# Translational Control in *Plasmodium* and *Toxoplasma* Parasites

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The life cycles of apicomplexan parasites such as *Plasmodium* spp. and *Toxoplasma gondii* are complex, consisting of proliferative and latent stages within multiple hosts. Dramatic transformations take place during the cycles, and they demand precise control of gene expression at all levels, including translation. This review focuses on the mechanisms that regulate translational control in *Plasmodium* and *Toxoplasma*, with a particular emphasis on the phosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2α). Phosphorylation of eIF2α (eIF2α~P) is a conserved mechanism that eukaryotic cells use to repress global protein synthesis while enhancing gene-specific translation of a subset of mRNAs. Elevated levels of eIF2α~P have been observed during latent stages in both *Toxoplasma* and *Plasmodium*, indicating that translational control plays a role in maintaining dormancy. Parasite-specific eIF2α kinases and phosphatases are also required for proper developmental transitions and adaptation to cellular stresses encountered during the life cycle. Identification of small-molecule inhibitors of apicomplexan eIF2α kinases may selectively interfere with parasite translational control and lead to the development of new therapies to treat malaria and toxoplasmosis.

**M**alaria is a devastating disease, with mortality in adults being higher than previously estimated. Malaria killed 1.24 million people in 2010 alone (1). Human malaria, transmitted by *Anopheles* mosquitoes, is caused by four species of *Plasmodium*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. Infection with the monkey malaria parasite *Plasmodium knowlesi* has been reported but remains rare. Malaria parasites undergo complex intracellular and extracellular stages in invertebrate vectors and vertebrate hosts (Fig. 1). The transition from one stage of the life cycle to the next is tightly regulated by gene expression at the level of epigenetics, transcription, translation, and posttranslational modification (2–4). These multiple levels of gene expression control help to ensure that parasite proliferation and differentiation take place appropriately within the diverse environments encountered by *Plasmodium*.

*Toxoplasma gondii* switches between proliferative (tachyzoite) and latent (bradyzoite) stages within its human host, giving rise to acute and chronic infection, respectively (5). *Toxoplasma* can chronically infect virtually any warm-blooded vertebrate, and the bradyzoite tissue cysts bestow a unique ability to transmit to new hosts through predation. In addition, infectious oocysts can be excreted by the definitive feline host (Fig. 2). Chronic infections with *Toxoplasma* are widespread among humans, placing individuals who become immunocompromised at great risk for recrudescence of acute infections. During acute infection, the parasite is also capable of crossing the placental barrier, a process which can result in spontaneous abortion or birth defects that include hydrocephalus, encephalitis, mental retardation, and ocular disease (6).

## TRANSLATIONAL CONTROL AND THE CELLULAR STRESS RESPONSE

During translation initiation, Met-tRNA interference (tRNAi), together with GTP, eukaryotic initiation factors (eIF), and the small ribosomal subunit (40S), forms a complex with the mRNA. eIF2 is composed of α (eIF2α), β, and γ subunits. An inactive eIF2-GDP leaves the 40S ribosomal subunit after translation initiation. eIF2-GDP is exchanged to eIF2-GTP by a guanine nucle-

otide exchange factor (eIF2B), at which point a new round of translation is able to commence. In response to cellular stress, phosphorylation of a regulatory serine residue on eIF2α (Ser51 in yeast and mammals) increases the binding affinity of eIF2 to eIF2B, favoring the formation of an inactive eIF2-eIF2B complex. eIF2B is present in limited amounts compared to eIF2; therefore, a small increase in phosphorylation of eIF2α (eIF2α~P) blocks the recycling of eIF2-GTP and downregulates global protein synthesis (7). However, in mammalian cells, a subset of mRNAs, including those that code for "master regulator" activating transcription factor 4 (ATF4) or general control nonderepressible 4 (GCN4) in metazoans or yeast, respectively, can be preferentially translated when eIF2α is phosphorylated. The preferential translation of ATF4 and GCN4 allows cells to alleviate stress damage by reprogramming gene expression (8–10). In mammalian cells, there are four signature eIF2α kinases activated upon exposure to different kinds of cellular stresses: GCN2 (general control nonderepressible 2) responds to amino acid deprivation, PERK (protein kinase PKR-like endoplasmic reticulum kinase) responds to endoplasmic reticulum (ER) stress, PKR (protein kinase R) responds to viral double-stranded RNA (dsRNA), and HRI (heme-regulated inhibitory kinase) responds to heme deficiency (10).

Translationally inactive mRNAs accumulate in stress granules, composed of stalled preinitiation complexes, or processing bodies (P bodies) associated with mRNA decay. P bodies and stress granules are morphologically indistinguishable nonmembranous cytoplasmic structures ranging in size from 0.1 to 2.0 μm. Both granules are in dynamic equilibrium with the translational pool, allowing rapid shifts between translation, storage, and decay (11). Stress granules are characterized by the presence of 40S ribosomal subunits, poly(A)-binding protein (PABP), and a subset of trans-
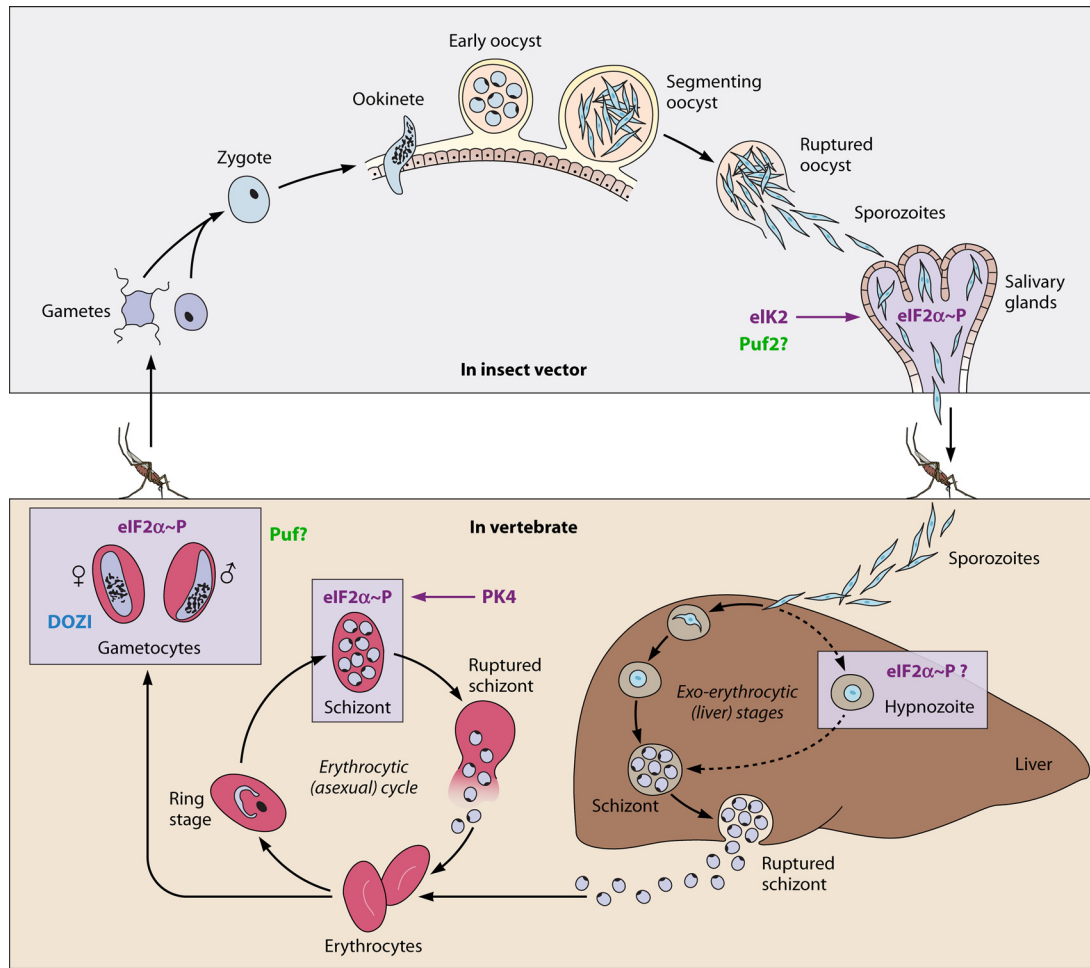
**FIG 1** Translational repression in the life cycle of *Plasmodium*. *P. falciparum* gametocytes maintain their infectivity for periods as long as 22 days (72). DOZI, a DDX6-class RNA helicase, binds to the ribonucleoprotein complex in female gametocytes and represses translation. These untranslated mRNAs are stored for translation after fertilization. RNA-binding proteins (Puf proteins) that repress translation by binding to the mRNA 3′ untranslated region are specifically upregulated in gametocytes (Puf1 and Puf2) (27, 28) and sporozoites (Puf2) (46, 47). The phosphorylation of eIF2α in salivary gland sporozoites, mature schizonts, and gametocytes induces translational repression and storage of untranslated mRNAs as stress granules (42, 52). *Plasmodium* sporozoites maintain infectivity for days in the salivary glands (69, 70). EIF2α kinase eIK2 controls the latency of sporozoites (42). Another eIF2α kinase, PK4, leads to the arrest of global protein synthesis in schizonts and is essential for the development of the erythrocytic cycle (52). In *P. vivax* and *P. ovale*, hypnozoites can persist in the liver for years and cause relapses (71). The mechanism behind the development into dormancy and the activation of hypnozoites is not known.

lation initiation factors (eIF2, eIF3, eIF4E, and eIF4G). In contrast, P bodies contain mRNAs associated with the decapping machinery (12, 13).

## TRANSLATION IN THE PARASITE MITOCHONDRIA AND APICOPLAST

In *Plasmodium* and *Toxoplasma*, RNA translation takes place in three subcellular compartments: the parasite cytosol, the single tubular mitochondrion, and a relict plastid called the apicoplast (14). While thousands of genes encoded in *Apicomplexa* genomes are translated in the cytoplasm, fewer than 50 are translated in the apicoplast and only 3 are translated in the mitochondrion. *Toxoplasma* and *Plasmodium* are susceptible to prokaryotic translation inhibitors, including clindamycin, macrolides, and tetracyclines, as these drugs interfere with translation in the bacterium-derived apicoplast (15, 16). Mutagenesis experiments have confirmed that clindamycin-resistant parasites result from a mutation within apicoplast rRNA that prevents clindamycin binding (17). Interest-

ingly, the mitochondrial genome lacks aminoacyl-tRNA genes, and charged tRNAs must be imported into the parasite mitochondria (18, 19). Nevertheless, translation in the mitochondrion is active and essential for parasite survival (20, 21). While translational regulation in the mitochondrion and apicoplast is essential for parasite viability and an intriguing area of investigation, the remainder of this review will focus on cytoplasmic control of protein synthesis and its role in regulating stress response and developmental transitions in *Plasmodium* and *Toxoplasma*.

## TRANSLATIONAL CONTROL IN *PLASMODIUM* SEXUAL STAGES

In contrast to other eukaryotes, the structure of rRNA differs among the various stages of the *Plasmodium* life cycle. The A type of rRNA is associated with the asexual blood stages and with liver stages, whereas the O and S types of rRNA predominate in mosquito stages (22). These striking structural changes suggest that rRNAs employed during the insect and mamma-
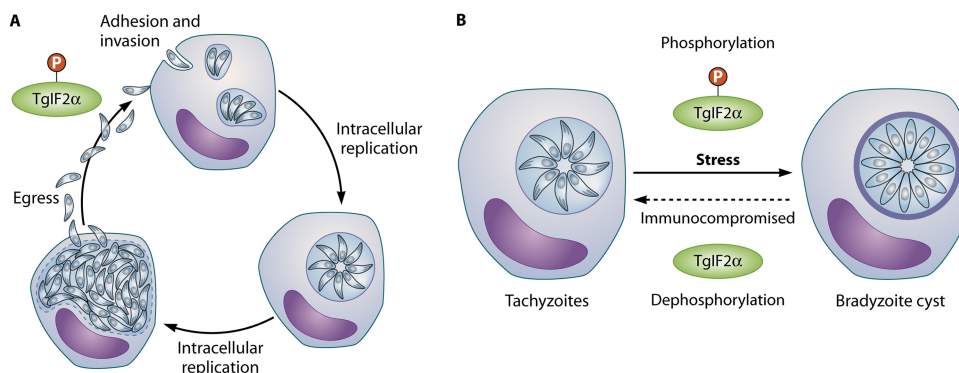
**FIG 2** Proliferation and development of *Toxoplasma*. (A) The *Toxoplasma* tachyzoite lytic cycle consists of the following three basic steps: attachment and invasion of a host cell, intracellular replication, and egress into the extracellular environment. Following egress, the parasites will repeat the process by invading a neighboring host cell and continuing to proliferate in the infected tissues. (B) During a primary infection, *Toxoplasma* tachyzoites quickly develop into latent bradyzoites, which are encased in a thick cyst wall inside host cells. Conversion from tachyzoite to bradyzoite can be mimicked *in vitro* through the application of cellular stress (e.g., heat shock, oxidative). *In vivo*, cyst generation and maintenance are reliant on the host immune response. Formation of bradyzoite cysts causes a chronic, incurable infection, as they can persist within the host indefinitely. If the host becomes immunocompromised, bradyzoites can reconvert to replicating tachyzoites, resulting in tissue destruction in cyst-prone areas of the body, such as the brain and heart. Dark-purple oval designates host cell nucleus.

lian stages may have varied functional properties, but confirmation of this idea awaits further investigation. It has been shown that the life cycles in mosquitoes and rodents of *Plasmodium berghei* parasites lacking the S-type genes are indistinguishable from those of the wild type, so it remains unclear why this unique organization of rRNA genes has been maintained in *Plasmodium* evolution (23).

Comparisons of *Plasmodium* transcriptomes and proteomes show that gene silencing via translational repression of mRNA is a frequent event during the life cycle. This was first described during development of the parasite's sexual stages. Male and female gametocytes circulate in the blood of the mammalian host. After being ingested by *Anopheles* mosquitoes, they differentiate into gametes and fertilization takes place in the lumen of the insect midgut. Soon the zygotes transform into the motile ookinetes that leave the hostile proteolytic environment of the lumen and take refuge in the wall of the mosquito stomach. Repression of mRNA translation encoding a surface protein of *P. berghei* (Pb21) was first documented during sexual development of gametocytes (24–26). Although the Pb21 mRNA is expressed in female gametocytes circulating in the vertebrate host, the synthesis of Pb21 protein starts only in the insect vector. Posttranscriptional silencing of several additional messages was revealed by comparing transcriptomes and proteomes of *Plasmodium*, suggesting that translational repression is an important regulatory mechanism (27). Notably, the 3′ untranslated regions of several repressed mRNAs contain a nucleotide motif known to bind to Pumilio (Puf) proteins, which repress translation and regulate RNA stability (27–29). The nontranslated mRNAs accumulate in cytoplasmic granules that contain an RNA-binding helicase named DOZI. The central role of DOZI in parasite development was revealed in a DOZI knockout. The mutants progressed normally through the blood cycle, but the complete absence in the female gametocytes led to an arrest of parasite development in mosquitoes (3). Analysis of the cytoplasmic granules in female gametocytes showed that they contain nontranslated mRNAs, eIF4E, and PABP but lack the RNA degradation elements that are characteristic of P bodies in higher eukaryotes (30).

## TRANSLATIONAL REGULATION OF *PLASMODIUM* SPOROZOITES

Thirty or more genes are upregulated in salivary gland sporozoites (*UIS* genes) compared to midgut sporozoites (31, 32). Among them, *UIS3* and *UIS4* are highly transcribed in the salivary glands, but the corresponding proteins are barely detectable (33). Both genes are expressed instead in early liver stages (34, 35). UIS3 is localized to the parasitophorous vacuolar membrane (PVM) surrounding the liver stage parasites. UIS3 binds to liver fatty acids and must participate in the acquisition of lipids that are essential for the growing parasite (36). *UIS4* encodes a small transmembrane localized in the PVM. In its absence, the late liver stages do not generate efficient blood stage infections (37).

Several other transcripts that accumulate in salivary gland sporozoites are translated only later in the life cycle. For example, liver stage-associated proteins 1 and 2 (LSAP-1/PFL0065w and LSAP-2/PFB0105c, respectively) are among the most abundant transcripts in the salivary gland transcriptome (33) but have not been detected in proteomic surveys of sporozoites (38, 39). Rather, these genes are translated only in liver stages (40). In contrast, the translation of the abundant proteins CSP and TRAP from the salivary gland sporozoites starts in oocyst sporozoites (41). In fact, a better correlation exists between levels of transcripts in oocyst sporozoites and protein abundance in salivary gland sporozoites than between transcripts and proteins from the salivary gland (42).

Three eIF2α serine/threonine kinases, eIK1, eIK2, and PK4, have been identified in *Plasmodium* (43). They are transcriptionally controlled. *eIK2* is predominantly transcribed in salivary gland sporozoites. *eIK1* and *PK4* are mostly transcribed in the asexual blood stages (42). *eIK2*, also called *UIS1*, is the most upregulated gene in salivary sporozoites relative to sporozoites from the midgut, in which its transcription is undetectable (31). eIK2 phosphorylates parasite eIF2α and inhibits global translation in the salivary sporozoites. Many stalled mRNAs encode proteins that are required during development of early-liver-stage forms. The nontranslated messages accumulate in the cytoplasm of the parasites as "stress" or RNA granules.

In the *P. berghei eIK2* knockout (*PbeIK2⁻*) sporozoites, eIF2α is not phosphorylated and translational repression is alleviated (42). The RNA granules disappear, the stalled liver stage messages are translated, and the salivary sporozoites prematurely transform into early liver stages. The sporozoite's inner membrane complex disassembles, the organelles redistribute in the cytoplasm, and a bulb-like structure appears in the center of the mutant parasite. The radical remodeling is proteasome dependent (42). The *PbeIK2⁻* sporozoites do not glide, and they lose infectivity. Comparison of transcribed genes between *PbeIK2⁻* sporozoites and the wild type showed that many genes found predominantly in liver stages, including some in the ubiquitin pathway, are upregulated in the mutant. This transformation of sporozoites into liver stages can start by incubation of the parasites at 37°C in the presence of serum (44).

Under physiological conditions, the latency of salivary sporozoites is disrupted only after their introduction into the mammalian host, when a phosphatase must be activated to dephosphorylate eIF2α~P. The parasite phosphatase has not been identified, but its activity can be inhibited by salubrinal, as first described for the eIF2α~P phosphatases in mammals (42, 45). Treatment of wild-type salivary sporozoites with salubrinal increases the phosphorylation of parasite eIF2α, implying that the phosphatase is present in salivary sporozoites. However, the kinase activity is predominant and leads to the latent state. It has been suggested that the phosphatase in the salivary glands may be repressed by the RNA-binding protein Puf-2. Indeed, when *Puf2* is knocked out in *P. berghei*, the sporozoites start transforming into liver stages while inside the mosquito salivary glands (46, 47).

Activation of *Plasmodium* sp. IK2 begins when midgut sporozoites enter the salivary sporozoites. This life cycle transition does not involve an obvious stress. However, the shift from a nutrient-rich midgut environment to the salivary glands may deprive the parasite of essential metabolic factors.

## TRANSLATIONAL REPRESSION IN *PLASMODIUM* ASEXUAL ERYTHROCYTIC STAGES

The few sporozoites that are injected into the host by mosquitoes develop into the clinically silent liver (or exoerythrocytic) stages. After a few days, a large number of membrane-bound sacs, named merosomes, containing infective merozoites, are released into the blood. The merozoites rapidly invade erythrocytes and begin the asexual intraerythrocytic life cycle that gives rise to malarial symptoms (48). The metabolism of the blood stages is largely dependent on the degradation of host cell hemoglobin, which produces a toxic monomeric α-hematin by-product. The hematin is a prooxidant and catalyzes the production of reactive oxygen species. Oxidative stress is believed to be generated during the conversion of heme to hematin. Free hematin can also bind and disrupt cell membranes, damaging cell structures and causing the lysis of the host erythrocyte (49). The malaria parasite detoxifies the hematin by biocrystallization, which converts it into insoluble and chemically inert β-hematin crystals (called hemozoin) (50, 51). Consequently, pigment appears as the parasite grows: it is absent in the ring stage and becomes detectable in the late trophozoite and the schizont.

Protein synthesis is more active in the *P. berghei* ring stage and young trophozoite, while translation is repressed in mature schizont. This is in accordance with the heavy phosphorylation of eIF2α in *P. berghei* schizonts and the accumulation of stalled mRNAs in stress granules (52). A schizont is the intraerythrocytic parasite that is undergoing or has undergone repetitive nuclear division. Nevertheless, the synthesis of some of the molecules needed for parasite multiplication, including DNA, starts in the trophozoite stage (53, 54).

The translational repression in schizonts is mediated by the eIF2α kinase PK4 (52, 55). Independent research groups have reported that PK4 is essential for the erythrocytic cycle in *P. berghei* and *P. falciparum* (52, 56). This conclusion is strongly supported by mutagenesis studies of the regulatory phosphorylation site Ser59 of eIF2α in *Plasmodium*, which corresponds to Ser51 of yeast/human eIF2α. Blood stage parasites bearing eIF2α Ser59Ala (nonphosphorylatable) or Ser59Asp (mimics a phosphorylated serine) mutations are not viable. However, Ser59Thr mutant parasites complete their life cycles normally (52). The implication of these studies is that drugs that inhibit PK4 are likely to alleviate malaria disease and inhibit transmission.

## IMPORTANCE OF TRANSLATION CONTROL IN *TOXOPLASMA*

Proteomic surveys have demonstrated that mRNA levels are not necessarily an accurate predictor of protein levels in *Toxoplasma* tachyzoites (57, 58). A large number of genes that are not detectable by microarray analysis and/or are in expressed sequence tag (EST) libraries can be detected at a protein level. Likewise, many abundant transcripts are not detected by current proteomic methodologies, suggesting that posttranscriptional regulation, including translational control, is an important factor regulating the proteome. Furthermore, proteomic analysis revealed that an attenuated parasite strain had markedly reduced levels of an eIF4A isoform relative to levels detected in the mouse-virulent strain (59). eIF4A is a DEAD box RNA helicase that facilitates translation initiation by unwinding the secondary structure within the 5′ untranslated region of the transcript to allow for ribosomal scanning. Reduced eIF4A in attenuated parasites suggests that eIF4A may be required for the translation of virulence factors.

## TRANSLATIONAL CONTROL DURING THE *TOXOPLASMA* LYTIC CYCLE

Cellular stress causes the phosphorylation of the *Toxoplasma* eIF2α homologue (TgIF2α) on a regulatory serine residue, Ser71, which is analogous to Ser59 in *Plasmodium* as well as Ser51 in yeast and humans (10, 52, 60). In contrast to *Plasmodium*, *Toxoplasma* tachyzoites harboring a point substitution at Ser71 to alanine (S71A) are viable (52, 61). However, parasites deficient in stress-induced TgIF2α phosphorylation (S71A-TgIF2α) showed a pronounced reduction in viability and slowed progression through the lytic cycle in the absence of exogenous cellular stress (61). *Toxoplasma* tachyzoites rapidly progress through a virus-like lytic cycle in any nucleated cell, which includes adherence and invasion of a host cell, multiple rounds of intracellular replication, and egress from the host cell (Fig. 2). Upon release into the extracellular milieu, the parasites search for a nearby host cell to repeat the process. Studies have shown that S71A-TgIF2α mutant parasites are ill-equipped to cope with the extracellular environment following egress from the host cell. Consequently, S71A-TgIF2α parasites suffer reduced viability in the extracellular environment and slowed replication following infection of a new host cell (61). Furthermore, parasites lacking the GCN2-like eIF2α kinase called TgIF2K-D fail to recover from extracellular stress as efficiently as the wild type, a defect that was also observed for the S71A-TgIF2α

mutant (62). Parasites lacking TgIF2K-D fail to phosphorylate TgIF2α during extracellular stress yet can still phosphorylate TgIF2α in response to ionophore treatment (62). It is well established that GCN2 eIF2α kinases are activated by nutrient deprivation, making it likely that nutrient starvation is the primary stress experienced by parasites outside their host cells. These results suggest that stress-induced TgIF2α phosphorylation and translational control are critical for *Toxoplasma* to conserve resources and to reprogram gene expression during conditions of nutrient stress.

Cytoplasmic ribonucleoprotein (RNP) granules containing the hallmark stress granule marker PABP were observed in parasites soon after host cell egress (63). The primary cause for the formation of these RNP granules is the change in the ionic conditions that occur when the parasites egress from the parasitophorous vacuole (63). Parasites released from the host cell in a high K⁺ buffer (which mimics the ionic conditions within the host cytosol) triggered the formation of stress granules at a higher rate and frequency than parasites released in a high Na⁺ buffer (which mimics the ionic conditions in the extracellular medium). Interestingly, numerous reports demonstrated that high K⁺ causes an increase in global protein synthesis, suggesting that during "externally triggered egress," a dynamic shift in translation results in the repression of a subset of mRNAs (which are targeted to stress granules) while allowing for the enhanced translation of mRNAs that are likely to be important for parasite survival upon exiting the host cell (64, 65). This is supported by the fact that granule-forming parasites demonstrated higher viability and an increased invasion and growth rate compared to non-granule-forming parasites (63). Exogenous cellular stress (sodium arsenite) and pharmacological agents that repress translation (salubrinal) also increase the size and number of these granules in extracellular parasites.

P bodies have not been well characterized in *Toxoplasma*, but a homologue of Argonaute (TgAGO) was localized to punctuate regions within the parasite cytosol that copurified with conventional P body markers, including numerous DEAD box helicases and a KH-type splicing regulatory protein (KSRP) that facilitates RNA decay (66). TgAGO did not appear to colocalize with the RNP granules observed in extracellular parasites, suggesting that stress granules and P bodies represent distinct sites of translation suppression and mRNA decay (63). Given the role of Puf proteins in *Plasmodium* RNP granules, it is likely that similar RNA-binding proteins regulate the repression and decay of mRNAs in *Toxoplasma*. *Toxoplasma* possesses two predicted Puf-containing proteins in its genome, but they have not been characterized to date.

*Toxoplasma* tachyzoites possess a PERK/PEK-like eIF2α kinase called TgIF2K-A that localizes to the parasite ER and is regulated through an association with the ER-resident chaperone BiP/GRP78 (67). Tunicamycin is a potent inducer of ER stress through its inhibition of N-linked protein glycosylation in the ER, and it induces robust phosphorylation of TgIF2α and bradyzoite formation *in vitro* (67). TgIF2K-B is another eIF2α kinase in *Toxoplasma*, and it appears to be unique to this parasite, devoid of any signature activation domain found on eIF2α kinases in other species. TgIF2K-C is a second GCN2-like eIF2α kinase, but its role has yet to be elucidated. It appears that TgIF2K-C is not entirely redundant to TgIF2K-D, as the TgIF2K-D knockout is defective in its response to extracellular stress (62). The expanded number of eIF2α kinases in these apicomplexan parasites is notable and may be reflective of their complex life cycles, which involve exposure to a multitude of different environments and potential stresses.

## TRANSLATIONAL CONTROL AND BRADYZOITE DEVELOPMENT IN *TOXOPLASMA*

Central to pathogenesis and transmission is the ability of *Toxoplasma* to convert from proliferative tachyzoites to latent bradyzoite cysts. The development and maintenance of bradyzoites during the primary infection may occur in response to various cellular stresses encountered in the new host, such as heat shock (fever), oxidative stress (reactive oxygen species produced from immune effector cells), and nutrient deprivation caused by gamma interferon (IFN-γ) stimulation of host macrophage (5). It was established that stresses, including those that trigger bradyzoite development, induce the phosphorylation of TgIF2α (60). Additionally, mature bradyzoite cysts induced *in vitro* have significantly elevated levels of TgIF2α~P compared to those of tachyzoites (67). These data indicate that translational control mediated by TgIF2α~P may be important for the development and maintenance of bradyzoite cyst forms. Consistent with this idea, we found that salubrinal, a pharmacological inhibitor of TgIF2α dephosphorylation, leads to increased levels of phosphorylated TgIF2α, activation of bradyzoite gene expression, and cyst wall formation (67). These findings implicate translational control as an important factor involved in the establishment of chronic toxoplasmosis. While the specific kinase(s) required for the development of bradyzoite cysts remains unclear, TgIF2K-B is an attractive candidate, as it shares the highest degree of sequence homology with IK2, which is required for regulating developmental transitions of *Plasmodium*.

It is of great interest to determine the mRNAs that are preferentially translated during the developmental transition to form bradyzoites. This may be achieved through the purification of polyribosomal fractions from parasites exposed to various bradyzoite induction triggers over a time course. The mRNAs may be identified from polysomes via hybridization to microarrays or through ribosome profiling techniques (68). Similar approaches may be employed to identify preferentially translated mRNAs during key transitions during the *Plasmodium* life cycle.

## CONCLUSIONS AND OUTLOOK

*Plasmodium* and *Toxoplasma* belong to *Apicomplexa*, a phylum of diverse obligate intracellular parasites that includes a number of medically and agriculturally significant pathogens. Translational repression mediated by phosphorylation of eIF2α plays important roles in *Plasmodium* and *Toxoplasma* life cycles. Elevated levels of eIF2α~P have been observed *in vitro* when *Toxoplasma* tachyzoites are subjected to stress and transform into bradyzoites. These are latent tissue cyst forms and can reconvert back into the rapidly replicating tachyzoites. Bradyzoites also appear spontaneously during culture of tachyzoites. The mechanisms that lead to bradyzoite formation *in vivo* are not clear, but they are maintained in a latent stage by the immune system. *Plasmodium* eIF2α is highly phosphorylated in sporozoites, which are quiescent and persist in mosquito salivary glands for the long term without significantly changing their transcriptional program while maintaining their infectivity (69, 70). We do not know the nature of the physiological signal that triggers the stress response in salivary gland sporozoites. Perhaps there are insufficient nutrients in the

gland or the parasites are subjected to oxidative stress. Elevated levels of eIF2α~P have also been observed in *Plasmodium* gametocytes and mature schizonts. In these stages, the host erythrocyte hemoglobin is likely expended, leading to a nutritional stress.

The hypnozoites are dormant hepatic forms present in *P. vivax* and *P. ovale*. Some hypnozoites can remain dormant in the liver for months or even years before reactivation. There is no morphological marker for hypnozoites, but it is assumed that they are small round forms. After reactivation, they transform into normal liver stages and release merozoites that infect the patient's red blood cells, causing relapse, clinically indistinguishable from the primary attacks (71). Arrest of translation is a conserved mechanism used by *Apicomplexa* (*Plasmodium* and *Toxoplasma*) to achieve latency. Therefore, it is not farfetched to speculate that the phosphorylation of eIF2α is involved in the generation of the still-enigmatic dormant stages of the malaria parasite of humans, *P. vivax*.

## ACKNOWLEDGMENTS

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