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REVIEW

Toxoplasma Effectors Targeting Host Signaling and Transcription

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SUMMARY Early electron microscopy studies revealed the elaborate cellular features that define the unique adaptations of apicomplexan parasites. Among these were bulbous rhoptry (ROP) organelles and small, dense granules (GRAs), both of which are secreted during invasion of host cells. These early morphological studies were followed by the exploration of the cellular contents of these secretory organelles, revealing them to be comprised of highly divergent protein families with few conserved domains or predicted functions. In parallel, studies on host-pathogen interactions identified many host signaling pathways that were mysteriously altered by infection. It was only with the advent of forward and reverse genetic strategies that the connections between individual parasite effectors and the specific host pathways that they targeted finally became clear. The current repertoire of parasite effectors includes ROP kinases and pseudokinases that are secreted during invasion and that block host immune pathways. Similarly, many secretory GRA proteins alter host gene expression by activating host transcription factors, through modification of chromatin, or by inducing small noncoding RNAs. These effectors highlight novel mechanisms by which *T. gondii* has learned to harness host signaling to favor intracellular survival and will guide future studies designed to uncover the additional complexity of this intricate host-pathogen interaction.

KEYWORDS chromatin remodeling, epigenetics, immune evasion, innate immunity, intracellular pathogen, serine/threonine kinases, signal transduction, transcription factors

INTRODUCTION

Toxoplasma gondii is a widespread parasite that infects many species of animals, including mammals, marsupials, and birds (1). A member of the phylum Apicomplexa, *Toxoplasma gondii* belongs to a diverse assemblage of organisms originally estimated to comprise ~5,000 species (2), although recent estimates based on genomic bar coding suggest much greater diversity (3). The majority of apicomplexans are obligate intracellular parasites, while others only partially enter their host cell to reside in an "epicellular" state (4). Apicomplexans infect many different hosts, including invertebrates (i.e., insects, worms, and mollusks), where gregarines predominate (http://tolweb.org/Gregarina/124806), as well as both cold-blooded and warm-blooded vertebrates (4). Intracellular and epicellular lifestyles are thought to have arisen multiple times during evolution of apicomplexans (4, 5). Apicomplexans are unified by structural similarities at the apical end, including the conoid that organizes the cytoskeleton and several groups of secretory organelles (6). Comparison of apicomplexans to their closest sister taxa, the free-living photosynthetic chromerids and predatory colpodellids, reveals many common adaptations, while apicomplexan-specific features are limited to a few conserved secretory proteins found in apical organelles called rhoptries and the unique class XIV myosin and associated components of the glideosome (7). Analyses of more than 60 *T. gondii* genomes in comparison with closely related parasites expand on this theme by showing that the amplification of polymorphic secretory proteins is associated with the diversification of apicomplexans within their respective vertebrate hosts (8).

Apicomplexans are best studied where they cause disease in warm-blooded hosts, including domestic animals and humans. Notable groups that frequently cause serious disease in humans include *Plasmodium* spp., responsible for malaria (9); *Cryptosporidium* spp., an agent of diarrheal disease (10); and *T. gondii*, which causes toxoplasmosis (11, 12). Unlike some groups of apicomplexans (e.g., *Sarcocystis* spp.) in which there are many distinct species, each infecting a discrete number of hosts (13), *T. gondii* is unified as a single species that infects many species of animals from diverse geographic regions (1). As described further below, *T. gondii* has a relatively young population structure, and isolates collected from diverse hosts around the world comprise a small number of clades of closely related strains (14, 15).

Toxoplasma gondii belongs to the tissue cyst-forming branch of the enteric coccidians, which contains important animal parasites such as *Eimeria* spp., which cause severe economic losses in agricultural animals (16). In contrast to the direct oral-fecal route of spread of enteric coccidians, *T. gondii* is transmitted by an alternating two-host life cycle, termed a heteroxenous cycle, relying on a definitive host for sexual transmission while undergoing asexual transmission in its alternative host. Different species of cats serve as definitive hosts for *T. gondii* (17). Sexual development takes place in enterocytes of the gut, resulting in the shedding of oocysts that contaminate the environment, whereupon they undergo meiosis in a process called sporulation (18). Oocysts are infectious to many animals, including a variety of rodents (19). During initial infection in the intermediate host, the parasite replicates in a variety of host cell types as tachyzoites, which expand dramatically in numbers and spread to many tissues in the body (20). Following a potent immune response, the parasite differentiates to a slow-growing bradyzoite, which remains semidormant within tissue cysts that reside in long-lived cells, including neurons and skeletal muscle cells (21). Bradyzoites divide slowly and asynchronously (22), consistent with the fact that tissue cysts grow over time, and cysts are thought to undergo multiple rounds of growth, rupture, and reinfection to sustain chronic infection (23). Ingestion of tissue cysts by the definitive host completes the cycle, giving rise to oocyst shedding (24). Although the life cycle of

T. gondii is remarkably flexible, transmission between hosts is most efficient when it follows the natural life cycle: oocysts are highly infectious for intermediate hosts such as rodents (20) and agricultural animals (25), and while they can also infect cats, the prepatent period before oocyst shedding is much longer (26). Bradyzoites found in tissue cysts can infect other rodents when orally ingested albeit much less efficiently than the definitive cat host (24).

The life cycle of a close relative of *T. gondii*, *Hammondia hammondi*, is an example of a very restrictive, obligatory heteroxenous cycle (27). *Hammondia hammondi* naturally infects rodents as intermediate hosts and undergoes sexual development and oocyst formation in cats, although it does not readily propagate *in vitro* and is not transmissible between intermediate hosts (28). In contrast to this restrictive cycle, *T. gondii* exhibits flexibility at several key steps in its life cycle that contributes to its successful expansion into many other hosts. First, the differentiation of tachyzoites into bradyzoites is reversible in *T. gondii*, allowing the reemergence of chronic infections in immunocompromised hosts (29). This trait allows the reisolation of *T. gondii* from chronic infections by inoculating tissue homogenates onto host cells cultured *in vitro* and subsequent cultivation of tachyzoites (30), making *T. gondii* a model for cellular and molecular studies (see below). Second, when tissue cysts of *T. gondii* are ingested by another intermediate host, they are infectious (31). Oral transmission may facilitate spread by asexual means through carnivorous or omnivorous feeding by hosts, bypassing the requirement for a definitive host. Strict herbivores must still be infected via oocysts shed from cats, but once infected, they can serve as intermediate hosts for asexual transmission through the food chain without the need for sexual development in cats. Although related parasites such as *Neospora caninum* are not transmitted between successive intermediate hosts via omnivorous or carnivorous feeding, they can be vertically transmitted (32), a trait shared by *T. gondii* in mice (33) and domestic animals such as sheep (34). The flexible nature of the *T. gondii* life cycle may be responsible for its widespread success as a parasite of so many diverse types of animals.

Transmission to Humans and Opportunistic Disease

Humans are accidental hosts for *T. gondii* and play little role in its natural life cycle. Oocysts are highly infectious when orally ingested (25, 35), and they remain infectious in the environment for extended time periods, as they are resistant to many conditions (36–39), including chlorine sterilization procedures used on many domestic water supplies (40). Humans can become infected by ingesting contaminated water that contains oocysts (41), and there have been a number of documented outbreaks of toxoplasmosis due to contamination of water supplies in British Columbia (42) and in different regions of Brazil (43, 44). In addition to waterborne outbreaks, oocyst infections have been responsible for aerosol exposures (45) and can cause infection by contamination of garden vegetables (46). Humans can also become infected by eating tissue cysts found in undercooked meat of infected animals (47). Finally, vertical transmission can result in congenital infection when a mother is newly infected during pregnancy (48). In all of these manifestations, humans act as an intermediate host, where the initial infection is propagated by the dissemination of tachyzoites that then convert to semidormant bradyzoites in long-lived tissue cysts. Globally, the serological prevalence of toxoplasmosis, largely reflecting subclinical chronic infections, is highly variable, ranging from $<$10 to 15% in the United States to $>$60% in South and Central America, parts of the Mediterranean, Europe, and Southeast Asia (49). Overall, $\sim$25% of the world's human population may be chronically infected with *T. gondii*.

In healthy adults, toxoplasmosis produces a relatively mild infection, with elevated fever, enlarged lymph nodes, and muscle weakness (12). Normally, acute infection resolves rapidly, leaving the individual with a chronic, subclinical infection (12). More severe outcomes can occur with congenital infections, where, depending on the timing of infection, the developing fetus can experience symptoms that range from severe (usually due to infection in the first trimester) to mild (more common when infection occurs later during pregnancy) (48). Severe forms of congenital toxoplasmosis can

result in hydrocephaly, microcephaly, intracranial calcification, and even loss of life (50). Milder infections may result in few symptoms at birth (51) but can be responsible for ocular toxoplasmosis later in life (52). Although ocular toxoplasmosis in North America and Europe seems to be due largely to a recurrence of congenital infection, a very different situation occurs in South America (53). In regions of southern Brazil, recurrent and severe ocular toxoplasmosis cases have been documented in healthy adults due to newly acquired infection (54). These episodes are associated with significant ocular inflammation, which requires treatment with corticosteroids in addition to antibiotic therapy to reduce the risk of recurrence (55). Whether the difference in disease severity is due to underlying differences in parasite strains (56, 57) (see below) or exposure burden (55, 58) is unclear, but it serves as a reminder that not all *T. gondii* infections in healthy adults are benign. Importantly, ocular toxoplasmosis patients in Europe show high levels of interleukin-17 (IL-17) and interferon gamma (IFN-γ), while the levels of these cytokines are much lower, with associated higher parasite loads, in South American patients (59). In immunocompromised adults, severe infections typically result from a reactivation of chronic infection, as seen in AIDS, organ transplant, or chemotherapy patients (60). These severe outcomes are all consistent with a lack of sustained immunity and the ability of the parasite to reemerge from tissue cysts and convert back to rapidly growing tachyzoites, which leads to tissue damage. As such, toxoplasmosis was considered a defining opportunistic infection of the AIDS epidemic (61). The introduction of highly active antiretroviral therapy (HAART) led to a drop in the incidence of cases of reactivated toxoplasmosis (62). However, this is still a problem in may regions due to a lack of available antiviral therapy or inadequate enrollment (63). The problem of chronic burden persists in the population, as available therapies, primarily pyrimethamine combined with sulfadiazine, do not eradicate the semidormant bradyzoites (64). Chronic infections in humans have also been associated with an elevated risk of psychiatric illnesses, including schizophrenia (65). Although the causality of this association has not been established, it is nonetheless worthy of further study.

Genetic Diversity and Population Structure

Early studies of genetic diversity based on restriction fragment length polymorphisms (RFLPs) (66) or isoenzyme markers (67) revealed differences in *T. gondii* strains, which are otherwise highly similar. These molecular typing studies also uncovered a striking pattern of clonality among North American strains (68), which were remarkably similar to those found in Europe (69, 70). Sampling from animal and human infections revealed three strongly clonal genotypes that were closely related to each other (71). Type 2 strains are most commonly associated with human infections in Europe in both cases of congenital infection (70) and immunocompromised patients (72–74), and this pattern is also seen in North America (71). Type 1 strains are relatively rare, although they are distinguished by their high level of acute virulence in the mouse model (68, 71), and they show elevated frequencies in some groups of immunocompromised patients (75). Finally, type 3 strains are relatively common in domestic and wild animals in North America and yet are rarely found in human infection (71, 76).

The distribution of single nucleotide polymorphisms (SNPs), which were revealed by sequencing of large numbers of cDNAs from the three clonal lineages (77), suggested a recent common inheritance of long haploblocks across the genome (78). This pattern is most easily explained by just a few genetic crosses that occurred in the wild between highly similar parental strains (78). The frequency of SNPs in selectively neutral loci (introns of housekeeping genes) was used to extrapolate the last common ancestor of the three strain types to within 10,000 years (31). This estimate is remarkably short considering the relatively long time span that apicomplexan parasites have been evolving within their vertebrate hosts, estimated at 400 million years (79). The recent ancestry of clonal *T. gondii* strains roughly coincides with the domestication of animals (80), the adoption of cats as pets (81), and the intrusion of house mice as pests (82). Hence, it appears that the convergence of definitive and intermediate hosts brought

together favorable conditions for transmission and provided opportunities for zoonotic infection of humans.

A number of animal and human pathogens show evidence of a loss of genetic diversity coincident with the rise of agriculture, and this pattern may reflect the lower genetic diversity of domesticated animals, which in turns selected for particular pathogen genotypes that were well suited in this niche (80). Why just three dominant genotypes of *T. gondii* emerged in North America and Europe after the recent genetic bottleneck is unclear, although it suggests that these genotypes are endowed with some selective advantage. One feature that the clonal lineages share is the common inheritance of similar forms of chromosome 1a, which has been linked to greater transmission in domestic cats (83–86). Additionally, the flexibility of the life cycle mentioned above allows *T. gondii* to bypass cats when transmitted by omnivorous or carnivorous feeding, thus potentially reinforcing this clonal population pattern. The ability of *T. gondii* to pass vertically, which occurs across repeated generations in rodents, may also contribute to asexual transmission in the wild (34). In addition to these original three clonal genotypes, more recent studies documented the existence of another clonal lineage in North America (87). Interestingly, strains of this fourth type are most often found in wild animals, and they show evidence of recent genetic exchange with type 2 strains (87). Together with their distribution in wild animals, this suggests that this type may represent the ancestral North American lineage prior to the emergence of the predominant clonal types.

In comparison to strains in North America and Europe, strains of *T. gondii* in South America are much more genetically diverse, lack evidence of a clonal population structure, and show greater evidence of genetic recombination (88, 89). When South American isolates were first genotyped by using RFLP markers developed from northern strains, they appeared similar to type 1 strains or as hybrid strains (90, 91), owing to the fact that they share some ancestral SNPs with the northern lineages (85). Deeper analysis of the patterns of SNP inheritance from sequenced genomic regions revealed that South American strains comprise distinct lineages that are not found in the north (85). Shared nucleotide patterns establish a common ancestry of 1 million to 2 million years, while much more recently derived regional patterns define groups that have evolved in the north versus those that are restricted to the south (85). Analysis of the population structure revealed 6 major clades containing ~16 haplogroups of *T. gondii* (15). These haplogroups show distinct geographical patterns, with some predominating in North America and Europe, others being unique to South America or Asia, and at least one showing a broad distribution globally (15). Network and population structure analyses of the relationships among these strains suggest sporadic gene flow between them at different times in the recent past (15), a conclusion supported by data from genome-wide SNP studies using transcriptome sequencing (RNA-Seq) (92) and whole-genome sequencing of more than 60 diverse lineages (8). These broader patterns are supported by specific examples of recombination in the wild generating hybrid strains (87, 93). The potential for the spread of pathogenicity genes via recombination is an important consideration in South America, where some genotypes have been associated with recurrent ocular disease (56, 57) or severe outcomes and even death in healthy adults (94–96).

Collectively, these studies indicate that the current population structure of *T. gondii* is derived from mosaic patterns of inheritance of blocks of conserved regions of the genome (8). Although these studies have enriched our view of the population structure of *T. gondii*, many regions of the world are still inadequately sampled (i.e., Africa, Asia, and Southeast Asia), suggesting that greater diversity will likely be uncovered by future studies. Importantly, next-generation (NexGen) sequencing studies have revealed that the genome of *T. gondii* is distinguished from its close relatives by the amplification of gene families that encode surface and secretory protein families of pathogenesis determinants (8). The pattern of inheritance of these secretory protein gene families, which occurs in conserved blocks that define the population structure, suggests that they impart important biological attributes to the major lineages. As described further

below, many of these secretory proteins play specific roles pathogenesis, while the function of others remains to be defined.

HOST-PATHOGEN INTERACTIONS

Invasion and Intracellular Survival

Toxoplasma gondii is an obligate intracellular parasite, and host cell entry is paramount to survival. The intracellular phase of the life cycle is followed by active egress from the host cell and then rapid reentry, as the parasite does not divide when it is extracellular (97). The zoites of most apicomplexans show marked apical specialization, with the formation of a microtubule-organizing center that organizes the apical end (6). The apical end also contains a set of secretory organelles termed micronemes (98) and rhoptries (99), which comprise different families of regulated secretory proteins. This apical specialization gives the zoite an elongated shape that imparts physical rigidity for supporting substrate-dependent motility.

Apicomplexan zoites display substrate-dependent gliding motility that relies on an actin-myosin motor complex located beneath the plasma membrane, which translocates adhesive microneme proteins from the apical end toward the posterior end of the cell, similar to a conveyor belt (100). Although the biological details have been worked out primarily for *T. gondii*, a very similar process drives sporozoite and merozoite invasion in *Plasmodium* (101). Motility is dependent on filamentous actin assembly in the parasite (102) as well as MyoA, a class XIV myosin located in the inner membrane complex (103). MyoA is associated with light chains involved in regulation as well as a complex of proteins called the glideosome that helps anchor the motor in the inner membrane complex (104), a system of flattened membranes beneath the plasma membrane (6). The interaction between the cytoplasmic tails of adhesins and the actin cytoskeleton is mediated by a novel connector containing a pleckstrin homology domain and a series of armadillo repeats (105). Although the parasite actin-myosin cytoskeleton is required for entry, and likely provides the majority of force generation, other studies have emphasized a role for host actin remodeling during invasion (106). Many of the parasite motor complex proteins are essential for efficient cell entry, as shown by the dramatic loss-of-function phenotypes when genes encoding these proteins are disrupted. However, it has also been argued that there may be alternative pathways for entry, as these mutants show some residual, albeit limited, ability to enter cells (107, 108). In some cases, the apparent dispensability of genes previously thought to be essential was due to functional redundancy (109) or due to the functional ability of low levels of protein that remain after gene deletion (110). Collectively, these studies support a model where the actin-myosin-based motor complex is of primary importance for efficient cell entry by *T. gondii* and related apicomplexans.

Motility and host cell invasion by apicomplexans are tightly coupled to protein secretion. The secretory system is streamlined and specialized for polarized anterior secretion (111). The endoplasmic reticulum (ER) is continuous with the nuclear envelop, and budding from the anterior region of the nucleus directs vesicles to the single-stacked Golgi apparatus (112). From here, secretory proteins are sorted into specific organelles prior to discharge. In addition to the anteriorly localized rhoptries and micronemes, which have distinct forward-directed sorting signals, a third secretory compartment, called the dense granule (GRA), is dispersed though the cytosol and provides a default pathway for export (111). The endocytic pathway of apicomplexans is highly devolved and serves functions in protein processing for the export of microneme and rhoptry proteins rather than canonical endocytic processes (111). The timing of expression may also be important for sorting, as the genes encoding proteins destined for each compartment are coordinately regulated during the cell cycle (113). Overall, this system provides streamlined processes for the synthesis and export of proteins that are destined for distinct secretory organelles.

During host cell invasion by *T. gondii*, regulated secretion from three different compartments releases the contents of micronemes, rhoptries, and dense granules (Fig. 1) (114). Initially, micronemes secrete their contents from the apical tip upon

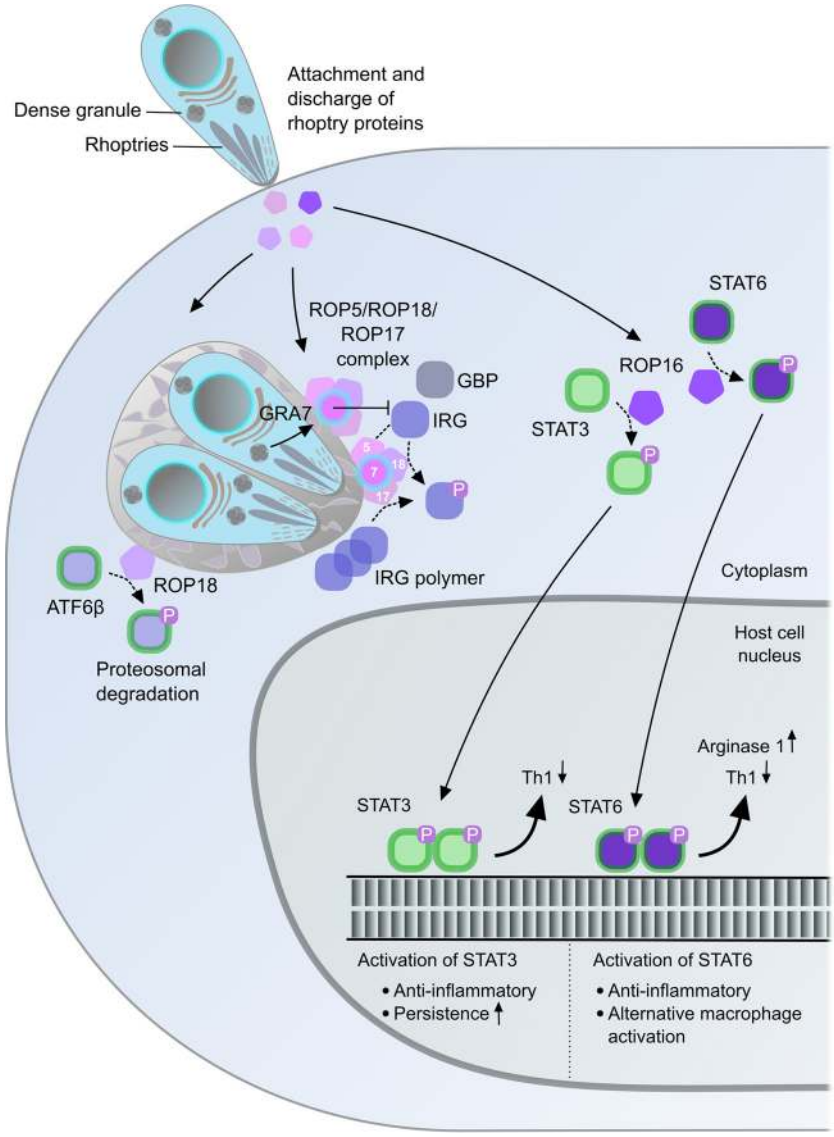

FIG 1 Rhoptry effectors that target host pathways. Following attachment to the host cell, rhoptry (ROP) effector proteins are released into the host cell cytosol prior to the entry of the parasite into the parasitophorous vacuole (PV). ROP proteins are found in the cytosol, traffic to the host nucleus, and also decorate the surface of the vacuole. The secreted kinase ROP18 is assembled on the PV membrane, where it forms complexes with another kinase, ROP17, and the pseudokinase ROP5. By the phosphorylation of IRG monomers/dimers (ROP18) and polymers (ROP17), these kinases prevent the accumulation of IRGs on the PV membrane. The pseudokinase ROP5 binds Irga6 directly and enhances the kinase activity of ROP18. The ROP5/ROP18/ROP17 complex also contains the transmembrane protein GRA7, originating from a parasite organelle named the dense granule. GRA7 can also bind directly to IRG polymers, and it accelerates their turnover. ROP18 has also been shown to phosphorylate the transcription factor ATF6β, marking it for proteosomal degradation. Another secretory rhoptry kinase, ROP16, activates the transcription factors STAT3 and STAT6 by direct phosphorylation, thus altering host transcription.

contact with host cells (115), in a process that is regulated by intracellular calcium (116). Micronemes contain a number of proteins that contain adhesive domains involved in recognizing glycoconjugates on the host cell surface (98). Second, rhoptries secrete proteins from the neck region, so-called RON proteins, which insert into the host membrane to form an anchoring point for the moving junction (117, 118). The contents of the rhoptry (ROP) bulbs are also secreted into the host cell at a very early step in invasion, in some cases releasing proteins directly into the host cytosol as well as the lumen of the parasitophorous vacuole (PV) (119) (Fig. 1). Although the significance of

the release of ROP proteins into the host cytosol was not determined until much later, it was originally suggested that this was a simple way to deliver proteins to the external surface of the PV membrane (PVM) (120). Rhoptry proteins can also be secreted into cells by a noninvading parasite, and hence, the delivery of effectors may alter host cell function even in the absence of invasion (121). The final wave of secretion occurs with the release of dense granule proteins, many of which occupy the lumen of the vacuole and decorate an elaborate array for membranous tubules called the intravacuolar network (122) (Fig. 1). Several GRA proteins are also anchored in the PV membrane and extend at least partly into the host cytosol, where they may interact with host proteins (123–125). GRA proteins also form part of the cyst wall that surrounds bradyzoites (126, 127), and many GRA proteins play important roles at this stage of development despite being dispensable during *in vitro* growth as tachyzoites (128). Recently, is was suggested that there may be more than one population of dense granules based on the fact that GRA proteins that occupy the lumen or PV membrane are released in a bolus early in invasion, while those that traffic outside the PV may be released more slowly over time (129).

During invasion, the PV forms by invagination of host cells, as shown by electrophysiology studies (130) as well as tracers for plasma membrane lipids (131). Despite being formed from the host plasma membrane, the process of forming the PV is fundamentally different from phagocytosis, as it occurs with minimal rearrangement of the host cytoskeleton and independently of Tyr phosphorylation that normally accompanies phagocytic uptake (132). Many host plasma membrane proteins are excluded from the vacuole during entry, and this exclusion is based on physical constraints and lipid partitioning (131, 133). These finding suggest that the moving junction may form a physical barrier for sieving proteins, and this in turn may affect the fate of the vacuole. The *T. gondii* PV is nonfusogenic with host lysosomes and endosomes (134), and it maintains a neutral pH (135). Excluded from contact with the host endomembrane and cytosol, the parasite remains confined within the PV. The parasite likely acquires nutrients by making the vacuole membrane permeable to small metabolites (136).

Innate Immunity in Mice

Laboratory mice have been used for studying infection caused by *T. gondii* based on the fact that rodents are a natural host and thus a reasonable model for studying immune responses and pathogenesis. During initial infection of mice, the parasite rapidly disseminates from the site of inoculation and reaches many tissues in the body (20, 137). Interaction with innate immune cells triggers the production of IL-12 by CD8α dendritic cells (DCs) (138, 139), plasmacytoid DCs (140, 141), macrophages (142), and neutrophils (143). One of the major pathways for trigging IL-12 is the detection of profilin, an actin binding protein, by Toll-like receptor 11 (TLR11) and TLR12 (144, 145). This pathway is highly important for the control of infection, as shown by the susceptibility of MyD88$^{-/-}$ mice (146), which lack the major adaptor for TLR signaling. IL-12 induces the production of IFN-γ, initially from natural killer (NK) cells (147, 148) and later from CD4 (149) and CD8 (150) T cells. The IL-12–IFN-γ axis is critical for controlling infection, as shown by the enhanced susceptibility of mice lacking IFN-γ due to antibody neutralization (151) or genetic ablation of IFN-γ (151), IFN-γ receptors (152), or IL-12 p40 (153). IFN-γ signaling proceeds through STAT1 phosphorylation and translocation to the nucleus to induce a set of interferon-stimulated genes (ISGs) (154, 155). Not surprisingly, STAT1 is also essential for the control of infection in mice (156, 157). However, when infection precedes the activation signal, *T. gondii* is able to block STAT1 signaling, leading to the downregulation of inducible nitric oxide synthase (iNOS) (158) and major histocompatibility complex class II (MHC-II) (159), among other ISGs (160). The mechanism of this block is described further below.

A number of other innate pathways are important for the control of chronic infection but are not essential during acute infection, including tumor necrosis factor alpha (TNF-α) (161), its receptors (162, 163), and iNOS (164). Type I IFN-β plays a much more modest role in the control of infection of the type 2 ME49 strain in mice (165) and

has also been shown to induce modest control in human macrophages (166). Type I interferons may play a greater role in some strains that have been shown to drive very high expression levels *in vitro* (167). Collectively, interferons likely lead to restricted parasite growth through the induction of iNOS, an enhanced respiratory burst, restriction of nutrients such as iron, and upregulation of specific pathways that target intravacuolar pathogens (168). The potent activation of the Th1 responses can also be detrimental to host survival, as shown in models of oral challenge with type 2 strains (169) or during acute challenge with type 1 strains (170, 171), where enhanced levels of proinflammatory cytokines result in pathology. Consistent with this, the induction of a potent Th1 response is modulated by IL-10, the absence of which leads to greater immunopathology (172). IL-27 also promotes regulatory T (Treg) cells that limit Th1 cell-mediated immunity in order to dampen inflammation (173).

Among the earliest and most strongly upregulated interferon-stimulated genes in mice is a family of guanylate binding proteins (GBPs) called immunity-related GTPases (IRGs) (174). The IRG family is expanded in mice, where it is important for the control of *T. gondii* as well as other intracellular pathogens (175–177). Like other GTPases, IRGs cycle between GDP-bound inactive and GTP-bound active forms (178). Normally, IRG proteins are thought to remain sequestered to IrgM proteins, which act as stabilizers by preventing GDP dissociation and thus preventing activation (179). Upon the recognition of a pathogen-containing vacuole, IRGs oligomerize and are recruited to the vacuolar membrane, where they result in vesiculation and stripping of the vacuolar membrane (180). The loss of the vacuole membrane results in the rapid killing of the released tachyzoites (181). It is not certain how pathogen-containing vacuoles are recognized, but several mechanisms have been suggested, including the absence of self, due to a lack of IrgM proteins (182), or altered composition of membrane lipids (183). A second group of immunity effectors that is also upregulated in response to IFN-γ is the large GTPase family known as GBPs (184). GBPs are not recruited directly to the PV but rather cluster in proximity to the PV membrane, where they occupy clusters of membrane vesicles (185). Deletion of GBP1 (185), GBP2 (186), or a locus on chromosome 3 (Chr3), which contains a cluster of six GBPs (187), compromises the control of *T. gondii* in IFN-γ-treated cells *in vitro* and increases the susceptibility of mice to infection. Recent studies suggest that GBPs may be involved in this second step of targeting to the parasite, directly leading to its destruction (188). Strains of *T. gondii* are differentially susceptible to destruction by IRGs (189, 190) and GBPs (185, 191), and as summarized below, this is due to active mechanisms of avoidance.

Autophagy contributes to innate immunity by capturing intracellular pathogens and routing them for destruction by a process called xenophagy (192). In a somewhat different role, the recruitment of IRGs and GBPs to the *T. gondii*-containing PV depends on a core set of autophagy proteins, including Atg7, Atg5, Atg16, and Atg12, but not the upstream activation steps or the degradation part of the pathway (180, 193, 194). In the absence of these core Atg proteins, IRGs and GBPs form aggregates that are spontaneously activated and cannot be recruited to the PV (185, 195). It has been argued that this core group of Atg proteins is needed for homeostasis (185), such that IRGs and GBPs are unstable in the absence of this pathway, similar to the loss of stability in IrgM mutants (179). Alternatively, Atg proteins may be directly involved in the recruitment of the IRG/GBP effectors to the PV membrane, as suggested by the early delivery of LC3 to a portion of susceptible parasite-containing vacuoles (193). Regardless of the exact mechanism, the requirement for Atg proteins in the IFN-γ response represents an intriguing link between innate immunity and cellular homeostasis pathways in host defense.

Innate Immunity in Humans

Humans are relatively resistant to infection despite the occasional occurrence of disease, as described above. Human cells also rely on IFN-γ and STAT1 signaling to control parasite replication *in vitro* (196). Although many of the ISGs regulated by IFN-γ are similar in mouse and human, human cells control intracellular *T. gondii* by very

different mechanisms than those described for mouse cells (197). First, humans lack most IRGs and express only two forms, one of which is constitutively expressed in testis (i.e., IRGC) and the other of which (i.e., IRGM) is truncated and likely does not function in a manner analogous to that in mice (198). Second, although human cells express a wide repertoire of GBPs (199), it has been questioned whether they participate in host defense, as a clustered regularly interspersed short palindromic repeat (CRISPR) deletion of the locus containing GBPs failed to show a role for these proteins in the control of the intracellular replication of parasites in IFN-γ-treated cells (194). However, other reports suggest that some GBPs may contribute to the control of infection in some human cells (200). Nonetheless, it appears that two of the main mechanisms of innate resistance in IFN-γ-stimulated mouse cells are not highly active in human cells. Additional mechanisms that have been described for IFN-γ-treated human cells include the increased production of reactive oxygen species (201), tryptophan limitation due to the upregulation of indole amine oxidase (202), the sequestration of iron (203), and the induction of the NALP1 inflammasome, leading to cell death and the loss of the replicative niche (204). These mechanisms do not appear to act universally in all cell types, and the disruption of any single pathway results in only a partial loss of IFN-γ-mediated control. These features suggest that each pathway may operate in parallel, such that the control of parasite replication depends on their additive contributions. Alternatively, these findings suggest that there are other important mechanisms that operate in human cells.

Autophagy pathways have also been implicated in the control of *T. gondii* infection in human cells, and interestingly, the same core set of ATG5, ATG12, and ATG16 proteins is required. In IFN-γ-treated HeLa cells, type 2 and 3 strain parasites are susceptible to ubiquitination, the accumulation of the adaptors NDP52 and p62, and the recruitment of LC3 (205), a canonical early marker for autophagosomes (206). Type 1 strain parasites avoid this ubiquitination-autophagy recruitment pathway by an unknown mechanism (205). The accumulation of autophagy adaptors and LC3 leads to engulfment of the PV in host membranes and restricted growth of type 2 parasites, although the compartment does not fuse with lysosomes (205). Similarly to the mouse system, this pathway requires pretreatment with IFN-γ, and the upstream activation steps in the pathway (i.e., Beclin1 and Atg14) are not required (205). In addition to the IFN-γ-dependent ATG pathway that has been described for HeLa cells, a lysosome-shunting mechanism that does not rely on ATG proteins has been described for human umbilical vein endothelial cells (HUVECs) (207). Type 2 parasites are susceptible to this IFN-γ-induced pathway, which results in ubiquitination, p62 recruitment, and shunting to lysosomes (207). Additionally, direct ligation of CD40 results in the recruitment of LC3 to *T. gondii*-containing vacuoles, which are subsequently delivered to lysosomes in a manner that is not IFN-γ dependent but relies on autophagy, including the upstream activating steps (208, 209). The CD40 pathway is also important in mice, where it is essential for the control of chronic infections in the central nervous system (210). It has been argued that this pathway may be more important in humans, as genetic mutations in STAT1 do not appear to render humans susceptible to toxoplasmosis, yet mutations in CD154 (the CD40 receptor) cause X-linked hyper-IgM syndrome and result in susceptibility to toxoplasmosis (211).

Defining Pathogenesis Determinants

The mouse is a natural host, and as such, it provides an excellent model for understanding innate and adaptive immunity to *T. gondii* (212, 213). Moreover, differences in strain-dependent phenotypes, combined with forward genetic analysis, have led to an understanding of pathogenicity determinants that act by disrupting the host immune system. These studies have taken advantage of the capacity for genetic crosses along with linkage analysis (214) to identify genes that underlie phenotypes that differ among the major strains types of *T. gondii*. This approach has been exploited to identify genes that mediate acute virulence as well as augment immune signaling (197, 215).

One of the most striking phenotypic differences among strains is their ability to cause lethal infection with a low inoculum. Type 1 strains show a 100% lethal dose (LD_{100}) of a single organism in laboratory mice, independent of the mouse strain, while those of type 2 show intermediate virulence, where LD_{50}s can be defined in outbred or inbred mice, and the highly avirulent type 3 strains typically do not cause lethal infection (68, 216). These differences were analyzed by using genetic crosses between the type 1 GT-1 strain and the type 3 CTG strain, mapping a single quantitative trait locus (QTL) on chromosome VIIa (217). Finer mapping of this locus and transcriptional analysis of genes that were differentially expressed led to the identification of ROP18, a polymorphic secretory protein that encodes a serine/threonine kinase (217). ROP18 is secreted from rhoptries during invasion, and it occupies small evacuoles that are discharged into the host cytosol before becoming associated with the PV membrane (217) (Fig. 1). The kinase activity as well as membrane anchorage are essential for the virulence-enhancing properties of ROP18 (218). Parallel studies also identified ROP18 as one of several QTLs that mediate differences between type 2 strain ME49 and type 3 strain CTG (219). Comparison of the expression profiles of ROP18 revealed that the type 3 lineage underexpresses this protein by ~100-fold, and virulence can be restored by overexpressing either the type 1 or the type 2 allele in the type 3 background (217, 219). Subsequent functional studies revealed that ROP18 targets the IRG family of proteins, phosphorylating conserved threonine residues that lie in switch region 1 of the GTPase domain (220, 221) (Fig. 1). Structural studies indicate that the hydroxyl residues of these threonine residues interact with the phosphate groups of GTP (222). Mutation of these residues to alanine prevents GTP hydrolysis and therefore blocks oligomerization (221). By analogy, it is likely that the phosphorylation of these threonine residues by ROP18 prevents GTP hydrolysis and oligomerization. In cells that express ample levels of ROP18, IRGs fail to accumulate on the vacuole, and the parasite survives, while a low level or absence of ROP18 leads to IRG recruitment and clearance of the parasite. Hence, ROP18 can explain the resistance of type 1 parasites and the susceptibility of type 3 strains to clearance by the IRG pathway.

Genetic mapping of a cross between the highly virulent type 1 GT-1 strain and the intermediately virulent type 2 ME49 strain identified a new QTL on chromosome XII, and subsequent fine mapping revealed a cluster of repeated genes encoding a polymorphic pseudokinase, ROP5 (223). The ROP5 locus was also implicated in phenotypic differences between type 2 and type 3 strains; in this case, the type 3 genotype was associated with virulence enhancement, while that of type 2 was associated with virulence suppression (224). From a genetic standpoint, the combination of alleles at these two loci explains the high-virulence trait of type 1 (both ROP18 and ROP5 are virulence enhancing), the intermediate phenotype of type 2 (ROP18 is virulence enhancing, while ROP5 is inhibitory), and the low virulence of type 3 (ROP18 is underexpressed, while a virulence-enhancing form of ROP5 is present but not sufficient alone). The identification of the biochemical functions of ROP5 helped explain the basis for these phenotypes (Fig. 1). ROP5 acts both to cooperatively enhance the kinase activity of ROP18 (223) and to bind the substrate Irga6 (225), holding it in a conformation that prevents assembly and that facilitates phosphorylation. Extension of these studies to analyses of South American strains revealed that ROP18 is also highly expressed and can rescue the normally nonvirulent type 3 lineage by complementation (226). Most strains from South America have alleles at ROP18 and ROP5 that are related to the type 1 forms (227). A genetic cross between the type 10 VAND strain from South America and the type 2 ME49 strain from North America confirmed the role of ROP18 and ROP5 in acute virulence, and this was further extended by using CRISPR-Cas9 to delete these genes from strains of several additional South American lineages (228).

Overexpression of ROP5 as a tandem affinity-tagged protein in *T. gondii* confirmed that it binds to ROP18 in complex with several other pseudokinases and also led to the identification of a new active kinase called ROP17 (229) (Fig. 1). ROP17 is not highly polymorphic, and hence, it was not mapped in any of the genetic crosses. However, a deletion of ROP17 is synergistic with a loss of ROP18, and the double mutant pheno-

copies the very strong defect of the Δ*rop5* mutant (229). ROP17 prefers to phosphorylate IRG oligomers, accelerating their turnover in the process (229) (Fig. 1). ROP18 and ROP17 have slightly different substrate preferences that are optimized for the two different conserved threonine residues found in many IRG proteins (229). The ROP5-ROP18-ROP17 complex(es) also contains other ROP pseudokinases of unknown function and a dense granule protein, GRA7, that also helps to disrupt IRG function (125) (Fig. 1). GRA7 is a transmembrane protein that spans the PV and extends into the cytosol, where it interacts with the ROP complexes (125). GRA7 also binds directly to IRGs, and it accelerates their assembly and GTP hydrolysis *in vitro* (125). Collectively, this complex of parasite secretory proteins targets the IRG system by accelerating turnover (GRA7 and ROP17), binding to IRG monomers to prevent assembly (ROP5), and phosphorylating IRGs to prevent their assembly (ROP17 and ROP18) (Fig. 1). ROP18 and ROP5 are also implicated in resistance to GBPs in mice (185), as is the pseudokinase ROP45 (230). The level of complexity exhibited by this set of parasite effectors speaks to the importance of thwarting the IRG and GBP host defense systems in mouse for the survival of *T. gondii*. Furthermore, evidence that a large GTPase of this family can overcome the ROP5 complex of normally virulent type 1 strains, leading to resistance to infection in naturally resistant wild house mice (231), supports a model of coevolution of virulence factors and host defense mechanisms.

Despite the importance of the ROP5-ROP18-ROP17 complex(es) in thwarting innate immunity in IFN-γ-activated mouse macrophages, these effectors appear to play little role in human cells (227). This difference likely reflects the fact that human cells express few IRGs and that GBPs may not play a major role in IFN-γ resistance. Nonetheless, there is some evidence that ROP18 alleles correlate with the severity of ocular disease in Colombia, and the type 1 allele is associated with greater inflammation (232). ROP18 has also been shown to target ATF6β, a transcription factor that is part of the unfolded-protein response, resulting in decreased antigen presentation by DCs to $CD8^+$ T cells in mice (233). Hence, ROP18 may have other roles in adaptive immunity that are also relevant for human toxoplasmosis.

TOXOPLASMA EFFECTORS THAT HIJACK HOST GENE EXPRESSION

From ROP Effectors to New Roles for GRA Proteins

Over the last decade, a wealth of studies has established that once intracellular, *T. gondii* actively reprograms the gene expression of its host cell by subverting host cell transcriptional machinery. To achieve this end, *T. gondii* has designed an arsenal of molecular hijackers that take control of host cell gene expression. With this repertoire of molecular weapons, it can target gene expression at both the transcriptional and posttranscriptional levels by regulating the amount of mRNAs encoding proteins and affecting noncoding RNAs, e.g., microRNAs, respectively (234, 235). An extra layer of complexity comes with the specificity in the reshaping of gene transcription for given host cell types and parasite strains. For instance, when macrophages and dendritic cells are infected by the same *T. gondii* strain type, they undergo distinct programming, while two different strain types trigger distinct transcript patterns in the same host cell type (236).

Initial studies focused on differences in transcriptional responses following infection of human or murine cells with different strain types. For instance, the use of gene expression profiles to map the pathways that were differentially induced led to the identification of the secretory ROP kinase ROP16 that targets STAT3 and STAT6 (237) (Fig. 1). The phosphorylation of STAT3 and STAT6 results in their activation and transcription of a number of genes, including IL-4, polarizing the response to Th2 while downregulating IL-12 production (237). Strain types 1 and 3 share a highly similar allele of ROP16 that is highly active in phosphorylating STAT3 and STAT6, and the difference in activity from the inactive type 2 allele was subsequently mapped to a single polymorphic residue (238). *T. gondii* preferentially targets cell-specific transcription factors that act as coordinating hubs in host defenses (i.e., NF-κB, interferon regulatory factor [IRF], and JAK/STAT) by regulating intrinsic activities and expression levels, which

is often achieved through differential phosphorylation (239–242). Additionally, the parasite can also drive changes in the host epigenetic landscape by co-opting chromatin-modifying enzymes to selectively switch on/off gene transcription (160).

Identifying the parasite-derived molecular switches or effectors at play during the reprogramming of gene transcription in infected cells and understanding further their mode of action once delivered into the host cell cytoplasm are highly challenging tasks. Rhoptry organelles known to release products concomitantly with cell invasion were initially identified as the main source of such effectors (197). Hence, the recent discovery of a still increasing repertoire of GRA proteins with host-modulating activities expanded our understanding of host-pathogen interactions. Unlike ROP effectors that are released in a burst during invasion (114), some GRA effectors are released in the host cell over the entire period of *T. gondii* intracellular development. Below, we review *T. gondii* GRA effector proteins that have convincingly been shown to contribute to the building of functional networks in infected cells by interfacing with the host signaling pathway or co-opting host transcription factors.

The Vacuole-Restricted GRA Effectors

The discovery of GRA15 and its extravacuolar activity defined a new class of effectors beyond the previous functions of GRA proteins that contribute to the biogenesis and maturation of the PV and to nutrient acquisition (Fig. 2). Indeed, GRA15 from the type 2 background was shown to decorate the PV membrane following secretion and to subsequently cause the activation and nuclear translocation of host p50/p65 NF-κB heterodimers, promoting the release of proinflammatory cytokines, including IL-12 (243). NF-κB activation is dependent on TRAF6 and the IκB kinase (IKK) complex but is not dependent on MyD88 and TRIF (243). Type 1 strains drive an opposite pattern in that they express a form of ROP16 that contributes to the suppressive effects of *T. gondii* infection on lipopolysaccharide (LPS)-induced cytokine synthesis in macrophages (244) (Fig. 1). Together, these two effectors determine the polarization of macrophages, with the type 1/3 form of ROP16 contributing to the induction of alternative activation, while the type 2 form of GRA15 drives classically activated macrophages (236). GRA15 has also been implicated in inducing IL-1β production by human cells through the activation of caspase 1 (245, 246).

The mechanism by which GRA15 from the type 2 background activates NF-κB has yet to be determined. The diversity of the NF-κB family members, which in mammals include five proteins, p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1), and p100/52 (NF-κB2), that can further assemble into a combination of homodimeric and heterodimeric species, provides high selectivity in the NF-κB-mediated transcriptional response. Despite the fact that p65 and p50 translocate to the nucleus in a GRA15-dependent manner (243), their relocation did not contribute to altering the levels of miR-146a and miR-155, two NF-κB-dependent microRNAs (234). On the other hand, miR-146a expression is impaired specifically in *c-Rel*$^{-/-}$ mice (247). Additionally, c-Rel is activated upon *T. gondii* infection but in a GRA15-independent fashion (234), thereby suggesting that an effector(s) other than GRA15 could account for the activation of specific members of the NF-κB family, including c-Rel. The subversion of the NF-κB pathway by *T. gondii* remains to be clarified, since type 1 strains were claimed to transiently block NF-κB nuclear translocation regardless of the host cell infected (248, 249), while type 2 strains have opposite effects through the activity of GRA15 (243) and additional as-yet-unidentified factors.

GRA6 is another secreted protein that displays a vacuole-restricted localization and activates the host transcription factor NFAT4 (nuclear factor of activated T cells 4) in a strain-specific manner, which promotes the synthesis of the chemokines Cxcl2 and Ccl2 (250) (Fig. 2). These chemokines attract inflammatory monocytes and neutrophils to the infection site, where they control parasite spreading (250). NFAT activation requires a conformational change that allows the exposure of the nuclear localization signal (NLS) and subsequent NFAT nuclear translocation. Although the dephosphorylation of specific serine residues by calcineurin is responsible for this change, *T. gondii* GRA6 was

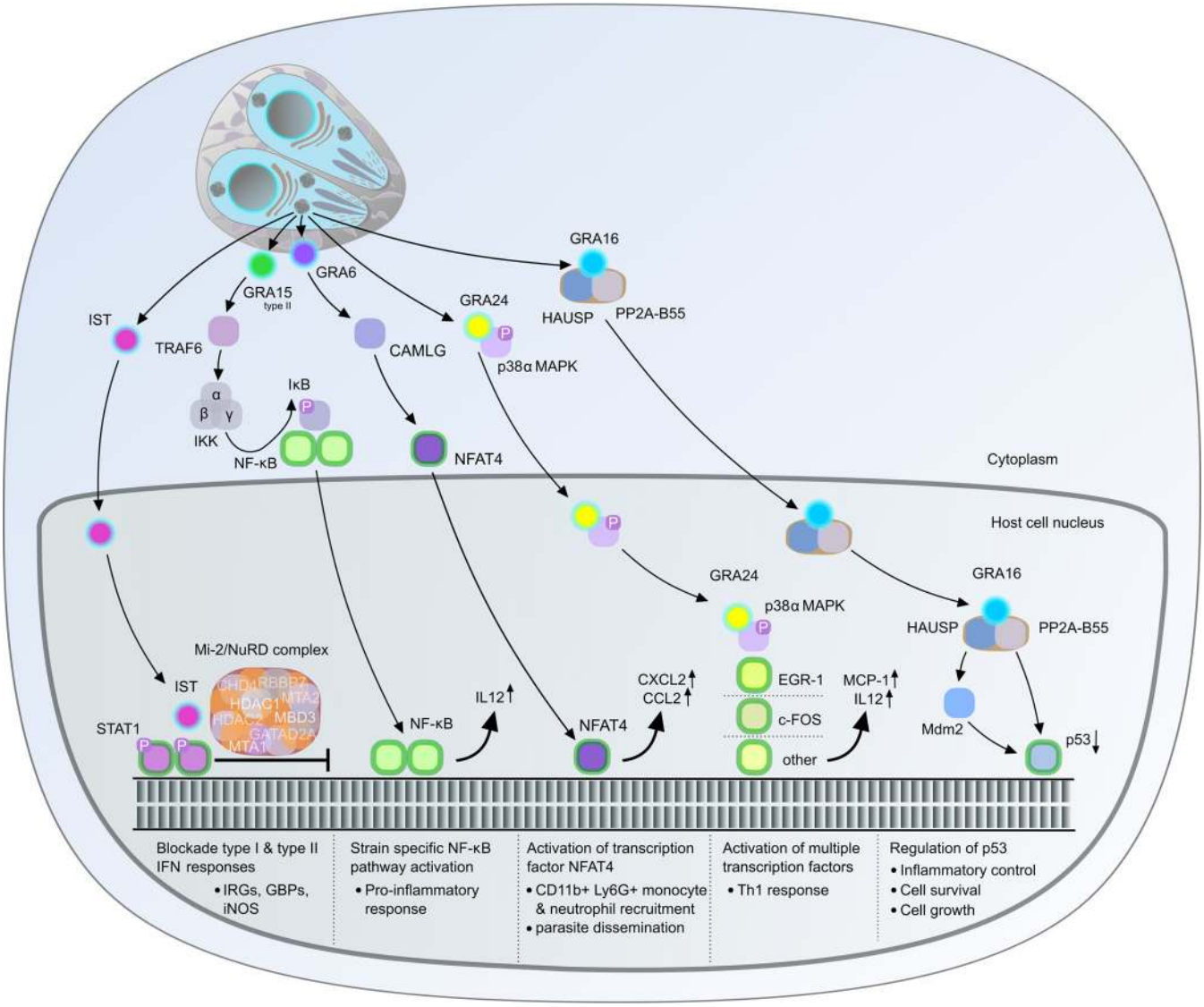

FIG 2 Secreted *T. gondii* effectors from dense granules transform host cell signaling pathways. After invasion of the host cell, *T. gondii* uses a large variety of effector proteins originating from dense granule organelles to manipulate host signaling pathways and gene expression. Some of these proteins translocate to the host nucleus (TgIST, GRA24, and GRA16), while others localize to the PV membrane (GRA15) or only partially interact with the host cell cytosol while residing in the PV (GRA6). Present in all *T. gondii* type stains, the dense granule protein TgIST globally blocks the interferon (IFN) response by the recruitment of the Mi-2/NuRD repressor complex to STAT1 binding sites in promoter regions of responsive genes. GRA24 bypasses the classical MAPK phosphorylation cascades by forming a complex with p38α, which is able to activate transcription factors such as EGR1 and c-Fos. GRA16 shuttles to the host nucleus while bound to a high-molecular-weight complex, including PP2A-B55 and HAUSP, to control p53 levels. In type 2 strains, GRA15 activates the NF-κB pathway by the activation of TRAF6, which subsequently activates IKK, leading to the phosphorylation and degradation of IκB. GRA6 has a vacuole-restricted location, and its cytosolic region interacts with CAMLG to activate calcineurin and stimulate the transcription factor NFAT4.

shown to promote the activation of the phosphatase by a direct interaction with the calcineurin activator calcium-modulating ligand (CAMLG) (250).

GRA16: Beyond the Vacuole Space

The PV membrane has been regarded as a sieve limiting the delivery of proteins secreted by the parasite beyond the vacuolar space. However, the discovery of GRA16 and its remarkable ability to cross the PV membrane and to accumulate in the host cell nucleus has changed this paradigm (Fig. 2). GRA16 was shown to traffic to the host cell nucleus together with a high-molecular-weight complex connecting the host phosphatase PP2A-B55 and the herpesvirus-associated ubiquitin-specific protease (HAUSP) (251). Through its interactions with HAUSP, GRA16 provokes alterations in steady-state protein levels of the tumor suppressor p53, while it induces the nuclear translocation

of the PP2A holoenzyme. GRA16 positively modulates the expression of host genes involved in metabolism, cell cycle progression, and the p53 tumor suppressor pathway (251).

The transcription factor p53 normally turns over rapidly, and it is maintained at low levels in normal cells by Mdm2-mediated ubiquitination and proteolysis. The stabilization of p53 in response to oncogene signaling is thought to result from deubiquitination by HAUSP. This pathway is also targeted by virus infection, as illustrated by the Epstein-Barr virus protein EBNA1, which sequesters HAUSP from p53 and leads to its degradation (252). GRA16 acts in an opposite manner by markedly increasing p53 levels in a HAUSP-dependent manner (251). Other studies show that p53 is also an important sensor of metabolic stress. For instance, upon glutamine deprivation, p53 is activated to support cell survival in a B55a-dependent manner (253). It is probably not a coincidence that GRA16 binds to PP2A-B55 and promotes its nuclear translocation, nor is it a coincidence that glutaminase 2, a p53 target gene involved in glutamine metabolism, is regulated by GRA16 in infected cells (251). Collectively, these results support a role for GRA16 in promoting host cell survival under stress conditions by simultaneously forming a complex with both HAUSP and PP2A-B55 to control p53 protein levels.

GRA24 and Molecular Mimicry

GRA24 shares with GRA16 the ability to reach the host nucleus and to regulate gene expression (Fig. 2). GRA24 acts as a parasite-derived agonist that bypasses the classical mitogen-activated protein kinase (MAPK) phosphorylation cascade and induces sustained p38α autophosphorylation, forming a complex that is able to activate transcription factors such as EGR1 or c-Fos (254). Therefore, GRA24 elicits a strong inflammatory response by turning on the production of proinflammatory cytokines, in particular CCL2/monocyte chemoattractant protein 1 (MCP-1) and IL-12, that enhance macrophage phagocytic activity at the site of infection and accordingly limit parasite burden (254). GRA24 is an intrinsically disordered protein (IDP) that operates through two atypical kinase-interacting motifs (KIMs), which combine attributes of docking domains from multiple MAPK partners to maximize binding. GRA24 is capable of binding, scaffolding, allosterically activating, and translocating p38α MAPK to the nucleus. GRA24 interacts with two molecules of p38α via KIMs in its C terminus. The binding of KIM1 to p38α alters the kinase domain conformation to activate the kinase. As shown by small-angle X-ray scattering and atomic force microscopy, GRA24 scaffolds two molecules of p38α in a flexible manner but with enough proximity to enable autoactivation via transphosphorylation (255). By adapting the KIM motif to bind to p38α in a way that provides sustained activation while preventing the binding of regulatory phosphatases (255), GRA24 is a prime example of how molecular mimicry contributes to host-parasite relationships.

GRA24 was also found to trigger the activation of a gene network under the control of the transcription factor CREB, but how it functions remains an open issue, since the phosphorylation of CREB Ser133 correlated with *T. gondii* infection but was independent of the GRA24/p38α pathway (254). CREB-Ser133 phosphorylation is involved in the recruitment of the histone-modifying enzymes CBP (CREB binding protein) and its paralog p300, which in turn were shown to facilitate transcription by catalyzing histone acetylation (256). Because GRA24 copurified with both CBP and p300 (M.-A. Hakimi, unpublished data), it is plausible that GRA24 shortcuts the phospho-CREB activation step and operates directly to remodel the chromatin structure in the vicinity of CREB-regulated genes.

TgIST Modifies Host Chromatin and Acts as an Epigenator

As detailed above, the cytokine IFN-γ acts at the frontline of defense against *T. gondii*. Early studies proposed that *T. gondii* counters this defense by remodeling the host cell to be unresponsive to IFN-γ at the transcriptional level in both humans and mice (240). Next, it was convincingly argued that *T. gondii* infection inhibits STAT1

transcriptional activity by blocking nuclear-cytoplasmic cycling (241). Only recently has the missing link between the two layers been elucidated with the discovery of another protein stored in dense granule-like organelles, which was identified as *T. gondii* inhibitor of STAT transcription (TgIST), based on its negative regulatory activity on the IFN-γ-dependent signaling pathway (257, 258) (Fig. 2). This pathway starts when a signal is transduced through the IFN-γ receptor and successively leads to the phosphorylation of STAT1 on the Y701 residue (Y701-P), the dimerization of STAT1, and its nuclear translocation. Nuclear STAT1 then regulates gene expression by binding to gamma-activated sequence (GAS) elements in the promoters of genes that respond to IFN-γ (e.g., IRF1). The transcriptional activity of STAT1 increases with a second independent phosphorylation event on S727. The dual Y701-S727 phosphorylation of STAT1 that typifies the chromatin-bound pool of STAT1 (259) promotes chromatin opening through a partnership with enzymes such as histone acetyltransferase (HAT) and p300/CBP, which together stimulate gene expression (260).

In cells infected by *T. gondii*, TgIST translocates across the parasitophorous vacuole and accumulates in the host cell nucleus, where it binds firmly to both activated STAT1 Y701-P and chromatin-modifying proteins found in the nucleosome-remodeling and deacetylase (NuRD) complex (257, 258) (Fig. 2). This complex contains the chromatin-remodeling ATPase (CHD3 and CHD4) and deacetylation (histone deacetylase 1 [HDAC1] and HDAC2) enzymes and the transcriptional corepressors C-terminal binding protein 1 (CtBP1) and CtBP2 (257, 258). In the context of *Stat1*-deficient U3A cells, the association of TgIST with NuRD and CtBPs was shown to be STAT1 independent, suggesting that TgIST bears distinct domains for binding to NuRD/CtBP and STAT1. Moreover, on the basis of assays using IRF1 mRNA and protein levels to monitor STAT1-mediated transcription, TgIST is the primary parasite protein responsible for inhibiting the STAT1-dependent responsiveness of the host cell to IFN-γ (257, 258). Upon IFN-γ stimulation, STAT1 nuclear relocation occurs normally, but this host transcription factor remains silent due to its sequestration with the NuRD complex by TgIST. Intriguingly, TgIST promotes STAT1 Y701 phosphorylation and nuclear translocation in the absence of IFN-γ treatment (257, 258). The ectopic expression of TgIST was sufficient to trigger this unusual IFN-γ-independent STAT1 Y701 phosphorylation, most likely through the recruitment of host kinases qualified to shortcut the JAK/STAT pathway by TgIST (257). Of note, in the absence of IFN-γ stimulation, *T. gondii* infection still drives the phosphorylation of S727 and subsequent STAT1 nuclear translocation in a TgIST-dependent fashion (257), suggesting that a chromatin-bound pool of STAT1 could be present in the vicinity of IFN-γ-inducible genes. Chromatin immunoprecipitation-quantitative PCR (ChIP-qPCR) analysis provided such evidence, since dual Y701-S727 phosphorylation of STAT1 was found to be markedly enriched at GAS-containing promoters under these conditions (257).

Despite detailed studies demonstrating that TgIST mediates the transcriptional repression of IFN-γ-inducible genes, the precise molecular mechanism for this alteration remains uncertain. HDAC enzymes embedded in the NuRD complex to which TgIST binds were first thought to be involved, but HDAC inhibitors targeting both class I and II enzymes were inefficient at preventing TgIST from inhibiting the IFN-γ-induced expression of IRF1 (257), as previously reported (241). Meanwhile, histone modification profiling led to discordant results, pointing to the need to investigate TgIST properties further. Indeed, while Gay et al. (257) reported that TgIST was not involved in modulating the acetylation state of histones, Olias et al. (258) found that *T. gondii* infection significantly reduced the acetylation status of histone H3 at HLA-E and GBP1 loci in a TgIST-dependent fashion. An alternative hypothesis would be that TgIST-bound HDACs compete with HAT to prevent STAT1 acetylation and DNA dissociation, thereby compromising STAT1 recycling, in line with a model proposed by Krämer and Heinzel (261). Assuming that HDACs might not be involved, the NuRD-associated ATP-dependent chromatin-remodeling enzymes CHD3 and CHD4 could play a critical role in TgIST-mediated transcriptional repression by influencing nucleosome positioning to create a

nonpermissive chromatin state (262). Further studies will be required to unravel these complexities.

Further profiling of histone modifications in *T. gondii*-infected cells revealed that H3K4me3, a hallmark of activation, was sustainably enriched at repressed STAT1 binding loci in a TgIST-dependent fashion and regardless of IFN-γ stimulation (257). Remarkably, this paradox mirrors those of stem cells that are typified by a repressive H3K27me3 (histone 3, lysine 27, trimethyl mark) combined with an activating H3K4me3 (histone 3, lysine 4, trimethyl mark) that provides a bimodal signature to silence developmental genes while keeping them poised for rapid activation (263, 264). If the analogy is pushed further, TgIST could be considered an epigenator, according to the definition of Berger et al. (265), suggesting that it acts as a repressive "memory mark."

At the cellular level, TgIST plays its major role early during infection by protecting the first wave of invading tachyzoites within naive cells by blocking potent ISG-mediated parasite killing (257, 258). However, this STAT1 silencing mechanism loses efficiency when myeloid cells are already primed by previous exposure to IFN-γ (258). This time-restricted activity of TgIST is consistent with the control of parasite expansion, although not complete clearance, as shown when mice were infected with TgIST-deficient *T. gondii* (257, 258). Hence, while TgIST can protect parasites within naive cells, there are other STAT1-dependent pathways that overcome this block and that are required to control toxoplasmosis (152, 156, 266).

Patterns That Emerge from GRA Effectors

Although there are as yet only a few GRA effectors characterized, we can make some general conclusions about their modes of action. First, there are two classes of effectors, those acting locally, near their secretion site (i.e., GRA6 and GRA15), and those acting at a longer distance, with the host nucleus as a final destination (i.e., GRA16, GRA24, and TgIST). Second, although the former effectors seem to interfere indirectly with their dedicated pathways, the latter ones seem to form hyperstable interactions with host proteins and to reshuffle the host interactome by gathering together enzymes/proteins that are usually not associated in uninfected cells. For instance, there is no precedent for any interaction of the NuRD complex with STAT transcription factors. Finally, different proteins can operate in effector communities by the convergent targeting of a common host cell pathway, for instance, M1 activation by GRA15 (243) and GRA24 (254). These effectors may adopt at least three alternative, although not mutually exclusive, strategies to subvert host gene expression. They may (i) modulate upstream signaling pathways (i.e., GRA6 and GRA24), (ii) directly target host transcription factor protein levels/activity (i.e., GRA15, GRA16, and TgIST), and/or (iii) affect histone packing and chromatin configuration (i.e., GRA24 and TgIST). However, and unlike the transcription activator-like (TAL) proteins secreted by phytopathogenic *Xanthomonas* and *Ralstonia* species (267), none of the *T. gondii* nucleus-targeted proteins described so far were able to mimic eukaryotic transcription factors and to bind directly to host cell DNA.

PROTEIN TRAFFIC WITHIN AND BEYOND THE VACUOLE

Protein Order and Function: Disordered To Conquer

The high abundance of intrinsically disordered regions (IDRs) combined with short linear motifs (SLIMs) in dense granule-derived effectors strongly suggests positive evolutionary selection of these structural features (268). The secretion of IDR proteins across membranes does not require active unfolding and thus would be advantageous. Additionally, structural flexibility may have other advantages beyond the ease of secretion and trafficking. In the context of the coevolutionary arms race between host and pathogen, the evolutionary time scales of the IDR are shorter than those required for globular domains (e.g., DNA binding domains) (269–272). IDR proteins exhibit higher rates of point mutations and repeat expansions than do folded proteins, and these properties could facilitate the evasion of immune recognition. Part of the effector strain specificity could stem from the evolution of their internal SLIM. For instance,

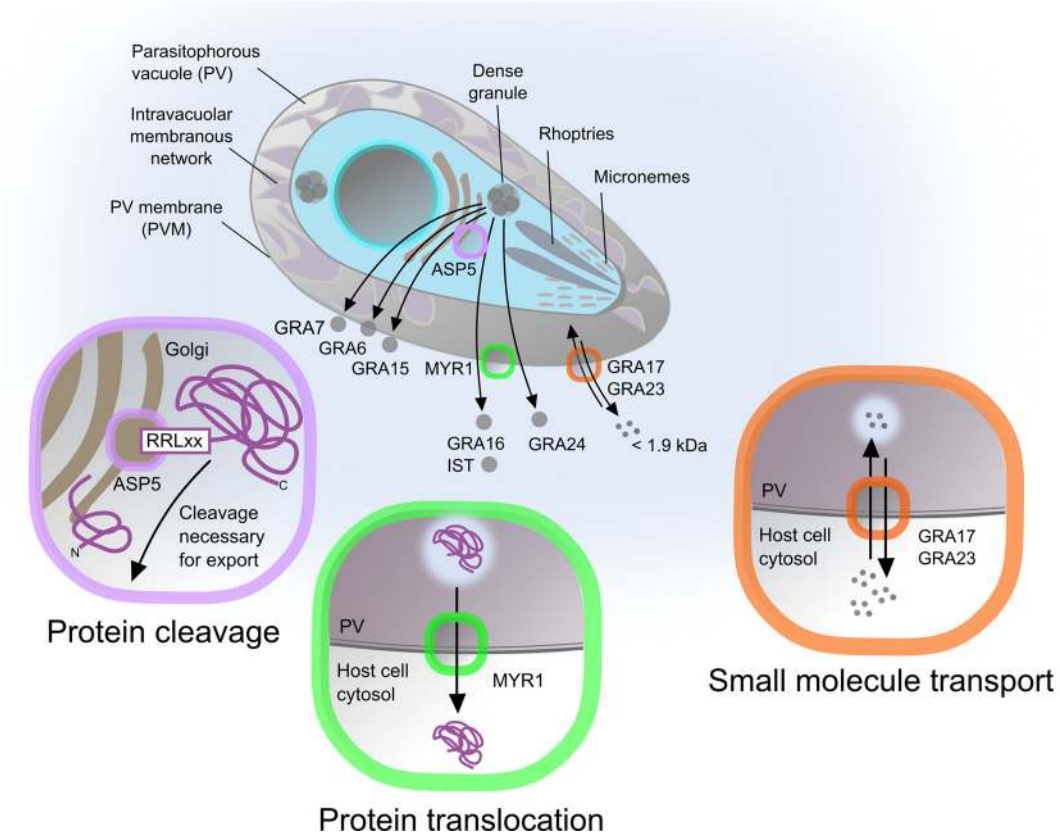

FIG 3 Mechanisms of protein export and traffic beyond the PV. The aspartyl protease ASP5 is situated at the Golgi apparatus of the parasite. The cleavage of some (i.e., GRA15, GRA16, and TgIST) but not all (i.e., GRA24) dense granule proteins in their recognition signal by ASP5 is necessary for the export of these proteins. MYR1 is part of a protein complex located at the PV membrane, where it is involved in the export of intrinsically disordered dense granule proteins across the vacuole membrane and into the host cytosol. A number of these export substrates are processed by ASP5. Two additional dense granule proteins, GRA17 and GRA23, which are also located at the PV membrane, are responsible for small-molecule transport between the host cytosol and the vacuole lumen.

C-terminal polymorphisms on GRA6 control strain-specific NFAT4 activation (250). Furthermore, IDR proteins are able to accommodate multiple protein partners and thereby maximize functional complexity with a reduced parasite "effectome." While mimicking host cell proteins, parasite effectors can gain efficacy over their native counterparts at modifying host cell signaling pathways, as demonstrated by GRA24 (255). Studies of these dynamic regions and their quick evolution to optimize their function inside host cells as a pathogenic scaffold/hub may help predict new parasite effectors that are yet to be identified. We have attempted to highlight a few examples that illustrate how some degree of disorder is required for parasite effectors to perform their prescribed functions in the infected cell, and this feature may also influence their trafficking. Such features are not restricted to parasites, as IDR proteins are quite common in eukaryotic proteomes. For instance, IDR proteins are frequently observed in nucleic acid binding proteins and in the proteins that interact with them (273). Going forward, studies of these IDR proteins will aid in gaining general insight into how a protein's flexibility enables its function.

Comparisons to *Plasmodium* Export

Communication between the parasite and the host cell takes place across the PV membrane, which is porous to small molecules (136) but resistant to fusion with endomembranes of the host (168) (Fig. 3). The current molecular understanding of export beyond the PV is fueled mainly by *Plasmodium* studies, which revealed that exported proteins traffic through the secretory pathway and are exported to the

vacuolar lumen before crossing the PV membrane (reviewed in reference 274). The majority of these proteins are typified by a signal peptide for ER entry followed by a conserved sequence motif (RxLxE/Q/D), referred to as the host targeting (HT) motif or the *Plasmodium* export element (PEXEL), which is required for export across the PV membrane into the host cell. The HT/PEXEL motif is recognized by a wide repertoire of known *Plasmodium* proteins by the ER-resident aspartyl protease plasmepsin V (PMV), which cleaves after the leucine, followed by N-terminal acetylation (reviewed in reference 274). Once posttranslationally modified, these proteins have all the attributes to be targeted by a parasite translocon located at the PV membrane, named *Plasmodium* translocon of exported proteins (PTEX). Although the PV membrane represents a barrier that exported proteins must cross before entering the host cytoplasm, protein unfolding is also necessary for HT/PEXEL proteins to cross the vacuolar membrane before being refolded and trafficked to their final destination, implicating an ATP-powered step in export (reviewed in reference 274). In line with these requirements, the PTEX complex was described as a multiple-protein complex, including a chaperone, HSP101, which facilitates the translocation process; thioredoxin 2, which reduces disulfide bonds; and a single-membrane protein named EXP2, which is predicted to form a protein-conducting channel (reviewed in reference 274).

Trafficking of GRA Proteins in *T. gondii*

Initially, the export mechanism was thought to be phylogenetically conserved across the phylum. Nonetheless, while the translocon that mediates the transport of proteins across the PV membrane remains enigmatic, the recent discovery of exported GRA proteins offers new insights into trafficking through the PV membrane in *T. gondii*. An early study identified sorting signal sequences reminiscent of the HT/PEXEL motif in the *T. gondii* GRA19, GRA20, and GRA21 proteins (275). However, while their motifs were proteolytically processed, they do not cross the PV membrane and are instead relocated to the PV membrane (276). Meanwhile, GRA16 and GRA24 offer more suitable molecular markers to monitor protein export and to evaluate whether the export machinery is similar to the *Plasmodium* system.

The role of ASP5, the *T. gondii* homolog of *Plasmodium* plasmepsin V, has recently been assessed (Fig. 3). In the absence of ASP5, the *T. gondii* proteins GRA19 and GRA20 fail to localize to the PV membrane. Notably, in Δ*asp5* mutants, GRA16 and GRA24 were no longer exported in the host cell nucleus but were retained in the vacuolar space (276–278). The HT/PEXEL-like motifs of GRA16, GRA19, and GRA20 were shown to be directly processed by ASP5 (276–278). Unexpectedly, GRA24, which does not have a conserved HT/PEXEL motif, was processed in an ASP5-independent fashion, although ASP5 was required for its export (278). An alternative pathway for the export of the HT/PEXEL-negative exported proteins (PNEPs), which involves a different protease(s) than PMV, has been described in *Plasmodium* (reviewed in reference 274) and may also exist in *T. gondii*. The export of TgIST required maturation by ASP5; however, whether its predicted HT/PEXEL motif corresponds to a direct cleavage site for ASP5 remains to be determined (257).

Unlike *Plasmodium* PMV, ASP5 is not essential, but its deletion triggers a decrease of parasite fitness and multiple phenotypes, including the loss of the intravacuolar network, and impairment of host mitochondrial recruitment at the PVM (276, 277). As expected, *asp5*-deficient parasites showed a greatly diminished ability to modulate host cell gene expression and were more susceptible to immune responses during infection (276, 277). As predicted by their pleiotropic phenotypes, *asp5*-deficient parasites were severely attenuated in a murine model of infection (276, 277).

The HT/PEXEL motif does not sufficiently typify the *T. gondii* exported GRA proteins identified so far. However, structural analysis revealed that, unlike ROP proteins, they are predicted to be devoid of a known catalytic domain (e.g., kinase domain) and are natively unfolded (268), as illustrated by GRA24 (255). Their intrinsically disordered nature (i.e., the lack of a stable tertiary structure) would be of an inherent advantage for PV membrane crossing, as it would not require active unfolding. Support for this

hypothesis was provided by the observation that epitope tagging of either GRA16 or GRA24 with fluorescent proteins (mCherry or green fluorescent protein [GFP]) inhibited PV membrane crossing, yet similar reporter proteins are routinely used in *Plasmodium* studies. In agreement with these data, it was shown that applying structural constraints to GRA16, for example, by fusing any folded protein fragments (e.g., dihydrofolate reductase [DHFR]) to the protein, led to an impairment of its export outside the PV membrane (278). Conversely, the addition of a disordered protein fragment to GRA16 did not alter the trafficking of the protein to its final destination (278). More importantly, the size of the polypeptide does not limit progression through the membrane, as illustrated by GRA28, a disordered and high-molecular-mass (>200-kDa) protein that crosses the PVM to accumulate in the host cell nucleus (279). These data contrast deeply with those for *Plasmodium* showing that protein unfolding is necessary during the export of proteins bearing PEXEL and PNEP motifs and that HSP101 provides the power source for unfolding (reviewed in reference 274). Thus, *T. gondii* has independently evolved an elementary export machinery that preferentially accommodates disordered proteins as a way to save energy and most likely to evolve SLIM-mediated interactions with the host cell.

Transport Complexes in the PV Membrane

If protein folding is not a limiting factor for export, the question of the existence of a protein-conducting channel remains. *Plasmodium* EXP2 was suspected to oligomerize and to form a pore based on similarity to *Escherichia coli* hemolysin E in modeling studies. Phylogenetic analysis revealed that *T. gondii* encodes two proteins with homology to EXP2, namely, GRA17 and GRA23, both of which localize to the PV membrane and, when expressed in *Xenopus laevis* oocysts, can form a large membrane pore similarly to hemolysin (280) (Fig. 3). Their deletion in *T. gondii* resulted in abnormal morphology with swelling of the PV (280). This phenotype was explained by their ability to mediate the transport of small molecules (<3,000 Da) but not protein export across the PV membrane, as neither Δ*gra17* nor Δ*gra23* mutants altered the export of GRA16 or GRA24 to the host cell (280). Interestingly, *Plasmodium* EXP2 functionally fully complements solute transport that is reduced in a *T. gondii gra17*-deficient mutant, raising the possibility that EXP2 may play a dual role as a nutrient pore and a protein channel (280).

The *Plasmodium* model of protein translocation through the PV membrane is evidently challenged by insights from *T. gondii*, whether it concerns the requirement of a PEXEL addressing signal, ATP-powered unfolding, or an EXP2-forming pore for a *T. gondii* GRA protein to cross the PV membrane. Therefore, simple comparative analogies may not readily reveal the diversity of protein export mechanisms present in this phylum. In this regard, a genetic screen originally set up to identify the effector protein responsible for c-Myc induction by *T. gondii* led to the identification of MYR1 and an alternative translocation pathway (281) (Fig. 3). MYR1 is processed by ASP5 into two stable portions (277), both of which are located in the PV and associate with the PV membrane (281). The export of GRA16, GRA24, and TgIST was impaired in cells infected by *myr1*-deficient parasites, while MAF1 and GRA15 functions were not altered, suggesting that MYR1-dependent export is devoted only to dense granule proteins that physically translocate across the PV membrane and accumulate in host cell compartments (281). This is a major difference from ASP5, which embraces a wider repertoire of GRA proteins. Phylogenetic analysis revealed that no convincing homolog of MYR1 was detectable in *Sarcocystis*, a coccidian that lacks a PV but instead develops in the host cell cytoplasm, or in the more distantly related genera *Eimeria* and *Plasmodium*, suggesting that the MYR1 pathway is specific to a subset of tissue cyst-forming coccidia.

ADDITIONAL PATHWAYS ALTERED BY *T. GONDII* INFECTION

In addition to the examples cited above, there are several host transcription factors whose activities are regulated by *T. gondii* infection but for which no effectors have yet

been identified. Infection by *T. gondii* activates hypoxia-inducible factor (HIF), and this pathway is important for the optimal growth of the parasite under hypoxic conditions (282). Although upstream regulators of this pathway have been identified, including activin-like receptor kinase (283), the parasite mediator that triggers this pathway remains uncharacterized. Additionally, *T. gondii* induces the phosphorylation of CREB-Ser133 and ATF2-Thr71, and this occurs independently of GRA24 (254). The parasite also promotes the induction of c-Myc, an unknown effector that relies on MYR1 for export (281), and EGR2 (284), and these responses are downstream of the serum response factor, which is triggered by *T. gondii* infection (242). Infection is also associated with the noncanonical activation of mTOR and the phosphorylation of the ribosomal protein S6 (285).

Host cells are equipped with several pathways for inducing cell death, including apoptosis and pyroptosis, and these pathways often function in host defense. Infection by *T. gondii* blocks both intrinsic (i.e., cytotoxic stress and DNA damage) and extrinsic (i.e., death receptor activation) pathways triggering apoptosis (286–292). Although the ability to block cell death may be important for ensuring a stable intracellular niche, the effectors that disrupt this pathway have not been identified. Infection by *T. gondii* has also been described to activate inflammasome activation in murine (245), rat (293), and human (204) cells. Although GRA15 from type 2 strains contributes to this pathway by activating NF-κB and inducing IL-1β induction in human cells (246), the second signal that directs inflammasome activation remains uncharacterized. Inflammasome activation likely plays a role in host defense, as the resulting cell death limits the replicative niche for parasite survival, making this an attractive pathway for the parasite to manipulate.

As mentioned above, components of the autophagy pathway are involved in regulating innate resistance to *T. gondii* in mouse and human cells. Although the role for ATG proteins in murine cells can be accounted for by IRGs (180, 193) and GBPs (185–187), these effectors are unlikely to play a role in human cells (194). Thus, the strain-dependent avoidance of ATG-mediated control by type 1 strains is likely due to a strain-specific mediator that blocks either this pathway or a susceptibility factor that allows the restriction of type 2 and 3 strains (205, 207). The role of ATG proteins in innate immunity is dependent on prior activation with IFN-γ. Under nonstimulated conditions, infection has been associated with the induction of host cell autophagy leading to enhanced parasite growth, although the mechanism by which this is triggered remains unknown (294).

CONCLUSIONS AND FUTURE DIRECTIONS

Although the last decade has seen significant progress in identifying parasite effectors, there are many ROP and GRA effectors for which the cellular targets and biological significance remain unknown. For example, there are more than 20 active ROP kinases and an equal number of pseudokinases (295), yet we know of functions for only a few of them (197). Similarly, there are a large number of GRA proteins for which functions have not been assigned, including the vacuolar protein GRA25, which influences immune responses in mice (296), and the host nucleus-targeted GRA28 protein (279). The diversity of these effectors may reflect the large number of different hosts infected by *T. gondii*, and the functions of other effectors may be revealed by studying a broader range of hosts.

Additionally, where effectors have been identified, they may not act alone. For example, while studying GRA16, we noticed that while GRA16 modulates p53 protein levels, paradoxically, the levels of the cell cycle inhibitor p21cip1/waf, a direct p53 downstream target, were not fully regulated by GRA16 (251), suggesting that another effector(s) may be involved in this pathway. A similar conclusion was drawn while investigating the activation of miR-146a and miR-155 by parasite infection: despite the fact that these microRNAs are regulated by NF-κB, they were not controlled by GRA15, a known activator of this transcription factor (234). Hence, *T. gondii* effectors may

inform us about the regulation and interaction of intrinsic cellular signaling pathways by serving as probes to dissect their functions.

Our understanding of protein export pathways has recently expanded with the recognition that ASP5 plays a role in *T. gondii* (276–278) parallel to that previously discovered in *Plasmodium* (reviewed in reference 274). Also, there are clearly novel activities on the PV, including the MYR1 complex involved in protein export (281) and the GRA17/23 complex implicated in import (280). The currently described components do not seem sufficiently complex to be solely responsible for such intricate activities, and it is likely that additional components of these systems will be discovered. Among the as-yet-unanswered questions are "What mechanism is involved in the recognition of proteins for export beyond the PV?" and "Does this depend primarily on their intrinsically disordered structure, or are there escort proteins that shuttle cargo through this pathway?" Additionally, it is possible that some components of the GRA17/23 complex implicated in nutrient import may also participate in protein export.

Although most effectors that target host transcription do this by altering proteins that then interact with transcription factors, TgIST acts in a novel way to alter host chromatin (257, 258). The TgIST-mediated alterations that are seen in the host epigenome raise the question of whether *T. gondii* effectors are able to promote "epigenetic memory" in resident cells at the site of infection that lasts far beyond the immunological clearance of the infecting pathogen. It also raises the possibility that there are other effectors that act to modify chromatin marks on host genes, thereby affecting gene expression, a theme that is common among bacterial pathogens but as yet largely unexplored in eukaryotic pathogens (297).

With the rapid identification of effectors in *T. gondii*, it seems surprising that they remained anonymous for as long as they did. The delay in recognizing effectors might be attributed to the fact that the genes and the proteins that they encode are highly divergent, which is itself a clue that they are under strong selective pressure to evolve. The advent of unbiased genetic systems led to the discovery and validation of secretory effectors in *T. gondii*. However, neither the pathways disrupted by *T. gondii* nor all the effectors identified are conserved in closely related parasites such as *Hammondia* or *Neospora*. This pattern may underlie the broader host range of *T. gondii*, perhaps due to a greater range of effectors that target the host, or may simply reflect the diversity of adaptation among different parasites. Regardless of the degree of conservation versus novelty, the value in defining these pathways may be in modulating the ability of the parasite to disrupt host pathways, thereby augmenting immune responses and perhaps dampening immune pathology.

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REFERENCES

1. Dubey JP. 2010. Toxoplasmosis of animals and humans. CRC Press, Boca Raton, FL.
2. Levine ND. 1988. The protozoan phylum Apicomplexa, vol 1 and 2. CRC Press, Boca Raton, FL.
3. Pawlowski J, Audic S, Adl S, Bass D, Belbahri L, Berney C, Bowser SS, Cepicka I, Decelle J, Dunthorn M, Fiore-Donno AM, Gile GH, Holzmann M, Jahn R, Jirku M, Keeling PJ, Kostka M, Kudryavtsev A, Lara E, Lukes J, Mann DG, Mitchell EA, Nitsche F, Romeralo M, Saunders GW, Simpson AG, Smirnov AV, Spouge JL, Stern RF, Stoeck T, Zimmermann J, Schindel D, de Vargas C. 2012. CBOL protist working group: barcoding eukaryotic richness beyond the animal, plant, and fungal kingdoms. PLoS Biol 10:e1001419. https://doi.org/10.1371/journal.pbio.1001419.
4. Bartosova-Sojkova P, Oppenheim RD, Soldati-Favre D, Lukes J. 2015. Epicellular apicomplexans: parasites "on the way in." PLoS Pathog 11:e1005080. https://doi.org/10.1371/journal.ppat.1005080.
5. Barta JR. 1989. Phylogenetic analysis of the class Sporozoea (phylum Apicomplexan Levine 1970): evidence for the independent evolution of heteroxenous life cycles. J Parasitol 75:195–206. https://doi.org/10.2307/3282766.
6. Morrissette NS, Sibley LD. 2002. Cytoskeleton of apicomplexan parasites. Microbiol Mol Biol Rev 66:21–38. https://doi.org/10.1128/MMBR.66.1.21-38.2002.
7. Janouskovec J, Tikhonenkov DV, Burki F, Howe AT, Kolisko M, Mylnikov AP, Keeling PJ. 2015. Factors mediating plastid dependency and the origins of parasitism in apicomplexans and their close relatives. Proc Natl Acad Sci U S A 112:10200–10207. https://doi.org/10.1073/pnas.1423790112.

8. Lorenzi H, Khan A, Behnke MS, Namasivayam S, Swapna LS, Hadjithomas M, Karamycheva S, Pinney D, Brunk BP, Ajioka JW, Ajzenberg D, Boothroyd JC, Boyle JP, Darde ML, Diaz-Miranda MA, Dubey JP, Fritz HM, Gennari SM, Gregory BD, Kim K, Saeij JP, Su C, White MW, Zhu XQ, Howe DK, Rosenthal BM, Grigg ME, Parkinson J, Liu L, Kissinger JC, Roos DS, Sibley LD. 2016. Local admixture of amplified and diversified secreted pathogenesis determinants shapes mosaic Toxoplasma gondii genomes. Nat Commun 7:10147. https://doi.org/10.1038/ncomms10147.
9. Miller LH, Ackerman HC, Su XZ, Wellems TE. 2013. Malaria biology and disease pathogenesis: insights for new treatments. Nat Med 19:156–167. https://doi.org/10.1038/nm.3073.
10. Checkley W, White AC, Jr, Jaganath D, Arrowood MJ, Chalmers RM, Chen XM, Fayer R, Griffiths JK, Guerrant RL, Hedstrom L, Huston CD, Kotloff KL, Kang G, Mead JR, Miller M, Petri WA, Jr, Priest JW, Roos DS, Striepen B, Thompson RC, Ward HD, Van Voorhis WA, Xiao L, Zhu G, Houpt ER. 2015. A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for cryptosporidium. Lancet Infect Dis 15:85–94. https://doi.org/10.1016/S1473-3099(14)70772-8.
11. Weiss LM, Dubey JP. 2009. Toxoplasmosis: a history of clinical observations. Int J Parasitol 39:895–901. https://doi.org/10.1016/j.ijpara.2009.02.004.
12. Petersen E. 2007. Toxoplasmosis. Semin Fetal Neonatal Med 12:214–223. https://doi.org/10.1016/j.siny.2007.01.011.
13. Fayer R, Esposito DH, Dubey JP. 2015. Human infections with Sarcocystis species. Clin Microbiol Rev 28:295–311. https://doi.org/10.1128/CMR.00113-14.
14. Sibley LD, Ajioka JW. 2008. Population structure of *Toxoplasma gondii*: clonal expansion driven by infrequent recombination and selective sweeps. Annu Rev Microbiol 62:329–351. https://doi.org/10.1146/annurev.micro.62.081307.162925.
15. Su CL, Khan A, Zhou P, Majumdar D, Ajzenberg D, Dardé ML, Zhu XQ, Ajioka JW, Rosenthal B, Dubey JP, Sibley LD. 2012. Globally diverse *Toxoplasma gondii* isolates comprise six major clades originating from a small number of distinct ancestral lineages. Proc Natl Acad Sci U S A 109:5844–5849. https://doi.org/10.1073/pnas.1203190109.
16. Chapman HD, Barta JR, Blake D, Gruber A, Jenkins M, Smith NC, Suo X, Tomley FM. 2013. A selective review of advances in coccidiosis research. Adv Parasitol 83:93–171. https://doi.org/10.1016/B978-0-12-407705-8.00002-1.
17. Frenkel JK, Dubey JP, Miller NL. 1970. *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. Science 167:893–896. https://doi.org/10.1126/science.167.3919.893.
18. Dubey JP, Frenkel JF. 1972. Cyst-induced toxoplasmosis in cats. J Protozool 19:155–177. https://doi.org/10.1111/j.1550-7408.1972.tb03431.x.
19. Dubey JP, Frenkel JK. 1973. Experimental *Toxoplasma* infection in mice with strains producing oocysts. J Parasitol 59:505–512. https://doi.org/10.2307/3278784.
20. Dubey JP, Speer CA, Shen SK, Kwok OCH, Blixt JA. 1997. Oocyst-induced murine toxoplasmosis: life cycle, pathogenicity, and stage conversion in mice fed *Toxoplasma gondii* oocysts. J Parasitol 83:870–882. https://doi.org/10.2307/3284282.
21. Dubey JP. 1997. Tissue cyst tropism in *Toxoplasma gondii*: a comparison of tissue cyst formation in organs of cats, and rodents fed oocysts. Parasitology 115:15–20. https://doi.org/10.1017/S0031182097008949.
22. Watts E, Zhao Y, Dhara A, Eller B, Patwardhan A, Sinai AP. 2015. Novel approaches reveal that Toxoplasma gondii bradyzoites within tissue cysts are dynamic and replicating entities in vivo. mBio 6:e01155-15. https://doi.org/10.1128/mBio.01155-15.
23. Frenkel JK, Escajadillo A. 1987. Cyst rupture as a pathogenic mechanism of toxoplasmic encephalitis. Am J Trop Med Hyg 36:517–522.
24. Dubey JP. 2001. Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. J Parasitol 87:215–219. https://doi.org/10.2307/3285204.
25. Dubey JP, Lunney JK, Shen SK, Kwok OCH, Ashford DA, Thulliez P. 1996. Infectivity of low numbers of *Toxoplasma gondii* oocysts to pigs. J Parasitol 82:438–443. https://doi.org/10.2307/3284082.
26. Freyre A, Dubey JP, Smith DD, Frenkel JK. 1989. Oocyst-induced *Toxoplasma gondii* infections in cats. J Parasitol 75:750–755. https://doi.org/10.2307/3283060.
27. Frenkel JK, Dubey JP. 2000. The taxonomic importance of obligate heteroxeny: distinction of *Hammondia hammondi* from *Toxoplasma gondii*—another opinion. Parasitol Res 86:783–786. https://doi.org/10.1007/s004360000261.
28. Dubey JP, Sreekumar C. 2003. Redescription of *Hammondia hammondi* and its differentiation from *Toxoplasma gondii*. Int J Parasitol 33:11437–11453. https://doi.org/10.1016/S0020-7519(03)00141-3.
29. Odaert H, Soete M, Fortier B, Camus D, Dubremetz JF. 1996. Stage conversion of Toxoplasma gondii in mouse brain during infection and immunodepression. Parasitol Res 82:28–31. https://doi.org/10.1007/BF03035408.
30. Soête M, Fortier B, Camus D, Dubremetz JF. 1993. *Toxoplasma gondii*: kinetics of bradyzoite-tachyzoite interconversion *in vitro*. Exp Parasitol 76:259–264. https://doi.org/10.1006/expr.1993.1031.
31. Su C, Evans D, Cole RH, Kissinger JC, Ajioka JW, Sibley LD. 2003. Recent expansion of Toxoplasma through enhanced oral transmission. Science 299:414–416. https://doi.org/10.1126/science.1078035.
32. Goodswen SJ, Kennedy PJ, Ellis JT. 2013. A review of the infection, genetics, and evolution of Neospora caninum: from the past to the present. Infect Genet Evol 13:133–150. https://doi.org/10.1016/j.meegid.2012.08.012.
33. Owen MR, Trees AJ. 1998. Vertical transmission of *Toxoplasma gondii* from chronically infected house (*Mus musculus*) and field (*Apodemus sylvaticus*) mice determined by polymerase chain reaction. Parasitology 116:299–304. https://doi.org/10.1017/S003118209700231X.
34. Hide G. 2016. Role of vertical transmission of Toxoplasma gondii in prevalence of infection. Expert Rev Anti Infect Ther 14:335–344. https://doi.org/10.1586/14787210.2016.1146131.
35. Were SR, Bowman DD, Mohammed HO, Jenkins MB, Quimby FW, Horton KM, Dubey JP. 1999. Transmission to guinea pigs of very low doses of oocysts of *Toxoplasma gondii* in drinking water. J Eukaryot Microbiol 46:71S–72S.
36. Frenkel JK, Dubey JP. 1973. Effects of freezing on the viability of *Toxoplasma* oocysts. J Parasitol 59:587–588. https://doi.org/10.2307/3278803.
37. Frenkel JK, Ruiz A, Chinchilla M. 1975. Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. Am J Trop Med Hyg 24:439–443.
38. Lindsay DS, Blagburn BL, Dubey JP. 2002. Survival of nonsporulated Toxoplasma gondii oocysts under refrigerator conditions. Vet Parasitol 103:309–313. https://doi.org/10.1016/S0304-4017(01)00554-4.
39. Lindsay DS, Dubey JP. 2009. Long-term survival of Toxoplasma gondii sporulated oocysts in seawater. J Parasitol 95:1019–1020. https://doi.org/10.1645/GE-1919.1.
40. Wainwright KE, Miller MA, Barr BC, Gardner IA, Melli AC, Essert T, Packham AE, Truong T, Lagunas-Solar M, Conrad PA. 2007. Chemical inactivation of Toxoplasma gondii oocysts in water. J Parasitol 93:925–931. https://doi.org/10.1645/GE-1063R.1.
41. Jones JL, Dubey JP. 2010. Waterborne toxoplasmosis—recent developments. Exp Parasitol 124:10–25. https://doi.org/10.1016/j.exppara.2009.03.013.
42. Bowie WR, King AS, Werker DH, Issac-Renton JL, Eng SB, Marion SA. 1997. Outbreak of toxoplasmosis associated with municipal drinking water. The BC Toxoplasma investigation team. Lancet 19:173–177. https://doi.org/10.1016/S0140-6736(96)11105-3.
43. Bahia-Oliveira LM, Jones JL, Azevedo-Silva J, Alves CC, Orefice F, Addiss DG. 2003. Highly endemic, waterborne toxoplasmosis in North Rio de Janeiro state, Brazil. Emerg Infect Dis 9:55–62. https://doi.org/10.3201/eid0901.020160.
44. de Moura L, Bahia-Oliveira LM, Wada MY, Jones JL, Tuboi SH, Carmo EH, Ramalho WM, Camargo NJ, Trevisan R, Graca RM, da Silva AJ, Moura I, Dubey JP, Garrett DO. 2006. Waterborne toxoplasmosis, Brazil, from field to gene. Emerg Infect Dis 12:326–329. https://doi.org/10.3201/eid1202.041115.
45. Teutsch SM, Juranek DD, Sulzer A, Dubey JP, Sikes RK. 1979. Epidemic toxoplasmosis associated with infected cats. N Engl J Med 300:695–699. https://doi.org/10.1056/NEJM197903293001302.
46. Jones JL, Muccioli C, Belfort R, Jr, Holland GN, Roberts JM, Silveira C. 2006. Recently acquired *Toxoplasma gondii* infection, Brazil. Emerg Infect Dis 12:582–587. https://doi.org/10.3201/eid1204.051081.
47. Jones JL, Dubey JP. 2012. Foodborne toxoplasmosis. Clin Infect Dis 55:864–851. https://doi.org/10.1093/cid/cis508.
48. Pfaff AW, Liesenfeld O, Candolfi E. 2007. Congenital toxoplasmosis, p 93–110. *In* Ajioka JW, Soldati D (ed), *Toxoplasma*: molecular and cellular biology. Horizon Bioscience, Norfolk, United Kingdom.
49. Pappas G, Roussos N, Falagas ME. 2009. Toxoplasmosis snapshots: global status of *Toxoplasma gondii* seroprevalence and implications for

pregnancy and congenital toxoplasmosis. Int J Parasitol 39:1385–1394. https://doi.org/10.1016/j.ijpara.2009.04.003.
50. McLeod R, Van Tubbergen C, Montoya JG, Petersen E. 2014. Human toxoplasma infection, p 99–159. *In* Weiss LM, Kim K (ed), Toxoplasma gondii: a model apicomplexan—perspectives and methods. Elsevier, Amsterdam, Netherlands.
51. McLeod R, Boyer K, Roizen N, Stein L, Swisher C, Holfels E, Hopkins J, Mack D, Karrison T, Patel D, Pfiffner L, Remington J, Withers S, Meyers S, Aitchison V, Mets M, Rabiah P, Meier P. 2000. The child with congenital toxoplasmosis. Curr Clin Top Infect Dis 20:189–208.
52. Melamed J, Eckert GU, Spadoni VS, Lago EG, Uberti F. 2009. Ocular manifestations of congenital toxoplasmosis. Eye (Lond) 24:528–534. https://doi.org/10.1038/eye.2009.140.
53. Arantes TE, Silveira C, Holland GN, Muccioli C, Yu F, Jones JL, Goldhardt R, Lewis KG, Belfort R, Jr. 2015. Ocular involvement following postnatally acquired Toxoplasma gondii infection in southern Brazil: a 28-year experience. Am J Ophthalmol 159:1002.e2–1012.e2. https://doi.org/10.1016/j.ajo.2015.02.015.
54. Glasner PD, Silveira C, Kruszon-Moran D, Martins MC, Burnier M, Silveira S, Camargo ME, Nussenblatt RB, Kaslow RA, Belfort R. 1992. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. Am J Ophthalmol 114:136–144. https://doi.org/10.1016/S0002-9394(14)73976-5.
55. Pfaff AW, de la-Torre A, Rochet E, Brunet J, Sabou M, Sauer A, Bourcier T, Gomez-Marin JE, Candolfi E. 4 November 2013. New clinical and experimental insights into Old World and neotropical ocular toxoplasmosis. Int J Parasitol https://doi.org/10.1016/j.ijpara.2013.09.007.
56. Vallochi AL, Muccioli C, Martins MC, Silveira C, Belfort R, Jr, Rizzo LV. 2005. The genotype of *Toxoplasma gondii* strains causing ocular toxoplasmosis in humans in Brazil. Am J Ophthalmol 139:350–351. https://doi.org/10.1016/j.ajo.2004.07.040.
57. Khan A, Jordan C, Muccioli C, Vallochi AL, Rizzo LV, Belfort R, Jr, Vitor RW, Silveira C, Sibley LD. 2006. Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, Brazil. Emerg Infect Dis 12:942–949. https://doi.org/10.3201/eid1206.060025.
58. Gilbert RE, Freeman K, Lago EG, Bahia-Oliveira LM, Tan HK, Wallon M, Buffolano W, Stanford MR, Petersen E. 2008. Ocular sequelae of congenital toxoplasmosis in Brazil compared with Europe. PLoS Negl Trop Dis 2:e277. https://doi.org/10.1371/journal.pntd.0000277.
59. de-la-Torre A, Sauer A, Pfaff AW, Bourcier T, Brunet J, Speeg-Schatz C, Ballonzoli L, Villard O, Ajzenberg D, Sundar N, Grigg ME, Gomez-Marin JE, Candolfi E. 2013. Severe South American ocular toxoplasmosis is associated with decreased Ifn-gamma/Il-17a and increased Il-6/Il-13 intraocular levels. PLoS Negl Trop Dis 7:e2541. https://doi.org/10.1371/journal.pntd.0002541.
60. Montoya JG, Liesenfeld O. 2004. Toxoplasmosis. Lancet 363:1965–1976. https://doi.org/10.1016/S0140-6736(04)16412-X.
61. Luft BJ, Remington JS. 1992. Toxoplasmic encephalitis in AIDS. Clin Infect Dis 15:211–222. https://doi.org/10.1093/clinids/15.2.211.
62. Gannon P, Khan MZ, Kolson DL. 2011. Current understanding of HIV-associated neurocognitive disorders pathogenesis. Curr Opin Neurol 24:275–283. https://doi.org/10.1097/WCO.0b013e32834695fb.
63. Dennis AM, Napravnik S, Sena AC, Eron JJ. 2011. Late entry to HIV care among Latinos compared with non-Latinos in a southeastern US cohort. Clin Infect Dis 53:480–487. https://doi.org/10.1093/cid/cir434.
64. McCabe RE. 2001. Antitoxoplasma chemotherapy, p 319–359. *In* Joynson DHM, Wreghitt TG (ed), Toxoplasmosis: a comprehensive clinical guide. Cambridge University Press, Cambridge, United Kingdom.
65. Torrey EF, Yolken RH. 2003. Toxoplasma gondii and schizophrenia. Emerg Infect Dis 9:1375–1380. https://doi.org/10.3201/eid0911.030143.
66. Sibley LD, LeBlanc AJ, Pfefferkorn ER, Boothroyd JC. 1992. Generation of a restriction fragment length polymorphism linkage map for *Toxoplasma gondii*. Genetics 132:1003–1015.
67. Dardé ML, Bouteille B, Pestre-Alexandre M. 1992. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. J Parasitol 78:786–794. https://doi.org/10.2307/3283305.
68. Sibley LD, Boothroyd JC. 1992. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. Nature 359:82–85. https://doi.org/10.1038/359082a0.
69. Ajzenberg D, Bañuls AL, Tibayrenc M, Dardé ML. 2002. Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int J Parasitol 32:27–38. https://doi.org/10.1016/S0020-7519(01)00301-0.
70. Ajzenberg D, Cogné N, Paris L, Bessieres MH, Thulliez P, Fillisetti D, Pelloux H, Marty P, Dardé ML. 2002. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis and correlation with clinical findings. J Infect Dis 186:684–689. https://doi.org/10.1086/342663.
71. Howe DK, Sibley LD. 1995. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J Infect Dis 172:1561–1566. https://doi.org/10.1093/infdis/172.6.1561.
72. Ajzenberg D, Yera H, Marty P, Paris L, Dalle F, Menotti J, Aubert D, Franck J, Bessieres MH, Quinio D, Pelloux H, Delhaes L, Desbois N, Thulliez P, Robert-Gangneux F, Kauffmann-Lacroix C, Pujol S, Rabodonirina M, Bougnoux ME, Cuisenier B, Duhamel C, Duong TH, Filisetti D, Flori P, Gay-Andrieu F, Pratlong F, Nevez G, Totet A, Carme B, Bonnabau H, Darde ML, Villena I. 2009. Genotype of 88 *Toxoplasma gondii* isolates associated with toxoplasmosis in immunocompromised patients and correlation with clinical findings. J Infect Dis 199:1155–1167. https://doi.org/10.1086/597477.
73. Howe DK, Honoré S, Derouin F, Sibley LD. 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. J Clin Microbiol 35:1411–1414.
74. Honoré S, Couvelard A, Garin YJ, Bedel C, Hénin D, Dardé ML, Derouin F. 2000. Genotyping of *Toxoplasma gondii* strains from immunocompromised patients. Pathol Biol (Paris) 48:541–547.
75. Khan A, Su C, German M, Storch GA, Clifford D, Sibley LD. 2005. Genotyping of *Toxoplasma gondii* strains from immunocompromised patients reveals high prevalence of type I strains. J Clin Microbiol 43:5881–5887. https://doi.org/10.1128/JCM.43.12.5881-5887.2005.
76. Mondragon R, Howe DK, Dubey JP, Sibley LD. 1998. Genotypic analysis of *Toxoplasma gondii* isolates in pigs. J Parasitol 84:639–641. https://doi.org/10.2307/3284743.
77. Ajioka JA, Boothroyd JC, Brunk BP, Hehl A, Hillier L, Manger ID, Overton GC, Marra M, Roos D, Wan KL, Waterston RH, Sibley LD. 1998. Gene discovery by EST sequencing in *Toxoplasma gondii* reveals sequences restricted to the Apicomplexa. Genome Res 8:18–28.
78. Boyle JP, Rajasekar B, Saeij JPJ, Ajioka JW, Berriman M, Paulsen I, Sibley LD, White M, Boothroyd JC. 2006. Just one cross appears capable of dramatically altering the population biology of a eukaryotic pathogen like *Toxoplasma gondii*. Proc Natl Acad Sci U S A 103:10514–10519. https://doi.org/10.1073/pnas.0510319103.
79. Berney C, Pawlowski J. 2006. A molecular time-scale for eukaryote evolution recalibrated with the continuous microfossil record. Proc Biol Sci 273:1867–1872. https://doi.org/10.1098/rspb.2006.3537.
80. Rosenthal BM. 2009. How has agriculture influenced the geography and genetics of animal parasites? Trends Parasitol 25:67–70. https://doi.org/10.1016/j.pt.2008.10.004.
81. Driscoll CA, Menotti-Raymond M, Roca AL, Hupe K, Johnson WE, Geffen E, Harley EH, Delibes M, Pontier D, Kitchener AC, Yamaguchi N, O'Brien SJ, Macdonald DW. 2007. The near eastern origin of cat domestication. Science 317:519–523. https://doi.org/10.1126/science.1139518.
82. Boursot P, Auffray JC, Britton-Davidian J, Bonhomme F. 1993. The evolution of house mice. Annu Rev Ecol Syst 24:119–152. https://doi.org/10.1146/annurev.es.24.110193.001003.
83. Khan A, Ajzenberg D, Mercier A, Demar M, Simon S, Darde ML, Wang Q, Verma SK, Rosenthal BM, Dubey JP, Sibley LD. 2014. Geographic separation of domestic and wild strains of *Toxoplasma gondii* in French Guiana correlates with a monomorphic version of chromosome1a. PLoS Negl Trop Dis 8:e3182. https://doi.org/10.1371/journal.pntd.0003182.
84. Khan A, Bohme U, Kelly KA, Adlem E, Brooks K, Simmonds M, Mungall K, Quail MA, Arrowsmith C, Chillingworth T, Churcher C, Harris D, Collins M, Fosker N, Fraser A, Hance Z, Jagels K, Moule S, Murphy L, O'Neil S, Rajandream MA, Saunders D, Seeger K, Whitehead S, Mayr T, Xuan X, Watanabe J, Suzuki Y, Wakaguri H, Sugano S, Sugimoto C, Paulsen I, Mackey AJ, Roos DS, Hall N, Berriman M, Barell B, Sibley LD, Ajioka JW. 2006. Common inheritance of chromosome Ia associated with clonal expansion of *Toxoplasma gondii*. Genome Res 16:1119–1125. https://doi.org/10.1101/gr.5318106.
85. Khan A, Fux B, Su C, Dubey JP, Darde ML, Ajioka JW, Rosenthal BM, Sibley LD. 2007. Recent transcontinental sweep of *Toxoplasma gondii* driven by a single monomorphic chromosome. Proc Natl Acad Sci U S A 104:14872–14877. https://doi.org/10.1073/pnas.0702356104.
86. Khan A, Miller N, Roos DS, Dubey JP, Ajzenberg D, Darde ML, Ajioka JW, Rosenthal B, Sibley LD. 2011. A monomorphic haplotype of chromosome Ia is associated with widespread success in clonal and nonclonal

populations of Toxoplasma gondii. mBio 2:e00228-11. https://doi.org/10.1128/mBio.00228-11.
87. Khan A, Dubey JP, Su C, Ajioka JW, Rosenthal BM, Sibley LD. 2011. Genetic analyses of atypical *Toxoplasma gondii* strains reveals a fourth clonal lineage in North America. Int J Parasitol 41:645–655. https://doi.org/10.1016/j.ijpara.2011.01.005.
88. Lehmann T, Marcet PL, Graham DH, Dahl ER, Dubey JP. 2006. Globalization and the population structure of *Toxoplasma gondii*. Proc Natl Acad Sci U S A 103:11423–11428. https://doi.org/10.1073/pnas.0601438103.
89. Pena HF, Gennari SM, Dubey JP, Su C. 2008. Population structure and mouse-virulence of *Toxoplasma gondii* in Brazil. Int J Parasitol 38:561–569. https://doi.org/10.1016/j.ijpara.2007.09.004.
90. Belfort-Neto R, Nussenblatt V, Rizzo L, Muccioli C, Silveira C, Nussenblatt R, Khan A, Sibley LD, Belfort RJ. 2007. High prevalence of unusual genotypes of *Toxoplasma gondii* infection in pork meat samples from Erechim, Southern Brazil. An Acad Bras Cienc 79:111–114. https://doi.org/10.1590/S0001-37652007000100013.
91. Ferreira ADM, Vitor RWA, Carneiro ACAV, Brandão GP, Melo MN. 2004. Genetic variability of Brazilian *Toxoplasma gondii* strains detected by random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) and simple sequence repeat anchored-PCR (SSR-PCR). Infect Genet Evol 4:131–142. https://doi.org/10.1016/j.meegid.2004.03.002.
92. Minot S, Melo MB, Li F, Lu D, Niedelman W, Levine SS, Saeij JP. 2012. Admixture and recombination among *Toxoplasma gondii* lineages explain global genome diversity. Proc Natl Acad Sci U S A 109:13458–13463. https://doi.org/10.1073/pnas.1117047109.
93. Dubey JP, Van Why K, Verma SK, Choudhary S, Kwok OC, Khan A, Behinke MS, Sibley LD, Ferreira LR, Oliveira S, Weaver M, Stewart R, Su C. 2014. Genotyping Toxoplasma gondii from wildlife in Pennsylvania and identification of natural recombinants virulent to mice. Vet Parasitol 200:74–84. https://doi.org/10.1016/j.vetpar.2013.11.001.
94. Carme B, Bissuel F, Ajzenberg D, Bouyne R, Aznar C, Demar M, Bichat S, Louvel D, Bourbigot AM, Peneau C, Neron P, Dardé ML. 2002. Severe acquired toxoplasmosis in immunocompetent adult patients in French Guiana. J Clin Microbiol 40:4037–4044. https://doi.org/10.1128/JCM.40.11.4037-4044.2002.
95. Dardé ML, Villena I, Pinon JM, Beguinot I. 1998. Severe toxoplasmosis caused by a *Toxoplasma gondii* strain with a new isotype acquired in French Guyana. J Clin Microbiol 36:324.
96. Demar M, Hommel D, Djossou F, Peneau C, Boukhari R, Louvel D, Bourbigot AM, Nasser V, Ajzenberg D, Darde ML, Carme B. 2012. Acute toxoplasmoses in immunocompetent patients hospitalized in an intensive care unit in French Guiana. Clin Microbiol Infect 18:E221–E231. https://doi.org/10.1111/j.1469-0691.2011.03648.x.
97. Sibley LD. 2010. How apicomplexan parasites move in and out of cells. Curr Opin Biotechnol 21:592–598. https://doi.org/10.1016/j.copbio.2010.05.009.
98. Carruthers VB, Tomley FM. 2008. Microneme proteins in apicomplexans. Subcell Biochem 47:33–45. https://doi.org/10.1007/978-0-387-78267-6_2.
99. Bradley PJ, Ward C, Cheng SJ, Alexander DL, Coller S, Coombs GH, Dunn JD, Ferguson DJ, Sanderson SJ, Wastling JM, Boothroyd JC. 2005. Proteomic analysis of rhoptry organelles reveals many novel constituents for host-parasite interactions in *T. gondii*. J Biol Chem 280:34245–34258. https://doi.org/10.1074/jbc.M504158200.
100. Sibley LD. 2004. Invasion strategies of intracellular parasites. Science 304:248–253. https://doi.org/10.1126/science.1094717.
101. Sharma P, Chitnis CE. 2013. Key molecular events during host cell invasion by apicomplexan pathogens. Curr Opin Microbiol 16:432–437. https://doi.org/10.1016/j.mib.2013.07.004.
102. Dobrowolski JM, Sibley LD. 1996. Toxoplasma invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. Cell 84:933–939. https://doi.org/10.1016/S0092-8674(00)81071-5.
103. Meissner M, Schluter D, Soldati D. 2002. Role of *Toxoplasma gondii* myosin A in powering parasite gliding and host cell invasion. Science 298:837–840. https://doi.org/10.1126/science.1074553.
104. 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 8:343–357. https://doi.org/10.1016/j.chom.2010.09.002.
105. Jacot D, Tosetti N, Pires I, Stock J, Graindorge A, Hung YF, Han H, Tewari R, Kursula I, Soldati-Favre D. 2016. An apicomplexan actin-binding protein serves as a connector and lipid sensor to coordinate motility and invasion. Cell Host Microbe 20:731–743. https://doi.org/10.1016/j.chom.2016.10.020.
106. Gonzalez V, Combe A, David V, Malmquist NA, Delorme V, Leroy C, Blazquez S, Menard R, Tardieux I. 2009. Host cell entry by apicomplexa parasites requires actin polymerization in the host cell. Cell Host Microbe 5:259–272. https://doi.org/10.1016/j.chom.2009.01.011.
107. Egarter S, Andenmatten N, Jackson AJ, Whitelaw JA, Pall G, Black JA, Ferguson DJ, Tardieux I, Mogilner A, Meissner M. 2014. The Toxoplasma Acto-MyoA motor complex is important but not essential for gliding motility and host cell invasion. PLoS One 9:e91819. https://doi.org/10.1371/journal.pone.0091819.
108. Andenmatten N, Egarter S, Jackson AJ, Jullien N, Herman JP, Meissner M. 23 December 2012. Conditional genome engineering in *Toxoplasma gondii* uncovers alternative invasion mechanisms. Nat Methods https://doi.org/10.1038/nmeth.2301.
109. Frenal K, Soldati-Favre D. 2015. Plasticity and redundancy in proteins important for Toxoplasma invasion. PLoS Pathog 11:e1005069. https://doi.org/10.1371/journal.ppat.1005069.
110. Drewry LL, Sibley LD. 2015. Toxoplasma actin is required for efficient host cell invasion. mBio 6:e00557-15. https://doi.org/10.1128/mBio.00557-15.
111. 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. https://doi.org/10.1371/journal.ppat.1003629.
112. Pelletier L, Stern CA, Pypaert M, Sheff D, Ngo HM, Roper N, He CY, Hu K, Toomre D, Coppens I, Roos DS, Joiner KA, Warren G. 2002. Golgi biogenesis in *Toxoplasma gondii*. Nature 418:548–552. https://doi.org/10.1038/nature00946.
113. Behnke M, Wooten JC, Lehmann M, Radke J, Lucas O, Nawas J, Sibley LD, White M. 2010. Coordinated progression through two subtranscriptions underlies the tachyzoite cycle of Toxoplasma gondii. PLoS One 5:e12354. https://doi.org/10.1371/journal.pone.0012354.
114. Carruthers VB, Sibley LD. 1997. Sequential protein secretion from three distinct organelles of *Toxoplasma gondii* accompanies invasion of human fibroblasts. Eur J Cell Biol 73:114–123.
115. Carruthers VB, Giddings OK, Sibley LD. 1999. Secretion of micronemal proteins is associated with Toxoplasma invasion of host cells. Cell Microbiol 1:225–236. https://doi.org/10.1046/j.1462-5822.1999.00023.x.
116. Carruthers VB, Moreno SNJ, Sibley LD. 1999. Ethanol and acetaldehyde elevate intracellular [Ca^{2+}] calcium and stimulate microneme discharge in *Toxoplasma gondii*. Biochem J 342:379–386. https://doi.org/10.1042/bj3420379.
117. Besteiro S, Dubremetz JF, Lebrun M. 2011. The moving junction of apicomplexan parasites: a key structure for invasion. Cell Microbiol 13:797–805. https://doi.org/10.1111/j.1462-5822.2011.01597.x.
118. Shen B, Sibley LD. 2012. The moving junction, a key portal to host cell invasion by apicomplexan parasites. Curr Opin Microbiol 15:449–455. https://doi.org/10.1016/j.mib.2012.02.007.
119. Bradley PJ, Sibley LD. 2007. Rhoptries: an arsenal of secreted virulence factors. Curr Opin Microbiol 10:582–587. https://doi.org/10.1016/j.mib.2007.09.013.
120. Håkansson S, Charron AJ, Sibley LD. 2001. *Toxoplasma* evacuoles: a two-step process of secretion and fusion forms the parasitophorous vacuole. EMBO J 20:3132–3144. https://doi.org/10.1093/emboj/20.12.3132.
121. Koshy AA, Dietrich HK, Christian DA, Melehani JH, Shastri AJ, Hunter CA, Boothroyd JC. 2012. Toxoplasma co-opts host cells it does not invade. PLoS Pathog 8:e1002825. https://doi.org/10.1371/journal.ppat.1002825.
122. Cesbron-Delauw MF, Gendrin C, Travier L, Ruffiot P, Mercier C. 2008. Apicomplexa in mammalian cells: trafficking to the parasitophorous vacuole. Traffic 9:657–664. https://doi.org/10.1111/j.1600-0854.2008.00728.x.
123. Lecordier L, Mercier C, Sibley LD, Cesbron-Delauw MF. 1999. Transmembrane insertion of the *Toxoplasma gondii* GRA5 protein occurs following soluble secretion into the host cell. Mol Biol Cell 10:1277–1287. https://doi.org/10.1091/mbc.10.4.1277.
124. Dunn JD, Ravindran S, Kim SK, Boothroyd JC. 2008. The *Toxoplasma gondii* dense granule protein GRA7 is phosphorylated upon invasion and forms an unexpected association with the rhoptry proteins ROP2 and ROP4. Infect Immun 76:5853–5861. https://doi.org/10.1128/IAI.01667-07.
125. Alaganan A, Fentress SJ, Tang K, Wang Q, Sibley LD. 2013. Toxoplasma GRA7 effector increases turnover of immunity-related GTPases and

contributes to acute virulence in the mouse. Proc Natl Acad Sci U S A 111:1126–1131. https://doi.org/10.1073/pnas.1313501111.
126. Fox BA, Falla A, Rommereim LM, Tomita T, Gigley JP, Mercier C, Cesbron-Delauw MF, Weiss LM, Bzik DJ. 2011. Type II Toxoplasma gondii KU80 knockout strains enable functional analysis of genes required for cyst development and latent infection. Eukaryot Cell 10:1193–1206. https://doi.org/10.1128/EC.00297-10.
127. Torpier G, Charif H, Darcy F, Liu J, Dardé ML, Capron A. 1993. *Toxoplasma gondii*: differential localization of antigens secreted from encysted bradyzoites. Exp Parasitol 77:13–22. https://doi.org/10.1006/expr.1993.1056.
128. Rommereim LM, Bellini V, Fox BA, Petre G, Rak C, Touquet B, Aldebert D, Dubremetz JF, Cesbron-Delauw MF, Mercier C, Bzik DJ. 2016. Phenotypes associated with knockouts of eight dense granule gene loci (GRA2-9) in virulent Toxoplasma gondii. PLoS One 11:e0159306. https://doi.org/10.1371/journal.pone.0159306.
129. Mercier C, Cesbron-Delauw MF. 2015. Toxoplasma secretory granules: one population or more? Trends Parasitol 31:60–71. https://doi.org/10.1016/j.pt.2014.12.002.
130. 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 fusion pore. Proc Natl Acad Sci U S A 93:8413–8418. https://doi.org/10.1073/pnas.93.16.8413.
131. Charron AJ, Sibley LD. 2004. Molecular partitioning during host cell penetration by *Toxoplasma gondii*. Traffic 5:855–867. https://doi.org/10.1111/j.1600-0854.2004.00228.x.
132. Morisaki JH, Heuser JE, Sibley LD. 1995. Invasion of *Toxoplasma gondii* occurs by active penetration of the host cell. J Cell Sci 108:2457–2464.
133. Mordue DG, Desai N, Dustin M, Sibley LD. 1999. Invasion by *Toxoplasma gondii* establishes a moving junction that selectively excludes host cell plasma membrane proteins on the basis of their membrane anchoring. J Exp Med 190:1783–1792. https://doi.org/10.1084/jem.190.12.1783.
134. Mordue D, Håkansson 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. https://doi.org/10.1006/expr.1999.4412.
135. Sibley LD, Weidner E, Krahenbuhl JL. 1985. Phagosome acidification blocked by intracellular *Toxoplasma gondii*. Nature 315:416–419. https://doi.org/10.1038/315416a0.
136. Schwab JC, Beckers CJM, Joiner KA. 1994. The parasitophorous vacuole membrane surrounding intracellular *Toxoplasma gondii* functions as a molecular sieve. Proc Natl Acad Sci U S A 91:509–513. https://doi.org/10.1073/pnas.91.2.509.
137. Dubey JP. 1998. Comparative infectivity of *Toxoplasma gondii* bradyzoites in rats and mice. J Parasitol 84:1279–1282. https://doi.org/10.2307/3284691.
138. Mashayekhi M, Sandau MM, Dunay IR, Frickel EM, Khan A, Goldszmid RS, Sher A, Ploegh HL, Murphy TL, Sibley LD, Murphy KM. 2011. CD8alpha(+) dendritic cells are the critical source of interleukin-12 that controls acute infection by *Toxoplasma gondii* tachyzoites. Immunity 35:249–259. https://doi.org/10.1016/j.immuni.2011.08.008.
139. Reis e Sousa C, Hieny S, Scharton-Kersten T, Jankovic D, Charest H, Germain RN, Sher A. 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. J Exp Med 186:1819–1829. https://doi.org/10.1084/jem.186.11.1819.
140. Bierly AL, Shufesky WJ, Sukhumavasi W, Morelli AE, Denkers EY. 2008. Dendritic cells expressing plasmacytoid marker PDCA-1 are Trojan horses during *Toxoplasma gondii* infection. J Immunol 181:8485–8491. https://doi.org/10.4049/jimmunol.181.12.8485.
141. Pepper M, Dzierszinski F, Wilson E, Tait E, Fang Q, Yarovinsky F, Laufer TM, Roos D, Hunter CA. 2008. Plasmacytoid dendritic cells are activated by *Toxoplasma gondii* to present antigen and produce cytokines. J Immunol 180:6229–6236. https://doi.org/10.4049/jimmunol.180.9.6229.
142. Robben PM, Mordue DG, Truscott SM, Takeda K, Akira S, Sibley LD. 2004. Production of IL-12 by macrophages infected with *Toxoplasma gondii* depends on the parasite genotype. J Immunol 172:3686–3694. https://doi.org/10.4049/jimmunol.172.6.3686.
143. Bliss SK, Butcher BA, Denkers EY. 2000. Rapid recruitment of neutrophils containing prestored IL-12 during microbial infection. J Immunol 165:4515–4521. https://doi.org/10.4049/jimmunol.165.8.4515.
144. Andrade WA, Souza MDC, Ramos-Martinez E, Nagpal K, Dutra MS, Melo MB, Bartholomeu DC, Ghosh S, Golenbock DT, Gazzinelli RT. 2013. Combined action of nucleic acid-sensing Toll-like receptors and TLR11/TLR12 heterodimers imparts resistance to Toxoplasma gondii in mice. Cell Host Microbe 13:42–53. https://doi.org/10.1016/j.chom.2012.12.003.
145. Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, Sher A. 2005. TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science 308:1626–1629. https://doi.org/10.1126/science.1109893.
146. Scanga CA, Aliberti J, Jankovic D, Tilloy F, Bennouna S, Denkers EY, Medzhitov R, Sher A. 2002. Cutting edge: MyD88 is required for resistance to *Toxoplasma gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. J Immunol 168:5997–6001. https://doi.org/10.4049/jimmunol.168.12.5997.
147. Gazzinelli RT, Heiny S, Wynn TA, Wolf S, Sher A. 1993. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell-deficient mice. Proc Natl Acad Sci U S A 90:6115–6119. https://doi.org/10.1073/pnas.90.13.6115.
148. Hunter CA, Subauste CS, Van Cleave VH, Remington JS. 1994. Production of gamma interferon by natural killer cells from *Toxoplasma gondii*-infected SCID mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha. Infect Immun 62:2818–2824.
149. Gazzinelli R, Wysocka M, Hayashi S, Denkers E, Hieny S, Caspar P, Trinchieri G, Sher A. 1994. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with *Toxoplasma gondii*. J Immunol 153:2533–2543.
150. Wilson DC, Matthews S, Yap GS. 2008. IL-12 signaling drives $CD8^+$ T cell IFN-gamma production and differentiation of $KLRG1^+$ effector subpopulations during *Toxoplasma gondii* infection. J Immunol 180:5935–5945. https://doi.org/10.4049/jimmunol.180.9.5935.
151. Scharton-Kersten TM, Wynn TA, Denkers EY, Bala S, Grunvald E, Hieny S, Gazzinelli RT, Sher A. 1996. In the absence of endogenous IFN-gamma, mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. J Immunol 157:4045–4054.
152. Yap GS, Sher A. 1999. Effector cells of both nonhemopoietic and hemopoietic origin are required for interferon (IFN)-gamma- and tumor necrosis factor (TNF)-alpha-dependent host resistance to the intracellular pathogen, *Toxoplasma gondii*. J Exp Med 189:1083–1091. https://doi.org/10.1084/jem.189.7.1083.
153. Yap G, Pesin M, Sher A. 2000. Cutting edge: IL-12 is required for the maintenance of IFN-gamma production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii*. J Immunol 165:628–631. https://doi.org/10.4049/jimmunol.165.2.628.
154. Darnell JE, Jr, Kerr IM, Stark GR. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264:1415–1421. https://doi.org/10.1126/science.8197455.
155. Schindler C, Darnell JE, Jr. 1995. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. Annu Rev Biochem 64:621–651. https://doi.org/10.1146/annurev.bi.64.070195.003201.
156. Gavrilescu LC, Butcher BA, Del Rio L, Taylor GA, Denkers EY. 2004. STAT1 is essential for antimicrobial effector function but dispensable for gamma interferon production during Toxoplasma gondii infection. Infect Immun 72:1257–1264. https://doi.org/10.1128/IAI.72.3.1257-1264.2004.
157. Lieberman LA, Banica M, Reiner SL, Hunter CA. 2004. STAT1 plays a critical role in the regulation of antimicrobial effector mechanisms, but not in the development of Th1-type responses during toxoplasmosis. J Immunol 172:457–463. https://doi.org/10.4049/jimmunol.172.1.457.
158. Luder CG, Algner M, Lang C, Bleicher N, Gross U. 2003. Reduced expression of the inducible nitric oxide synthase after infection with *Toxoplasma gondii* facilitates parasite replication in activated murine macrophages. Int J Parasitol 33:833–844. https://doi.org/10.1016/S0020-7519(03)00092-4.
159. Lüder CG, Walter W, Beuerle B, Maeurer MJ, Gross U. 2001. *Toxoplasma gondii* down-regulates MHC class II gene expression and antigen presentation by murine macrophages via interference with nuclear translocation of STAT1a. Eur J Immunol 31:1475–1484. https://doi.org/10.1002/1521-4141(200105)31:5<1475::AID-IMMU1475>3.0.CO;2-C.
160. 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-gamma. PLoS Pathog 8:e1002483. https://doi.org/10.1371/journal.ppat.1002483.

161. Gazzinelli RT, Eltoum I, Wynn TA, Sher A. 1993. Acute cerebral toxoplasmosis is induced by *in vivo* neutralization of TNF-alpha and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. J Immunol 151:3672–3681.
162. Deckert-Schlüter M, Bluethmann H, Rang A, Hof H, Schluter D. 1998. Crucial role of TNF receptor type 1 (p55), but not of TNF receptor type 2 (p75), in murine toxoplasmosis. J Immunol 160:3427–3436.
163. Yap GS, Scharton-Kersten T, Charest H, Sher A. 1998. Decreased resistance of TNF receptor p55- and p75-deficient mice to chronic toxoplasmosis despite normal activation of inducible nitric oxide synthase in vivo. J Immunol 160:1340–1345.
164. Scharton-Kersten TM, Yap G, Magram J, Sher A. 1997. Inducible nitric oxide is essential for host control of persistent but not acute infection with the intracellular pathogen *Toxoplasma gondii*. J Exp Med 185:1261–1273. https://doi.org/10.1084/jem.185.7.1261.
165. Han SJ, Melichar HJ, Coombes JL, Chan SW, Koshy AA, Boothroyd JC, Barton GM, Robey EA. 2014. Internalization and TLR-dependent type I interferon production by monocytes in response to Toxoplasma gondii. Immunol Cell Biol 92:872–881. https://doi.org/10.1038/icb.2014.70.
166. Schmitz JL, Carlin JM, Borden EC, Byrne GI. 1989. Beta interferon inhibits Toxoplasma gondii growth in human monocyte-derived macrophages. Infect Immun 57:3254–3256.
167. Melo MB, Nguyen QP, Cordeiro C, Hassan MA, Yang N, McKell R, Rosowski EE, Julien L, Butty V, Darde ML, Ajzenberg D, Fitzgerald K, Young LH, Saeij JP. 2013. Transcriptional analysis of murine macrophages infected with different Toxoplasma strains identifies novel regulation of host signaling pathways. PLoS Pathog 9:e1003779. https://doi.org/10.1371/journal.ppat.1003779.
168. Sibley LD. 2011. Invasion and intracellular survival by protozoan parasites. Immunol Rev 240:72–91. https://doi.org/10.1111/j.1600-065X.2010.00990.x.
169. Liesenfeld O. 2002. Oral infection of C57BL/6 mice with *Toxoplasma gondii*: a new model of inflammatory bowel disease? J Infect Dis 185:S96–S101. https://doi.org/10.1086/338006.
170. Gavrilescu LC, Denkers EY. 2001. IFN-gamma overproduction and high level apoptosis are associated with high but not low virulence *Toxoplasma gondii* infection. J Immunol 167:902–909. https://doi.org/10.4049/jimmunol.167.2.902.
171. Mordue DG, Monroy F, La Regina M, Dinarello CA, Sibley LD. 2001. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. J Immunol 167:4574–4584. https://doi.org/10.4049/jimmunol.167.8.4574.
172. Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kuhn R, Muller W, Trinchieri G, Sher A. 1996. In the absence of endogenous IL-10, mice acutely infected with *Toxoplasma gondii* succumb to a lethal immune response dependent of $CD4^+$ T cells and accompanied by overproduction of IL-12, IFN-gamma, and TNF-alpha. J Immunol 157:798–805.
173. Hall AO, Beiting DP, Tato C, John B, Oldenhove G, Lombana CG, Pritchard GH, Silver JS, Bouladoux N, Stumhofer JS, Harris TH, Grainger J, Wojno ED, Wagage S, Roos DS, Scott P, Turka LA, Cherry S, Reiner SL, Cua D, Belkaid Y, Elloso MM, Hunter CA. 2012. The cytokines interleukin 27 and interferon-gamma promote distinct Treg cell populations required to limit infection-induced pathology. Immunity 37:511–523. https://doi.org/10.1016/j.immuni.2012.06.014.
174. Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, Taylor LS, Eagleson B, Secrest L, Southon EA, Reid SW, Tessarollo L, Bray M, McVicar DW, Komschlies KL, Young HA, Biron CA, Sher A, Vande Woude GF. 2000. Pathogen-specific loss of host resistance in mice lacking IFN-gamma-inducible gene IGTP. Proc Natl Acad Sci U S A 97:751–755. https://doi.org/10.1073/pnas.97.2.751.
175. Collazo CM, Yap GS, Hieny S, Caspar P, Feng CG, Taylor GA, Sher A. 2002. The function of gamma interferon-inducible GTP-binding protein IGTP in host resistance to *Toxoplasma gondii* is Stat1 dependent and requires expression in both hematopoietic and nonhematopoietic cellular compartments. Infect Immun 70:6933–6939. https://doi.org/10.1128/IAI.70.12.6933-6939.2002.
176. Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tessarollo L, Woude GF, Sher A, Taylor GA. 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 194:181–188. https://doi.org/10.1084/jem.194.2.181.
177. Feng CG, Weksberg DC, Taylor GA, Sher A, Goodell MA. 2008. The p47 GTPase Lrg-47 (Irgm1) links host defense and hematopoietic stem cell proliferation. Cell Stem Cell 2:83–89. https://doi.org/10.1016/j.stem.2007.10.007.
178. Papic N, Hunn JP, Pawlowski N, Zerrahn J, Howard JC. 2008. Inactive and active states of the interferon-inducible resistance GTPase, Irga6, in vivo. J Biol Chem 283:32143–32151. https://doi.org/10.1074/jbc.M804846200.
179. Hunn JP, Koenen-Waisman S, Papic N, Schroeder N, Pawlowski N, Lange R, Jaiser F, Zerrahn J, Martens S, Howard JC. 2008. Regulatory interactions between IRG resistance GTPases in the cellular response to *Toxoplasma gondii*. EMBO J 27:2495–2509. https://doi.org/10.1038/emboj.2008.176.
180. Zhao YO, Khaminets A, Hunn JP, Howard JC. 2009. Disruption of the *Toxoplasma gondii* parasitophorous vacuole by IFN-gamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. PLoS Pathog 5:e1000288. https://doi.org/10.1371/journal.ppat.1000288.
181. Martens S, Parvanova I, Zerrahn J, Griffiths G, Schell G, Reichmann G, Howard JC. 2005. Disruption of *Toxoplasma gondii* parasitophorous vacuoles by the mouse p47-resistance GTPases. PLoS Pathog 1:e24. https://doi.org/10.1371/journal.ppat.0010024.
182. Haldar AK, Saka HA, Piro AS, Dunn JD, Henry SC, Taylor GA, Frickel EM, Valdivia RH, Coers J. 2013. IRG and GBP host resistance factors target aberrant, "non-self" vacuoles characterized by the missing of "self" IRGM proteins. PLoS Pathog 9:e1003414. https://doi.org/10.1371/journal.ppat.1003414.
183. Tiwari S, Choi HP, Matsuzawa T, Pypaert M, MacMicking JD. 2009. Targeting of the GTPase Irgm1 to the phagosomal membrane via PtdIns(3,4)P(2) and PtdIns(3,4,5)P(3) promotes immunity to mycobacteria. Nat Immunol 10:907–917. https://doi.org/10.1038/ni.1759.
184. Kim BH, Shenoy AR, Kumar P, Bradfield CJ, MacMicking JD. 2012. IFN-inducible GTPases in host cell defense. Cell Host Microbe 12:432–444. https://doi.org/10.1016/j.chom.2012.09.007.
185. Selleck EM, Fentress SJ, Beatty WL, Degrandi D, Pfeffer K, Virgin HW, III, Macmicking JD, Sibley LD. 2013. Guanylate-binding protein 1 (Gbp1) contributes to cell-autonomous immunity against *Toxoplasma gondii*. PLoS Pathog 9:e1003320. https://doi.org/10.1371/journal.ppat.1003320.
186. Degrandi D, Kravets E, Konermann C, Beuter-Gunia C, Klumpers V, Lahme S, Wischmann E, Mausberg AK, Beer-Hammer S, Pfeffer K. 2013. Murine guanylate binding protein 2 (mGBP2) controls *Toxoplasma gondii* replication. Proc Natl Acad Sci U S A 110:294–299. https://doi.org/10.1073/pnas.1205635110.
187. Yamamoto M, Okuyama M, Ma JS, Kimura T, Kamiyama N, Saiga H, Ohshima J, Sasai M, Kayama H, Okamoto T, Huang DCS, Soldati-Favre D, Horie K, Takeda J, Takeda K. 2012. A cluster of interferon-gamma-inducible p65 GTPases plays a critical role in host defense against *Toxoplasma gondii*. Immunity 37:302–313. https://doi.org/10.1016/j.immuni.2012.06.009.
188. Kravets E, Degrandi D, Ma Q, Peulen TO, Klumpers V, Felekyan S, Kuhnemuth R, Weidtkamp-Peters S, Seidel CA, Pfeffer K. 2016. Guanylate binding proteins directly attack Toxoplasma gondii via supramolecular complexes. eLife 5:e11479. https://doi.org/10.7554/eLife.11479.
189. Khaminets A, Hunn JP, Konen-Waisman S, Zhao YO, Preukschat D, Coers J, Boyle JP, Ong YC, Boothroyd JC, Reichmann G, Howard JC. 2010. Coordinated loading of IRG resistance GTPases on to the *Toxoplasma gondii* parasitophorous vacuole. Cell Microbiol 12:939–961. https://doi.org/10.1111/j.1462-5822.2010.01443.x.
190. Zhao Y, Ferguson DJ, Wilson DC, Howard JC, Sibley LD, Yap GS. 2009. Virulent *Toxoplasma gondii* evade immunity-related GTPase-mediated parasite vacuole disruption within primed macrophages. J Immunol 182:3775–3781. https://doi.org/10.4049/jimmunol.0804190.
191. Virreira Winter S, Niedelman W, Jensen KD, Rosowski EE, Julien L, Spooner E, Caradonna K, Burleigh BA, Saeij JP, Ploegh HL, Frickel EM. 2011. Determinants of GBP recruitment to Toxoplasma gondii vacuoles and the parasitic factors that control it. PLoS One 6:e24434. https://doi.org/10.1371/journal.pone.0024434.
192. Huang J, Brumell JH. 2014. Bacteria-autophagy interplay: a battle for survival. Nat Rev Microbiol 12:101–114. https://doi.org/10.1038/nrmicro3160.
193. Choi J, Park S, Biering S, Selleck EM, Liu C, Zhang X, Fujita N, Saitoh T, Akira S, Yoshimori T, Sibley LD, Hwang S, Virgin HW. 2014. The parasitophorous vacuole membrane of *Toxoplasma gondii* is targeted for disruption by ubiquitin-like conjugation systems of autophagy. Immunity 40:924–935. https://doi.org/10.1016/j.immuni.2014.05.006.

194. Ohshima J, Lee Y, Sasai M, Saitoh T, Su Ma J, Kamiyama N, Matsuura Y, Pann-Ghill S, Hayashi M, Ebisu S, Takeda K, Akira S, Yamamoto M. 2014. Role of mouse and human autophagy proteins in IFN-gamma-induced cell-autonomous responses against *Toxoplasma gondii*. J Immunol 192:3328–3335. https://doi.org/10.4049/jimmunol.1302822.
195. Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, Miller BC, Cadwell K, Delgado MA, Ponpuak M, Green KG, Schmidt RE, Mizushima N, Deretic V, Sibley LD, Virgin HW. 2008. Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. Cell Host Microbe 4:458–469. https://doi.org/10.1016/j.chom.2008.10.003.
196. Ceravolo IP, Chaves AC, Bonjardim CA, Sibley D, Romanha AJ, Gazzinelli RT. 1999. Replication of *Toxoplasma gondii*, but not *Trypanosoma cruzi*, is regulated in human fibroblasts activated with gamma interferon: requirement of a functional JAK/STAT pathway. Infect Immun 67:2233–2240.
197. Hunter CA, Sibley LD. 2012. Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. Nat Rev Microbiol 10:766–778. https://doi.org/10.1038/nrmicro2858.
198. Howard JC, Hunn JP, Steinfeldt T. 2011. The IRG protein-based resistance mechanism in mice and its relation to virulence in *Toxoplasma gondii*. Curr Opin Microbiol 14:414–421. https://doi.org/10.1016/j.mib.2011.07.002.
199. MacMicking JD. 2012. Interferon-inducible effector mechanisms in cell-autonomous immunity. Nat Rev Immunol 12:367–382. https://doi.org/10.1038/nri3210.
200. Johnston AC, Piro A, Clough B, Siew M, Virreira Winter S, Coers J, Frickel EM. 2016. Human GBP1 does not localize to pathogen vacuoles but restricts Toxoplasma gondii. Cell Microbiol 18:1056–1064. https://doi.org/10.1111/cmi.12579.
201. Murray HW, Byrne GI, Rothermel CD, Cartelli DM. 1983. Lymphokine enhances oxygen-independent activity against intracellular pathogens. J Exp Med 158:234–239. https://doi.org/10.1084/jem.158.1.234.
202. Pfefferkorn ER. 1984. Interferon-gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host to degrade tryptophan. Proc Natl Acad Sci U S A 81:908–912. https://doi.org/10.1073/pnas.81.3.908.
203. Dimier IH, Bout DT. 1997. Inhibition of Toxoplasma gondii replication in IFN-gamma-activated human intestinal epithelial cells. Immunol Cell Biol 75:511–514. https://doi.org/10.1038/icb.1997.80.
204. Witola WH, Mui E, Hargrave A, Liu S, Hypolite M, Montpetit A, Cavailles P, Bisanz C, Cesbron-Delauw MF, Fournié GJ, McLeod R. 2011. NALP1 influences susceptibility to human congenital toxoplasmosis, proinflammatory cytokine response, and fate of Toxoplasma gondii-infected monocytic cells. Infect Immun 79:756–766. https://doi.org/10.1128/IAI.00898-10.
205. Selleck EM, Orchard RC, Lassen KG, Beatty WL, Xavier RJ, Levine B, Virgin HW, Sibley LD. 2015. A noncanonical autophagy pathway restricts Toxoplasma gondii growth in a strain-specific manner in IFN-gamma-activated human cells. mBio 6:e01157-15. https://doi.org/10.1128/mBio.01157-15.
206. Levine B, Mizushima N, Virgin HW. 2011. Autophagy in immunity and inflammation. Nature 469:323–335. https://doi.org/10.1038/nature09782.
207. Clough B, Wright JD, Pereira PM, Hirst EM, Johnston AC, Henriques R, Frickel EM. 2016. K63-linked ubiquitination targets Toxoplasma gondii for endo-lysosomal destruction in IFNgamma-stimulated human cells. PLoS Pathog 12:e1006027. https://doi.org/10.1371/journal.ppat.1006027.
208. Andrade RM, Wessendarp M, Subaste CS. 2003. CD154 activates macrophage anti-microbial activity in the absence of IFN-gamma through a TNF-alpha dependent mechanism. J Immunol 171:6750–6756. https://doi.org/10.4049/jimmunol.171.12.6750.
209. Andrade RM, Wessendarp M, Gubbels JM, Striepen B, Subaste CS. 2006. CD40 induces macrophage anti-*Toxoplasma gondii* activity by triggering autophagy-dependent fusion of pathogen-containing vacuoles and lysosomes. J Clin Invest 116:2366–2377. https://doi.org/10.1172/JCI28796.
210. Portillo JA, Van Grol J, Zheng L, Okenka G, Gentil K, Garland A, Carlson EC, Kern TS, Subauste CS. 2008. CD40 mediates retinal inflammation and neurovascular degeneration. J Immunol 181:8719–8726. https://doi.org/10.4049/jimmunol.181.12.8719.
211. Subauste CS. 2009. Autophagy in immunity against Toxoplasma gondii. Curr Top Microbiol Immunol 335:251–265. https://doi.org/10.1007/978-3-642-00302-8_12.
212. Dupont CD, Christian DA, Hunter CA. 2012. Immune response and immunopathology during toxoplasmosis. Semin Immunopathol 34:793–813. https://doi.org/10.1007/s00281-012-0339-3.
213. Yarovinsky F. 2014. Innate immunity to Toxoplasma gondii infection. Nat Rev Immunol 14:109–121. https://doi.org/10.1038/nri3598.
214. Khan A, Taylor S, Su C, Mackey AJ, Boyle J, Cole RH, Glover D, Tang K, Paulsen I, Berriman M, Boothroyd JC, Pfefferkorn ER, Dubey JP, Roos DS, Ajioka JW, Wootton JC, Sibley LD. 2005. Composite genome map and recombination parameters derived from three archetypal lineages of *Toxoplasma gondii*. Nucleic Acids Res 33:2980–2992. https://doi.org/10.1093/nar/gki604.
215. Behnke MS, Dubey JP, Sibley LD. 2016. Genetic mapping of pathogenesis determinants in Toxoplasma gondii. Annu Rev Microbiol 70:63–81. https://doi.org/10.1146/annurev-micro-091014-104353.
216. Su C, Howe DK, Dubey JP, Ajioka JW, Sibley LD. 2002. Identification of quantitative trait loci controlling acute virulence in *Toxoplasma gondii*. Proc Natl Acad Sci U S A 99:10753–10758. https://doi.org/10.1073/pnas.172117099.
217. Taylor S, Barragan A, Su C, Fux B, Fentress SJ, Tang K, Beatty WL, Hajjj EL, Jerome M, Behnke MS, White M, Wootton JC, Sibley LD. 2006. A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. Science 314:1776–1780. https://doi.org/10.1126/science.1133643.
218. Fentress SJ, Sibley LD. 2012. An arginine-rich domain of ROP18 is necessary for vacuole targeting and virulence in *Toxoplasma gondii*. Cell Microbiol 14:1921–1933. https://doi.org/10.1111/cmi.12022.
219. Saeij JPJ, Boyle JP, Coller S, Taylor S, Sibley LD, Brooke-Powell ET, Ajioka JW, Boothroyd JC. 2006. Polymorphic secreted kinases are key virulence factors in toxoplasmosis. Science 314:1780–1783. https://doi.org/10.1126/science.1133690.
220. Fentress SJ, Behnke MS, Dunay IR, Moashayekhi M, Rommereim LM, Fox BA, Bzik DJ, Tayor GA, Turk BE, Lichti CF, Townsend RR, Qiu W, Hui R, Beatty WL, Sibley LD. 2010. Phosphorylation of immunity-related GTPases by a parasite secretory kinase promotes macrophage survival and virulence. Cell Host Microbe 16:484–495. https://doi.org/10.1016/j.chom.2010.11.005.
221. Steinfeldt T, Konen-Waisman S, Tong L, Pawlowski N, Lamkemeyer T, Sibley LD, Hunn JP, Howard JC. 2010. Phosphorylation of mouse immunity-related GTPase (IRG) resistance proteins is an evasion strategy for virulent *Toxoplasma gondii*. PLoS Biol 8:e1000576. https://doi.org/10.1371/journal.pbio.1000576.
222. Ghosh A, Uthaiah R, Howard J, Herrmann C, Wolf E. 2004. Crystal structure of IIGP1: a paradigm for interferon-inducible p47 resistance GTPases. Mol Cell 15:727–739. https://doi.org/10.1016/j.molcel.2004.07.017.
223. Behnke MS, Fentress SJ, Mashayekhi M, Li LL, Taylor GA, Sibley LD. 2012. The polymorphic pseudokinase ROP5 controls virulence in *Toxoplasma gondii* by regulating the active kinase ROP18. PLoS Pathog 8:e1002992. https://doi.org/10.1371/journal.ppat.1002992.
224. Reese ML, Zeiner GM, Saeij JP, Boothroyd JC, Boyle JP. 2011. Polymorphic family of injected pseudokinases is paramount in Toxoplasma virulence. Proc Natl Acad Sci U S A 108:9625–9630. https://doi.org/10.1073/pnas.1015980108.
225. Reese ML, Shah N, Boothroyd JC. 2014. The Toxoplasma pseudokinase ROP5 is an allosteric inhibitor of the immunity-related GTPases. J Biol Chem 289:27849–27858. https://doi.org/10.1074/jbc.M114.567057.
226. Khan A, Taylor S, Ajioka JW, Rosenthal BM, Sibley LD. 2009. Selection at a single locus leads to widespread expansion of *Toxoplasma gondii* lineages that are virulent in mice. PLoS Genet 5:e1000404. https://doi.org/10.1371/journal.pgen.1000404.
227. Niedelman W, Gold DA, Rosowski EE, Sprokholt JK, Lim D, Farid Arenas A, Melo MB, Spooner E, Yaffe MB, Saeij JP. 2012. The rhoptry proteins ROP18 and ROP5 mediate *Toxoplasma gondii* evasion of the murine, but not the human, interferon-gamma response. PLoS Pathog 8:e1002784. https://doi.org/10.1371/journal.ppat.1002784.
228. Behnke MS, Khan A, Lauron EJ, Jimah JR, Wang Q, Tolia NH, Sibley LD. 2015. Rhoptry proteins ROP5 and ROP18 are major murine virulence factors in genetically divergent South American strains of Toxoplasma gondii. PLoS Genet 11:e1005434. https://doi.org/10.1371/journal.pgen.1005434.
229. Etheridge RD, Alagan A, Tang K, Turk BE, Sibley LD. 2014. ROP18 and ROP17 kinase complexes synergize to control acute virulence of Tox-

oplasma in the mouse. Cell Host Microbe 15:537–550. https://doi.org/10.1016/j.chom.2014.04.002.
230. Kim EW, Nadipuram SM, Tetlow AL, Barshop WD, Liu PT, Wohlschlegel JA, Bradley PJ. 2016. The rhoptry pseudokinase ROP54 modulates Toxoplasma gondii virulence and host GBP2 loading. mSphere 1:e00045–16. https://doi.org/10.1128/mSphere.00045-16.
231. Lilue J, Muller UB, Steinfeldt T, Howard JC. 2013. Reciprocal virulence and resistance polymorphism in the relationship between Toxoplasma gondii and the house mouse. eLife 2:e01298. https://doi.org/10.7554/eLife.01298.
232. Sanchez V, de-la-Torre A, Gomez-Marin JE. 2014. Characterization of ROP18 alleles in human toxoplasmosis. Parasitol Int 63:463–469. https://doi.org/10.1016/j.parint.2013.10.012.
233. Yamamoto M, Ma JS, Mueller C, Kamiyama N, Saiga H, Kubo E, Kimura T, Okamoto T, Okuyama M, Kayama H, Nagamune K, Takashima S, Matsuura Y, Soldati-Favre D, Takeda K. 2011. ATF6-beta is a host cellular target of the *Toxoplasma gondii* virulence factor ROP18. J Exp Med 208:1533–1546. https://doi.org/10.1084/jem.20101660.
234. Cannella D, Brenier-Pinchart M-P, Braun L, van Rooyen JM, Bougdour A, Bastien O, Behnke MS, Curt R-L, Curt A, Saeij JPJ, Sibley LD, Pelloux H, Hakimi M-A. 2014. miR-146a and miR-155 delineate a microRNA fingerprint associated with Toxoplasma persistence in the host brain. Cell Rep 6:928–937. https://doi.org/10.1016/j.celrep.2014.02.002.
235. Zeiner GM, Norman KL, Thomson JM, Hammond SM, Boothroyd JC. 2010. Toxoplasma gondii infection specifically increases the levels of key host microRNAs. PLoS One 5:e8742. https://doi.org/10.1371/journal.pone.0008742.
236. Jensen KDC, Wang Y, Wojno EDT, Shastri AJ, Hu K, Cornel L, Boedec E, Ong Y-C, Chien Y, Hunter CA, Boothroyd JC, Saeij JPJ. 2011. Toxoplasma polymorphic effectors determine macrophage polarization and intestinal inflammation. Cell Host Microbe 9:472–483. https://doi.org/10.1016/j.chom.2011.04.015.
237. Saeij JPJ, 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 445:324–327. https://doi.org/10.1038/nature05395.
238. Yamamoto M, Standley DM, Takashima S, Saiga H, Okuyama M, Kayama H, Kubo E, Ito H, Takaura M, Matsuda T, Soldati-Favre D, Takeda K. 2009. A single polymorphic amino acid on Toxoplasma gondii kinase ROP16 determines the direct and strain-specific activation of Stat3. J Exp Med 206:2747–2760. https://doi.org/10.1084/jem.20091703.
239. Kim L, Del Rio L, Butcher BA, Mogensen TH, Paludan SR, Flavell RA, Denkers EY. 2005. p38 MAPK autophosphorylation drives macrophage IL-12 production during intracellular infection. J Immunol 174:4178–4184. https://doi.org/10.4049/jimmunol.174.7.4178.
240. Kim S-K, 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 178:5154–5165. https://doi.org/10.4049/jimmunol.178.8.5154.
241. Rosowski EE, Nguyen QP, Camejo A, Spooner E, Saeij JPJ. 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 82:706–719. https://doi.org/10.1128/IAI.01291-13.
242. Wiley M, Teygong C, Phelps E, Radke J, Blader IJ. 2011. Serum response factor regulates immediate early host gene expression in Toxoplasma gondii-infected host cells. PLoS One 6:e18335. https://doi.org/10.1371/journal.pone.0018335.
243. Rosowski EE, Lu D, Julien L, Rodda L, Gaiser RA, Jensen KDC, Saeij JPJ. 2011. Strain-specific activation of the NF-kappaB pathway by GRA15, a novel Toxoplasma gondii dense granule protein. J Exp Med 208:195–212. https://doi.org/10.1084/jem.20100717.
244. Butcher BA, Fox BA, Rommereim LM, Kim SG, Maurer KJ, Yarovinsky F, Herbert DBR, Bzik DJ, Denkers EY. 2011. Toxoplasma gondii rhoptry kinase ROP16 activates STAT3 and STAT6 resulting in cytokine inhibition and arginase-1-dependent growth control. PLoS Pathog 7:e1002236. https://doi.org/10.1371/journal.ppat.1002236.
245. Gorfu G, Cirelli KM, Melo MB, Mayer-Barber K, Crown D, Koller BH, Masters S, Sher A, Leppla SH, Moayeri M, Saeij JP, Grigg ME. 2014. Dual role for inflammasome sensors NLRP1 and NLRP3 in murine resistance to Toxoplasma gondii. mBio 5:e01117-13. https://doi.org/10.1128/mBio.01117-13.
246. 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. https://doi.org/10.1128/mBio.00255-13.
247. Yang L, Boldin MP, Yu Y, Liu CS, Ea C-K, Ramakrishnan P, Taganov KD, Zhao JL, Baltimore D. 2012. miR-146a controls the resolution of T cell responses in mice. J Exp Med 209:1655–1670. https://doi.org/10.1084/jem.20112218.
248. Butcher BA, Kim L, Johnson PF, Denkers EY. 2001. Toxoplasma gondii tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF-kappa B. J Immunol 167:2193–2201. https://doi.org/10.4049/jimmunol.167.4.2193.
249. Shapira S, Speirs K, Gerstein A, Caamano J, Hunter CA. 2002. Suppression of NF-kappaB activation by infection with Toxoplasma gondii. J Infect Dis 185(Suppl 1):S66–S72. https://doi.org/10.1086/338000.
250. Ma JS, Sasai M, Ohshima J, Lee Y, Bando H, Takeda K, Yamamoto M. 2014. Selective and strain-specific NFAT4 activation by the Toxoplasma gondii polymorphic dense granule protein GRA6. J Exp Med 211:2013–2032. https://doi.org/10.1084/jem.20131272.
251. Bougdour A, Durandau E, Brenier-Pinchart M-P, Ortet P, Barakat M, Kieffer S, Curt-Varesano A, Curt-Bertini R-L, Bastien O, Coute Y, Pelloux H, Hakimi M-A. 2013. Host cell subversion by Toxoplasma GRA16, an exported dense granule protein that targets the host cell nucleus and alters gene expression. Cell Host Microbe 13:489–500. https://doi.org/10.1016/j.chom.2013.03.002.
252. Saridakis V, Sheng Y, Sarkari F, Holowaty MN, Shire K, Nguyen T, Zhang RG, Liao J, Lee W, Edwards AM, Arrowsmith CH, Frappier L. 2005. Structure of the p53 binding domain of HAUSP/USP7 bound to Epstein-Barr nuclear antigen 1 implications for EBV-mediated immortalization. Mol Cell 18:25–36. https://doi.org/10.1016/j.molcel.2005.02.029.
253. Reid MA, Wang W-I, Rosales KR, Welliver MX, Pan M, Kong M. 2013. The B55α subunit of PP2A drives a p53-dependent metabolic adaptation to glutamine deprivation. Mol Cell 50:200–211. https://doi.org/10.1016/j.molcel.2013.02.008.
254. Braun L, Brenier-Pinchart M-P, Yogavel M, Curt-Varesano A, Curt-Bertini R-L, Hussain T, Kieffer-Jaquinod S, Coute Y, Pelloux H, Tardieux I, Sharma A, Belrhali H, Bougdour A, Hakimi M-A. 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 210:2071–2086. https://doi.org/10.1084/jem.20130103.
255. Pellegrini E, Palencia A, Braun L, Kapp U, Bougdour A, Belrhali H, Bowler MW, Hakimi M-A. 2017. Structural basis for the subversion of MAP kinase signaling by an intrinsically disordered parasite secreted agonist. Structure 25:16–26. https://doi.org/10.1016/j.str.2016.10.011.
256. Andrisani OM. 1999. CREB-mediated transcriptional control. Crit Rev Eukaryot Gene Expr 9:19–32.
257. Gay G, Braun L, Brenier-Pinchart M-P, Vollaire J, Josserand V, Bertini R-L, Varesano A, Touquet B, De Bock P-J, Coute Y, Tardieux I, Bougdour A, Hakimi M-A. 2016. Toxoplasma gondii TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-γ-mediated host defenses. J Exp Med 213:1779–1798. https://doi.org/10.1084/jem.20160340.
258. Olias P, Etheridge RD, Zhang Y, Holtzman MJ, Sibley LD. 2016. Toxoplasma effector recruits the Mi-2/NuRD complex to repress STAT1 transcription and block IFN-γ-dependent gene expression. Cell Host Microbe 20:72–82. https://doi.org/10.1016/j.chom.2016.06.006.
259. Sadzak I, Schiff M, Gattermeier I, Glinitzer R, Sauer I, Saalmüller A, Yang E, Schaljo B, Kovarik P. 2008. Recruitment of Stat1 to chromatin is required for interferon-induced serine phosphorylation of Stat1 transactivation domain. Proc Natl Acad Sci U S A 105:8944–8949. https://doi.org/10.1073/pnas.0801794105.
260. Wojciak JM, Martinez-Yamout MA, Dyson HJ, Wright PE. 2009. Structural basis for recruitment of CBP/p300 coactivators by STAT1 and STAT2 transactivation domains. EMBO J 28:948–958. https://doi.org/10.1038/emboj.2009.30.
261. Krämer OH, Heinzel T. 2010. Phosphorylation-acetylation switch in the regulation of STAT1 signaling. Mol Cell Endocrinol 315:40–48. https://doi.org/10.1016/j.mce.2009.10.007.
262. Xue Y, Wong J, Moreno GT, Young MK, Côté J, Wang W. 1998. NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell 2:851–861. https://doi.org/10.1016/S1097-2765(00)80299-3.
263. Matsumura Y, Nakaki R, Inagaki T, Yoshida A, Kano Y, Kimura H, Tanaka T, Tsutsumi S, Nakao M, Doi T, Fukami K, Osborne TF, Kodama T,

Aburatani H, Sakai J. 2015. H3K4/H3K9me3 bivalent chromatin domains targeted by lineage-specific DNA methylation pauses adipocyte differentiation. Mol Cell 60:584–596. https://doi.org/10.1016/j.molcel.2015.10.025.
264. Voigt P, Tee W-W, Reinberg D. 2013. A double take on bivalent promoters. Genes Dev 27:1318–1338. https://doi.org/10.1101/gad.219626.113.
265. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. 2009. An operational definition of epigenetics. Genes Dev 23:781–783. https://doi.org/10.1101/gad.1787609.
266. Silva NM, Rodrigues CV, Santoro MM, Reis LFL, Alvarez-Leite JI, Gazzinelli RT. 2002. Expression of indoleamine 2,3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with Toxoplasma gondii: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. Infect Immun 70:859–868. https://doi.org/10.1128/IAI.70.2.859-868.2002.
267. Doyle EL, Stoddard BL, Voytas DF, Bogdanove AJ. 2013. TAL effectors: highly adaptable phytobacterial virulence factors and readily engineered DNA-targeting proteins. Trends Cell Biol 23:390–398. https://doi.org/10.1016/j.tcb.2013.04.003.
268. Hakimi M-A, Bougdour A. 2015. Toxoplasma's ways of manipulating the host transcriptome via secreted effectors. Curr Opin Microbiol 26:24–31. https://doi.org/10.1016/j.mib.2015.04.003.
269. Davey NE, Van Roey K, Weatheritt RJ, Toedt G, Uyar B, Altenberg B, Budd A, Diella F, Dinkel H, Gibson TJ. 2012. Attributes of short linear motifs. Mol Biosyst 8:268–281. https://doi.org/10.1039/C1MB05231D.
270. Fuxreiter M, Tompa P, Simon I. 2007. Local structural disorder imparts plasticity on linear motifs. Bioinformatics 23:950–956. https://doi.org/10.1093/bioinformatics/btm035.
271. Hagai T, Azia A, Babu MM, Andino R. 2014. Use of host-like peptide motifs in viral proteins is a prevalent strategy in host-virus interactions. Cell Rep 7:1729–1739. https://doi.org/10.1016/j.celrep.2014.04.052.
272. Marín M, Uversky VN, Ott T. 2013. Intrinsic disorder in pathogen effectors: protein flexibility as an evolutionary hallmark in a molecular arms race. Plant Cell 25:3153–3157. https://doi.org/10.1105/tpc.113.116319.
273. Dyson HJ. 2012. Roles of intrinsic disorder in protein-nucleic acid interactions. Mol Biosyst 8:97–104. https://doi.org/10.1039/C1MB05258F.
274. Spillman NJ, Beck JR, Goldberg DE. 2015. Protein export into malaria parasite-infected erythrocytes: mechanisms and functional consequences. Annu Rev Biochem 84:813–841. https://doi.org/10.1146/annurev-biochem-060614-034157.
275. Hsiao C-HC, Hiller NL, Haldar K, Knoll LJ. 2013. A HT/PEXEL motif in Toxoplasma dense granule proteins is a signal for protein cleavage but not export into the host cell. Traffic 14:519–531. https://doi.org/10.1111/tra.12049.
276. Hammoudi P-M, Jacot D, Mueller C, Di Cristina M, Dogga SK, Marq J-B, Romano J, Tosetti N, Dubrot J, Emre Y, Lunghi M, Coppens I, Yamamoto M, Sojka D, Pino P, Soldati-Favre D. 2015. Fundamental roles of the Golgi-associated toxoplasma aspartyl protease, ASP5, at the host-parasite interface. PLoS Pathog 11:e1005211. https://doi.org/10.1371/journal.ppat.1005211.
277. Coffey MJ, Sleebs BE, Uboldi AD, Garnham A, Franco M, Marino ND, Panas MW, Ferguson DJ, Enciso M, O'Neill MT, Lopaticki S, Stewart RJ, Dewson G, Smyth GK, Smith BJ, Masters SL, Boothroyd JC, Boddey JA, Tonkin CJ. 2015. An aspartyl protease defines a novel pathway for export of Toxoplasma proteins into the host cell. eLife 4:e10809. https://doi.org/10.7554/eLife.10809.
278. Curt-Varesano A, Braun L, Ranquet C, Hakimi M-A, Bougdour A. 2016. The aspartyl protease TgASP5 mediates the export of the Toxoplasma GRA16 and GRA24 effectors into host cells. Cell Microbiol 18:151–167. https://doi.org/10.1111/cmi.12498.
279. Nadipuram SM, Kim EW, Vashisht AA, Lin AH, Bell HN, Coppens I, Wohlschlegel JA, Bradley PJ. 2016. In vivo biotinylation of the Toxoplasma parasitophorous vacuole reveals novel dense granule proteins important for parasite growth and pathogenesis. mBio 7:e00808-16. https://doi.org/10.1128/mBio.00808-16.
280. Gold DA, Kaplan AD, Lis A, Bett GCL, Rosowski EE, Cirelli KM, Bougdour A, Sidik SM, Beck JR, Lourido S, Egea PF, Bradley PJ, Hakimi M-A, Rasmusson RL, Saeij JPJ. 2015. The Toxoplasma dense granule proteins GRA17 and GRA23 mediate the movement of small molecules between the host and the parasitophorous vacuole. Cell Host Microbe 17:642–652. https://doi.org/10.1016/j.chom.2015.04.003.
281. Franco M, Panas MW, Marino ND, Lee M-CW, Buchholz KR, Kelly FD, Bednarski JJ, Sleckman BP, Pourmand N, Boothroyd JC. 2016. A novel secreted protein, MYR1, is central to Toxoplasma's manipulation of host cells. mBio 7:e02231-15. https://doi.org/10.1128/mBio.02231-15.
282. 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 8:339–352. https://doi.org/10.1111/j.1462-5822.2005.00628.x.
283. Wiley M, Sweeney KR, Chan DA, Brown KM, McMurtrey C, Howard EW, Giaccia AJ, Blader IJ. 2010. Toxoplasma gondii activates hypoxia-inducible factor (HIF) by stabilizing the HIF-1alpha subunit via type I activin-like receptor kinase receptor signaling. J Biol Chem 285:26852–26860. https://doi.org/10.1074/jbc.M110.147041.
284. 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 76:4703–4712. https://doi.org/10.1128/IAI.01447-07.
285. Wang Y, Weiss LM, Orlofsky A. 2009. Intracellular parasitism with Toxoplasma gondii stimulates mammalian-target-of-rapamycin-dependent host cell growth despite impaired signalling to S6K1 and 4E-BP1. Cell Microbiol 11:983–1000. https://doi.org/10.1111/j.1462-5822.2009.01305.x.
286. Carmen JC, Hardi L, Sinai AP. 2006. *Toxoplasma gondii* inhibits ultraviolet light-induced apoptosis through multiple interactions with the mitochondrion-dependent programmed cell death pathway. Cell Microbiol 8:301–315. https://doi.org/10.1111/j.1462-5822.2005.00622.x.
287. Goebel S, Gross U, Lüder CGK. 2001. Inhibition of host cell apoptosis by *Toxoplasma gondii* is accompanied by reduced activation of the caspase cascade and alterations of the poly(ADP-ribose) polymerase expression. J Cell Sci 114:3495–3505.
288. Goebel S, Luder CG, Gross U. 1999. Invasion by *Toxoplasma gondii* protects human-derived HL-60 cells from actinomycin D-induced apoptosis. Med Microbiol Immunol 187:221–226. https://doi.org/10.1007/s004300050096.
289. Kim L, Denkers EY. 2006. Toxoplasma gondii triggers Gi-dependent PI 3-kinase signaling required for inhibition of host cell apoptosis. J Cell Sci 119:2119–2126. https://doi.org/10.1242/jcs.02934.
290. Luder CG, Gross U. 2005. Apoptosis and its modulation during infection with *Toxoplasma gondii*: molecular mechanisms and role in pathogenesis. Curr Top Microbiol Immunol 289:219–237.
291. Nash PB, Purner MB, Leon RP, Clarke P, Duke RC, Curiel TJ. 1998. *Toxoplasma gondii*-infected cells are resistant to multiple inducers of apoptosis. J Immunol 160:1824–1830.
292. Payne TM, Molestina RE, Sinai AP. 2003. Inhibition of caspase activation and a requirement for NF-kB function in the *Toxoplasma gondii*-mediated blockade of host apoptosis. J Cell Sci 116:4345–4358. https://doi.org/10.1242/jcs.00756.
293. Cavailles P, Sergent V, Bisanz C, Papapietro O, Colacios C, Mas M, Subra JF, Lagrange D, Calise M, Appolinaire S, Faraut T, Druet P, Saoudi A, Bessieres MH, Pipy B, Cesbron-Delauw MF, Fournie GJ. 2006. The rat *Toxo1* locus directs toxoplasmosis outcome and controls parasite proliferation and spreading by macrophage-dependent mechanisms. Proc Natl Acad Sci U S A 103:744–749. https://doi.org/10.1073/pnas.0506643103.
294. Wang Y, Weiss LM, Orlofsky A. 2009. Host cell autophagy is induced by Toxoplasma gondii and contributes to parasite growth. J Biol Chem 284:1694–1701. https://doi.org/10.1074/jbc.M807890200.
295. Talevich E, Kannan N. 2013. Structural and evolutionary adaptation of rhoptry kinases and pseudokinases, a family of coccidian virulence factors. BMC Evol Biol 13:117. https://doi.org/10.1186/1471-2148-13-117.
296. Shastri AJ, Marino ND, Franco M, Lodoen MB, Boothroyd JC. 2014. GRA25 is a novel virulence factor of Toxoplasma gondii and influences the host immune response. Infect Immun 82:2595–2605. https://doi.org/10.1128/IAI.01339-13.
297. Silmon de Monerri NC, Kim K. 2014. Pathogens hijack the epigenome: a new twist on host-pathogen interactions. Am J Pathol 184:897–911. https://doi.org/10.1016/j.ajpath.2013.12.022.

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