Toxoplasma gondii: Presentations at the 10th International Workshops on Opportunistic Protists: 100 Years and Counting

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Toxoplasma gondii, an apicomplexan parasite of mammals, was first identified over 100 years ago (in 1908) by Nicolle and Manzecaux, who isolated tachyzoites from the gundi, a North African rodent (34). Splendore also identified this parasite in the tissue of a rabbit in 1908 (46). The genus Toxoplasma was named for its bow-like shape (from the Greek “toxo,” for bow or arc, and “plasma,” for creature). The presence of a tissue cyst (bradyzoite) life stage was rapidly recognized, but it was not until almost 60 years later that this organism was recognized to be a coccidian and that felines were identified as being the definitive hosts by several groups working independently, including Dubey and Frenkel in 1970 (16).

The association of T. gondii with food-borne and waterborne transmission has resulted in its classification as a National Institute of Allergy and Infectious Diseases (NIAID) category B priority agent. Due to the extensive repertoire of applicable experimental techniques available for this pathogen, it has become a model organism for the study of intracellular pathogens. Research on T. gondii continues to move rapidly, and this review will address information related to recent advances in our understanding of the biology of T. gondii presented at the IWOP-10 held in Boston, MA, 28 to 31 May 2008, and the symposium entitled “Centenary Celebration of Toxoplasma Discovery” held at that meeting.

History, epidemiology, and life cycle. The details of the history of the discovery of T. gondii were reviewed at IWOP-10 by Dubey (15) and were also described in recent reviews (1, 14). T. gondii is estimated to infect about one-third of the world’s human population and is a significant zoonotic and veterinary pathogen. In humans and veterinary hosts, T. gondii is frequently associated with congenital infection and abortion. This parasite can be transmitted by the vertical transmission of the rapidly growing tachyzoite form if an immunologically naive mother acquires a new infection during pregnancy. In addition, T. gondii is an opportunistic pathogen associated with encephalitis or systemic infections in immunocompromised hosts such as individuals with advanced human immunodeficiency virus infection (i.e., AIDS). Tachyzoites divide rapidly within host cells and are thought to be responsible for the clinical manifestations of infection.

In humans, T. gondii is most commonly acquired by the oral ingestion of tissue cysts containing bradyzoites in meat. In Portugal, the location of the 2006 IWOP meeting, it was reported that 9.1% of pigs were found to be seropositive for T. gondii, which suggests that they are a significant source of infected meat products (37). Improved animal husbandry practices and increased awareness of the risk of eating undercooked meat have resulted in a decreased prevalence of toxoplasmosis (50). T. gondii infection can also be acquired by the ingestion of oocysts containing sporozoites that are the product of the sexual cycle in cat intestines. In Lisbon, Portugal, a recent survey demonstrated that 26.2% of cats were seropositive, which is a decrease from previous surveys in 1984 and 1992 (13). Once tissue cysts or the environmental oocysts are ingested, their contents, the bradyzoites and sporozoites, respectively, invade host cells and differentiate into tachyzoites. Oocysts are very resistant to harsh environmental conditions and are highly infectious. As illustrated by studies in Canada (7) and South America (3, 5), oocysts transmitted via water or other environmental sources are a significant source of T. gondii infection. A method has been developed to extract high-quality RNA from T. gondii oocysts that can be used to study the biology of this least-well-understood life stage using microarray or similar studies (42).

T. gondii is unusual in that its propagation does not require passage through the definitive host (felids in whose intestinal tissues the sexual cycle occurs). It is an obligate intracellular parasite and cannot be propagated axenically. Tachyzoites differentiate into latent bradyzoite forms, which are surrounded by a carbohydrate-rich cyst wall within the parasitophorous vacuole. These forms were first recognized in 1928 by Levaditi, and the term tissue cyst was defined by Dubey and Beattie in 1988 (14, 15, 50). This differentiation can be increased by the exposure of the organism to stress conditions in culture and the developing immune response to the tachyzoites in vivo. These tissue cysts can persist indefinitely for the life of the host, perhaps due to a cycle of reactivation and reinfection.

Analysis using enzyme zymodemes and single-nucleotide polymorphisms suggests that most T. gondii isolates from North America and Europe can be grouped into one of three genotypes, e.g., types I, II, and III (22, 44). Most likely, these lineages are related to the domestication of animals by humans about 10,000 years ago (48). Type I strains grow rapidly in vitro, are hypervirulent in mice, and are frequently associated with ocular toxoplasmosis and acute outbreaks (19). Type II and type III isolates are less virulent in mice and readily form cysts in vitro, and type II strains are commonly isolated from clinical cases of toxoplasmosis, particularly in immunodeficient hosts. Data indicate that other genotypes are predominant in other parts of the world (29), which may represent zoonotic
isolates related to nondomesticated animal species rather than
clonal expansion and involvement of the sexual life cycle of this
parasite (43). It is estimated that 11 major lineages exist (43).

Cell biology and immunology. The characteristic apical se-
cretory organelles for which the Apicomplexa were named,
micronemes, rhoptries, and dense granules, are specialized for
the invasion and remodeling of the host. Studies over the past
two decades have revealed many of the proteins that are in-
volved in adhesion and the subsequent formation of the mov-
ing junction (2, 11). Proteomic analysis of rhoptries has re-
sulted in the identification of rhoptry neck proteins in addition
to traditional rhoptry proteins (ROPs) (8, 9). Rhopty neck
proteins are involved in the formation of the moving junction
during invasion (2, 28). During invasion, the parasite forms a
parasitophorous vacuole that enables the efficient procure-
ment of nutrients and evasion of host immune defenses (8, 32).
The parasitophorous vacuole membrane (PVM) is modified
extensively by the parasite and contains multiple proteins that
interact with host cell organelles including the host cell mito-
chondria and endoplasmic reticulum (30, 45). The parasite
modulates host signaling pathways including the apoptosis
pathway (10) and coopts specialized aspects of vesicular trans-
port machinery related to lipid acquisition (12).

*T. gondii* injects various signaling molecules into the host
cell, resulting in an extensive remodeling of the host cell gene
expression profile and metabolic pathways (6). The *T. gondii*
proteins involved in these host cell interactions have been
found to be predominantly rhoptry derived and are implicated
in organelle recruitment as well as the direct regulation of host
gene transcription within the nucleus (8). ROP16 and ROP18
(43) are related kinases that are secreted into the host cell
cyttoplasm during invasion and localized to the PVM, respec-
tively (38, 39, 49). ROP16 alters STAT signaling in the host cell
nucleus (39), while the target(s) of ROP18 remains elusive.
Genetic studies have linked these genes to parasite virulence
(43), and there are phenotypic differences among the three
major genotypes (38, 39, 49). Dense-granule proteins facilitate
the formation of specialized tubules that enable nutrient ac-
quision by the parasite (12), and some of these proteins, most
notably GRA7, are secreted into host cells (12). It was recently
demonstrated that in *T. gondii*, the phytohormone abscisic acid
induced the formation of the second-messenger cyclic ADP
ribose, stimulated calcium-dependent protein secretion, and
induced parasite egress from the infected host cell in a density-
dependent manner (33).

*T. gondii*, like most members of the Apicomplexa, has a
nonphotosynthetic chloroplast-like organelle termed the
apicoplast, which is essential (47). The isoprenoid pathway is
important for the synthesis of the signaling molecule ab-
scisic acid, which regulates calcium-dependent signaling in
host invasion and egress (33). It is believed to have arisen
via secondary endosymbiosis, as evidenced by the presence
of four membranes around this organelle. *T. gondii* ana-
logues of Tic22 and Tic20 have been identified and charac-
terized (47). Using a novel split green fluorescent protein
vector system, the localization of different proteins to the
apicoplast four-membrane system has been investigated
(47).

The immune response to *T. gondii* involves both traditional
effector cells of the immune system as well as somatic cells such
as astrocytes. These interactions can be investigated by exam-
ining cell migration in organ explant cultures using newly de-
veloped microscopy techniques (24). Those studies demon-
strated the dynamic nature of cell interactions in the brain
during *T. gondii* infection. It was previously demonstrated that
the ability of gamma interferon-treated astrocytes to inhibit
*T. gondii* growth is dependent on IGTP (20). IGTP is a member
of the IRG proteins (p47GTPases), which have recently been
recognized as being one of the strongest resistance systems
against intracellular pathogens in mice, acting in a cell-auton-
omous manner (21). Previous studies have found that gamma
interferon inhibition involves the disruption of the parasito-
phorous vacuole in both macrophages and astrocytes, although
the role of the IRG proteins is unclear (30, 31). In astrocytes,
parasitophorous vacuole disruption appears to be due to the
disruption of the PVM via an IGTP-dependent mechanism
(36).

Genomics, epigenetics, and proteomics. Data on the *T. gon-
dii* genome are available at http://www.toxodb.org. Currently,
genome sequences are available for ME49, a commonly used
type II strain, as well as GT-1 (a type I strain that is able to
complete the entire life cycle) and VEG (a type III strain that
is also able to complete the life cycle) (27). The availability of
the genome sequence and expressed sequence tags has directly
facilitated the application of high-throughput techniques to the
study of *T. gondii* biology. The patterns of gene expression
during development have been studied using serial analysis of
gen expression (35) and DNA microarrays. More recently,
whole-genome arrays have been used to understand the role
of epigenomic modifications in gene expression (17, 18, 26; M.
Gissot and K. Kim, unpublished). These arrays have the
advantage that they are unbiased and therefore have the po-
tential for new gene discovery as well as an understanding of
the relationship of epigenetic modifications to gene expression.
As *T. gondii* does not appear to have detectable DNA cytosine
methylation (18), the modification of chromatin structure, par-
cularly posttranslational modification of histones, is likely to
be essential for the coordinated regulation of gene expression.
Epigenetic gene regulation provides a mechanism by which an
organism can maintain a type of short-term memory of its most
recent environment and can respond quickly to changing con-
ditions. Peaks of histone modifications (H3K4me3, H3K9ac,
and H4ac) are coincident in the genome and represent epige-
netic modifications that correlate strongly with gene activation
(18, 40). These peaks can be used to predict regions of the
genome that drive gene expression (17, 18). The correlation of
these peaks with cDNA expression enables a comprehensive
survey of genes that are active and the epigenetic marks that
identify active genes. Chromatin immunoprecipitation analysis
of individual loci has been reported for individual epitope-
tagged chromatin remodelers (40, 41), and genome-wide chro-
matin immunoprecipitation-chip experiments are feasible (17,
18, 40, 41; Gissot and Kim, unpublished).

Proteomics efforts for *T. gondii* have included general sur-
veys of the proteome of *T. gondii* tachyzoites (available at
www.toxodb.org) as well as other significant subproteomes
such as membrane-associated proteins and the cytoskeleton
(23) (available at the Albert Einstein College of Medicine
Biodefense Proteomics Center [http://toro.aecom.yu.edu/
biodefense/]). There have also been focused studies character-
izing particular cellular compartments or types of proteins. It has become clear with these studies that many of the gene prediction algorithms are imperfect. Thus, in addition to providing important insights into the protein repertoire, the cataloging of proteins expressed by T. gondii provides a unique opportunity to refine gene annotations and gene prediction algorithms with experimental data (L. M. Weiss, K. Kim, and A. Fiser, unpublished data; 26).

A new family of plant-like transcription factors (the AP2 family) has been identified by a bioinformatics approach (4). The AP2 family members can be grouped according to their conserved DNA-binding domains, and several members appear to be conserved in several species of the Apicomplexa (25). T. gondii has over 50 members of the AP2 family, most of which are expressed in T. gondii tachyzoites (M. Gissot and K. Kim, unpublished data; 26). Intriguingly, AP2 family members are known to regulate developmental transitions and the stress response in plants.

Summary. Significant advances have been made in our understanding of the life cycle of T. gondii in the 100 years since its discovery, but many questions remain regarding the control of its developmental life cycle and the intimate relationship of its discovery, but many questions remain regarding the control of its developmental life cycle and the intimate relationship of its life cycle and the intimate relationship of its development with its host. The availability of high-throughput technologies for sequencing, proteomics, transcriptomics, epigenomics, and metabolomics is allowing the development of global research approaches that are hypothesis generating rather than hypothesis driven. The next several years promise fascinating insights into how T. gondii has evolved into such a successful parasite as these new data sets are integrated into systematic approaches to intracellular parasitism and developmental biology.

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