Lytic Cycle of *Toxoplasma gondii*: 15 Years Later

Ira J. Blader,[1] Bradley I. Coleman,[2] Chun-Ti Chen,[2] and Marc-Jan Gubbels[2]

[1]Department of Microbiology and Immunology, University at Buffalo, Buffalo, New York 14127; email: iblader@buffalo.edu

[2]Department of Biology, Boston College, Chestnut Hill, Massachusetts 02467; email: bradley.coleman@bc.edu, chunti.chen@bc.edu, gubbelsj@bc.edu

Annu. Rev. Microbiol. 2015. 69:463–85

First published online as a Review in Advance on August 28, 2015

The *Annual Review of Microbiology* is online at micro.annualreviews.org

This article's doi: 10.1146/annurev-micro-091014-104100

Keywords

Apicomplexa, invasion, egress, endodyogeny, IRG, inflammasome

Abstract

Toxoplasmosis is the clinical and pathological consequence of acute infection with the obligate intracellular apicomplexan parasite *Toxoplasma gondii*. Symptoms result from tissue destruction that accompanies lytic parasite growth. This review updates current understanding of the host cell invasion, parasite replication, and eventual egress that constitute the lytic cycle, as well as the ways *T. gondii* manipulates host cells to ensure its survival. Since the publication of a previous iteration of this review 15 years ago, important advances have been made in our molecular understanding of parasite growth and mechanisms of host cell egress, and knowledge of the parasite's manipulation of the host has rapidly progressed. Here we cover molecular advances and current conceptual frameworks that include each of these topics, with an eye to what may be known 15 years from now.

Contents

INTRODUCTION 464
Toxoplasma gondii 464
Toxoplasmosis 465
HOST CELL EGRESS AND INVASION 466
Triggers and Signaling Toward Egress 466
Gliding Motility 469
Invasion 470
PARASITE REPLICATION 471
Cell Cycle Progression 471
Centrosome as Organization Hub 471
Mitosis 473
Cortical Cytoskeleton 473
Internal Budding 474
PARASITE-HOST INTERACTION (VACUOLE) 475
Immune Evasion 475
CD40 476
Host Cell Death Pathways (Apoptosis and Pyroptosis) 476
Host Cell Manipulation to Promote Replicative Niche Development 477

INTRODUCTION

A review of the *Toxoplasma gondii* lytic cycle first appeared under the same title as this review 15 years ago (17). At that time the lytic cycle was differentiated into five steps: attachment, invasion, vacuole formation, replication, and egress, with calcium signaling identified as a critical regulator. Although these basic steps have not changed, new findings facilitated by advances in technology and the improved experimental access to *Toxoplasma* have improved our understanding of their connections and details within them. First, egress is now viewed as linked to and immediately preceding invasion since many shared features have become apparent. Second, the understanding of the structure and function of the parasitophorous vacuole and its interface with the host cell has seen a tumultuous advance over the last 15 years. Finally, molecular detail on signaling pathways has permeated each step of the lytic cycle; each of the following sections discusses these pathways.

Toxoplasma gondii

IMC (inner membrane complex): the peripheral alveolar membrane cytoskeleton system defining the superphylum Alveolata, which comprises the dinoflagellates, ciliates, and Apicomplexa

Toxoplasma is a member of the Apicomplexa, a phylum comprising more than 5,000 protozoa that are almost exclusively obligate intracellular pathogens of both vertebrate and invertebrate animals. Together with the ciliates and dinoflagellates, the Apicomplexa make up the superphylum Alveolata. All alveolates share a peripheral alveolar membrane system; in the Apicomplexa it is known as the inner membrane complex (IMC). The parasitic lifestyle of the Apicomplexa contrasts with the variety of lifestyles found among the ciliates and dinoflagellates. These evolutionary distinctions suggest that the Apicomplexa evolved from free-living, photosynthetic organisms into the diverse extant obligate intracellular parasites. Within the Apicomplexa, *Toxoplasma* is a member of the cyst-forming coccidia.

Because an excellent review chronicling the discovery of *Toxoplasma* is available (43), we focus here on the key aspects of the parasite's life cycle. The asexual, or vegetative, stages of *Toxoplasma*

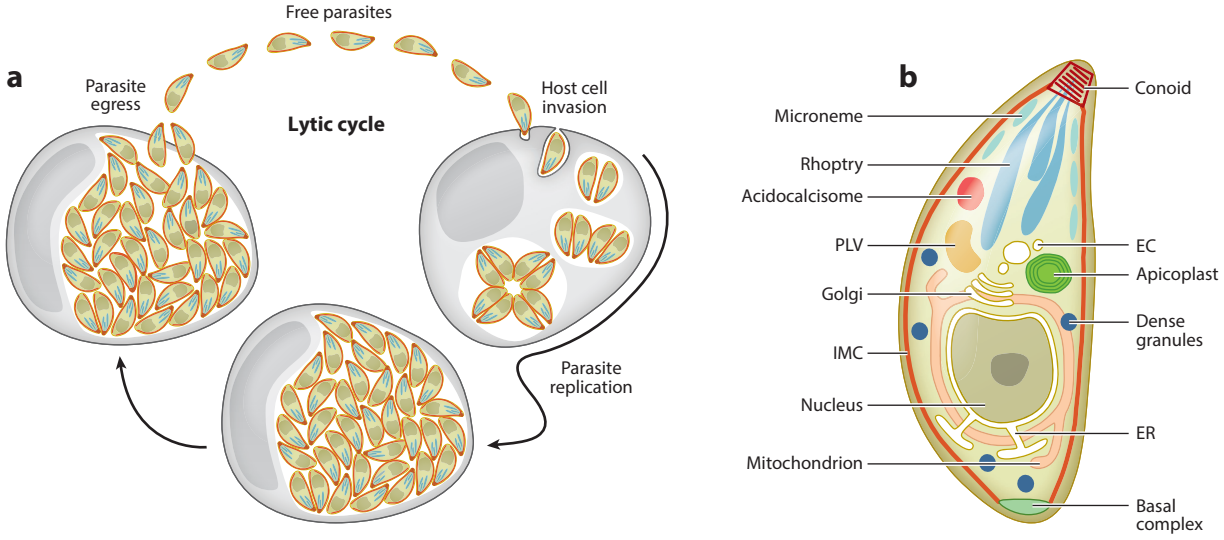

Figure 1

The *Toxoplasma* lytic cycle and basic tachyzoite organization. (*a*) The lytic cycle of invasion, replication, and egress. (*b*) Organelles in the secretory pathway are shown in white and the secretory organelles themselves in three shades of blue. Abbreviations: EC, endosome compartment; ER, endoplasmic reticulum; IMC, inner membrane complex; PLV, plant-like vacuole.

have been encountered in virtually every mammal and bird tested, whereas the coccidian, or sexual development, stages are exclusive to cats of any sort (Felids). The typical route of infection for coccidian parasites like *Toxoplasma* is through sporozoites developing within the oocysts shed in the feces of the definitive host. When an intermediate host ingests a sporulated oocyst the sporozoites will excyst in the small intestine and infect epithelial cells. Subsequently, these sporozoites will develop into tachyzoites that replicate inside the parasitophorous vacuole established upon invasion. Following replication they will actively egress from the host cell, which will be destroyed in the process. Freed tachyzoites will continue this cycle of invasion, replication, and egress—the lytic cycle (**Figure 1*a***). Tachyzoites spread throughout all tissues in the body until they are controlled by a potent host immune response that triggers the parasite to differentiate into the bradyzoite stage. Bradyzoites are the persistent stage of *Toxoplasma* infection and are maintained for the life span of the host. Consumption of bradyzoite tissue cysts from infected animals is a second route of horizontal transmission and is the route of infection for the definitive (feline) host. Within a host, bradyzoites can differentiate back into tachyzoites upon loss of a potent immune response, which is a major cause of *Toxoplasma*-associated morbidity and mortality in immunocompromised humans. Finally, unlike most coccidians, *Toxoplasma* is capable of vertical transmission during which tachyzoites cross the placenta and infect the developing fetus (congenital infection).

Toxoplasmosis

Clinical manifestations associated with *Toxoplasma* infection are very diverse and range from myocarditis, ocular toxoplasmosis (retinochoroiditis, choroiditis), encephalitis, and hydrocephalus to mental diseases (102). Although local rates vary tremendously, on a global scale one-third of the human population is infected with *Toxoplasma*. Clinical disease due to *Toxoplasma* is not common because it is predominantly the fast-replicating tachyzoite stage that is responsible for tissue

destruction resulting in clinical manifestations, and this stage is typically well controlled by the host immune response. Bradyzoite infection is latent and does not generate any clear clinical disease. Two conditions confer heightened risks for clinical disease: (*a*) immunosuppression, which typically results in bradyzoites converting back to the tachyzoite stage, and (*b*) primary infection during pregnancy, which can result in congenital infection of the fetus, where lack of a mature immune system facilitates uncurbed tachyzoite replication.

Moving junction: protein complex at the host-parasite interface of invasion; composed of rhoptry neck proteins and microneme-secreted AMA1

HOST CELL EGRESS AND INVASION

Intracellular and extracellular parasites represent two distinct biological states. Extracellular parasites do not divide, are highly motile, extrude the conoid, and secrete the contents of their microneme organelles. Intracellular parasites divide, are nonmotile, and do not secrete micronemes or extrude their conoid (see **Figure 1*b*** for identity of the organelles). The switch between states is accompanied by many dramatic changes in gene expression (54, 83), mRNA availability for translation (86), and translocation of the glycolytic enzymes from the cytoplasm to the cortex (120). Motility, and therefore secretion of motility-associated motors and adhesins from the micronemes, is required for the portion of the parasite life cycle that spans egress, movement to a new host cell, and subsequent invasion. Because of these similar requirements, egress is considered the first step in invasion (66). However, our molecular understanding of invasion and egress has begun to indicate that, although gliding and secretion are required for both processes, several aspects are specifically tailored to only either egress or invasion. Host cell calpains, for example, disassemble the host cell's cytoskeleton to forge a path out for the parasite and are only required for egress (32). The formation of the moving junction (MJ) (described in detail below) is likewise required only for invasion.

Triggers and Signaling Toward Egress

Activation toward the extracellular state can be considered the first step of the lytic cycle. The proposed triggers can be divided into those that occur in the presence of a host immune response and those that occur in the absence of a host immune response (summarized in **Figure 2*a***). Several arms of host immunity respond to *Toxoplasma* infection, and some are paths toward egress (98, 115, 142). Best understood are perforin and death-receptor (Fas/FasL)-mediated damage to the host cell following a cytotoxic $CD8^+$ T cell response (116). Damage to the host cell leads to a drop in intracellular K^+, which triggers egress (105).

In the absence of an immune response, parasites replicate to maturity and initiate egress through several different mechanisms. One such mechanism involves abscisic acid (ABA), which the parasite continuously produces during intracellular replication. When the concentration of ABA rises above a threshold (quorum sensing), the parasite egresses (108). It is unclear how ABA is sensed or how its signal is transduced, but interestingly, pharmacological interference with ABA production leads to enhanced bradyzoite differentiation, indicating ABA may play multiple roles during the parasite's life cycle.

In addition, parasite replication leads to acidification of the parasitophorous vacuole. Egress is also triggered when decreasing pH overcomes the suppression of microneme secretion and motility by K^+ ions (123). This implicates K^+ in egress, though its principal role appears to be during invasion when the parasite is in the intracellular environment, as discussed below. The pH change is especially relevant to perforin-like protein 1 (PLP1), a microneme protein (MIC protein) that inserts itself in membranes at low pH and is critical to the formation of pores in the vacuolar and host cell's plasma membranes to forge a path out of the host cell (74). The mechanisms of

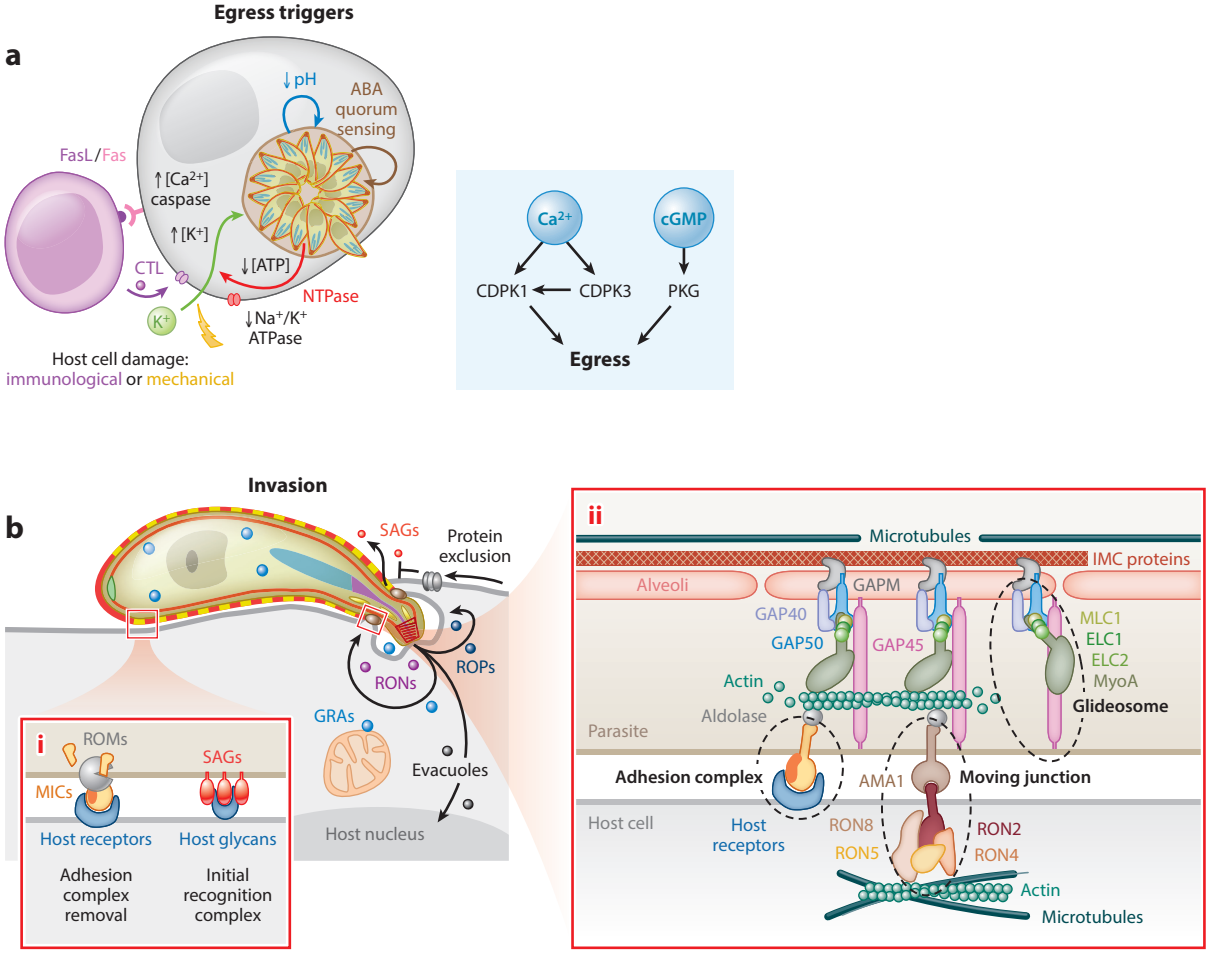

Figure 2

Activation of egress, gliding motility, and host cell invasion. (*a*) Schematic overview of described egress stimuli. The cytotoxic T cell response releases perforin, which inserts itself into the plasma membrane. Fas receptor activation induces host cell necrosis rather than apoptosis. (*Inset*) Summary of Ca^{2+} and cGMP secondary messenger signaling following triggers to egress. (*b*) Invasion gliding motility and glideosome composition (modified from Reference 15 with permission): (*i*) initial attachment and adhesion release; (*ii*) molecular composition and mechanism of adhesion, gliding motility, the glideosome, and the moving junction. The family of actin-binding proteins is not depicted. Abbreviations: ABA, abscisic acid; AMA1, apical membrane antigen 1; CDPK, Ca^{2+}-dependent protein kinase; cGMP, cyclic guanosine monophosphate; ELC, essential light chain; GAP, glideosome-associated protein; IMC, inner membrane complex; MIC, microneme protein; MLC, myosin light chain; MyoA, myosin A; PKG, protein kinase G; RON, rhoptry neck protein; ROM, rhoptry bulb protein; SAG, glycosylphosphatidylinositol (GPI)-anchored surface antigen.

vacuole acidification are currently unknown. Several parasite H^+-ATPases have been described, but their role in this process is poorly understood. The relative topologies of the pH and ABA pathways are also unknown, and they may intersect or run parallel to one another (123).

Yet another player associated with egress is a family of nucleotide triphosphate–degrading enzymes (NTPases) that the parasite secretes into the vacuole. It has been postulated that the NTPases increasingly deplete host cell ATP, leading to the Na^+/K^+-ATPase pumps running dry, a drop in K^+, and eventually egress. It is unclear, however, whether the redox state within the

vacuole activates the NTPases, and thus it remains unclear whether the NTPases act as a primary sensory pathway or are secondary effectors (134).

Egress of *T. gondii* from the host cell clearly involves various mechanisms. Individual pathways are tuned to different environmental cues, and multiple pathways may communicate and converge. Genetic and pharmacological manipulations can trigger dramatically inflated vacuole sizes that eventually physically rupture the host cell in the absence of regulated egress (e.g., 32, 46, 74). Hence, timing of egress is controlled by the parasite and is not due to a simple physical rupture of the host cell.

CDPKs (Ca^{2+}-dependent protein kinases): apicomplexan protein family that includes both a calmodulin-like Ca^{2+}-binding domain and a Ser/Thr protein kinase domain

Ca^{2+} and cGMP. The signals triggering egress are transduced by two secondary messengers: cytoplasmic Ca^{2+} and cyclic guanosine monophosphate (cGMP). The essential role of Ca^{2+} in egress and invasion is well documented (for recent reviews see 109, 119). Chelation of intracellular Ca^{2+} abrogates motility and microneme secretion, whereas increase of cytoplasmic Ca^{2+} concentration by ionophores triggers egress, enhances microneme secretion and motility, and increases invasion capacity (146). Ca^{2+} stored in the acidocalcisome and the endoplasmic reticulum can be released upon stimulation, whereas Ca^{2+} stored in the mitochondrion plays a minor role (109, 146). Pharmacological evidence indicates the presence of 1,4,5-triphosphate inositol (IP_3)-responsive channels (28) and cADPR (produced by ADP-ribosyl cyclase)-sensing, ryanodine-response channels in the endoplasmic reticulum (34), but molecular evidence for these channels is lacking in the genome (91). In addition, there is a putative role for Ca^{2+} from outside the parasite (112). The failure to bioinformatically identify these Ca^{2+} channels, which are almost certainly present, suggests that the primary structure of *T. gondii* Ca^{2+} channels deviates dramatically from the well-studied model organism channels. Despite this gap in knowledge, the identities of some other operators acting on Ca^{2+} in the parasite—such as sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) channels in the endoplasmic reticulum (107), a vacuolar Ca^{2+}-dependent ATPase pump in the acidocalcisome (92), and a Na^+/H^+ exchanger that is involved in Ca^{2+} homeostasis (8)—have been established.

Although the upstream regulators are unknown, cGMP is produced by membrane-bound guanylyl cyclases and degraded by cyclic nucleotide phosphodiesterases. The only known effector of cGMP in apicomplexans is protein kinase G (PKG). A recent study in *Plasmodium berghei* found that the production of lipids—notably, the production of phosphatidylinositol (4,5)-bisphosphate [$PI(4,5)P_2$] by PIP5K (22)—is controlled by PKG activity. In turn, $PI(4,5)P_2$ is the substrate for phosphoinositol protein lipase C (PI-PLC), which then produces the secondary messenger IP_3, triggering the release of intracellular Ca^{2+} through IP_3-responsive Ca^{2+} channels. These data identify an upstream connection between PKG and Ca^{2+} levels that likely applies to *Toxoplasma*. This extrapolation is supported by inhibition of *Toxoplasma* P-glycoprotein transporters, which results in reduced or aberrant lipid synthesis and defects in Ca^{2+} signaling, although it cannot be excluded that this effect originates in the residence of the transporters in the acidocalcisome (19). PKG activation also leads to phosphorylation of IMC proteins, glideosome (motility) proteins, and vesicular trafficking proteins, any or all of which may be important for parasite egress.

Global phosphoproteomic studies (110, 143) and specific studies of two Ca^{2+}-dependent protein kinases, CDPK1 (88) and CDPK3 (144), have generated insights into specific phosphorylation events associated with Ca^{2+} release. The global studies have identified hundreds of phosphoproteins involved in signal transduction cascades (kinases, phosphatases), the regulation of exocytosis, the cortical cytoskeleton (IMC), and the control of parasite motility, as well as many hypothetical proteins with as yet unknown functions. The identification of the IMC in both cGMP- and Ca^{2+}-dependent processes is intriguing, as this structure is the platform for motility.

CDPKs are kinases not found in the mammalian host and have therefore garnered significant interest as potential drug targets. CDPK1 is required for microneme secretion (77, 89), while

CPDK3 is involved in the K^+-mediated egress pathway (55, 90, 95). Target identification for CDPK1 did not immediately provide intuitive insights into its mechanism of action (88), whereas the CDPK3 target identification hinted at roles upstream of other Ca^{2+}-dependent signaling pathways, in ion homeostasis and metabolism (144).

Gliding motility: adhesion-dependent, actinomyosin-based motility underlying tissue migration and host cell invasion of Apicomplexa

Gliding Motility

Gliding motility is considered critical to *Toxoplasma* tachyzoite invasion of host cells. However, the role of gliding in invasion is not uniquely conserved across apicomplexan parasites, and the bigger common denominator is that the innovation of gliding permitted the crossing of biological barriers, greatly expanding host range and tissue tropism (59). Although tachyzoites demonstrate three different forms of motility on slides—circular gliding (full circles), helical gliding (half-circle followed by a flip), and twirling (parasite spins its apex while standing on its base) (63)—this translates into spiral movements in a 3D gel matrix (84). The concept of apicomplexan gliding motility is based on the following principles: (*a*) microneme secretion of adhesion molecules on the apical end and their insertion into the parasite's plasma membrane; (*b*) anchoring of the adhesion molecules on the cytoplasmic side to short actin filaments; (*c*) apical-to-basal translocation of actin filaments by a myosin motor; (*d*) anchoring of the myosin motor in the IMC membrane; and (*e*) release of MIC proteins on the basal end by intramembrane cutting rhomboid proteases [**Figure 2*b(i)***] (References 96 and 129 are recent reviews).

Microneme secretion and microneme proteins. Micronemes are located on the apical end of the parasite (**Figure 1**) and are secreted in a Ca^{2+}-dependent fashion. Although an attractive model was that the micronemes merge with the rhoptry neck to purge their contents into the outside milieu, genetic ablation of rhoptries does not impair microneme secretion, suggesting a direct fusion with the plasma membrane (11). Germane to motility, several microneme protein complexes recognize a variety of substrates—notably, glycosaminoglycans present in the extracellular matrix and on the surface of host cells (for a recent review see 29).

The glideosome. The engine powering gliding motility is myosin A (MyoA) (97), a class XIVa myosin contained in a protein complex known as the glideosome [**Figure 2*b(ii)***]. MyoA is in complex with glideosome-associated proteins 45 and 50 (GAP45, GAP50) (71). GAP50 is the main anchor in the IMC, whereas GAP45 spans the space between the plasma membrane and the IMC outer membrane through lipid modifications anchoring it in both membranes (52), which likely secures the distance between the two membranes so that the motor can reach the actin filaments associated with the MIC proteins. Furthermore, the glideosome contains a myosin light chain (MLC1) and two essential light chains (ELC1, ELC2) that regulate the activity of the motor: The MLC1 phosphorylation and Ca^{2+} binding by the ELCs change their conformations and modulate MyoA activity (110, 140). In addition, the phosphorylation state of MyoA itself is critical (140), underscoring the multiple levels of Ca^{2+}-mediated control that prevent premature motor activation, thereby curbing premature egress.

Actin polymerization. The spatiotemporal control of actin polymerization is critical for activation of motility. Compared to mammals, Apicomplexa encode a limited set of a dozen actin-interacting proteins (9). The cytoplasmic concentration of G-actin is relatively high (8–10 μM), whereas F-actin is not readily detectable (40). Actin only polymerizes in very short (100 nm), unstable filaments (128, 132). F-actin in normal parasites is observed in cables or bundles restricted to areas where there is contact between the parasite and host cell or substrate (147). Branched

networks are not observed, which is consistent with the genetic absence of ARP2/3, required to form actin branches in other systems. These observations fit a model wherein filaments are only formed transiently and where the spatiotemporal dynamics of filament formation likely controls the proper directionality and timing of motility. A key question relevant to the Apicomplexa is how the actin filaments are connected with the cytoplasmic domain of the MIC adhesion protein complexes. For several years the glycolytic enzyme aldolase was a strong candidate, but recently, genetic deletion of aldolase was demonstrated not to block invasion, disproving this model (131). Therefore, the connection of actin to the adhesive proteins secreted from the micronemes is still an open question.

Rhomboid proteases. Transportation of MIC protein complexes to the basal end would lead to a pileup, unless the complexes were released from the parasite. This release is facilitated by several rhomboid proteases localized at the basal end of the parasite that have the ability to cleave transmembrane domains inside the membrane-spanning sequence (24, 42). Hence, when gliding, the parasites leave behind trails of MIC proteins.

Invasion

Following egress, tachyzoites glide around in the extracellular environment. Compared with the time spent intracellularly this condition is of relatively short duration, but it provides the parasite opportunities to disseminate to new tissues.

Attachment. The first contact of the parasite with a potentially new host cell is through its surface protein coat. The parasite is covered in glycosylphosphatidylinositol (GPI)-anchored surface antigens known as SAGs. The SAG1 structure recognizes sulfated proteoglycans on the host cell (65). This model is supported by the inhibition of host cell attachment in cells with a reduced amount of SAG1 on their surface (16), or upon addition of exogenous glycans (27).

Moving junction. Following initial recognition, the parasite is triggered to engage in a tighter interaction. This is mediated by the secreted MIC proteins and subsequently by the formation of the MJ (73). The MJ is a cooperative structure between the microneme protein apical membrane antigen 1 (AMA1) and proteins residing in the rhoptry neck (RON proteins). RON proteins are secreted before the proteins residing in the rhoptry bulb (ROPs) (3). The presence of rhoptry proteins in the host cell's cytoplasm implies that a breach in the host's plasma membrane is made (62), but the molecular details of this breach are as yet unclear. In contrast to the Ca^{2+}-based regulation of micronemes, the signals for rhoptry release remain obscure. It has been shown that AMA1 (99), MIC8 (76), and RON5 (10) are required for rhoptry release, but the underlying mechanisms are unknown. In the MJ, AMA1 is anchored in the parasite's plasma membrane and interacts with the RON complex anchored in the host cell's plasma membrane [**Figure 2*b(ii)***]. Direct interaction between AMA1 and the transmembrane domain protein RON2 has been resolved at the molecular level. In addition, RON8 mediates the contact of the complex with the host cytoskeleton and is required for proper interaction (135), whereas RON4 and RON5 appear to have a more structural function in organizing the architecture of the complex (15).

Entry. After establishing the MJ, the parasite lunges forward, thereby invaginating the host cell plasma membrane, which becomes the parasitophorous vacuole membrane (PVM). Interestingly, the MJ functions as a sieve for proteins in the host cell's plasma membrane, excluding all transmembrane proteins and proteins in lipid rafts (102). In effect, this results in a vacuolar compartment

lacking receptors for the intracellular host cell machinery and yields a nonfusogenic membrane that is refractory to fusion with the host cell membrane trafficking machinery.

Motility. One of the unanswered questions surrounding host cell invasion is whether motility is a strict requirement. It has been shown that many of the glideosome components are required for efficient invasion but that a fraction of the parasites are still able to invade and develop normally. This suggests a potential alternative mechanism powering the entry of the host cell (44); plasticity between different myosins has also been demonstrated (51). Therefore, the mechanism of host cell invasion is currently a dynamic area of research.

Endodyogeny: cell division through internal budding of two daughter parasites within the confinement of the parent parasite

ApiAP2 (apicomplexan apetala 2): family of transcription factors unique to the Apicomplexa; each contains 1–4 AP2 DNA-binding domains

PARASITE REPLICATION

The parasite employs a unique mode of cell division known as endodyogeny. Endodyogeny unfolds through the formation of two daughter cells within the boundaries of a mature parent parasite that is consumed at the end of the process (49, 130, 136) (**Figure 3**). As discussed in detail below, *Toxoplasma*'s cell division process differs at several levels from how its mammalian host divides.

Cell Cycle Progression

The tachyzoite has a haploid genome (1N). During S phase the DNA is replicated, but duplication pauses at an ~1.8N DNA content, at which time the mitotic spindle starts to appear. Therefore, a true G2 phase, intervening between the S phase and mitosis, is missing in these parasites; however, the slowdown in the S phase upon reaching 1.8N might be the functional equivalent. Another distinct feature is that daughter budding, and as such cytokinesis, starts when 1.8N DNA content is reached.

The molecular machinery underlying cell cycle progression is, as in other systems, based on cyclins and cyclin-dependent kinases (Cdks). Although cyclins and Cdks have been identified in *Toxoplasma*, it has not been established which pairs control which step of the cell cycle (81). Cell cycle progression is characterized by two different subtranscriptomes: one with a G1 signature (biosynthetic and metabolic genes turned on), and one with an S/M signature (DNA replication, cytoskeleton, and invasion organelle genes turned on). In fact, one-third of genes are expressed in bimodal patterns throughout the cell cycle, controlled by a unique family of ApiAP2 transcription factors, many of which are active in sequential waves themselves (14).

The genome harbors candidates for the spindle assembly checkpoint controlled by the anaphase-promoting complex (APC/C), but these have not been studied in detail. In summary, we have identified the major cell cycle progression regulators, but the molecular mechanism is still largely undefined.

Centrosome as Organization Hub

Toxoplasma cell division has been approached by forward genetics (60). This has drawn the attention to a pivotal role for the centrosome in coordinating the mitotic, karyokinetic, and cytokinetic events (33, 47, 48, 138). The centrosome resides in cytoplasm and is always in close apposition to the spindle pole embedded in the nuclear envelope. Late in G1, the centrosome migrates to the basal end of the nucleus and divides (64). At this point the microtubules for the spindle are assembled, nucleating from the centrosome. Following migration back to the apical side, the spindle is established to facilitate mitosis, and the first daughter cytoskeleton elements start to accumulate on the duplicated centrosomes.

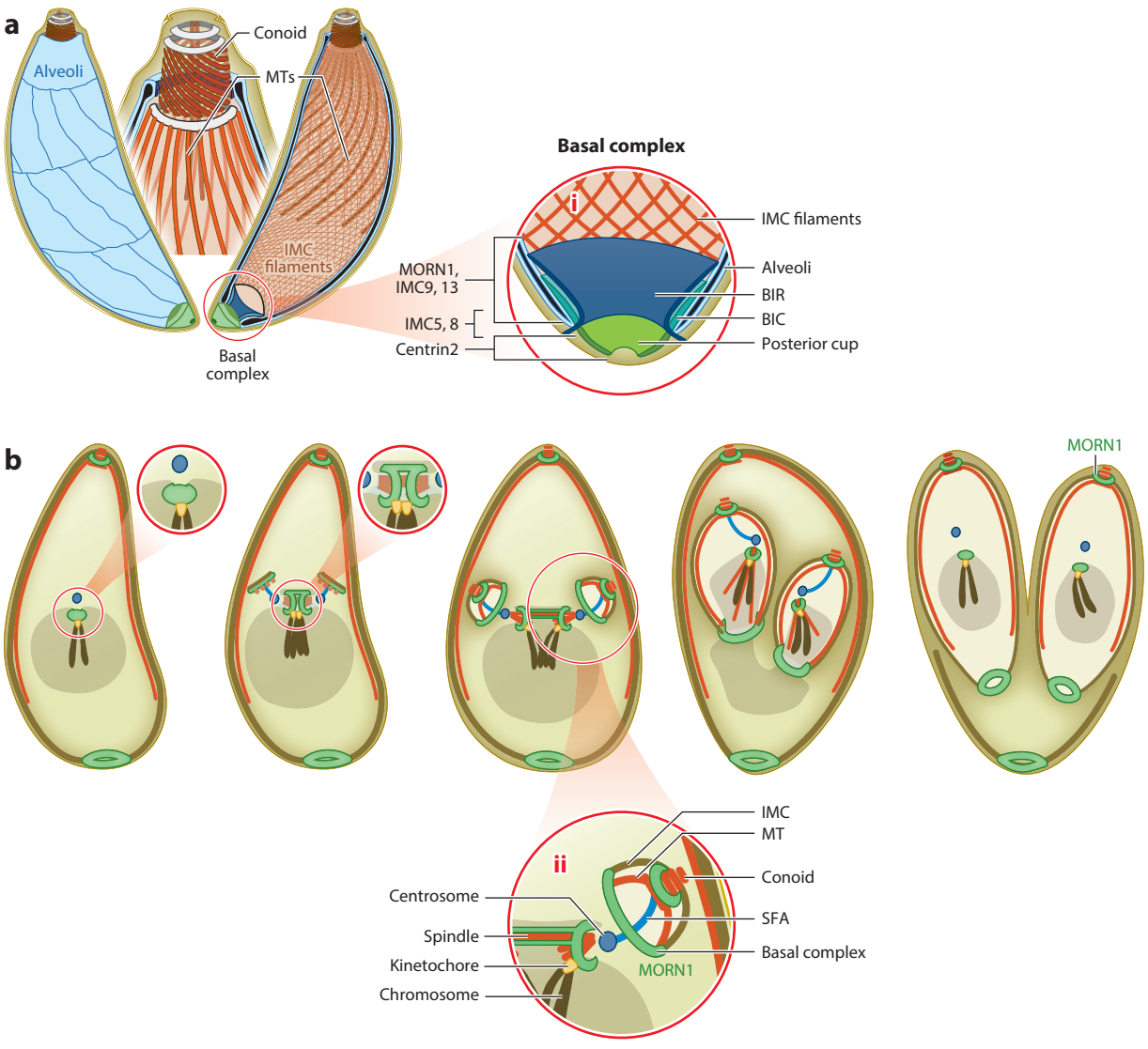

Figure 3

The cortical cytoskeleton and cell division. (*a*) A patchwork of alveoli (flattened vesicles) underlies the plasma membrane (*left*). The alveoli are supported by a meshwork of intermediate filament–like IMC proteins (*right*). At the apical end (*enlarged in center*), the conoid composed of microtubules is an extrudable basket, whereas 22 cortical, subpellicular microtubules emanate from the apical end to about two-thirds the length of the parasite. At the basal end, the cytoskeleton is capped by the basal complex and posterior cup, as magnified in inset *i*. (*b*) Concurrent mitosis and cytokinesis are coordinated by the centrosome. Mitosis is closed without chromosome condensation, whereas the spindle resides eccentrically at the apical side of the nucleus. Throughout the cell cycle the 14 chromosomes are clustered at the centromeres and anchored at the spindle pole (centrocone) by the kinetochore. Daughter bud assembly starts before completion of mitosis and is driven by assembly of the cortical cytoskeleton in an apex-to-base direction. The nucleus is anchored in the daughter scaffolds through the centrosome and SFA fiber. Constriction of the basal complex drives tapering of the daughter bud toward the basal end and is required to separate the daughter parasites. Inset *ii* magnifies the interaction between nucleus, centrosome, and daughter cytoskeleton. Abbreviations: BIC, basal inner complex; BIR, basal inner ring; IMC, inner membrane complex; MORN1, membrane occupation and recognition nexus protein 1; MT, microtubule; SFA, striated fiber assemblin. Panel *b* adapted from Reference 61 with permission.

At the heart of the *Toxoplasma* centrosome resides a pair of atypical parallel centrioles (104). Only recently have researchers begun to elucidate the protein composition in detail, by identification of orthologs found in higher eukaryote centrosomes (138). The striking observation was that the *Toxoplasma* centrosome comprises two functionally distinct regions: a proximal region that appears to organize mitosis and a distant region that is associated with initiating cell division. This functional separation of the centrosome's role in mitosis from its role in cytokinesis permits the uncoupling of the mitotic cycle from the cell division cycle, which is used during merogony in the cat gut, where not every mitosis is followed by daughter budding.

The centrosomal cycle is coordinated by a variety of kinases. Although the genome lacks the typical Polo-like kinases, various Aurora-like and NIMA-related kinases are present. The NIMA-related kinase Nek1 controls centrosome splitting, whereas the three Ark kinases are associated with various aspects of the centrosome—though their exact role has not yet been pinpointed (138; C.-T. Chen, M.-J. Gubbels, C. Doerig, W. Daher, manuscript in preparation). Furthermore, two kinases not associated with mammalian centrosome biology have been associated with the *Toxoplasma* centrosome. The first is a Ca^{2+}-dependent kinase, CDPK7, required for centrosomal positioning and integrity (103). The second is a MAPK (mitogen-activated protein kinase)-like protein, MAPK-L1 (25), which in mammals works in the signal transduction cascade but in *Toxoplasma* is required for anchoring the daughter bud to the centrosome (138).

Mitosis

The *Toxoplasma* genome is maintained in 14 chromosomes totaling 65 Mb. Mitosis is closed, meaning that segregation of duplicated chromosomes occurs within the confines of the nuclear envelope (130). The spindle pole is embedded in the nuclear envelope in a structure known as the centrocone, which appears to be a modified or specialized nuclear pore (M.E. Francia & B. Striepen, personal communication) (**Figure 3*b***). The centrocone, but not the spindle pole microtubules, is retained throughout the cell cycle. As in other eukaryotes, the chromosomes attach to the spindle microtubules via the kinetochore protein complex assembled on the centromere (47). The centromeres are always clustered together and anchored to the centrocone (23), which in interphase is independent of the presence of microtubules (47). The reason for this persistent clustering is not well understood, but it may be related to maintaining genome integrity.

Cortical Cytoskeleton

In the last 15 years much progress has been made in our understanding of the cortical cytoskeleton, which drives the assembly of the daughter parasites (Reference 4 is a detailed, recent review). The cortical *Toxoplasma* cytoskeleton is a complex, layered structure comprising an outer plasma membrane and an underlying IMC (130). The IMC is composed of a double membrane system assembled from a series of alveolar vesicles (**Figure 3*a***). On the cytoplasmic side a family of IMC subcompartment proteins (ISPs) differentiates the various alveolar subcompartments (12). In addition, a 10-nm filamentous network of intermediate filament–like IMC proteins lines the cytoplasmic side of the IMC membranes and endows shape to the parasite (5, 94). The IMC rests on a final cytoskeletal layer composed of 22 subpellicular microtubules emanating from the apical end of the parasite and ending at two-thirds the length of the parasite. Specialized cytoskeletal structures are present at the extreme anterior and posterior ends of the parasite, which are known as the apical complex and basal complex, respectively. At the apical end the IMC is connected to a tubulin basket known as the conoid, which is extruded out of the parasite upon egress in a Ca^{2+}-dependent fashion. The conoid is a complex structure bound by several apical rings (68, 75).

TOXOPLASMA CELL DIVISION BY INTERNAL BUDDING IS RELATED TO THE FLAGELLA-FORMING MACHINERY

The composition of the division apparatus of *Toxoplasma* suggests it is mechanistically related to the flagellar machinery (see 49 for a recent review). Although tachyzoites do not carry flagella, the male microgametes have two; the most likely ancestor also carries two flagella (49). Indeed, assembly of the tubulin-containing conoid follows recruitment of the striated fiber assemblin (SFA) fiber, which is also present in algal flagellates such as *Chlamydomonas* (48). Furthermore, the conoid harbors additional evidence that the cytoskeleton is derived from the flagellar apparatus in the presence of a SAS-6-like protein residing at its bases, next to a normal SAS6 in the centrosome; SAS6 proteins function in microtubule organization (39). Finally, a centrin molecule, TgCentrin2 (67), and a myosin VI (TgMyosinJ) (K. Engelberg and M.-J. Gubbels, unpublished data) are present at both the apicomplexan basal complex and the flagellar transition zone.

At the basal end of the IMC lies the basal complex, a multilayered structure containing MORN1 and a set of dedicated IMC proteins (4, 61, 87). At the extreme basal end of the parasite lies the posterior cup, which is composed of Centrin2 (67, 68, 94). As discussed below, the basal complex functions as the contractile ring in completing cell division.

Internal Budding

The division cycle starts with duplication of the Golgi apparatus, followed by centrosome duplication (64). Budding of daughter cells is driven by assembly of the cortical cytoskeleton, which forms the scaffold in which all subsequently formed or divided organelles will be anchored (49, 111). Several aspects of budding are shared with the machinery assembling flagella, suggesting shared ancestry (see sidebar, *Toxoplasma* Cell Division by Internal Budding Is Related to the Flagella-Forming Machinery). The first daughter elements recruited to the (outer) centrosome are IMC15 and Rab11B (5, 138); the latter trafficks Golgi-derived vesicles into the daughter alveoli (1). In addition, striated fiber assemblin (SFA) proteins are recruited to form an SFA fiber that anchors the centrosome in the developing cytoskeleton at the base of the conoid [**Figure 3*b*(*ii*)**] (48). The next critical cytoskeleton elements to be incorporated are MORN1 and the ISPs. MORN1 is associated with the early alveolar vesicles and transitions to the apical and basal complexes (61, 67), whereas ISP2 is essential for early IMC development (12). From this time on, the cytoskeleton grows from the basal end (the basal complex) by deposition of various IMC proteins and growth of subpellicular microtubules into a widening cone. Coinciding with the completion of karyokinesis, which also concludes division of the endoplasmic reticulum, the subpellicular microtubules stop growing when they reach two-thirds the ultimate length of the daughter cell.

Halfway through the assembly of the daughter scaffold several notable changes occur. A subset of IMC proteins initially assembled in the daughter scaffold transition to the basal complex, where, at the same time, Centrin2 is recruited. Upon continuing growth of the bud the basal complex starts to contract, resulting in tapering of the parasite toward the basal end (67). Because constriction is independent of actin/myosin in the parasite (in contrast to mammalian cells), it has been postulated that Centrin2, which can form contractile fibers, drives constriction (67).

At this point, the two daughter cells are trapped within the parent cell and further growth is facilitated by the organized disassembly of the parent cell's cytoskeleton (**Figure 3*b***). Disassembly progresses in the direction of apex to base. Where the parent cell's IMC retracts, the plasma membrane is zippered onto the IMC by the deposition of GAP45 in a Rab11a-dependent process

(2, 52, 104). Interestingly, the basal complex does not completely separate the parasites, and a cytoplasmic bridge remains.

The apical secretory organelles (micronemes and rhoptries) assemble de novo in the daughter buds (111) in a process requiring the alveolate-specific, dynamin-related protein DrpB (21). Protein trafficking during assembly to the secretory organelles, apicoplast, and IMC proceeds through the secretory pathway using a repurposed endosomal system to cover the wide variety of destinations (141). Vesicular targeting is mediated by the Rab family of GTPases (80). However, a specific Rab could not be identified for each organelle, suggesting additional sorting mechanisms. An extended family of palmitoyltransferases could potentially function here, whereas organelle-specific palmitoyltransferases do also partake in organelle-specific protein targeting (53).

The apicoplast divides by association with the centrosome, and its scission concludes by the action of another dynamin-related protein, DrpA (145). Just before basal complex constriction concludes, one mitochondrion enters each daughter cell (111). Taken together, the timing of daughter budding with respect to the cell cycle and the intimate relationship between daughter budding and mitosis are the hallmark of tachyzoite cell division.

IRGs (IFN-γ-regulated GTPases): enzymes able to sense and destroy the intracellular parasite in mice

PARASITE-HOST INTERACTION (VACUOLE)

When the previous review of the *T. gondii* lytic cycle was published, 15 years ago, few details regarding the role of the host cell were known (17). We now have a better (yet still incomplete) understanding of how the host cell contributes to the parasite's life cycle. The preceding sections highlight how the host cell contributes to invasion and egress. Below, we discuss how the parasite manipulates host cell processes to evade host cell defenses and to facilitate its replication.

Immune Evasion

The interplay between the host immune response and parasite immune evasion strategies is critical in balancing host survival with parasite survival. To this end the parasite interferes at various levels with the host immune response.

Host cell autophagic degradation of the parasitophorous vacuole. Innate and adaptive immune responses are required for resistance to *Toxoplasma*, and IFN-γ is critical for both (139). IFN-γ kills *Toxoplasma* by upregulating expression of antiparasitic genes that utilize a variety of mechanisms to kill the parasite. These include autophagy-dependent degradation of the PVM by the p47 family of IFN-γ-regulated GTPases (IRGs) (36) and degradation of essential nutrients, such as tryptophan by indoleamine dioxygenase (117). *Toxoplasma* has developed two primary ways of evading these IFN-γ effectors.

First, *Toxoplasma* secretes proteins into the host cell cytoplasm that directly inactivate some effectors. The best-studied example of this mechanism is the inactivation of the IFN-γ-inducible GTPases by ROP, ROP1, and ROP17. Interestingly, ROP5, ROP18, and ROP17 are polymorphic between parasite strains and ROP5 is the major virulence factor in mice (13, 122). Given that higher primates do not express functional IRGs, it is tempting to speculate that *Toxoplasma* polymorphic virulence factors coevolved with a specific clade of host species to ensure parasite survival (69).

Toxoplasma also avoids IFN-γ-dependent killing by interfering with IFN-γ-regulated gene expression (79). IFN-γ activates gene expression by stimulating phosphorylation of the STAT1 transcription factor, which allows STAT1 to translocate to the nucleus and bind DNA (121). *Toxoplasma* does not inhibit STAT1 phosphorylation or nuclear localization. Rather, it blocks STAT1 from binding to the promoters of its target genes by disrupting chromatin remodeling

and by preventing its recycling between promoters (82, 126). The STAT1-inhibiting parasite factor or factors are unknown, but given that all known *Toxoplasma* strains inhibit STAT1, this factor is likely nonpolymorphic (79).

Inflammasome: multiprotein complex that detects microbe-derived factors and promotes IL-1β and IL-18 synthesis and secretion and pyroptotic host cell death

NLRPs [nucleotide-binding domain and leucine-rich repeat (NLR)-containing pyrin domains]: family of proteins that function in the inflammasome

CD40

The IRGs are not the only proteins that can degrade the PV via autophagy. CD40 is a ubiquitously expressed member of the TNF receptor superfamily, and genetic disruption of this gene as well as its ligand, CD154, decreases murine resistance to *Toxoplasma* (7). Upon CD154 binding, CD40 kills *Toxoplasma* by inducing accumulation of autophagy-associated proteins on the PV surface, and this is followed by degradation of the PV and the parasite (6). But *Toxoplasma* can evade CD40-triggered autophagy by activating epidermal growth factor receptor signaling, thereby preventing autophagosome formation (106).

Host Cell Death Pathways (Apoptosis and Pyroptosis)

Killing of an infected host cell is an important innate defense strategy, and inflammasome-dependent pyroptosis has emerged as a cell death pathway critical for resistance (30, 35). The inflammasome is a multiprotein complex that assembles after a sensor protein binds a microbe-derived molecule. The complex then activates caspase 1, whose activity leads to IL-1β and IL-18 secretion and triggers formation of large pores in the plasma membrane, resulting in cell lysis and death. The inflammasome was demonstrated to be important for resistance to *Toxoplasma*: Polymorphisms in human and rat genes for the inflammasome sensor NLRP1 correlated with resistance to *Toxoplasma* (31, 149).

Although humans and rats use the inflammasome to control parasite growth, they do so by different mechanisms. In rats, inflammasome activation leads to rapid death of the infected host cells (30). Infected human host cells, however, do not die, suggesting a role for IL-1β/IL-18 signaling or a novel mechanism (58). Another key difference between humans and rats is that inflammasome activation in humans requires GRA15, which is a dense granule protein secreted into the host cell that regulates NF-κB (59). In mice, *Toxoplasma* also activates the inflammasome via both NLRP1 and NLRP3; and as with human cells, mouse cells do not undergo pyroptosis following infection (45, 57).

Three critical questions remain regarding the interaction between *Toxoplasma* and the host inflammasome. First, how do polymorphisms in NLRP1 alter resistance to *Toxoplasma*? Second, which parasite factors are sensed by the inflammasome? Third, why does inflammasome activation fail to activate pyroptosis in human and murine cells, and what impact does this have on virulence? One possibility is that after caspase 1 is activated, a rhoptry or dense granule factor is secreted into the host cell and this factor prevents pyroptosis but has no effect on cytokine synthesis.

Apoptosis is a second cell death pathway that is activated by intrinsic (e.g., mitochondrial damage) and extrinsic (e.g., Fas ligand) stimuli that culminate in activation of caspase 3. *Toxoplasma* inhibits caspase 3 activation as well as several upstream caspases that regulate either the intrinsic or extrinsic pathways (56). It has been proposed that *Toxoplasma* inhibits host cell apoptosis via increased expression of antiapoptotic proteins, activation of cell survival signaling pathways, and inhibition of proapoptotic proteins (26, 78, 100, 113). But unlike pyroptosis in humans and rodents, which genetic studies have linked to susceptibility to *Toxoplasma* infection, apoptosis has not been linked to toxoplasmosis. The importance of host cell apoptosis in host resistance remains to be established.

Host Cell Manipulation to Promote Replicative Niche Development

To obtain the resources it needs to grow and replicate, the parasite manipulates and redirects pathways in the host cell in various ways.

Membrane trafficking. During parasite invasion, the PV is formed using lipids derived from the host plasma membrane and most GPI-linked and peripheral plasma membrane proteins but not transmembrane proteins (102, 137). These data supported a model that the PV was in a nonfusogenic state due to its ability to avoid host endosomes and lysosomes. This was challenged when the parasite was shown to scavenge lipids such as cholesterol and sphingolipids by redirecting host endolysosomal- and Golgi-derived vesicles to the PV (37, 124). How these lipids cross the PV and are taken up by the parasite is unknown. A dense granule protein, GRA7, may function in the former process (38), whereas receptor-mediated or bulk flow endocytic pathways may be used for the latter (41).

It has long been recognized that large host organelles such as mitochondria and endoplasmic reticulum are closely apposed to the PV, in contrast to endolysosomal trafficking (72). How host endoplasmic reticulum is recruited to the PV remains unknown. Host mitochondrial recruitment was recently discovered to only occur in cells infected with type I parasites; this is due to a dense granule protein, MAF1, that is secreted into the host cytosol and appears to modulate host cytokine expression (114). Important questions regarding MAF1 remain, including how MAF1 (or any dense granule or rhoptry protein) enters the host cell and how it recruits mitochondria.

Transcription and signaling. Early transcriptomic studies revealed that regardless of the host cell type, infection induces large-scale changes to host cell transcription, which likely leads to global changes in the cell's physiology (18). Not surprisingly, immune response genes (e.g., those encoding chemokines and cytokines) are one clade of these genes. The others, including those involved in metabolism, cell growth, and signaling, provide a glimpse at host cell processes needed to support parasite growth. As an example, host mevalonate metabolic genes are upregulated, most likely due to parasite scavenging of host isoprenoids (85).

The large numbers of host cell genes modulated by infection also suggested that infection modulated the activity of a diverse array of host cell transcription factors. Some of these (NF-κB, STAT3, STAT6, NFAT4, and STAT5) are regulated by polymorphic factors and appear to be important not for parasite replication but rather for shaping the resulting immune response (70, 93, 125, 127). However, other host cell transcription factors are activated [including hypoxia-inducible factor 1 (HIF-1), c-myc, and early growth response factor (EGR)], apparently in a nonpolymorphic manner (118, 133, 148). The mechanisms by which these factors are activated appear to be diverse. EGR is dependent on p38 MAPK activation, and this is mediated by GRA24, which is a dense granule protein that is injected into the host cell (20). c-myc is activated by host JNK MAPK signaling, although it remains to be determined how *Toxoplasma* activates this kinase (50). Finally, HIF-1 is activated by signaling through members of the host serine/threonine kinase receptor family, ALK4,5,7, although the parasite factor that activates ALK4,5,7 is also unknown (148). Of these host transcription factors, parasite growth is dependent on only HIF-1, although HIF-1 is primarily required at physiological oxygen levels (133).

SUMMARY POINTS

1. The lytic cycle is associated with the most severe pathology.
2. The intracellular and extracellular parasites represent two distinct biological states.

3. Ca^{2+} and cGMP are secondary messengers in transition between the two states.
4. Internal budding and mitosis are coordinated by the centrosome.
5. Parasite division concludes by constriction of the basal complex.
6. A diverse array of proteins secreted by the parasite (in some cases tailored to the specific mammalian host) manipulate the host cell's physiology and the immune response at numerous levels.

FUTURE ISSUES

1. How are the triggers for egress sensed by the parasite, and how are these signals transduced to release Ca^{2+} and produce cGMP?
2. How is rhoptry secretion triggered, and how are the contents released in the host cell's cytoplasm?
3. What controls and mediates the specific disassembly of the parasite parent cell structures in the late stages of cell division?
4. What is the contractile mechanism driving basal complex constriction?
5. How is the wide variety in host tropism covered by the parasite on the level of specific parasite-host interaction following invasion?
6. Which parasite factors are sensed by the inflammasome sensor and which modulate the inflammasome?
7. How does *Toxoplasma* regulate host membrane trafficking?
8. Which host proteins are required for parasite growth?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Work in the Gubbels lab is sponsored by National Institutes of Health (NIH) grants R01AI081924, R03AI107475, R21AI099658, and F32AI108251 (to B.I.C.), and American Cancer Society grant RSG-12-175-01-MPC. I.J.B. is supported by NIH grants R01AI069986 and R21AI107257.

LITERATURE CITED

1. Agop-Nersesian C, Egarter S, Langsley G, Foth BJ, Ferguson DJ, Meissner M. 2010. Biogenesis of the inner membrane complex is dependent on vesicular transport by the alveolate specific GTPase Rab11B. *PLOS Pathog.* 6:e1001029
2. Agop-Nersesian C, Naissant B, Ben Rached F, Rauch M, Kretzschmar A, et al. 2009. Rab11A-controlled assembly of the inner membrane complex is required for completion of apicomplexan cytokinesis. *PLOS Pathog.* 5:e1000270

3. Alexander DL, Mital J, Ward GE, Bradley P, Boothroyd JC. 2005. Identification of the moving junction complex of *Toxoplasma gondii*: a collaboration between distinct secretory organelles. *PLOS Pathog.* 1:e17
4. Anderson-White BR, Beck JR, Chen CT, Meissner M, Bradley PJ, Gubbels MJ. 2012. Cytoskeleton assembly in *Toxoplasma gondii* cell division. *Int. Rev. Cell Mol. Biol.* 298:1–31
5. Anderson-White BR, Ivey FD, Cheng K, Szatanek T, Lorestani A, et al. 2011. A family of intermediate filament-like proteins is sequentially assembled into the cytoskeleton of *Toxoplasma gondii*. *Cell Microbiol.* 13:18–31
6. Andrade RM, Wessendarp M, Gubbels MJ, Striepen B, Subauste CS. 2006. CD40 induces macrophage anti-*Toxoplasma gondii* activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. *J. Clin. Investig.* 116:2366–77
7. Andrade RM, Wessendarp M, Subauste CS. 2003. CD154 activates macrophage antimicrobial activity in the absence of IFN-gamma through a TNF-alpha-dependent mechanism. *J. Immunol.* 171:6750–56
8. Arrizabalaga G, Ruiz F, Moreno S, Boothroyd JC. 2004. Ionophore-resistant mutant of *Toxoplasma gondii* reveals involvement of a sodium/hydrogen exchanger in calcium regulation. *J. Cell Biol.* 165:653–62
9. Baum J, Papenfuss AT, Baum B, Speed TP, Cowman AF. 2006. Regulation of apicomplexan actin-based motility. *Nat. Rev. Microbiol.* 4:621–28
10. Beck JR, Chen AL, Kim EW, Bradley PJ. 2014. RON5 is critical for organization and function of the *Toxoplasma* moving junction complex. *PLOS Pathog.* 10:e1004025
11. Beck JR, Fung C, Straub KW, Coppens I, Vashisht AA, et al. 2013. A *Toxoplasma* palmitoyl acyl transferase and the palmitoylated Armadillo Repeat protein TgARO govern apical rhoptry tethering and reveal a critical role for the rhoptries in host cell invasion but not egress. *PLOS Pathog.* 9:e1003162
12. Beck JR, Rodriguez-Fernandez IA, Cruz de Leon J, Huynh MH, Carruthers VB, et al. 2010. A novel family of *Toxoplasma* IMC proteins displays a hierarchical organization and functions in coordinating parasite division. *PLOS Pathog.* 6:e1001094
13. Behnke MS, Khan A, Wootton JC, Dubey JP, Tang K, Sibley LD. 2011. Virulence differences in *Toxoplasma* mediated by amplification of a family of polymorphic pseudokinases. *PNAS* 108:9631–36
14. Behnke MS, Wootton JC, Lehmann MM, Radke JB, Lucas O, et al. 2010. Coordinated progression through two subtranscriptomes underlies the tachyzoite cycle of *Toxoplasma gondii*. *PLOS ONE* 5:e12354
15. Besteiro S, Dubremetz JF, Lebrun M. 2011. The moving junction of apicomplexan parasites: A key structure for invasion. *Cell. Microbiol.* 13:797–805
16. Bishop JR, Crawford BE, Esko JD. 2005. Cell surface heparan sulfate promotes replication of *Toxoplasma gondii*. *Infect. Immun.* 73:5395–401
17. Black MW, Boothroyd JC. 2000. Lytic cycle of *Toxoplasma gondii*. *Microbiol. Mol. Biol. Rev.* 64:607–23
18. Blader IJ, Manger ID, Boothroyd JC. 2001. Microarray analysis reveals previously unknown changes in *Toxoplasma gondii*-infected human cells. *J. Biol. Chem.* 276:24223–31
19. Bottova I, Sauder U, Olivieri V, Hehl AB, Sonda S. 2010. The P-glycoprotein inhibitor GF120918 modulates Ca^{2+}-dependent processes and lipid metabolism in *Toxoplasma gondii*. *PLOS ONE* 5:e10062
20. Braun L, Brenier-Pinchart MP, Yogavel M, Curt-Varesano A, Curt-Bertini RL, et al. 2013. A *Toxoplasma* dense granule protein, GRA24, modulates the early immune response to infection by promoting a direct and sustained host p38 MAPK activation. *J. Exp. Med.* 210:2071–86
21. Breinich MS, Ferguson DJ, Foth BJ, van Dooren GG, Lebrun M, et al. 2009. A dynamin is required for the biogenesis of secretory organelles in *Toxoplasma gondii*. *Curr. Biol.* 19:277–86
22. Brochet M, Collins MO, Smith TK, Thompson E, Sebastian S, et al. 2014. Phosphoinositide metabolism links cGMP-dependent protein kinase G to essential Ca^{2+} signals at key decision points in the life cycle of malaria parasites. *PLOS Biol.* 12:e1001806
23. Brooks CF, Francia ME, Gissot M, Croken MM, Kim K, Striepen B. 2011. *Toxoplasma gondii* sequesters centromeres to a specific nuclear region throughout the cell cycle. *PNAS* 108:3767–72
24. Brossier F, Jewett TJ, Sibley LD, Urban S. 2005. A spatially localized rhomboid protease cleaves cell surface adhesins essential for invasion by *Toxoplasma*. *PNAS* 102:4146–51
25. Brown KM, Suvorova E, Farrell A, Wiley BB, Marth G, et al. 2014. Forward genetic screening identifies a small molecule that blocks *Toxoplasma gondii* growth by inhibiting both host- and parasite-encoded kinases. *PLOS Pathog.* 10:e1004180

26. Carmen JC, Sinai AP. 2011. The differential effect of *Toxoplasma gondii* infection on the stability of BCL2-family members involves multiple activities. *Front. Microbiol.* 2:1
27. Carruthers VB, Hakansson S, Giddings OK, Sibley LD. 2000. *Toxoplasma gondii* uses sulfated proteoglycans for substrate and host cell attachment. *Infect. Immun.* 68:4005–11
28. Carruthers VB, Moreno SN, Sibley LD. 1999. Ethanol and acetaldehyde elevate intracellular [Ca2+] and stimulate microneme discharge in *Toxoplasma gondii*. *Biochem. J.* 342(Part 2):379–86
29. Carruthers VB, Tomley FM. 2008. Microneme proteins in apicomplexans. *Subcell. Biochem.* 47:33–45
30. Cavailles P, Flori P, Papapietro O, Bisanz C, Lagrange D, et al. 2014. A highly conserved Toxo1 haplotype directs resistance to toxoplasmosis and its associated caspase-1 dependent killing of parasite and host macrophage. *PLOS Pathog.* 10:e1004005
31. Cavailles P, Sergent V, Bisanz C, Papapietro O, Colacios C, et al. 2006. The rat Toxo1 locus directs toxoplasmosis outcome and controls parasite proliferation and spreading by macrophage-dependent mechanisms. *PNAS* 103:744–49
32. Chandramohanadas R, Davis PH, Beiting DP, Harbut MB, Darling C, et al. 2009. Apicomplexan parasites co-opt host calpains to facilitate their escape from infected cells. *Science* 324:794–97
33. Chen CT, Gubbels MJ. 2013. The *Toxoplasma gondii* centrosome is the platform for internal daughter budding as revealed by a Nek1 kinase mutant. *J. Cell Sci.* 126:3344–55
34. Chini EN, Nagamune K, Wetzel DM, Sibley LD. 2005. Evidence that the cADPR signalling pathway controls calcium-mediated microneme secretion in *Toxoplasma gondii*. *Biochem. J.* 389:269–77
35. Cirelli KM, Gorfu G, Hassan MA, Printz M, Crown D, et al. 2014. Inflammasome sensor NLRP1 controls rat macrophage susceptibility to *Toxoplasma gondii*. *PLOS Pathog.* 10:e1003927
36. Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tessarollo L, et al. 2001. Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. *J. Exp. Med.* 194:181–88
37. Coppens I. 2006. Contribution of host lipids to *Toxoplasma* pathogenesis. *Cell Microbiol.* 8:1–9
38. Coppens I, Dunn JD, Romano JD, Pypaert M, Zhang H, et al. 2006. *Toxoplasma gondii* sequesters lysosomes from mammalian hosts in the vacuolar space. *Cell* 125:261–74
39. de Leon JC, Scheumann N, Beatty W, Beck JR, Tran JQ, et al. 2013. A SAS-6-like protein suggests that the *Toxoplasma* conoid complex evolved from flagellar components. *Eukaryot. Cell* 12:1009–19
40. Dobrowolski JM, Niesman IR, Sibley LD. 1997. Actin in the parasite *Toxoplasma gondii* is encoded by a single copy gene, *ACT1* and exists primarily in a globular form. *Cell Motil. Cytoskeleton.* 37:253–62

41. Discovery of an endocytic pathway in *Toxoplasma*.

41. Dou Z, McGovern OL, Di Cristina M, Carruthers VB. 2014. *Toxoplasma gondii* ingests and digests host cytosolic proteins. *mBio* 5:e01188-14

42. Dowse TJ, Pascall JC, Brown KD, Soldati D. 2005. Apicomplexan rhomboids have a potential role in microneme protein cleavage during host cell invasion. *Int. J. Parasitol.* 35:747–56
43. Dubey JP. 2008. The history of *Toxoplasma gondii*—the first 100 years. *J. Eukaryot. Microbiol.* 55:467–75
44. Egarter S, Andenmatten N, Jackson AJ, Whitelaw JA, Pall G, et al. 2014. The *Toxoplasma* Acto-MyoA motor complex is important but not essential for gliding motility and host cell invasion. *PLOS ONE* 9:e91819
45. Ewald SE, Chavarria-Smith J, Boothroyd JC. 2014. NLRP1 is an inflammasome sensor for *Toxoplasma gondii*. *Infect. Immun.* 82:460–68
46. Farrell A, Thirugnanam S, Lorestani A, Dvorin JD, Eidell KP, et al. 2012. A DOC2 protein identified by mutational profiling is essential for apicomplexan parasite exocytosis. *Science* 335:218–21
47. Farrell M, Gubbels MJ. 2014. The *Toxoplasma gondii* kinetochore is required for centrosome association with the centrocone (spindle pole). *Cell. Microbiol.* 16:78–94

48. First report on the shared ancesty between endodyogeny and the machinery underlying flagellum construction.

48. Francia ME, Jordan CN, Patel JD, Sheiner L, Demerly JL, et al. 2012. Cell division in apicomplexan parasites is organized by a homolog of the striated rootlet fiber of algal flagella. *PLOS Biol.* 10:e1001444

49. Francia ME, Striepen B. 2014. Cell division in apicomplexan parasites. *Nat. Rev. Microbiol.* 12:125–36
50. Franco M, Shastri AJ, Boothroyd JC. 2014. Infection by *Toxoplasma gondii* specifically induces host c-Myc and the genes this pivotal transcription factor regulates. *Eukaryot. Cell* 13:483–93

51. Frenal K, Marq JB, Jacot D, Polonais V, Soldati-Favre D. 2014. Plasticity between MyoC- and MyoA-glideosomes: an example of functional compensation in *Toxoplasma gondii* invasion. *PLOS Pathog.* 10:e1004504
52. Frenal K, Polonais V, Marq JB, Stratmann R, Limenitakis J, Soldati-Favre D. 2010. Functional dissection of the apicomplexan glideosome molecular architecture. *Cell Host Microbe* 8:343–57
53. Frenal K, Tay CL, Mueller C, Bushell ES, Jia Y, et al. 2013. Global analysis of apicomplexan protein S-acyl transferases reveals an enzyme essential for invasion. *Traffic* 14:895–911
54. Gaji RY, Behnke MS, Lehmann MM, White MW, Carruthers VB. 2011. Cell cycle-dependent, intercellular transmission of *Toxoplasma gondii* is accompanied by marked changes in parasite gene expression. *Mol. Microbiol.* 79:192–204
55. Garrison E, Treeck M, Ehret E, Butz H, Garbuz T, et al. 2012. A forward genetic screen reveals that calcium-dependent protein kinase 3 regulates egress in *Toxoplasma*. *PLOS Pathog.* 8:e1003049
56. Goebel S, Gross U, Luder CG. 2001. Inhibition of host cell apoptosis by *Toxoplasma gondii* is accompanied by reduced activation of the caspase cascade and alterations of poly(ADP-ribose) polymerase expression. *J. Cell Sci.* 114:3495–505
57. Gorfu G, Cirelli KM, Melo MB, Mayer-Barber K, Crown D, et al. 2014. Dual role for inflammasome sensors NLRP1 and NLRP3 in murine resistance to *Toxoplasma gondii*. *mBio* 5:e01117-13
58. Gov L, Karimzadeh A, Ueno N, Lodoen MB. 2013. Human innate immunity to *Toxoplasma gondii* is mediated by host caspase-1 and ASC and parasite GRA15. *mBio* 4:e00255-13
59. Gubbels MJ, Duraisingh MT. 2012. Evolution of apicomplexan secretory organelles. *Int. J. Parasitol.* 42:1071–81
60. Gubbels MJ, Lehmann M, Muthalagi M, Jerome ME, Brooks CF, et al. 2008. Forward genetic analysis of the apicomplexan cell division cycle in *Toxoplasma gondii*. *PLOS Pathog.* 4:e36
61. Gubbels MJ, Vaishnava S, Boot N, Dubremetz JF, Striepen B. 2006. A MORN-repeat protein is a dynamic component of the *Toxoplasma gondii* cell division apparatus. *J. Cell Sci.* 119:2236–45
62. Hakansson S, Charron AJ, Sibley LD. 2001. *Toxoplasma* evacuoles: A two-step process of secretion and fusion forms the parasitophorous vacuole. *EMBO J.* 20:3132–44
63. Hakansson S, Morisaki H, Heuser J, Sibley LD. 1999. Time-lapse video microscopy of gliding motility in *Toxoplasma gondii* reveals a novel, biphasic mechanism of cell locomotion. *Mol. Biol. Cell* 10:3539–47
64. Hartmann J, Hu K, He CY, Pelletier L, Roos DS, Warren G. 2006. Golgi and centrosome cycles in *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* 145:125–27
65. He XL, Grigg ME, Boothroyd JC, Garcia KC. 2002. Structure of the immunodominant surface antigen from the *Toxoplasma gondii* SRS superfamily. *Nat. Struct. Biol.* 9:606–11
66. Hoff EF, Carruthers VB. 2002. Is *Toxoplasma* egress the first step in invasion? *Trends Parasitol.* 18:251–55
67. Hu K. 2008. Organizational changes of the daughter basal complex during the parasite replication of *Toxoplasma gondii*. *PLOS Pathog.* 4:e10
68. Hu K, Johnson J, Florens L, Fraunholz M, Suravajjala S, et al. 2006. Cytoskeletal components of an invasion machine—the apical complex of *Toxoplasma gondii*. *PLOS Pathog.* 2:e13
69. Hunn JP, Feng CG, Sher A, Howard JC. 2011. The immunity-related GTPases in mammals: a fast-evolving cell-autonomous resistance system against intracellular pathogens. *Mamm. Genome* 22:43–54
70. Jensen KD, Hu K, Whitmarsh RJ, Hassan MA, Julien L, et al. 2013. *Toxoplasma gondii* rhoptry 16 kinase promotes host resistance to oral infection and intestinal inflammation only in the context of the dense granule protein GRA15. *Infect. Immun.* 81:2156–67
71. Johnson TM, Rajfur Z, Jacobson K, Beckers CJ. 2007. Immobilization of the type XIV myosin complex in *Toxoplasma gondii*. *Mol. Biol. Cell* 18:3039–46
72. Jones TC, Hirsch JG. 1972. The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *J. Exp. Med.* 136:1173–94
73. Kafsack BF, Carruthers VB, Pineda FJ. 2007. Kinetic modeling of *Toxoplasma gondii* invasion. *J. Theor. Biol.* 249:817–25
74. Kafsack BF, Pena JD, Coppens I, Ravindran S, Boothroyd JC, Carruthers VB. 2009. Rapid membrane disruption by a perforin-like protein facilitates parasite exit from host cells. *Science* 323:530–33

72. First report showing that host organelles interact with the PV.

75. Katris NJ, van Dooren GG, McMillan PJ, Hanssen E, Tilley L, Waller RF. 2014. The apical complex provides a regulated gateway for secretion of invasion factors in *Toxoplasma*. *PLOS Pathog.* 10:e1004074
76. Kessler H, Herm-Gotz A, Hegge S, Rauch M, Soldati-Favre D, et al. 2008. Microneme protein 8—a new essential invasion factor in *Toxoplasma gondii*. *J. Cell Sci.* 121:947–56
77. Kieschnick H, Wakefield T, Narducci CA, Beckers C. 2001. *Toxoplasma gondii* attachment to host cells is regulated by a calmodulin-like domain protein kinase. *J. Biol. Chem.* 276:12369–77
78. Kim L, Denkers EY. 2006. *Toxoplasma gondii* triggers G_i-dependent PI 3-kinase signaling required for inhibition of host cell apoptosis. *J. Cell Sci.* 119:2119–26
79. Kim SK, Fouts AE, Boothroyd JC. 2007. *Toxoplasma gondii* dysregulates IFN-gamma-inducible gene expression in human fibroblasts: insights from a genome-wide transcriptional profiling. *J. Immunol.* 178:5154–65
80. Kremer K, Kamin D, Rittweger E, Wilkes J, Flammer H, et al. 2013. An overexpression screen of *Toxoplasma gondii* Rab-GTPases reveals distinct transport routes to the micronemes. *PLOS Pathog.* 9:e1003213
81. Kvaal CA, Radke JR, Guerini MN, White MW. 2002. Isolation of a *Toxoplasma gondii* cyclin by yeast two-hybrid interactive screen. *Mol. Biochem. Parasitol.* 120:187–94
82. Lang C, Hildebrandt A, Brand F, Opitz L, Dihazi H, Luder CG. 2012. Impaired chromatin remodelling at STAT1-regulated promoters leads to global unresponsiveness of *Toxoplasma gondii*-infected macrophages to IFN-γ. *PLOS Pathog.* 8:e1002483
83. Lescault PJ, Thompson AB, Patil V, Lirussi D, Burton A, et al. 2010. Genomic data reveal *Toxoplasma gondii* differentiation mutants are also impaired with respect to switching into a novel extracellular tachyzoite state. *PLOS ONE* 5:e14463
84. Leung JM, Rould MA, Konradt C, Hunter CA, Ward GE. 2014. Disruption of TgPHIL1 alters specific parameters of *Toxoplasma gondii* motility measured in a quantitative, three-dimensional live motility assay. *PLOS ONE* 9:e85763
85. Li ZH, Ramakrishnan S, Striepen B, Moreno SN. 2013. *Toxoplasma gondii* relies on both host and parasite isoprenoids and can be rendered sensitive to atorvastatin. *PLOS Pathog.* 9:e1003665
86. Lirussi D, Matrajt M. 2011. RNA granules present only in extracellular *Toxoplasma gondii* increase parasite viability. *Int. J. Biol. Sci.* 7:960–67

87. Demonstrates that the basal complex is the functional ortholog of the contractile ring in cell division.

87. Lorestani A, Sheiner L, Yang K, Robertson SD, Sahoo N, et al. 2010. A *Toxoplasma* MORN1 null mutant undergoes repeated divisions but is defective in basal assembly, apicoplast division and cytokinesis. *PLOS ONE* 5:e12302

88. Lourido S, Jeschke GR, Turk BE, Sibley LD. 2013. Exploiting the unique ATP-binding pocket of *Toxoplasma* calcium-dependent protein kinase 1 to identify its substrates. *ACS Chem. Biol.* 8:1155–62

89. First proof of a Ca^{2+}-dependent protein kinase, a protein family absent from mammals, with an essential role in invasion.

89. Lourido S, Shuman J, Zhang C, Shokat KM, Hui R, Sibley LD. 2010. Calcium-dependent protein kinase 1 is an essential regulator of exocytosis in *Toxoplasma*. *Nature* 465:359–62

90. Lourido S, Tang K, Sibley LD. 2012. Distinct signalling pathways control *Toxoplasma* egress and host-cell invasion. *EMBO J.* 31:4524–34
91. Lovett JL, Marchesini N, Moreno SN, Sibley LD. 2002. *Toxoplasma gondii* microneme secretion involves intracellular Ca^{2+} release from inositol 1,4,5-triphosphate (IP_3)/ryanodine-sensitive stores. *J. Biol. Chem.* 277:25870–76
92. Luo S, Ruiz FA, Moreno SN. 2005. The acidocalcisome Ca2+-ATPase (TgA1) of *Toxoplasma gondii* is required for polyphosphate storage, intracellular calcium homeostasis and virulence. *Mol. Microbiol.* 55:1034–45
93. Ma JS, Sasai M, Ohshima J, Lee Y, Bando H, et al. 2014. Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6. *J. Exp. Med.* 211:2013–32
94. Mann T, Beckers C. 2001. Characterization of the subpellicular network, a filamentous membrane skeletal component in the parasite *Toxoplasma gondii*. *Mol. Biochem. Parasitol.* 115:257–68
95. McCoy JM, Whitehead L, van Dooren GG, Tonkin CJ. 2012. TgCDPK3 regulates calcium-dependent egress of *Toxoplasma gondii* from host cells. *PLOS Pathog.* 8:e1003066
96. Meissner M, Ferguson DJ, Frischknecht F. 2013. Invasion factors of apicomplexan parasites: essential or redundant? *Curr. Opin. Microbiol.* 16:438–44

97. Shows that myosin A, a class XIVa myosin, is the main motor driving motility and invasion.

97. Meissner M, Schluter D, Soldati D. 2002. Role of *Toxoplasma gondii* myosin A in powering parasite gliding and host cell invasion. *Science* 298:837–40

98. Melzer T, Duffy A, Weiss LM, Halonen SK. 2008. The gamma interferon (IFN-γ)-inducible GTP-binding protein IGTP is necessary for *Toxoplasma* vacuolar disruption and induces parasite egression in IFN-γ-stimulated astrocytes. *Infect. Immun.* 76:4883–94
99. Mital J, Meissner M, Soldati D, Ward GE. 2005. Conditional expression of *Toxoplasma gondii* apical membrane antigen-1 (TgAMA1) demonstrates that TgAMA1 plays a critical role in host cell invasion. *Mol. Biol. Cell* 16:4341–49
100. Molestina RE, Payne TM, Coppens I, Sinai AP. 2003. Activation of NF-κB by *Toxoplasma gondii* correlates with increased expression of antiapoptotic genes and localization of phosphorylated IκB to the parasitophorous vacuole membrane. *J. Cell Sci.* 116:4359–71
101. Montoya JG, Liesenfeld O. 2004. Toxoplasmosis. *Lancet* 363:1965–76
102. Mordue DG, Hakansson S, Niesman I, Sibley LD. 1999. *Toxoplasma gondii* resides in a vacuole that avoids fusion with host cell endocytic and exocytic vesicular trafficking pathways. *Exp. Parasitol.* 92:87–99
103. Morlon-Guyot J, Berry L, Chen CT, Gubbels MJ, Lebrun M, Daher W. 2014. The *Toxoplasma gondii* calcium dependent protein kinase 7 is involved in early steps of parasite division and is crucial for parasite survival. *Cell. Microbiol.* 16:95–114
104. Morrissette NS, Sibley LD. 2002. Disruption of microtubules uncouples budding and nuclear division in *Toxoplasma gondii*. *J. Cell Sci.* 115:1017–25
105. Moudy R, Manning TJ, Beckers CJ. 2001. The loss of cytoplasmic potassium upon host cell breakdown triggers egress of *Toxoplasma gondii*. *J. Biol. Chem.* 276:41492–501
106. Muniz-Feliciano L, Van Grol J, Portillo JA, Liew L, Liu B, et al. 2013. *Toxoplasma gondii*-induced activation of EGFR prevents autophagy protein-mediated killing of the parasite. *PLOS Pathog.* 9:e1003809
107. Nagamune K, Beatty WL, Sibley LD. 2007. Artemisinin induces calcium-dependent protein secretion in the protozoan parasite *Toxoplasma gondii*. *Eukaryot. Cell* 6:2147–56
108. Nagamune K, Hicks LM, Fux B, Brossier F, Chini EN, Sibley LD. 2008. Abscisic acid controls calcium-dependent egress and development in *Toxoplasma gondii*. *Nature* 451:207–10
109. Nagamune K, Moreno SN, Chini EN, Sibley LD. 2008. Calcium regulation and signaling in apicomplexan parasites. *Subcell. Biochem.* 47:70–81
110. Nebl T, Prieto JH, Kapp E, Smith BJ, Williams MJ, et al. 2011. Quantitative in vivo analyses reveal calcium-dependent phosphorylation sites and identifies a novel component of the *Toxoplasma* invasion motor complex. *PLOS Pathog.* 7:e1002222
111. Nishi M, Hu K, Murray JM, Roos DS. 2008. Organellar dynamics during the cell cycle of *Toxoplasma gondii*. *J. Cell Sci.* 121:1559–68
112. Pace DA, McKnight CA, Liu J, Jimenez V, Moreno SN. 2014. Calcium entry in *Toxoplasma gondii* and its enhancing effect of invasion-linked traits. *J. Biol. Chem.* 289:19637–47
113. Payne TM, Molestina RE, Sinai AP. 2003. Inhibition of caspase activation and a requirement for NF-κB function in the *Toxoplasma gondii*-mediated blockade of host apoptosis. *J. Cell Sci.* 116:4345–58
114. Pernas L, Adomako-Ankomah Y, Shastri AJ, Ewald SE, Treeck M, et al. 2014. *Toxoplasma* effector MAF1 mediates recruitment of host mitochondria and impacts the host response. *PLOS Biol.* 12:e1001845
115. Persson CM, Lambert H, Vutova PP, Dellacasa-Lindberg I, Nederby J, et al. 2009. Transmission of *Toxoplasma gondii* from infected dendritic cells to natural killer cells. *Infect. Immun.* 77:970–76
116. Persson EK, Agnarson AM, Lambert H, Hitziger N, Yagita H, et al. 2007. Death receptor ligation or exposure to perforin trigger rapid egress of the intracellular parasite *Toxoplasma gondii*. *J. Immunol.* 179:8357–65
117. Pfefferkorn ER. 1984. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *PNAS* 81:908–12
118. Phelps ED, Sweeney KR, Blader IJ. 2008. *Toxoplasma gondii* rhoptry discharge correlates with activation of the early growth response 2 host cell transcription factor. *Infect. Immun.* 76:4703–12
119. Plattner H, Sehring IM, Mohamed IK, Miranda K, De Souza W, et al. 2012. Calcium signaling in closely related protozoan groups (Alveolata): non-parasitic ciliates (*Paramecium*, *Tetrahymena*) versus parasitic Apicomplexa (*Plasmodium*, *Toxoplasma*). *Cell Calcium* 51:351–82
120. Pomel S, Luk FC, Beckers CJ. 2008. Host cell egress and invasion induce marked relocations of glycolytic enzymes in *Toxoplasma gondii* tachyzoites. *PLOS Pathog.* 4:e1000188

105. First comprehensive analysis of the egress mechanism by innovative assays.

121. Ramana CV, Gil MP, Schreiber RD, Stark GR. 2002. Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. *Trends Immunol.* 23:96–101
122. Reese ML, Zeiner GM, Saeij JP, Boothroyd JC, Boyle JP. 2011. Polymorphic family of injected pseudokinases is paramount in *Toxoplasma* virulence. *PNAS* 108:9625–30
123. Roiko MS, Svezhova N, Carruthers VB. 2014. Acidification activates *Toxoplasma gondii* motility and egress by enhancing protein secretion and cytolytic activity. *PLOS Pathog.* 10:e1004488
124. Romano JD, Sonda S, Bergbower E, Smith ME, Coppens I. 2013. *Toxoplasma gondii* salvages sphingolipids from the host Golgi through the rerouting of selected Rab vesicles to the parasitophorous vacuole. *Mol. Biol. Cell* 24:1974–95
125. Rosowski EE, Lu D, Julien L, Rodda L, Gaiser RA, et al. 2011. Strain-specific activation of the NF-κB pathway by GRA15, a novel *Toxoplasma gondii* dense granule protein. *J. Exp. Med.* 208:195–212
126. Rosowski EE, Nguyen QP, Camejo A, Spooner E, Saeij JP. 2014. *Toxoplasma gondii* inhibits gamma interferon (IFN-γ)- and IFN-β-induced host cell STAT1 transcriptional activity by increasing the association of STAT1 with DNA. *Infect. Immun.* 82:706–19
127. Saeij JP, Coller S, Boyle JP, Jerome ME, White MW, Boothroyd JC. 2007. *Toxoplasma* co-opts host gene expression by injection of a polymorphic kinase homologue. *Nature* 445:324–27
128. Sahoo N, Beatty W, Heuser J, Sept D, Sibley LD. 2006. Unusual kinetic and structural properties control rapid assembly and turnover of actin in the parasite *Toxoplasma gondii*. *Mol. Biol. Cell* 17:895–906
129. Sharma P, Chitnis CE. 2013. Key molecular events during host cell invasion by apicomplexan pathogens. *Curr. Opin. Microbiol.* 16:432–37
130. Sheffield HG, Melton ML. 1968. The fine structure and reproduction of *Toxoplasma gondii*. *J. Parasitol.* 54:209–26
131. Shen B, Sibley LD. 2014. *Toxoplasma* aldolase is required for metabolism but dispensable for host-cell invasion. *PNAS* 111:3567–72
132. Skillman KM, Diraviyam K, Khan A, Tang K, Sept D, Sibley LD. 2011. Evolutionarily divergent, unstable filamentous actin is essential for gliding motility in apicomplexan parasites. *PLOS Pathog.* 7:e1002280
133. Spear W, Chan D, Coppens I, Johnson RS, Giaccia A, Blader IJ. 2006. The host cell transcription factor hypoxia-inducible factor 1 is required for *Toxoplasma gondii* growth and survival at physiological oxygen levels. *Cell Microbiol.* 8:339–52
134. Stommel EW, Ely KH, Schwartzman JD, Kasper LH. 1997. *Toxoplasma gondii*: Dithiol-induced Ca^{2+} flux causes egress of parasites from the parasitophorous vacuole. *Exp. Parasitol.* 87:88–97
135. Straub KW, Peng ED, Hajagos BE, Tyler JS, Bradley PJ. 2011. The moving junction protein RON8 facilitates firm attachment and host cell invasion in *Toxoplasma gondii*. *PLOS Pathog.* 7:e1002007
136. Striepen B, Jordan CN, Reiff S, van Dooren GG. 2007. Building the perfect parasite: cell division in apicomplexa. *PLOS Pathog.* 3:e78
137. Suss-Toby E, Zimmerberg J, Ward GE. 1996. *Toxoplasma* invasion: The parasitophorous vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *PNAS* 93:8413–18
138. Suvorova ES, Francia M, Striepen B, White MW. 2015. A novel bipartite centrosome coordinates the apicomplexan cell cycle. *PLOS Biol.* 13:e1002093
139. Suzuki Y, Orellana MA, Schreiber RD, Remington JS. 1988. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* 240:516–18
140. Tang Q, Andenmatten N, Triana MA, Deng B, Meissner M, et al. 2014. Calcium-dependent phosphorylation alters Class XIVa myosin function in the protozoan parasite *Toxoplasma gondii*. *Mol. Biol. Cell* 25:2579–91
141. Tomavo S, Slomianny C, Meissner M, Carruthers VB. 2013. Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion. *PLOS Pathog.* 9:e1003629
142. Tomita T, Yamada T, Weiss LM, Orlofsky A. 2009. Externally triggered egress is the major fate of *Toxoplasma gondii* during acute infection. *J. Immunol.* 183:6667–80
143. Treeck M, Sanders JL, Elias JE, Boothroyd JC. 2011. The phosphoproteomes of *Plasmodium falciparum* and *Toxoplasma gondii* reveal unusual adaptations within and beyond the parasites' boundaries. *Cell Host Microbe* 10:410–19

138. Explains the mechanical basis for how mitosis and daughter budding are coordinated by the centrosome.

144. Treeck M, Sanders JL, Gaji RY, LaFavers KA, Child MA, et al. 2014. The calcium-dependent protein kinase 3 of *Toxoplasma* influences basal calcium levels and functions beyond egress as revealed by quantitative phosphoproteome analysis. *PLOS Pathog.* 10:e1004197
145. van Dooren GG, Reiff SB, Tomova C, Meissner M, Humbel BM, Striepen B. 2009. A novel dynamin-related protein has been recruited for apicoplast fission in *Toxoplasma gondii*. *Curr. Biol.* 19:267–76
146. Wetzel DM, Chen LA, Ruiz FA, Moreno SN, Sibley LD. 2004. Calcium-mediated protein secretion potentiates motility in *Toxoplasma gondii*. *J. Cell Sci.* 117:5739–48
147. Wetzel DM, Hakansson S, Hu K, Roos D, Sibley LD. 2003. Actin filament polymerization regulates gliding motility by apicomplexan parasites. *Mol. Biol. Cell* 14:396–406
148. Wiley M, Sweeney KR, Chan DA, Brown KM, McMurtrey C, et al. 2010. *Toxoplasma gondii* activates hypoxia-inducible factor (HIF) by stabilizing the HIF-1α subunit via type I activin-like receptor kinase receptor signaling. *J. Biol. Chem.* 285:26852–60
149. Witola WH, Mui E, Hargrave A, Liu S, Hypolite M, et al. 2011. NALP1 influences susceptibility to human congenital toxoplasmosis, proinflammatory cytokine response, and fate of *Toxoplasma gondii*-infected monocytic cells. *Infect. Immun.* 79:756–66

Annual Review of Microbiology

Volume 69, 2015

Contents

My Lifelong Passion for Biochemistry and Anaerobic Microorganisms
Rudolf Kurt Thauer 1

The Pyromaniac Inside You: *Salmonella* Metabolism in the Host Gut
Fabian Rivera-Chávez and Andreas J. Bäumler 31

Regulation of Transcript Elongation
Georgiy A. Belogurov and Irina Artsimovitch 49

Candida albicans Biofilms and Human Disease
Clarissa J. Nobile and Alexander D. Johnson 71

Transcription Factors That Defend Bacteria Against Reactive Oxygen Species
James A. Imlay 93

Bacterial Proteasomes
Jordan B. Jastrab and K. Heran Darwin 109

The Organellar Genomes of *Chromera* and *Vitrella*, the Phototrophic Relatives of Apicomplexan Parasites
Miroslav Oborník and Julius Lukeš 129

The Gut Microbiota of Termites: Digesting the Diversity in the Light of Ecology and Evolution
Andreas Brune and Carsten Dietrich 145

Microsporidia: Eukaryotic Intracellular Parasites Shaped by Gene Loss and Horizontal Gene Transfers
Nicolas Corradi 167

Anthrax Pathogenesis
Mahtab Moayeri, Stephen H. Leppla, Catherine Vrentas, Andrei P. Pomerantsev, and Shihui Liu 185

CRISPR-Cas: New Tools for Genetic Manipulations from Bacterial Immunity Systems
Wenyan Jiang and Luciano A. Marraffini 209

Perception and Homeostatic Control of Iron in the Rhizobia and Related Bacteria
Mark R. O'Brian 229

Thymineless Death Lives On: New Insights into a Classic Phenomenon
Arkady Khodursky, Elena C. Guzmán, and Philip C. Hanawalt 247

Membrane-Coupled mRNA Trafficking in Fungi
Carl Haag, Benedikt Steuten, and Michael Feldbrügge 265

Molecular Pathogenesis of *Ehrlichia chaffeensis* Infection
Yasuko Rikihisa 283

Microbiology Meets Big Data: The Case of Gut Microbiota–Derived Trimethylamine
Gwen Falony, Sara Vieira-Silva, and Jeroen Raes 305

The Unique Molecular Choreography of Giant Pore Formation by the Cholesterol-Dependent Cytolysins of Gram-Positive Bacteria
Rodney K. Tweten, Eileen M. Hotze, and Kristin R. Wade 323

Ion Regulation in the Malaria Parasite
Kiaran Kirk 341

Physics of Intracellular Organization in Bacteria
Ned S. Wingreen and Kerwyn Casey Huang 361

Stochastic Switching of Cell Fate in Microbes
Thomas M. Norman, Nathan D. Lord, Johan Paulsson, and Richard Losick 381

Assembly of the Mycobacterial Cell Wall
Monika Jankute, Jonathan A.G. Cox, James Harrison, and Gurdyal S. Besra 405

Mechanisms of Bacterial Colonization of the Respiratory Tract
Steven J. Siegel and Jeffrey N. Weiser 425

Interactions Between the Gastrointestinal Microbiome and *Clostridium difficile*
Casey M. Theriot and Vincent B. Young 445

Lytic Cycle of *Toxoplasma gondii*: 15 Years Later
Ira Blader, Bradley Coleman, Chun-Ti Chen, and Marc-Jan Gubbels 463

Septins and Generation of Asymmetries in Fungal Cells
Anum Khan, Molly McQuilken, and Amy S. Gladfelter 487

How Is Fe-S Cluster Formation Regulated?
Erin L. Mettert and Patricia J. Kiley 505

Protein Phosphatases of Pathogenic Bacteria: Role in Physiology and Virulence
Andaleeb Sajid, Gunjan Arora, Anshika Singhal, Vipin C. Kalia, and Yogendra Singh 527

Index

Cumulative Index of Contributing Authors, Volumes 65–69 549

Errata

An online log of corrections to *Annual Review of Microbiology* articles may be found at http://www.annualreviews.org/errata/micro