Simposio

BIOQUÍMICA DE PARÁSITOS PROTOZOARIOS

Reconstruction and constraints based modelling of *Toxoplasma gondii* metabolism

Carl Song[1], Stacy Hung[1,2], James Wasmuth[1], Alexandra Gast[2], John Parkinson[1], Michael Grigg[2]

[1] Molecular Structure and Function, The Hospital for Sick Children, Toronto, ON, Canada
[2] Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, MD, USA

Metabolic enzymes do not operate in isolation rather they form components of an integrated biochemical network. To acquire a more complete understanding of the complex relationships between enzymes and pathways, constraints based modeling frameworks such as flux balance analysis (FBA) have been developed.

Here we present an extensively 'curated' genome scale metabolic reconstruction (*i*CS352) for *Toxoplasma gondii*, which includes 470 metabolic and transport reactions. Three hundred and seventy three reactions could be accounted for by 352 genes. The remaining reactions represent either experimentally verified reactions in *T. gondii* for which no gene has yet been identified or reactions predicted to be essential for parasite growth.

An objective function based on current literature and optimized by FBA simulations was formulated to describe the biomass constituents required for growth. Using available microarray data, reaction constraints were introduced to reflect the relative expression of each enzyme. FBA was performed to monitor the effects of gene deletions in six different strains (Me49, CTG, RH1, GT1, VEG and Prugniaud).

Applied to *T. gondii* Me49, our studies predict 224 essential reactions that represent potential targets for therapeutic intervention. Across the six strains we identified nine reactions whose deletions had variable impact on parasite growth and are predicted to mediate critical roles in virulence. Flux variability analysis also revealed changes in flux limits across the six strains, suggesting reliance on alternate pathways.

Based on these analyses, we have begun targeting enzymes in the pantothenate biosynthesis pathway for further biochemical characterization and *in vivo* knockout studies in *Toxoplasma*.

• • •

Pyrimidine biosynthesis in *Toxoplasma gondii*

Miryam Andrea Hortúa, Gabriel L. Lozano, Heidy Y. Narváez, María Consuelo Rocha, Enrique A. Camargo, Manuel F. Garavito, Barbara H. Zimmermann

Bioquímica y Biología Molecular de Parásitos, Departamento de Biología, Universidad de los Andes, Bogotá, D.C., Colombia

Toxoplasma gondii is an obligate intracellular parasite from the phylum Apicomplexa with worldwide distribution. Infections are usually asymptomatic; however life-threatening illness occurs in immunocompromised patients and in the fetus (1). Current drugs cause severe side effects in some patients, and are not effective against the latent stage of the parasite (2). Thus, there is a need to identify new targets for the design of less toxic and more effective drugs.

Targeted gene disruption experiments demonstrate that the pyrimidine biosynthetic pathway of *T. gondii* is essential for parasite replication and virulence, and its enzymes are potential drug targets (3,4). Bioinformatic analyses suggest that structural differences between *T. gondii* and human enzymes may be exploited to produce parasite-specific inhibitors.

Our lab has cloned, overexpressed, and purified the following pyrimidine biosynthetic enzymes from *T. gondii*: aspartate transcarbamoylase (catalyzing step 2 of the pathway), dihydroorotase (step 3), dihydroorotate dehydrogenase (step 4), orotate phosphoribosyl transferase (step 5), orotidine-5'-

monophosphate decarboxylase decarboxylase (step 6), and cytidine triphosphate synthase (step 8).

Our goal is to use these active recombinant enzymes to test potential inhibitors, and to develop a full strategy against the entire pathway. Various sources of inhibitors are being used, including the National Cancer Institute Chemotherapeutic Agents Repository, and inhibitors of *Plasmodium* pyrimidine biosynthetic enzymes donated by other laboratories.

Kinetic parameters have been determined for four of the purified recombinant proteins, and inhibitor studies are in progress. Kinetic characterizations of the remaining enzymes are expected shortly. To complement the *in vitro* inhibitor studies, we have generated models of the *T. gondii* enzymes based on structures of enzymes from other organisms for use in *in silico* docking experiments. Testing of promising inhibitors in *T. gondii* cultures is planned. The availability of active recombinant proteins makes possible future crystallization experiments of selected enzymes that may lead to collaborative structure determinations.

References

1. Weiss LM, Dubey JP. Toxoplasmosis: A history of clinical observations. Int J Parasitol. 2009;39:895-901.
2. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet. 2004;363:1965-76.
3. Fox BA, Bzik DJ. *De novo* pyrimidine biosynthesis is required for virulence of *Toxoplasma gondii*. Nature. 2002;415:926-9.
4. Fox BA, Bzik DJ. Avirulent uracil auxotrophs based on disruption of orotidine-5'-monophosphate decarboxylase elicit protective immunity to *Toxoplasma gondii*. Infect Immun. 2010;78:3744-52.

• • •

Toxoplasma uses a novel nutrient pathway to internalize and degrade host-derived proteins

Zhicheng Dou, Vern B. Carruthers

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

As an obligate intracellular parasite, *Toxoplasma* sp. critically relies on the acquisition of host metabolites as nutrients to support its replication. Although it was reported that *Toxoplasma* sp. parasites adopt several strategies to usurp simple metabolites and lipids from host cells, none of these existing pathways readily permit access to host derived polypeptides as a nutrient source. While recent studies showed that *Toxoplasma* sp. cathepsin L protease (TgCPL) plays a specialized role as a maturase for secretory proteins, whether it functions in a more classic role as a resident catabolic enzyme in the lysosome-like compartment (VAC/PLV) remains unknown.

Here, we show that a TgCPL-deficient mutant replicates slowly, possibly reflecting a defect in acquiring and/or utilizing host nutrients. Consistent with this, we identified host cytosolic GFP within the VAC/PLV of TgCPL-deficient parasites, suggesting that the parasite can internalize host polypeptides. Interestingly, host cytosolic GFP was not seen inside WT parasites.

Since cathepsins such as TgCPL are classically associated with protein degradation, we suggest that the failure to detect GFP in WT parasites is because TgCPL and possibly other endosomal proteases rapidly digest it. Host ER-derived GFP was not detected within WT or TgCPL-deficient parasites, indicating either that it is not internalized or is below the level of detection. Moreover, we provide evidence of at least two additional proteases in the parasite endosomal system that might also contribute to host protein degradation.

Mutants deficient in the known nutrient pathways and the parasite endocytic pathway are being used to dissect the mechanism of internalization and trafficking. Comprehensive characterization of host cell protein uptake by the parasite endocytic pathway should inform how other metabolites are assimilated by *Toxoplasma* sp. parasites, which will pave the way for attenuating parasite replication.

• • •

Toxoplasma's great escape: regulation of a pore forming protein for rapid parasite

Marijo Roiko, Vern B. Carruthers

Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI, USA

The opportunistic pathogen *Toxoplasma gondii* secretes an arsenal of proteins to actively invade and exit host cells. While most characterized secretory proteins function in cell invasion, we discovered that a secreted perforin-like protein (PLP1) is essential for rapid host cell egress, revealing a mechanism for cytolytic exit. PLP1-deficient parasites are profoundly virulence attenuated, underscoring a relationship between pathogenicity and egress capacity.

Our recent findings suggest that PLP1 uses pH and membrane receptor availability to selectively destroy host membranes during egress while limiting damage to itself and the host cell during subsequent cell invasion. Low pH boosts PLP1 cytolytic activity, implying a means of environmental regulation within the parasitophorous vacuole during egress.

The parasite appears to also exploit a specific type of phosphatidyl inositol that is exposed on the internal leaflet of the host plasma membrane to selectively destroy this barrier to the parasite's freedom. PLP1 membrane specificity is also dictated by cholesterol, which is minimal in the parasite plasma membrane, suggesting a means of self-protection from PLP1 cytolysis.

Together, our findings reveal novel mechanisms that regulate parasite cytolytic egress from an expiring cell without compromising parasite integrity or invasion of the next target cell.

• • •