The Role of miRNAs in Regulation of Chromatin Bivalency

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Abstract

Sperm and eggs have the unique ability to generate a new embryo at fertilization, but the factors that allow them to do so are still unknown. The simultaneous presence of activating and repressing histone chemical modifications, known as bivalent chromatin, have been identified throughout spermatogenesis(1). The evolutionarily conserved occurrence of this unique state at genes central to embryo development suggests essential involvement in embryonic development of mammals. The regulatory role of bivalency and the mechanisms which control its variation during sperm production have been scarcely studied. Our lab has discovered that Ago2, a key protein regulator of the microRNA (miRNA) pathway, binds to specific regions of nuclear chromatin in spermatogenesis. Based on this preliminary data, we hypothesize that one of the key factors in regulating bivalency throughout sperm maturation is miRNAs. There are no known protein regulators that control the entire bivalent state suggesting that a noncoding molecule such as miRNAs could play a role. We will test the presence of Ago2 in the nucleus of spermatogenic cells using immunohistochemistry (IHC) paired with immunofluorescence (IF). We will then use crosslinking immunoprecipitation (CLIP) to identify the miRNAs binding to Ago2 in the nucleus. We expect the results from these experiments to help us understand the role of bivalency and miRNA regulation in human male fertility.

Methods

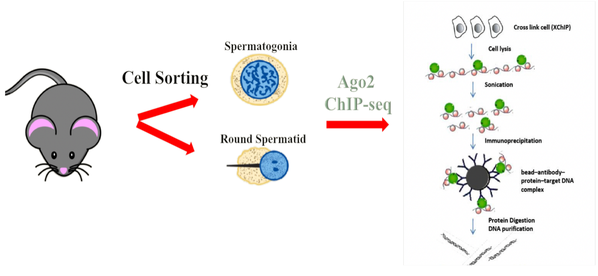


Figure 1. **Sample preparation workflow.**
Cell sorting was used to separate round spermatids and spermatogonia from whole testes sample.
- Spermatogonia- Antibody specific to the C-Kit protein was used to identify spermatogonia.
- Round Spermatid- Spermatids have half the DNA content of a somatic cell so dye was used to identify DNA concentration in round spermatids.

Ago2 ChIP-seq: Allows for the analysis of interactions between Ago2 and the genome(3).

Background

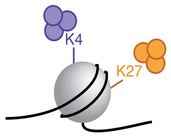


Figure 2. **Bivalent state.**
The activating H3K4me3 and repressing H3K27me3 chemical modifications simultaneously present on the same nucleosome

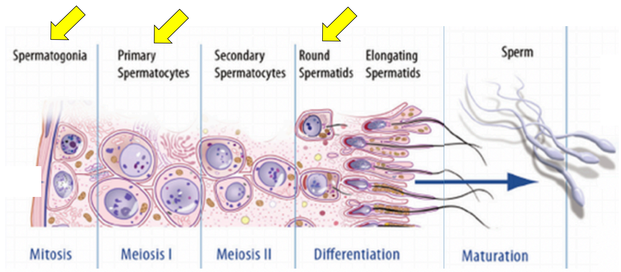


Figure 3. **Spermatogenesis.**
Our study compares different stages of sperm development- spermatogonia, pachytene spermatocytes, and round spermatids.

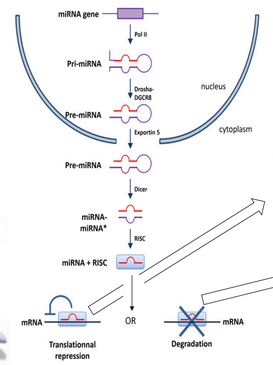
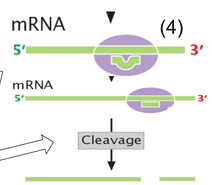


Figure 4. **microRNA biogenesis pathway.**
After processing, miRNAs are bound by Ago2 to perform various functions(2).



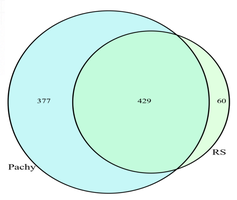


Figure 5. **Dynamic bivalency.**
Bivalency has been found to be dynamic throughout spermatogenesis.
Pachytene Spermatocytes- Shows early stage spermatogenic (Pachy) genes marked by bivalency
Round Spermatids- Shows late stage spermatogenic (RS) genes marked by bivalency

Results

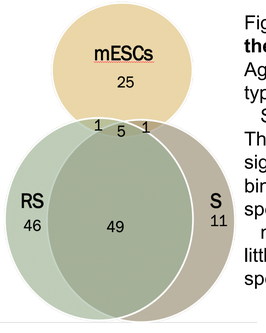


Figure 6. **Ago2 localization in the nucleus.**
Ago2 binding patterns are cell type specific.
Spermatogenic Cells- There is a significant overlap in Ago2 binding throughout spermatogenesis.
mESCs- The control shows little overlap in Ago2 binding with sperm cells.

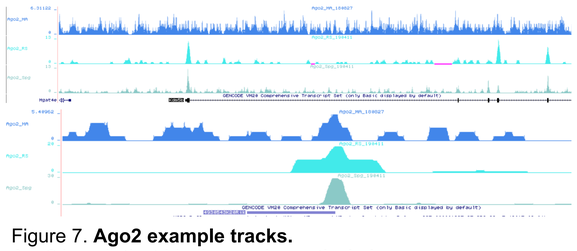


Figure 7. **Ago2 example tracks.**
Ago2 has distinct binding peaks particularly at the transcription start sites of genes.
Top- mESCs
Middle- Round Spermatids
Bottom- Spermatogonia

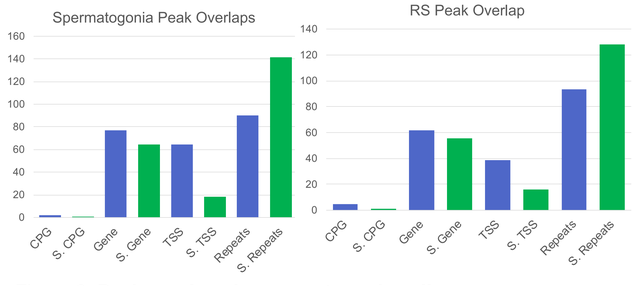


Figure 8. **Peak overlaps in spermatogenic cells.**
Enrichment at transcription start sites is shown to be significant.

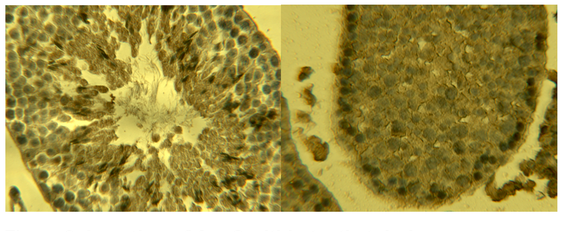


Figure 9. **Location of Ago2 within testis tubule.**
Left- PRM1 as positive control
Right- Presence of Ago2 within the nucleus of spermatogenic cells.

Summary

- Ago2 is present and interacting with nuclear chromatin in both spermatogonia and round spermatids
- Ago2 is present and interacting with the genome
- Binding patterns differ between the two cell types with more overlap occurring between spermatogenic populations relative to mESCs
- There is enrichment at transcription start sites of genes

Discussion

- Binding patterns change throughout spermatogenesis which may be a result of differences in the miRNAs binding to Ago2
- The significant binding of Ago2 at transcription start sites suggests that Ago2 is interacting with mRNAs as they are transcribed from DNA.
- If miRNAs and Ago2 are regulating bivalency then the various binding patterns between cell types can explain why bivalency is dynamic throughout spermatogenesis.

Future Directions

- Repeat ChIP-seq to further validate binding sites
- Run IF with IHC to look at localization of Ago2 in the nucleus
- Preform CLIP to determine which miRNAs are being bound
- Inhibit miRNAs to see if it effects bivalency by: a) knocking out Ago2 or b) directly inhibit specific miRNAs with miRNA sponges

References

1. Lesch, B J, et al. “Parallel Evolution of Male Germline Epigenetic Poising and Somatic Development in Animals.” Nature Genetics., Aug. 2016.
2. Devaux, Yvan & Stammet, Pascal & Friberg, Hans & Hassager, Christian & Kuiper, Michael & Wise, Matt & Nielsen, Niklas. (2015). MicroRNAs: New biomarkers and therapeutic targets after cardiac arrest?
3. Bettegowda, A. and M. F. Wilkinson (2010). "Transcription and post-transcriptional regulation of spermatogenesis." Philosophical Transactions of the Royal Society B: Biological Sciences 365(1546): 1637-1651.
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