Reassessing the mechanics of parasite motility and host-cell invasion

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The capacity to migrate is fundamental to multicellular and single-celled life. Apicomplexan parasites, an ancient protozoan clade that includes malaria parasites (*Plasmodium*) and *Toxoplasma*, achieve remarkable speeds of directional cell movement. This rapidity is achieved via a divergent actomyosin motor system, housed within a narrow compartment that lies underneath the length of the parasite plasma membrane. How this motor functions at a mechanistic level during motility and host cell invasion is a matter of debate. Here, we integrate old and new insights toward refining the current model for the function of this motor with the aim of revitalizing interest in the mechanics of how these deadly pathogens move.

Introduction

The ancient phylum Apicomplexa includes many of the world's preeminent pathogens (Woo et al., 2015), such as *Plasmodium*, the genus responsible for more than half a million malaria-related deaths each year (White et al., 2014), and *Toxoplasma gondii,* one of the most prevalent pathogens of humankind (Torgerson and Mastroiacovo, 2013). At some point in their life cycle, most Apicomplexa are obligate intracellular parasites (Gubbels and Duraisingh, 2012) that develop and proliferate inside a surrogate host cell before being released to infect new host cells. Although each pathogen has evolved highly specialized strategies for coopting host cells during infection (Marsolier et al., 2015; Coffey et al., 2016; Curt-Varesano et al., 2016), it is the cellular biology of their extracellular forms, often referred to as *zoites*, that defines many of the key hallmarks of apicomplexan cell biology, not least their ability to move and invade host cells. The classic apicomplexan zoite cell is ideally adapted for motility and invasion; for example, the *Plasmodium* sporozoite (see Glossary of terms) glides at speeds an order of magnitude faster than the fastest of human cells (Münter et al., 2009), whereas *Plasmodium* or *Babesia* merozoites penetrate host cells within a few tens of seconds (Dvorak et al., 1975; Asada et al., 2012). Each motile zoite has a distinctive apical complex consisting of secretory organelles and microtubule-based structures, from which the phylum derives its name (Fig. 1 a). The secretory organelles contain protein constituents or lipids that are released via the apex, which then take one of several routes: passing rearward over the zoite plasma membrane; spewed out into the surrounding milieu; or injected directly into the host cell (Bradley and Sibley, 2007; Boothroyd and Dubremetz, 2008; Bargieri et al., 2014). The stepwise release of each constituent (and their inherent subcompartmentalization or differentiation into different subsets) likely defines a hierarchy of steps in zoite motility and invasion (Zuccala et al., 2012; Kremer et al., 2013).

Apicomplexan cell motility, broadly referred to as gliding, is unlike conventional strategies used by most eukaryotic cells to move, differs from amoeboid movement, and is not reliant on tubulin-dependent appendages, such as cilia or flagella (Sibley, 2004). Gliding is instead intimately involved with a highly organized outer pellicle of the zoite cell. This outer pellicle consists of the plasmalemma and the underlying organized cortex of the parasite cell (Fig. 1). Underpinning the entire structure is a static microtubule and intermediate filament-based cytoskeleton (Morrissette and Sibley, 2002). This nondynamic, rigid structure gives the zoite its fixed but flexible shape (Russell and Sinden, 1982; Cyrklaff et al., 2007; Hanssen et al., 2013; Kan et al., 2014). Built over the cytoskeleton is a double-membraned, flattened compartment called the inner membrane complex (IMC), above which sits the parasite plasma membrane (PPM). These together create a three-layered appearance (Fig. 1) that is broadly conserved across the infrakingdom Alveolata (Gould et al., 2011). It is within the 20- to 30-nm lumen between the IMC and PPM that apicomplexan zoite motility is thought to be generated.

Currently, the most widely accepted model for a how a subcortical actomyosin motor might function in apicomplexan cell motility envisages a complex of proteins, called the glideosome, anchoring the divergent apicomplexan class XIV myosin to the outer IMC membrane, linked to the surface via actin filaments, bridging proteins and secreted surface proteins. Force from the motor drives actin filaments and linked adhesins rearward, creating a traction force that drives the parasite forward or into the host cell (Soldati et al., 2004). The model is based on several molecular and microscopy-based studies localizing key glideosome components to the IMC and immunoprecipitating partner proteins to provide a linkage between the zoite and the

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Abbreviations used: F-actin, filamentous actin; GAP, glideosome-associated protein; IMC, inner membrane complex; PPM, parasite plasma membrane.

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 214 No. 5 507–515
www.jcb.org/cgi/doi/10.1083/jcb.201605100

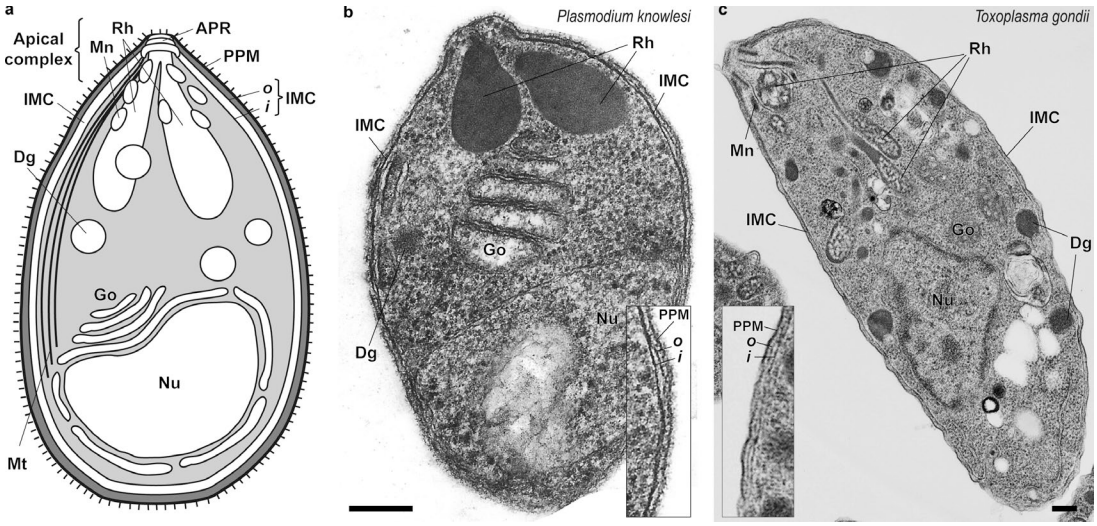

Figure 1. **Motile apicomplexan zoite cells designed for invasion and motility.** (a) Schematic of an apicomplexan zoite cell (here a merozoite) showing key cellular structures and apical complex, characteristic of motile zoites. (b and c) Electron micrographs of a *P. knowlesi* merozoite (b) and a *Toxoplasma* tachyzoite (c). Apicomplexan zoites are generally polarized and elongated, with either a crescent or oval shape. Each has a distinctive apical complex, which consists of secretory organelles called micronemes, rhoptries, and dense granules. Micronemes (oval or pear-shaped organelles) secrete their contents at the anterior tip of motile zoites during motility/invasion. Rhoptries (club-shaped organelles) fuse and release their contents concomitantly with host-cell invasion (Carruthers and Tomley, 2008; Counihan et al., 2013; Hanssen et al., 2013). Dense granules (a mixed grouping of secretory vesicles) are released via fusion with the plasma membrane before or after invasion (also called exonemes; Yeoh et al., 2007). Insets highlight the triple-layered appearance of the parasite pellicle at higher magnification (double-membraned IMC, lying under the PPM). The myosin motor is thought to lie between the outer (*o*) IMC membrane and the PPM. APR, apical polar (tubulin-rich) rings; Dg, dense granules; Go, Golgi apparatus; *i*, inner membrane of the IMC; Mn, micronemes; Mt, subpellicular microtubules; Nu, nucleus; Rh, rhoptries. Bars, 200 nm. Micrograph images courtesy of L.H. Bannister (Kings College London, London, England, UK) and D. Ferguson (University of Oxford, Oxford, England, UK).

extracellular environment (Buscaglia et al., 2003; Jewett and Sibley, 2003; Gaskins et al., 2004; Jones et al., 2006; Frénal et al., 2010; Fig. 2 a). Although a great deal of recent work has extended our understanding of the constituent molecular components of the glideosome and related structures (Alexander et al., 2005; Frénal et al., 2010; Riglar et al., 2011; Srinivasan et al., 2011; Weiss et al., 2015), the model is still incomplete in its ability to fully explain the mechanics of how zoites move on substrates or enter cells. Some of this deficit arises from limitations in the ability of immunoprecipitation, as a technique, to determine precise motor topology. Similarly, several recent studies that have viably knocked out key proteins associated with glideosome function, previously thought of as essential to the motor model, challenge our ability to completely understand how each protein is involved (Andenmatten et al., 2013; Egarter et al., 2014; Kehrer et al., 2016). Some residual motility from such mutants can certainly be explained by a redundancy in the expression of paralogs, as is the case for *Toxoplasma* apical membrane antigen 1 (AMA1) (Lamarque et al., 2014), or by prolonged protein stability over successive generations, as might be the situation with *Toxoplasma* actin (Drewry and Sibley, 2015). However, many of the nuances of each mutant phenotype and ongoing debates about the essential or nonessential role each factor might play (reviewed extensively by Meissner et al. [2013]) remain unresolved. For example, such explanations still require the definition of mechanisms that can trigger paralogue gene expression or the ability of proteins to remain stable over generations and across parasite systems (i.e., not just in *Toxoplasma*). Irrespective of these key debates, which we do not attempt to address here, it is our opinion that robust resolution for a detailed molecular basis of gliding motility is still left wanting.

Because several reviews have catalogued the molecular and cellular components of the gliding machinery or assessed essential versus nonessential roles for proteins involved (Daher and Soldati-Favre, 2009; Skillman et al., 2011; Cowman et al., 2012; Boucher and Bosch, 2015; Heintzelman, 2015), here we have instead focused our discussion on the fundamental mechanics of the motor. Our review, drawing predominantly on work from *Plasmodium* and *Toxoplasma*, aims to integrate new and old work to try and build an understanding of the motor's architecture and topology and how its force might be transmitted to the extracellular environment. Looking to the future, we also point out outstanding questions, gaps in our understanding, and types of experiments that might yield insights to resolve gliding motor mechanics.

Mechanistic insights into gliding and invasion: The macroscopic level

For more than 100 years, parasitologists have observed apicomplexan gliding motility (Schewiakoff, 1894; Crawley, 1902; Freyvogel, 1966; Vanderberg, 1974), with the first movies of host-cell invasion taken more than 50 years ago (Hirai et al., 1966; Bannister et al., 1975; Dvorak et al., 1975). These original, premolecular descriptions, together with their detailed illustration by electron microscopy (Ladda et al., 1969; Bannister et al., 1975; Aikawa et al., 1977, 1978; Stewart et al., 1986) have played a key role in informing how we understand apicomplexan motility.

Continuous motion. The first detailed observations of *Gregarina* or *Eimeria* zoites (and later those of *Plasmodium* or *Toxoplasma*) reported the capping of surface markers, such as pigment (Crawley, 1902; Russell and Sinden, 1981) or antibodies (Speer et al., 1985; Stewart and Vanderberg, 1988), at the posterior of motile cells or, in some cases, their dynamic movement rearward over the motile parasite's length. In live studies, surface-bound markers were seen to move backward along the

Glossary

Sporozoite. A motile life cycle stage of the Plasmodium parasite. Sporozoites develop within the oocyst on the mosquito midgut wall and release into the hemocoel of the mosquito, whereupon they migrate to the salivary glands. Upon mosquito biting, the sporozoites are released from the salivary glands into the mammalian dermis, where they enter capillaries that take them to the liver. After hepatocyte invasion, the parasite then develops to produce the blood stage merozoite form. Sporozoites are the fastest-moving lifecycle stages, able to reach speeds of several micrometers per second.

Merozoite. A motile life cycle stage of the Plasmodium parasite specifically adapted for invasion of the erythrocyte. Merozoites develop within the liver cell after its infection by the sporozoite and are released into the bloodstream after hepatocyte rupture. Merozoites are invasive but not traditionally thought of as being able to glide, although this remains relatively untested.

Tachyzoite. The rapidly multiplying stage of Toxoplasma development, highly motile and promiscuous in its ability to invade almost all nucleated cell types.

Inner membrane complex (IMC). Membranous vesicles, or alveoli, that are located beneath the plasma membrane of apicomplexan cells. These vesicles are subtended by the microtubule-based cytoskeleton and, in motile forms, house key components of the glideosome complex believed to anchor myosin XIV, required for motility.

Reflection interference contrast microscopy (RICM). A type of microscopy that determines a specimen's shape and distance from a nearby, usually flat, reflective surface by using the interference pattern produced by light reflecting from both the object and the surface. The interference patterns that arise permit assessment of differences in proximity between cell membrane and substrate surface; in the case of a motile zoite, the cell body adhering to and lying just above the glass slide.

Traction force microscopy (TFM). When undertaken on soft elastic substrates with embedded marker beads, TFM provides spatially resolved measurements of the motion of beads around the motile cell within an in vitro elastic environment (e.g., Matrigel), permitting estimation of the traction forces applied by a moving cell on the extracellular milieu.

Tight junction. In the case of apicomplexan cells, the tight junction is used to define a structure that forms between the invading apicomplexan zoite and the host cell. By electron microscopy this is seen as a region of close approximation between the cells (it is electron dense, too). The rhoptry neck protein, RON4, is thought to be a core component of the tight junction, secreted within the host cell, where it binds plasma membrane–inserted RON2, which itself interacts with micronemal secreted protein AMA1 on the surface of the invading zoite (Baum and Cowman, 2011).

zoite surface at the same rate as that at which the entire cell progressed forward (Russell and Sinden, 1981). Crucially, this motility-dependent capping was sensitive to inhibitors of filamentous actin (F-actin) turnover, such as cytochalasin, but not to microtubule inhibitors (Russell and Sinden, 1981; Stewart and Vanderberg, 1988; Bumstead and Tomley, 2000). This indicated the existence of subcortical oriented actin filaments (located in the IMC space; Fig. 1) that, when moved by myosin motors, would translocate surface ligands along the cell body to drive gliding. The concept of this motor and models for its organization were most clearly formulated first by King (1988), with a topology originally presented with myosin linked to the PPM (Fig. 2 b); however, as discussed in Gliding motor organization at the molecular level, later models reversed this.

Host cell invasion. At the same time that actin inhibitors were shaping our understanding of gliding, invasion studies with *Toxoplasma* and *Plasmodium* parasites (Ryning and Remington, 1978; Miller et al., 1979; Dobrowolski and Sibley, 1996) consolidated the view that a subcortical linear motor was also responsible for driving host cell entry (Bannister et al., 1975; Dvorak et al., 1975; Pinder et al., 1998). These works proposed that invasion proceeded via distinct steps, from loose to intimate attachment between zoite and host cell, followed by subsequent apical reorientation and then invasion. Most strikingly, it appeared that the parasite was active in driving this process, as illustrated by *Plasmodium* merozoites that were seen to literally pull the target erythrocyte when attempting invasion (Dvorak et al., 1975). This process was also found to be sensitive to cytochalasin (Miller et al., 1979), providing further support for a parasite-centric process. Parasite actin-dependent invasion was then described for *Toxoplasma* (Nguyen and Stadtsbaeder, 1979; Morisaki et al., 1995), with tachyzoites arresting on cytochalasin treatment when attempting to invade cytochalasin-resistant fibroblasts (Dobrowolski and Sibley, 1996). These findings helped to define apicomplexan host-cell entry as being distinct from the induced phagocytic-dependent entry mechanism that characterizes invasion strategies of other intracellular pathogens (Sibley, 2004).

Gliding motor organization at the molecular level

One of the most important insights to shape our molecular understanding of gliding motor topology was the identification of a complex of proteins (glideosome-associated proteins or GAPs) that localize to the IMC, where they were demonstrated to (indirectly) anchor the myosin motor inside the zoite cell (Gaskins et al., 2004). Combined with an additional study that linked surface secreted adhesins, such as MIC2, with aldolase (itself binding F-actin; Jewett and Sibley, 2003), these works helped propose a reversed molecular topology of the gliding motor (King, 1988; Opitz and Soldati, 2002), leading to the currently envisaged linear surface motor (Fig. 2 a). The role of aldolase has since been shown to be nonessential (Shen and Sibley, 2014), leaving a gap between actin and its connection with the PPM surface. It is worth stressing, however, that the assumption about topology derives largely from immunoprecipitation data linking individual interactions: MIC2/M-TRAP/TRAP with aldolase (Buscaglia et al., 2003; Jewett and Sibley, 2003; Baum et al., 2006); aldolase with actin (Jewett and Sibley, 2003); actin with myosin; myosin with its light chains (Nebl et al., 2011); and light chains with GAP proteins (Gaskins et al., 2004). Although immunoprecipitation can show direct interactions, topology prediction of (for example) MIC2 through GAP protein remains unproven.

Fundamentally, the motor itself has not been visualized in situ, in part because of the challenges involved in visualizing parasite actin microfilaments. Apicomplexan actin is markedly divergent from that of other eukaryotes (Wesseling et al., 1988). It forms very short dynamic filaments (Schmitz et al., 2005; Sahoo et al., 2006), likely specifically evolved for a nonstructural but instead dynamic motile function (Skillman et al., 2011, 2013). As such their short length and transient nature preclude their definitive visualization, even using the best current cryo–electron microscopy techniques (Kudryashev et al., 2010). Complementary studies using electron microscopy of freeze-dried replica trails from moving *Toxoplasma* tachyzoites (Wetzel et al., 2003), superresolution fluorescent microscopy on various parasite stages, and biochemical association of actin

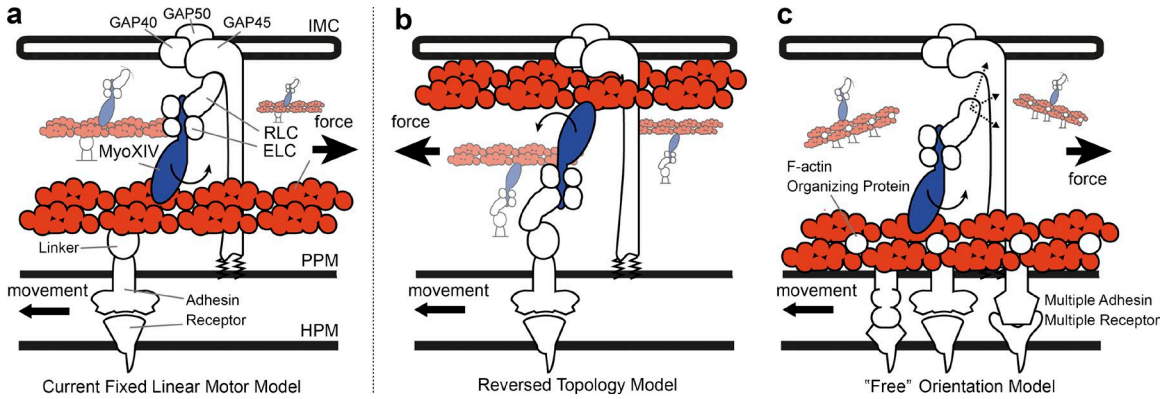

Figure 2. **Alternative models for the apicomplexan gliding motor organization.** Schematics for three alternative gliding motor models. (a) The fixed linear motor model, based on Soldati et al. (2004), the most widely accepted current model responsible for the mechanics of gliding and invasion. (b) An alternative reversed topology model from King (1988) developed before isolation of the GAP protein complex and now less widely considered. (c) A "free" motor model that the authors support, in which actin filaments are bound to the underlying PPM directly (avoiding a linking protein) and myosin motor orientation is not fixed along the anterior–posterior axis. Proteins labeled include the glideosome-associated proteins, GAP40, 45, and 50. GAP45 spans the IMC space embedded in both membranes via palmitoylation and myristoylation. In each model, myosin XIV is shown with its neck bound by essential and regulatory light chains (ELC and RLC, the latter also called MTIP in *Plasmodium* or MLC in *Toxoplasma*). F-actin is shown as a red doublet helical polymer. In model a, and with aldolase no longer believed to play a mechanical role (Shen and Sibley, 2014), an unknown linker is shown connecting actin filaments to the cytoplasmic domain of a surface-bound adhesin (e.g., a TRAP-like protein). Model b requires a reversed polarity of actin filaments so that myosin-generated motor force correlates with forward motion. In our alternative model, c, actin is arranged into filament bundles by organizing proteins, such as Coronin (Olshina et al., 2015; Bane et al., 2016). Because the direct interaction between the myosin XIV motor complex and GAP45/GAP-protein complex is unknown, this is presented with dashed arrows and question marks. This means the direction of motor force is not fixed to the anterior–posterior axis. Instead directional movement would rely on other factors such as cell shape (restricting direction of movement) or be entirely reliant on F-actin orientation (restricted to those myosin heads oriented to produce viable motor force). An additional innovation of this model is that motor force generated by myosin XIV moves a patch of membrane (F-actin bound) in which multiple adhesins are embedded. Links to the extracellular substrate are left intentionally ambiguous in light of conflicting evidence for the role played by previously implicated candidates in transmitting force (Bargieri et al., 2013; Riglar et al., 2016). HPM, host plasma membrane.

with the PPM (Angrisano et al., 2012) all certainly converge to support the presence of filamentous structures compatible with actin filaments positioned underneath the PPM. Similarly, recent colocalization of the actin-filament binding and bundling protein coronin with F-actin (Olshina et al., 2015; Bane et al., 2016) reinforce this architecture and the prevailing IMC-motor PPM-F-actin topology (Fig. 2 a). However, it is the view of the present authors that a reversed topology (Fig. 2 b) has never been formally disproven.

Is motor topology simply a choice between the anchorage of myosin to the IMC (Soldati et al., 2004) versus its anchorage to the PPM (King, 1988; Opitz and Soldati, 2002; Fig. 2, a vs. b)? One GAP protein (GAP45) clearly spans the IMC and PPM compartment (Frénal et al., 2010). It is, as yet, not determined where the myosin motor complex binds this protein, which suggests that myosin anchorage need not necessarily be restricted to either side but could be almost anywhere within the space (which is only 20–30 nm wide; Raibaud et al., 2001). Although a myosin head and actin filament need to be precisely aligned to interact, the actual directionality or organization of motor force (precisely which direction myosin pushes) similarly remain unknown beyond the net movement of many myosin heads being rearward. The architecture of the motor could, therefore, be much more loosely organized (as we suggest in Fig. 2 c), wherein individual motors push in a general rearward direction (perhaps oriented by actin filaments kept according to a particular polarity at the PPM) but not precisely guided by GAP proteins or other cytoskeletal features. Such a motor would still result in rearward motor force and zoite forward motion. Either way, testing the transduction of motor force to the extracellular milieu (e.g., as per Münter et al. [2009]) is critical for resolving precisely whether and how the glideosome is organized and thereby the mechanics of how motility is achieved.

Cell shape and surface adhesion forces in cell movement

An underappreciated area of study has been the role of shape in determining the mechanics of parasite motility. Two- and three-dimensional time-lapse imaging of zoites from *Plasmodium*, *Toxoplasma*, and *Eimeria* (Freyvogel, 1966; Russell and Sinden, 1981; Frixione et al., 1996; Håkansson et al., 1999; Harker et al., 2014; Kan et al., 2014; Leung et al., 2014) has revealed important observations. For example, *Toxoplasma* and *Plasmodium* zoites move with a largely conserved left-handed gait (Kan et al., 2014; Leung et al., 2014). This likely arises as a direct result of the chiral zoite cytoskeleton, which guides a twisted cell shape (Kudryashev et al., 2012; Battista et al., 2014; Kan et al., 2014) that can itself define the mode of movement or even the tissue to be infected, by virtue of the physical proportions of the parasite cell and target structure (Battista et al., 2014; Hopp et al., 2015). Following this train of thought, it is possible to envisage that a spiral shape might then relax the need for a precisely organized motor (approaching Fig. 2 c). In a twisted cell (like a corkscrew), motor forces exerted from the parasite surface in any direction, from perpendicular to parallel (although not toward the cell's anterior), will always result in forward rotating motion (Kan et al., 2014). Under such conditions, the motor could theoretically be loosely organized within the pellicle, able to rely on chance interactions between randomly oriented microfilaments and myosin or between other organizing factors. Under certain circumstances, parasites have been seen to move with opposing gaits (Håkansson et al., 1999), perhaps indicative of changes in the orientation of the underlying

microtubule-based cytoskeleton or changes in torsional forces in the zoite cell. Greater efforts are clearly required to connect these macro concepts of a twisted chiral shaped cell, torsional forces experienced during motion (Frixione et al., 1996), and internal motor organization.

How surface forces and retrograde flow interact to drive motility

A series of analyses with motile *Plasmodium berghei* sporozoites on elastic substrates have attempted to address the question of how forces generated internally connect with extracellular substrates and where they concentrate on the moving zoite (Münter et al., 2009). Sporozoites imaged using reflection interference contrast microscopy and traction force microscopy moved in what has been called a "stick-slip" manner, with adhesive sites first forming at the anterior, then at the posterior, followed by movement of adhesion over the sporozoite middle region. Adhesion sites at the poles showed less turnover (stick), providing stable attachment to substrates (a stall force). Mid-regions, in contrast, were associated with high turnover and slip-like (rapid) movement once the stall force at the poles was removed. Disrupting adhesion sites, via perturbing actin dynamics or removing key adhesins such as the TRAP protein, revealed that zoite adhesion turnover is critical for sporozoite movement, indicating that the regulation of gliding motility in general might have as much to do with coordinating de-adhesion as it does with adhesion (Münter et al., 2009).

To distinguish between actomyosin rearward motor force and retrograde flow (the constant movement of material, such as membrane and associated proteins, over the surface of motile *Plasmodium* sporozoites), Quadt et al. (2016) measured the rates of bead movement versus the force required to displace a surface-bound bead under different conditions. Foremost among their observations was that retrograde flow (bead movement over the surface) was faster than overall parasite speed (i.e., >1–2 µm/s; Münter et al., 2009; Quadt et al., 2016). This suggests that retrograde flow and force generation need not be the same thing—the rate of retrograde flow does not directly translate to forward migration, as is often implied. In terms of forces, normal parasites can produce 70–190 pN force, equivalent to ±20–50 myosin motor heads, depending on the force potential of class XIV myosins (currently unknown) in comparison to better-studied myosins such as those from human skeletal muscle (Finer et al., 1994). Parasites lacking a well-studied surface adhesin, TRAP-like protein (TLP) produce a much weaker force than wild-type parasites, but remarkably demonstrate retrograde flow at twice the rate (12–15 µm/s). Similar observations are seen when actin is disturbed using jasplakinolide, a compound that elongates F-actin into disordered arrays (Wetzel et al., 2003). These data suggest that TLP (and possibly other surface adhesins such as TRAP) and organized short actin filaments might act like a molecular clutch (Swaminathan and Waterman, 2016), stabilizing and slowing retrograde flow to permit force production, or disengaging to let the flow run free. What is the actual nature of retrograde flow? Either it arises by the natural secretion of membrane material (e.g., from micronemes) onto the parasite surface, sloughing off at the parasite's rear (Stewart and Vanderberg, 1988; Wetzel et al., 2003), or it is the result of free-wheeling interactions between the myosin motor and membrane-attached filaments of actin. The engagement of surface adhesins would then be expected to link motor activity to retrograde flow, slowing it down for effective force engagement. As to the role of retrograde flow in the absence of motility: like speed regulation by de-adhesion, it currently remains unclear, although resolution of its role is clearly of critical importance.

As if the complexity of these studies were not enough, surveying the history of bead studies often turns up further issues that sit less comfortably with a linear gliding motor. For example, and as exemplified by time-lapse imaging with *Plasmodium* sporozoites, surface-bound material does not always move rearward. Sometimes particles are seen to move back and forth (Münter et al., 2009), indicative of a loosely ordered motor (Fig. 2 c). How such observations fit with a simplified, linear motor, however, is often ignored in reviews on gliding.

Forces involved in invasion at the host–zoite interface

Attempts to bring similar detail to the process of invasion has also benefited from advanced microscopy techniques, such as the use of optical tweezers to study invading *Plasmodium* merozoites (Crick et al., 2014) or the intensive analysis of videos of *Toxoplasma* tachyzoites invading host cells under different conditions (Bichet et al., 2014). By using optical traps to bring *Plasmodium* merozoites to erythrocytes, Crick et al. (2014) showed that the adhesive force of a normal merozoite–erythrocyte interaction could withstand up to 40 pN of force; this adhesion force lessened when discrete populations of receptors were removed (using chymotrypsin). Despite the size of the force and its origins in receptor–ligand interactions, the adhesiveness of a merozoite did not necessarily correlate with whether it was competent for invasion (Crick et al., 2014). This suggests that adhesiveness alone (determined by the lifetime of a secreted adhesin on the surface) cannot define whether invasion occurs, leaving other interfaces, such as the junction (Bargieri et al., 2014), to determine functional entry. If the optical trap system could be used to measure the contribution to invasion from actomyosin motor forces, it could be used to test a recent biophysical estimation of the number of motors required for a merozoite to penetrate a target cell (Dasgupta et al., 2014), their orientation in the cell (along the anterior–posterior axis or circumferential), and any potential contribution from host cell forces (as discussed in the next section).

In addition to assessing the contribution of the motor, a major challenge remaining is determination of where motor force is actually applied during an invasion event. This again speaks to the need to understand motor organization and how continued force is applied at a specific point of entry/traction (in contrast to gliding, which might require traction to be applied across the surface of the zoite or in distinct but changeable areas as required). Predictions from imaging of fixed parasites midentry (Riglar et al., 2011; Angrisano et al., 2012) indicate that actomyosin force is applied consistently at, or proximal to, the tight junction, a key host–parasite interface around which invasion is organized (see Glossary). Compelling evidence for just such a traction force exerted at the junction has come from live observations of *Toxoplasma* tachyzoites which, having encountered host resistance to entry, continue to pull on the junction, effectively tracking a nascent vacuole onto themselves (Bichet et al., 2014).

These findings together suggest that to understand gliding motility and invasion, we will need to integrate the currently separate concepts of retrograde flow across the zoite surface, actomyosin motor force potential, and the engagement of motor

force by surface-bound adhesins. It appears from these results that linked adhesins play a central anchoring role in gliding, effectively slowing the (retrograde-dependent) flow of adhesion sites. This anchoring, when linked to the motor, maximizes traction forces, causing the net forward motion of the zoite. For invasion, links between the host and parasite membranes/cytoskeletons (through the tight junction) could then provide force transmission to a motile traction point (rather than to fixed adhesion site) that scans the zoite surface.

Finally, is invasion all about the parasite?

A large body of data has accumulated over the years on the parasitic components required for motility, but it is important not to overlook a potential contribution from the host cell to invasion. Several studies have investigated this role (Bargieri et al., 2013; Bichet et al., 2014), highlighting the energetic contributions that host cells might make to invasion, whether a fibroblast in *Toxoplasma,* a liver cell in *Plasmodium* sporozoites, or even an erythrocyte for *Plasmodium* merozoites (Koch and Baum, 2016). A key concept to emerge from these studies is that of membrane wrapping, wherein the wrapping forces of the host cell membrane reduce the energetic requirements for invasion, which if validated would reduce the number of myosin motor heads required for entry (Dasgupta et al., 2014). As an example, if invasive zoites can initiate host cell changes that increase membrane wrapping, such changes would account for a sizeable energetic contribution beyond that of the parasite's motor. Evidence is certainly accumulating for host cell changes that precede or correlate with invasion (Gonzalez et al., 2009; Zuccala et al., 2016). Although it is challenging to disentangle a process such as actin polymerization (or other cytoskeletal remodeling events) specifically in response to parasite entry versus its cycles of formation and deformation under normal cellular conditions, if proven to reduce the energy barrier for invasion, it might indicate that combined host–parasite energetic contributions bring about host-cell entry across apicomplexan genera (as is already well described for the apicomplexan genus *Theileria* [Shaw, 2003]).

Future challenges

Reassessing the literature on gliding motility and invasion gives rise to many new questions that need to be answered if we are to achieve a comprehensive mechanistic model of actomyosin force generation and transmission in apicomplexan zoites. We have highlighted potential areas for future research below. Many of these will be advanced by the acceleration of technologies and methods, which have flourished in the apicomplexan cellular sciences in recent years.

Imaging technologies, for example, will likely be a source of key future insights. Advances in structural cryo–electron microscopy will undoubtedly prove very powerful for exploring the architecture of the zoite pellicle, which lies at the heart of motility (Mahamid et al., 2016). Similarly, as live-imaging platforms improve in resolution and speed (Chen et al., 2014; Li et al., 2015), we will be able to explore in space and in real time the forces at play and the molecules involved during gliding. Calculating force measurements from 3D displacement of beads by traction force microscopy could yield profound insights but will require advances in computational biology to process the huge amounts of information from beads moving as a result of distinct forces (such as elastic forces in the medium, retrograde flow on the parasite surface, and parasite-derived forces from the motor). Other techniques, such as atomic force microscopy (to make precise measurements of the adhesive force of parasite surface proteins; del Rio et al., 2009), optical tweezers applied to merozoites (Crick et al., 2014), and imaging approaches in the course of real-time in vivo infection (Vlachou et al., 2004; Amino et al., 2006; Hopp et al., 2015), will each no doubt help to resolve some of the key outstanding questions concerning parasite motion.

Above all, the insights gained to date point to the need for a more integrative and less reductionist approach to understanding the motile and invasive mechanics among the Apicomplexa. The journey will be long, and we should resist the temptation to arrive too early, only to find that our understanding has taken us, unlike the parasites, in the wrong direction.

Future areas of research into the mechanics of apicomplexan gliding motility and host-cell invasion

As technological advances continue, they can be used to address unresolved questions concerning the mechanics of apicomplexan cell motility and host-cell invasion. Potential future research directions could include the following.

High-resolution imaging to resolve the organization of myosin, actin filaments, and their accessory proteins (and their respective stoichiometry) along the length of the motile apicomplexan zoite, possibly making use of advances in either superresolution microscopy (Chen et al., 2014; Li et al., 2015) or cryo–electron microscopy (Mahamid et al., 2016).

Structural understanding of how motor force is transmitted in an organized way to the extracellular milieu, together with continued molecular interrogation of the role that adhesive proteins, for example (following Quadt et al. [2016]), motor regulatory proteins (including the role of posttranslational modifications) and retrograde flow play in this process.

Detailed traction force exploration of motile zoites in three dimensions to explore how force within the parasite is exerted to the extracellular milieu, with extension to parasites within an in vivo context (e.g., Hopp et al. [2015]).

Exploration of the links between parasite and host cell at the tight junction, which permit force to be stably transmitted from the internal actomyosin motor. New technological advances need to address whether the linkages at this key interface are entirely parasite derived or whether host proteins anchor the complex as well.

Detailed dissection of the signaling pathways (e.g., host kinases or signaling networks) that are activated in host cells and that might facilitate invasion to understand how cytoskeletal reorganization (e.g., Gonzalez et al. [2009]) or host-protein phosphorylation events (e.g., Zuccala et al. [2016]) contribute to parasite entry.

Detailed assessments of host and parasite lipids during invasion to understand where membrane curvature of the host cell might contribute to invasion and to resolve the contribution of host- versus parasite-derived lipids to the nascent vacuolar membrane.

Acknowledgments

We thank L. Bannister and D. Ferguson for electron micrographs, H. Delowar for help with motor schematics, and L. Zuccala and M. Koch for critical reading of the manuscript.

J. Baum is funded through a Wellcome Trust investigator award (100993/Z/13/Z). I. Tardieux is funded by the Fondation pour la Recherche Médicale agency (FRM-DEQ20100318279) and Fondation Innovations en Infectiologie–Rhone Alpes.

The authors declare no competing financial interests.

Submitted: 26 May 2016
Accepted: 9 August 2016

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