TATA-Box Binding Protein1 is Essential for *Toxoplasma gondii*

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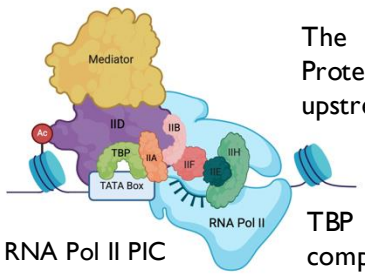
Precise transcription allows apicomplexan parasites, like *Toxoplasma,* to adapt changes to its environment. Transcription is the process of converting genetic material into RNA, allowing for production of the necessary proteins needed for survival.



RNA Pol II PIC

The process is initiated by TATA-Box Binding Protein (TBP) binding to unique sequences upstream of genes called promoters.

TBP binding facilitates formation of pre-initiation complexes specific to each RNA Polymerase.

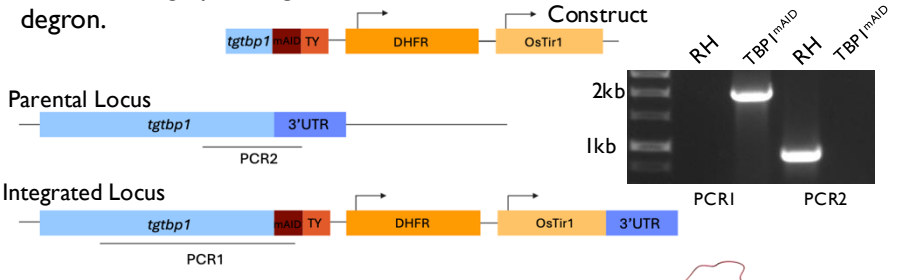
***Interestingly, Toxoplasma* encodes three TBP homologs, TgTBP1-III.** Each protein is predicted to be essential for tachyzoite fitness with dissimilar expression profiles throughout the *Toxoplasma* life cycle, indicating distinct functional roles.

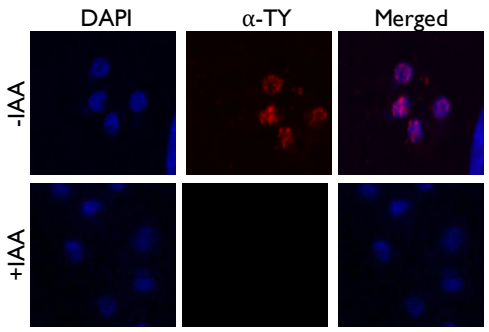
However, little is known about their individual roles in transcription initiation. Further, our knowledge about the sequences that make up promoters and the pre-initiation complexes in *Toxoplasma* is similarly misunderstood.

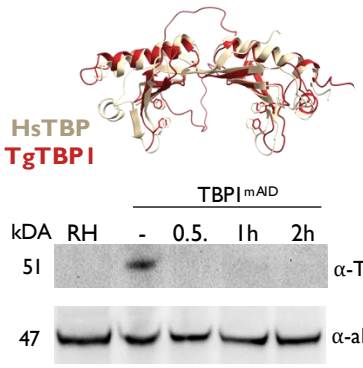
What is the functional significance for maintaining three TBP homologs when TBP diversity is largely restricted to multicellular organisms?

TgTBP1 Tagging Strategy

To determine the role of TgTBP1 on parasite fitness, we targeted the C-terminus of *tgtbp1* using CRISPR/Cas9 and introduced a mini-auxin inducible degron.

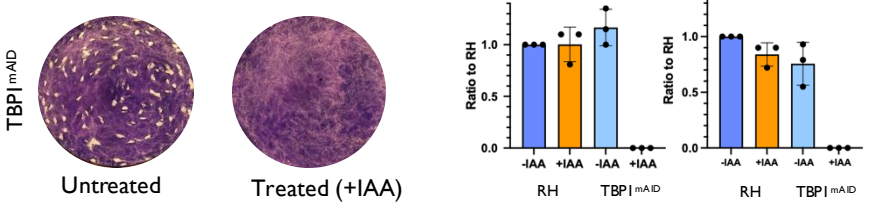




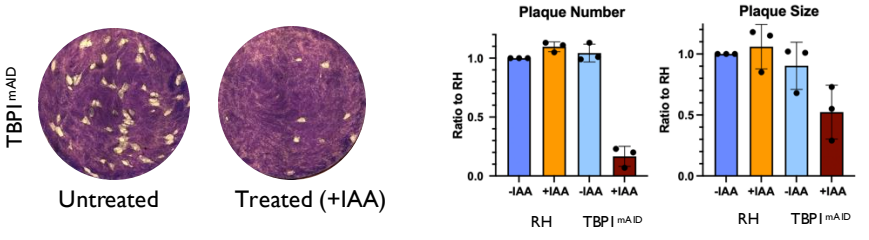


Short-term depletion is lethal to tachyzoites

To assess growth in the absence of TgTBP1, parasites were grown on human foreskin fibroblasts for 6-days.



No defects were observed in the wildtype or untreated TgTBP1 knockdown line but TgTBP1 knockdown was lethal. To investigate if short term depletion similarly affected parasite fitness, TgTBP1 parasites were treated with IAA then grown with standard culture media for 6-days.

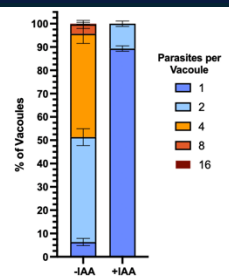


Six hours of TgTBP1 depletion resulted in significant reduction in plaque number and size, and extended treatment for 12 hours (data not shown) completely ablated parasite viability.

Tachyzoite replication is severely affected

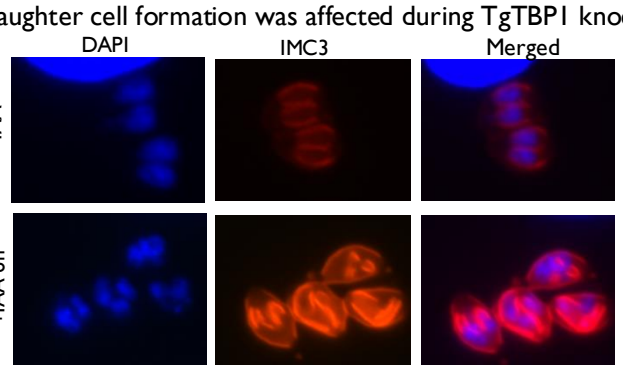
To determine the point in the lytic cycle that is inhibited by the loss of TgTBP1, we monitored parasite replication 12-hours post-knockdown.

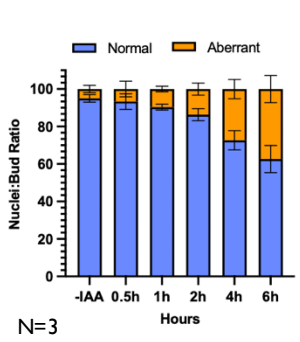
No defects were observed in the wildtype or untreated TgTBP1 line (data not shown). However, the loss of TgTBP1, even for 12 hours, severely affected intracellular replication (right).



Daughter cell formation is also severely affected

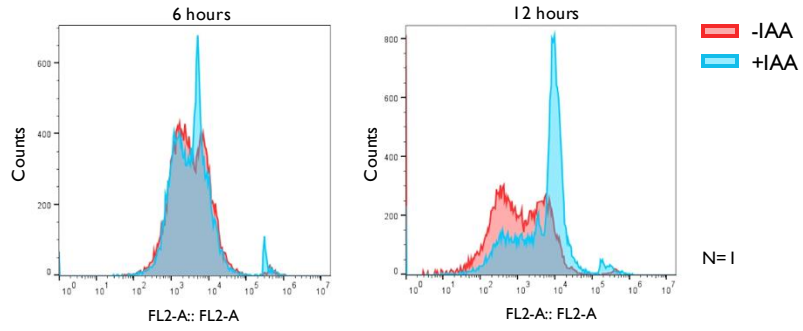
Toxoplasma divides by endodyogeny, an internal budding process that forms two daughter parasites per round of division. This process driven by the assembly of the inner membrane complex (IMC) which is supported by an intermediate filament network and microtubules. We aimed to determine if daughter cell formation was affected during TgTBP1 knockdown.





As expected, in the absence of IAA, parasites divided by endodyogeny. In contrast, multiple nuclei and budding defects were observed within mother cells during knockdown.

To further examine the multinucleate phenotype during TgTBP1 knockdown, we used propidium iodide staining to assess if the observed phenotype reflected an increase in DNA content.



Our preliminary results indicate that DNA content was elevated in the 6-hours post-knockdown relative to untreated TgTBP1, and was substantially elevated 12 hours post-knockdown. Although mitosis is proceeding, cytokinesis is severely impaired, however, additional work is required to validate this observation.

Future Directions

Interestingly, our CO-IP and CUT&TAG results indicate TgTBP1 interacts with promoters and the basal transcriptional machinery in a non-canonical manner. To better understand the mechanism underlying this phenotype, our ongoing research will determine how the loss of the TgTBP1 affects global gene expression.

Citations and Acknowledgments

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