Comparative Analysis Of Epigenetic Gene Regulation Between Mice and Humans At A Candidate Gene

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Introduction

All cells in the human body contain the same DNA, however, this does not mean every gene is expressed.

- Epigenetic traits are one of many regulation mechanisms that modify DNA structure to turn genes "on" or "off".
- **Developmental genes have an important epigenetic trait called bivalency that simultaneously turn it "on" *and* "off".**
- Bivalency, or the bivalent state, is not well understood.
- Our lab is focused on furthering our understanding of the bivalent state and its importance on development.
- The candidate gene *TRAF6* was selected because of its role in immunity and development.
- By comparing *TRAF6* between mice and other mammals, we can have a better understanding of bivalency and its role on development and disease.

Background

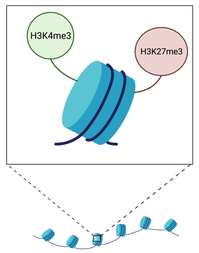


Figure 1. Bivalent state
Bivalent state is the simultaneous existence of the activating and repressing modifications on the same subunit of DNA.

H3K4me3 - activating
H3K27me3 - repressing

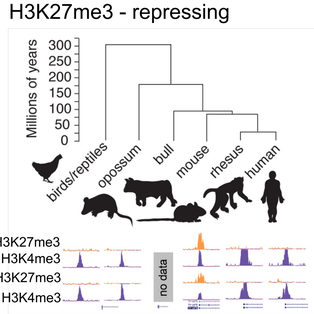


Figure 2. *TRAF6* epigenetic state across evolution
TRAF6 is bivalent and inactive in mice whereas in other mammals, it is active and not bivalent (H3K4me3 only).

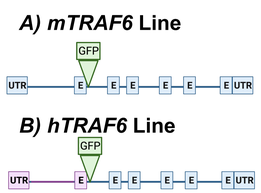


Figure 3. Transgenic mouse line

A) mTRAF6 is the control group with only a green fluorescent protein (GFP) sequence.

B) hTRAF6 is the experimental group with the initial *TRAF6* segment replaced with the correlating human sequence and GFP sequence.

GFP sequence allows us to track any changes to gene expression. Both lines have one normal wild-type allele.

Project Aims

To identify and characterize the role of DNA sequence on the bivalent state:

1. **Characterize the epigenetic state change in humanized *TRAF6* mouse line.**
2. **Qualitatively analyze GFP levels of humanized *TRAF6* mouse line.**
3. **Quantitatively analyze GFP levels of humanized *TRAF6* mouse line.**

Methods

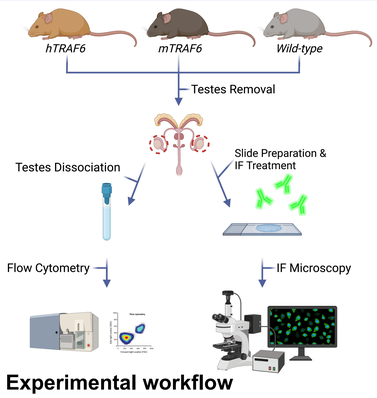


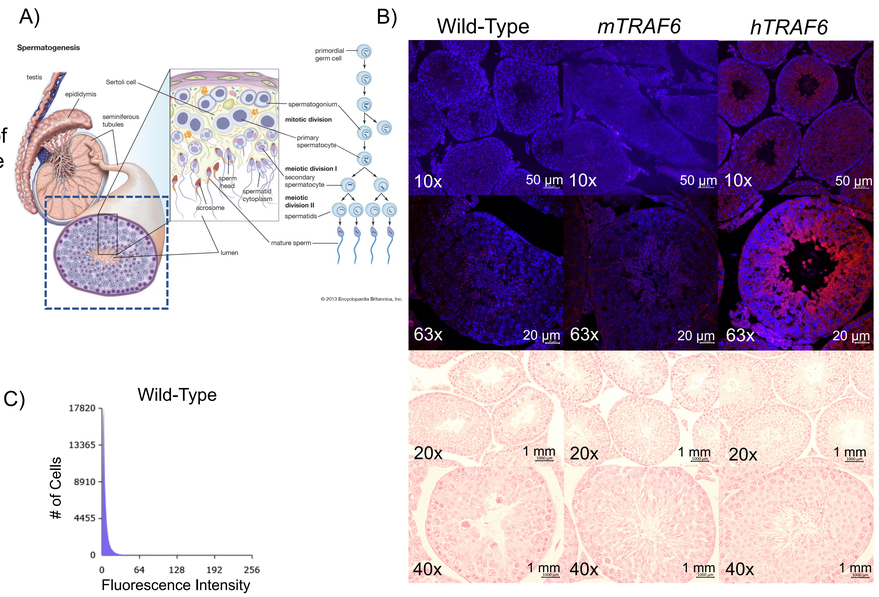
Figure 4. Experimental workflow
Mice testes were removed from 3 mouse lines: ***hTRAF6, mTRAF6,* and wild-type.** One testis was analyzed via flow cytometry to quantitatively characterize GFP levels. The other testis was prepared into a slide via immunofluorescence (IF) treatment to allow for visual characterization of GFP levels.

Results

Figure 5. Microscopy of GFP expression

A) Cross-section diagram of testis tubules. Reference for images.

B) Top 2 Rows- IF images
Blue- all cell nuclei
Red- GFP
Bottom 2 Rows- Stained images



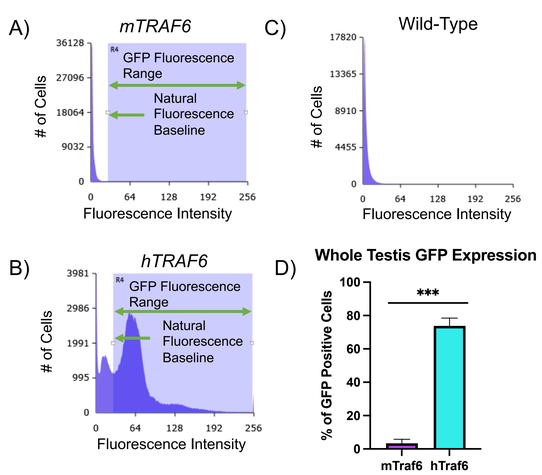


Figure 6. Flow cytometry for GFP expression
A, B, and C show the cell count relative to the fluorescence intensity for each mouse line.

D shows the raw percentage of GFP positive cells from two *mTRAF6* mice samples and three *hTRAF6* mice samples.
***p<0.0002 (Welch's t-test)

Conclusion

- **Altering the initial sequence of *TRAF6* in mice resulted in an epigenetic state change.**
- **The *hTRAF6* line lost its bivalency at *TRAF6* and became active.**
- **Immunofluorescence microscopy displayed an overall increase in GFP levels in the *hTRAF6* line.**
- **Flow cytometry quantified an overall increase of ~70% in GFP levels in the *hTRAF6* line.**

Discussion and Future Directions

TRAF6 plays an important role in immunity and embryonic development. Studying the bivalent mechanisms involved allows us to understand how DNA sequence affects the epigenetic state and *TRAF6* itself.

- In the future, we will investigate other body parts giving a more robust understanding of its role in development.
- We will also look for other epigenetic factors that are involved in impacting the epigenetic state.

References

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6. Figure 1,3, and 4 created in BioRender. Figure 5A is from Encyclopædia Britannica.

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