Evaluating TDP-43 Targets in Amyotrophic Lateral Sclerosis Using *Drosophila* and Patient Spinal Cords

Alexander Blythe[1], Erik Lehmkuhl[1], Suvitha Loganathan[1], Eric Alsop[4], Dianne Barrameda[1], Bhavani B. Siddegowda[1], Archi Joardar[1], Kendall Jensen[4], Daniela C. Zarnescu[1,2,3]

[1]Department of Molecular and Cellular Biology, [2]Department of Neuroscience, [3]Department of Neurology, [4]Translational Genomics Research Institute

Introduction and Background

In 97% of ALS cases and about 45% of frontotemporal dementia cases, insoluble cytoplasmic aggregates containing highly phosphorylated TDP-43 have been identified as pathological hallmarks[1]. Considering these complexes and their noted neurotoxicity, a current hypothesis for TDP-43's potential role in modifying neurodegenerative phenotypes during ALS is the ribostasis hypothesis, which posits that TDP-43 binds to and sequesters mRNAs into cytoplasmic insoluble complexes in motor neurons. These aggregates result in translational repression of affected mRNAs and severely dysregulate protein homeostasis, leading to motor neuron stress and eventually death[2].

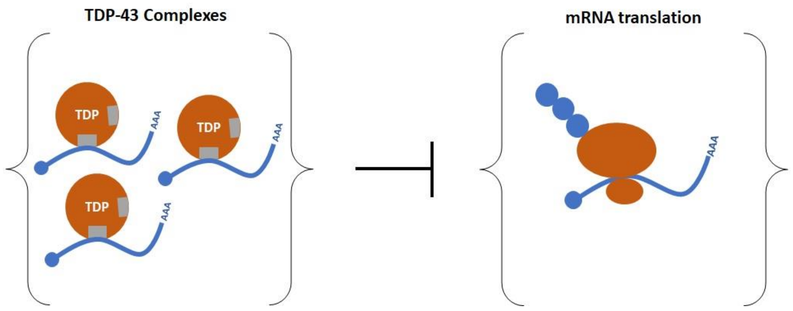


Figure 1. ***Ribostasis hypothesis***: Cytoplasmic TDP-43-induced sequestration of mRNAs into insoluble complexes results in translational repression of affected mRNAs, thereby lowering protein levels and disrupting proteostasis in motor neurons.

A *Drosophila* model of ALS has been developed by overexpressing either wild-type or mutant TDP-43 in motor neurons, using a motor neuron-specific D42 driver with different versions of the TDP-43 bound to UAS promoters. These models have demonstrated ALS phenotypes including cytoplasmic TDP-43 aggregates, reduced lifespan, sleep fragmentation, and locomotor dysfunction[3]. Following tagged ribosome affinity purifications (TRAP) and RNA immunoprecipitations (RIP), potential mRNA candidates with both low ribosome association and co-precipitation with TDP-43 have been identified[4]. One such candidate is **dally-like protein (*DLP*)**, a glypican present in the extracellular matrix of developing motor neurons. By determining if changed *DLP* levels exacerbate or rescue ALS phenotypes in developing larvae and observing if *DLP* protein levels are altered in CNS tissue and muscle-neuron junctions in disease models, we hope to determine if *DLP* is translationally repressed by TDP-43 proteinopathy in ALS. We also hope to determine if these results are consistent in human spinal tissue when observing the human orthologs of *DLP*, **glypicans 4 and 6 (*GPC4* and *GPC6*)**.

Effect of *DLP* mRNA Levels on Larval Motor Function

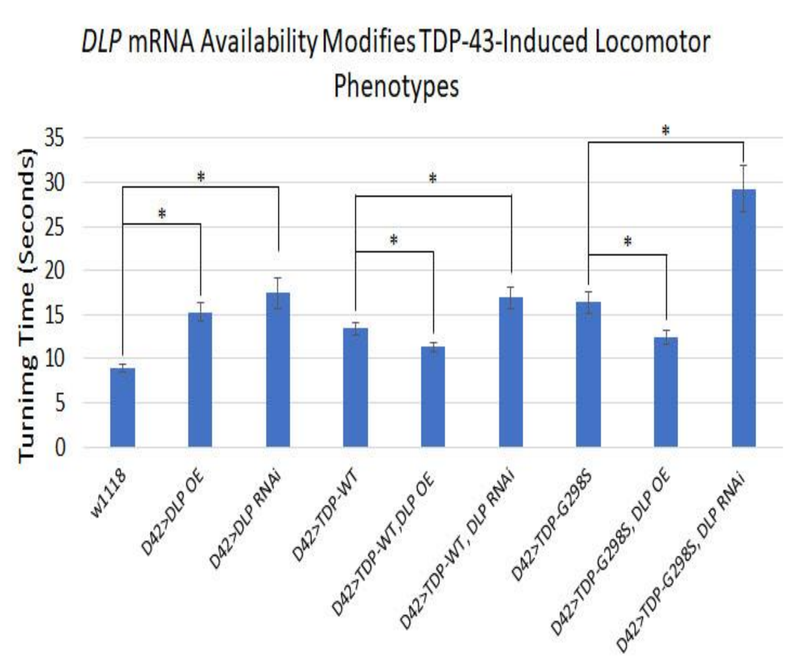


Figure 2. Larval turning times were used to assay locomotor function in 3rd instar larvae. *DLP* overexpression (OE) resulted in a rescue of locomotor function while DLP knockdown (RNAi) exacerbated TDP-43-induced locomotor dysfunction. N=33-40. Asterisks (*) indicate p-values less than 0.05. Significance tests were performed using the Mann U Whitney Test.

DLP Visualized at the Larval Neuromuscular Junction

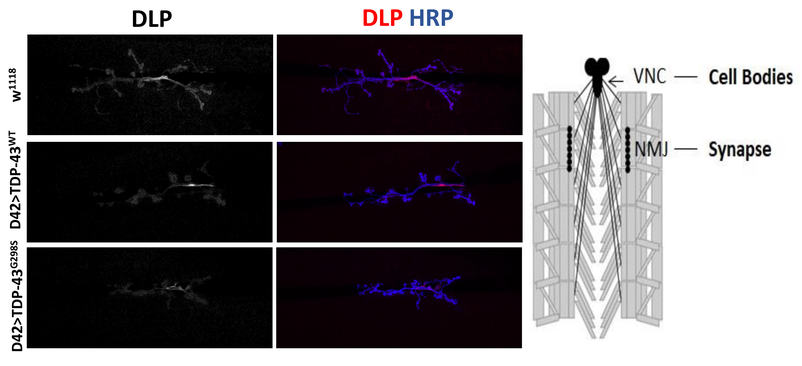


Figure 3. Images of dissected larval neuromuscular junctions (NMJ) treated with immunofluorescent staining. Horseradish peroxidase (HRP) is used as a control to emphasize changes in *DLP* levels. An additional diagram showing the different nervous tissue types in developing Drosophila larvae is shown.

DLP Levels in VNCs and NMJs During TDP-43 Proteinopathy

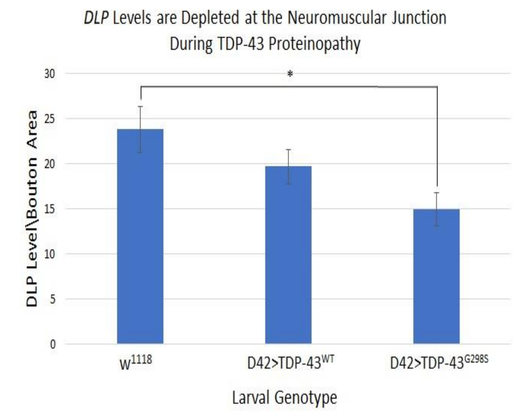


Figure 4. Quantification of *DLP* levels in 4 terminal boutons at the NMJ of 3rd instar larvae relative to bouton size. N=3. Comparison of w^{1118} to D42>TDP-43^{G298S} yields a p-value of 0.044. Asterisks (*) denote p-values less than 0.05.

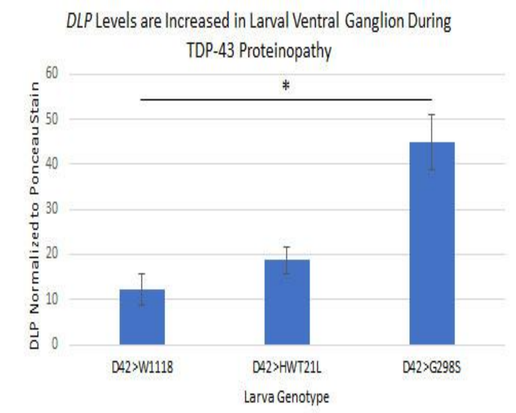


Figure 5. Quantification of *DLP* levels in the ventral nerve cords (VNCs) of 3rd instar larvae. Western blots were run on homogenized VNCs from control larvae and larvae expressing TDP-43^{WT} and TDP-43^{G298S} in the motor neurons. 10 VNCs were used in each sample. DLP intensity was normalized to Ponceau stain.

Effect of *DLP* mRNA Levels on Adult *Drosophila* Lifespan

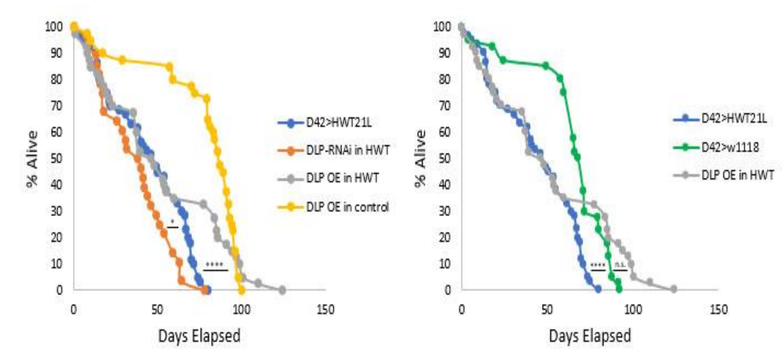
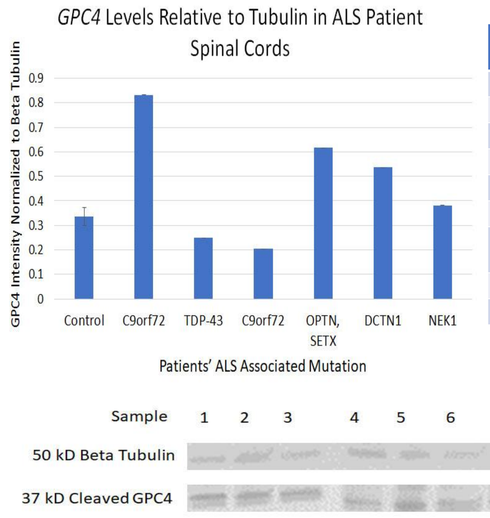


Figure 6. Lifespan assay for flies kept inside a fridge set at 22 °C in vials containing yeast-based fly food. Flies were flipped into new vials every week. An insignificant amount (<8) flies expressing motor neuron-specific TDP-43^{G298S} survived past the pupation stage, regardless of *DLP* overexpression or knockdown, and were thus not included. A single asterisk (*) denotes p-values of less than 0.05, and four asterisks (****) denote p-values of less than 0.0001. **DLP OE in D42>HWT21L resulted in a significant rescue** of lifespan compared to the controls.

DLP Human Ortholog (*GPC4*) Levels in Spinal Tissue



Sample Type	Spinal Region	TDP-43 Pathology	Disease Mutation
Control	Thoracic	No	NA
Control	Lumbar	No	NA
Control	Cervical	No	NA
Disease	Thoracic	Unknown	C9orf72
Disease	Lumbar	Yes	TDP-43
Disease	Cervical	Yes	C9orf72
Disease	Lumbar	Yes	OPTN, SETX
Disease	Lumbar	Yes	DCTN1
Disease	Lumbar	Yes	NEK1

Figure 6. Quantification of *GPC4* levels in human spinal cord tissue from Western blots performed on both ALS- and non-ALS-afflicted patients. Beta tubulin was used as a normalizing gene. *GPC4* levels are highly variable across spinal region and disease mutation.

Conclusions

- ***DLP* overexpression (OE) resulted in a rescue of locomotor function while DLP knockdown (RNAi) exacerbated TDP-43-induced locomotor dysfunction.**
- ***GPC4* levels are highly variable across spinal region and disease mutation, but levels are slightly lowered in spinal tissue with the TDP-43 disease mutation.**
- ***DLP* levels are significantly depleted at the neuromuscular junction but increased in the ventral ganglion in 3rd instar larvae expressing the mutant form of TDP-43 compared to the w^{1118} control.**
- **Motor neuron-specific *DLP* overexpression appears to result in increased lifespan in adult flies expressing wild-type TDP-43, while DLP knockdown appears deleterious to lifespan.**
- **Manipulation of *DLP* mRNA levels in *Drosophila* overexpressing the G298S mutant form of TDP-43 appears to be lethal at the pupal stage.**

References

1. Ling, S., Polymenidou, M., & Cleveland, D. W. (2015). Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis. *Neuron*, *79*(3), 416–438.
2. Coyne, A. N., Zaepfel, B. L., & Zarnescu, D. C. (2017). Failure to Deliver and Translate—New Insights into RNA Dysregulation in ALS. *Frontiers in Cellular Neuroscience*, *11*(August), 1–13.
3. Yang, C., Wang, H., Qiao, T., Yang, B., Aliaga, L., Qiu, L., … Xu, Z. (2014). Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(12), 1121–1129.
4. Lehmkuhl, E. M., & Zarnescu, D. C. (2012). Lost in Translation: Evidence for Protein Synthesis Deficits in ALS/FTD and Related Neurodegenerative Diseases. *Current Chemical Biology*, *5*(2), 90–98.

Acknowledgements

Funding for this project was provided by the Undergraduate Biology Research Program at the University of Arizona. Spinal tissue was provided by the United States Veteran's Affairs Department of Southern Arizona. Additional funding was provided by NIH T32-GM00869 (to Erik Lehmkuhl), NIH NS091299, MDA RG "Metabolic Dysregulation in ALS," Sandra Harsha Estate (to Daniela Zarnescu).