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*Cell Mol Life Sci.* 2018 July ; 75(13): 2355–2373. doi:10.1007/s00018-018-2808-x.**A Latent Ability to Persist: Differentiation in *Toxoplasma gondii***Victoria Jeffers<sup>1,\*</sup>, Zoi Tampaki<sup>2</sup>, Kami Kim<sup>2,3</sup>, and William J. Sullivan Jr<sup>1,4</sup><sup>1</sup>Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA<sup>2</sup>Departments of Medicine, Microbiology & Immunology, and Pathology; Albert Einstein College of Medicine, Bronx, NY 10461, USA<sup>3</sup>Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA<sup>4</sup>Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202, USA**Abstract**

A critical factor in the transmission and pathogenesis of *Toxoplasma gondii* is the ability to convert from an acute disease-causing, proliferative stage (tachyzoite), to a chronic, dormant stage (bradyzoite). The conversion of the tachyzoite-containing parasitophorous vacuole membrane into the less permeable bradyzoite cyst wall, allows the parasite to persist for years within the host to maximize transmissibility to both primary (felids) and secondary (virtually all other warm-blooded vertebrates) hosts. This review presents our current understanding of the latent stage, including the factors that are important in bradyzoite induction and maintenance. Also discussed are the recent studies that have begun to unravel the mechanisms behind stage switching.

**Keywords**

*Toxoplasma*; toxoplasmosis; differentiation; encystation; tachyzoite; bradyzoite; latency; gene regulation; epigenetics; immunity

**INTRODUCTION**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite capable of infecting warm-blooded vertebrates world-wide [1]. Although the parasite cannot survive outside the host cell, it has a significantly high transmissibility. It is estimated that approximately one third of the human population is infected with *Toxoplasma* [2]. It is one of the most widespread parasites due to its large range of host species and ability to transmit between hosts through diverse routes [3].

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

*Toxoplasma* belongs to the phylum *Apicomplexa*, which includes other important pathogens such as *Plasmodium*, *Eimeria*, *Babesia*, *Neospora*, *Sarcocystis* and *Cryptosporidium* [4–6]. Upon initial infection of an immunocompetent host with *Toxoplasma*, an often asymptomatic acute infection is followed by establishment of chronic infection, which is marked by the formation of intracellular tissue cysts. These cysts can persist within the host tissues throughout the life of the host, maintained in the quiescent state by its immune system. Upon immune suppression, the bradyzoites in the cyst will transition back into proliferating tachyzoites and can lead to devastating tissue destruction. Reactivated toxoplasmosis is a major concern in HIV-infected individuals and other immunocompromised patients [7]. In most cases, due to the proclivity of the latent bradyzoite to reside in central nervous system tissues, encephalitis is usually the main cause of death after reactivation of infection [8]. In addition, *Toxoplasma* tachyzoites are capable of crossing the placental blood barrier to be transmitted vertically from mother to fetus. Vertical transmission can occur if an immunologically naïve woman becomes infected with the parasite during her pregnancy, and can cause serious congenital birth defects or spontaneous abortion [6, 9]. Ocular toxoplasmosis is also a common manifestation of *Toxoplasma*, in which the retina of the eye may be destroyed in both healthy and immunocompromised individuals [10]. Ocular toxoplasmosis was traditionally thought to stem from reactivation of a congenital infection, but acquired infection is now also appreciated to be a main cause of this disease [11], particularly in South America. *Toxoplasma* has also been linked with water borne outbreaks in humans and deaths in marine wildlife due to contamination of water with oocysts [12–14], which has led the parasite to being classified as a Category B Biodefense pathogen by NIAID.

A crucial event for parasite transmission and pathogenesis is the stage conversion between the actively replicating tachyzoites and the encysted bradyzoites. Conversion to a dormant tissue cyst augments parasite persistence inside the host, which in turn, increases the chances of transmission through predation. Bradyzoites therefore provide *Toxoplasma* with extraordinary flexibility with respect to transmission, allowing the parasite to bypass its sexual stage in order to disseminate [3]. Of clinical relevance, the bradyzoite cysts appear to be refractory to current available drug treatments, giving rise to chronic infection that presently cannot be eradicated.

## CHARACTERISTICS OF THE BRADYZOITE CYST

Tissue cysts are intracellular structures that may develop in any visceral organ; however, they exhibit tropism for neural and muscle tissues. *In vivo*, cysts are most commonly detected in the brain, the eye, and in both skeletal and cardiac muscles [8]. Within the central nervous system (CNS), cysts have been detected in neurons, astrocytes, and microglia [15–17], although recent studies show that parasites primarily interact with neurons in mice [18, 19].

The size of immature cysts has been estimated to be about 5 µm with at least two parasites inside, indicating that cyst wall formation is an early event in bradyzoite development [20–23]. Furthermore, using bioluminescence imaging technology in combination with parasites expressing luciferase under the control of a bradyzoite-specific gene promoter, it was

demonstrated that *in vivo* formation of bradyzoites commences as early as one day post-infection [24]. As the cyst matures, changes in its size may be observed due to bradyzoite replication within the cyst [25]. Mature tissue cyst size and shape is variable; on average a mature brain cyst is spherical, about 10–70  $\mu\text{m}$  in diameter and the number of parasites within the cyst may reach the thousands. In muscle tissues, cysts are generally more elongated in length and may reach a size up to 100  $\mu\text{m}$  [8].

A number of major ultrastructural differences have been observed between the tachyzoite and bradyzoite stages. Bradyzoites are more slender than tachyzoites and have a pronounced crescent shape [26]. Bradyzoites contain a nucleus located at their posterior end and have electron dense rhoptry organelles and numerous micronemes [8, 16, 27]. Furthermore, bradyzoites contain amylopectin granules that stain red with periodic acid Schiff (PAS) [8]. It has been reported that *in vitro* differentiated bradyzoites may lose the parasite's apicoplast, a non-photosynthetic plastid-like organelle [22, 28]. Mature bradyzoites represent a growth-arrested stage of the parasite with a uniform 1N content nuclear DNA [29]. Although this stage is generally considered to be quiescent, it may still be motile. *In vitro* bradyzoites have been observed exiting from an existing cyst to invade a new host cell and establish a new cyst [22]. This feature could explain the clusters of cysts that have been observed *in vivo* as well as the increase in cyst burdens in chronically infected mice. Recently, a novel method for isolating tissue cysts from brains of infected mice has been developed and has permitted a detailed study of *in vivo* cyst characteristics [30]. Analysis of these enriched cysts by immunostaining for a parasite surface protein, found that clusters of parasites within a subset of cysts were undergoing cell division in an asynchronous manner, providing some of the first direct evidence that tissue cysts are more dynamic than previously believed [30].

### Transformation of the parasitophorous vacuole membrane into the cyst wall

The formation of the cyst wall and the matrix material provide a physical barrier to host immune factors. The cyst wall is visible as a reinforcement of the parasitophorous vacuole membrane (PVM) that occurs beneath this membrane [16]. Typically the PVM that contains the cyst is elastic and thin (<0.5  $\mu\text{m}$ ), consisting of chitin or similar polysaccharides and glycoproteins that can be detected using various fluorescently labeled lectins [31–33]. The agglutinin from *Dolichos biflorus* (DBA) that recognizes  $\alpha$ -linked N-acetyl galactosamine has high affinity for the bradyzoite cyst wall structure and is regularly used to detect cysts in tissue culture (Fig. 1). Currently, only a few components of the bradyzoite cyst wall have been identified and their molecular functions remain unclear. CST1, a glycoprotein recognized by DBA, localizes in the cyst wall under the PVM [33, 34]. CST1 was recently identified as being a O-glycosylated protein with a mucin domain, previously annotated as SRS44 and disruption of the gene results in impaired cyst formation and extremely fragile cysts [28]. Dense granule proteins (GRA proteins) have also been found in the bradyzoite cyst wall [34]. At the bradyzoite stage, GRA5 is predominantly localized to the modified PVM rather than the granular material that precipitates underneath this membrane [23, 34]. The other GRA proteins that have been observed in the cyst wall so far are GRA1, GRA2, GRA3, GRA6 and GRA7 [23, 34–36]. MAG1 (Matrix antigen 1) is another cyst wall and matrix specific antigen [37]. Notably, MAG1 is also detected inside the parasitophorous

vacuole of the tachyzoite stage but is present in significantly lower abundance, suggesting that it has an important role in the formation of the bradyzoite cyst wall [34, 38]. Recently, three novel cyst wall proteins were identified: a bradyzoite pseudokinase (BPK1), a microneme adhesive repeat domain-containing protein 4 (MCP4) and a proteophosphoglycan [39–41], but the functions of these proteins have yet to be resolved. Further characterization of the cyst wall and its components are required to understand the molecular mechanisms of tissue cyst development.

### Identification of genes contributing to bradyzoite development

Bradyzoites express certain genes exclusive to this stage of the life cycle. The findings that bradyzoites express specific antigens such as surface antigen 4 (SAG4); bradyzoite surface antigen, BSR4; surface antigen 2/2D (SAG2/2D), matrix antigen 1 (MAG1); 116-kDa cyst wall antigen (CST1); bradyzoite antigens 1–5 (BAG 1/5), specific metabolic enzyme isoforms (lactate dehydrogenase 2, LDH2; enolase 2, ENO1) [42–51] have led to extensive research focused on describing the function of bradyzoite-specific genes.

Initial attempts tried to identify genes that are specifically expressed or induced during *Toxoplasma* stage conversion. In an early study, a cDNA library was constructed from mRNA isolated from brain cysts in chronically infected mice. Screening of this expression library in bacteria with a pool of polyclonal antibodies against bradyzoite antigens led to the identification of several bradyzoite-related genes such as *mag1* [37], *bag1* [45] and *ldh2* [46, 47]. Parallel studies using material from *in vitro* differentiated bradyzoites confirmed the presence of the bradyzoite marker BAG1 [43] as well as other proteins, such as SAG4 [52]. Expressed sequence tag (EST) libraries from both *in vitro*- and *in vivo*-prepared bradyzoites have also been used for the detection of numerous stage specific genes [53]. Another approach employed a subtractive cDNA library to identify genes that were exclusively expressed in bradyzoites, many of which are involved in crucial cellular processes such as metabolism, protein folding, and DNA repair [49]. Promoter trapping screens have also been effective for the identification of bradyzoite-specific genes [32, 54, 55]. These screens have led to the detection of several bradyzoite gene loci, for example the bradyzoite surface antigen *bsr4* (p36) [55]. More recently, deep RNA sequencing has allowed for a more global, non-biased approach to determining the patterns of gene expression in tachyzoites versus bradyzoites. Analysis of the total RNA from forebrains from both acute and chronically infected mice identified 547 parasite genes significantly regulated between the two stages of disease. In addition to previously reported alterations to metabolic gene expression, this approach also identified many novel stage-specific markers. From these results, it appears that the parasites downregulate one set of sag-related surface markers in the tachyzoite to upregulate another set of similar genes in the bradyzoite. A large proportion (~50%) of the significantly upregulated genes in the bradyzoite were hypothetical genes, hinting at a number of parasite-specific processes that could be targeted with inhibitors as therapies against chronic toxoplasmosis [56].

Improvements in the tools facilitating molecular genetics analyses in *Toxoplasma* have allowed the construction of an increasing number of bradyzoite-specific gene knockouts. The construction of the *bag1* knockout strain was one of the first attempts to disrupt a

bradyzoite-specific gene [57, 58]. Other knockouts that have been described include the membrane proton-ATPase (PMA1) [59], the bradyzoite surface antigen SRS9 [60], the dense granule proteins GRA4 and GRA6 [61], the genetic locus ROP4/7 [61], the plant-like Apetala-2 transcription factors AP2XI-4, AP2IX-9, AP2IX-4, AP2IV-4 and AP2IV-3 [62–66], and the bradyzoite secreted pseudokinase 1 (BPK1) [39]. In all knockouts made to date, the production of cyst-like structures was observed *in vitro*, suggesting that individually these genes are not required for initiating bradyzoite switching. Nevertheless, defects in cyst morphology, cyst number, *in vivo* cyst formation and transmission of infection, indicate that many of these genes contribute significantly to cyst development.

In one study, chemical mutagenesis was used to identify tachyzoite to bradyzoite interconversion defective (Tbd<sup>-</sup>) mutants [67]. Other approaches applied insertional mutagenesis and reverse genetic screens [41, 68–70]. Finally, another study employed a signature-tagged insertional mutagenesis screen designed to isolate generally avirulent parasites. These clones were subsequently screened for reduced *in vivo* cyst formation in order to identify genes required only for pathogenesis [71]. Despite differences in the type of mutagenesis performed and the methods of selection of defective parasites, this work from different groups has collectively identified factors that may contribute to bradyzoite differentiation. However, most of the candidates have yet to be verified in independent studies as having a clear role in cyst formation. Moreover, some study designs may have isolated mutants resistant to *in vitro* differentiation stresses, and the genes may have more to do with stress-resistance than differentiation. Despite these caveats, the genes and their associated bradyzoite phenotypes that have been identified from these screens are discussed in Table 1.

Further investigation is necessary to describe the molecular mechanisms of *Toxoplasma* differentiation. Individual studies of the genes implicated in bradyzoite development have been informative but the complete picture of this developmental pathway is still elusive. Nevertheless, the advent of techniques such as mRNA deep sequencing will allow full-scale expression profiling to compare both the tachyzoite and bradyzoite developmental stages and will refine our current understanding of this clinically relevant developmental pathway. Importantly, new *in vitro* models need to be pursued to delineate the phenomenon of stress-induced and spontaneous differentiation, and candidate genes need to be supported by *in vivo* data.

### Metabolism in the bradyzoite stage

As the parasites transform from replicating tachyzoites to latent bradyzoites, changes in parasite metabolism have been observed. It may be assumed that these changes allow the parasites to remain dormant for long periods of time and are mediated by upregulation of bradyzoite-specific forms of various metabolic enzymes, particularly those involved in dealing with oxygen radical metabolism and carbohydrate metabolism. The formation of the amylopectin storage granules upon differentiation to bradyzoites indicates that the parasites modulate carbohydrate metabolism during stage switching. Tight regulation of amylopectin levels is required for maintaining chronic infection in mice [30]. Site-directed mutagenesis of an enzyme responsible for amylopectin digestion, the *Toxoplasma* glycogen

phosphorylase (TgGP), was performed to determine the importance of amylopectin metabolism in both tachyzoite and bradyzoite stages. Mutation of a phosphorylated serine to inactivate (S25A) TgGP led to excessive accumulation of amylopectin. Conversely, mutation of this residue to a phosphoserine-mimetic (S25E) caused amylopectin deficiency. Although both mutant lines were capable of differentiation *in vitro*, they both displayed significantly reduced cyst burdens in the brains of chronically infected mice compared to the parental line, indicating that maintaining the appropriate levels of amylopectin is necessary for establishing a persistent chronic infection [30, 72].

It also appears that bradyzoites rely on anaerobic glycolysis for generation of ATP since they do not retain a functional TCA cycle [48]. Further evidence for changes in carbohydrate metabolism comes from differential regulation of bradyzoite-specific isoforms of lactate dehydrogenase (LDH2), glucose-6-phosphate dehydrogenase (G6-PI), and enolase-1 (ENO1), all of which display different stability and enzymatic properties compared to their tachyzoite counterparts [48, 73]. The bradyzoite-specific LDH2 isoform is hypothesized to be important for establishing chronic infection in the host, since mice infected with LDH2-knockdown parasites displayed much lower numbers of brain cysts compared to the parental line [74].

Persistence of chronic *Toxoplasma* infection also relies on continued autophagy and turnover of old organelles or cellular components. Cathepsin protease L is not required for tachyzoite replication or the initial stages of bradyzoite development, but is critical for viability of the mature tissue cyst. Genetic or chemical ablation of cathepsin protease activity caused an accumulation of undigested autophagosomes and loss of bradyzoite viability indicating that the parasites must recycle proteins and organelles to persist [75].

The long-lived latent cysts must also be capable of dealing with exposure to various radicals and reactive metabolic byproducts; consequently, enzymes that cope with this stress are upregulated during bradyzoite formation e.g. thioredoxin and glutathione peroxidase [53].

## EXPERIMENTAL MODELS OF CYST FORMATION

### Strain differences

Genetic analysis of *Toxoplasma* isolates from Europe and North America has identified three predominant lineages of the parasite designated Types I, II, and III, which resulted from a single genetic cross approximately 10,000 years ago [76]. The three strains differ in their growth rate, virulence, their ability to traverse or to pass through epithelial layers (transmigration), and their capacity to form cysts [77, 78].

Type I strains generally replicate faster and, consequently, are more virulent in mice. A Type I strain isolated in 1939, designated “RH” after the patient from which it was isolated [79], is the most common laboratory strain in use. Today’s RH strain has a rapid replication rate and is highly amenable to genetic manipulation, making it a favored tool for the study of tachyzoite biology. However, RH parasites are limiting in the study of conversion from tachyzoite to bradyzoite. Although it has been reported that under stress conditions RH tachyzoites will upregulate specific bradyzoite genes and, in some reports, produce



bradyzoite-specific proteins or cyst wall components [80–82], the parasites are largely incapable of forming mature bradyzoite cysts either *in vitro* (in cell culture) or *in vivo* (during mouse infection). However, low-passage type I strains are capable of switching to bradyzoites [83], which means that extensive passage *in vitro* is associated with the loss of developmental competency [84]. Type II and Type III strains replicate at a slower rate, readily convert to bradyzoites, and are hypovirulent in mice, but these characteristics change with prolonged *in vitro* passage. Differences in the developmental capacity among these strains are influenced by adaptation to *in vitro* growth in HFF cells.

Genome sequence data from parasite lines of all three types has been collected and can be accessed at the *Toxoplasma* genome database ([www.toxodb.org](http://www.toxodb.org)). This genome information has proven essential in dissection of the mechanisms and pathways for conversion from tachyzoite to bradyzoite forms.

### Stress-induced bradyzoite formation

The early stages of the transition from tachyzoite to bradyzoite may be studied using *in vitro* culture and conditions shown to induce the conversion. These methods have been used to examine potential pathways associated with the early stages of bradyzoite development. Different kinds of stress applied to proliferating tachyzoites have been demonstrated to induce cyst formation.

The most commonly used method to induce bradyzoite cysts for *in vitro* culture is continuous alkaline treatment of parasite-infected host cells by adjusting the growth medium to pH 8.0–8.2 [80]. A brief alkaline treatment of extracellular tachyzoites prior to invasion has also been demonstrated to increase frequency of cyst development [85]. A number of other methods that involve adjustments to the culture medium or culture conditions to trigger stress-induced bradyzoite formation include various forms of nutrient deprivation through arginine starvation [86], axenic incubation [49], and depletion of pyrimidine in uracil phosphoribosyltransferase (UPRT)-deficient parasites grown in 0.03% CO<sub>2</sub> [22, 81].

Bradyzoite formation may also be enhanced using heat shock as an exogenous stress [80]. Soete *et al.* (1994) observed bradyzoite formation after heat shocking host cells at 43°C before allowing parasites to invade at 37°C and applying an additional heat shock to the infected cells [80].

Chemical treatment of the parasites and host cells has also been used to apply stress and trigger cyst formation. Tunicamycin treatment of parasite-infected cells causes endoplasmic reticulum (ER) stress and subsequent bradyzoite development [87]. Chemical stress may also be applied using sodium arsenite [80]. Chemical inhibition of calcium-induced egress with the herbicide fluridone has also been demonstrated to result in a larger proportion of bradyzoite cysts in culture [88].

Compounds that inhibit mitochondrial function and subsequently induce oxidative stress (i.e. sodium nitroprusside or atovaquone) will also trigger bradyzoite development [89, 90]. Interferon - gamma (IFN- $\gamma$ ) treatment of tachyzoite infected fibroblasts will not induce bradyzoite development [80], but when murine macrophages are infected with *Toxoplasma*

and subsequently treated with IFN- $\gamma$ , bradyzoite development commences, likely as a result of nitric oxide production from the activated macrophage [91].

Cyclic adenosine monophosphate (cAMP)-mediated signaling regulates a number of parasite processes including differentiation from tachyzoite to bradyzoite [92]. Using an engineered optogenetic *Toxoplasma* strain in which cAMP levels could be controlled by photo-activation, Hartmann and colleagues (2013) demonstrated that a minimum level of intra-parasitic cAMP was required for initiation of bradyzoite formation, but increased levels of cAMP impaired the process indicating that cAMP-responsive factors are involved in the differentiation signaling pathway [92]. The importance for tightly regulated cAMP levels in parasite differentiation is underscored by a recent study on a cAMP-dependent protein kinase A homologue, TgPKA3. Knock-out of the TgPKA3 catalytic subunit led to a growth defect in tachyzoites and an increase in spontaneous cyst formation, in both Type I and Type II backgrounds. The role of TgPKA3 in cAMP levels was confirmed by rescue of the aberrant growth and differentiation phenotypes with the compound 3-isobutyl-1-methylxanthine, which restores cAMP levels in the parasite [93].

### Host cell determinants and spontaneous bradyzoite development

Factors in the host cell may also act as determinants of cyst development. Reviewed in [94]. *Toxoplasma* tachyzoites replicate rapidly and require sufficient levels of nutrients that are scavenged from the host cell to support this proliferation. If host cell metabolic conditions limit the supply of essential nutrients to the parasites, it will slow the replication rate and favor differentiation to the bradyzoite stage [91, 95]. This has been demonstrated in *in vitro* parasite culture when removal of essential nutrients such as arginine or lipoprotein (a source of cholesterol) from the culture medium favors bradyzoite differentiation [86, 96]. The host cell glycolytic flux can also influence the rate of parasite initiation of differentiation. Dividing host cells in high glucose conditions have an enhanced rate of glycolysis. This generates higher levels of lactate which was shown to favor tachyzoite replication and to inhibit conversion to bradyzoites [97]. Treatment of human host cells with a trisubstituted pyrrole called “Compound 1” prior to tachyzoite infection slowed parasite replication and led to the upregulation of bradyzoite-specific gene expression [98]. Although compound 1 was originally developed as an inhibitor of cyclic guanosine monophosphate (cGMP) kinase [99, 100], it was found to exert its effect through host cell factors [98]. The same study also demonstrated that pre-stressing host cells with alkaline pH before parasite infection was capable of enhancing cyst formation [98].

While cellular stress is a strong mediator of tachyzoite to bradyzoite conversion, other factors may play a role in initiating the development of cysts. Tachyzoites of cystogenic Type II and Type III strains will spontaneously convert to bradyzoites in *in vitro* culture, particularly when host cells have been inoculated with a low multiplicity of infection (MOI) or if egressed tachyzoites are continuously removed from the culture [101]. Although *Toxoplasma* tachyzoites are capable of infecting any nucleated cell from a warm-blooded animal, certain cell types are more conducive to cyst formation than others [102]. Transcriptomic analysis of parasite gene expression during infection of a number of different cell types indicated greater bradyzoite specific gene expression in certain cell types such as



neurons or muscle cells, even in the absence of extrinsic stress. These data suggest that a subset of cell types are more conducive to bradyzoite formation [103]. The cell cycle stage of host cells that are more permissive to bradyzoite formation may be a strong influence. Terminally differentiated mouse myotubes (skeletal muscle fibres) restricted tachyzoite replication and promoted bradyzoite differentiation compared to their dividing myoblast progenitors [104]. Neurons are in a similar state of terminal differentiation to skeletal muscle cells and it is tempting to speculate that this explains the enrichment of tissue cysts in the CNS and muscle tissue in infected animals.

### Host immunity factors and tissue cysts

Since the initiation of cyst development has been largely studied in the context of stress, it has been proposed that *in vivo* the stress applied by immune system factors during host infection is crucial in triggering tachyzoites to transform into bradyzoites [105]. The host immune system can effectively control *Toxoplasma* infection (for a recent review see [106]) but it appears that rather than specific immune effectors actively inducing chronic infection, a functional immune response maintains parasite in the latent bradyzoite state and that a dysfunctional immune response simply allows acute infection to go unchecked.

Cell-mediated immunity is vital for the restriction of *Toxoplasma* and the development of chronic infection in an immunocompetent host (reviewed in [106, 107]). Increased susceptibility to *Toxoplasma* has been observed in patients with impaired T-cells as well as in mice that lack B-cells, CD8<sup>+</sup>, or CD4<sup>+</sup> T-cells [108–110]. CD4<sup>+</sup> cells, which can activate CD8<sup>+</sup> cells, have been proven to be necessary for the maintenance of CD8<sup>+</sup> functions during the chronic stage of infection [111, 112].

Notably, it appears that highly virulent *Toxoplasma* strains have evolved strategies to inhibit the functions of CD8<sup>+</sup> cells. A recent study showed that CD8<sup>+</sup>-mediated immunity is seriously compromised during infection by the RH strain [113]. The kinase activity of ROP18 in the virulent Type I strain was found to target the host activating transcription factor 6β (ATF6-β) for degradation [114], which resulted in downregulation of CD8<sup>+</sup> cell-mediated immune responses. Although RH parasites are capable of developing tissue cysts, infection never progresses to the chronic stage because the host immune system is rapidly overwhelmed by ROP18 function and tachyzoite proliferation leads to systemic infection, organ failure and death.

The major product of activated CD8<sup>+</sup> [115], CD4<sup>+</sup> T-cells [116] as well as of non-T and non-NK cells [117–119] is IFN-γ, a cytokine that is well-documented to be a key mediator of *Toxoplasma* infection. In contrast to wild-type mice that survive and develop chronic infection, IFN-γ knock-out (KO) mice succumb to infection with avirulent *Toxoplasma* strains [120]. Moreover, chronic parasite infection does not develop in mice that have been treated with monoclonal antibodies against IFN-γ [115, 121]. Depending on the host species and the cell type, IFN-γ has been proven to activate a variety of effector mechanisms that are required for resistance during chronic infection.

After *Toxoplasma* infection, IFN-γ induces the formation of reactive oxygen and nitric oxide (NO) metabolites in macrophages, microglia, and astrocyte cells of mice and humans

[122–124]. It appears that the NO by-product is a key factor for suppressing parasite replication and inducing bradyzoite development [89, 125–127], similarly to *in vitro* induction of bradyzoites with sodium nitroprusside, as previously discussed. However, NO is not an essential factor for *in vivo* formation of bradyzoites. Tissue cysts could develop in inducible nitric oxide synthase KO mice [120] as well as in mice that had been treated with aminoguanidine, the inhibitor of the enzyme that produces NO [128].

An IFN- $\gamma$ -mediated tryptophan degradation response has been observed in human brain endothelial cells, HFF cells, and macrophages infected with *Toxoplasma* [129–131]. Additionally, IFN- $\gamma$ -treatment of *Toxoplasma*-infected cells results in upregulation of indoleamine 2,3-dioxygenase 1 and 2 (IDO1 and IDO2), which are host enzymes that are responsible for degradation of tryptophan [132–134]. Since *Toxoplasma* is auxotrophic for tryptophan, upregulation of IDO1 and IDO2 restricts availability of this essential amino acid and thus inhibits parasite replication [135]. Parasite replication may be restored with the addition of exogenous tryptophan [136]. *In vivo* inhibition of IDO1 and IDO2 with a chemical inhibitor did not affect the levels of IFN- $\gamma$  produced after *Toxoplasma* challenge, but treatment of chronically infected mice with IDO inhibitors led to reactivation of the disease and increased *Toxoplasma* cyst burden, demonstrating an important role for IDO during latent infection [134]. Notably, IDO has also recently been shown to be involved in host immunity against *Trypanosoma cruzi* and malaria [137, 138].

The host immune effector Tumor Necrosis Factor  $\alpha$  (TNF $\alpha$ ) has also been shown to play an important role in establishing chronic *Toxoplasma* infection [139, 140]. IFN- $\gamma$  may function in synergy with TNF $\alpha$  since treatment of *Toxoplasma*-infected murine macrophages induces the expression of bradyzoite markers [89]. Interleukin 6 (IL-6) is another inflammatory cytokine that causes the increase of cyst numbers produced *in vitro* in HFF cells [141]. It also appears that control of both acute and chronic stages of infection depends on IL-12, a cytokine that drives the production of IFN- $\gamma$  [115, 142, 143].

In addition to host cell background influencing spontaneous bradyzoite conversion, the particular *in vivo* distribution of cysts in brain and muscle tissues may be facilitated by specific local immune responses [144, 145] or potentially ineffective immune responses within these tissues [146]. Alternatively, organ or cell type-specific factors that trigger high levels of stage conversion and tissue cyst formation may contribute to enhance parasite persistence in these tissues. As previously mentioned, treatment of host cells with Compound 1 modulates host cell factors, making them more permissive to bradyzoite formation [98]. Treatment of host cells with Compound 1 prior to infection with *Toxoplasma* induced the upregulation of the human cell division autoantigen-1 gene (CDA-1). The increased expression levels of CDA-1 affected parasite replication, arresting the parasite cell cycle and inducing bradyzoite gene expression, directly implicating host cell factors in the differentiation process [98]. Furthermore, it was recently noted that the metabolic state of a cell, as well as non-immune secreted metabolites, could designate host cells as either permissive or resistant to bradyzoite development [97, 147].

Although the host immune responses play critical roles in elimination of tachyzoites and contribute to host environmental changes that foster tachyzoite to bradyzoite conversion and

maintenance of tissue cysts, it remains unclear how these signaling pathways compare to induction methods currently used for *in vitro* differentiation models. The growing convenience of RNA sequencing will be valuable for comparing the transcriptional responses of parasites during the early stages of bradyzoite development during *in vivo* infection and the commonly used *in vitro* induction models. Pinpointing the commonalities within the different models will identify the core signaling molecules that drive the transition to the chronic stage.

### Reactivation of infection: bradyzoites to tachyzoites

The tissue cysts remain largely quiescent for the life of a host, but tachyzoites can re-emerge and cause toxoplasmosis in immunocompromised patients. The reactivation mechanisms have not been studied extensively, but it is generally assumed that abrogation of the host immune response results in bradyzoite to tachyzoite conversion [105]. Human immunodeficiency virus (HIV) patients with reduced numbers of CD4<sup>+</sup> cells acquire toxoplasmosis [7, 148]. A similar course of infection occurs in chronically infected mice that lack CD4<sup>+</sup> cells [109], further supporting that cell-mediated immunity is important for suppressing bradyzoite reactivation. Additionally, it has been demonstrated that *in vivo* depletion of CD8<sup>+</sup> and CD4<sup>+</sup> cells in chronically infected mice results in reactivation of *Toxoplasma* infection [149]. Suppressing IFN- $\gamma$  production also facilitates bradyzoite reactivation during chronic infection in mice [150–152]. In long-term murine astrocyte cultures of brain-derived *Toxoplasma* cysts, the presence of IFN- $\gamma$  is necessary in order to suppress reactivation. Removal of IFN- $\gamma$  from the culture, 120 days post-infection, resulted in cyst rupture and the appearance of cells heavily infected with tachyzoites predominating in the culture [153]. One current hypothesis of the mechanism that links IFN- $\gamma$  with suppression of reactivation is that IFN- $\gamma$  may stimulate continuous anti-parasitic activity in the astrocytes, causing cell cycle arrest, which has been shown to be associated with bradyzoite differentiation [29]. A direct role of the host immune system in repression of reactivation remains elusive since even in healthy mice tissue cysts can occasionally rupture, releasing invasive parasites that infect new cells [17].

## MOLECULAR MECHANISMS

### Translational control – downregulation of global translation and preferential translation of transcription factors

Given that virtually any type of cellular stress can induce bradyzoite differentiation, and can do so independently of the host cell, attention has been directed to the study of stress response pathways in the parasite to illuminate the mechanisms driving stage conversion. A common response to cellular stress in all eukaryotic organisms is the control of global translation via phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2 $\alpha$ ). Depending on the type of stress the cell is experiencing, one of a number of specific kinases is activated to phosphorylate eIF2 $\alpha$ , leading to global downregulation of translation with concurrent, preferential translation of a subset of mRNAs that code for factors required to deal with the stress insult [154]. Recent evidence points to translational control via the *Toxoplasma* homologue of eIF2 $\alpha$ , TgIF2 $\alpha$ , as a contributing factor in regulating the transition of tachyzoite to bradyzoite, maintenance of the dormant bradyzoite, as well as the

reactivation of bradyzoites into proliferating tachyzoites, reviewed in [155]. When *Toxoplasma* tachyzoites are subjected to different cellular stresses that are known to induce bradyzoite development, TgIF2 $\alpha$  becomes phosphorylated [156]. In contrast to intracellular replicating tachyzoites, TgIF2 $\alpha$  is phosphorylated to a high degree in mature bradyzoites [87]. Dephosphorylation of mammalian eIF2 $\alpha$  is specifically inhibited by treatment with salubrinal or guanabenz [157, 158]. These compounds also inhibit dephosphorylation of TgIF2 $\alpha$  and treatment of tachyzoites with either salubrinal or guanabenz triggers bradyzoite differentiation *in vitro* [87, 159]. Furthermore, cultured bradyzoite cysts treated with salubrinal or guanabenz were no longer able to reactivate into replicating tachyzoites [159].

Once TgIF2 $\alpha$  has been phosphorylated, translation is dramatically downregulated [87]. The decrease in protein synthesis conserves resources and allows the parasite to reprogram its genome to respond accordingly to the stress, which in the case of *Toxoplasma*, may include switching to the latent bradyzoite stage [3]. Discerning which mRNAs are preferentially translated while TgIF2 $\alpha$  is phosphorylated would be of great use in understanding the precise sequence of events and the essential components required for the transition from tachyzoite to bradyzoite. Polysome profiling has been used to identify the mRNAs that are undergoing translation at a specific timepoint. We have developed a polysome fractionation protocol for *Toxoplasma* combined with microarray analyses, which allowed the identification of a subset of mRNAs that are suggested to undergo preferential translation in parasites experiencing ER stress as a result of tunicamycin treatment [160]. A number of these preferentially translated mRNAs encode proteins containing plant-like AP2 domains, which have been identified as the major family of transcription factors in *Apicomplexa* [161]. This set of preferentially translated transcription factors are prime candidates for the main effectors upregulating bradyzoite-specific genes and the transition to the dormant cysts.

### Transcriptional regulation of bradyzoite-specific genes

Once the switch to cyst development has been triggered, upregulation of bradyzoite-specific genes has been observed with a concurrent downregulation of tachyzoite-specific genes. A number of studies using different techniques have sought to identify the genome-wide changes in gene expression during the bradyzoite developmental program [40, 53, 56, 162, 163]. Cascades of gene expression are observed as the parasites transition from one stage to the next, indicating that transcriptional regulation plays an important role in the regulation of stage-switching. A number of these studies also report distinct functional groups of genes that are differentially regulated, with surface antigens, metabolic proteins, and secreted proteins distinguished with significant differences in expression levels [40, 162]

The initial results from genome sequencing of *Toxoplasma* and related apicomplexan parasites suggested a surprisingly small number of putative transcription factors, prompting focus on epigenetic means for gene regulation [164]. However, a growing body of evidence indicates that gene expression is regulated by conventional eukaryotic mechanisms. Much of the conventional general transcriptional machinery appears to be conserved in *Toxoplasma* [165]. There also appears to be specific DNA motifs for recruitment of factors controlling stage-specific gene expression. The promoters of 16 bradyzoite-specific genes were mapped to high resolution, which led to the identification of *cis*-acting elements that appear to be

specific to genes induced during differentiation. These elements from the two bradyzoite-specific genes *bag1* and *B-NTPase* were inserted into the constitutive DHFR-TS promoter and converted it into a promoter that enhanced expression of a reporter under bradyzoite-induction conditions [83].

To identify DNA-associated factors that could function as repressors of bradyzoite gene expression during tachyzoite proliferation, pull-downs using the bradyzoite-specific promoter *ENO1* as bait were performed. This study identified different regulatory factors such as an RNA helicase, kinases, phosphatases, and heat shock proteins, as well as a number of hypothetical proteins containing domains with high homology to known chromatin-association factors. One of the proteins pulled down was an FK506 binding protein (FK506BP) homologue called *Toxoplasma* nuclear factor 3 (TgNF3). TgNF3 displays a nuclear localization in tachyzoites, but was found to be predominantly cytoplasmic in bradyzoites. TgNF3 was shown to associate with both nucleosomes and free histones to repress *ENO1* and 18S ribosomal RNA genes [166].

The characterization of the ApiAP2 family of plant-like transcription factors in *Toxoplasma* and related apicomplexan parasites [161] has prompted efforts to identify the specific AP2 factors that may be responsible for coordinating the response to the different signals that trigger bradyzoite differentiation (reviewed in [167]). Collation of the various gene expression analyses that have been performed on differentiating parasites has identified a handful of AP2 factors that are specifically upregulated during bradyzoite development and are dampened once the mature tissue cyst is formed [65, 168]. Genetic disruption of these AP2 factors is revealing unexpected complexity in the stage transition from tachyzoite to bradyzoite, which appears to be mediated by interplay between both activators and repressors of bradyzoite genes.

At least two of these AP2 factors, AP2IV-3 and AP2XI-4, function as activators of bradyzoite genes [63, 65]. Deletion of AP2XI-4 in both Type I and Type II backgrounds generated mutant lines that were unable to initiate expression of bradyzoite marker genes during stress [63]. The Type II mutant also displayed a significant defect in cyst formation during *in vivo* infection in mice [63]. AP2IV-3 is also induced early during bradyzoite development to promote expression of bradyzoite genes [65]. Knockout of AP2IV-3 reduced the rate of cyst formation in response to alkaline stress, while inducible overexpression of this factor increased the rate of cyst formation, even in the absence of extrinsic stress. Gene expression analysis of the AP2IV-3 overexpressing parasites confirmed upregulation of a subset of known alkaline stress-responsive genes [65].

Conversely, another AP2 factor that is upregulated early during bradyzoite development AP2IX-9, acts as a repressor of bradyzoite formation. AP2IX-9 knockout parasites have a higher rate of spontaneous cyst conversion in normal growth medium and overexpression of AP2IX-9 decreased the rate of differentiation under alkaline stress [62, 65]. Both AP2IV-3 and AP2IX-9 appear to regulate the same target genes and were found to directly associate with the chromatin in at least one promoter region (*bag1*), however further studies are required to understand how the action of these antagonistic factors culminates in a decision to either continue replication as a tachyzoite, or commit to bradyzoite development.

Adding to the complexity of tissue cyst development at the molecular level are findings on two other AP2 factors that are cell cycle-regulated in tachyzoites. Although not essential for tachyzoite replication, AP2IX-4 knockout mutants displayed a defect in cyst formation *in vitro* and *in vivo* [64]. Localization of AP2IX-4 protein revealed that it is cell-cycle regulated and expressed only in dividing parasites, both tachyzoites and bradyzoites. Gene expression analysis reveals that AP2IX-4 operates as a repressor of bradyzoite genes during alkaline stress, consistent with AP2IX-4 being present in dividing parasites [64]. Similarly, another cell cycle regulated factor that peaks at the S/M stage in tachyzoites, AP2IV-4, was also identified as a repressor of bradyzoite genes when gene expression analysis on the knockout parasites revealed many bradyzoite-specific transcripts to be upregulated [66]. Although this mutant line displayed a comparable cyst formation rate to the parental line under alkaline stress *in vitro*, it was completely deficient in tissue cyst formation during mouse infection, suggesting that the aberrant expression of bradyzoite proteins early during infection primed the host immune response for efficient clearing of the parasite load before chronic infection could be established [66]. Collectively, these studies argue against a single master regulator of bradyzoite differentiation and suggest that multiple transcription factors are involved in fine-tuning gene expression at multiple checkpoints during the transition to latency.

### The cell cycle and bradyzoite formation

The cyclic regulation of many of the regulators involved in bradyzoite formation suggests that this differentiation process is intimately linked to the parasite cell cycle. Early experiments showed that when the cell cycle is blocked, differentiation does not proceed [169]. Subsequent studies have attempted to elucidate the point in the cell cycle in which the tachyzoite switches to the bradyzoite developmental program and which factors play a role at this transition point. Gene Set Enrichment Analysis (GSEA) on a number of independent gene expression datasets from differentiation experiments provides strong evidence that bradyzoite formation and cell cycle regulation are coupled. Genes associated with the S/M phase of the cell cycle were more highly enriched in bradyzoites, while genes corresponding to the G1 phase were more enriched in tachyzoites [170, 171]. Cell cycle comparison between replicating and differentiating tachyzoites identified an enrichment of parasites in late S and M stages when bradyzoite development was triggered [95]. Concurrent with this observation, microarray analysis at different points in the tachyzoite replication cycle found that the levels of a number of bradyzoite-specific transcripts peaked at the late mitotic phase of the cell cycle [168]. This was observed in unstressed parasite populations, suggesting that even under normal conditions certain factors that are capable of initiating bradyzoite development are present, but the master signal for the transition is either absent or overpowered by other regulators that drive tachyzoite replication. This evidence supports a model whereby tachyzoites pass through a point in the cell cycle in which they are poised for development into a bradyzoite. If the signal for bradyzoite development is present, the gene expression program for cyst formation proceeds, but in the absence of such stress signals, tachyzoite replication continues.



## Changes in the epigenetic landscape for tachyzoite versus bradyzoite genes

A series of studies have demonstrated that epigenetics and histone post-translational modifications (PTMs) play a vital role in protozoan parasite biology [63]. More specifically, *Toxoplasma* epigenetics has been linked with the regulation of genes expressed during oxidative stress responses, cell cycle, and DNA repair mechanisms, as well as during tachyzoite to bradyzoite stage conversion [172–177].

Mining of the *Toxoplasma* genome has identified a wealth of factors homologous to the chromatin remodeling machinery of other well-characterized eukaryotes [164, 176]. Additionally, chromatin immunoprecipitation coupled to microarray chip (ChIP-chip) experiments have been utilized to determine that H3K4 tri-methylation and H3K9 acetylation are markers of active promoters in *Toxoplasma* [178]. The first evidence that linked epigenetic regulation with bradyzoite development was the observation that nucleosomes present in inactive bradyzoite-specific promoters are hypoacetylated during the tachyzoite stage but become acetylated under bradyzoite differentiation conditions [172, 179]. The converse was shown for tachyzoite-specific genes, whereas constitutively expressed genes were hyperacetylated in both developmental stages [172]. The differential association of histone deacetylase TgHDAC3 and histone acetyltransferase TgGCN5a at stage-specific promoters was also observed using ChIP assays [172]. Specifically, TgGCN5a is highly enriched at the promoter regions of transcribed genes [172, 173, 180] while TgHDAC3 is found at promoter regions of bradyzoite-specific genes that are silent during tachyzoite replication [172]. Moreover, analysis of the transcriptional profile of a TgGCN5a KO strain suggested a role for TgGCN5a in triggering bradyzoite gene expression in response to alkaline stress. TgGCN5a KO parasites under alkaline treatment did not upregulate *bag1* or *ldh2*, and these parasites were deficient in stress recovery [173]. *Toxoplasma* also expresses a second GCN5 histone acetyltransferase homologue, TgGCN5b which appears to be crucial during tachyzoite replication, but a role for this enzyme in mediating the transition to bradyzoite cyst has yet to be determined [29]. The involvement of specific HDACs in differentiation was revealed through the use of the compound FR235222, which specifically inhibits TgHDAC3 [174]. Treatment of *Toxoplasma*-infected cells with FR235222 induced bradyzoite differentiation [174]. In addition, comparisons of the transcriptional profiles of tachyzoites in the presence or absence of apicidin, another histone deacetylase inhibitor, suggested that the tachyzoite to bradyzoite conversion is regulated in part through histone acetylation and deacetylation modifications [181]. While these studies support the role of histone modifying enzymes in bradyzoite differentiation, it should be noted that “histone” modifying enzymes have numerous other substrates whose activity could be regulated by acetylation [182, 183].

Chemical inhibitors have also been used to determine the role of methyltransferases in *Toxoplasma* stage differentiation. Specifically, pre-treatment of extracellular tachyzoites with AMI-I, an inhibitor of the arginine methyltransferase TgCARM1, induced cyst formation [172]. ChIP assays were used to confirm that AMI-I treatment resulted in a reduction of H3R17 methylation in the nucleosomes at promoter regions of tachyzoite-specific genes, suggesting an important function of the H3R17 mark for tachyzoites [172]. However, methylation of H3R17 can also be observed in *in vitro* bradyzoites, pointing to a

key function for TgCARM1 in both stages. A significant gene duplication event and subsequent divergence in the SET-containing domain histone lysine methyltransferases has been reported in *Toxoplasma* [175]. TgSET8 was shown to be enriched in heterochromatic regions such as silenced rRNA genes, satellite repeats, and telomeric sites, with the repressive methylation marks H3K9 and H4K20 [175]. Notably, increased H4K20me1 levels have been detected in bradyzoite containing cysts purified from mice [175], indicating that TgSET8 may facilitate the maintenance of the chronic stage inside the host.

An interesting family of chromatin remodelers that has not been studied extensively, but has been linked to epigenetic regulation of stage conversion, is the SWI/SNF ATP-dependent nucleosome remodeling complexes. Intriguingly, the *Toxoplasma* genome has 17 predicted members of this family [176]. The mRNA from one member of this family, *TgSRCAP* (Snf2-Related CBP Activator Protein), is upregulated during *in vitro* bradyzoite stage conversion [184]. As previously mentioned, another member of this family, *TgRSC8*, was identified in an insertional mutagenesis screen designed to select for mutants defective in bradyzoite formation [41, 185].

Changing nucleosome composition is an additional mechanism that may be used to control gene expression during stage conversion in *Toxoplasma*. In addition to the canonical histones H2A and H2B, the *Toxoplasma* genome encodes a number of histone H2 variants, denoted H2AX, H2AZ, and H2Bv [164]. H2Bv forms dimers with H2A.Z on the promoter regions of active genes [186]. Notably, the incidence of H2A.Z changes in relation to the transcriptional status of a gene; preliminary data showed that H2A.Z is positioned within the coding region of silent bradyzoite-specific genes and within promoter regions but not coding regions of actively expressed genes [187]. In contrast, H2AX is enriched at promoters of repressed genes as well as within silent genomic regions in tachyzoites [186]. Furthermore, the expression levels of H2AX are increased during *in vitro* bradyzoite formation, suggesting that H2AX may facilitate global gene repression during the latent bradyzoite stage [186].

Multiple approaches have demonstrated the importance of epigenetic regulation during the transition from tachyzoite to bradyzoite. Many of the fundamentals of epigenetic regulatory mechanisms appear to be well preserved in parasites. However, there is a surprisingly high diversity within the proteins that constitute the chromatin remodeling machinery in *Toxoplasma*. PTM-histone mapping studies [187] as well as characterization of “species-specific” and “stage-specific” epigenetic modifications of *Toxoplasma* will assist in elucidating the complete mechanism of the complex developmental pathway driving bradyzoite formation.

## CONCLUSION AND FUTURE DIRECTIONS

The ability of *Toxoplasma* to form latent cysts and persist in the host for many years is the key to the parasite’s successful dissemination throughout a large proportion of the human population and numerous animal hosts worldwide.

We are beginning to understand the early stages of the transition from tachyzoite to bradyzoite induced by stress, but further research is required to flesh out the mechanisms involved. Importantly, these mechanisms need to be compared to newer models of in vitro differentiation that do not require the application of exogenous stress (i.e. spontaneous differentiation). Recent advances have identified a number of AP2 and chromatin remodeling factors that are responsible for controlling the gene expression program as the parasites switch from tachyzoite to bradyzoite, but how they are engaged and how they interplay remains largely unresolved. Many elements are at work in this complex, multifactorial process and advances in the molecular tools for cystogenic *Toxoplasma* strains will help to address remaining questions.

An area that has been largely neglected to date is the process of reactivation and how the parasite re-enters the proliferative tachyzoite cycle after dormancy. Phosphorylation of TgIF2 $\alpha$  appears to play a crucial role in perpetuating quiescence of the bradyzoites within the cyst and tantalizing new studies demonstrate that pharmacological interference with translational control can impede tachyzoite growth and the reactivation of infection [159].

Ultimately, we will require a detailed understanding of the signaling processes required for bradyzoite formation to interrupt the tachyzoite-bradyzoite transition. Definition of these pathways may enable us to chemically target central mediators of the differentiation and latency processes, and allow development of novel therapies for the individuals at risk of toxoplasmosis.

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## References

1. Hutchison WM, Dunachie JF, Siim JC, Work K. Life cycle of *Toxoplasma gondii*. *British medical journal*. 1969; 4:806.
2. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. *International journal for parasitology*. 2000; 30:1217–58. [PubMed: 11113252]
3. Sullivan WJ Jr, Jeffers V. Mechanisms of *Toxoplasma gondii* persistence and latency. *FEMS microbiology reviews*. 2012; 36:717–33. [PubMed: 22091606]
4. Luft BJ, Remington JS. AIDS commentary. Toxoplasmic encephalitis. *The Journal of infectious diseases*. 1988; 157:1–6. [PubMed: 3121758]
5. McLeod R, Mack D, Brown C. *Toxoplasma gondii*—new advances in cellular and molecular biology. *Experimental parasitology*. 1991; 72:109–21. [PubMed: 1993460]
6. Wong SY, Remington JS. Biology of *Toxoplasma gondii*. *AIDS*. 1993; 7:299–316. [PubMed: 8471191]
7. Luft BJ, Brooks RG, Conley FK, McCabe RE, Remington JS. Toxoplasmic encephalitis in patients with acquired immune deficiency syndrome. *JAMA: the journal of the American Medical Association*. 1984; 252:913–7. [PubMed: 6748191]
8. Dubey JP. Advances in the life cycle of *Toxoplasma gondii*. *International journal for parasitology*. 1998; 28:1019–24. [PubMed: 9724872]

9. Jones J, Lopez A, Wilson M. Congenital toxoplasmosis. *American family physician*. 2003; 67:2131–8. [PubMed: 12776962]
10. Wallace GR, Stanford MR. Immunity and *Toxoplasma* retinochoroiditis. *Clinical and experimental immunology*. 2008; 153:309–15. [PubMed: 18549442]
11. Vasconcelos-Santos DV. Ocular manifestations of systemic disease: toxoplasmosis. *Current opinion in ophthalmology*. 2012; 23:543–50. [PubMed: 23047172]
12. Bowie WR, King AS, Werker DH, Isaac-Renton JL, Bell A, Eng SB, Marion SA. Outbreak of toxoplasmosis associated with municipal drinking water. The BC *Toxoplasma* Investigation Team. *Lancet*. 1997; 350:173–7. [PubMed: 9250185]
13. Aramini JJ, Stephen C, Dubey JP, Engelstoft C, Schwantje H, Ribble CS. Potential contamination of drinking water with *Toxoplasma gondii* oocysts. *Epidemiology and infection*. 1999; 122:305–15. [PubMed: 10355797]
14. Miller MA, Gardner IA, Kreuder C, Paradies DM, Worcester KR, Jessup DA, Dodd E, Harris MD, Ames JA, Packham AE, Conrad PA. Coastal freshwater runoff is a risk factor for *Toxoplasma gondii* infection of southern sea otters (*Enhydra lutris nereis*). *International journal for parasitology*. 2002; 32:997–1006. [PubMed: 12076629]
15. Ferguson DJ, Hutchison WM. The host-parasite relationship of *Toxoplasma gondii* in the brains of chronically infected mice. *Virchows Archiv A, Pathological anatomy and histopathology*. 1987; 411:39–43. [PubMed: 3107207]
16. Ferguson DJ, Hutchison WM. An ultrastructural study of the early development and tissue cyst formation of *Toxoplasma gondii* in the brains of mice. *Parasitology research*. 1987; 73:483–91. [PubMed: 3422976]
17. Ferguson DJ, Hutchison WM, Pettersen E. Tissue cyst rupture in mice chronically infected with *Toxoplasma gondii*. An immunocytochemical and ultrastructural study. *Parasitology research*. 1989; 75:599–603. [PubMed: 2771928]
18. Cabral CM, Tuladhar S, Dietrich HK, Nguyen E, MacDonald WR, Trivedi T, Devineni A, Koshy AA. Neurons are the Primary Target Cell for the Brain-Tropic Intracellular Parasite *Toxoplasma gondii*. *PLoS pathogens*. 2016; 12:e1005447. [PubMed: 26895155]
19. Koshy AA, Dietrich HK, Christian DA, Melehani JH, Shastri AJ, Hunter CA, Boothroyd JC. *Toxoplasma* co-opts host cells it does not invade. *PLoS pathogens*. 2012; 8:e1002825. [PubMed: 22910631]
20. Kim K, Weiss LM. *Toxoplasma gondii*: the model apicomplexan. *International journal for parasitology*. 2004; 34:423–432. [PubMed: 15003501]
21. Soete M, Dubremetz JF. *Toxoplasma gondii*: kinetics of stage-specific protein expression during tachyzoite-bradyzoite conversion in vitro. *Current topics in microbiology and immunology*. 1996; 219:76–80. [PubMed: 8791691]
22. Dzierszynski F, Nishi M, Ouko L, Roos DS. Dynamics of *Toxoplasma gondii* differentiation. *Eukaryotic cell*. 2004; 3:992–1003. [PubMed: 15302832]
23. Lane A, Soete M, Dubremetz JF, Smith JE. *Toxoplasma gondii*: appearance of specific markers during the development of tissue cysts in vitro. *Parasitology research*. 1996; 82:340–6. [PubMed: 8740550]
24. Di Cristina M, Marocco D, Galizi R, Proietti C, Spaccapelo R, Crisanti A. Temporal and spatial distribution of *Toxoplasma gondii* differentiation into Bradyzoites and tissue cyst formation in vivo. *Infection and immunity*. 2008; 76:3491–501. [PubMed: 18505811]
25. van der Waaij D. Formation, growth and multiplication of *Toxoplasma gondii* cysts in mouse brain. *Trop Geogr Med*. 1959; 11:345–360.
26. Mehlhorn H, Frenkel JK. Ultrastructural comparison of cysts and zoites of *Toxoplasma gondii*, *Sarcocystis muris*, and *Hammondia hammondi* in skeletal muscle of mice. *The Journal of parasitology*. 1980; 66:59–67. [PubMed: 6767834]
27. Weiss LM, Kim K. The development and biology of bradyzoites of *Toxoplasma gondii*. *Frontiers in bioscience : a journal and virtual library*. 2000; 5:D391–405. [PubMed: 10762601]
28. Tomita T, Bzik DJ, Ma YF, Fox BA, Markillie LM, Taylor RC, Kim K, Weiss LM. The *Toxoplasma gondii* Cyst Wall Protein CST1 Is Critical for Cyst Wall Integrity and Promotes Bradyzoite Persistence. *PLoS pathogens*. 2013; 9:e1003823. [PubMed: 24385904]

29. Wang J, Dixon SE, Ting LM, Liu TK, Jeffers V, Croken MM, Calloway M, Cannella D, Hakimi MA, Kim K, Sullivan WJ Jr. Lysine acetyltransferase GCN5b interacts with AP2 factors and is required for *Toxoplasma gondii* proliferation. *PLoS pathogens*. 2014; 10:e1003830. [PubMed: 24391497]
30. Watts E, Zhao Y, Dhara A, Eller B, Patwardhan A, Sinai AP. Novel Approaches Reveal that *Toxoplasma gondii* Bradyzoites within Tissue Cysts Are Dynamic and Replicating Entities In Vivo. *mBio*. 2015; 6:e01155–15. [PubMed: 26350965]
31. Wang T, Gao JM, Yi SQ, Geng GQ, Gao XJ, Shen JL, Lu FL, Wen YZ, Hide G, Lun ZR. *Toxoplasma gondii* infection in the peritoneal macrophages of rats treated with glucocorticoids. *Parasitology research*. 2014; 113:351–8. [PubMed: 24248630]
32. Knoll LJ, Boothroyd JC. Molecular Biology's Lessons about *Toxoplasma* Development: Stage-specific Homologs. *Parasitol Today*. 1998; 14:490–3. [PubMed: 17040862]
33. Zhang YW, Halonen SK, Ma YF, Wittner M, Weiss LM. Initial characterization of CST1, a *Toxoplasma gondii* cyst wall glycoprotein. *Infection and immunity*. 2001; 69:501–7. [PubMed: 11119543]
34. Ferguson DJ. Use of molecular and ultrastructural markers to evaluate stage conversion of *Toxoplasma gondii* in both the intermediate and definitive host. *International journal for parasitology*. 2004; 34:347–60. [PubMed: 15003495]
35. Torpier G, Charif H, Darcy F, Liu J, Darde ML, Capron A. *Toxoplasma gondii*: differential location of antigens secreted from encysted bradyzoites. *Experimental parasitology*. 1993; 77:13–22. [PubMed: 8344403]
36. Lemgruber L, Lupetti P, Martins-Duarte ES, De Souza W, Vommario RC. The organization of the wall filaments and characterization of the matrix structures of *Toxoplasma gondii* cyst form. *Cellular microbiology*. 2011; 13:1920–32. [PubMed: 21899696]
37. Parmley SF, Yang S, Harth G, Sibley LD, Sucharczuk A, Remington JS. Molecular characterization of a 65-kilodalton *Toxoplasma gondii* antigen expressed abundantly in the matrix of tissue cysts. *Molecular and biochemical parasitology*. 1994; 66:283–96. [PubMed: 7808478]
38. Parmley S, Slifer T, Araujo F. Protective effects of immunization with a recombinant cyst antigen in mouse models of infection with *Toxoplasma gondii* tissue cysts. *The Journal of infectious diseases*. 2002; 185(Suppl 1):S90–5. [PubMed: 11865445]
39. Buchholz KR, Bowyer PW, Boothroyd JC. Bradyzoite Pseudokinase 1 Is Crucial for Efficient Oral Infectivity of the *Toxoplasma gondii* Tissue Cyst. *Eukaryotic cell*. 2013; 12:399–410. [PubMed: 23291621]
40. Buchholz KR, Fritz HM, Chen X, Durbin-Johnson B, Rocke DM, Ferguson DJ, Conrad PA, Boothroyd JC. Identification of tissue cyst wall components by transcriptome analysis of in vivo and in vitro *Toxoplasma gondii* bradyzoites. *Eukaryotic cell*. 2011; 10:1637–47. [PubMed: 22021236]
41. Craver MP, Rooney PJ, Knoll LJ. Isolation of *Toxoplasma gondii* development mutants identifies a potential proteophosphoglycan that enhances cyst wall formation. *Molecular and biochemical parasitology*. 2010; 169:120–3. [PubMed: 19879901]
42. Tomavo S, Fortier B, Soete M, Ansel C, Camus D, Dubremetz JF. Characterization of bradyzoite-specific antigens of *Toxoplasma gondii*. *Infection and immunity*. 1991; 59:3750–3. [PubMed: 1894373]
43. Bohne W, Gross U, Ferguson DJ, Heesemann J. Cloning and characterization of a bradyzoite-specifically expressed gene (*hsp30/bag1*) of *Toxoplasma gondii*, related to genes encoding small heat-shock proteins of plants. *Molecular microbiology*. 1995; 16:1221–30. [PubMed: 8577255]
44. Bohne W, Parmley SF, Yang S, Gross U. Bradyzoite-specific genes. *Current topics in microbiology and immunology*. 1996; 219:81–91. [PubMed: 8791692]
45. Parmley SF, Weiss LM, Yang S. Cloning of a bradyzoite-specific gene of *Toxoplasma gondii* encoding a cytoplasmic antigen. *Molecular and biochemical parasitology*. 1995; 73:253–7. [PubMed: 8577335]
46. Yang S, Parmley SF. A bradyzoite stage-specifically expressed gene of *Toxoplasma gondii* encodes a polypeptide homologous to lactate dehydrogenase. *Molecular and biochemical parasitology*. 1995; 73:291–4. [PubMed: 8577343]

47. Yang S, Parmley SF. *Toxoplasma gondii* expresses two distinct lactate dehydrogenase homologous genes during its life cycle in intermediate hosts. *Gene*. 1997; 184:1–12. [PubMed: 9016946]
48. Denton H, Roberts CW, Alexander J, Thong KW, Coombs GH. Enzymes of energy metabolism in the bradyzoites and tachyzoites of *Toxoplasma gondii*. *FEMS microbiology letters*. 1996; 137:103–8. [PubMed: 8935663]
49. Yahiaoui B, Dzierszinski F, Bernigaud A, Slomianny C, Camus D, Tomavo S. Isolation and characterization of a subtractive library enriched for developmentally regulated transcripts expressed during encystation of *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 1999; 99:223–35. [PubMed: 10340486]
50. Toursel C, Dzierszinski F, Bernigaud A, Mortuaire M, Tomavo S. Molecular cloning, organellar targeting and developmental expression of mitochondrial chaperone HSP60 in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2000; 111:319–32. [PubMed: 11163440]
51. Holpert M, Luder CG, Gross U, Bohne W. Bradyzoite-specific expression of a P-type ATPase in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2001; 112:293–6. [PubMed: 11223136]
52. Odberg-Ferragut C, Soete M, Engels A, Samyn B, Loyens A, Van Beeumen J, Camus D, Dubremetz JF. Molecular cloning of the *Toxoplasma gondii* sag4 gene encoding an 18 kDa bradyzoite specific surface protein. *Molecular and biochemical parasitology*. 1996; 82:237–44. [PubMed: 8946389]
53. Manger ID, Hehl A, Parmley S, Sibley LD, Marra M, Hillier L, Waterston R, Boothroyd JC. Expressed sequence tag analysis of the bradyzoite stage of *Toxoplasma gondii*: identification of developmentally regulated genes. *Infection and immunity*. 1998; 66:1632–7. [PubMed: 9529091]
54. Bohne W, Wirsing A, Gross U. Bradyzoite-specific gene expression in *Toxoplasma gondii* requires minimal genomic elements. *Molecular and biochemical parasitology*. 1997; 85:89–98. [PubMed: 9108551]
55. Knoll LJ, Boothroyd JC. Isolation of developmentally regulated genes from *Toxoplasma gondii* by a gene trap with the positive and negative selectable marker hypoxanthine-xanthine-guanine phosphoribosyltransferase. *Molecular and cellular biology*. 1998; 18:807–14. [PubMed: 9447977]
56. Pittman KJ, Aliota MT, Knoll LJ. Dual transcriptional profiling of mice and *Toxoplasma gondii* during acute and chronic infection. *BMC genomics*. 2014; 15:806. [PubMed: 25240600]
57. Zhang YW, Kim K, Ma YF, Wittner M, Tanowitz HB, Weiss LM. Disruption of the *Toxoplasma gondii* bradyzoite-specific gene BAG1 decreases in vivo cyst formation. *Molecular microbiology*. 1999; 31:691–701. [PubMed: 10027984]
58. Bohne W, Hunter CA, White MW, Ferguson DJ, Gross U, Roos DS. Targeted disruption of the bradyzoite-specific gene BAG1 does not prevent tissue cyst formation in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 1998; 92:291–301. [PubMed: 9657333]
59. Holpert M, Gross U, Bohne W. Disruption of the bradyzoite-specific P-type (H<sup>+</sup>)-ATPase PMA1 in *Toxoplasma gondii* leads to decreased bradyzoite differentiation after stress stimuli but does not interfere with mature tissue cyst formation. *Molecular and biochemical parasitology*. 2006; 146:129–33. [PubMed: 16375982]
60. Kim SK, Fouts AE, Boothroyd JC. *Toxoplasma gondii* dysregulates IFN-gamma-inducible gene expression in human fibroblasts: insights from a genome-wide transcriptional profiling. *J Immunol*. 2007; 178:5154–65. [PubMed: 17404298]
61. Fox BA, Falla A, Rommereim LM, Tomita T, Gigley JP, Mercier C, Cesbron-Delauw MF, Weiss LM, Bzik DJ. Type II *Toxoplasma gondii* KU80 knockout strains enable functional analysis of genes required for cyst development and latent infection. *Eukaryotic cell*. 2011; 10:1193–206. [PubMed: 21531875]
62. Radke JB, Lucas O, De Silva EK, Ma Y, Sullivan WJ Jr, Weiss LM, Llinas M, White MW. ApiAP2 transcription factor restricts development of the *Toxoplasma* tissue cyst. *Proceedings of the National Academy of Sciences of the United States of America*. 2013
63. Walker R, Gissot M, Croken MM, Huot L, Hot D, Kim K, Tomavo S. The *Toxoplasma* nuclear factor TgAP2XI-4 controls bradyzoite gene expression and cyst formation. *Molecular microbiology*. 2013; 87:641–55. [PubMed: 23240624]



64. Huang S, Holmes MJ, Radke JB, Hong DP, Liu TK, White MW, Sullivan WJ Jr. *Toxoplasma gondii* AP2IX-4 Regulates Gene Expression during Bradyzoite Development. *mSphere*. 2017; 2
65. Hong DP, Radke JB, White MW. Opposing Transcriptional Mechanisms Regulate *Toxoplasma* Development. *mSphere*. 2017; 2
66. Radke JB, Worth D, Hong DP, Huang S, Sullivan WJ, Wilson EH, White M. Transcriptional repression by ApiAP2 factors is central to chronic toxoplasmosis. *bioRxiv*. 2017
67. Singh U, Brewer JL, Boothroyd JC. Genetic analysis of tachyzoite to bradyzoite differentiation mutants in *Toxoplasma gondii* reveals a hierarchy of gene induction. *Molecular microbiology*. 2002; 44:721–33. [PubMed: 11994153]
68. Matrajt M, Donald RG, Singh U, Roos DS. Identification and characterization of differentiation mutants in the protozoan parasite *Toxoplasma gondii*. *Molecular microbiology*. 2002; 44:735–47. [PubMed: 11994154]
69. Vanchinathan P, Brewer JL, Harb OS, Boothroyd JC, Singh U. Disruption of a locus encoding a nucleolar zinc finger protein decreases tachyzoite-to-bradyzoite differentiation in *Toxoplasma gondii*. *Infection and immunity*. 2005; 73:6680–8. [PubMed: 16177345]
70. Anderson MZ, Brewer J, Singh U, Boothroyd JC. A pseudouridine synthase homologue is critical to cellular differentiation in *Toxoplasma gondii*. *Eukaryotic cell*. 2009; 8:398–409. [PubMed: 19124578]
71. Frankel MB, Mordue DG, Knoll LJ. Discovery of parasite virulence genes reveals a unique regulator of chromosome condensation 1 ortholog critical for efficient nuclear trafficking. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:10181–6. [PubMed: 17535896]
72. Sugi T, Tu V, Ma Y, Tomita T, Weiss LM. *Toxoplasma gondii* Requires Glycogen Phosphorylase for Balancing Amylopectin Storage and for Efficient Production of Brain Cysts. *mBio*. 2017; 8
73. Dzierszynski F, Mortuaire M, Dendouga N, Popescu O, Tomavo S. Differential expression of two plant-like enolases with distinct enzymatic and antigenic properties during stage conversion of the protozoan parasite *Toxoplasma gondii*. *Journal of molecular biology*. 2001; 309:1017–27. [PubMed: 11399076]
74. Al-Anouti F, Tomavo S, Parmley S, Ananvoranich S. The expression of lactate dehydrogenase is important for the cell cycle of *Toxoplasma gondii*. *The Journal of biological chemistry*. 2004; 279:52300–11. [PubMed: 15459194]
75. Di Cristina M, Dou Z, Lunghi M, Kannan G, Huynh MH, McGovern OL, Schultz TL, Schultz AJ, Miller AJ, Hayes BM, van der Linden W, Emiliani C, Bogyo M, Besteiro S, Coppens I, Carruthers VB. *Toxoplasma* depends on lysosomal consumption of autophagosomes for persistent infection. *Nature microbiology*. 2017; 2:17096.
76. Su C, Evans D, Cole RH, Kissinger JC, Ajioka JW, Sibley LD. Recent expansion of *Toxoplasma* through enhanced oral transmission. *Science*. 2003; 299:414–6. [PubMed: 12532022]
77. Howe DK, Sibley LD. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *The Journal of infectious diseases*. 1995; 172:1561–6. [PubMed: 7594717]
78. Barragan A, Sibley LD. Migration of *Toxoplasma gondii* across biological barriers. *Trends in microbiology*. 2003; 11:426–30. [PubMed: 13678858]
79. Sabin AB. Isolation of a Filtrable, Transmissible Agent with “Neurolytic” Properties from *Toxoplasma*-Infected Tissues. *Science*. 1938; 88:189–91. [PubMed: 17741514]
80. Soete M, Camus D, Dubremetz JF. Experimental induction of bradyzoite-specific antigen expression and cyst formation by the RH strain of *Toxoplasma gondii* in vitro. *Experimental parasitology*. 1994; 78:361–70. [PubMed: 8206135]
81. Bohne W, Roos DS. Stage-specific expression of a selectable marker in *Toxoplasma gondii* permits selective inhibition of either tachyzoites or bradyzoites. *Molecular and biochemical parasitology*. 1997; 88:115–26. [PubMed: 9274873]
82. Lescault PJ, Thompson AB, Patil V, Lirussi D, Burton A, Margarit J, Bond J, Matrajt M. Genomic data reveal *Toxoplasma gondii* differentiation mutants are also impaired with respect to switching into a novel extracellular tachyzoite state. *PloS one*. 2010; 5:e14463. [PubMed: 21209930]

83. Behnke MS, Radke JB, Smith AT, Sullivan WJ Jr, White MW. The transcription of bradyzoite genes in *Toxoplasma gondii* is controlled by autonomous promoter elements. *Molecular microbiology*. 2008; 68:1502–18. [PubMed: 18433450]
84. Frenkel JK, Dubey JP, Hoff RL. Loss of stages after continuous passage of *Toxoplasma gondii* and *Besnoitia jellisoni*. *The Journal of protozoology*. 1976; 23:421–4. [PubMed: 823327]
85. Weiss LM, Ma YF, Takvorian PM, Tanowitz HB, Wittner M. Bradyzoite development in *Toxoplasma gondii* and the hsp70 stress response. *Infection and immunity*. 1998; 66:3295–302. [PubMed: 9632598]
86. Fox BA, Gigley JP, Bzik DJ. *Toxoplasma gondii* lacks the enzymes required for de novo arginine biosynthesis and arginine starvation triggers cyst formation. *International journal for parasitology*. 2004; 34:323–31. [PubMed: 15003493]
87. Narasimhan J, Joyce BR, Naguleswaran A, Smith AT, Livingston MR, Dixon SE, Coppens I, Wek RC, Sullivan WJ Jr. Translation regulation by eukaryotic initiation factor-2 kinases in the development of latent cysts in *Toxoplasma gondii*. *The Journal of biological chemistry*. 2008; 283:16591–601. [PubMed: 18420584]
88. Nagamune K, Hicks LM, Fux B, Brossier F, Chini EN, Sibley LD. Abscisic acid controls calcium-dependent egress and development in *Toxoplasma gondii*. *Nature*. 2008; 451:207–10. [PubMed: 18185591]
89. Bohne W, Heesemann J, Gross U. Reduced replication of *Toxoplasma gondii* is necessary for induction of bradyzoite-specific antigens: a possible role for nitric oxide in triggering stage conversion. *Infection and immunity*. 1994; 62:1761–7. [PubMed: 8168938]
90. Tomavo S, Boothroyd JC. Interconnection between organellar functions, development and drug resistance in the protozoan parasite, *Toxoplasma gondii*. *International journal for parasitology*. 1995; 25:1293–9. [PubMed: 8635881]
91. Bohne W, Heesemann J, Gross U. Induction of bradyzoite-specific *Toxoplasma gondii* antigens in gamma interferon-treated mouse macrophages. *Infection and immunity*. 1993; 61:1141–5. [PubMed: 8432596]
92. Hartmann A, Arroyo-Olarte RD, Imkeller K, Hegemann P, Lucius R, Gupta N. Optogenetic modulation of an adenylate cyclase in *Toxoplasma gondii* demonstrates a requirement of the parasite cAMP for host-cell invasion and stage differentiation. *The Journal of biological chemistry*. 2013
93. Sugi T, Ma YF, Tomita T, Murakoshi F, Eaton MS, Yakubu R, Han B, Tu V, Kato K, Kawazu S, Gupta N, Suvorova ES, White MW, Kim K, Weiss LM. *Toxoplasma gondii* Cyclic AMP-Dependent Protein Kinase Subunit 3 Is Involved in the Switch from Tachyzoite to Bradyzoite Development. *mBio*. 2016; 7
94. Luder CGK, Rahman T. Impact of the host on *Toxoplasma* stage differentiation. *Microb Cell*. 2017; 4:203–211. [PubMed: 28706936]
95. Radke JR, Guerini MN, Jerome M, White MW. A change in the premitotic period of the cell cycle is associated with bradyzoite differentiation in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2003; 131:119–27. [PubMed: 14511810]
96. Ihara F, Nishikawa Y. Starvation of low-density lipoprotein-derived cholesterol induces bradyzoite conversion in *Toxoplasma gondii*. *Parasites & vectors*. 2014; 7:248. [PubMed: 24885547]
97. Weilhammer DR, Iavarone AT, Villegas EN, Brooks GA, Sinai AP, Sha WC. Host metabolism regulates growth and differentiation of *Toxoplasma gondii*. *International journal for parasitology*. 2012; 42:947–59. [PubMed: 22940576]
98. Radke JR, Donald RG, Eibs A, Jerome ME, Behnke MS, Liberator P, White MW. Changes in the expression of human cell division autoantigen-1 influence *Toxoplasma gondii* growth and development. *PLoS pathogens*. 2006; 2:e105. [PubMed: 17069459]
99. Gurnett AM, Liberator PA, Dulski PM, Salowe SP, Donald RG, Anderson JW, Wiltsie J, Diaz CA, Harris G, Chang B, Darkin-Rattray SJ, Nare B, Crumley T, Blum PS, Misura AS, Tamas T, Sardana MK, Yuan J, Biftu T, Schmatz DM. Purification and molecular characterization of cGMP-dependent protein kinase from Apicomplexan parasites. A novel chemotherapeutic target. *The Journal of biological chemistry*. 2002; 277:15913–22. [PubMed: 11834729]

100. Donald RG, Zhong T, Wiersma H, Nare B, Yao D, Lee A, Allocco J, Liberator PA. Anticoccidial kinase inhibitors: identification of protein kinase targets secondary to cGMP-dependent protein kinase. *Molecular and biochemical parasitology*. 2006; 149:86–98. [PubMed: 16765465]
101. McHugh TD, Gbewonyo A, Johnson JD, Holliman RE, Butcher PD. Development of an in vitro model of *Toxoplasma gondii* cyst formation. *FEMS microbiology letters*. 1993; 114:325–32. [PubMed: 8288109]
102. Ferreira da Silva Mda F, Barbosa HS, Gross U, Luder CG. Stress-related and spontaneous stage differentiation of *Toxoplasma gondii*. *Molecular bioSystems*. 2008; 4:824–34. [PubMed: 18633484]
103. Swierzy IJ, Handel U, Kaever A, Jarek M, Scharfe M, Schluter D, Luder CGK. Divergent co-transcriptomes of different host cells infected with *Toxoplasma gondii* reveal cell type-specific host-parasite interactions. *Scientific reports*. 2017; 7:7229. [PubMed: 28775382]
104. Swierzy IJ, Luder CG. Withdrawal of skeletal muscle cells from cell cycle progression triggers differentiation of *Toxoplasma gondii* towards the bradyzoite stage. *Cellular microbiology*. 2015; 17:2–17. [PubMed: 25131712]
105. Gross U, Bohne W, Soete M, Dubremetz JF. Developmental differentiation between tachyzoites and bradyzoites of *Toxoplasma gondii*. *Parasitol Today*. 1996; 12:30–3. [PubMed: 15275305]
106. Dupont CD, Christian DA, Hunter CA. Immune response and immunopathology during toxoplasmosis. *Seminars in immunopathology*. 2012; 34:793–813. [PubMed: 22955326]
107. Denkers EY, Gazzinelli RT. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clinical microbiology reviews*. 1998; 11:569–88. [PubMed: 9767056]
108. Kang H, Remington JS, Suzuki Y. Decreased resistance of B cell-deficient mice to infection with *Toxoplasma gondii* despite unimpaired expression of IFN-gamma, TNF-alpha, and inducible nitric oxide synthase. *J Immunol*. 2000; 164:2629–34. [PubMed: 10679102]
109. Johnson LL, Sayles PC. Deficient humoral responses underlie susceptibility to *Toxoplasma gondii* in CD4-deficient mice. *Infection and immunity*. 2002; 70:185–91. [PubMed: 11748181]
110. Denkers EY, Sher A. Role of natural killer and NK1+ T-cells in regulating cell-mediated immunity during *Toxoplasma gondii* infection. *Biochemical Society transactions*. 1997; 25:699–703. [PubMed: 9191186]
111. Lutjen S, Soltek S, Virna S, Deckert M, Schluter D. Organ- and disease-stage-specific regulation of *Toxoplasma gondii*-specific CD8-T-cell responses by CD4 T cells. *Infection and immunity*. 2006; 74:5790–801. [PubMed: 16988257]
112. Combe CL, Curiel TJ, Moretto MM, Khan IA. NK cells help to induce CD8(+)-T-cell immunity against *Toxoplasma gondii* in the absence of CD4(+) T cells. *Infection and immunity*. 2005; 73:4913–21. [PubMed: 16041005]
113. Tait ED, Jordan KA, Dupont CD, Harris TH, Gregg B, Wilson EH, Pepper M, Dzierszynski F, Roos DS, Hunter CA. Virulence of *Toxoplasma gondii* is associated with distinct dendritic cell responses and reduced numbers of activated CD8+ T cells. *J Immunol*. 2010; 185:1502–12. [PubMed: 20592284]
114. 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. ATF6beta is a host cellular target of the *Toxoplasma gondii* virulence factor ROP18. *The Journal of experimental medicine*. 2011; 208:1533–46. [PubMed: 21670204]
115. Suzuki Y, Orellana MA, Schreiber RD, Remington JS. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science*. 1988; 240:516–8. [PubMed: 3128869]
116. Gazzinelli RT, Hakim FT, Hieny S, Shearer GM, Sher A. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. *J Immunol*. 1991; 146:286–92. [PubMed: 1670604]
117. Suzuki Y, Claflin J, Wang X, Lengi A, Kikuchi T. Microglia and macrophages as innate producers of interferon-gamma in the brain following infection with *Toxoplasma gondii*. *International journal for parasitology*. 2005; 35:83–90. [PubMed: 15619519]

118. Kang H, Suzuki Y. Requirement of non-T cells that produce gamma interferon for prevention of reactivation of *Toxoplasma gondii* infection in the brain. *Infection and immunity*. 2001; 69:2920–7. [PubMed: 11292707]
119. Wang X, Suzuki Y. Microglia produce IFN-gamma independently from T cells during acute toxoplasmosis in the brain. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*. 2007; 27:599–605.
120. Scharton-Kersten TM, Wynn TA, Denkers EY, Bala S, Grunvald E, Hieny S, Gazzinelli RT, Sher A. In the absence of endogenous IFN-gamma, mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. *J Immunol*. 1996; 157:4045–54. [PubMed: 8892638]
121. Gazzinelli RT, Wysocka M, Hayashi S, Denkers EY, Hieny S, Caspar P, Trinchieri G, Sher A. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with *Toxoplasma gondii*. *J Immunol*. 1994; 153:2533–43. [PubMed: 7915739]
122. Langermans JA, van der Hulst ME, Nibbering PH, van Furth R. Endogenous tumor necrosis factor alpha is required for enhanced antimicrobial activity against *Toxoplasma gondii* and *Listeria monocytogenes* in recombinant gamma interferon-treated mice. *Infection and immunity*. 1992; 60:5107–12. [PubMed: 1452344]
123. Chao CC, Anderson WR, Hu S, Gekker G, Martella A, Peterson PK. Activated microglia inhibit multiplication of *Toxoplasma gondii* via a nitric oxide mechanism. *Clinical immunology and immunopathology*. 1993; 67:178–83. [PubMed: 8519093]
124. Peterson PK, Gekker G, Hu S, Chao CC. Human astrocytes inhibit intracellular multiplication of *Toxoplasma gondii* by a nitric oxide-mediated mechanism. *The Journal of infectious diseases*. 1995; 171:516–8. [PubMed: 7844409]
125. Adams LB, Hibbs JB Jr, Taintor RR, Krahenbuhl JL. Microbiostatic effect of murine-activated macrophages for *Toxoplasma gondii*. Role for synthesis of inorganic nitrogen oxides from L-arginine. *J Immunol*. 1990; 144:2725–9. [PubMed: 2319133]
126. Luder CG, Algnier M, Lang C, Bleicher N, Gross U. Reduced expression of the inducible nitric oxide synthase after infection with *Toxoplasma gondii* facilitates parasite replication in activated murine macrophages. *International journal for parasitology*. 2003; 33:833–44. [PubMed: 12865083]
127. Ibrahim HM, Bannai H, Xuan X, Nishikawa Y. *Toxoplasma gondii* cyclophilin 18-mediated production of nitric oxide induces Bradyzoite conversion in a CCR5-dependent manner. *Infection and immunity*. 2009; 77:3686–95. [PubMed: 19564392]
128. Hayashi S, Chan CC, Gazzinelli R, Roberge FG. Contribution of nitric oxide to the host parasite equilibrium in toxoplasmosis. *J Immunol*. 1996; 156:1476–81. [PubMed: 8568250]
129. Daubener W, Spors B, Hucke C, Adam R, Stins M, Kim KS, Schrotten H. Restriction of *Toxoplasma gondii* growth in human brain microvascular endothelial cells by activation of indoleamine 2,3-dioxygenase. *Infection and immunity*. 2001; 69:6527–31. [PubMed: 11553600]
130. Gupta SL, Carlin JM, Pyati P, Dai W, Pfefferkorn ER, Murphy MJ Jr. Antiparasitic and antiproliferative effects of indoleamine 2,3-dioxygenase enzyme expression in human fibroblasts. *Infection and immunity*. 1994; 62:2277–84. [PubMed: 8188349]
131. Daubener W, Gutsche M, Nockemann S, MacKenzie C, Seghrouchni S, Hadding U. Protamine enhances the activity of human recombinant interferon-gamma. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research*. 1996; 16:531–6.
132. Pfefferkorn ER. Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. *Proceedings of the National Academy of Sciences of the United States of America*. 1984; 81:908–12. [PubMed: 6422465]
133. Murray HW, Szuro-Sudol A, Wellner D, Oca MJ, Granger AM, Libby DM, Rothermel CD, Rubin BY. Role of tryptophan degradation in respiratory burst-independent antimicrobial activity of gamma interferon-stimulated human macrophages. *Infection and immunity*. 1989; 57:845–9. [PubMed: 2492973]
134. Divanovic S, Sawtell NM, Trompette A, Warning JI, Dias A, Cooper AM, Yap GS, Arditi M, Shimada K, Duhadaway JB, Prendergast GC, Basaraba RJ, Mellor AL, Munn DH, Aliberti J,

- Karp CL. Opposing biological functions of tryptophan catabolizing enzymes during intracellular infection. *The Journal of infectious diseases*. 2012; 205:152–61. [PubMed: 21990421]
135. Sibley LD, Messina M, Niesman IR. Stable DNA transformation in the obligate intracellular parasite *Toxoplasma gondii* by complementation of tryptophan auxotrophy. *Proceedings of the National Academy of Sciences of the United States of America*. 1994; 91:5508–12. [PubMed: 8202518]
136. Schmitz JL, Carlin JM, Borden EC, Byrne GI. Beta interferon inhibits *Toxoplasma gondii* growth in human monocyte-derived macrophages. *Infection and immunity*. 1989; 57:3254–6. [PubMed: 2506136]
137. Knubel CP, Martinez FF, Fretes RE, Diaz Lujan C, Theumer MG, Cervi L, Motran CC. Indoleamine 2,3-dioxygenase (IDO) is critical for host resistance against *Trypanosoma cruzi*. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2010; 24:2689–701. [PubMed: 20233946]
138. Tetsutani K, To H, Torii M, Hisaeda H, Himeno K. Malaria parasite induces tryptophan-related immune suppression in mice. *Parasitology*. 2007; 134:923–30. [PubMed: 17316473]
139. Yap GS, Scharton-Kersten T, Charest H, Sher A. 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*. 1998; 160:1340–5. [PubMed: 9570552]
140. Schluter D, Kwok LY, Lutjen S, Soltek S, Hoffmann S, Korner H, Deckert M. Both lymphotoxin-alpha and TNF are crucial for control of *Toxoplasma gondii* in the central nervous system. *J Immunol*. 2003; 170:6172–82. [PubMed: 12794148]
141. Weiss LM, Laplace D, Takvorian PM, Tanowitz HB, Cali A, Wittner M. A cell culture system for study of the development of *Toxoplasma gondii* bradyzoites. *The Journal of eukaryotic microbiology*. 1995; 42:150–7. [PubMed: 7757057]
142. Yap GS, Sher A. 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*. *The Journal of experimental medicine*. 1999; 189:1083–92. [PubMed: 10190899]
143. Gazzinelli RT, Hieny S, Wynn TA, Wolf S, Sher A. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon gamma by an intracellular parasite and induces resistance in T-cell-deficient hosts. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:6115–9. [PubMed: 8100999]
144. Streilein JW. Ocular immune privilege in the immunosuppressive intraocular microenvironment. *Ocular immunology and inflammation*. 1995; 3:139–44. [PubMed: 22827281]
145. Wiendl H, Hohlfeld R, Kieseier BC. Immunobiology of muscle: advances in understanding an immunological microenvironment. *Trends in immunology*. 2005; 26:373–80. [PubMed: 15922662]
146. Jones LA, Alexander J, Roberts CW. Ocular toxoplasmosis: in the storm of the eye. *Parasite immunology*. 2006; 28:635–42. [PubMed: 17096642]
147. Mahamed DA, Mills JH, Egan CE, Denkers EY, Bynoe MS. CD73-generated adenosine facilitates *Toxoplasma gondii* differentiation to long-lived tissue cysts in the central nervous system. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:16312–7. [PubMed: 22988118]
148. Israelski DM, Remington JS. Toxoplasmic encephalitis in patients with AIDS. *Infectious disease clinics of North America*. 1988; 2:429–45. [PubMed: 3060527]
149. Gazzinelli R, Xu Y, Hieny S, Cheever A, Sher A. Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J Immunol*. 1992; 149:175–80. [PubMed: 1351500]
150. Suzuki Y, Remington JS. A method for obtaining large numbers of trophozoites of avirulent strains of *Toxoplasma gondii* using an antibody to interferon-gamma. *The Journal of parasitology*. 1989; 75:174–6. [PubMed: 2493090]
151. Suzuki Y, Joh K. Effect of the strain of *Toxoplasma gondii* on the development of toxoplasmic encephalitis in mice treated with antibody to interferon-gamma. *Parasitology research*. 1994; 80:125–30. [PubMed: 8202451]

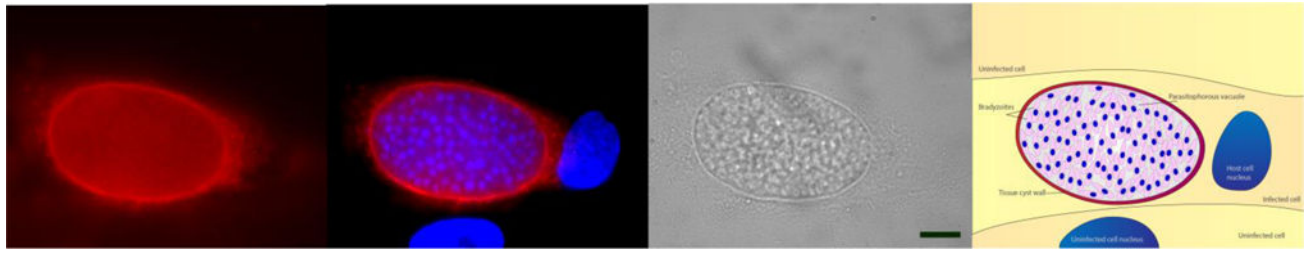


152. Dunay IR, Chan WC, Haynes RK, Sibley LD. Artemisone and artemiside control acute and reactivated toxoplasmosis in a murine model. *Antimicrobial agents and chemotherapy*. 2009; 53:4450–6. [PubMed: 19635951]
153. Jones TC, Bienz KA, Erb P. In vitro cultivation of *Toxoplasma gondii* cysts in astrocytes in the presence of gamma interferon. *Infection and immunity*. 1986; 51:147–56. [PubMed: 3079728]
154. Wek RC, Jiang HY, Anthony TG. Coping with stress: eIF2 kinases and translational control. *Biochemical Society transactions*. 2006; 34:7–11. [PubMed: 16246168]
155. Holmes MJ, Augusto LDS, Zhang M, Wek RC, Sullivan WJ Jr. Translational Control in the Latency of Apicomplexan Parasites. *Trends in parasitology*. 2017; 33:947–960. [PubMed: 28942109]
156. Sullivan WJ Jr, Narasimhan J, Bhatti MM, Wek RC. Parasite-specific eIF2 (eukaryotic initiation factor-2) kinase required for stress-induced translation control. *The Biochemical journal*. 2004; 380:523–31. [PubMed: 14989696]
157. Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. *Science*. 2005; 307:935–9. [PubMed: 15705855]
158. Tsaytler P, Harding HP, Ron D, Bertolotti A. Selective inhibition of a regulatory subunit of protein phosphatase 1 restores proteostasis. *Science*. 2011; 332:91–4. [PubMed: 21385720]
159. Konrad C, Queener SF, Wek RC, Sullivan WJ Jr. Inhibitors of eIF2alpha dephosphorylation slow replication and stabilize latency in *Toxoplasma gondii*. *Antimicrobial agents and chemotherapy*. 2013
160. Joyce BR, Tampaki Z, Kim K, Wek RC, Sullivan WJ Jr. The Unfolded Protein Response in the Protozoan Parasite *Toxoplasma gondii* Features Translational and Transcriptional Control. *Eukaryotic cell*. 2013; 12:979–89. [PubMed: 23666622]
161. Painter HJ, Campbell TL, Llinas M. The Apicomplexan AP2 family: integral factors regulating *Plasmodium* development. *Molecular and biochemical parasitology*. 2011; 176:1–7. [PubMed: 21126543]
162. Cleary MD, Singh U, Blader IJ, Brewer JL, Boothroyd JC. *Toxoplasma gondii* asexual development: identification of developmentally regulated genes and distinct patterns of gene expression. *Eukaryotic cell*. 2002; 1:329–40. [PubMed: 12455982]
163. Radke JR, Behnke MS, Mackey AJ, Radke JB, Roos DS, White MW. The transcriptome of *Toxoplasma gondii*. *BMC biology*. 2005; 3:26. [PubMed: 16324218]
164. Sullivan WJ Jr, Hakimi MA. Histone mediated gene activation in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2006; 148:109–16. [PubMed: 16644030]
165. Meissner M, Soldati D. The transcription machinery and the molecular toolbox to control gene expression in *Toxoplasma gondii* and other protozoan parasites. *Microbes and infection / Institut Pasteur*. 2005; 7:1376–84.
166. Olguin-Lamas A, Madec E, Hovasse A, Werkmeister E, Callebaut I, Slomianny C, Delhaye S, Mouveaux T, Schaeffer-Reiss C, Van Dorsselaer A, Tomavo S. A novel *Toxoplasma gondii* nuclear factor TgNF3 is a dynamic chromatin-associated component, modulator of nucleolar architecture and parasite virulence. *PLoS pathogens*. 2011; 7:e1001328. [PubMed: 21483487]
167. White MW, Radke JR, Radke JB. *Toxoplasma* development - turn the switch on or off? *Cellular microbiology*. 2014; 16:466–72. [PubMed: 24438211]
168. Behnke MS, Wootton JC, Lehmann MM, Radke JB, Lucas O, Nawas J, Sibley LD, White MW. Coordinated progression through two subtranscriptomes underlies the tachyzoite cycle of *Toxoplasma gondii*. *PloS one*. 2010; 5:e12354. [PubMed: 20865045]
169. Radke JR, Striepen B, Guerini MN, Jerome ME, Roos DS, White MW. Defining the cell cycle for the tachyzoite stage of *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2001; 115:165–75. [PubMed: 11420103]
170. Croken MM, Ma Y, Markillie LM, Taylor RC, Orr G, Weiss LM, Kim K. Distinct strains of *Toxoplasma gondii* feature divergent transcriptomes regardless of developmental stage. *PloS one*. 2014; 9:e111297. [PubMed: 25393307]



171. Croken MM, Qiu W, White MW, Kim K. Gene Set Enrichment Analysis (GSEA) of *Toxoplasma gondii* expression datasets links cell cycle progression and the bradyzoite developmental program. *BMC genomics*. 2014; 15:515. [PubMed: 24962434]
172. Saksouk N, Bhatti MM, Kieffer S, Smith AT, Musset K, Garin J, Sullivan WJ Jr, Cesbron-Delauw MF, Hakimi MA. Histone-modifying complexes regulate gene expression pertinent to the differentiation of the protozoan parasite *Toxoplasma gondii*. *Molecular and cellular biology*. 2005; 25:10301–14. [PubMed: 16287846]
173. Naguleswaran A, Elias EV, McClintick J, Edenberg HJ, Sullivan WJ Jr. *Toxoplasma gondii* lysine acetyltransferase GCN5-A functions in the cellular response to alkaline stress and expression of cyst genes. *PLoS pathogens*. 2010; 6:e1001232. [PubMed: 21179246]
174. Bougdour A, Maubon D, Baldacci P, Ortet P, Bastien O, Bouillon A, Barale JC, Pelloux H, Menard R, Hakimi MA. Drug inhibition of HDAC3 and epigenetic control of differentiation in Apicomplexa parasites. *J Exp Med*. 2009; 206:953–66. [PubMed: 19349466]
175. Sautel CF, Cannella D, Bastien O, Kieffer S, Aldebert D, Garin J, Tardieux I, Belrhali H, Hakimi MA. SET8-mediated methylations of histone H4 lysine 20 mark silent heterochromatic domains in apicomplexan genomes. *Molecular and cellular biology*. 2007; 27:5711–24. [PubMed: 17562855]
176. Dixon SE, Stilger KL, Elias EV, Naguleswaran A, Sullivan WJ Jr. A decade of epigenetic research in *Toxoplasma gondii*. *Molecular and biochemical parasitology*. 2010; 173:1–9. [PubMed: 20470832]
177. Bougdour A, Braun L, Cannella D, Hakimi MA. Chromatin modifications: implications in the regulation of gene expression in *Toxoplasma gondii*. *Cellular microbiology*. 2010; 12:413–23. [PubMed: 20109158]
178. Gissot M, Kelly KA, Ajioka JW, Grealley JM, Kim K. Epigenomic modifications predict active promoters and gene structure in *Toxoplasma gondii*. *PLoS pathogens*. 2007; 3:e77. [PubMed: 17559302]
179. Sullivan WJ Jr, Smith AT, Joyce BR. Understanding mechanisms and the role of differentiation in pathogenesis of *Toxoplasma gondii*: a review. *Memorias do Instituto Oswaldo Cruz*. 2009; 104:155–61. [PubMed: 19430637]
180. Bhatti MM, Livingston M, Mullapudi N, Sullivan WJ Jr. Pair of unusual GCN5 histone acetyltransferases and ADA2 homologues in the protozoan parasite *Toxoplasma gondii*. *Eukaryotic cell*. 2006; 5:62–76. [PubMed: 16400169]
181. Boyle JP, Rajasekar B, Saeij JP, Ajioka JW, Berriman M, Paulsen I, Roos DS, Sibley LD, White MW, Boothroyd JC. Just one cross appears capable of dramatically altering the population biology of a eukaryotic pathogen like *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:10514–9. [PubMed: 16801557]
182. Jeffers V, Sullivan WJ Jr. Lysine acetylation is widespread on proteins of diverse function and localization in the protozoan parasite *Toxoplasma gondii*. *Eukaryotic cell*. 2012; 11:735–42. [PubMed: 22544907]
183. Xue B, Jeffers V, Sullivan WJ, Uversky VN. Protein intrinsic disorder in the acetylome of intracellular and extracellular *Toxoplasma gondii*. *Molecular bioSystems*. 2013; 9:645–57. [PubMed: 23403842]
184. Sullivan WJ Jr, Monroy MA, Bohne W, Nallani KC, Chrivia J, Yaciuk P, Smith CK 2nd, Queener SF. Molecular cloning and characterization of an SRCAP chromatin remodeling homologue in *Toxoplasma gondii*. *Parasitology research*. 2003; 90:1–8. [PubMed: 12743798]
185. Rooney PJ, Neal LM, Knoll LJ. Involvement of a *Toxoplasma gondii* chromatin remodeling complex ortholog in developmental regulation. *PloS one*. 2011; 6:e19570. [PubMed: 21655329]
186. Dalmasso MC, Onyango DO, Naguleswaran A, Sullivan WJ Jr, Angel SO. *Toxoplasma* H2A variants reveal novel insights into nucleosome composition and functions for this histone family. *Journal of molecular biology*. 2009; 392:33–47. [PubMed: 19607843]
187. Nardelli SC, Che FY, Silmon de Monerri NC, Xiao H, Nieves E, Madrid-Aliste C, Angel SO, Sullivan WJ Jr, Angeletti RH, Kim K, Weiss LM. The histone code of *Toxoplasma gondii* comprises conserved and unique posttranslational modifications. *mBio*. 2013; 4:e00922–13. [PubMed: 24327343]

188. Lekutis C, Ferguson DJ, Grigg ME, Camps M, Boothroyd JC. Surface antigens of *Toxoplasma gondii*: variations on a theme. *International journal for parasitology*. 2001; 31:1285–92. [PubMed: 11566296]
189. Lekutis C, Ferguson DJ, Boothroyd JC. *Toxoplasma gondii*: identification of a developmentally regulated family of genes related to SAG2. *Experimental parasitology*. 2000; 96:89–96. [PubMed: 11052867]
190. Saeij JP, Arrizabalaga G, Boothroyd JC. A cluster of four surface antigen genes specifically expressed in bradyzoites, SAG2CDXY, plays an important role in *Toxoplasma gondii* persistence. *Infection and immunity*. 2008; 76:2402–10. [PubMed: 18347037]
191. Schwarz JA, Fouts AE, Cummings CA, Ferguson DJ, Boothroyd JC. A novel rhoptry protein in *Toxoplasma gondii* bradyzoites and merozoites. *Molecular and biochemical parasitology*. 2005; 144:159–66. [PubMed: 16182390]
192. Lecordier L, Moleon-Borodowsky I, Dubremetz JF, Tourvieille B, Mercier C, Deslee D, Capron A, Cesbron-Delauw MF. Characterization of a dense granule antigen of *Toxoplasma gondii* (GRA6) associated to the network of the parasitophorous vacuole. *Molecular and biochemical parasitology*. 1995; 70:85–94. [PubMed: 7637717]
193. Patil V, Lescault PJ, Lirussi D, Thompson AB, Matrajt M. Disruption of the Expression of a Non-Coding RNA Significantly Impairs Cellular Differentiation in *Toxoplasma gondii*. *International journal of molecular sciences*. 2012; 14:611–24. [PubMed: 23275028]
194. Milligan-Myhre KC, Rooney PJ, Knoll LJ. Examination of a virulence mutant uncovers the ribosome biogenesis regulatory protein of *Toxoplasma gondii*. *The Journal of parasitology*. 2011; 97:1173–7. [PubMed: 21736491]
195. Joyce BR, Queener SF, Wek RC, Sullivan WJ Jr. Phosphorylation of eukaryotic initiation factor-2{alpha} promotes the extracellular survival of obligate intracellular parasite *Toxoplasma gondii*. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:17200–5. [PubMed: 20855600]



**Figure 1.**

A five day old, *in vitro* bradyzoite cyst induced under alkaline conditions, stained with fluorophore-linked lectin from *Dolichos biflorus* (red), which binds to the polysaccharide-rich cyst wall. Blue: 4',6-diamidino-2-phenylindole, DAPI (nucleus). Scale bar, 10 $\mu$ m. Labelled schematic depicts the stained tissue cyst in the infected host cell.

Table 1

Genes associated with bradyzoite development or maintenance in *Toxoplasma*.

Name	ToxoDB ID	Function	Reference
CST1	TGME49_264660 (SRS44)	Cyst wall glycoprotein, recognized by <i>Dolichos biflorus</i> lectin.	[28, 33, 34]
MAG1	TGME49_270240	Cyst wall and matrix protein, bradyzoite specific marker.	[37]
BPK1	TGME49_253330	Cyst wall protein. In <i>bpk1</i> strain cysts are smaller and more sensitive to pepsin-acid <i>bpk1</i> strain has reduced ability to cause oral infection. treatment;	[39]
MCP4	TGME49_208730	Cyst wall protein.	[40]
PPG1	TGME49_297520	Serine/proline-rich proteophosphoglycan (GU182879), this gene has a role in cyst wall formation.	[41]
SAG4 (or SRS35B)	TGME49_280570	SAG-family related surface protein of bradyzoite.	[52]
BSR4 (or SRS16C)	TGME49_320180	SAG-family related surface protein of bradyzoite.	[55]
SAG2C (or SRS49D)	TGME49_207160	SAG-family related surface protein of bradyzoite.	[188, 189]
SAG2D (or SRS49C)	TGME49_207150	SAG-family related surface protein of bradyzoite.	[188, 189]
SAG2Y (or SRS49A)	TGME49_207130	SAG-family related surface protein of bradyzoite. The transcript is upregulated in bradyzoites. <i>sag2y</i> forms cysts both <i>in vitro</i> and <i>in vivo</i> but demonstrates a defect in persistence <i>in vivo</i> .	[190]
SAG2X (or SRS49B)	TGME49_207140	SAG-family related surface protein of bradyzoite. The transcript is upregulated in bradyzoites. <i>sag2x</i> forms cysts both <i>in vitro</i> and <i>in vivo</i> but demonstrates a defect in persistence <i>in vivo</i> .	[190]
SRS9 (or SRS16B)	TGME49_320190	SAG-family related surface protein of bradyzoite.	[60]
BRP1	TGME49_314250	Bradyzoite rhoptry protein 1, localized in rhoptries of bradyzoites and found to be secreted in PV of <i>in vitro</i> bradyzoites. <i>brp1</i> strain develops and establishes both acute and chronic infection normally.	[191]
HSP60	TGME49_247550	Mitochondrial heat shock protein, member of mitochondria chaperone family. HSP60 transcript is highly increased in bradyzoites. The protein is localized in two vesicular bodies distinct from the mitochondrion in encysted bradyzoites.	[50]
hsp30/BAG1 (BAG1)	TGME49_259020	Small heat shock protein, bradyzoite specific marker, not necessary for cyst formation.	[43, 45]
LDH2	TGME49_291040	Lactate dehydrogenase 2 is a glycolytic enzyme upregulated in bradyzoites. <i>ldh2</i> parasites form fewer brain cysts.	[46, 47, 74]
ENO1	TGME49_268860	Bradyzoite-specific Enolase 1, functions in glycolysis	[73]
PMA1	TGME49_252640	P type ATPase, upregulated in bradyzoite stage. The protein is localized in the membrane of mature bradyzoites purified from brain tissue cysts of chronically infected mice. The <i>pma-1</i> strain has reduced ability to form bradyzoites in response to a number of different <i>in vitro</i> triggers, but is still capable of establishing chronic infection in mice.	[51, 59]
AP2XII-6	TGME49_249190	Putative transcription factor. The insertional mutant on this locus is defective in upregulating bradyzoite markers during <i>in vitro</i> induction.	[82]
AP2XI-4	TGME49_315760	Putative transcription factor. <i>ap2xi-4</i> parasites are unable to express many genes including some bradyzoite markers during pH stress differentiation, and have defects in cyst formation in mice.	[63]
AP2IX-9	TGME49_306620	Transcription factor that represses bradyzoite formation. <i>ap2ix-9</i> parasites have enhanced ability to form cysts <i>in vitro</i> . Overexpression of AP2IX-9 inhibits bradyzoite formation.	[62, 65]

Name	ToxoDB ID	Function	Reference
AP2IX-4	TGME49_288950	Cell cycle regulated transcription factor that represses expression of bradyzoite genes. <i>ap2ix-4</i> parasites have reduced ability to form cysts under alkaline stress <i>in vitro</i> .	[64]
AP2IV-3	TGME49_318610	Transcription factor that is induced during bradyzoite development and promotes expression of bradyzoite specific genes. Overexpression of TgAP2IV-3 enhances bradyzoite formation <i>in vitro</i> .	[65]
AP2IV-4	TGME49_318470	Cell cycle-regulated transcription factor that represses bradyzoite-specific transcripts during tachyzoite development. Parasites lacking this factor do not form cysts in mouse brain tissue.	[66]
GRA1	TGME49_270250	Dense granule protein. In the cyst, GRA1 is localized in the cyst wall and the cyst matrix. Unknown function.	[23, 34, 35]
GRA3	TGME49_227280	Dense granule protein. In the cyst, GRA3 is localized in the cyst wall. Unknown function.	[23, 34, 35]
GRA5	TGME49_286450	Dense granule protein. In the cyst, GRA5 is localized at the cyst wall membrane. Unknown function.	[23, 34, 42, 192]
GRA6	TGME49_275440	Dense granule protein. In the cyst, GRA6 is localized in the cyst wall. Unknown function.	[23, 34, 35]
GRA7	TGME49_203310	Dense granule protein. In the cyst, GRA6 is localized in the cyst wall. Unknown function.	[36]
Splicing factor, arginine/serine rich	TGME49_213635	Putative splicing factor. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
OWP1	TGME49_204420	Annotated as putative oocyst wall protein. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
Replication factor A protein 3	TGME49_238110	Function unknown. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
PUS1	TGME49_202640	Pseudouridine synthase homolog. Two insertional mutants on this locus are defective in tachyzoite to bradyzoite differentiation <i>in vitro</i> . Infection with <i>pus-1</i> parasites results in higher mortality and increased cyst burden.	[70]
ZFP1	TGME49_218362	Nucleolar CCHC motif zinc-finger protein 1. Both the insertional mutant and the <i>zfp1</i> strain have impaired differentiation <i>in vitro</i> and fail to upregulate bradyzoite specific transcripts.	[69]
Tg-ncRNA-1	TGME49_238110	Non coding RNA 1. Insertional mutants on this locus show differentiation defects and reduced cyst loads.	[71, 193]
TgRRS1	TGME49_320450	Ribosome biogenesis regulatory protein 1 that it localized to the nucleolus. Two insertional mutants in <i>tgrs1</i> are defective in bradyzoite formation <i>in vitro</i> .	[194]
DNA primase subunit, large subunit	TGME49_297840	Function unknown. The insertional mutant on this locus is defective in bradyzoite <i>in vitro</i> differentiation	[82]
TBC domain containing protein	TGME49_285730	Function unknown. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
HECT-domain (ubiquitin-transferase) domain-containing protein	TGME49_270580	Function unknown. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
Supt5	TGME49_233300	Rho-GAP domain containing protein, transcription elongation factor. The insertional mutant on this locus is defective in bradyzoite differentiation <i>in vitro</i> .	[82]
TgSRCAP	TGME49_280800	SWI/SNF-like factor, which is upregulated during bradyzoite differentiation.	[184]
TgRSC8	TGME49_286920	SWI/SNF-like factor. An insertional mutant with reduced protein levels of TgRSC8 expresses lower levels of some bradyzoite specific transcripts during <i>in vitro</i> induction.	[41, 185]

Name	ToxoDB ID	Function	Reference
eIF2- $\alpha$	TGME49_286420	Homologue of the alpha subunit of the eukaryotic initiation factor 2. eIF2- $\alpha$ is highly phosphorylated in bradyzoites. Important factor in regulation of bradyzoites differentiation and maintenance as well as in reactivation of bradyzoites to tachyzoites.	[87, 155, 156, 195]
HDAC3	TGME49_227290	Histone deacetylase 3, the protein is highly enriched in promoters of silent genes. Chemical inhibition of HDAC3 induced bradyzoite differentiation <i>in vitro</i> .	[172, 174]
GCN5a	TGME49_254555	Histone lysine acetyltransferase A. Transcriptional analysis of <i>gcn5a</i> parasites demonstrates a role for TgGCN5a in upregulating bradyzoite transcripts <i>in vitro</i> .	[172, 173]

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