Identification of Ribbon Transcriptional Targets in the Adult *Drosophila* Testis

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Abstract

Past research has demonstrated that the the transcription factor Ribbon (Rib) plays an essential role in *Drosophila* testis development and function. Loss of function of *rib* results in a failure of somatic cells to coalesce to form a gonad in the developing embryo. More recent work from our lab has shown that increased expression of *rib* throughout development results in significant morphology defects in the adult testis. If overexpression is limited to germ cells and somatic cells in the adult, more mild defects are observed. In order to better understand how Rib regulates spermatogenesis, we are interested in identifying its transcriptional targets in this context. We compared gene expression in control testes and testes in which *rib* was overexpressed in somatic cells by RNA-Sequencing. Utilizing the Kallisto-Sleuth pipeline, gene expression levels of *rib* overexpression flies were compared to the control, resulting in the identification of 301 genes with statistically significant changes in gene expression. In order to filter results, the differentially-expressed genes were compared to a data set of genes that are known to be expressed in the somatic cells of testes.. Additionally, differentially-expressed genes underwent gene ontology analysis. Given Rib's previously described roles in cell morphogenesis and cell-cell interactions during development, we were particularly interested in genes involved in cell adhesion and regulation of the cytoskeleton. Of these 301 genes, approximately 20 genes of interest were selected for additional analysis due to their implications in cell adhesion or the cytoskeleton. Follow-up experiments are currently being conducted to validate select genes of interest as targets of Rib.

Background on Ribbon (Rib)

- Transcription factor with a BTB domain and Pipsqueak (Psq) motif
- Helps direct cell migration

Figure 1. Molecular Structure of Ribbon. Silva D, Olsen KW, Bednarz MN, Droste A, Lenkeit CP, Chaharbakhshi E, et al. (2016). Regulation of Gonad Morphogenesis in Drosophila melanogaster by BTB Family Transcription Factors. PLoS ONE 11(11): e0167283. doi:10.1371/journal. pone.0167283

rib Overexpression is Detrimental to Testes Development

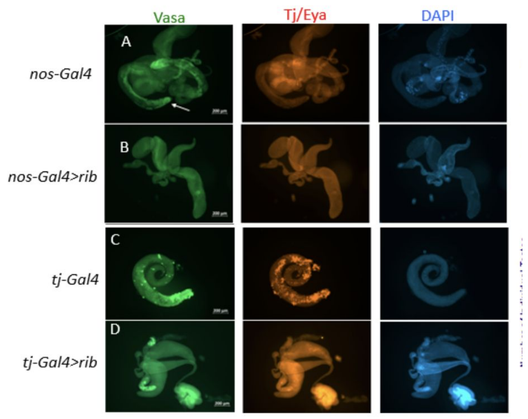


Figure 2. Overexpression of *rib* in germ cells using *nanos (nos)-Gal4* and somatic cells using *traffic jam (tj)-Gal4*. A-D. 1-3 day old males. Germ cells (Vasa, green), somatic cells (Tj/Eya, red), and DAPI (blue). Alvarez, Khan, Moqeet, unpublished.

The *Drosophila* Testis

Figure 3. The *Drosophila* Testis. Gleason RJ, Anand A, Kai T, Chen X. Protecting and Diversifying the Germline. Genetics. 2018 Feb;208(2):435-471. doi: 10.1534/genetics.117.300208. PMID: 29378808; PMCID: PMC5788515.

Step One: Overexpressing *rib* in the Testis

Control: *w^{118}; tj-Gal4; gal80^{ts}/ +*

Experimental: *w^{118}; tj-Gal4; gal80^{ts}/ UAS-rib*

- Flies raised at 18℃
  - Gal80 is functional at low temps (inhibits Gal4, which prevents the transcription of *rib*)
- Moved to 29℃
  - "Turns off" Gal80, allowing for the transcription of *rib*

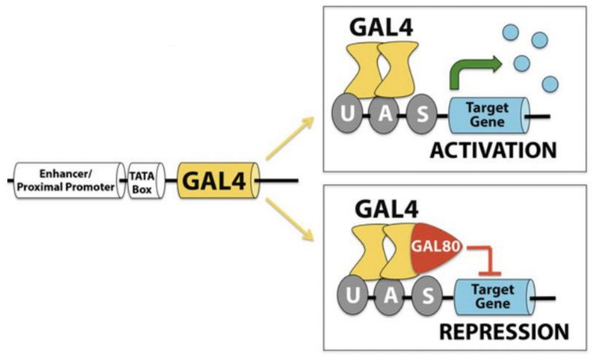


Figure 4. Function of GAL4 and GAL80. Silva, D., & Jemc, J. C. (2015). Sorting Out Identities: An Educational Primer for Use with "Novel Tools for Genetic Manipulation of Follicle Stem Cells in the Drosophila Ovary Reveal an Integrin-Dependent Transition from Quiescence to Proliferation." *Genetics*, *201*(1), 13–22. https://doi.org/10.1534/genetics.115.179911

Step Two: RNA Extraction and Sequencing

- Mature testes from both *rib* overexpression and control flies were dissected
- RNA was isolated using TRIzol and purified using a Zymo Direct-zol RNA kit
- Sequenced at Novogene using an Illumina Hi-Seq
  - 150 b.p., paired-end reads
- Experiment was done in triplicate, with 50 pairs of testes used per sequencing run

Step Three: Differential Expression Analysis

- Reads were quantified using Kallisto
- After quantification, DE analysis was conducted using Sleuth
  - Genes with p-value > 0.05 or FC < 1 were filtered out
- 1497 DE genes were identified through this process

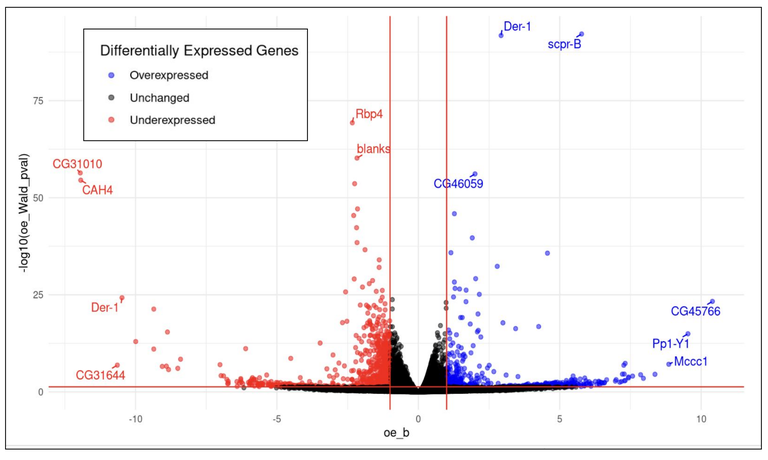


Figure 5. Volcano Plot of Differentially-Expressed Genes Under *rib* Overexpression

Step 4: Comparisons With Adashev et al.

- Initial DE analysis produced an unmanageable list of genes
  - Needed to narrow down the list
- In a different lab, Adashev *et al.* compared RNA transcript abundances between early and late somatic cyst cells in *Drosophila*
  - Strong baseline to which our data could be compared
- Reanalyzed the Adashev data using the Kallisto/Sleuth pipeline
- Compared the Adashev output to our output
  - Narrowed the initial 1497 genes to 301 potential targets

Step 5: Selecting Genes of Interest

- Plan to validate that certain genes of interest are targets of *rib*
  - Immunohistochemistry
  - qRT-PCR
- Identified genes in the list of 301 potential targets that have implications in epidermal growth or cell morphology (cytoskeleton, adhesion)
  - Determined which of these genes had available reagents
- Selected approximately 20 genes of interest for follow-up testing——

Table 1. Examples of Selected Genes of Interest

Gene	Known Function(s)
Mmp1	Matrix metalloprotease/ECM/cell migration
ten-m	Interacts intracellularly with cytoskeleton regulatory proteins
shot	Binds both actin and microtubules
rhea	Encodes fly talin (essential for all adhesive functions of integrins)
chic	Actin monomer binding protein
mys	□ subunit of the integrin dimer

Conclusion & Next Steps

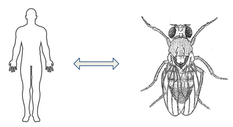
Ultimately, we hope to identify transcriptional targets of Rib to understand how Rib and its homologs regulate spermatogenesis. At this time, RNA-Seq analysis has been completed, and wet-lab testing is being planned to validate select genes of interest as targets of Rib.

Next Steps:

1. Immunohistochemistry and qRT-PCR experiments
2. Time permitting, genes of interest with unknown functions may undergo RNAi knockdown for basic characterization of gene function

Broader Impacts

- *Drosophila melanogaster* is a model organism
- Findings in *Drosophila* impact our understanding of other organisms, including humans
- Humans have a homolog of Rib called BTBD18
  - The results of this study may improve our understanding of BTBD18's role in human testis development
- Understanding testis development is foundational to understanding reproduction, one of the most essential biological processes



Acknowledgements

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