A Comparative Study of Bivalent Gene Expression of the Traf6 Promoter Gene and Changes in Epigenetic State between Mice and Humans

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Introduction

- Epigenetics is the study of how your behaviors, environment or other factors outside the DNA sequence that can affect the way your genes work.
- During the process of cellular growth, cells have instructions from genes that dictate what traits will be present, and what traits will be 'silent.'
- Some developmental genes have a special trait called **bivalency** that has a gene poised to turn "on" or "off, allowing them to develop in different ways
- ***Traf6*** is our gene of study because it is a bivalent developmental gene present in germ cells of some species and not others.
- *Traf6*, as it has a major role in hair, skin, and nail development, as well as immune function.
- We compare the *Traf6* gene between mice and other mammals to gather a better understanding of bivalency and its role in gene regulation.

Background

Figure 1. The bivalent state
The bivalent state is both activating and repressing epigenetic information on the same gene.
H3K4me3 – activating
H3K27me3 – repressing

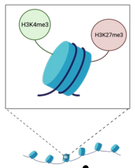

Figure 2. A core set of genes marked by the bivalent state in all five species indicated in red while the ones in grey have the bivalent epigenetic state in only one species

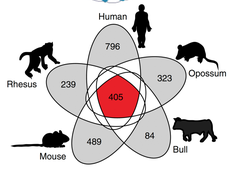

Figure 3. The epigenetic state of TRAF6 across mammalian evolution.
Traf6 is marked only by H3K4me3 in most species but is bivalent in the mouse making it an ideal cell line for study.
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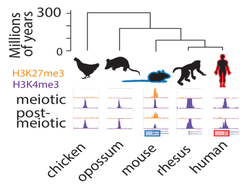
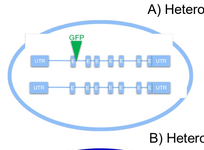


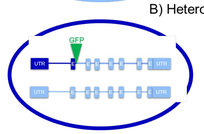
Figure 4. Transgenic stem cells and mouse lines.
We generated two sets of transgenic mouse cells .
A) The control group "**mTraf6**" only has GFP inserted after the first exon of TRAF6.

B) The experimental group "**hTraf6**" contains the human segment of TRAF6

A) Heterozygous *mTRAF6*



B) Heterozygous h*TRAF6*



Specific Aims

- Quantitively analyze GFP levels of humanized *Traf6* mouse line
- Quantitively analyze CTFC of specific locations of humanized *Traf6* mouse line
- Understand the regulation of the epigenetic state change in humanized *TRAF6* mouse line

Methods

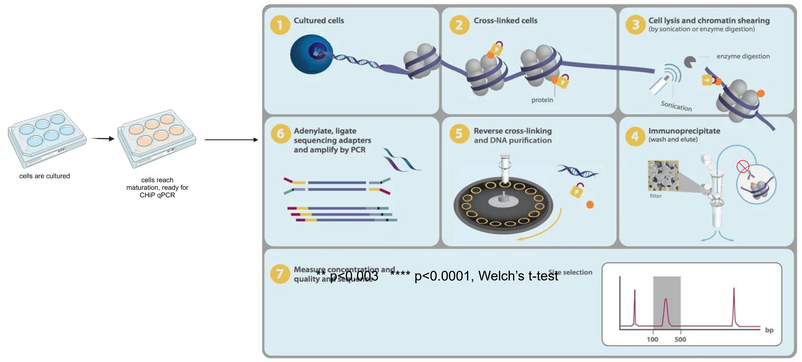


Figure 5. Experimental workflow
Mice testes were removed from two mouse lines: ***hTRAF6*** and ***mTRAF6***. The samples were then sorted into early-stage cells of interest via flow cytometry by measuring detectable levels of GFP. Cells were cultivated and prepared for ChIP qPCR to quantitatively measure H3K4me3 and H3K27me3.

Results

Figure 6. Alteration of *hTRAF6*
The diagram depicts the hTRAF6 gene, and the alterations done for **knockout lines** (KO1 and KO2.)

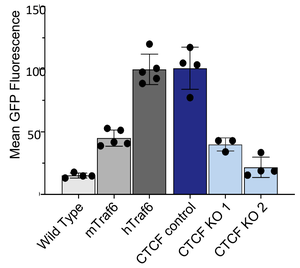


Figure 7. Flow Cytometry Results
Using flow cytometry, we can detect and identify the amount of GFP to analyze knockout lines (KO1 and KO2) of *hTRAF6*. GFP acts as a proxy for detection of *TRAF6* expression, which decreases following the removal of the DNA sequence bound by CTCF.

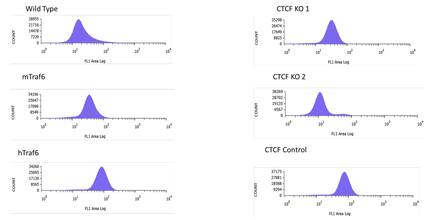


Figure 8. Histogram of Results
Using a histogram, we are able to identify baseline control metrics of expected GFP. This enables our experiment to proceed with the knowledge that our KO lines indeed have detectable levels of GFP.

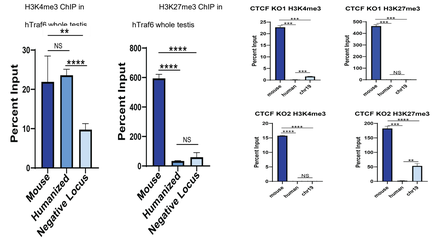


Figure 9. Measurement of CTCF for KO1 and KO2
A.) Shown is the enrichment of histone marks in the Traf6 promoter in hTraf6 testes.
B.) Shown is the enrichment of histone marks in promoter of CTCF knockout lines determined by ChIP qPCR. Both KO lines show a decrease in H3K4me3 and H3K27me3. While a decrease in H3K4me3 was and expected, **the decrease in H3K27me3 is contradicting the hypothesis** of our experiment.

Discussion and Future Considerations

- *hTraf6* allows for more expression of GFP and more binding of **CTCF**, a transcription factor that serves as a gene regulator and formation of chromatin loops.
- The cell lines selected in our study yielded less GFP expression, indicating that the deletion of the CTCF binding site play a role in regulating *TRAF6* expression.

Figure 10. CTCF and TRAF6
GFP uptake is limited or impossible with the deletion of CTCF.

- Further ChIP qPCR testing will be conducted to include KO lines that include the deletion of CTCF to replicate our discovered data.
- Future studies about the relationship with CTCF deletion and how it affects the epigenetic state of *hTraf6.*
- Testing other tissue samples of *hTRAF6* mice for changes in morphology and establishing baseline information
- Comparative analysis of different mammalian germ cell lines for *hTRAF6*

References

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7.Figure 5 created in BioRender.

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