Jennie Vallis/NPG

NEURODEGENERATIVE DISEASE

The plot thickens

> “synthesis of new proteins was markedly reduced in the axon terminals of *Xenopus laevis* neurons expressing full-length $FUS^{ALS–FTD}$”

Some forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are characterized by intraneuronal assemblies of mutant variants of RNA-binding proteins, such as FUS. However, whether and how these assemblies might contribute to neurotoxicity in ALS and FTD is unknown. Now, Murakami *et al.* show that pathological *FUS* mutations promote the transition of liquid droplets of FUS into a gel that can trap ribonucleoproteins (RNPs), thereby disrupting RNP granule function and protein synthesis.

The authors generated *Caenorhabditis elegans* that expressed either wild-type human FUS (FUS^{WT}) or one of five variants of mutant human FUS that have been associated with ALS or FTD ($FUS^{ALS–FTD}$) under a neuron-specific promoter. In contrast to FUS^{WT}-expressing worms, which showed no neurotoxicity, $FUS^{ALS–FTD}$-expressing worms exhibited age-dependent motor deficits and reduced lifespans, and the severity of this phenotype correlated with the abundance of intraneuronal $FUS^{ALS–FTD}$ assemblies.

Previous work had indicated that the amino-terminal ‘low-complexity’ (LC) domain of FUS enables the protein to aggregate. Here, worms in which truncated $FUS^{ALS–FTD}$ variants that lacked the LC domain were specifically expressed in neurons had fewer FUS assemblies and showed less-severe motor impairment and reductions in lifespan than did worms expressing full-length $FUS^{ALS–FTD}$ variants. Moreover, neuronal expression of the $FUS^{ALS–FTD}$ LC domains led to more-severe neurotoxicity than did expression of the FUS^{WT} LC domain. Therefore, the LC domains of pathological FUS mediate neurotoxicity.

Next, the authors characterized the biophysical nature of the $FUS^{ALS–FTD}$ assemblies. Live imaging of cultured mammalian neurons expressing yellow-fluorescent protein-tagged variants of FUS revealed that FUS^{WT} resides as liquid droplets in the nucleus, whereas $FUS^{ALS–FTD}$ droplets are present not only in the nucleus but also in the cytoplasm. The authors were able to form liquid droplets of FUS *in vitro* by cooling solutions of recombinant FUS variants to 4 °C. On warming back to 23 °C, the droplets of FUS^{WT} almost all dissolved, whereas more than half of the droplets of $FUS^{ALS–FTD}$ remained. When droplets of FUS LC domains were cooled, they formed a gel that could be ‘melted’ by warming. However, whereas FUS^{WT} LC domains could undergo four or five of these liquid–gel cycles before irreversibly gelling, droplets of $FUS^{ALS–FTD}$ LC domains formed an irreversible gel that was more viscous than the FUS^{WT} LC domain gel after only two cycles. Thus, $FUS^{ALS–FTD}$ variants have a higher propensity than FUS^{WT} for phase transitioning from a monomeric, dissolved form to an irreversible fibrous gel form.

The authors investigated the functional effects of $FUS^{ALS–FTD}$ gel formation on RNP granules. They cooled mixtures of $FUS^{ALS–FTD}$ or FUS^{WT} and the RNPs survival motor neuron (SMN) and STAU1, and used single-molecule imaging to track the diffusion of SMN and STAU1 particles. On melting the gels, SMN and STAU1 in the FUS^{WT} mixture could diffuse freely. By contrast, the $FUS^{ALS–FTD}$ mixture contained irreversible gel assemblies that prevented SMN and STAU1 from moving, suggesting that the $FUS^{ALS–FTD}$ gel may ‘trap’ RNPs. This effect seems to disrupt RNA translation, as the synthesis of new proteins was markedly reduced in the axon terminals of *Xenopus laevis* neurons expressing full-length $FUS^{ALS–FTD}$ compared with neurons expressing FUS^{WT}.

This study provides evidence that, whereas reversible liquid droplets and gels formed by FUS^{WT} support physiological RNP granule function, pathological variants of FUS can form irreversible gel assemblies that may trap RNPs, and thus could impair RNP granule function and disrupt protein synthesis. This represents a possible mechanism for neurodegeneration in the forms of ALS and FTD that are associated with mutant RNA-binding proteins.

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ORIGINAL RESEARCH PAPER Murakami, T. *et al.* ALS/FTD mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* http://dx.doi.org/10.1016/j.neuron.2015.10.030 (2015)