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Intracellular Parasite Invasion Strategies
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Bacterial Invasion: In Vivo Veritas

A major issue is to validate, in vivo, the molecular and cellular events analyzed in vitro. If one focuses on invasion of the intestinal barrier, it is clear that *L. monocytogenes*, *Shigella*, *Salmonella*, and *Yersinia*, despite their shared capacity to invade epithelial cells in vitro, differ with regard to (i) the capacity to disrupt, invade, and eventually cause the inflammatory destruction of the epithelium; and (ii) the possibility of proceeding to systemic dissemination and possibly colonization of organs at a distance.

A major handicap to studying the respective invasive phenotypes in vivo has been the lack of a mouse model simulating the intestinal and systemic diseases observed in humans (*67*). This was particularly the case for *L. monocytogenes*, until a transgenic mouse line expressing the human E-cadherin receptor of internalin became available, thus unlocking the transintestinal route for this pathogen, i.e., via invasion of enterocytes (*68*). A relevant animal model has yet to be found for *Shigella* because, unlike infected humans, mice do not undergo extensive invasion and inflammatory destruction of their rectal and colonic mucosae. Despite these limitations, a picture is emerging (Fig. 5) concerning the various strategies used by these pathogens.

In conclusion, although current work aims to elucidate the in vivo relevance of the now well-understood mechanisms used by invasive bacteria in vitro, future efforts should focus on understanding both bacterial and host cell transcription and translation programs during infection, in various cells and tissues. This information should provide vital clues in the ongoing battle against bacterial disease and for elaborating new therapeutic strategies.

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REVIEW

Intracellular Parasite Invasion Strategies

L. D. Sibley

Intracellular parasites use various strategies to invade cells and to subvert cellular signaling pathways and, thus, to gain a foothold against host defenses. Efficient cell entry, ability to exploit intracellular niches, and persistence make these parasites treacherous pathogens. Most intracellular parasites gain entry via host-mediated processes, but apicomplexans use a system of adhesion-based motility called "gliding" to actively penetrate host cells. Actin polymerization–dependent motility facilitates parasite migration across cellular barriers, enables dissemination within tissues, and powers invasion of host cells. Efficient invasion has brought widespread success to this group, which includes *Toxoplasma*, *Plasmodium*, and *Cryptosporidium*.

Parasites exist in virtually every conceivable niche, but none is so specialized as that of the obligate intracellular parasite, which must gain entry into the cells of its host to survive. Most intracellular parasites are protozoans, many of which are responsible for lethal and debilitating diseases in animals and humans. Our defenses present an array of barriers to infection, including skin, mucosa, connective tissue, and an active surveillance system to detect and destroy foreign objects. Overcoming these defenses and breaching the final barrier imposed by the cell membrane is a formidable challenge. By entering into the confines of a host cell, the parasite assures itself of both a ready source of nutrients and a potential means to avoid immune clearance. Parasites that practice this life-style have typically given up the capacity for extracellular growth, which leaves them vulnerable if entry is impeded. Defining how parasites gain entry into their host cells is thus important for rational design of improved therapies. Parasites are among the earliest branching eukaryotes (*1*); their study expands our knowl-

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edge of biological diversity and may also provide insights into the origins and workings of our own systems. The wide range of different intracellular niches exploited by parasites attests to the advantages of this life-style. A diversity of strategies is practiced for cell entry, ranging from phagocytosis (e.g., *Leishmania*) and induced uptake (e.g., *Trypanosoma cruzi*) to active penetration (e.g., *Toxoplasma* and related apicomplexans) (Table 1). The strategies used by *Leishmania* (*2*) and *T. cruzi* (*3*) to invade cells and to evade the immune response have recently been reviewed. Here, I will focus on recent advances in our understanding of how apicomplexan parasites gain entry and establish an intracellular niche within host cells.

Apicomplexans: Active Motility Leads to Cell Penetration

A highly successful strategy for cell entry by protozoan parasites is practiced by a diverse group of parasites in the phylum Apicomplexa, exemplified by *Toxoplasma* (Table 1). Invasion is an active process mediated by the parasite's cytoskeleton, and the host cell does not play an active role in uptake (*4*). Unlike phagocytosis, an entry strategy used by other intracellular parasites, apicomplexan entry occurs without alteration to the host actin cytoskeleton and without phosphorylation of tyrosine residues on host proteins. Entry is also independent of host cell calcium and instead relies on a calcium-regulated secretion pathway in the parasite (*5*, *6*). Active cell entry by apicomplexans is mechanistically distinct from the uptake of bacteria, viruses, and other parasites.

Actin-dependent motility and active cell penetration are highly conserved among apicomplexans, and these processes are inextricably linked by their reliance on actin polymerization in the parasite (*4*). Although polymerization is required for their unique form of motility called "gliding," actin is almost exclusively globular in *Toxoplasma* and possibly other apicomplexans (*7*). Moreover, because apicomplexans do not display the crawling movements characteristic of mammalian cells or amoebas, the common paradigms for actin-regulated motility in animal cells do not adequately explain how these parasites move. Recent research developments in *Toxoplasma* and *Plasmodium* have led to an improved understanding of the mechanism of gliding and a better appreciation of how this phenomenon is important in cell entry, in passage across biological barriers, and in facilitating tissue dissemination that occurs during infection.

Advancing Across the Terrain

The ancient phylum Apicomplexa contains some ~5000 members that are all obligate intracellular parasites and includes the human pathogens, *Plasmodium* (malaria), *Toxoplasma*, and *Cryptosporidium* (Fig. 1). Related to dinoflagellates and ciliates, apicomplexans lack normal appendages for locomotion such as cilia and flagella. Instead, apicomplexans move by gliding across the substrate without major changes in cell shape (*8*). Gliding by apicomplexans is distinct from that of bacteria, a process that relies on type IV pili, structures that are not found in apicomplexans. Pennate diatoms, which are related to the Apicomplexa, also display substrate-dependent gliding that is actin-based. Whereas diatom gliding is associated with secretion of mucin, apicomplexans deposit trails consisting of major surface proteins (Fig. 1). Trails can be visualized easily by using specific antibody staining, which provides a convenient static assay to study length, rate, and periodicity and to evaluate inhibitors that affect motility. When examined by time-lapse video microscopy, gliding occurs at rates of 1 to 10 μm per second, more than 10 times the speed of crawling by most amoeboid cells. *Toxoplasma* motility has a well-defined choreography consisting of circular gliding that always occurs counterclockwise and helical gliding that invariably proceeds clockwise (*9*) (movies S1 and S2). Gliding by *Toxoplasma*, *Cryptosporidium*, and *Plasmodium* is highly conserved, and a similar form of gliding motility is exhibited by gregarines, an

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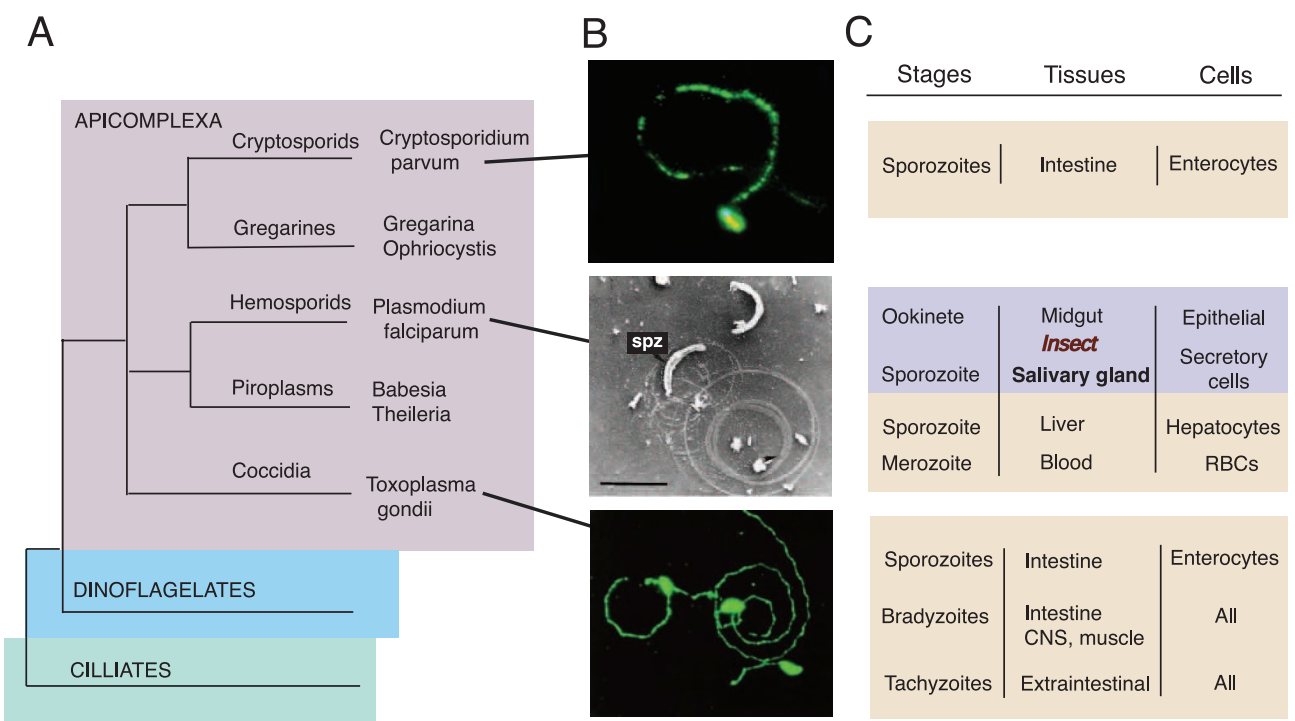

Fig. 1. Gliding motility is a conserved feature of apicomplexans. (**A**) Phylogenetic relations are depicted on the basis of life histories and small subunit RNA phylogeny. (**B**) Gliding motility by apicomplexans deposits characteristic trails on the substrate (SOM text and movies S1 to S10). *Toxoplasma* (*23*) and *Plasmodium* (*52*) images used with permission. *Cryptosporidium* trail image revealed by staining with monoclonal antibody 3E3 against a 25-kD surface protein and fluorescently conjugated secondary antibodies (provided by D. Wetzel). (**C**) Summary of motile life-cycle stages, tissues involved in migration, and cells invaded by apicomplexan human pathogens.

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early branching member of the phylum [movies S1 to S10 and supporting online material (SOM) text]. Video microscopy has revealed the erratic nature of gliding movements, with characteristic halting and restarting at seemingly random intervals. This behavior is consistent with the small size of the organism and hence low Reynolds number that predicts that inertia contributes little to forward movement (*8*). The term "gliding" is thus somewhat misleading, as continual turnover of the force-generating system is necessary to drive forward movement.

Breaching Cellular Membranes and Barriers

Although apicomplexans share a common mechanism of motility and invasion, they infect many different hosts and parasitize a variety of cell types (Fig. 1). For example, whereas *Cryptosporidium* only infects enterocytes of the intestine, *Toxoplasma* successfully enters virtually all nucleated cells of its host. *Plasmodium* has an even more complex life cycle, requiring specific interactions with host cells in both the mosquito and vertebrate hosts. The wide range of host cell types that are susceptible to invasion is, in part, dictated by interactions between the parasite's cell surface adhesins and host cell receptors. Binding to receptors on the host cell surface is a critical component of cell entry, because such interactions provide a foothold for forward movement.

Apicomplexans are not only adept at getting into cells, but many migrate extensively within their invertebrate and vertebrate hosts (summarized in Fig. 1). For example, *Toxoplasma* infects via the mouth and, after entry into intestinal epithelial cells, is capable of migrating across the basement membrane and penetrating deep into the submucosa (*10*). Transmigration across polarized epithelial monolayers is highly dependent on the parasite genotype and strongly linked with acute virulence (*10*). Active migration may facilitate dissemination to deep tissues, such as the retina, central nervous system, and placenta, where toxoplasmosis causes its most severe symptoms.

Malaria also undergoes extensive migrations during its life cycle. In the insect, ookinetes migrate across the midgut epithelium (movie S8), whereas sporozoites disseminate in the hemolymph before penetrating the salivary glands. In the vertebrate host, sporozoites are injected with the bite of a mosquito then navigate the circulatory system before homing in on the liver. Multiple rounds of cell invasion appear to be necessary to potentiate the intracellular survival of malaria sporozoites within liver hepatocytes (*11*, *12*). Recent studies have revealed that specific sporozoite proteins are necessary to facilitate passage across liver sinusoidal Kupffer cells, possibly as a prerequisite to entry into hepatocytes (*13*).

Table 1. Summary of entry mechanisms and survival niches of intracellular parasites. Abbreviations: BR(2), bradykinin receptor 2; C1, complement receptor 1; C3, complement receptor 3; TGF-β, transforming growth factor–β.

Property	Intracellular parasite		
	Leishmania	*T. cruzi*	*Toxoplasma*
Cell types	Primarily macrophages	Variety	All nucleated
Receptors	C1, C3, Scavenging	G protein, BR(2), TGF-β	GAGs, sialic acid
Entry mechanism	Phagocytosis	Calcium-induced lysosome fusion	Direct penetration
Host actin	Yes	No	No
Parasite actin	No	No	Yes
Niche	Lysosomal	Cytosolic	Nonfusogenic vacuole

Stealthy Entry

Active penetration carries *Toxoplasma* into the host cell, where the parasite resides in a specially modified vacuole derived primarily from the host cell plasma membrane (*14*). *Toxoplasma*-containing vacuoles completely avoid fusion with normal host endocytic and exocytic vesicles, and the parasite replicates within this protected intracellular niche. During entry, apical organelles called rhoptries discharge their contents, which contribute to formation the vacuole membrane (*15*). One such component, ROP2, is a transmembrane (TM) protein that traffics to the vacuole, where it mediates association of host cell mitochondria and endoplasmic reticulum (*16*). Parasite proteins are also discharged from the rhoptries directly into the host cytosol (*15*), which indicates that this route may release multiple components that affect the host cell.

Invasion by *Toxoplasma* is a rapid process taking about 15 to 20 s, during which, host cell plasma TM proteins but not glycosylphosphatidylinosi-tol (GPI) lipid–anchored proteins are largely excluded from the forming vacuole (*17*). Sorting of host membrane proteins also occurs during invasion of red blood cells by *Plasmodium* merozoites. The *Plasmodium*-containing vacuole contains host proteins found in detergent-resistant membranes (i.e., Duffy receptor, GPI-anchored proteins, and the parasite protein EXP1), but excludes most TM proteins (*18*). Although the entry of malaria parasites into red cells occurs by a parasite-driven process, signaling through host cell β_2-adenergic receptors via heterotrimeric GTP-binding proteins (G proteins) is important for successful entry (*19*). Activation of this pathway may be important for exclusion of cytoskeletal and TM proteins from the vacuole.

Although apicomplexans are all intracellular parasites, *Cryptosporidium* offers a special case. Rather than residing deep within the cytosol, *Cryptosporidium* enters the apical surface of intestinal epithelial cells where it rests on a bed of host actin filaments and associated actin-binding proteins (such as talin, ezrin, vinculin, α-actinin) (*20*). The host cytoskeleton is reorganized with the aid of actin-polymerizing factors such as N-WASP, VASP, and Arp2/3 (*20*, *21*). However, *Cryptosporidium* exhibits actin-dependent gliding and probably uses a parasite-driven process to gain entry into the host cell initially.

Providing Traction and Recognition

Unlike bacterial and viral pathogens, which display adhesins statically on their surfaces, apicomplexans conceal their ligands for host receptors inside and selectively release them when encountering the host cell. Apicomplexan adhesins are stored in apically located secretory organelles called micronemes (*22*). Constant low-level constitutive secretion from these organelles probably suffices to promote substrate interactions necessary for gliding. However, on cell contact, microneme secretion is strongly up-regulated through a calcium-mediated release pathway (*23*). Although the signaling events that trigger microneme secretion are incompletely understood, recent studies demonstrate that apicomplexans contain both inositol 1,4,5-trisphosphate–regulated and ryanodine-type calcium release channels that are important for regulated secretion (*5*). Regulated secretion of cell surface adhesins accomplishes two important goals: (i) release from the apical end of the parasite assures polarized attachment to the host cell, a necessary prerequisite for productive entry, and (ii) restricting availability to the zone of host cell contact may prevent antibodies from neutralizing attachment (*24*).

Microneme proteins contain a variety of well-recognized adhesive motifs including epidermal growth factor (EGF)–like repeats, Apple domains, thrombospondin type 1 repeats (TSR), and integrin A-like domains (A domains) typified by von Willebrand factor (vWF) domain (*22*). A variety of microneme proteins have been shown to bind host cells;

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however, by far the best-studied example is the TRAP (thrombospondin anonymous repeat protein) family of proteins, first characterized in *Plasmodium* and present in a variety of apicomplexans (Fig. 2). TRAP family proteins are type 1 TM proteins that provide a crucial link, between extracellular adhesion to receptors and the parasite cytoskeleton, that is necessary for motility and invasion of apicomplexans (*25*). A variety of conserved sequence motifs in TRAP proteins have been shown to participate in host cell binding, in trafficking to the micronemes, and in linking to the cytoskeleton (Fig. 2).

TRAP homologs contain one or more A domains (except in *Cryptosporidium*) followed by one or more TSR domains (Fig. 2). vWF or A domains are found in a variety of integrins, collagens, and complement factors, where they mediate cell and matrix binding via a divalent metal–binding domain known as MIDAS (*26*). Although most specialization of vWF domains arose in metazoans (*26*), their presence in apicomplexans suggests an older origin for this domain in mediating extracellular adhesion. The A domains in TRAP and MIC2 bind to heparin-like molecules, and interactions with glycosaminoglycans (GAGs) are important for parasite recognition of host cells (*27*, *28*). In mammalian systems, A domains mediate binding to a variety of matrix and cell surface proteins, which raises the possibility that similar interactions may be discovered with parasite A-domain proteins. TSR domains are also widespread in extracellular matrix and the developing nervous system of mammals, where they mediate binding to GAGs, sulfatides, and matrix proteins (*29*). TSR domains in TRAP and the circumsporozoite protein CS have been shown to interact with GAGs, and this interaction partially mediates sporozoite internalization [summarized in (*28*)].

A Moving Conveyor Belt

After secretion onto the anterior end of the parasite cell, MIC2 in *Toxoplasma* (*30*) and TRAP in *Plasmodium* (*31*) are translocated to the posterior pole. Translocation requires the parasite's actin cytoskeleton and is inhibited by cytochalasin-D. Biochemical studies based on MIC2 reveal that the protein is ultimately released from the cell surface by proteolysis that occurs within the membrane (*32*, *33*). Mutations just outside the TM domain also block processing, and parasites expressing these defective forms of the protein have a dominant-negative phenotype, promoting adhesion but inhibiting invasion (*34*). Thus, apical secretion, posterior translocation, and proteolytic release of adhesin-receptor complexes must be carefully coordinated to promote motility and invasion.

TRAP homologs are widely expressed in apicomplexans, which suggests that this conserved family of adhesins serves an important function in cell invasion. Gene disruption experiments in *P. berghei* demonstrate that TRAP is necessary for motility and invasion of sporozoites into both salivary glands in the mosquito and liver hepatocytes in the mouse (*25*). Specific point mutations in conserved residues of the vWF (A) domains or TSR domains of TRAP implicate these conserved motifs in mediating entry into both mammalian and insect cell types (*35*, *36*). *Plasmodium* ookinetes also express a TRAP homolog called CTRP, and disruption of this gene prevents normal passage across the midgut epithelial cells in the insect (*37*). In *Toxoplasma*, MIC2 is expressed in all invasive stages, and indirect evidence suggests it is essential for invasion. MIC2 is tightly coupled to an accessory protein M2AP, and deletion of this partner leads to inefficient targeting to the micronemes and failure to up-regulate MIC2 secretion, which results in impaired invasion (*38*).

Plasmodium merozoites do not express TRAP homologs or display gliding motility on substrates, yet they penetrate red blood cells by process that is inhibited by cytochalasin (*39*). A variety of cell surface proteins have been implicated in the recognition of red blood cells by merozoites including MSP-1, AMA-1, Duffy-binding proteins (i.e., EBA-175), reticulocyte-binding proteins (i.e., RBPs in *P. vivax*); proteolytic processing is also required for efficient invasion (*40*). Although specific recognition is a necessary prerequisite, binding itself does not lead to internalization in the absence of a parasite-driven motor process. Recent studies have shown that the cytoplasmic domain of EBA-175, a major merozoite surface protein implicated in invasion of red cells, can be replaced with that of TRAP from sporozoites (*41*). Thus, despite having a different repertoire of receptors for recognition of the host cell, merozoites use actin-myosin machinery for cell invasion similar to that described above.

Grabbing Motility by the Tail

Observations that cell surface adhesins are translocated in an actin-dependent manner suggest they somehow interact with the cytoskeleton through their cytoplasmic domains. The cytoplasmic domain (C domain) of TRAP homologs shows only modest conservation at the sequence level with the most obvious features being overall length, a cluster of acidic residues, and a terminal tryptophan residue (Fig. 2). In *P. berghei*, the C domain is essential for normal motility, and parasites expressing a mutant version of the TRAP tail (TrpAsn changed to AlaSer) can only move through a partial arc before falling back to the original starting position (*31*). Despite the modest overall sequence conservation, the C domain of MIC2 from *Toxoplasma* can replace that of TRAP, which indicates that that the function of these domains is conserved (*31*). The C domain of MIC2 is also essential for infection by *Toxoplasma* tachyzoites (*42*).

The C domains of MIC2 and TRAP bind to a complex that contains the cytoplasmic glycolytic enzyme aldolase (*43*). Aldolase is a tetrameric enzyme that cross-links actin filaments through binding to specific conserved residues in the core of the enzyme. Binding between the MIC2 or TRAP C domains and aldolase is direct, and this complex recruits actin both in vitro and in cell lysates of *Toxoplasma* and *Plasmodium* (*43*, *44*). Binding between the cytoplasmic domain of the GLUT-4 receptor and the actin cytoskeleton is also mediated by aldolase binding, and there is a striking similarity

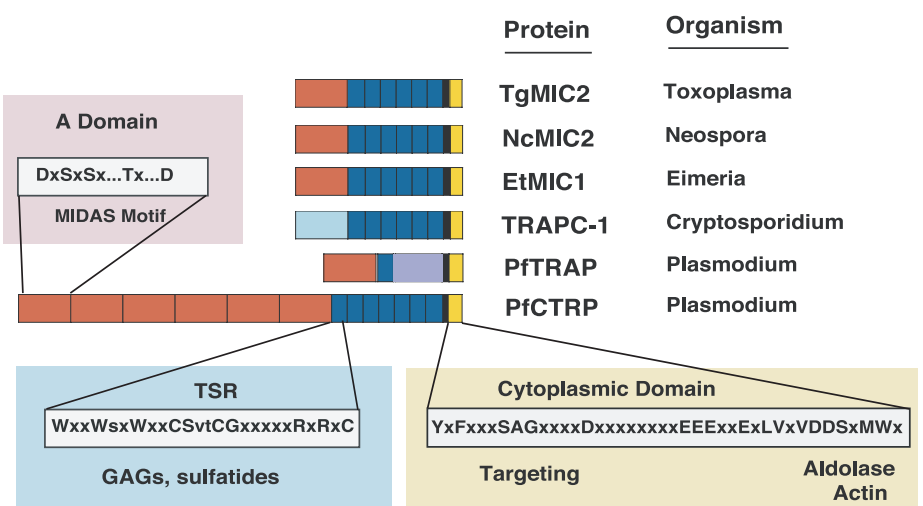

Fig. 2. TRAP family homologs are expressed by a variety of apicomplexan parasites. TRAP homologs have one or more integrin A domains containing a conserved MIDAS motif (*26*) followed by one or more TSRs (*29*). The C-terminal cytoplasmic domain contains sorting motifs defined in MIC2 (*52*) and a region that links to the actin cytoskeleton via bridging with aldolase (*43*, *44*). Consensus residues are shown in capitals; x indicates any amino acid.

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between their C domains: both contain a cluster of acidic charges and an aromatic residue (Trp or Phe). The binding of aldolase to the C domains of MIC2 or TRAP is abolished in mutants where the Trp is changed to Ala (*43*, *44*). These findings provide a molecular explanation for the earlier observations that mutations in the Trp residue disrupted normal gliding in *Plasmodium* sporozoites (*31*). In TRAP, additional acidic residues in the tail are also important for the interaction with aldolase (*44*). Thus, binding of the C domain of TRAP homologs to aldolase forms a bridge to the actin cytoskeleton, coupling adhesion to motility.

Motors, Mechanisms, and Models

Perhaps dating to their common origins with ciliates and dinoflagellates, apicomplexans have retained a complex cytoskeleton and are enclosed by multiple membranes (Fig. 3A) (SOM text and fig. S1). Their crescent cell shape is maintained by a corset of singlet microtubules that underlies the inner membrane complex (IMC), which is composed of both flattened membrane cisternae and a filamentous network of proteins (*45*, *46*). The IMC effectively divides the main cytosol from a smaller cortical cytoplasmic compartment contained by the outer plasma membrane. Within this cortical space lies the motor complex that drives motility.

Myosins in apicomplexans belong to class XIV, an unusual group of small motors with degenerate regulatory domains and very short tails. TgMyoA is the major myosin expressed by tachyzoites of *Toxoplasma* where it is localized beneath the plasma membrane (*47*). A newly developed regulated expression system confirmed that TgMyoA is responsible for gliding and cell invasion by *Toxoplasma* (*48*). TgMyoA is a relatively fast, plus-end-directed motor that is not processive, which indicates that it requires relatively abundant local concentration of filaments to provide productive work (*49*). Recent studies with *Plasmodium* reveal that PfMyoA, a homolog of TgMyoA, is not found in the plasma membrane, as previously thought, but instead is anchored to the IMC in association with a light chain (*50*). A similar complex exists in *Toxoplasma* consisting of TgMyoA, a light chain, and several 50-kD associated proteins that are likely responsible for localizing the motor in the IMC (*49*, *51*) (see Fig. 3, inset). Thus anchored in the IMC, MyoA is positioned to propel actin filaments in the space between the inner membrane and plasma membrane

Several recent observations combine to provide an improved understanding of gliding motility in apicomplexans (Fig. 3): (i) Calcium-mediated exocytosis releases adhesins at the apical end of the parasite, (ii) binding between the C domain of the adhesin and aldolase tethers them to the actin cytoskeleton, and (iii) MyoA translocates the adhesin–aldolase–actin complex rearward. Attachment between the extracellular domains of the adhesin and the substratum, combined with the concerted action of the motor complex, propels the parasite body forward. Finally, the parasite must break the interaction with the substrate to maintain forward movement; this step is accomplished by proteolytic processing, probably involving a rhomboid type protease that cleaves the adhesin within the TM domain (*32*, *33*).

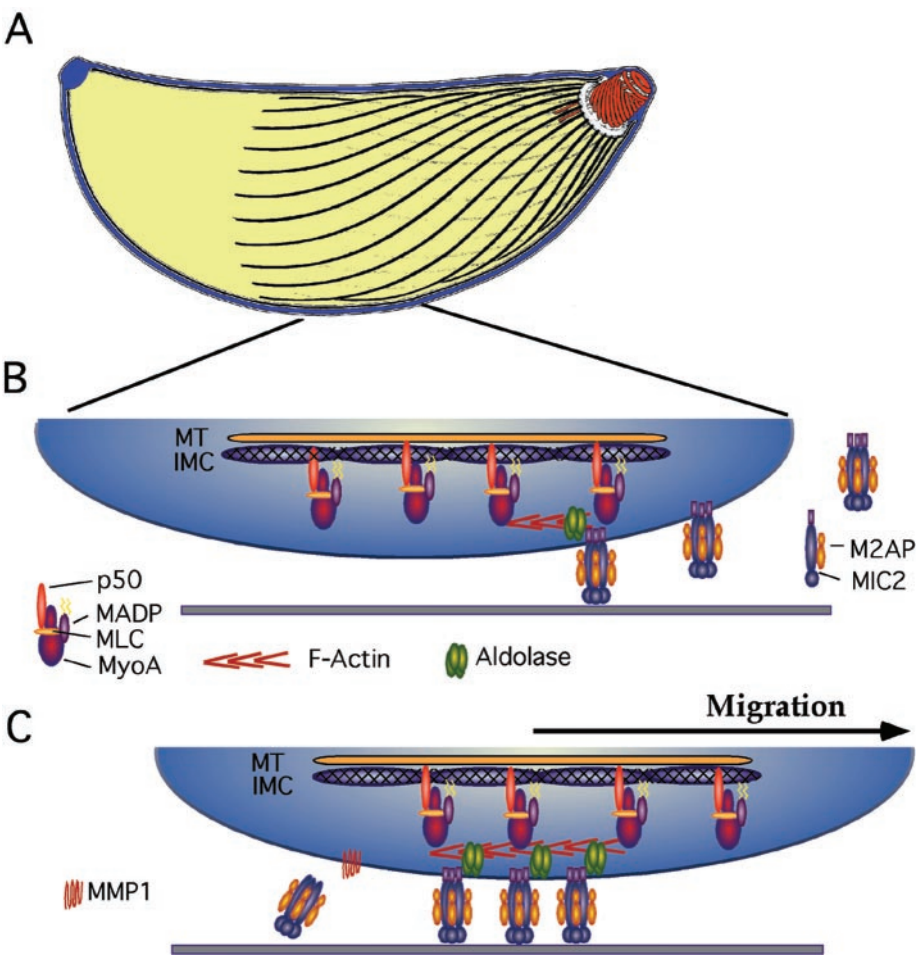

Fig. 3. Model for gliding motility by apicomplexans. (**A**) The crescent-shaped parasite maintains its rigid shape by an underlying cortex of microtubules (MTs) and an IMC [modified from (*46*) with permission], (supporting text online and fig. S1). (**B**) Expanded view of the cell surface membrane and underlying IMC/MT complex. The myosin motor complex is anchored in the IMC and is composed of MyoA; myosin light chain (MLC); an acylated, myosin-associated docking protein (MADP); and a p50 TM subunit (*48*, *49*, *51*). MIC2/M2AP adhesin complex is discharged at the anterior end of the cell as a hexameric complex in the parasite membrane (*42*). The adhesin complex binds to aldolase through the C domain and links to the actin cytoskeleton (*43*, *44*). (**C**) Actin filaments are polymerized beneath the plasma membrane and provide a scaffold for translocation by MyoA. Progressive movement of F-actin–aldolase–MIC2/M2AP complexes along the cell surface propels the parasite forward. MIC2 is released after an intramembrane processing event, likely mediated by a rhomboid-type protease called MMP1 (*33*).

Remaining Questions and Challenges

Despite recent advances, there remain a number of puzzling questions about apicomplexan motility and invasion. First, because motility is highly directional actin filaments must be assembled in a directional manner and at a precise time and place. It is unclear whether an Arp2/3-like process drives F-actin assembly or if a completely separate mechanism is responsible. Whether filament turnover is due to a highly active actin-depolymerizing factor or to unusual polymerization kinetics is also uncertain. How myosins are anchored to the underlying cytoskeleton and how their motor activity is regulated are areas for further investigation. The separate duties of aldolase in glycolysis and adhesin-complex formation suggest that cellular partitioning is necessary to maintain these functions, yet the details of this process are unknown.

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Moreover, the binding of the extracellular domains of adhesins to host cell surface receptors remains incompletely characterized, as are the molecular interactions that govern processing by proteases.

Judging from their deep branching evolutionary position and present-day success, apicomplexans are likely to be with us for some time. Thus far, our glimpses into parasite motility have revealed a very different process than that used by mammalian cells. Although these differences may explain the tremendous success of apicomplexans, their understanding may also enable selective disruption of parasite motility. If we are to thwart these ancient and mysterious parasites, our attention should be focused on defining their unique biology.

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REVIEW

Ancient Invasions: From Endosymbionts to Organelles

Sabrina D. Dyall, Mark T. Brown, Patricia J. Johnson*

The acquisitions of mitochondria and plastids were important events in the evolution of the eukaryotic cell, supplying it with compartmentalized bioenergetic and biosynthetic factories. Ancient invasions by eubacteria through symbiosis more than a billion years ago initiated these processes. Advances in geochemistry, molecular phylogeny, and cell biology have offered insight into complex molecular events that drove the evolution of endosymbionts into contemporary organelles. In losing their autonomy, endosymbionts lost the bulk of their genomes, necessitating the evolution of elaborate mechanisms for organelle biogenesis and metabolite exchange. In the process, symbionts acquired many host-derived properties, lost much of their eubacterial identity, and were transformed into extraordinarily diverse organelles that reveal complex histories that we are only beginning to decipher.

Analyses of mitochondrial genes and their genomic organization and distribution indicate that mitochondrial genomes are derived from an α-proteobacterium–like ancestor, probably due to a single ancient invasion (Fig. 1) of an Archea-type host that occurred >1.5 billion years ago (Ga) (*1*). Whether the host cell was already eukaryotic is unclear (Fig. 1), although all contemporary eukaryotes examined contain some genes contributed by this symbiont (*2*).

How the proto-mitochondrial ancestor invaded and avoided elimination by the host has generated many hypotheses since the symbiosis theory was revived by Margulis (*3*). Some account for the concurrent origin of eukaryotes and mitochondria (*4*, *5*). These hypotheses propose a metabolically driven symbiosis where the host is a methanogenic archaean that associated with a methanotrophic proteobacterium to obtain essential compounds, e.g., hydrogen (*4*). The hydrogen hypothesis accounts for both mitochondrial aerobic pathways and anaerobic pathways in organelles of possible mitochondrial ancestry, e.g., hydrogenosomes (*4*). Notably, these scenarios posit the invasion to have occurred under anoxic conditions because both host and symbiont were capable of anaerobic metabolism. In contrast, an "aerobic" origin theory hypothesizes that the symbiosis was driven by an aerobic proteobacterium relieving

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