TATA-Binding Protein 1 Is Essential for Intracellular Infection by the Protozoan Parasite *Toxoplasma gondii*

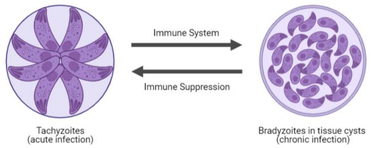
Malorie Nitz, Samantha Swartz, Krista Fleck, PhD and Victoria Jeffers, PhD
University of New Hampshire, Molecular, Cellular and Biomedical Sciences, Durham, NH

Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular parasite capable of infecting most cells in warm-blooded animals, including humans. Approximately 30-50% of the world's population is infected with the parasite.

Healthy individuals are typically asymptomatic upon infection. Immunocompromised individuals are at risk because current treatments do not treat the chronic form of the pathogen.

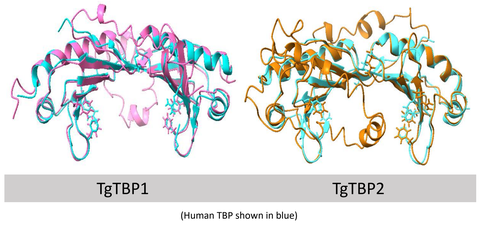
The parasite utilizes strict gene expression for differentiation between its acute and chronic stages, host cell invasion, and pathogenesis.



TATA-Binding Proteins

For transcription to begin, a TATA binding protein (TBP) must recognize the TATA box (TATAAA) or other motifs to recruit the transcription preiniation complex (PIC) and RNA polymerase to the promoter.

Toxoplasma contains two TBPs, TgTBP1 and TgTBP2. While this is peculiar, it is more interesting to note that *Toxoplasma* does not possess any TATA boxes or other conserved motifs enriched in promoter regions within its genome. **Little is known about the role of TBPs in *Toxoplasma*.**



Several residues are important for TBP-DNA interaction. While TgTBP1 and TgTBP2 are missing some key residues for binding, structural predictions show structures similar to other TBPs.

Hypothesis:

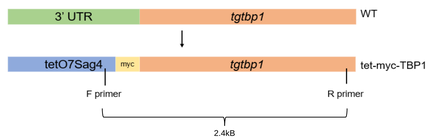
We hypothesize that TgTBP1 plays an essential role in transcriptional regulation and it critical for parasite viability.

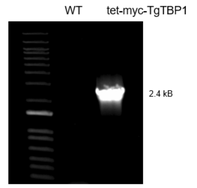
Tetracycline Inducible Knockdown of TgTBP1

To determine the role of TgTBP1 on parasite fitness, we generated a parasite line to knockdown *tgtbp1* by replacing its promoter with a tetracycline-regulatable promoter.

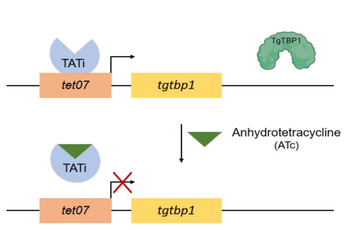
Promoter Replacement Strategy:

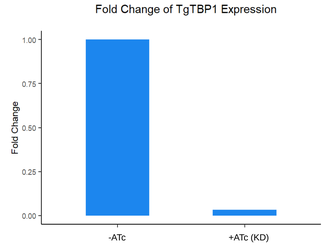
We utilized the Nucleofector® X Unit to transfect a plasmid construct containing a tetO7Sag4 promoter at the C-terminal end of *tgtbp1* into parasites. This replaces the endogenous *tgtbp1* promoter with a tetracycline-regulatable promoter (left). Integration was confirmed by genomic PCR (right).





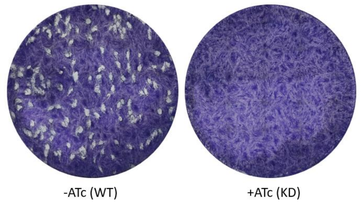
After 48 hours of incubation with anhydrotetracycline (ATc), *tgtbp1* expression decreases as quantified by qRT-PCR.

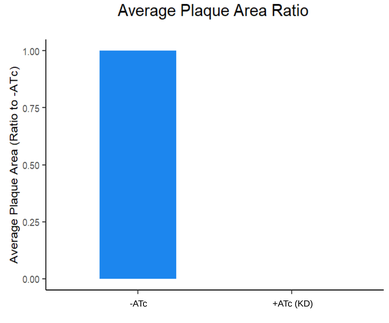




tgtbp1 knockdown is lethal to parasites

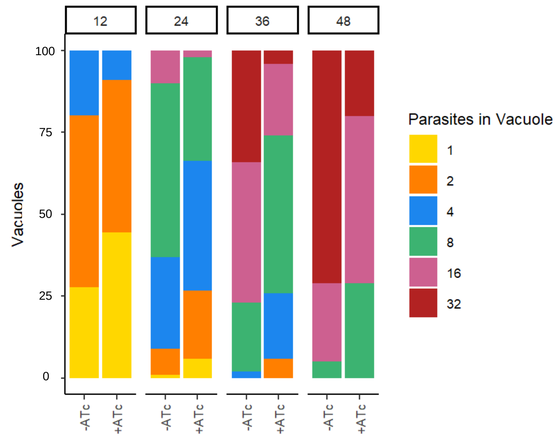
Upon *tgtbp1* knockdown, parasites do not grow. No plaques were found in an intracellular plaque assay to assess parasite growth in human foreskin fibroblasts (HFFs).





TgTBP1 is essential for replication

When TgTBP1 is lost, parasites replication is affected. Parasites grow more slowly over 48 hours upon TgTBP1 knockdown compared to WT parasites.



Summary

- We are investigating how the PIC and RNA polymerase are recruited to the promoter and how gene expression is regulated in *Toxoplasma*.
- We have shown that TgTBP1 is an essential protein for parasite viability.
- Our ongoing research will determine how TgTBP1 loss affects parasite invasion, cell division and morphology, and global gene expression in the parasite.
- With this information, we can identify unique features of the transcription initiation process to target with therapeutic drugs.

Acknowledgements

Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the NIH under grant number P20GM113131.
Dr. Krisztina Varga and Logan Brown, Department of Molecular, Cellular, and Biomedical Sciences
Sequences obtained from the *Toxoplasma gondii* genome database ToxoDB.org.