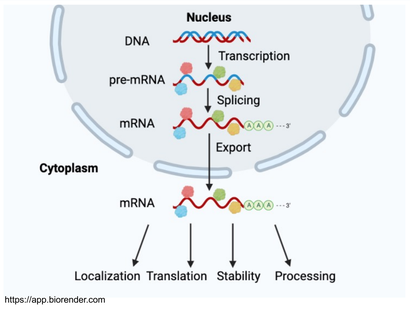
Investigation of splicing changes in dNab2 knockdown *Drosophila* neurons

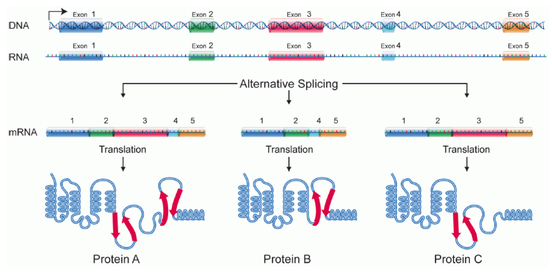
Authors: Katherine Shelmidine, Dr. Seth Kelly
Biochemistry and Molecular Biology Program - The College of Wooster

Gene splicing and expression



https://app.biorender.com

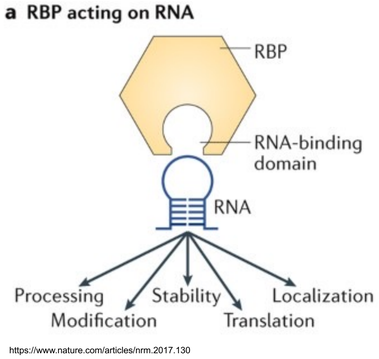
- Mutations in genes that encode for RNA binding proteins can lead to variations in splicing resulting in incorrect alternative splicing or the retention of certain introns.
- Intron retention can impact overall gene expression and potentially the proteins being produced (Vuong et al., 2017).



https://bioprinciples.biosci.gatech.edu/module-4-genes-and-genomes/4/-8-genomes

ZC3H14 as an RNA binding protein

- Mutations that cause changes in splicing are linked to a variety of muscular and neuronal conditions (Pak et al., 2011; Poulos et al., 2011).
- ZC3H14 is a gene in humans that is essential for brain development and neural function. It encodes for the ZC3H14 RNA binding protein which regulates poly(A) tail length control, brain development, and the production of synaptic proteins.
- Mutations in the ZC3H14 gene is linked to increased prevalence of intellectual disability (Kelly et al., 2014; Musante et al., 2014).



https://www.nature.com/articles/nrm.2017.130

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NIH National Institutes of Health

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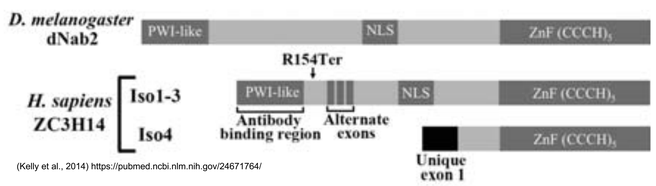
Drosophila dNab2 is an ortholog of ZC3H14

- ZC3H14 has a functionally comparable ortholog in *Drosophila melanogaster* called dNab2.
- Both play a role in polyadenylation control and splicing regulation.
- dNab2 and ZC3H14 have a similar set of domains including a zinc finger at the C-terminus end (Kelly at al., 2014).



(Kelly et al., 2014) https://pubmed.ncbi.nlm.nih.gov/24671764/

- Lack of dNab2 causes changes in gene expression, splicing, and observable phenotypes in behavior and neuronal function.
- Due to these developmental defects, flies that lack the dNab2 gene typically die before reaching adulthood (Kelly et al., 2014).

Genotype	% Adult survival
***dNab2* null** (*ex3/ex3*)	~3%
+ *dNab2* transgene alone (*ex3/ex3;UAS-dNab2^{Flag}*)	39%
+ *dNab2* expressed in neurons (*ex3/ex3,elav>Gal4,UAS-dNab2^{Flag}*)	93%
+ *Iso1 ZC3H14* expressed in neurons (*ex3/ex3,elav>Gal4,UAS-ZC3H14-iso1^{Flag}*)	78%

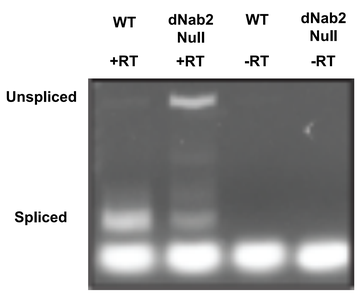
(Kelly et al., 2014) https://pubmed.ncbi.nlm.nih.gov/24671764/

- **This shows that ZC3H14 can compensate for dNab2 loss.**

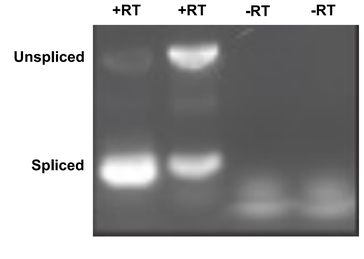
Previous splicing data

- Previously collected RT-PCR data from larval brain tissue shows that *Drosophila* lacking dNab2 experience altered splicing and what appears as a retained first intron in numerous genes responsible for proper CNS development.
- The dNab2 null flies also demonstrated higher relative gene expression levels compared to the wild-type.

Ca-Alpha1D isoforms (K,L,M,N,O)



CASK isoforms (D,E,G)



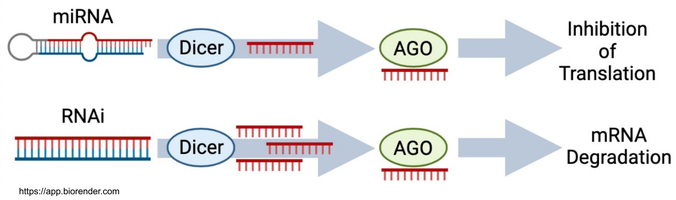
Results: Loss of dNab2 alters splicing of Ca-Alpha1D and CASK, exhibiting significantly more unspliced transcript compared to the wild-type. This indicates possible retention of the first intron.

Research Question

- It is unknown what dNab2 specifically interacts with that results in this defect in gene splicing and expression due to dNab2 loss.
- The previous experiments examined all cell types of larval brain tissue and did not examine which specific brain cell types undergo this altered splicing.
- This study also looks at adult flies in addition to larvae to see if different stages of development potentially impact splicing.
- **Will the splicing defect due to knocked down levels of dNab2 occur in neurons of adult flies and larvae?**

Using RNAi to knock down dNab2 levels

- RNA interference (RNAi) produces small RNA fragments called inverted repeats (IR) that are complementary to the target mRNA to reduce translation and therefore overall protein production (Mohr, 2014).



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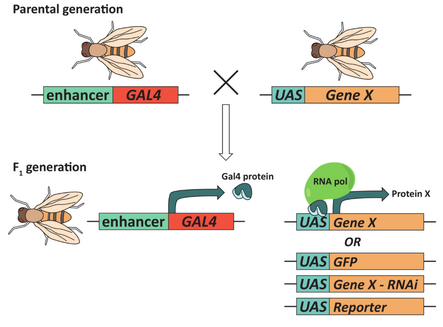
Gal4/UAS System

- Gal4 is a transcription factor used to produce mRNA in a specific tissue type (Brand et al., 1993).
- The Gal4/UAS system targeted mRNA knockdown of dNab2 in neurons.
- I used flies containing elav-Gal4 to drive neuronal gene expression and promote Gal4 transcription.
- These flies were crossed with those containing an upstream activating sequence (UAS) for Gal4 to bind to and initiate transcription of the desired genes.
- These flies also contain an IR sequence that is used to create short hairpin RNAs to decrease dNab2 expression.



Kelly, et, al. 2017 https://www.jove.com/t/56174/dissection-immunofluorescent-staining-mushroom-body-photoreceptor

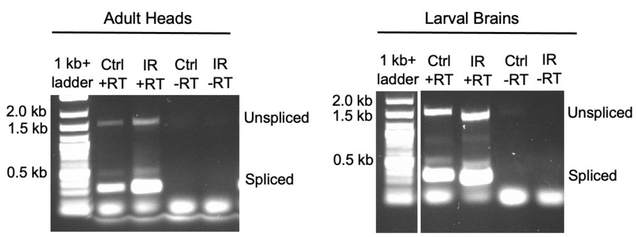
Methods

RNA → **cDNA** → **RT-PCR**

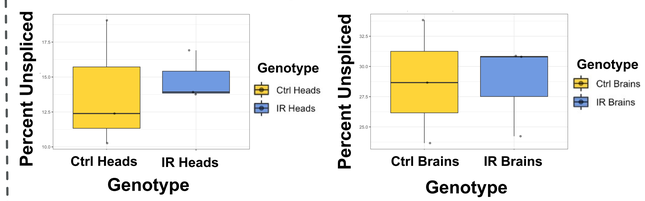
- RNA was isolated and purified from the adult fly heads and larval brain tissue in the Gal4 control and dNab2-IR experimental groups.
- cDNA (complementary DNA) was synthesized from the collected RNA samples using reverse transcriptase.
- Semi-Quantitative RT-PCR was run on the cDNA with gene specific primers to target and amplify the first intron in Ca-Alpha1D and CASK.

Gel Electrophoresis Quantification

CASK isoforms (D,E,G)

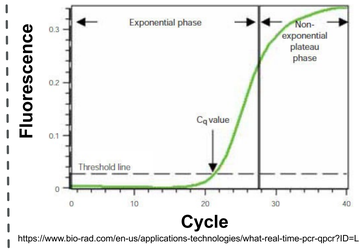


Percent Unspliced for CASK



Results: Knocked down levels of dNab2 did not significantly alter splicing of CASK, only raised the average percentage of unspliced transcript.

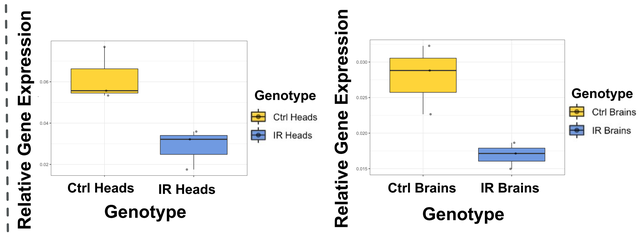
qRT-PCR quantification of dNab2 levels



https://www.bio-rad.com/en-us/applications-technologies/what-real-time-pcr-qpcr?ID=LUSO4W8UU

- qRT-PCR uses a fluorescent tag to examine if there is a change in the amount of the transcript.

dNab2 mRNA Levels



Results: The adult fly heads showed a fold change value of 0.46 indicating dNab2 expression levels were knocked down by about 54% in the IR compared to the control ($p<0.05$). The larval brains had a fold change of 0.61 and dNab2 knockdown by 39% ($p<0.05$).

Conclusion

- The dNab2-IR flies did not demonstrate the same level of unspliced transcript as previous data with dNab2 null flies.
- Potentially, the level of dNab2 was not knocked down enough to produce the splicing defect.
- Alternatively, neurons might not experience altered splicing of Ca-Alpha1D and CASK when dNab2 is decreased.

Future Directions:

- Increase the amount of dNab2 knockdown using a UAS-dicer.
- Use a different Gal4 to target another tissue type (ex. glia).