REVIEW

Toxoplasma gondii: determinants of tachyzoite to bradyzoite conversion

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Abstract Apicomplexa are primarily obligate intracellular protozoa that have evolved complex developmental stages important for pathogenesis and transmission. *Toxoplasma gondii*, responsible for the disease toxoplasmosis, has the broadest host range of the Apicomplexa as it infects virtually any warm-blooded vertebrate host. Key to *T. gondii's* pathogenesis is its ability to differentiate from a rapidly replicating tachyzoite stage during acute infection to a relatively non-immunogenic, dormant bradyzoite stage contained in tissue cysts. These bradyzoite cysts can reconvert back to tachyzoites years later causing serious pathology and death if a person becomes immune-compromised. Like the sexual stage sporozoites, bradyzoites are also orally infectious and a major contributor to transmission. Because of the critical role of stage conversion to pathogenesis and transmission, a major research focus is aimed at identifying molecular mediators and pathways that regulate differentiation. Tachyzoite to bradyzoite development can occur spontaneously in vitro and be induced in response to exogenous stress including but not limited to host immunity. The purpose of this review is to explore the potential contributors to stage differentiation in infection and how a determination is made by the parasite to differentiate from tachyzoites to bradyzoites.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It is a highly successful parasite infecting approximately 30% of people worldwide in addition to infecting warm-blooded vertebrates and avian species. Like many protozoa including other Apicomplexa, its life cycle requires switching between distinct developmental stages. In the case of *T. gondii*, the stages are the rapidly replicating tachyzoite, the slower growing bradyzoites within tissue cysts, and the sporozoites within oocysts. The sexual cycle or oocyst stage occurs only within the intestine of its feline definitive host. Oocysts get shed in the cat's feces where they persist through adverse conditions and sporulate to become infectious. However, a key to the parasite's success is that it has also evolved a second infectious route independent of its sexual cycle to infect intermediate hosts through oral ingestion of bradyzoite tissue cysts.

Infection is most often initiated through the ingestion of oocysts containing sporozoites or cysts containing bradyzoites in contaminated food or water. Following ingestion, the sporozoites or bradyzoites invade the intestinal epithelium and differentiate to tachyzoites, which disseminate and replicate within the new host. An interferon-gamma (IFN-γ)-dependent cell-mediated immune response eventually kills off the majority of the tachyzoites but in some sites, most often the CNS and muscle tissue including the heart, the tachyzoites convert to bradyzoites. The bradyzoites reside within cysts inside host cells. The cyst wall is created by parasite modifications to the parasitophorous vacuole membrane. These cysts are relatively non-immunogenic persisting long term in a relatively dormant state. However, tissue cysts do occasionally rupture and release bradyzoites that convert to tachyzoites and these can replicate, disseminate, and cause severe pathology and death in the absence of an intact IFN-γ-dependent cell-mediated immune response. This is referred to as reactivation. It is a serious and life-threatening condition in immune-compromised individuals.

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Since tachyzoite to bradyzoite conversion is the key to establishment of a chronic infection and the reverse conversion from bradyzoites to tachyzoites is responsible for life-threatening pathology, understanding how stage conversion is regulated both by the parasite and its host cell environment is a critical ongoing area of research.

Natural cyst development during infection

Tissue cysts containing bradyzoites can be detected within 6 to 7 days following infection with oocysts or tissues cysts and are thought to persist throughout the life of the host (Dubey et al. 1998). However, they are not entirely dormant and appear to breakdown periodically releasing bradyzoites that may convert to tachyzoites, invade adjacent cells, and transform back to bradyzoites creating new tissue cysts even in immune competent hosts. Although cysts appear capable of forming in virtually any cell type in vitro, in infected individuals they are most prevalent in neural and muscular tissues including the central nervous system (CNS), the eyes, and skeletal and cardiac muscles. However, cysts can also be detected in visceral organs including the lungs, liver, and kidneys as well as the bone marrow. The species of intermediate host may also affect the ability of tachyzoites to either convert to bradyzoites or to persist as tissue cysts. For example, tissue cysts are rarely found in beef or buffalo meat although antibodies to *T. gondii* from these species suggest a prior infection rate of 92% and 20%, respectively (Tenter et al. 2000). In contrast, "tissue cysts are more often found in tissues from infected pigs, sheep and goats and less often in infected poultry, rabbits, dogs and horses" (Tenter et al. 2000). Tissue cysts are also more prevalent in some species of wild animals although this is likely due in large part to epidemiological factors such as risk of infection in the wild compared to livestock in regions with intensive hygiene measures (Tenter et al. 2000).

Bradyzoite differentiation is tightly correlated to the parasite's cell cycle

It is unclear what intrinsic or extrinsic environmental signals are critical for initiating stage conversion during actual in vivo infection. However, it is clear that both endogenous and exogenous factors are important for bradyzoite differentiation in vitro. How the parasite senses environmental signals to differentiate, or to respond in other ways to changes in its intracellular environment, is also not known. As yet, there is no evidence the parasite has two-component regulatory systems similar to those in prokaryotes and a few eukaryotes responsible for sensing changes

in its intracellular environment. In *Plasmodium*, genes regulated by the parasite's developmental cycle are produced in a "just in time" manner, meaning the timing of messenger RNA (mRNA) expression for a given gene during the parasite's lytic and developmental cycle correlates well with its function (Bozdech et al. 2003). *T. gondii's* developmental transition from tachyzoites to bradyzoites, and likely its lytic cycle as well, is similarly accompanied by a temporally ordered transcription of genes dispersed throughout the parasites' chromosomes (Radke et al. 2005). Parasite growth is intimately interlinked with stage conversion as slowing of the parasite cell cycle precedes entry into a bradyzoite differentiation program whether differentiation is induced by exogenous stressors or occurs spontaneously (Radke et al. 2003; Bohne et al. 1994). Differentiating parasite populations are enriched with parasites in late S/G2 with a 1.8–2 N DNA content in marked contrast to tachyzoites in which parasites in G2 are markedly absent (Jerome et al. 1998; Radke et al. 2003). Apparently, these parasites continue to replicate and once differentiation into bradyzoites in tissue cysts is complete enter into a quiescent G_0 state (Radke et al. 2003). Slowing of replication rather than an abrupt cessation of parasite growth also seems important for differentiation as cell cycle blocks do not lead to bradyzoite differentiation (Gubbels et al. 2008). Presumably, the parasite must be able to progress through its cell cycle to reach a premitotic checkpoint in order to enter a bradyzoite differentiation program (Radke et al. 2003). A slowing of the parasite cell cycle, whether induced by exogenous stress or "hard wired" in the parasite, seems to be an important initiator or contributor to bradyzoite conversion. However, it is likely that *T. gondii* has a yet undefined ability to respond to changes in its environment particularly during infection that is important for both bradyzoite conversion and other environmental adaptations as well.

Exogenous stress independent of host immunity can induce differentiation

Tachyzoite to bradyzoite differentiation can be triggered by exogenous stress in the absence of host immunity in vitro. Exogenous stress can effectively act directly on the parasite, the host cell, or the two concurrently to induce bradyzoite conversion. Stress-induced conversion seems to involve conserved parasite signaling pathways which include alterations in cyclic nucleotides important for responses to stress or nutrient starvation in other microorganisms including a role for cGMP and cAMP in stimulating differentiation (Eaton et al. 2006). Microarray (Manger et al. 1998; Cleary et al. 2002; Singh et al. 2002) and serial analysis of gene expression (SAGE) (Radke et al.

2005) during different stages of parasite differentiation have shown that many coordinately expressed genes are scattered across the chromosomes suggesting transcriptional regulation by *trans*-factors acting on *cis*-elements in both stress-induced and spontaneous bradyzoite differentiation. In fact, a *cis*-element in promoters for a small panel of bradyzoite-specific genes has been identified that bind to *trans*-factors in bradyzoite-enriched parasite lysates (Behnke et al. 2008). Critically, the minimal *cis*-element for bradyzoite expression for two of the genes has been mapped. The *cis*-element is also sufficient for bradyzoite expression as it is capable of converting a constitutively expressed gene into one that is developmentally expressed only in bradyzoites (Behnke et al. 2008). Differentiation also involves epigenetic and chromatin modifications and this is an area of active productive investigation (Sullivan and Hakimi 2006; Bougdour et al. 2009). The eukaryotic initiation factor-2, important for post-transcriptional stress responses in eukaryotes, has also been demonstrated to play a role in bradyzoite differentiation including reducing overall protein translation (Sullivan et al. 2004).

One of the earliest and most effective methods to induce tachyzoite to bradyzoite conversion in vitro is culturing infected human foreskin fibroblast (HFF) cells in alkaline culture pH of 8–8.2 for 3 to 4 days (Soete et al. 1993, 1994; Weiss et al. 1995). Exposure of parasites to an alkaline pH in the absence of host cells enhances bradyzoite differentiation when they are allowed to invade new HFF cells (Weiss et al. 1998). This shows that even a short exposure of extracellular parasites to appropriate stress environments can be sufficient to promote bradyzoite development independent of any action of the stressor on the host cell. Preconditioning HFF cells to an alkaline pH then returning them to a physiological pH prior to parasite invasion also stimulates stage conversion to bradyzoites (Radke et al. 2006). However, the most efficient conversion to bradyzoites occurs when the alkaline pH can be applied to infected cells suggesting that both effects on the host and parasite are important for optimal differentiation. Treating infected cells also allows longer exposure to the stress than can be performed with extracellular parasites.

Heat shock (43°C) of host cells for 2 h prior to invasion followed by parasite invasion for 2 h at 37°C and additional heat shock of infected cells for 12–48 h post-infection also enhances bradyzoite differentiation in vitro (Soete et al. 1994). *T. gondii* expresses multiple heat shock proteins (HSPs) and HSPs are important for adapting to a variety of stress responses as well as differentiation in diverse phyla (Heikkila 1993a, b). HSP30/Bag-1 is one of the most abundantly expressed bradyzoite-specific genes based on expressed sequence tag frequency (Manger et al. 1998; Bohne et al. 1995). HSP30 may contribute to cyst production in vivo but it is not required for differentiation

in vitro or in vivo (Bohne et al. 1995; Zhang et al. 1999). A homolog of HSP70 is also induced during differentiation whether from tachyzoites to bradyzoites or the reverse from bradyzoites to tachyzoites (Lyons and Johnson 1995; Silva et al. 1998; Weiss et al. 1998). HSP90 expression is upregulated and its cellular localization enriched for the parasite nucleus relative to cytoplasm in bradyzoites (Echeverria et al. 2005). Whether temperature during infection is capable of inducing tachyzoite to bradyzoite conversion is not clear. Most infections in immune-competent individuals at least are relatively asymptomatic suggesting that in the majority of human cases temperatures may not be sufficiently elevated to promote stage conversion to bradyzoites independent of other factors.

The rapidly replicating tachyzoite stage of the parasite is more metabolically active than bradyzoites and this is likely to result in higher overall nutrient demands. Therefore, it is not surprising that nutrient starvation can induce tachyzoite to bradyzoite conversion in vitro. Deprivation of the amino acid arginine which the parasite must salvage from host cells induces slowed growth and differentiation to bradyzoites (Fox et al. 2004). Simultaneous inhibition of pyrimidine de novo biosynthesis and salvage pathways also induced slowed growth and differentiation to bradyzoites (Bohne and Roos 1997). This was demonstrated by using parasites that had the uracil phosphoribosyltransferase (UPRT) gene deleted to prevent pyrimidine salvage and growing the gene-deleted parasites in reduced levels of CO_2 to inhibit de novo synthesis of pyrimidines as *T. gondii* relies on CO_2 to synthesize the pyrimidine ring. Surprisingly, CO_2 starvation even in wild type parasites with an intact UPRT gene was sufficient to induce slowed growth and bradyzoite differentiation (Dzierszinski et al. 2004). The stress of extended periods of time outside a host cell also induces significant stress on *T. gondii* as it is an obligate intracellular pathogen. Culturing tachyzoites for 12 h in the absence of host cells resulted in induction of bradyzoite genes and cyst-like structures when parasites were subsequently allowed to invade HFF cells (Yahiaoui et al. 1999). It is likely that the host immune response also induces changes in nutrient availability during in vivo infection (Appelberg 2006). Also, different types of host cells may vary in their availability of nutrients to the parasite within its parasitophorous vacuole during an ongoing immune response. With the advances in global approaches to examine *T. gondii* gene expression, it may be possible to use changes in the parasites' gene expression profile during infection to elucidate the intracellular nutrient environment it encounters due to the host immune response and in specific host cell microenvironments. It remains possible that limitations in nutrient availability during infection could contribute to a microenvironment conducive to bradyzoite conversion.

Host immunity can induce bradyzoite differentiation

Host immunity, specifically a strong IFN-γ-dependent cell-mediated immune response, is responsible for controlling parasite replication and actually killing the majority of tachyzoites during acute infection and in controlling chronic infection (Suzuki et al. 1988, 1989). In the absence of IFN-γ in mice, infection is uniformly fatal due to apparently uncontrolled parasite replication. Similarly, in humans, an impaired CD 4 T cell response and resultant deficiency in IFN-γ as what occurs in AIDS patients also results in "uncontrolled" parasite replication with a potentially fatal outcome without rapid intervention. Consequently, the critical role IFN-γ plays in control of *T. gondii* infection is unequivocal. However, its requisite role in directly triggering tachyzoite to bradyzoite differentiation during infection is more ambiguous. The primary role of IFN-γ and cell-mediated immunity during infection is certainly to eliminate the majority of tachyzoites during acute infection and to prevent pathological reactivation of cysts during chronic infection. In relation to bradyzoite differentiation, one possibility is that host immunity eliminates tachyzoites allowing bradyzoites to predominate in diverse locations including the CNS and muscle tissue. The other possibility is that the host immune response is also critical for directly signaling the parasites to differentiate to bradyzoites. In vitro IFN-γ, in the presence and absence of tumor necrosis factor alpha (TNF-α), inhibits parasite replication and induces bradyzoite conversion in some cell types including murine macrophages (Bohne et al. 1993, 1994). However, IFN-γ treatment in vitro of other cell types including *T. gondii*-infected murine astrocytes, rat neural cells, or HFF did not increase parasite expression of bradyzoite markers (Jones et al. 1986; Luder et al. 1999). A key determinant in whether IFN-γ induces slowed parasite replication and bradyzoite conversion in a particular cell type may be the capacity of IFN-γ to induce nitric oxide in that cell type. Nitric oxide-induced downstream of IFN-γ signaling through stimulation of inducible nitric oxide synthase has been implicated in parasite growth suppression and bradyzoite conversion in macrophages (Bohne et al. 1994; Luder et al. 2003; Ibrahim et al. 2009). The induction of bradyzoite antigen expression was closely linked to levels of nitric oxide that reduced but did not eliminate parasite replication (Bohne et al. 1994). This is consistent with the hypothesis that a moderate immune response may contribute to bradyzoite conversion by suppressing parasite replication but still allowing parasites to reach a requisite premitotic cell cycle checkpoint for entry into a bradyzoite differentiation program. In contrast, a strong immune response may completely cease cell cycle progression prior to the checkpoint or be microbicidal.

Nitric oxide is also sufficient to signal bradyzoite conversion in the absence of additional mediators of macrophage activation. Sodium nitroprusside (SNP), a nitric oxide donor, induces bradyzoite conversion in murine macrophages, rat brain cells, and HFF in vitro (Bohne et al. 1994; Weiss et al. 1998; Luder et al. 1999; Kirkman et al. 2001). NO exerts its effect on enzymes of the respiratory chain containing iron–sulfur centers [reviewed in (De Groote and Fang 1995; Fang 2004)] suggesting its action on bradyzoite conversion may be due to actions on the parasite and/or host cell mitochondrial electron transport. Consistent with this idea, inhibitors of mitochondrial transport, oligomycin, and antimycin A suppress parasite replication and induce bradyzoite antigen expression in vitro although not to the same extent as SNP. The inhibitors also enhanced bradyzoite differentiation in host cells with a nonfunctional mitochondrial respiratory chain showing they could act directly on the parasite to induce differentiation (Bohne et al. 1994). Although the host immune response is clearly capable of promoting tachyzoite to bradyzoite conversion in vitro, confirmation of an essential role for IFN-γ directly in bradyzoite conversion in vivo is elusive.

An IFN-γ-dependent cell-mediated immune response is also required to prevent pathological reactivation of infection when bradyzoites differentiate back to the rapidly replicating tachyzoite stage (reactivation) as occurs in immune-compromised individuals. Suppressing IFN-γ or CD 4 and CD 8 T cells enables bradyzoite reactivation during chronic infection in mice (Suzuki et al. 1989; Gazzinelli et al. 1992). Nitric oxide and TNF-α also contribute to control of chronic infection (Scharton-Kersten et al. 1997; Yap et al. 1998; Schlüter et al. 1999). Nitric oxide is also important for immunity to ocular toxoplasmosis (Hayashi et al. 1996; Roberts et al. 2000). Proof of a direct role for immunity in suppression of reactivation is complicated by the fact that while bradyzoites within tissue cysts are more quiescent than tachyzoites, they are not inert and occasionally rupture allowing reinvasion of adjacent host cells. Consequently, host immunity may directly suppress a reactivation pathway in bradyzoites or it may function strictly to rapidly kill parasites following cyst rupture and reactivation and prevent parasite spread to adjacent cells. In favor of a direct role for suppressing reactivation, IFN-γ in vitro suppressed reactivation of bradyzoites in murine astrocytes during long-term cultures following challenge with brain-derived *T. gondii* cysts. Removal of IFN-γ subsequently allowed reactivation of bradyzoites from cysts (Jones et al. 1986). This suggests that IFN-γ may act on some host cells to stabilize cysts in a G_0 state and to prevent reactivation during chronic infection in addition to killing off those tachyzoites that do emerge.

The host cell environment affects bradyzoite differentiation

A trisubstituted pyrrole 4-[2-(4-fluorophenyl)-5-(1-methyl-piperidine-4-yl)-1*H*-pyrrol-3-yl]pyridine, designated as compound 1, was discovered to have in vitro and in vivo activity against the apicomplexan parasites *T. gondii* and *Eimeria tenella* (Gurnett et al. 2002; Nare et al. 2002). The primary molecular target of this compound in *E. tenella* and *T. gondii* is a cyclic GMP-dependent protein kinase which appears to be an essential gene in the parasites (Donald et al. 2002; Donald and Liberator 2002; Gurnett et al. 2002). However, compound 1 also has activity against additional secondary parasite kinases as well (Donald et al. 2006). Compound 1 not only dramatically slows replication of *T. gondii* (Donald et al. 2002) but it potently induces bradyzoite-specific antigens bradyzoite-specific antigen 1 (BAG1) and cyst wall protein (Radke et al. 2006). Compound 1 induced bradyzoite conversion both when host cells were pretreated with the compound or when cells were treated after parasite infection, suggesting that compound 1 may also act on the host cell to induce expression by the parasite of bradyzoite-specific antigens. Analysis of the effect of compound 1 on the host cell transcriptome revealed that the compound specifically increased expression of human cell division autoantigen-1 (CDA-1). Inhibition of host cell CDA-1 by interfering RNA prevented both parasite growth inhibition and bradyzoite conversion triggered by compound 1. Ectopic expression of CDA-1 in HeLa cells was sufficient in the absence of exogenous stress or compound 1 to suppress parasite replication and induce bradyzoite antigens and cyst wall proteins (Radke et al. 2006). How CDA-1 expression regulates parasite differentiation to bradyzoites is not known. Compound 1 alters expression of other genes, in addition to CDA-1, implicated in regulating growth of HFF cells, leading to an intriguing hypothesis that the growth state of a host cell may contribute to bradyzoite conversion. This would fit with the observations from chronic infections that parasite tissue cysts are primarily distributed in differentiated long-lived host cells that include brain neurons (Ferguson and Hutchison 1987) and skeletal muscle (Dubey et al. 1998). Such long-lived cells also suit the needs for the parasite to form cysts and persist long term within a host cell. The genotype of *T. gondii* affects its replication rate, migration, propensity to differentiate to bradyzoites, and virulence [reviewed in (Sibley and Ajioka 2008; Sibley et al. 2009)]. Type II and III genotypes are generally less virulent and more cystogenic compared to type I genotypes. Compound 1 induced low passage (<12) type II (ME49) and III (VEG) strain parasites to differentiate to bradyzoites but not type I strain GT-1 (<12 passages). A genetic cross showed that the resistance of type I strains could be genetically inherited to type III strains implicating a specific genetic loci in the resistance of type I strains to the effect of compound 1 (Radke et al. 2006).

T. gondii can infect, survive, and differentiate within a broad range of host cells. The impact of the type of host cell on the initiation of bradyzoite conversion during infection is still an open question. One reason tissue cysts are often found within neurons in the brain and in muscle tissue may be because during infection these cells are not sufficiently activated to kill parasites leading instead to a moderate level of activation conducive for bradyzoite differentiation and cyst production. Since these cells are also long-lived, the tissue cysts within them may be more stable and persistent leading to a greater likelihood to find cysts in these locations over time. It is also possible that muscle cells and neurons have distinct microenvironments that can suppress parasite replication and induce bradyzoite conversion and ultimately tissue cysts with little to no exogenous stress applied compared to some other cell types. Tachyzoite to bradyzoite conversion occurs spontaneously in vitro within rat primary neurons, astrocytes, and microglia (Luder et al. 1999). Inoculation of tachyzoites directly into mouse brains results in bradyzoite differentiation within 2 to 3 days which is earlier than required for a fully activated immune response (Dubey and Frenkel 1976). However, comparisons between the kinetics of tachyzoite to bradyzoite differentiation following injection of parasites into the brain versus other anatomical locations have not been made. Recent studies have shown spontaneous and efficient tachyzoite to bradyzoite conversion in vitro for type III strain VEG parasites in primary skeletal muscle cells differentiated to mature myotubes as well as in a rat myoblast cell line L6.C10 at higher rates than in fibroblasts (Ferreira-da-Silva et al. 2009b). Recently, type I RH strain which is less cystogenic was discovered to also undergo spontaneous tachyzoite to bradyzoite conversion in these cells but at a low rate of approximately 3% (Ferreira-da-Silva et al. 2009a). These studies emphasize the potential importance of cell-type-specific microenvironments in bradyzoite development during an infection. Particularly, since the cell types where spontaneous bradyzoite conversion appears most efficient in vitro are also the cell populations that most commonly contain bradyzoite cysts following acute infection.

Freshly isolated sporozoites and bradyzoites spontaneously stage convert

Freshly isolated sporozoites (VEG strain type III) or bradyzoites inoculated into HFF cells in vitro follow a defined course of development (Jerome et al. 1998; Radke

et al. 2003, 2005). They proceed to a replicative tachyzoite stage and after approximately 20 division cycles from culture initiation uniformly slow their growth prior to spontaneous conversion to bradyzoites. This is characterized by a slowing of S phase leading to mature bradyzoites with cell cycle arrest in G1/G0 (1 N DNA). SAGE was used to define the *T. gondii* transcriptome at key stages following initiation of culture through bradyzoite development (Radke et al. 2005). By day 15 post-culture with sporozoites, SAGE analysis showed that the population was a mixture of tachyzoites and bradyzoites with reduced growth rates and a gene expression profile resembling alkaline pH switched bradyzoites. Interestingly, SAGE analysis also showed that parasites emerging after five divisions in the sporozoite-infected cell retain a gene expression profile that retains some sporozoite genes along with an emerging tachyzoite profile. Consequently, parasites grown routinely in vitro or in vivo as tachyzoites are likely to have a modified gene expression profile relative to those differentiating from sporozoites or even bradyzoites during natural transmission. These data strongly suggest that freshly isolated sporozoites or bradyzoites are genetically programmed to slow their growth and proceed down a bradyzoite differentiation program after a brief period of rapid division as tachyzoites. It also indicates that inherent growth regulation can be easily lost or overcome by continuous passage as tachyzoites. This has profound implications for our understanding of tachyzoite to bradyzoite differentiation and points out the importance of studies using freshly isolated sporozoites or bradyzoites in natural infection models to delineate or confirm mediators important for bradyzoite conversion during infection. The fact that tachyzoites differentiated from fresh sporozoite isolates retain expression of many sporozoite genes is also an important consideration in studies looking at early stages of in vivo infection, as these genes may play important roles which may be missed in infection studies using tachyzoites passed in vitro or in vivo. SAGE analysis during development also confirmed the dynamic nature of *T. gondii* gene transcription as large numbers of mRNAs were exclusively expressed during a single developmental stage or time frame. This is consistent with the just in time concept of gene expression for the related apicomplexan *Plasmodium* where 80% of transcripts are tightly regulated and expressed during a single developmental stage (Bozdech et al. 2003).

Summary

Understanding the molecular environment important in both the parasite and host cell that regulate development from tachyzoites to bradyzoites and back again during

reactivation of chronic infection remains an area of intense investigation due to its critical importance in both transmission and disease pathology. The recent ability to link global parasite transcriptome and protein analyses kinetically throughout parasite development has made it possible to begin genetic dissection of molecular regulatory networks important for parasite differentiation between stages. However, a linchpin for bradyzoite differentiation whether spontaneous or induced may be a requisite slowing of parasite replication that still allows parasites to proceed to a premitotic checkpoint for entry into a programmed bradyzoite differentiation pathway. The ability of the environment or parasite to appropriately slow parasite cell cycle progression may itself be a trigger for bradyzoite differentiation, and perhaps additional stress response pathways as well. It is also likely that the parasite has undefined mechanisms of sensing and responding to changes in its environment during infection that act upstream or independent of the parasite cell cycle. The fact that freshly isolated low passage sporozoites and bradyzoites appear programmed for a period of rapid replication, followed by slowed growth and differentiation to bradyzoites, suggest that major external inducers of bradyzoite differentiation may not be required during natural transmission and infection. However, it is also likely that differentiation into bradyzoites during infection is multifactorial with contributions from host immunity as well as effects of unique cell-type-specific microenvironments. A complication of dissecting the role of external and intrinsic factors in stage conversion during infection is that bradyzoite differentiation need not be particularly efficient during infection to generate sufficient bradyzoite-containing cysts to maintain a chronic infection capable of reactivation or for transmission through carnivorism. Consequently, multiple low efficiency contributors to bradyzoite differentiation in vitro may be sufficient during infection to establish chronic infection.

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