Published in final edited form as:
Int J Parasitol. 2004 March 9; 34(3): 423–432. doi:10.1016/j.ijpara.2003.12.009.

Toxoplasma gondii: the model apicomplexan

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Abstract

Toxoplasma gondii is an obligate intracellular protozoan parasite which is a significant human and veterinary pathogen. Other members of the phylum Apicomplexa are also important pathogens including *Plasmodium* species (i.e. malaria), *Eimeria* species, *Neospora*, *Babesia*, *Theileria* and *Cryptosporidium*. Unlike most of these organisms, *T. gondii* is readily amenable to genetic manipulation in the laboratory. Cell biology studies are more readily performed in *T. gondii* due to the high efficiency of transient and stable transfection, the availability of many cell markers, and the relative ease with which the parasite can be studied using advanced microscopic techniques. Thus, for many experimental questions, *T. gondii* remains the best model system to study the biology of the Apicomplexa. Our understanding of the mechanisms of drug resistance, the biology of the apicoplast, and the process of host cell invasion has been advanced by studies in *T. gondii*. Heterologous expression of apicomplexan proteins in *T. gondii* has frequently facilitated further characterisation of proteins that could not be easily studied. Recent studies of Apicomplexa have been complemented by genome sequencing projects that have facilitated discovery of surprising differences in cell biology and metabolism between Apicomplexa. While results in *T. gondii* will not always be applicable to other Apicomplexa, *T. gondii* remains an important model system for understanding the biology of apicomplexan parasites.

Keywords

Apicomplexa; Invasion; Heterologous expression; Model system; *Toxoplasma gondii*

1. Introduction

The Apicomplexa consist of numerous genera of pathogenic protozoa that cause diseases in humans and a variety of economically important animal species. *Toxoplasma gondii* is an opportunistic pathogen that causes disease in the immunocompromised, in congenitally infected children, and also has been associated with outbreaks of acute ocular disease (Bowie et al., 1997). *Plasmodium* species, which cause malaria, are responsible for millions of deaths each year in the developing world. *Eimeria* and *Cryptosporidium* are important enteric pathogens while *Neospora* and *Theileria* are systemic veterinary pathogens. Both *T. gondii* and *Cryptosporidium parvum* have caused water-borne disease outbreaks.

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Of the Apicomplexa, *T. gondii* is the most experimentally tractable. Methodology for classic and reverse genetics is well established (Table 1). *T. gondii* pathogenic stages are easily propagated and easily quantitated in the laboratory; the mouse animal model is well-established; and reagents for study of the host response as well as basic biology of the parasite are widely available (see the other articles within this issue of the *International Journal for Parasitology* for examples of such studies). Because of these experimental advantages, *T. gondii* has emerged as a major model for the study of apicomplexan biology (Roos et al., 1999). Recent studies and the annotation of the genomes of various species within the genera of Apicomplexa have also revealed surprising differences in the biology of the Apicomplexa, particularly in some key metabolic pathways. Although these differences do not preclude the use of *T. gondii* as an experimental model, it is now clear that the unique niches occupied by the Apicomplexa have resulted in significant biological differences that were not previously appreciated.

2. Brief overview of *T. gondii* genetics

T. gondii undergoes its sexual cycle in feline species. Progeny from a genetic cross within cat intestines can be used to map genetic traits by classical genetic methods (Pfefferkorn and Kasper, 1983; Sibley et al., 1992; Su et al., 2002). This method, although both expensive and laborious, is the most definitive way to map complex traits such as those that are responsible for virulence of *T. gondii* (Su et al., 2002). Information from the *T. gondii* genome sequencing project is being used to develop a higher resolution genetic map to facilitate mapping of genetic traits.

Molecular analysis of *T.gondii* strains dispersed throughout the world suggests that most strains fall into one of the three genotypes that are due to clonal proliferation of three individual progeny resulting from a single genetic cross (Sibley and Boothroyd, 1992b; Grigg et al., 2001). Type I strains (e.g. RH strain) are highly virulent in mice with LD100 (100% lethal dose) of < 10 parasites. Type II and III strains are less virulent in mice and have an LD100 of > 1000 parasites. Type II strains (e.g. ME49/PLK) are the most prevalent clinical isolates. In general Type I strains form cysts less easily in culture and in animals, but this property is strain-specific and is influenced by how the strain has been propagated. The rapid dissemination of these three *T. gondii* genotypes has been proposed to be due to the development of a trait that allowed direct transmission from intermediate hosts, making the sexual cycle no longer obligatory for transmission of parasites (Su et al., 2003). *T. gondii* is unusual among the Apicomplexa in not requiring the sexual cycle for transmission.

Transfection of the Apicomplexa was first reported in *T. gondii* in 1993 (Donald and Roos, 1993; Kim et al., 1993; Soldati and Boothroyd, 1993). This rapidly led to the development of a variety of tools for genetic manipulation of *T. gondii*. Stable transformation can result from either homologous or random integration making gene disruption and stable expression of transgenes readily achievable. Although the obligatory growth of *T. gondii* in nucleated host cells has restricted the choice of selectable markers, there are sufficient numbers of markers available for selection of stable transfectants. Effective selection markers include chloramphenicol acetyltransferase (cat), dihydrofolate reductase (DHFR), hypoxanthine xanthine guanine phosphoribosyl transferase (HXGPRT), bleomycin (ble), and tryptophan (trp) (Donald and Roos, 1993; Kim et al., 1993; Sibley et al., 1994; Messina et al., 1995; Soldati et al., 1995; Donald et al., 1996). HGXPRT selection must be performed in mutants lacking HXGPRT (available from the NIH AIDS reagent repository, http://www.aidsreagent.org). HXGPRT can also be used for negative selection (Donald et al., 1996) as can uracil phosphoribosyltransferase (UPRT; Donald and Roos, 1995) and thymidine kinase (TK; Radke and White, 1998).

There are also reports of RNA-based techniques for reduction of gene expression in *T. gondii* and *Plasmodium falciparum* (Nakaar et al., 1999; Al-Anouti and Ananvoranich, 2002; Malhotra et al., 2002; Al-Anouti et al., 2003; Sheng et al., 2004), but these techniques are not as well established as in mammalian cells or in *Trypanosoma brucei*.

The techniques for transfection established in *T. gondii* are very similar to those later adapted to malaria, *Neospora* and *Eimeria* (Beckers et al., 1997; Howe and Sibley, 1997; Howe et al., 1997; Kelleher and Tomley, 1998). In general gene expression requires a genus-specific promoter. Many *T. gondii* promoters do work in *Neospora* (Howe and Sibley, 1997), and malaria promoters frequently also work in other *Plasmodium* species. *Neospora* transfection is very similar to *T. gondii*, but the field has a smaller population of investigators and therefore, fewer reagents are available.

Currently *T. gondii* remains the apicomplexan species most readily amenable to genetic manipulation. Transient transfection efficiency is high (routinely over 50%), and expression of epitope tags, reporter constructs and heterologous proteins is relatively uncomplicated. Among the reporters that are routinely used are cat, β-galactosidase, and green fluorescent protein (GFP; Soldati and Boothroyd, 1993; Seeber and Boothroyd, 1996; Striepen et al., 1998; Kim et al., 2001). Luciferase (luc) can also be used, but particular attention needs to be paid to the initial codons used for the *luc* gene (Matrajt et al., 2002). Some reporter genes may be better expressed as fusion proteins (Radke and White, 1998; Striepen et al., 1998; Matrajt et al., 2002). A variety of expression vectors have been developed, and many techniques used in mammalian molecular biology are readily transferable to *T. gondii*. Reporter parasites have been developed to facilitate non-radioactive high throughput screening protocols for potential chemotherapeutic agents (McFadden et al., 1997; Gubbels et al., 2003). Plasmids for complementation studies have also been described (Black and Boothroyd, 1998; Striepen et al., 2002), but reliable methods for complementation cloning are not yet established. A system for tetracycline-regulated expression was recently developed for *T. gondii* but prior to this, due to their haploid genomes, disruption of essential genes could not be performed in the Apicomplexa (Meissner et al., 2001, 2002).

Techniques for both transient and stable transformations of *Plasmodium* species are commonly used (van Dijk et al., 1995; Wu et al., 1995, 1996; Crabb and Cowman, 1996; van der Wel et al., 1997; Waters et al., 1997; Mamoun et al., 1999; de Koning-Ward et al., 2001; Mota et al., 2001). Although transfection of malaria species is now routine, creation of stable transfectants is a laborious process. Transfection efficiency is low, and malaria parasites are difficult to study in transient transfection assays. Stable transformants can take months to generate. Stable transformation of rodent malaria species is faster, but in vitro cultivation of *Plasmoidum yoelii* and *Plasmodium berghei* is still not possible.

Neither *Eimeria* nor *Cryptosporidium* can be sustained in tissue culture systems, seriously limiting the types of studies that can be performed readily in the laboratory. *Eimeria* species can be transiently transfected, but phenotypic studies of transformants are difficult to perform due to the lack of methods for in vitro cultivation (Kelleher and Tomley, 1998). Studies with *Theileria* are limited due to governmental restrictions in the availability of some life cycle stages for laboratory study.

3. Expression of genes from other Apicomplexa in *T. gondii*

Because of the difficulties in genetic manipulation of most Apicomplexa, *T. gondii* has been used as an expression system for these parasites. *T. gondii* has also been used for testing the biological or biochemical function of proteins that for one reason or other cannot be readily expressed in other organisms. One of the first effective strategies for stable transformation of *T. gondii* was genetic engineering of mapped mutations of malaria DHFR from lines

resistant to antifolates (Donald and Roos, 1993; Reynolds and Roos, 1998). This resulted in an effective selectable marker for *T. gondii*, validated the importance of the clinical mutations and provided the first effective stable selectable marker for *plasmodium* species (Roos et al., 1999).

Various *plasmodium* genes have now been expressed in *T. gondii*, most frequently for testing of *T. gondii* as a possible malaria vaccine delivery system. The malaria sporozoite circumsporozoite protein (CSP) was successfully expressed by two groups (Di Cristina et al., 1999; Charest et al., 2000). Anecdoctal data suggest that most attempts of expression of *plasmodium* genes in *T. gondii* require resynthesis of the gene so that the *P. falciparum* A/T codon bias is eliminated (Withers-Martinez et al., 1999). *T. gondii* codon bias is more like mammalian species and, in general, constructs that express in mammalian cells will express in *T. gondii*.

C. parvum cannot be grown in vitro and cannot be transfected. Its genome is also A/T rich, but less so than *P. falciparum*. Several groups have successfully used *T. gondii* as an expression system to explore the aspects of biology of *C. parvum*. O'Connor et al. (2003) expressed the *C. parvum* sporozoite surface gp40/15 in *T. gondii* tachyzoites by transient transfection. Gp40/15 is a major sporozoite surface antigen and vaccine candidate. Constructs were targeted to the parasite surface and recognised by glycotope-specific reagents for gp40/15 suggesting that unique post-translational glycosylation was conserved. Although very inefficient, the *C. parvum* gp40/15 proteolytic processing appeared to occur in *T. gondii*. The carbohydrate modifications of *C. parvum* surface proteins are critical for host cell interaction but have been very difficult to study due to the technical obstacles the *C. parvum* system presents. Candidate *N*-acetyl galactosamine transferases possibly involved in *O*-glycosylation of *T. gondii* proteins have been identified and expressed in *T. gondii* (Wojczyk et al., 2003; Stwora-Wojczyk et al., 2004).

4. Apicomplexan genomes

The availability of genome sequences has revolutionised the study of microbial pathogens (Table 2). Genome sequences for the Apicomplexa are in various stages of completion. The *T. gondii* genome is 80 Mb in size and has been sequenced at 10 × coverage (as of October 2003; http://www.toxodb.org). The codon bias of *T. gondii* genes is similar to mammalian species. In general the genes of *T. gondii* are much more intron-rich than those of *plasmodium* or *Cryptosporidium*. This makes identifying genes in databases problematic. As a general rule, genes for surface antigens and proteins secreted from micronemes, rhoptries and dense granules have had fewer introns than housekeeping genes. Many are also close to the ends of chromosomes. The genome project is sequencing a clone of ME49, a Type II strain of *T. gondii*. Efforts to link sequence data with previously obtained genetic data are actively being pursued in the Sibley laboratory (Sibley and Boothroyd, 1992a; Sibley et al., 1992; Su et al., 2002).

In October 2003, the annotated genomes of *P. falciparum* and *P. yoelii* were released (Carlton et al., 2002; Gardner et al., 2002). Other malaria species that are also being sequenced include *P. berghei*, *plasmodium chabaudi*, *plasmodium reichenowi*, *plasmodium vivax*, and *plasmodium knowlesi*. There appears to be a significant degree of synteny between malaria species (Carlton et al., 1999). The *P. falciparum* genome is 28 Mb in size, and most of the other *plasmodium* species have genomes of similar size. Although introns were originally thought to be rare, many more malaria genes have introns than originally appreciated.

Both bovine (Type II) and human (Type I, also called *Cryptosporidium hominis*) *C. parvum* strains have been sequenced and annotation is ongoing. The genome of *C. parvum* is

relatively small at 10.4 Mb. Sequence data for the human isolate and bovine (IOWA) strain are available (see Table 2).

Plans are in place for the eventual access of all these annotated apicomplexan genomes via a single web page able to support searches and phylogenetic comparisons. Other genomes in various stages of completion include *Theileria annulata*, *Theileria parva* and *Eimeria tenella*. Sequencing of the *Neospora* genome has also been proposed.

Expressed Sequence Tag (EST; single pass cDNA sequencing) projects are also ongoing for many Apicomplexa with results from most species available at ApiESTDB (http://www.cbil.upenn.edu/paradbs-servlet/index.html). A full list of EST projects and species is available at http://www.ncbi.nlm.nih.gov/dbEST/index.html. *T. gondii* EST contigs will be mapped onto genome sequence data in the future. Currently *T. gondii* has over 60,000 ESTs that have been sequenced from different life cycle stages and the three major genotypes (Ajioka et al., 1998; Li et al., 2003). Thus information about single nucleotide polymorphisms as well as cDNA sequence is available for highly expressed genes. Information about strain polymorphisms is being used for chromosome mapping of various genetic traits. The ESTs for *T. gondii* are particularly important due to large numbers of introns in the genome sequence. ESTs and annotation of experimentally verified protein expression data will facilitate development of algorithms for prediction of translation initiation sites and intron–exon junctions used for gene prediction.

ESTs for *E. tenella*, *Neospora caninum*, *P. falciparum*, *Sarcocystis neurona*, *T. gondii* and *Babesia bovis* are available (Table 2). Ultimately genome information will be linked to other data including gene expression data obtained from microarray analysis and proteomic analysis of life cycle stages. Searches of *Toxoplasma* or malaria genomes can be performed at http://www.toxodb.org/ or http://www.plasmodb.org/ (Kissinger et al., 2002, 2003). The results of genome sequencing efforts for all Apicomplexa and other protozoa can be searched at the microbial genomes web pages at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = Genome).

Comparative analyses of the genomes of the Apicomplexa have already led to insights into the biology of the phylum. Genes that are unique to the Apicomplexa have been identified as have genes that encode potential chemotherapeutic targets. With completion of the genomes and more extensive annotation, further advances are likely, particularly for those organisms like *C. parvum* and *Eimeria* where significant technical obstacles exist.

5. *Toxoplasma* as a model system for apicomplexan cell biology

T. gondii has been an important model system for the study of many aspects of the cell biology of the Apicomplexa. The genetic tractability of this organism, the ability to express proteins tagged with GFP or epitope tags such as haemagglutinin (HA), c-myc or BB2 (Bastin et al., 1996) has allowed researchers to follow protein trafficking, organellar targeting and organellar biogenesis. In addition, the intracellular morphology of *T. gondii* is very distinct, and organelles can be readily visualised using light microscopy and electron microscopy. Although *T. gondii* tachyzoites are small, $2 \times 7\ \mu m^2$, advanced deconvolution light microscopy techniques have made it possible to visualise individual microtubules and to reliably distinguish micronemes from rhoptries or other organelles in the apical region (Matthiesen et al., 2001; Swedlow et al., 2002).

5.1. The apicoplast

The apicoplast is a newly described organelle of the Apicomplexa that had its origins in a secondary endosym-biotic event. The apicoplast is thought to be derived from the

chloroplast of the original red algal species that was engulfed by the ancestor of the Apicomplexa. The few Apicomplexa, such as *C. parvum*, that do not have an apicoplast are thought to have lost it after divergence from other Apicomplexa. Since its discovery, the apicoplast has been a subject of intensive investigation, and several in-depth reviews of these studies are available (Roos et al., 2002; Foth and McFadden, 2003). *T. gondii* has been an important model for studies of apicoplast development and function.

The apicoplast appears to be essential but its exact role in parasites is not completely understood. The apicoplast has its own genome (previously known as the 35 kb element) that encodes number of genes including an RNA polymerase and ribosomal genes, but the vast majority of the apicoplast proteins are encoded in the nuclear genome. The existence of a non-mitochondrial extrachromosomal element in the Apicomplexa was described in the 1970s, as was the existence of an organelle bound by four membranes (the Golgi adjunct). These observations were first unified in *T. gondii* by the description, sequencing and localisation of the apicoplast genome in *T. gondii* (Kohler et al., 1997).

Although a relic chloroplast, apicoplasts are non-photosynthetic. Their primary function appears to be for fatty acid, isoprenoid and haem synthesis. Apicoplast pathways are more closely related to prokaryotic pathways rather than mammalian metabolic pathways. Therefore they have been explored as potential drug targets. Antibiotics that inhibit processes in the apicoplast include the fluoroquinolones, triclosan, chloramphenicol and the macrolide antibiotics.

The products of the nuclear encoded genes are targeted to the organelle via the secretory pathway. Targeting via a bipartite N-terminal leader sequence has been extensively characterised in *T. gondii* and *Plasmodium* species (Waller et al., 1998; DeRocher et al., 2000; Foth and McFadden, 2003; Foth et al., 2003). The bioinformatics approach has led to the identification of numerous genes that are likely to have been of cyanobacterial origin and are now nuclear encoded (Gardner et al., 2002; Foth et al., 2003). Approximately 10% of the nuclear encoded genes of *P. falciparum* have evidence of algal origin and may have originally derived from the endosymbiont. Molecular validation of apicoplast targeting signals has been performed most exhaustively for *T. gondii* apicoplast-targeted molecules. Expression of *P. falciparum* apicoplast targeting signals in *T. gondii* also targets reporter proteins to the apicoplast (Roos et al., 1999) suggesting that the signals and trafficking machinery are similar.

5.2. Invasion of host cells

The Apicomplexa are obligate intracellular parasites, and it is thought that the process of invasion is conserved throughout the phylum. Most, but not all, species have the apical organelles for which the Apicomplexa are named. Studies in *T. gondii* have revealed that invasion is a carefully orchestrated process that is accompanied by sequential release of micronemes, rhoptries and then dense granules (reviewed by Opitz and Soldati, 2002; Sibley, 2003). Not all life cycle stages of all species have a full complement of apical organelles. Microneme proteins are rich in adhesive domains that are similar to those in mammals. There is little strict homology among microneme proteins, but organisation of proteins into modules of adhesive motifs including lectin domains, epidermal growth factor (EGF) domains, and thrombospondin domains is common (Tomley and Soldati, 2001). Secreted microneme adhesins such as TgMIC2 are translocated on the parasite surface by a parasite actin myosin motor during parasite-mediated entry (Opitz and Soldati, 2002; Sibley, 2003).

Rhoptry contents are secreted during invasion and are thought to contribute to the formation of the parasitophorous vacuole. Rhoptry contents of *T. gondii* are also hypothesised to be

responsible for the non-fusogenic nature of the vacuole and recruitment of mitochondria and endoplasmic reticulum (Sinai et al., 1997; Hakansson et al., 2001; Sinai and Joiner, 2001).

Although the mechanics of invasion are probably largely conserved, host range, cell specificity of each species and even life cycle stages within species is quite different. Most of the current vaccine candidates for *T. gondii* and other Apicomplexa are either surface- or secreted antigens that appear to be essential for the invasion process.

Proteolysis of apical organelle contents during organellar formation or during invasion is a common theme seen in the Apicomplexa. After translocation to the posterior end of the parasite, parasite adhesins are proteolytically cleaved and shed from the parasite surface. Perturbation of this cleavage interferes with invasion. Studies in with a variety of protease inhibitors in *T. gondii* and *Plasmodium* species suggest that both cysteine and serine proteinases are involved in invasion (Conseil et al., 1999; Blackman, 2000; Que et al., 2002). *P. falciparum* falcipain I, a papain-family cysteine proteinase, appears to play an important role in merozoite invasion (Greenbaum et al., 2002). Further studies with the circumsporozoite protein (CSP) of *P. berghei* sporozoites indicate that cleavage of CSP by an unidentified cysteine proteinase is a critical step in sporozoite invasion (A. Coppi and P. Sinnis, personal communication). Subtilisin-like serine proteinases have been identified and localised to micronemes and rhoptries in *T. gondii* (Miller et al., 2001, 2003) and to apical dense granules in *P. falciparum* (Blackman et al., 1998; Barale et al., 1999), but a role for these proteases in invasion has not been established.

Although the mechanics of invasion may well be similar, the ligands that are recognised vary by species. The exact nature of the parasite-host interaction is not completely understood, but recognition of glycosyl groups such as heparin sulphate or chondroitin sulphate moieties on the host appears to be common in the Apicomplexa (Carruthers et al., 2000; Pinzon-Ortiz et al., 2001; Naguleswaran et al., 2003). Differences in recognition of glycoproteins may be partially responsible for some of the species or stage-specific differences in host cell specificity (Naguleswaran et al., 2003; Vonlaufen et al., 2004).

5.3. Motility and the cytoskeleton

Study of *T. gondii* using molecular genetic techniques has clarified the roles of actin and myosin in host cell invasion by the Apicomplexa. *T. gondii* and other Apicomplexa enter host cells using a unique actin–myosin-dependent mechanism termed gliding motility. Early studies with cytochalasins, inhibitors of actin polymerisation, suggested that the actin myosin motor was essential for host cell invasion by the Apicomplexa (Miller et al., 1979; Russell and Sinden, 1981; Russell, 1983). Genetic studies conclusively proved that parasite actin was essential for invasion (Dobrowolski and Sibley, 1996). Further studies have implicated parasite myosin in generating the force necessary for host cell invasion (Dobrowolski et al., 1997). The myosins of *T. gondii* and *Plasmodium* have been characterised and comprise a unique myosin family (Pinder et al., 1998; Hettmann et al., 2000). TgMyoA is a single-headed non-processive fast motor that is essential for parasite invasion of host cells (Herm-Gotz et al., 2002; Meissner et al., 2002). The glycolytic enzyme aldolase forms a bridge between the cytoplasmic tail of *Toxoplasma* microneme protein MIC2 and actin (Jewett and Sibley, 2003). A similar interaction occurs between TRAP, the *P. berghei* sporozoite MIC2 homologue, and aldolase (Buscaglia et al., 2003). Myosin is tethered to the inner membrane complex, flattened membranes that lie beneath the parasite plasma membrane (Bergman et al., 2003).

6. Toxoplasma as a model system for drug target validation

Avian coccidiosis is a disease of tremendous economic importance caused by *Eimeria* species. Identification of treatments for *Eimeria* is an area of antiparasitic drug development that is being pursued by the pharmaceutical industry. Because the *Eimeria* life cycle can be completed only partially in vitro, the type of studies that can be performed is limited. Many aspects of the biology of *Eimeria* are similar to *T. gondii*, and *T. gondii* has been used as a model system for validation of *Eimeria* drug targets (Donald and Liberator, 2002; Donald et al., 2002). This approach was used for study of compound 1. Compound 1 was a drug that was found to have anticoccidial activity in chickens (Gurnett et al., 2002). Radiolabeled compound 1 was used to purify the presumed target, a novel cGMP-dependent protein kinase (PKG). After other systems proved unsuccessful, expression of *Eimeria* PKG was performed in *T. gondii* (Gurnett et al., 2002). Further, *T. gondii* PKG, a gene that could not be disrupted, could be successfully knocked out in parasites expressing *Eimeria* PKG. Transfection with PKG-resistant mutants conferred compound 1 resistance to *T. gondii* tachyzoites, validating PKG as the major target for compound 1. Further exploration of the biological function of *Eimeria* and *T. gondii* PKG has been performed in *T. gondii*, and these studies reveal a likely role for PKG in parasite invasion and motility (Wiersma et al., 2004).

Striepen and colleagues have used *T. gondii* for complementation cloning and experimental validation of chemotherapy targets for C. parvum. By complementing *T. gondii* lacking HXGPRT with a *C. parvum* library and selecting with xanthine and mycophenolic acid, the *C. parvum* IMPDH was identified (Striepen et al., 2002). Further studies on *C. parvum* purine and pyrimidine metabolism have been performed using *T. gondii* as an expression system (Striepen et al., 2004). Similar complementation strategies for other metabolic pathways from apicomplexans should work if appropriate *T. gondii* mutants are available.

7. To each its own: purine and pyrimidine metabolism

Despite many similarities between the Apicomplexa, critical biological and metabolic differences have emerged. Most of the Apicomplexa have made critical adaptations to their host environment and most are quite restricted in their host specificity. There appear to have been several unique lateral transfers of genes (Huang et al., 2004), some involving genes in purine and pyrimidine metabolism (Striepen et al., 2004). The Apicomplexa, as is typical for protozoa, are unable to synthesise purines de novo. Most, but not all Apicomplexa can synthesise pyrimidines de novo, and some but not all can salvage pyrimidines as well.

Given this common biology, it had been assumed that purine salvage pathways would be identical in the Apicomplexa. Remarkably, the Apicomplexa are quite diverse in their complement of purine salvage enzymes. *T. gondii* has redundancy in its purine salvage pathways, being able to utilise both adenosine or hypoxanthine, the major purines in host tissue. Elegant genetic studies in *T. gondii* have demonstrated that neither adenosine kinase (AK) nor hypoxanthine xanthine guanine phosphoriboysl transferase (HXGPRT) is essential but both genes cannot be simultaneously disrupted (Donald et al., 1996; Sullivan et al., 1999).

Malaria species, in contrast, appear to utilise purines via the purine nucleoside phosphorylase (PNP)/HGXPRT pathway. AK cannot be identified in any malaria genome. Thus for malaria, it appears that all purine salvage is via hypoxanthine made by PNP from inosine (Kicska et al., 2002b). Remarkably the PNP present in *T. gondii* and malaria is a hexameric PNP that is similar to PNPs of the eubacteria, rather than a trimeric PNP as is classically seen in other eukaryotes (Kicska et al., 2002a). These structural differences result

in unique substrate specificity and inhibitor profiles that could potentially be exploited for chemotherapy design (Kicska et al., 2002a).

C. parvum is also unique. It appears to salvage both purines and pyrimidines as it lacks genes for pyrimidine synthesis. It instead has acquired uracil phophoribosyl transferase (UPRT), a fused uridine kinase–phosphoribosyltransferase (UK-UPRT) and thymidine kinase (TK; Striepen et al., 2004). The *C. parvum* thymidine kinase is a strong candidate for lateral transfer from proteobacteria and has not been described in any other Apicomplexa.

UPRT is also present in *T. gondii*, but *T. gondii* appears to require its pyrimidine biosynthetic enzymes for replication and virulence presumably because exogenous levels of pyrimidines are too low to sustain rapid cell division (Fox and Bzik, 2002). Malaria parasites have no UPRT and cannot salvage pyrimidines. Instead of relying upon hypoxanthine, *C. parvum* salvages adenosine exclusively with AK, and has no identifiable PNP or HGXPRT (Striepen et al., 2004). It is therefore particularly vulnerable to inhibition of IMPDH (Striepen et al., 2004). Although *T. gondii* purine metabolism differs from *C. parvum*, transfection studies with *T. gondii* mutants have been valuable in documenting the biological function of *C. parvum* purine salvage enzymes (Striepen et al., 2002, 2004).

8. Conclusion

The similarities between *T. gondii* and the other Apicomplexa have led to *T. gondii* being the experimental model of choice for many aspects of apicomplexan biology. There are a wide variety of genes of unknown function that are Apicomplexa-specific (Ajioka, 1998; Ajioka et al., 1998; Li et al., 2003), unique organelles in common, as well as many conserved metabolic pathways. The mechanics of host cell invasion also are generally conserved.

Despite their classification in the Apicomplexa, the various members also have significant biological differences. Thus use of *T. gondii* as a model system, therefore, requires careful formulation of the scientific questions to be answered. Many aspects of disease pathogenesis, host range and life cycle are not conserved. Further, many metabolic pathways have been adapted for survival in unique host cell environments. *Plasmodium* species and *Cryptosporidium*, which are Apicomplexa that are specialised for growth in only a subset of host cells, have less redundancy in some metabolic pathways than *T. gondii*, which infects many host types and many cells within each host. The differences in the Apicomplexa do not, however, preclude use of *T. gondii* as a model system, particularly since mutants are so readily created and analysed. Although the fundamentals of protein trafficking are not completely worked out in any of the Apicomplexa, heterologous expression of apicomplexan proteins in *T. gondii* has been successful in cases where expression in bacteria, yeast or mammalian cells does not yield protein that resembles the native protein of interest. The amenability of *T. gondii* to genetic manipulation has also enabled development of novel genetic or complementation strategies that may facilitate identification of molecules responsible for unique aspects of apicomplexan biology. Overall, there are many advantages in using *T. gondii* as a model for the study of other Apicomplexa and it will continue to provide insights into the biology of these important parasitic protozoa.

Acknowledgments

This review has its origins in a special session of the Seventh International Congress on Toxoplasmosis titled `*T. gondii*: Relevance to Other Systems' sponsored by the Ellison Medical Foundation held in May 2003 in Tarrytown, NY. The work in our laboratories has been supported by the National Institutes of Health (NIAID) (KK, LMW) and the Burroughs Wellcome Fund (KK).

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Table 1

Comparison of *Toxoplasma gondii* as an experimental model with other Apicomplexa

	T. gondii	Plasmodium	Cryptosporidium	Neospora	Eimeria
In vitro culture system	Yes	Yes	No	Yes	No
Classic genetics (host)	Yes (cat)	Yes (mosquito, *P. falciparum*)	No	No	No
Genome sequence	In progress	Yes	Yes	Proposed	In progress
Transient transfection	Yes	Yes	No	Yes	Yes
Stable transfection	Yes	Yes	No	Not reported, but probably similar to *T. gondii*	No
Genes expressed in *T. gondii*?	Yes	Yes (frequently with gene resynthesis)	Yes	Not reported, but *T. gondii* genes expressed in *Neospora*	Yes
Presence of mitochondrion	Yes	Yes	No	Yes	Yes
Presence of apicoplast	Yes	Yes	No	Yes	Yes

Table 2

Apicomplexan Expressed Sequence Tag and genome web resources

Parasite	Web address	Source
Toxoplasma gondii	http://www.toxodb.org, http://www.tigr.org/tdb/parasites/, http://www.sanger.ac.uk/Projects/Protozoa/	Genome
	http://www.cbil.upenn.educ/paradbs-servlet/, http://www.paradb.cis.upenn.edu/	EST
Plasmodium spp.	www.plasmodb.org, http://www.tigr.org/tdb/parasites/, http://www.sanger.ac.uk/Projects/Protozoa/, http://sequence-www.stanford.edu/group/malaria/index.html	Genomes and ESTs
Eimeria tenella	http://www.sanger.ac.uk/Projects/E_tenella/	Genome
Eimeria tenella	http://www.cbil.upenn.edu/paradbs-servlet/	ESTs
Babesia bovis	http://www.sanger.ac.uk/Projects/B_bovis/	ESTs
Cryptosporidium parvum	http://www.parvum.mic.vcu.edu/, http://www.cbc.umn.edu/ResearchProjects/AGAC/Cp/	Human and bovine strain
Theileria annulata	http://www.sanger.ac.uk/Projects/Protozoa/	Genome and ESTs
Theileria parva	http://www.tigr.org/tdb/e2k1/tpa1/	Genome
Neospora caninum	http://www.cbil.upenn.edu/paradbs-servlet/	ESTs
Sarcocystis neurona	http://www.cbil.upenn.edu/paradbs-servlet/	ESTs