein synthesis rather than microtubules
Taxol (stabilizer) ^j	0.1–0.5 μ M	Parasites are incapable of budding	No drug effect on extracellular parasites; host cell microtubules will also be affected (no microtubules in RBCs).
Docetaxel/taxotere (stabilizer) ^k	10 nm–50 μ M	Parasites are incapable of replication	No drug effect on extracellular parasites; host cell microtubules will also be affected (no microtubules in RBCs).
Epithalone A (stabilizer) ^l	0.1–0.5 μ M	Parasites are incapable of budding	No drug effect on extracellular parasites; host cell microtubules will also be affected (no microtubules in RBCs).
Myosin			
BDM (ATPase inhibitor) ^m	20–40 mM	Inhibits actin-based gliding motility (extracellular) and invasion	Will also act on host cell myosins (not present in red cells).
KT5926 (LC kinase inhibitor) ⁿ	1–5 μ M	Inhibits actin-based gliding motility (extracellular) and invasion	Most probably inhibits secretion of TRAP family adhesins rather than myosin.

^a Data from references 8, 25, 28, 35, 37, 39, 54, 56, 57, 64, 69, 85, 103, 104, 129, 131, 140, 143–145, 148, 152, 156, 163, and 170.

^b Data from reference 145.

^c Data from references 123, 145, and 147.

^d Data from references 11, 17, 22, 59, 78, 96, 110, 145, and 164.

^e Data from references 15, 23, 59, 85, 131, 156, 173, 185, 188, and 189.

^f Data from reference 16.

^g Data from references 16, 35, 120, 131, 156, 174, 188, and 189.

^h Data from references 35 and 174.

ⁱ Data from references 16, 35, and 36.

^j Data from references 59, 69, 85, 144, 145, 159, and 168.

^k Data from references 137 and 159.

^l Data from reference 168.

^m Data from references 37, 56, 57, 64, and 119.

ⁿ Data from reference 37.

tions. For these reasons, the most comprehensive examination of the effects of microtubule-disrupting drugs has been carried out with the *P. falciparum* erythrocytic stages in red cells (which lack microtubules). In vitro studies have established that replicating *P. falciparum* merozoites are susceptible to colchicine and colcemid, to vinblastine and vincristine, to the

dinitroanilines trifluralin and pendimethalin, and to tubulozoles, including tubulozole-T, an isomer that is inactive in mammalian systems (15, 33, 34, 178). In contrast, merozoites show only low sensitivity to the benzimidazoles (albendazole, thiabendazole, mebendazole, and omeprazole) (33, 163). The results for benzimidazole are consistent with the deduced

amino acid sequences of apicomplexan β -tubulins that lack Glu 198 and Phe 200, predictors of sensitivity to these compounds (44a, 81a). *Plasmodium* merozoites are also sensitive to microtubule-stabilizing drugs such as taxol, docetaxel, and epothilone A, which inhibit schizont nuclear division and budding (141, 162, 172). Colchicine, trifluralin, and taxol also perturb gametocyte and ookinete differentiation in *Plasmodium* (80, 88). Parallel experiments to examine the role of microtubules in other apicomplexans have been carried out with *Eimeria*, *Toxoplasma*, and *Cryptosporidium* by using dinitroaniline compounds such as oryzalin or trifluralin (10, 16, 168). These drugs specifically inhibit the microtubules of plants and protists and do not destabilize vertebrate microtubules. To assess the effects of other drugs such as colchicine and taxol, resistant host cells can be used. When colchicine- or taxol-resistant cells are used as host cells for *Toxoplasma* replication, the effects of colchicine and taxol are akin to what is observed in *Plasmodium* merozoites (115).

Tubulin and Microtubule-Associated Proteins

Apicomplexan tubulin genes have been cloned from *T. gondii*, *Eimeria tenella*, *Babesia bovis*, *P. yoelii*, *P. berghei*, *C. parvum*, and *P. falciparum* (8, 20, 21, 25, 29, 30, 74, 75, 118, 119, 145, 179, 197). In most of these apicomplexans, the α -tubulin and β -tubulin genes appear to be unlinked, single-copy genes containing up to three introns. The introns are in the same location and are similar in sequence in *E. tenella*, *C. parvum*, and *T. gondii*. The γ -tubulin gene has also been sequenced in *P. falciparum*. It is a single-copy gene and lacks introns (95). Curiously, *P. falciparum* and *P. yoelii* each have two α -tubulin genes, which are located on different chromosomes (8, 129). The α -tubulin-I gene is expressed throughout the parasite differentiation cycle, but the α -tubulin-II gene is specifically expressed in male gametes. The α -tubulin-II-specific monoclonal antibody 5E7 specifically labels stage III through mature male gametocytes and exflagellating and free male gametes (129). Immunoelectron microscopy using this antibody labels the axonemes of male gametes.

Microtubule-associated proteins (MAPs) are clearly critical to the highly organized structure of apicomplexan parasites. Bridges connecting the subpellicular microtubules to the inner membrane complex have been observed in thin sections of parasites (3, 131, 195). Isolated frozen-hydrated microtubules of *T. gondii* have a distinct 32-nm periodicity along their length as revealed by Fourier analysis (113). The periodicity most probably results from a MAP that heavily decorates these microtubules and that may account for their unusual stability after isolation. This MAP may coordinate the close interaction of the subpellicular microtubules with the IMC. The existence of a group of monoclonal antibodies that labels the subpellicular microtubules in *Toxoplasma* and cross-reacts with *Plasmodium* suggests that the MAPs may be conserved within the Apicomplexa (112, 114). The *Plasmodium* and *Cryptosporidium* genome databases and the *Toxoplasma*, *Eimeria*, and *Neospora* expressed sequence tag (EST) projects have sequences annotated as encoding putative kinesins and dyneins.

SUBPELLICULAR NETWORK OF THE APICOMPLEXA

Proteins of the Subpellicular Network

Deoxycholate extraction of *Toxoplasma* reveals a network of filamentous material that extends from the APR to the posterior of the parasite (98). The filaments have a diameter of 8 to 10 nm, and the network has the same general shape as the parasite, suggesting that these filaments may play a role in generating and maintaining cell shape. A polyclonal serum generated from the extracted pellicles has been used to identify two novel proteins that localize to the subpellicular network (98). These proteins, TgIMC-1 and TgIMC-2, are not homologous to any known proteins but are predicted to form regions of coiled coils and have weak similarity to the coiled-coil domains of cytoskeletal proteins such as myosin. TgIMC-1 is rich in valine and glutamic acid (together they constitute almost 30% of the protein). Interestingly, TgIMC-1 is similar to the articulins, a group of proteins that form a membrane skeleton in *Euglena* and other protists. Both the articulins and TgIMC-1 have a 12-amino-acid VPV repeat. Gold labeling with antisera to either TgIMC-1 or TgIMC-2 labels the cytoplasmic face of the pellicle between the subpellicular microtubules and the IMC (Fig. 3B). A homolog of TgIMC-1 is present in the *P. falciparum* genome. PfIMC-1 has an additional 220-amino-acid region near the C terminus, containing seven copies of a repeating sequence. The TgIMC-1 antiserum cross-reacts with *Plasmodium*, labeling late-stage schizonts during the formation of merozoites.

Organization of the Inner Membrane Complex and the Subpellicular Network

The IMC lies directly below the parasite plasma membrane and is closely associated with it, creating a three-layered pellicle characteristic of the Apicomplexa (26, 104, 183). In *Plasmodium* sporozoites, the IMC is constructed from a single large flattened vesicle joined by a single suture line traversing the long axis of the parasite (44). In other apicomplexans, it is made of many flattened vesicles aligned in longitudinal rows and joined in a patchwork fashion by sutures (43, 124). Glycerol-extracted, negatively stained pellicles from *Sarcocystis ovifelis*, *Besnoitia jellisoni*, and *Eimeria falciformis* all have a mesh-like pattern that runs along the full length of the IMC (32). This mesh-like structure is similar to the network of IMC-associated filaments observed in *Toxoplasma*. Freeze fracture of *Toxoplasma*, *Sarcocystis*, *Eimeria*, and *Plasmodium* has shown that apicomplexans share a striking organization of the IMC (6, 43, 44, 104, 124). The membranes of the IMC are characterized by parallel alignment of intramembranous particles (IMPs). The lines of IMPs extend down the long axis of the parasite and are organized as single rows interspersed with double rows. The double rows correspond in number and arrangement to the underlying subpellicular microtubules (Fig. 3B, inset). The rows show continuity across the plates of the IMC, which is remarkable because each plate is a topologically distinct vesicle. Fourier analysis of the IMPs shows that they have a distinct 32-nm longitudinal repeat creating a two-dimensional lattice, with the second dimension at an angle of approximately 75° to the rows (113). The integrity of the particle lattice is not destroyed by disruption of actin or microtu-

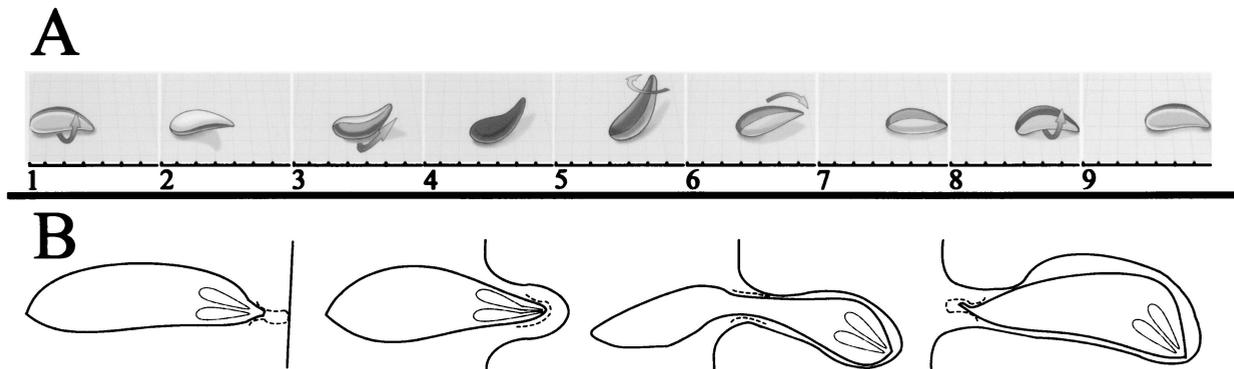


FIG. 5. Motility and invasion by the Apicomplexa. (A) Helical gliding is the only behavior associated with motility that leads to long-distance movement across a substrate. During this biphasic behavior, the parasite moves forward a body length and then repositions to repeat this cycle. This sequence illustrates the path taken by a gliding *Toxoplasma* tachyzoite to move forward one complete cycle. The parasite travels forward by moving a contact zone from its apex to its posterior in a helical path. Because the parasite is crescent shaped, it moves in a “corkscrew” fashion (frames 1 to 5). The crescent shape of the tachyzoite prevents the continuation of this motion because the parasite cannot contact the substrate with its concave face. To rectify this, the parasite rotates 180° without forward motion (frames 6 and 7) before initiating another cycle of ‘corkscrewing’ translational movement (frames 8 and 9). (Modified from reference (66) with the permission of the publisher.) (B) Apicomplexan invasion consists of attachment and apical orientation, induction of a parasitophorous vacuole, and translocation of the parasite into the vacuole. Apically secreted adhesins are capped along the moving junction to the posterior of the parasite. The moving junction is associated with a constriction of parasite shape that moves from the apex to the posterior.

bules; this suggests the existence of additional cytoskeletal filaments. Glycerol- and detergent-extracted freeze-dried replicas of *Toxoplasma* tachyzoites reveal a filament network with striking similarity to the IMP lattice (Fig. 3b). The recent discovery of intermediate filament-like proteins that localize to the subpellicular network most probably identifies the lattice-generating network elements. We hypothesize that the IMP lattice may represent the transmembrane domains of receptors that anchor the subpellicular network to the IMC. This lattice may also anchor the subpellicular microtubules since they are decorated with a MAP that binds at 32-nm intervals (see above) (113).

ACTIN AND MYOSIN IN THE APICOMPLEXA

Properties and Localization of Actin

A large fraction of the actin in parasites of the Apicomplexa appears to be in monomeric rather than polymerized form. Experiments with *Toxoplasma* have established that tachyzoites have strikingly small amounts of assembled actin (36). Approximately 98% of *Toxoplasma* actin is globular, and this distribution is not shifted by the addition of agents that drive actin polymerization, such as phalloidin, $MgCl_2$, exogenous actin, spermine, or phosphatidylinositol-4,5-bisphosphate (PIP_2). In fact, although some researchers have observed phalloidin binding (127, 187) or filament stabilization with phalloidin (126), others have concluded that phalloidin does not bind to apicomplexan microfilaments (27, 36, 55, 56, 111, 151). Until quite recently, apicomplexan microfilaments had not even been observed by electron microscopy (12). However, in the presence of the microfilament-polymerizing drug jasplakinolide, *Toxoplasma* will form microfilaments (Fig. 3C and c) (126, 151). Jasplakinolide induces actin polymerization, most notably at the apical end of extracellular *Toxoplasma* tachyzoites (Table 1). The actin filaments in these membrane-enclosed apical projections are aligned parallel to the long axis of the

parasite and can be up to 2 μM long (151). Jasplakinolide-induced filaments decorate with myosin subfragment 1, demonstrating that they are indeed actin; unfortunately, the filaments are too close together to determine polarity. Anti-actin antibodies label the apical projections in jasplakinolide treated parasites. In untreated parasites, heterologous actin antibodies label the apical region or appear as a diffuse distribution in the cytosol (47, 187, 196). A polyclonal antiserum directed against *Toxoplasma* actin labels a circumferential pattern in the tachyzoites, extending below the apical region (36). In hypotonically swelled parasites, a *Toxoplasma*-specific antiactin monoclonal antibody labels the region between the plasma membrane and the IMC (35).

Motility and Invasion

Most members of the Apicomplexa are motile. In these species, locomotion is intimately associated with host cell invasion and probably employs the same underlying cellular machinery. In fact, gliding locomotion and invasion often appear continuous and occur without a discernible change in speed. Apicomplexans (includinggregarines) move by gliding motility (38, 52, 77, 84, 133). This movement does not exploit a discrete organelle (such as a flagellum) or result from amoeboid deformations of the cell. It is, however, substrate dependent. Motility has been analyzed in *T. gondii* and consists of three behaviors: circular gliding, upright twirling, and helical gliding (61, 66). When a crescent-shaped parasite lies on its right side, it moves in counterclockwise circles. Twirling occurs when the parasite is attached to the substrate by its posterior end, producing a clockwise spinning. Lastly, helical gliding is similar to twirling but occurs in horizontal parasites (Fig. 5A). This biphasic behavior consists of an 180° clockwise revolution (resulting in a corkscrewing forward movement) coupled to a parasite flip to return the parasite to its original face, so that it can initiate helical motion anew. Helical gliding is the only

behavior that results in long-distance movement across a substrate. Actin-disrupting or -stabilizing drugs (cytochalasin D and jasplakinolide), as well as myosin inhibitors (butanedione monoxime [BDM]), disrupt *Toxoplasma* motility and invasion (35, 37, 126). This suggests that an actomyosin-based mechanism underlies these behaviors (Table 1). Cytochalasin inhibition of gliding motility and/or invasion has been demonstrated in *C. parvum*, *T. gondii*, *E. tenella*, *E. acervulina*, and *Plasmodium* species (37, 56, 77, 106, 133, 135). Motility and invasion are associated with translocation of secreted adhesive proteins from the apex to the parasite posterior and their shedding or deposition into a slime trail. Both adhesins and parasite surface antigens are deposited into a trail by gliding parasites (11, 22, 37, 48, 166, 167, 171). The presence of surface proteins in slime trails is likely to reflect the artificially sticky substrate used to capture adhesion trails.

Conserved adhesins have been found in *Plasmodium*, *Toxoplasma*, *Eimeria*, *Neospora*, and *Cryptosporidium* spp. (22, 24, 92, 132, 155, 171, 185). The thrombospondin-related anonymous protein (TRAP) family adhesins have common structural motifs and presumably an underlying mechanism of action. They contain a conserved adhesive domain consisting of a thrombospondin type 1 repeat that occurs in different numbers and locations within the individual apicomplexan adhesins. TRAP family proteins are localized to micronemes and are apically secreted during motility and invasion (22, 171). TRAP proteins are located on the surface of motile parasites in a transmembrane form and are capped from anterior to posterior in gliding parasites. The short cytoplasmic tail of these proteins is conserved and is thought to interact with the actomyosin cytoskeleton in order to translocate the protein backward. Adhesins are released as soluble protein by proteolytic cleavage at the posterior end of locomoting parasites (23). When the sporozoite-specific TRAP gene is knocked out in *P. berghei* merozoites, parasites behave normally until they differentiate to sporozoites within the mosquito. TRAP knockout sporozoites are immotile and noninvasive (171). Moreover, expression of a TRAP construct missing the last 14 amino acids of the cytoplasmic tail permits surface localization of the protein in knockout sporozoites (81). However, these sporozoites also display atypical behavior, repeatedly gliding one-third of a circle and snapping back to their original position. This suggests that the inability of TRAP to be appropriately translocated and/or released at the posterior end of locomoting parasites prevents their continued forward movement (81, 101). Menard has recently written a comprehensive review of gliding motility and the adhesins in the Apicomplexa (101).

The existence of an actomyosin-based mechanism for invasion was first implied by observations of the effect of cytochalasin B on *Plasmodium* invasion. Cytochalasin B blocks *Plasmodium knowlesi* merozoite invasion of red cells (106). Merozoites will attach irreversibly to red cells and form a vestigial parasitophorous vacuole but are inhibited from moving into the cell. Since red cells are unequivocally nonphagocytic, the effects of cytochalasin on *Plasmodium* invasion are likely to represent drug disruption of parasite (not host cell) microfilaments. However, for other apicomplexans, cytochalasin inhibition of invasion was sometimes attributed to inhibition of induced phagocytosis by the host cell. Active invasion of *Toxoplasma* tachyzoites can be distinguished from the phago-

cytic uptake of parasites because invasion of host cells (including macrophages) does not induce host cell membrane ruffling, actin microfilament reorganization, or tyrosine phosphorylation, which are all indicative of phagocytosis (111). Invasion is three to four times faster than phagocytosis (occurring within 25 to 40 s) and is characterized by parasite penetration into a tight-fitting vacuole formed by invagination of the plasma membrane. In contrast, phagocytosis of *Toxoplasma* involves membrane ruffling and the parasite is captured in a loose-fitting phagosome that forms over 2 to 4 min (78, 111, 121). Phagocytosis involves both reorganization of the host cytoskeleton and tyrosine phosphorylation of host proteins (111).

Experiments with cytochalasin D-resistant *T. gondii* have definitively established that invasion of *Toxoplasma* is critically dependent on actin filaments in the parasite but not in the host cell (37). Invasion of cytochalasin D-resistant host cells by wild-type (cytochalasin-sensitive) *Toxoplasma* tachyzoites is blocked by cytochalasin D. As observed with *Plasmodium*, attachment and apical orientation of *Toxoplasma* is normal in the presence of cytochalasin D. Cytochalasin D-resistant *Toxoplasma* mutants were isolated by chemical mutagenesis and selection for growth in cytochalasin D in resistant host cells. These resistant parasites have a point mutation in the single-copy actin gene *ACT1* (A136G) and can invade wild-type host cells in the presence of cytochalasin D. Transformation of the mutant *act1* allele into wild-type *Toxoplasma* confers cytochalasin D-resistant motility and invasion either as an allelic replacement or as a nonhomologous integration, generating a pseudodiploid parasite (37).

Apicomplexan invasion consists of three phases: (i) attachment with apical orientation, (ii) induction of a parasitophorous vacuole, and (iii) translocation of the parasite into the vacuole (Fig. 5B). Parasites attach to host cells and form an intimate connection through apical end contact (37, 53, 106). This results in sequential secretion from the parasite micronemes and rhoptries. Adhesins from the micronemes are translocated along the parasite length and are shed at the site of the moving junction; parasitophorous vacuole components from the rhoptries are secreted into this forming compartment (24). Both tight adhesion to the host cell and secretion into the host cell occur when parasites are immobilized early in invasion by cytochalasin treatment (37, 53, 65, 106).

The "moving junction" which forms during invasion is a circumferential zone of attachment at the orifice of the host cell invagination (7, 105). It is characterized by a markedly thickened host cell membrane with increased electron density and is frequently accompanied by a constriction in the parasite body. The parasite enters the nascent parasitophorous vacuole by capping the moving junction down its body. Ultimately, the parasite becomes enclosed within a cavity delimited by the invaginated host cell membrane. Formation of a moving junction that is capped to the posterior during invasion is likely to be a feature of invasion shared by many apicomplexans (*Theileria* is an exception [discussed below]). The moving junction is a highly specialized interface of the parasite with the host cell, presumably exploiting cytoskeletal proteins, signaling molecules, and receptors. Very little is known about this interface. *P. falciparum* MCP-1 (merozoite-capping protein 1) is a 60-kDa merozoite protein that moves from anterior to posterior with the moving junction during merozoite invasion of red cells

(86). MCP-1 lacks both a signal sequence and transmembrane domain and is located in the parasite cytosol. The precise role of MCP-1 in invasion remains obscure. The protein has an amino-terminal domain that is conserved in bacterial and eukaryotic oxidoreductases (76). There are also a group of microneme proteins that may play a role in this junction that localize to the moving junction and the (exposed) posterior region during invasion (24, 57, 62).

Actin and Actin Binding Proteins

Genes encoding actin have been cloned in several members of the Apicomplexa (36, 83, 119, 189–191). In *T. gondii* and *C. parvum*, actin is encoded by a single-copy gene (36, 83, 119). In *Cryptosporidium*, the actin gene is intronless, and in *Toxoplasma* it has one intron. In *P. falciparum*, the situation is akin to that for α -tubulin. There are two genes encoding actin (189–191). One gene (actin I) is intronless and is expressed throughout the parasite life cycle. In contrast, the *P. falciparum* actin II gene has an intron and is transcribed only in the sexual stages (191). The amino acid sequence of actin II is divergent from that of previously characterized actins. Additionally, relative to the high degree of conservation shown by most actins, the 79% amino acid sequence similarity between *Plasmodium* actin I and actin II is quite low. Actin-related proteins (arps) have not been characterized in the Apicomplexa yet, but sequences in the *Plasmodium* genome are annotated as putative arps.

F-actin affinity chromatography has been used to isolate actin binding proteins from *P. knowlesii* and *P. falciparum* merozoites and from *Toxoplasma* tachyzoites (54, 125, 173). In *P. knowlesii*, five major proteins with molecular masses of 75, 70, 48, 40, and 32/34 kDa are eluted from F-actin columns (173). The 70-kDa protein has been identified as heat shock protein 70 (HSC70). The 32/34-kDa doublet coelutes with HSC70 from columns or in gel filtration chromatography; however, the identity of these proteins remains unknown. Highly enriched fractions of the *Plasmodium* HSC70-HSC32-HSC34 complex inhibit rabbit skeletal muscle actin polymerization in vitro. Biochemical experiments have established that this is due to a capping activity that is Ca^{2+} independent and is inhibited by PIP_2 .

Homologs of two widely conserved actin-associated proteins, coronin and actin-depolymerizing factor (ADF), have been characterized in the Apicomplexa. Coronin is a WD repeat containing actin binding protein that was first characterized in *Dictyostelium discoideum*, where it is essential for phagocytosis and motility. WD repeats (a tryptophan-aspartic acid motif) are found in diverse proteins; this motif is thought to mediate protein-protein interactions. Homologs of coronin are found in a large variety of eukaryotes, ranging from humans to *C. elegans* to yeast. A coronin homolog has been described in *P. falciparum* and is encoded by a single-copy gene (174). Compared to *Dictyostelium* coronin, the *Plasmodium* protein has conserved residues throughout the entire protein. A monoclonal antibody to *D. discoideum* coronin detects a 42-kDa protein in Triton X-100-insoluble extracts of *P. falciparum* schizonts. ADF/actophorin/cofilin is a widely conserved low-molecular-weight actin monomer-sequestering protein with filament-severing activity. An ADF homolog has been

characterized in *T. gondii* (9). The single-copy gene encodes a 13.4-kDa protein. *Toxoplasma* ADF has a high degree of sequence similarity to other ADF homologs, particularly *Acanthamoeba* actophorin and plant ADFs. *Toxoplasma* ADF localizes to cytoplasm, especially under the plasma membrane. Recombinant *Toxoplasma* ADF purified from *E. coli* binds actin monomers and depolymerizes microfilaments in a pH-independent, concentration-dependent fashion.

One surprising finding is that a homolog of the actin monomer binding protein profilin has not been found in the Apicomplexa. In its place, *Toxoplasma* has apparently substituted a novel protein, toxofilin (125). Toxofilin is a 27-kDa actin monomer binding protein that was originally isolated from *Toxoplasma* extracts on G-actin affinity columns. In pyrene actin assays, toxofilin inhibits actin polymerization, acting as an actin-sequestering protein. It also slows microfilament disassembly through a filament end-capping activity. A single-copy gene encodes toxofilin. The protein has a pI of 9.63 and two coiled-coil domains and lacks consensus motifs or any similarity to known proteins. Overexpression of green fluorescent protein (GFP)-tagged toxofilin in vertebrate cells disrupts stress fibers and reduces microfilament levels by half. Toxofilin localizes to the apical cytoplasm in intracellular *Toxoplasma* but is found at the posterior of invading parasites. In motile parasites, toxofilin is localized throughout the entire cytoplasm.

Myosin

Evidence for myosins in the Apicomplexa was first suggested by observations that *T. gondii* gliding motility and host cell invasion are reversibly inhibited by the myosin inhibitors BDM (an ATPase inhibitor) and KT5926 (a myosin light-chain kinase inhibitor) (35). BDM also blocks motility and invasion in *Plasmodium* and in *Cryptosporidium* (56, 123). The effect of BDM is likely to be myosin specific; however, KT5926 blocks host cell attachment and motility by inhibiting the secretion of adhesins, proteins required for motility and cell attachment (Table 1). Immunofluorescence with heterologous myosin antibodies or antisera that recognizes the highly conserved myosin peptide LEAF localizes to the anterior pole or to a circumferential pattern that overlaps with the distribution of actin in *T. gondii* and *P. falciparum* (35, 144, 187). Immunoelectron microscopy of hypotonically swelled parasites (the plasma membrane is separated from the IMC) demonstrates that actin is associated with the IMC and that myosin is associated with the plasma membrane (Fig. 3C) (35). The LEAF peptide antiserum identifies a 90-kDa band in *T. gondii* lysates, and heterologous myosin antibodies label a *Plasmodium* protein of 86 kDa (187).

Apicomplexan myosins are highly atypical and were ultimately cloned in *Toxoplasma* by using degenerate PCR of conserved regions of the motor domain (70). A similar strategy has been used to identify myosins in *Plasmodium*, *Neospora*, *Eimeria*, *Sarcocystis*, *Babesia*, and *Cryptosporidium* (69). All apicomplexan myosins are extremely similar, suggesting that the diversity of myosins in these parasites is extremely limited (69). Phylogenetic analysis of the myosins places these motors in a novel, highly divergent class (XIV) in the myosin superfamily (69, 70). Apicomplexan myosins range from 91 to 125

kDa and include the smallest myosins characterized thus far (70, 73). There is a high degree of sequence conservation among all apicomplexan myosins throughout their whole length. All have short tails that do not have homology to any other myosin tails. However, these tails do contain a highly basic charge distribution similar to myosin I family members, suggesting that the apicomplexan myosins may interact with membranes. Generally, myosin motors have three domains; the amino terminus contains the motor domain, the central "neck" region binds light chains and acts as a lever arm, and the tail is diverse, carrying out different functions such as targeting to subcellular regions or binding to cargo (18, 102). The apicomplexan myosins do not contain the strictly conserved glycine residue at the fulcrum point of the lever arm and generally lack IQ motifs that bind calmodulin and calmodulin-related proteins (68, 70, 73). Additionally, the *Toxoplasma* myosins do not follow the TEDS rule, i.e., the presence of an acidic or phosphorylatable residue at a precise site close to the actin binding region (70). In lower eukaryotes, this residue is crucial for stimulation of the ATPase of class I myosins, but other exceptions to this rule have been described. Both the absence of an IQ motif and the nonadherence to the TEDS rule suggest that these motors may be regulated in a novel fashion.

T. gondii expresses five class XIV myosins: TgM-A, TgM-B, TgM-C, TgM-D, and TgM-E (68–70, 73). TgM-A is 93 kDa and lacks a discernible neck domain and IQ motifs (70). Epitope-tagged TgM-A localizes beneath the plasma membrane (73). Mutational analysis has established that a pair of arginine residues is essential to target TgM-A to the periphery (73). Since ectopically expressed TgM-A in HeLa cells does not target to the plasma membrane, peripheral localization in parasites may require a membrane-associated receptor. The *P. falciparum* homolog of TgM-A (PfM-A/Pf-myosin1) is synthesized in mature schizonts and is present in merozoites but vanishes after the parasite enters the red cell (123). PfM-A is associated with the particulate parasite fraction, and immunofluorescence and immunogold analysis shows that PfM-A localizes to the periphery of mature schizonts and merozoites.

TgM-B and TgM-C are the products of differential RNA splicing and are 114 and 125 kDa respectively (70). They are identical throughout their head and neck domains and diverge in their distal tail structures. Both contain a single IQ motif. TgM-B has not been localized, but TgM-C localizes to a juxtannuclear region toward the apical pole of the parasite, consistent with an association with the Golgi apparatus (70, 73). TgM-D is a 91-kDa protein that has a punctate peripheral localization (73). TgM-E is the most recently discovered myosin and is currently being characterized (69, 73). Biochemical studies have established that the myosins bind actin in the absence but not the presence of ATP and that they are tightly associated with membranes (68, 73). The peripheral localization of TgM-A and of the GFP–TgM-A tail fusion is not dependent on an intact F-actin cytoskeleton (73). Truncation of the tail domains of TgM-A or TgM-D abolishes their peripheral localization and tight membrane association; fusion of the TgM-A or TgM-D tail to GFP is sufficient to confer plasma membrane localization (73).

MANIPULATION OF THE HOST CYTOSKELETON BY APICOMPLEXAN PARASITES

Reorganization of the Microvilli of Intestinal Epithelia by *Cryptosporidium*

Like other apicomplexans, *C. parvum* resides in a parasitophorous vacuole within the host cell, in this case the intestinal epithelium. However, the *Cryptosporidium* intracellular vacuole is extracytoplasmic, remaining at the apical surface of infected cells, in the region of the microvilli. The parasite induces host cell cytoskeletal rearrangement including the formation of branched microvilli clustered around the parasitophorous vacuole (55, 93). Moreover, *Cryptosporidium* induces the formation of a junctional complex that lies between it and the cytoplasm of the infected epithelial cell, keeping the parasite in the region of the microvilli. The junctional complex is associated with a plaque containing host cell actin. Accumulation of host cell actin, arp2, arp3, neural Wiskott-Aldrich syndrome protein (N-WASP), vasodilator-stimulated phosphoprotein (VASP), and α -actinin begins before entry is complete and these proteins localize beneath the invading parasite (45, 46). The plaque does not contain other actin binding proteins found in the intestinal epithelium, such as the catenins, zyxin, or plakoglobin (45). As parasites grow within the host cell, α -actinin is lost from the plaque, but the plaque size continues to increase to accommodate the increasing size of the replicating parasites. In addition to the above results, others have described localization of phosphotyrosine and villin at the site of parasite attachment (55). Expression of dominant negative constructs of Scar1 or N-WASP in host cells blocks *Cryptosporidium* invasion, suggesting that parasite-induced host cell actin reorganization is required for invasion (45, 46).

Plasmodium Modification and Mimicry of Erythrocyte Cytoskeletal Proteins

Infection with *Plasmodium* merozoites results in dramatic changes to the shape and biochemical properties of the parasitized red cell. Red cells infected with *Plasmodium* have increased phosphorylation of band 4.1, and a cysteine protease from the parasite cleaves red cell ankyrin (96, 128). *P. falciparum* induces knob formation of the surface of red cells, and the normal discoid shape of these cells becomes spherical (176). These structural alterations contribute to sequestration of infected red cells in organ capillaries, preventing their circulation and exposure to the spleen (107). The *Plasmodium* proteins RESA, MESA, and HRP-1 are anchored to the red cell membrane by association with spectrin, band 4.1, and the band 3 binding domain of ankyrin, respectively (17, 94, 97, 122). *P. falciparum* growth is decreased in human erythrocytes containing abnormal spectrin or band 4.1 (142). Parasites invade these cells normally, suggesting that an intact red cell membrane skeleton is required for parasite growth.

Plasmodium erythrocytic stages also synthesize proteins which are similar to ankyrin and spectrin and which are hypothesized to play a role in reorganization of the cytoskeleton of red cells. *Plasmodium chabaudi* ROPE (repetitive organelar protein) has a structure similar to that of spectrin (188). This 229-kDa protein is localized to the apical end of merozoites, possibly in the rhoptries. ROPE has characteristics of a

cytoskeletal protein. A 364-amino-acid repetitive region based on 32 11-mer repeats suggests that the protein forms an helical coiled-coil triple helix containing a leucine-histidine zipper. Strikingly, this three-dimensional arrangement resembles the structure of spectrin. It has been postulated that ROPE may be involved in invasion, by interacting with the erythrocyte cytoskeleton via molecular mimicry of spectrin. *P. falciparum* expresses an 88-kDa phosphoprotein that is nearly identical to the amino-terminal region of ankyrin, a region of the protein that binds band 3 (170). This protein may also help the parasite reorganize the membrane skeleton via molecular mimicry.

***Theileria* Exploitation of Host Cell Microtubules**

With respect to behavior and morphology, *Theileria parva* sporozoites are certainly the most distinct members of the Apicomplexa and do not conform to many of the general properties of the Apicomplexa described in this review. *Theileria* sporozoites are bounded by a simple plasma membrane structure and lack both an inner membrane complex and subpellicular microtubules. Host cell entry does not require apical orientation of the *Theileria* sporozoite and occurs by a zippering rather than a moving-junction mechanism (49, 50, 79, 147, 148, 152). Once inside the host cell, the parasite escapes from the parasitophorous vacuole and takes up residence in the cytoplasm. The parasite plasma membrane becomes coated with a number of host cell-derived microtubules organized in arrays tangential to the sporozoite surface (49, 79, 117, 147, 175). Sporozoite-associated microtubules are highly resistant to nocodazole disruption (147, 175). These parasites also activate NF- κ B, specifically inducing clonal expansion of infected cells (193). Infected lymphocytes will proliferate indefinitely in culture until antiparasitic drugs halt unchecked replication (193). Sporozoites undergo nuclear divisions to form a multinucleate schizont. The host microtubules associated with the schizont are captured by the spindle of the proliferating host lymphocytes, pulling fragments of the schizont into each daughter lymphocyte (117, 175). The coupling of induction of host cell proliferation and association with host cell microtubules ensures that infected cells are specifically expanded and that the resulting progeny continue to harbor the parasite.

CONCLUSIONS

Although researchers studying apicomplexan parasites have amassed many data regarding the cytoskeleton, we still are missing explicit evidence linking cytoskeletal components to cellular properties. The following discussion suggests possible links between the cytoskeletal elements and behavioral traits that are common to the members of the Apicomplexa. One obvious generalization is that in these cells, the subpellicular network and the subpellicular microtubules are critical to cell shape while actin and myosin are essential for motility and invasion. This is not to say that future work will not uncover additional functions for the cytoskeleton.

Although apicomplexan parasites are profoundly deformed during host cell invasion, they retain membrane integrity during host cell entry. This may be ascribed to their robust arrangement of plasma membrane, IMC, and subpellicular network. The newly identified proteins IMC-1 and IMC-2 are

implicated in subpellicular network formation (98). In addition to the obvious questions of how these proteins form filaments and how filament assembly is regulated is the issue of how this network associates with the IMC. The lattice of intramembranous particles observed after freeze fracture of the pellicle could reflect the transmembrane domains of receptors for the subpellicular network; however, the identity of these highly organized particles remains undetermined, as does any physical connection between the particles and the lattice proteins (43, 113, 124).

Apicomplexan parasites multiply by endodyogeny or schizogeny. As described above, these processes require independent regulation of spindle and subpellicular microtubules. Perhaps consequently, subpellicular microtubules and spindle microtubules are organized by different MTOCs: the apical polar ring and the spindle pole plaque/centrioles, respectively. In endodyogeny, parasites must discriminate between maternal and daughter apical polar rings and between maternal and daughter subpellicular microtubules. In schizogeny, daughter cell budding is induced after movement of multiple nuclei to the periphery of a maternal cell that lacks subpellicular microtubules. It is likely that the spindle pole plaques then induce or coordinate the formation of the apical polar rings, coupling each nucleus with a set of subpellicular microtubules. Daughter cell budding is distinct from vertebrate cytokinesis. In fact, inhibitor studies suggest that parasite scission may not utilize microfilaments such as are required at the vertebrate cleavage furrow (149). However, a recent study (28a) of the alternatively spliced myosins MyoB and MyoC in *Toxoplasma* demonstrates that overexpression of MyoB causes defects in cell division, and the parasites make extremely large residual bodies. Tagged MyoB localizes in a punctate cytosolic pattern and tagged MyoC localizes to the apical and posterior polar rings of tachyzoites. These latter observations suggest that MyoB and MyoC may play a role in parasite cell division, implicating an acto-myosin ring in parasite scission. Recent microtubule inhibitor studies show that subpellicular microtubule assembly can be disconnected from nuclear division, creating *Toxoplasma* tachyzoites that lack nuclei, although budding and scission from the maternal mass is completed (115). Multiple MTOCs permit apicomplexans to control nuclear division independently from cell polarity and cytokinesis. Although this grants greater cell cycle flexibility to these parasites, it abolishes the checks for coregulation of nuclear division and cytokinesis that are found in other eukaryotes.

Rigidity, cell shape, and apical polarity are provided by the subpellicular microtubules, and the apical polar ring organizes these microtubules (120, 134, 168). The apical polar ring represents a MTOC that is unique to the Apicomplexa. We know very little about its genesis and nothing about its component proteins. If the apical polar ring controls daughter cell budding, it must be replicated in a highly regulated fashion. It will also be informative to understand how it nucleates the subpellicular microtubules. The number of subpellicular microtubules and their organization are invariable within a life cycle stage of a particular species of apicomplexan parasite. The apical polar ring is a highly ordered structure and may contain signals that determine the number and placement of subpellicular microtubules.

Like the subpellicular network, the subpellicular microtu-

bules also have intimate connections with the inner membrane complex (3, 113, 131, 195). To understand these interactions, it will be necessary to identify and characterize MAPs. MAPs may dictate subpellicular microtubule length and position under the pellicle. It is unclear why some apicomplexans distribute their microtubules uniformly beneath the pellicle while others center one microtubule beneath one-third of the circumference and evenly space the remainder below the other two-thirds of the pellicle. In studies of motility, it is clear that the convex and concave sides of the parasite are not equivalent. The asymmetry of microtubules in some apicomplexans may simply reflect areas that are more or less closely involved in force generation during motility or other essential functions. Subpellicular microtubules may contribute to motility by providing tracks that direct the acto-myosin-based capping activity. *Toxoplasma* tachyzoites and *Plasmodium* merozoites with shortened subpellicular microtubules (due to drug treatment) are noninvasive, supporting this notion (14, 115). However, it is not absolutely clear how microtubules could serve as tracks for the acto-myosin system, since actin and myosin are believed to act between the plasma membrane and the IMC while microtubules localize to the cytoplasmic face of the IMC (35, 120).

Many studies have implicated actin in apicomplexan motility, although apicomplexan microfilaments are apparently quite labile under most circumstances. Polymerized actin is observed only in the presence of jasplakinolide, and in untreated cells nearly all the actin is found as G-actin (36, 151). The apical actin filaments observed after treatment of *Toxoplasma* with jasplakinolide may reflect the location of actin regulators that nucleate or otherwise facilitate filament polymerization (151). Alternately, the apical localization of an F-actin projection after jasplakinolide treatment may represent the "path of least resistance" since the apical region is the only area of the pellicle not surrounded by three unit membranes and the subpellicular network. The short-lived nature of microfilaments suggests that actin assembly and disassembly are closely regulated. The *Plasmodium* HSC70 complex caps F-actin, limiting filament growth, and the apicomplexan homologs of ADF/cofilin are likely to sever filaments and sequester monomers, facilitating rapid disassembly of actin filaments (9, 173). Additionally, in *Toxoplasma*, actin may be kept monomeric by sequestration by toxofilin, a novel monomer binding protein (125). BLAST searches of the *Cryptosporidium* and *Plasmodium* genomes do not identify homologs of toxofilin, suggesting that distinct proteins may provide this function in other apicomplexans (unpublished data).

Myosin motors are also implicated in motility and invasion (35, 56, 66, 123). The apicomplexan myosins are quite divergent from myosins in other organisms, constituting a new class of motors in the myosin family (68–70, 73, 123). Apicomplexan myosins are all quite similar but have different subcellular localizations. Myosin-A is most likely to be involved in motility since it is found beneath the plasma membrane, whereas myosin-B is located to the Golgi and myosin-D is found on vesicles, consistent with roles for these latter motors in membrane traffic (68, 73, 123). Ectopic expression of myosin-A has shown that it does not localize to the plasma membrane in nonapicomplexans and therefore must be targeted to this region in parasites by additional proteins (73).

The precise mechanism by which parasites use an acto-myosin motor to generate motility is unclear. Since actin filaments are rare and since the apicomplexan myosins lack typical regulatory domains, it has been suggested that the movement of myosin is limited by filament generation. Consistent with this, jasplakinolide-treated parasites show increased motility, although drug treatment inhibits rather than enhances parasite invasiveness (151). For the myosin motors to have force-generating movement, the microfilaments must be tethered so that they remain in place. Actin filaments may be immobilized by interactions with the IMC, and the rigidity provided by the subpellicular network and the subpellicular microtubules could provide the extra stability required for myosin movement to transport adhesins to the posterior end of the parasite. Myosin movement along the actin filaments would lead to capping of adhesins down the length of the parasite and ultimately to gliding motility or invasion. Gliding motility is a trait shared with gregarines, apicomplexan parasites of invertebrates that are quite distinct from other members of the phylum described here (84, 85, 184).

This review has generalized the behavior of apicomplexans as a group. By doing so, we have undoubtedly glossed over differences, particularly with the more divergent members of this phylum. However, in many cases, atypical attributes are particular to the life cycle stage of the parasite rather than characteristic of the organism as a whole. This is most clearly illustrated with *P. falciparum* merozoites and *Theileria* sporozoites. Although most apicomplexans have many subpellicular microtubules and use an acto-myosin mechanism to glide and to invade cells, *Plasmodium* merozoites have reduced these traits and *Theileria* sporozoites have eliminated them. *P. falciparum* merozoites have a drastically scaled-back set of subpellicular microtubules that may reflect the greatly reduced size of merozoites relative to liver and insect stage parasites; both traits may be dictated by the smaller size of the host red cells. Merozoites do not display gliding motility, although they actively invade red cells in an actin-dependent fashion. Other *Plasmodium* stages are larger, have prominent subpellicular microtubules, and are clearly motile. Similarly, *Theileria* sporozoites lack subpellicular microtubules and infect lymphocytes after entering in a novel and nonmotile fashion. Tick-stage *Theileria* kinetes are larger and have subpellicular microtubules. Although very little work has been done on this stage, we assume that they are motile and actively invade host cells. Nonetheless, there are clearly dangers in overgeneralizing the behavior of apicomplexan parasites. Other exceptions to these generalizations will undoubtedly be found within this diverse group of parasites.

At present, the *P. falciparum* genome project is nearing completion and several genome projects are under way for other *Plasmodium* species. The *Cryptosporidium* genome is also nearing completion; genome projects for *Toxoplasma*, *Theileria*, and *Babesia* are under way; and there are ongoing EST projects for *Toxoplasma*, *Neospora*, *Eimeria*, *Sarcocystis*, and *Plasmodium*. In the past few years, researchers have developed techniques to transfect *Toxoplasma* and *Plasmodium*, to make targeted deletions, and to create gene replacements. With the amassed information about the cytoskeleton and these new resources and tools, we are truly poised to understand the mechanisms underlying cytoskeletal functions in the apicom-

plexan parasites. Disease caused by these protozoa has tremendous medical and economic impact worldwide. For the cell biologist, the unique biology of the Apicomplexa represents an intriguing departure from standard eukaryotic behaviors; for the clinician, these distinctions may represent unique drug targets.

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