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An Inside Job: Hacking into Janus Kinase/Signal Transducer and Activator of Transcription Signaling Cascades by the Intracellular Protozoan *Toxoplasma gondii*

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The intracellular protozoan *Toxoplasma gondii* is well known for its skill at invading and living within host cells. New discoveries are now also revealing the astounding ability of the parasite to inject effector proteins into the cytoplasm to seize control of the host cell. This review summarizes recent advances in our understanding of one such secretory protein called ROP16. This molecule is released from rhoptries into the host cell during invasion. The ROP16 molecule acts as a kinase, directly activating both signal transducer and activator of transcription 3 (STAT3) and STAT6 signaling pathways. In macrophages, an important and preferential target cell of parasite infection, the injection of ROP16 has multiple consequences, including downregulation of proinflammatory cytokine signaling and macrophage deviation to an alternatively activated phenotype.

Intracellular microorganisms, whether bacterial, fungal, or protozoal, are faced with special challenges to achieve a productive infection. They must survive in a potentially hostile intracellular environment, and to do so they employ strategies of host evasion, active interference with host cell machinery, or a combination of both. The payoff of this high-risk intracellular lifestyle is that the microorganism gains access to the nutrient-rich environment of the host cytosol and at the same time avoids extracellular effectors of host immunity. Unraveling how intracellular pathogens achieve this intracellular ecological niche is not only fascinating from a purely biological perspective but can also provide us with new targets to control infection. In addition, understanding mechanisms developed by intracellular pathogens to manipulate the host cell internal environment may provide new insights into controlling mammalian cell behavior.

Nowhere is this more the case than for the apicomplexan protozoan *Toxoplasma gondii*, a parasite that has emerged in recent years as the model obligate intracellular eukaryotic pathogen. *Toxoplasma* is transmitted by ingestion of infectious cysts as a result of carnivorism or predation. In the intestine of cats, *T. gondii* undergoes sexual reproduction, resulting in fecal shedding of highly infectious oocysts (22). While normally asymptomatic, *Toxoplasma* may cause severe disease in immunocompromised populations and during congenital infection (63). The astonishingly widespread geographical and biological distribution of *T. gondii* is a dramatic indication of the success of this parasite in living with its host and achieving successful transmission to new hosts.

Toxoplasma tachyzoites (the rapidly replicating form of the parasite responsible for acute toxoplasmosis) enter host cells through a well-studied process of active invasion involving parasite actin-based motility and establishment of a moving junction at the interface between host and parasite membranes (57, 77, 85). During invasion, the parasite creates a specialized parasitophorous vacuole that resists acidification and lysosomal fusion (58). The vacuole membrane consists of parasite and host lipids and a subset of parasite proteins but is largely devoid of host cell proteins (57, 80). The parasitophorous vacuole membrane (PVM) serves as a molecular sieve through which *T. gondii* scavenges host cell nutrients, including certain amino acids, nucleic acid precursors, and lipids, such as cholesterol (12, 16, 24, 25, 74). During invasion and creation of the PVM, apically oriented organelles (hence the term "apicomplexan" for this group of protozoa) called micronemes and rhoptries are discharged, followed later by release of dense granules (11, 35). Regulated secretion of parasite proteins originating from these organelles mediates adhesion, invasion, and creation of the mature PVM. Of direct relevance to the present review, it is also now clear that some of these secreted molecules are injected directly into the host cell cytoplasm during invasion (32). Moreover, some injected parasite molecules are directed to the host cell nucleus (6, 31). One of these parasite proteins is ROP16, a specialized kinase that hacks into host cell signaling cascades to modify the behavior of the parasite-infected host cell (71).

Contained within the parasitophorous vacuole, *Toxoplasma* evades elimination by the immune system. The parasite actively deploys an infection strategy that keeps the host alive to allow establishment of long-lasting latent infection, promoting the likelihood of transmission to new hosts. The latent phase of infection is characterized by the formation of quiescent cysts in the brain and skeletal muscle tissue. To prevent host death, *T. gondii* triggers a robust Th1 response characterized by early interleukin 12 (IL-12) production by cells, such as macrophages, dendritic cells (DC), and neutrophils (5, 29, 67). Early IL-12 production is followed by emergence of gamma interferon (IFN-γ)-producing $CD4^+$ and $CD8^+$ T lymphocytes, and these cell types are required

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TABLE 1 *Toxoplasma* molecules that interact with components of host defense

Parasite molecule	Host target/ligand	Major effect on host	Reference(s)
Profilin	TLR11	Induction of IL-12	90
Cyclophilin-18	CCR5	Induction of IL-12	1
GPI moeities	TLR2, TLR4	Induction of IL-12, TNF-α	19
ROP16	STAT3, STAT6	IL-12 downregulation, arginase 1 induction	8, 36, 61, 71, 89
ROP18	IRG proteins, ATF6β	PVM destruction, promotion of CD8 responses	23, 79
ROP5	Unknown	Contributes to virulence	3, 65
ROP38	Unknown	Suppression of MAPK signaling	62
GRA15	NF-κB	IL-12 induction	36, 69

for surviving acute infection and ultimately preventing potentially lethal reactivation events during chronic infection (20, 26, 28, 82). Both IL-12 and IFN-γ production are essential for the host to survive infection (72, 73, 81). Absence of either cytokine results in the inability to control parasite replication and dissemination, resulting in massive tissue necrosis and host death. Yet, parasite-induced production of both IL-12 and IFN-γ must also be tightly regulated to prevent cytokine-mediated mortality (the so-called "cytokine storm"). Control of these proinflammatory mediators is achieved initially by direct parasite-mediated suppression (further detailed below) and later by induction of anti-inflammatory cytokines such as IL-10. Evidence for the importance of the latter cytokine was revealed by now classic studies demonstrating that without IL-10, mice rapidly succumb to *Toxoplasma* infection as a result of dysregulated proinflammatory cytokines rather than loss of an ability to control the parasite itself (30, 83).

Recent years have witnessed the identification of several *Toxoplasma* effector molecules that directly interact with cells of the immune system to mediate many of the effects described above (Table 1). Early reports suggested a role for parasite cyclophilin-18 interacting with chemokine receptor CCR5 to induce IL-12 (1). This was followed by identification of *Toxoplasma* profilin as a ligand for Toll-like receptor 11 (TLR11) driving DC IL-12 induction (90). Recently, release of dense granule protein GRA15 into the host cell cytoplasm was found to mediate NF-κB activation and initiate IL-12 synthesis (69). The rhoptry kinase ROP18 was identified as a virulence determinant that phosphorylates and deactivates host immunity-related p47 GTPase (IRG) proteins that mediate IFN-γ-dependent destruction of the PVM (23, 79). A rhoptry pseudokinase, ROP5, is a major virulence determinant, although the host target molecule is not yet known (3, 65). Similarly, ROP38 has been identified as a rhoptry protein influencing host cell signaling (62). Finally, the ROP16 kinase is another determinant of virulence (71). As we review here, the ROP16 molecule is highly complex, because it directly activates at least two distinct transcription factors in the classical Janus kinase (JAK)/signal transducer and activator of transcription (STAT) cytokine signaling pathway.

LINKING *TOXOPLASMA*-DEPENDENT IMMUNOSUPPRESSION TO STAT3 ACTIVATION AND FUNCTION

Although *T. gondii* is known for its ability to infect diverse host cell types, macrophages, DC, and neutrophils, major effectors of innate immunity, are preferentially targeted during *in vivo* infection (4, 14, 17). This is of keen biological interest, because these cell types are well known for their ability to produce cytokines and proinflammatory antimicrobial effector molecules. How does *Toxoplasma* survive within these masters of antimicrobial defense? Groundbreaking advances in recent years are for the first time yielding fascinating molecular insight into how this eukaryotic parasitic pathogen lives within these cells. In particular, *Toxoplasma* seizes control of intracellular signaling pathways during invasion, specifically those transduction pathways involved in the JAK/STAT pathway of cytokine production.

While *in vivo* infection stimulates strong type 1 cytokine-based immunity in the host, closer examination of the response unexpectedly reveals another side to this host-parasite interaction. For example, from within the infected cell, the parasite efficiently blocks TLR-linked signaling pathways that originate via extracellular signals, such as lipopolysaccharide (LPS) (9, 21, 43). Similarly, parasite-infected cells become largely nonresponsive to IFN-γ activation (39, 40, 50). This results in their defective ability to activate T cells (51). DC infected with *Toxoplasma* are also nonresponsive to TLR activation, and cells are likewise defective in T cell activation (56). Importantly, DC targeted for infection *in vivo* are also nonresponsive to *ex vivo* TLR activation (4). Early on, it was recognized that suppression of host responses by *T. gondii* required live parasites and an ability to actively invade host cells (7). The studies collectively suggest that *Toxoplasma* has developed mechanisms to downregulate antimicrobial effector mechanisms despite (or perhaps because of) being immersed in an overwhelmingly proinflammatory cytokine environment. Many different mechanisms have been proposed to explain these downregulatory effects. These include inhibition of NF-κB and STAT1 nuclear translocation (9, 52, 76), degradation of STAT1 (92), inhibition of mitogen-activated protein kinase (MAPK) signaling (37), induction of suppressor of eytokine synthesis-1 (SOCS1) (92), and most recently interference with chromatin remodeling (45, 46). While each of these phenomena may indeed contribute to parasite-mediated suppression, a unifying mechanism of suppression is not apparent.

Insight into how *Toxoplasma* interferes with host signal transduction came from the observation that the parasite triggers rapid, strong, and sustained activation of STAT3 during infection (10). The response requires live parasites and is restricted to infected cells rather than noninfected bystander cells. Importantly, the ability of *T. gondii* to mediate suppression of TLR responsiveness in STAT3-null macrophages was severely curtailed, suggesting that the parasite actively exploits this transcription factor to inhibit proinflammatory responses.

As a member of the JAK/STAT signaling family, the canonical model of STAT3 function is that cytokines such as IL-10 and IL-6 bind to their heterodimeric receptors, resulting in tyrosine phosphorylation of receptor-associated JAK kinases, in turn resulting in STAT3 recruitment (66). After JAK-dependent STAT3 tyrosine

phosphorylation, STAT3 dimerizes and translocates to the nucleus as a functional transcription factor. Full activation of STAT3 and other STAT molecules also requires MAPK-mediated serine phosphorylation. Nevertheless, this model JAK/STAT pathway has also been called into question, and alternate models involving constitutive shuttling of STAT between the nucleus and cytoplasm have been recently proposed (75).

The effects of STAT3 activation are complex. On the one hand, IL-10 activation of STAT3 leads to anti-inflammatory responses and attenuation of cytokines such as tumor necrosis factor alpha (TNF-α) in response to LPS (41, 84). On the other hand, IL-6, a proinflammatory cytokine that triggers the acute-phase response, also acts though activation of STAT3 (2). What determines whether STAT3 is a pro- or anti-inflammatory transcription factor depends upon the activity of suppressor of cytokine synthesis 3 (SOCS3) during IL-6 signaling (18, 42, 91). Thus, in the absence of SOCS3, IL-6-stimulated STAT3 activation is prolonged and the cytokine acts like IL-10 and downregulates TNF-α and IL-12. Because the suppression of proinflammatory responses mediated by *T. gondii* is dependent on STAT3 and parasite invasion, the parasite appears to be exploiting the IL-10 signaling pathway. Nevertheless, it was recently found that parasite-induced STAT3 activation is enhanced in the absence of SOCS3 in a manner similar to that of IL-6 (86). The implication of this interesting result is that there is additional complexity to *Toxoplasma*-mediated STAT3 activation that we have yet to understand.

RHOPTRY PROTEIN ROP16 ACTIVATES STAT3 AND STAT6

In Europe and North America, *Toxoplasma* occurs predominantly as 3 clonal lineages (types I, II, and III) that display differences in disease pathogenesis in humans and animals (78). Thus, type I strains are highly virulent in mice, whereas types II and III are considerably less virulent and cause less pathology during infection. The stage was set for a major leap in understanding *Toxoplasma*-host cell interactions by the observation that type II but not type I or type III parasite strains triggered large amounts of macrophage IL-12 and that this was accompanied by strain-specific translocation of NF-κB (38, 68). By genetic examination of F1 progeny between type II and type III parasite strains, virulence loci were identified, and in this way several secretory rhoptry proteins emerged as important parasite effectors that controlled virulence (6). In particular, ROP16 was identified as a virulence determinant, and pathway analysis suggested that interaction with STAT signaling was required for the activity of this rhoptry protein (70, 71). Type I and type III ROP16 were found to be involved in activation of both STAT3 and STAT6, whereas type II ROP16 was defective in this regard. The tyrosine and serine phosphorylation stimulated by ROP16 occur on the same amino acid residues as that induced by cytokine-mediated signaling.

The ROP16 molecule contains a nuclear localization sequence (NLS), and during invasion it is injected into the host cell cytoplasm followed by rapid translocation into the nucleus. As such, ROP16 is one of a growing number of rhoptry proteins and dense granule molecules now known to gain access to the intracellular environment during invasion. The exact mechanism by which ROP16 is introduced into the host cytoplasm is not known. Although ROP16 contains an NLS which is required for ROP16 to enter the nucleus, NLS-mutated ROP16 is still capable of activating STAT6 and also probably STAT3, suggesting that this rhoptry molecule acts in the cytoplasm to phosphorylate STAT proteins (71). This raises the interesting possibility that ROP16 may also possess STAT3- and STAT6-independent activities that provide additional functions within the host nucleus.

The ROP16 protein was originally identified as a putative serine-threonine kinase, but more recently it was shown by *in vitro* assays to directly tyrosine phosphorylate STAT3 and STAT6 (61, 89). Parenthetically, the recent observation that virus and nucleic acids activate STAT molecules through endoplasmic reticulum IFN stimulator (ERIS or STING)-dependent recruitment to the endoplasmic reticulum provides another possible model for STAT3 and STAT6 activation by ROP16 (13). Consistent with the role of STAT3 in downregulating proinflammatory signaling, ROP16 knockout parasites induce larger amounts of IL-12 than wild-type parasites, and cells infected with ROP16 knockout parasites display a markedly reduced ability to suppress cellular responses triggered by LPS and IFN-γ (8).

Interestingly, using ROP16 knockout parasites, we found that immediate-early activation of STAT3 was normal in the absence of ROP16, but sustained phosphorylation of STAT3 required this rhoptry protein (8). In contrast, activation of STAT6 was completely dependent upon ROP16. The parasite or host molecules required for the immediate-early ROP16-independent STAT3 activation are not currently known. Although inhibitors of JAK signaling can block the immediate-early STAT3 response, interpretation of this result is difficult, because such inhibitors may also act on parasite kinases (61). Regardless, the observation that the kinetics of ROP16-dependent STAT3 and STAT6 activation are not directly parallel is further indication of the complexity of this rhoptry protein in the host-parasite interaction.

ROLE OF ROP16-DEPENDENT STAT6 ACTIVATION

Activation of STAT6 is normally associated with signaling driven by IL-4 or IL-13. In macrophages, this can lead to an M2 (or alternatively activated) phenotype that is associated with the production of anti-inflammatory mediators. In contrast, M1 (classically activated) macrophages are generated by proinflammatory cytokines and TLR ligands, such as LPS. One of the main products of M2 macrophages is arginase 1. This enzyme converts arginine into ornithine, which is a precursor of polyamines and collagen, in turn contributing to extracellular matrix generation. Thus, one of the main functions of alternatively activated macrophages is believed to be tissue repair, and for this reason these cells are also called wound-healing macrophages (59).

Type I ROP16 is a potent inducer of arginase 1, consistent with a specific role of ROP16 in sustained activation of STAT6 (8). In fact, an interesting antagonistic relationship has been proposed in which type I and type III parasites trigger ROP16-dependent and STAT6-dependent M2 activation, whereas type II parasites induce GRA15-dependent and NF-κB-dependent M1 activation (36). Two biologically opposing outcomes are possible following induction of arginase 1 in infected cells. On the one hand, *Toxoplasma* is a polyamine auxotroph; therefore, induction of arginase 1 could promote growth of type 1 parasites by supplying essential polyamine nutrients, as is seen in some cases (15, 36). Alternatively, the parasite is also an arginine auxotroph, and therefore induction of arginase 1 could act to limit growth of parasites by limiting the availability of this essential amino acid, which is also seen in some cases (8, 25). Possibly, use of different host cell types or amounts of arginine available underlies these disparate results. During *in vivo* infection, ROP16 knockout parasites display a

growth advantage over ROP16 competent parasites that depends on ROP16-dependent and STAT6-dependent host cell expression of arginase 1. The *in vivo* growth advantage observed in ROP16 knockout parasites suggests that arginine availability can become limiting during infection (8). Parasite control of host signaling pathways can regulate nutrient availability, perhaps allowing the parasite to regulate its own growth rate. This is a particularly interesting nutritional adaptation for ROP16, because *Toxoplasma* is unusual in lacking metabolic pathways for either synthesis of arginine (25), or for the degradation of this amino acid via a parasite-encoded arginase (24).

ROP16-mediated STAT6 activation and induction of arginase 1 may also decrease the availability of arginine as a substrate for inducible nitric oxide synthase (iNOS). Therefore, in addition to the potential of ROP16 to reduce arginine availability for parasite growth, ROP16 may also enhance parasite survival by decreasing the production of nitric oxide (NO). While iNOS and NO do not appear to be essential for the control of acute infection, these host responses are necessary for the control of chronic infection (72).

Additional functions for parasite-induced STAT6 activation are also possible. The chemokines CCL17 and CCL22 (whose receptor is CCR4) are strongly upregulated in macrophages infected with type I but not type II *T. gondii* (44). The *ccl17* gene is known to possess functional STAT6 binding sites, and it therefore seems likely that this chemokine is under the control of type I ROP16 (47, 87). Studies in *Ccr4*$^{-/-}$ mice suggest that this receptor and its chemokine ligands are involved in several aspects of innate immunity as well as in Th2-type adaptive immunity (60). It remains to be determined if ROP16-mediated induction of these chemokines plays a role in disease pathogenesis or initiation of immunity *in vivo*.

UNDERSTANDING ROP16 AND *IN VIVO* STAT3 AND STAT6 ACTIVATION

Studies on the function of ROP16 are revealing the complexity of this rhoptry molecule in the host-parasite interaction. STAT3-driven activation by type I ROP16 plays a role in downregulating inflammatory cytokines, an effect that is apparent *in vivo* as decreased IL-12 responses (8, 10). For the parasite, this could be a means to prevent or delay immune recognition and elimination. We found that mouse astrocytes and microglial cells produce microbicidal nitric oxide (NO) following IFN-γ activation but that this response was suppressed by *Toxoplasma* in a ROP16-dependent manner (8). The finding that ROP16 knockout parasites do not persist in activated astrocytes coupled with older reports showing that NO is an important defense mechanism against *T. gondii* in the brain suggests that ROP16-dependent STAT3 activation is a way to promote parasite persistence during chronic infection (27, 72). Alternatively, ROP16-dependent downregulation of proinflammatory responses could be a means to avoid proinflammatory cytokine pathology during infection. It is well known that *Toxoplasma* can cause lethal Th1 cytokine overproduction dependent on the host genotype and parasite dose (30, 49). This is not a desired outcome for the parasite, which seeks to establish latent infection to maximize chances of transmission to a new host. ROP16 could function to dampen down the inflammatory response enough to avoid pathology without leading to uncontrolled parasite replication and host death.

Evidence for an anti-inflammatory function for type I ROP16 comes from models of oral infection. High-dose type II *Toxoplasma* infection of C57BL/6 mice results in lesions in the small intestine mediated by overproduction of IL-12, TNF-α, and IFN-γ (48, 49). The parasite seems to act as a trigger, causing tissue damage, emergence of enteroadhesive *Escherichia coli*, and bacterial subepithelial translocation culminating in fulminant pathology (33, 34). However, type II parasites expressing type I ROP16 appear to cause less inflammation in the intestine (36). This might be seen as evidence for an anti-inflammatory effect of type I ROP16, but it is also possible that this is due to type I ROP16-dependent limitation in parasite replication.

An important role for ROP16 may be its capacity to influence nutrient acquisition by *Toxoplasma*. The parasite relies on the host for many nutrients, including amino acids and nucleic acid precursors. In particular, arginine is an essential amino acid that *T. gondii* must scavenge from the host cell (25). Limiting arginine availability through increased arginase 1 expression could be a host's defense response to limit infection. Alternatively, by limiting parasite replication, ROP16-driven STAT6-dependent arginase 1 induction could allow cells to carry parasites longer, effectively functioning as Trojan horses to spread infection. Yet, it has also been proposed that induction of arginase 1 could have the opposite effect, promoting production of polyamines that the parasite also requires for survival (36). In this respect, ROP16 might function to increase parasite dissemination by promoting increased cycles of infection and lysis. However, the observation that type I ROP16-null parasites expand more rapidly *in vivo* tends to argue against this (8).

We do not fully understand the role of ROP16 during infection, but clearly the biology of this rhoptry kinase is complex (Fig. 1). ROP16 appears to be a central regulator of the host-parasite interaction that controls inflammatory cytokine production, suppression of TLR and IFN-γ responses in infected cells, and nutrient availability that determines both parasite growth rate and host ability to produce nitric oxide. This complexity is not surprising given that ROP16 activates (at least) two distinct transcription factors with their own independent activities. Determining whether ROP16 mediates its effects through STAT3 or STAT6 *in vivo* is complicated by the lethality of global STAT3 knockout, as well as bystander STAT activation due to the parasite-induced cytokine response. The predominant activity of ROP16 may depend on the cell type hosting the parasite, amount of arginine available in the host microenvironment, or on the parasite life cycle stage. The concept that a single rhoptry molecule may have multiple distinct functions has recent precedent, inasmuch as rhoptry kinase ROP18 deactivates IRG proteins to prevent destruction of the PVM while at the same time targeting transcription factor ATF6β for proteasomal degradation that affects DC antigen presentation to CD8$^+$ T lymphocytes (23, 79, 88). The multifunctional activities of these secreted parasite effector molecules that function within infected cells provide new challenges to understanding their functions in cells and animals.

PROTOZOAN TYROSINE KINASES—EVOLUTIONARY CONSIDERATIONS

Protein tyrosine kinases are an evolutionary innovation that accompanied emergence of multicellular organisms. They function to create high-affinity binding sites for proteins containing Src homology-2 (SH2) domains, and they are regulated by protein tyrosine phosphatases. With the exception of choanoflagellates (which are believed to be the most closely related of unicellular

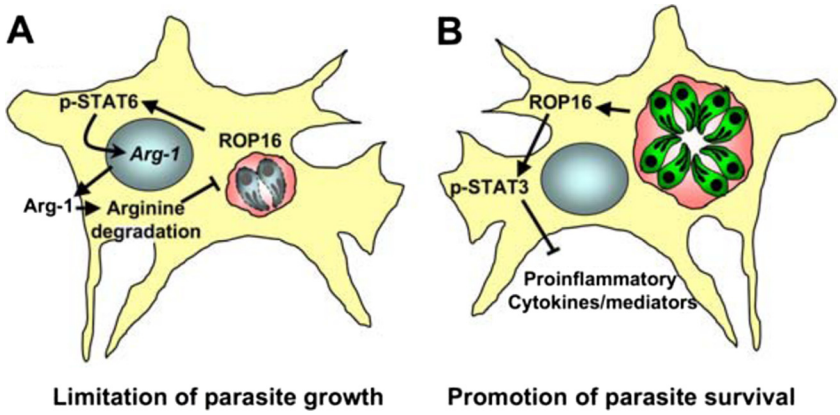

FIG 1 ROP16 is a multifunctional kinase with diverse effects on the host. (A) ROP16 activates STAT6, resulting in arginase-1 induction. Degradation of arginine limits replication of *Toxoplasma*, which is auxotrophic for this amino acid. Limiting parasite growth is beneficial for the host. It could also assist in the spread of the parasite, which uses cells such as macrophages and dendritic cells in dissemination during *in vivo* infection. (B) ROP16 and tyrosine phosphorylate STAT3. This transcription factor can have proinflammatory or anti-inflammatory activity, depending on the context of infection. During *Toxoplasma* infection, the predominant activity appears to be anti-inflammatory, although the exact nuclear targets of STAT3 are not known. For the parasite, this may be a way to evade antimicrobial immunity. Anti-inflammatory STAT3 function may also be a means to downmodulate harmful proinflammatory pathology. This benefits the host but is also advantageous to *Toxoplasma*, which seeks to keep its host alive to permit establishment of latent infection that is required for parasite transmission to new hosts. Which of these activities predominates may depend upon host cell type and life cycle stage of the parasite.

organisms to metazoans), there are few recognizable tyrosine kinases, protein tyrosine phosphatases, or SH2 domain-containing proteins in the protozoa (54, 55, 64). Then how is it that ROP16 can be a tyrosine kinase for this signaling module? ROP16 was originally identified as a putative serine-threonine kinase, molecules that are abundant throughout evolution (53). There is evidence in some fungi that serine-threonine kinases can also mediate inefficient tyrosine phosphorylation (64). The function of ROP16 would be consistent with a view that this was originally a functional serine-threonine kinase that acquired tyrosine kinase activity. It is therefore possible that ROP16 is a promiscuous kinase, maintaining functional serine-threonine activity on still-to-be-discovered host or parasite protein targets while also maintaining functional tyrosine kinase activity on host STAT3 and STAT6 signaling cascades. The unusual adaptation of ROP16 into a functional tyrosine kinase in mammalian cells suggests that ROP16 is important for the successful global expansion of this parasite.

FUTURE DIRECTIONS

Research on *T. gondii* is leading the way in our understanding of how intracellular eukaryotes manipulate the invaded host cell, but the field is still in its infancy. The consequences of ROP16 intracellular injection in other host species, such as humans and cats (the definitive host), are not yet known. We do not completely understand how ROP16 affects the mucosal immune response during initiation of infection, nor do we know if this molecule plays a role during establishment of chronic infection or during toxoplasmic encephalitis. Because of the promiscuous nature of *T. gondii*, the consequences of ROP16-mediated STAT activation need to be considered in other cells that the parasite invades. It has been estimated based on phylogenomic analysis that there are at least 44 rhoptry kinase family genes (62). We understand something of the function of two (ROP16, ROP18). The functions of ROP5 and ROP38 kinases, in particular, can be expected to be revealed in the near future. It seems certain that other important rhoptry molecules that interact with the host cell await discovery.

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