TDP-43 is a conserved RNA binding protein with known roles in mRNA splicing and stability. Cytoplasmic deposition of TDP-43 has been linked to multiple neurodegenerative diseases, including ALS and frontotemporal lobar dementia (FTLD). We have engineered pan-neuronal expression of human TDP-43 protein in C. elegans, with the goal of generating a convenient in vivo model of TDP-43 neurotoxicity. Full-length wild-type human TDP-43 expressed in C. elegans is nuclear as is observed in human cells. Transgenic worms with neuronal human TDP-43 expression exhibit an uncoordinated phenotype and have abnormal motorneuron synapses. By using this uncoordinated phenotype as a read-out of TDP-43 neurotoxicity, we have investigated the contribution of specific TDP-43 domains as well as TDP-43 sub-cellular localization to toxicity. Deletion of either RNA recognition domain (RRM1 or RRM2) completely blocks neurotoxicity, as does deletion of the C-terminal region. These deleted TDP-43 variants still accumulate in the nucleus, although their subnuclear distribution is altered. In contrast, N-terminal deletions result in the formation of toxic cytoplasmic aggregates. Mutation of the TDP-43 nuclear localization signal (NLS) results in cytoplasmic deposition of full-length TDP-43, which is not toxic. Mutations that alter two TDP-43 caspase cleavage sites (DBD219E), however, do not rescue TDP-43 toxicity. Our results demonstrate that TDP-43 neurotoxicity can result from either nuclear activity of the full-length protein or accumulation of cytoplasmic aggregates composed of C-terminal fragments. These results suggest that there may be (at least) two different mechanisms of TDP-43 neurotoxicity.

### Summary of Results

- Pan-neuronal expression of full length nuclear hTDP-43 in C. elegans produces uncoordinated movement.
- GABAergic motorneuron synaptic dysregulation and axonal fasciculation is observed, but not motorneuron loss.
- This phenotype is alleviated by deletion of the functional domains RRM1, RRM2 and the C terminal domain.
- This phenotype is alleviated by mutagenesis of the TDP-43 NLS.
- The unc phenotype is recapitulated by pan-neuronal cytoplasmic expression of the ALS relevant C terminal fragment TDP-25.
- The mechanisms of nuclear hTDP-43 and cytoplasmic hTDP-25 derived neurotoxicity can be disseminated from each other using the caspase-cleavage resistant construct hTDP-43.D89E, D219E.

### Conclusions

We report two mechanisms of hTDP-43 neurotoxicity in C. elegans, from:

1. Pan-neuronal expression of full length nuclear hTDP-43.
2. Cytoplasmic aggregation of the ALS relevant C terminal fragment TDP-25.