Genetic Variation and Phylogenetics of Histone Variants of the protozoan parasite, *Toxoplasma gondii*

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GEN 711

Toxoplasma gondii & Toxoplasmosis

- Protozoan parasite that can infect every warm-blooded animal
- Symptoms: fever, confusion, headache, nausea, malaise, very harmful if in eyes
- Especially harmful for pregnant people and Immunocompromised patients
- Transition between lifecycle stages of T. gondii is essential for it to cause disease[2]
- Transition is mediated by gene regulation
- Understanding gene development in T. gondii can lead to new treatments for toxoplasmosis[5]

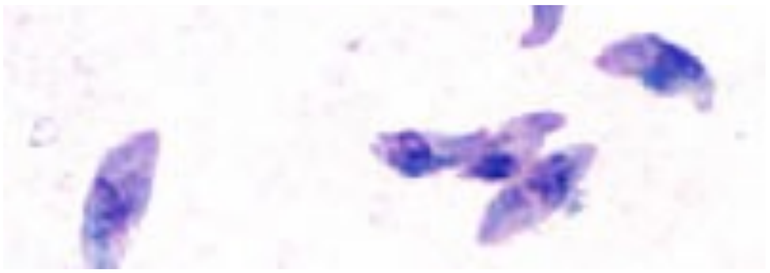

Figure 1: *Toxoplasma gondii* tachyzoites[1]

Histones

- Exist as octamers packaging the genome
- Multiple variants associated with either activation or repression (epigenetic regulation)
- H2A.Z: associated with activation (~45% similar to human)[4]
- H2A.X: associated with gene silencing and DNA repair[5]
- H2A.1: Interacts with H2B, most conserved variant[3]
- H2Bv: interacts with H2A.Z during gene activation[5]
- H4: Many functions, highly conserved. Differs from human by 6 amino acids[5]

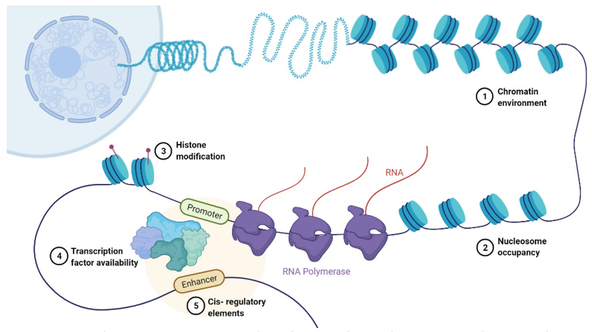

Figure 2: Chromatin organization and regulation within nucleus. Generated in Biorender

My Thesis: Genetically Engineering a Parasite Line

- H2A.Z gene was tagged so the gene can be tracked with an antibody
- Tagged gene was transfected into a BDP-1 inducible knockdown parasite line
- Plasmid introduced to *T. gondii*
  - incorporated next to existing H2A.Z
  - CAT allows selection via antibiotics
  - 3xHA is visible under fluorescent microscope

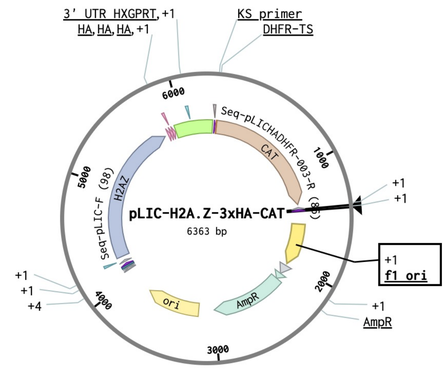

Figure 3: pLIC H2A.Z 3xHA CAT plasmid diagram, Generated in Benchling

Hypothesis

H2A.Z and H2A.1 will be the most genetically similar because they both interact with H2Bv during gene activation

Results

Alignment:

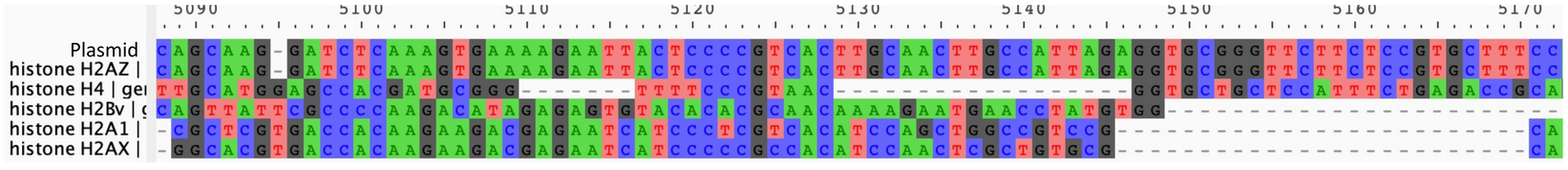

Figure 4: Alignment of FASTA sequences, Generated in AliView

- Plasmid and H2A.Z align (top two lines)
- H2A.1 and H2A.X align well to conserved chunks (bottom two lines)
- H4 and H2Bv align in some places (middle two lines)

Phylogenetic Tree:

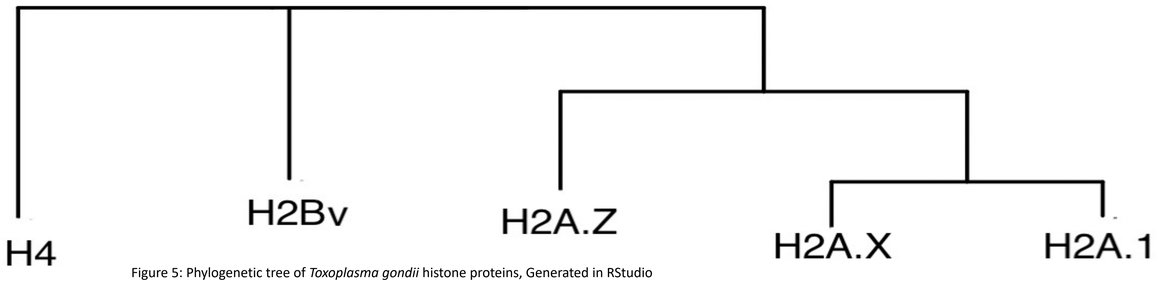

Figure 5: Phylogenetic tree of *Toxoplasma gondii* histone proteins, Generated in RStudio

- H2A.X and H2A.1 are the most similar of the group
- H2A.Z is more closely related to H2A.X and H2A.1 than the other histones
- H4 is the least similar to the other tested proteins
- Bootstrap Values: TBD?

Methods

- Used bioconda package
  conda install –c bioconda
- Put all my fasta into one file
  cat tgme* > all_seq.fasta
- Converted to an ali file using mafft
  mafft –auto all_seq.fasta >alignment.fasta
- Alignment file was visualized using AliView
- Used RAxML to make the tree (5 bootstraps)
  - GTRGAMMA option: specific model of nucleotide substitution rates
    raxmlHPC_PTHREADS –T 8 –N 5 –s alignment_no_plasmid.ali -m GTRGAMMA –p 12345 –n histone_tree.tre
- Tree visualize using Rstudio ggtree

```
tree <- read.tree("RAxML_bestTree.histone_tree2.tre")
tree
rooted_tree <- root(tree, outgroup = "TGME49_239260",
node=NULL,                resolve.root=TRUE,
interactive=FALSE,edgelabels=FALSE)
rooted_tree
ggtree(rooted_tree, layout = "rectangular") +
geom_tiplab()+
expand_limits(x=5)
```

Acknowledgements & Citations

Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the NIH under grant number P20GM113131

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