Investigating the Effect of the Nab2 RNA-Binding Protein on CircMbl Biogenesis in *Drosophila melanogaster*

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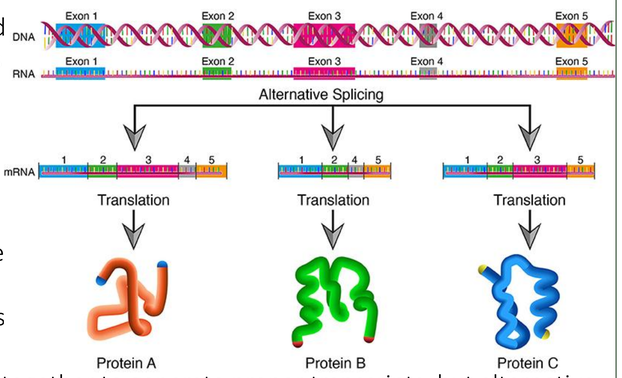
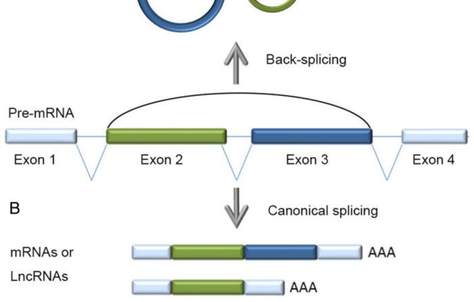
Introduction

- DNA is transcribed into RNA, which is translated into protein.
- Proteins carry out functions in cells.
- Within genes, coding regions are exons, and noncoding regions are introns.

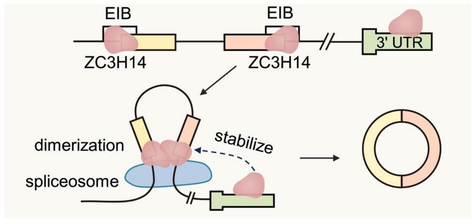


- Exons are spliced together to generate proper transcripts, but alternative splicing can lead to non-canonical transcripts.



- One type of alternative splicing forms circRNAs via back-splicing.
- CircRNAs rarely encode any protein and are resilient to degradation.
- Other functional roles have remained unclear since their discovery.
- Several mechanisms can lead to back-splicing.

- One common mechanism is assisted by the formation of temporary base-pairs in introns adjacent to a circularizing exon, bringing the splice sites into close proximity.
- The RNA-binding protein Nab2/ZC3H14 can facilitate back-splicing by binding to intron-exon boundaries and bring them into proximity for splicing.



ZC3H14 facilitates backsplicing

Research Question

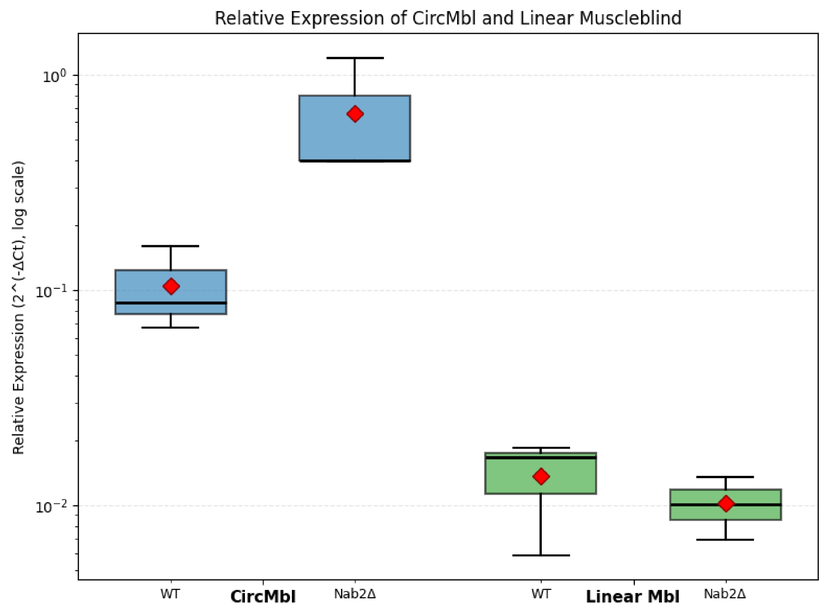
Does the presence of the Nab2 RNA-binding protein affect the biosynthesis and abundance of circMbl from *Muscleblind* transcripts?

Aims:

- Compare circMbl abundance levels in Nab2-knockout and wild-type *Drosophila* brain tissue
- Analyze sequence conservation of the back-spliced region of circMbl

Results

CircMbl expression was ~9-fold greater in larval brain tissue of Nab2-knockout mutants compared to wild type, a substantial difference which demonstrates practical significance (p = 0.1038, d = 1.7, n=3).



- RNA was isolated from ~60 larval fly brains per biological replicate and used to generate cDNA libraries.
- QPCR data was collected from three biological replicates using three primer pairs, targeting circMbl, linear Muscleblind, and RP49.
- Data was normalized using RP49, followed by a fold change calculation to find relative expression.
- Statistical analysis included a two-tailed t-test and effect size calculation.

Synthesis of circMbl via back-splicing occurs at the canonical intron-exon boundary in both wild type and Nab2-knockout

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                    131       140       150       160       170       180       190       200
                    |---------+---------+---------+---------+---------+---------+---------+
Nab2Δ Forward       GCAAGGTCACCGCCTGCTACGACAGCATCAAG-ATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
WT Forward          GCAAGGTCACCGCCTGCTACGACAGCATCAAG-ATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
Nab2Δ Reverse       GCAAGGTCACCGCCTGCTACGACAGCATCAAG-ATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
WT Reverse          GCAAGGTCACCGCCTGCTACGACAGCATCAAG-ATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
Exon 2 Boundary     GCAAGGTCACCGCCTGCTACGACAGCATCAAGNATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
Consensus           GCAAGGTCACCGCCTGCTACGACAGCATCAAG.ATAATGTAAACTCAGCTTACACACAAAAAAGCAGTAAAATATA
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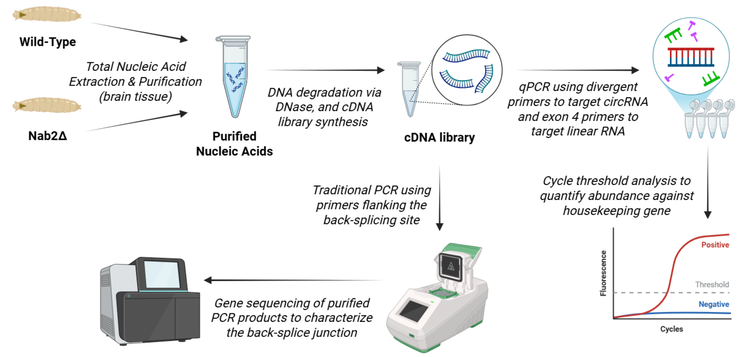
- The back-spliced region of circMbl was amplified using divergent primers, generating PCR amplicons of this site.
- These amplicons were sequenced and aligned, demonstrating complete conservation as shown above.
- The sequenced PCR product was consistent with canonical exon 2 boundaries.

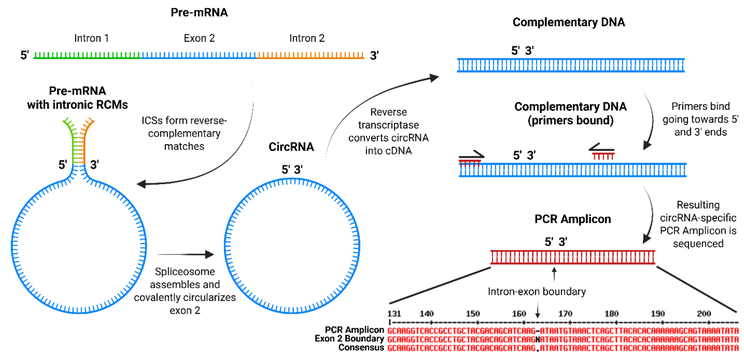
Methodology

Hypothesis: The presence of Nab2 affects the frequency of circMbl biogenesis, and therefore circMbl abundance.



- Dissected and extracted DNA & RNA from fly larvae brain tissue
- Purified RNA and synthesized a complementary cDNA library
- Tested abundances of linear and circMbl using quantitative PCR
- Amplified and sequenced the back-spliced region of circMbl

Divergent Primers, Circularization, and Reverse Transcriptase:



- Reverse transcriptase makes a DNA copy of RNA sequences
- Divergent primers bind to the DNA copy but only amplify if the RNA was circularized

Conclusions and Future Work

Conclusion: CircMbl biosynthesis in *Drosophila* is upregulated when Nab2 is absent, which is the opposite of Nab2's typical effect on circularization.

Future aims:

- Testing the binding specificity of the Nab2 protein
- Determining localization and potential sequestration of Nab2 and Muscleblind by excess circMbl

Works Cited: Yu, C.-Y.; Kuo, H.-C. The Emerging Roles and Functions of Circular RNAs and Their Generation. J Biomed Sci 2019, 26 (1), 29. ; Li, Q.; Yang, et.al. ZC3H14 Facilitates Backsplicing by Binding to Exon-Intron Boundary and 3' UTR. Molecular Cell 2024, 84 (22).