Targeting TDP-43 Aggregation with Novel Small RNA Chaperones Using the FIT System

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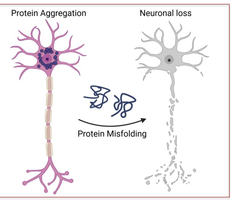
Introduction

TAR DNA-binding protein 43 (TDP-43) is a protein that regulates mRNA expression and gene transcription. Protein aggregation of TDP-43 is a hallmark in many neurodegenerative diseases such as ALS, FTD, and Alzheimer's disease. RNA-bound TDP-43 remains soluble and prevents phase-shifts into the solid aggregate form of TDP-43. Recent findings prove that short RNA chaperones (34 nucleotides) can bind to TDP-43 to prevent aggregation. Project is focused on finding reduced-size RNAs consisting of only 20 nucleotides that prevent TDP-43 aggregation in both wild-type and disease-associated mutant variants.



Methods

100,000 randomized RNA sequences were screened using a feedback inhibition of translation (FIT) system in yeast to determine promising small RNAs. The FIT System sorts through small 20 nucleotide RNAs that have the potential to reduce translation through a protein-mediated negative feedback loop. All small RNAs, RNA 1, RNA Q and RNA K were selected through this process. RNAs were collected through competent cell transformation, and purified RNAs were tested in vitro through aggregation assays against 5 µM TDP-43 variants for a 2-hour period. Additionally, yeast transformation was performed to test the effectiveness of the small RNAs in the cells. WT TDP-43 was harvested, purified, and then used in a serial dilution experiment with RNA 1 in *Figure 4D.*

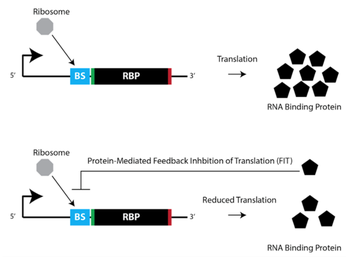


Figure 1. Feedback Inhibition of Translation (FIT) System in Yeast

Protein Purification

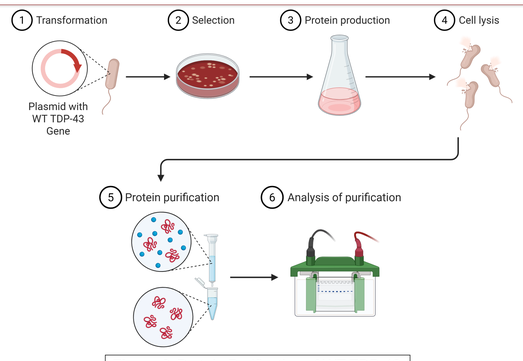


Figure 2. Protein Purification of WT TDP-43

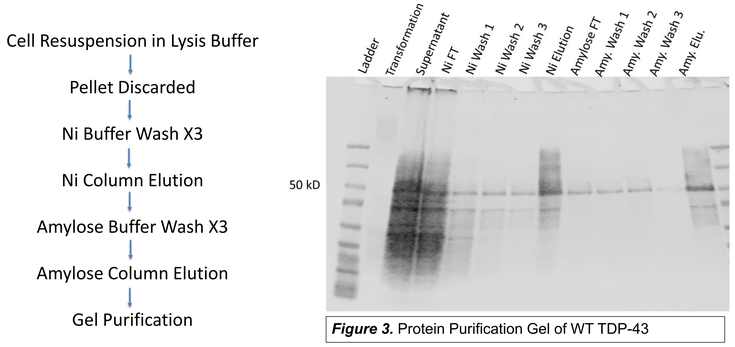


Figure 3. Protein Purification Gel of WT TDP-43

Small RNA vs TDP-43 Aggregation

Small RNAs, RNA 1 and RNA K, are able to prevent aggregation at 5 µM concentrations in WT and Q331K TDP-43 variants. Additionally, at concentrations of 2.5 and 1.25 µM, RNA 1 and RNA K delay aggregation of WT, Q331K, and K181E TDP-43 variants. RNA Q did not prevent aggregation against all TDP-43 variants but has demonstrated to delay aggregation in concentrations of 1.25 µM. Serial dilution of RNA 1 demonstrates that aggregation increases at lower concentrations of RNA. However, at concentrations of 1.25 µM, aggregation is shown to significantly increase.

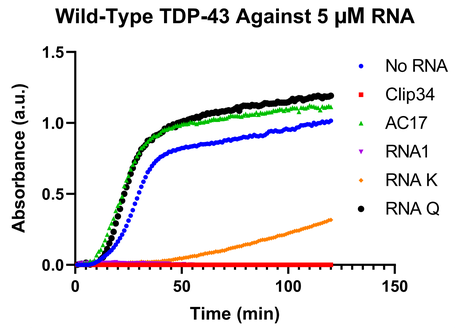


Figure 4. Aggregation assay of WT TDP-43 during a 2-hour period. Normalized to 395 nm.

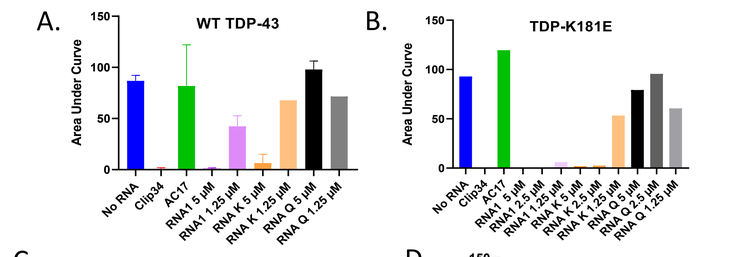


Figure 5. Aggregation of variants TDP-43 against 5, 2.5, 1.25 µM concentrations of RNA.

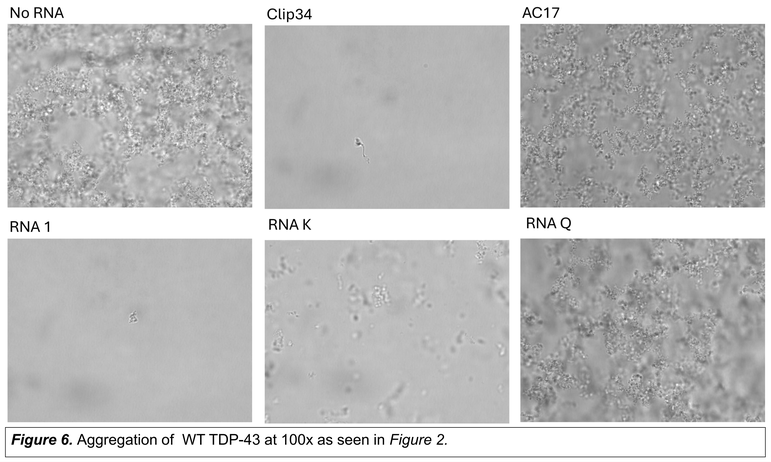


Figure 6. Aggregation of WT TDP-43 at 100x as seen in *Figure 2.*

Yeast Spotting of Small RNA

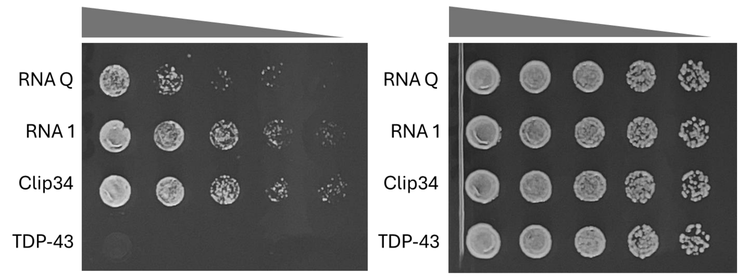


Figure 7. Yeast spotting assay of small RNA 1 and RNA Q

Clip34 and all RNA variants worked against preventing TDP-43 aggregation as demonstrated by growth on the galactose induced plate. Interestingly, RNA-Q demonstrated prevention of TDP-43 toxicity in yeast cells while being unable to prevent TDP-43 aggregation in vitro. While further tests are required to determine the reasoning for this effect, this result demonstrates there could be intracellular interactions that increase the effectiveness of RNA-Q.

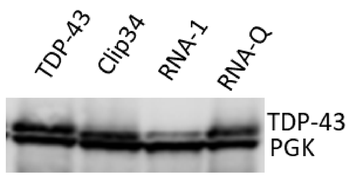


Figure 8. Western Blot of Yeast Spotting Assay Samples with housekeeping gene PGK

Conclusion

- Small RNA 1 and RNA K prevent TDP-43 aggregation at 5 µM and delay aggregation at 1.25 µM concentrations, while RNA-Q did not prevent aggregation.
- Yeast spotting assays suggest that RNA 1 and RNA Q inhibit toxic wild-type TDP-43 aggregation.
- 20 nucleotide RNAs may be a viable therapeutic approach for treating TDP-43 proteinopathy.
- Future directions: 50% inhibitory concentration values, testing in human cells, and fluorescence polarization tests.

Acknowledgements

- Special thanks to Dr. Edward Barbieri, Dr. James Shorter, and the extended Shorter Lab for their endless support and contributions to this research and poster presentation.
- Extended gratitude for the University of Pennsylvania's Summer Undergraduate Internship Program (SUIP) for sponsoring this experience.

Shorter Lab:
- Jim Shorter, Ph.D.
- Edward Barbieri, Ph.D.
- Hana Odeh, Ph.D.
- Linamarie Miller, Ph.D.
- Miriam Linsenmeier, Ph.D.
- Raju Roy, Ph.D.
- JiaBei Lin, Ph.D.
- Zarin Tabassum
- Sabrina Lin
- Tala Qarage
- Zhuoyi Chen
- Johanna Bergstrom
- Nicole Muravsky

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