Human TDP-43 toxicity in fly is modified by diverse pathways

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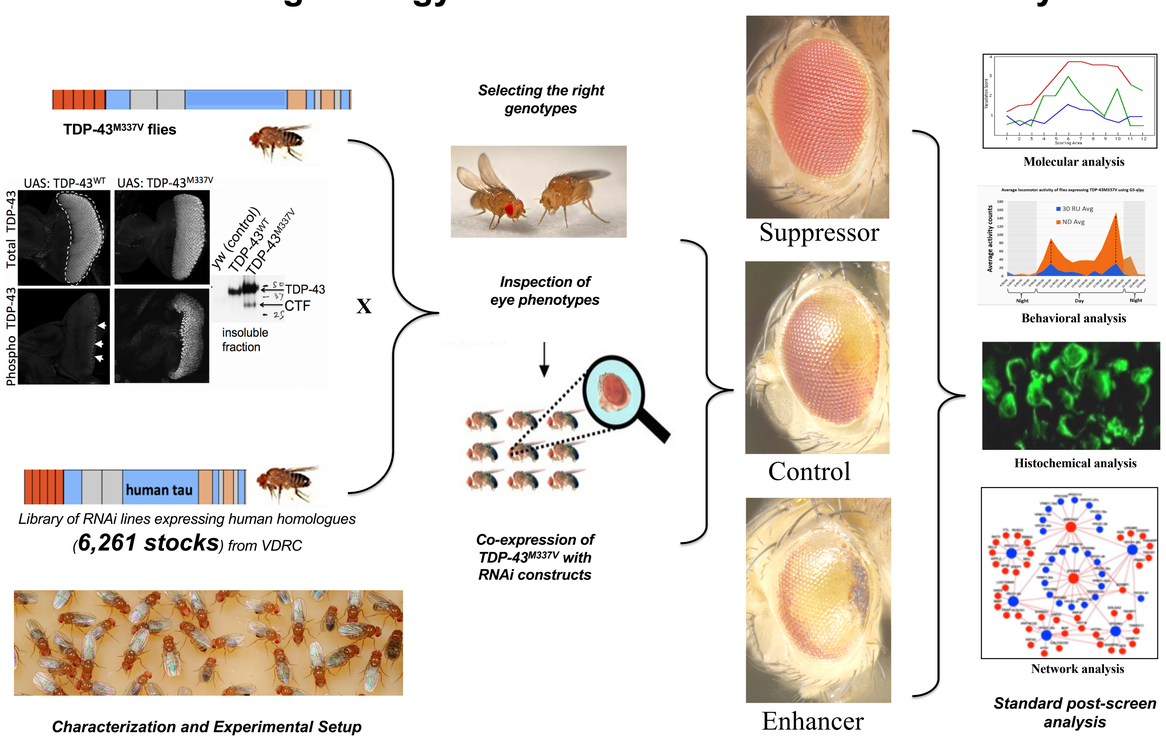
Introduction

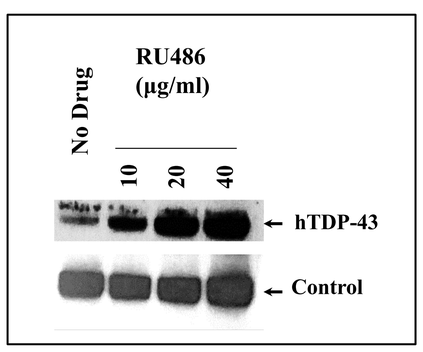
TAR DNA-binding protein 43 (TDP-43) is a highly conserved DNA/RNA binding protein with primarily nuclear distribution. However, it translocates to the cytoplasm and forms pathological aggregates in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) Despite considerable effort to investigate the physiological role of TDP-43, we still have a very limited understanding of the molecular and cellular mechanisms of pathogenesis underlying TDP-43 proteinopathies. A key challenge, therefore, is to identify critical proteins and pathways mediating neuronal degeneration. To shed light on this issue, we searched for genetic modifiers of neurotoxicity in transgenic flies expressing human TDP-43^{M337V}, which display a very reliable phenotype in the eye. Thus, we crossed these flies with a library of 6,261 RNAi strains obtained from the Vienna Drosophila RNAi Center. In a primary screen, we identified more than 200 modifiers of mutant TDP-43 toxicity. Then we verified the results of the primary screen to eliminate variability and account for different observers. We also examined the specificity of the enhancers by making sure they do not induce abnormal eyes on their own. Interestingly, many suppressors are linked to RNA related functions. We also confirmed the role of genes involved in nucleocytoplasmic shuttling. Furthermore, we have found a number of modifiers linked to other functions such as neurogenesis, syntaxin binding, mitochondrial transport, ubiquitin activity, phosphatidylinositol signaling, and protein quality control to name a few. In summary, this loss-of function screen has led to the identification of several genes and molecular pathways not previously known to be associated with TDP-43 pathologies.

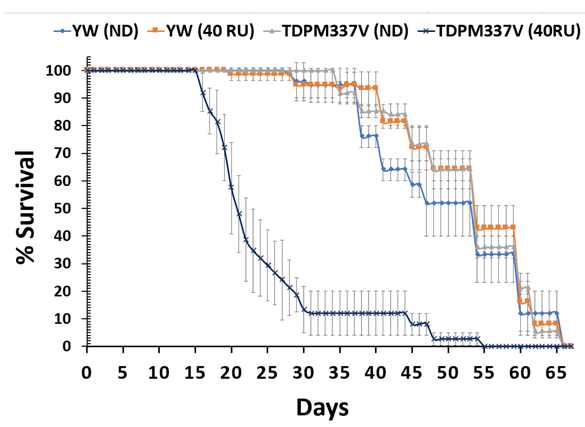
Screening strategy to find modifiers of TDP-43 toxicity



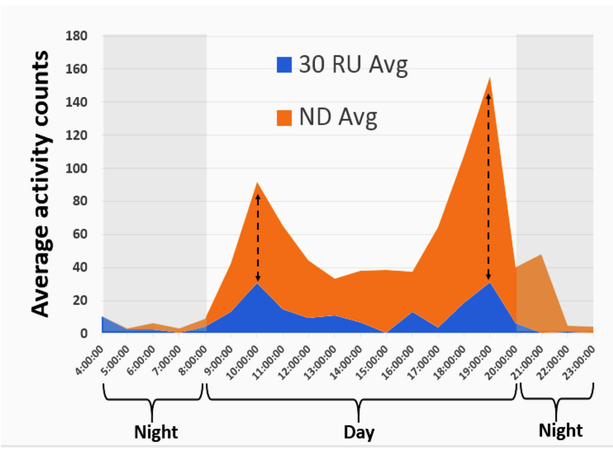
Characterization and paradigms for functional validation

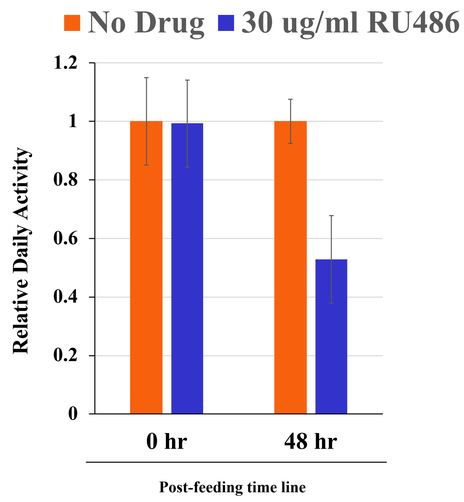
1. Flies expressing TDP-43^{M337V}, using GS-elav show shorter lifespan





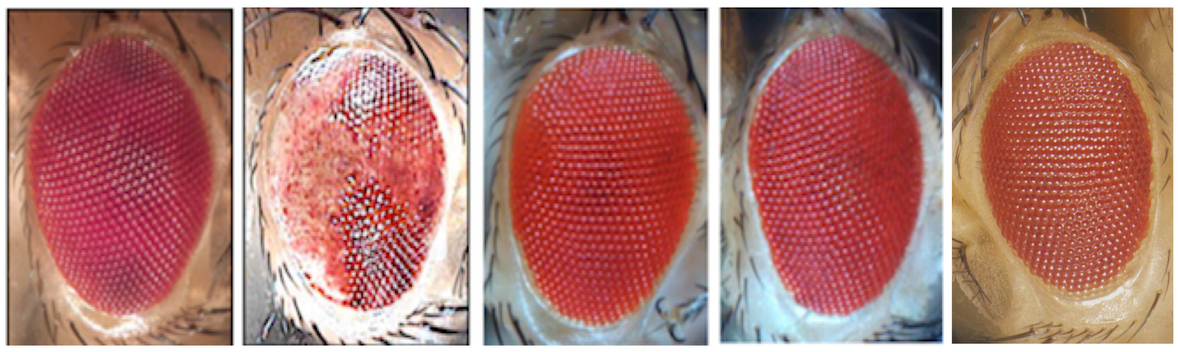
2. Locomotion defects in flies expressing TDP-43^{M337V}





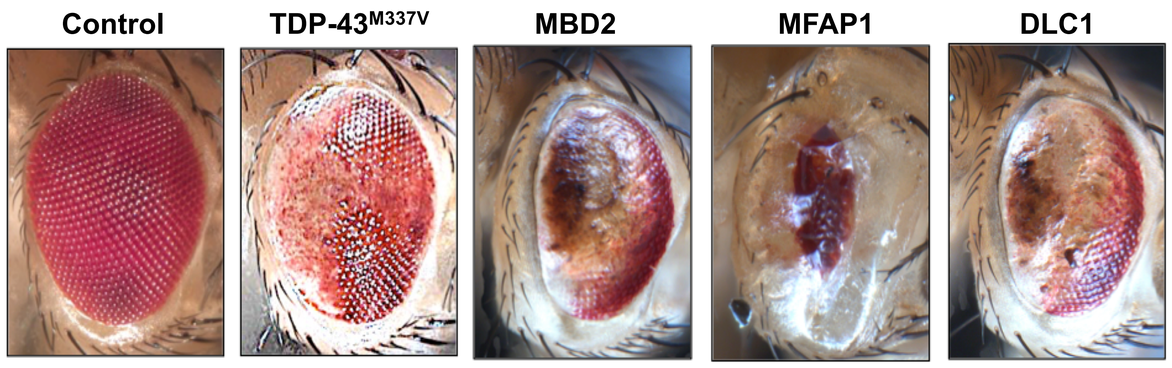
ND: No drug control; 30.40 RU: 30/40µg/ml RU486

Selected suppressors of mutant TDP-43 toxicity



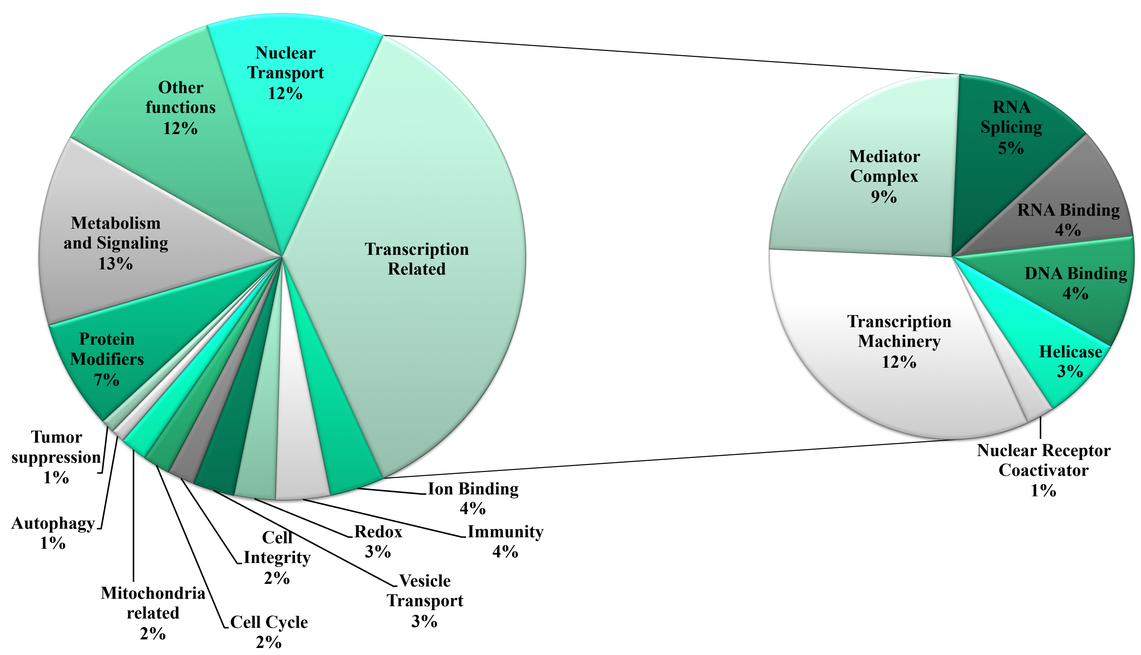
SZRD1- SUZ RNA binding domain containing 1
SCAF6- SCAF6 RNA binding protein
RPII15- an RNA polymerase complex II subunit

Selected enhancers of mutant TDP-43 toxicity



MBD2- Methyl-CpG binding domain protein 2
MFAP1- Microfibrillar associated protein 1
DLC1- DLC1 Rho GTPase activating protein

Gene Ontology of TDP-43^{M337V} suppressors



Future Directions

- Define the interacting network and pathways of modifiers through bioinformatics.
- Understand the mechanisms associated with TDP-43 suppressors.
- Assess the effect of modifiers in other fly models of ALS/FTD.
- Validate targets in mammalian models of TDP-43 proteinopathies.

Acknowledgements

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