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Interplay between TDP-43 and FUS in protein assemblies

Influence of mRNA on the aggregation process

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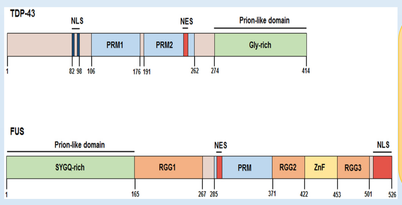
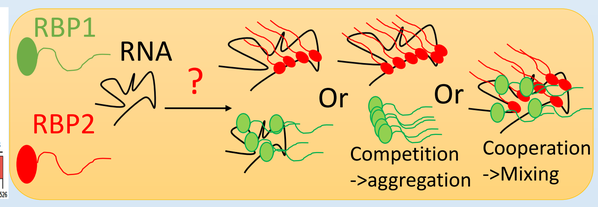
1 Introduction

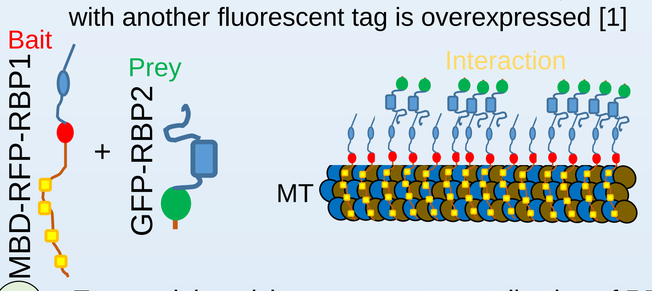
TDP43 and FUS are 2 RNA Binding Proteins (RBP) involved in neurodegenerative diseases. These 2 nuclear proteins are very similar in structure and function; they both harbor RRM (RNA Recognitions Motif) to bind to RNA, and a domain of low complexity promoting protein-protein interaction and potentially, aggregation. They are involved in several mRNA-related process as RNA biogenesis, transport, and splicing. TDP-43 targets more than 6000 mRNA thanks to its RRMs with specificity towards GU motif while FUS interact with broad varieties of mRNA through its ZnF, RGG motifs and RRM without any specific nucleotide sequence. Despite this difference in RNA interaction mode, these proteins promote the formation of mRNA rich compartments like stress granules, drosha complexes, splicing complexes… via the process of liquid-liquid phase separation (LLPS), and within these structures, they accumulate on common RNA targets. Such proximity in functions and localization point toward an interplay between FUS and TDP-43. Here, we analyze their ability to mix or phase separate in cellular context using the microtubule bench technology. Then we reveal the ultrastructure of pure and mixed TDP43 and FUS assemblies as well as the influence of RNA.



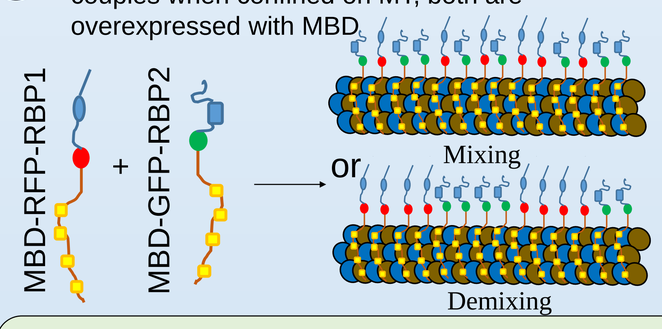


2,1 The Microtubule Bench

a To detect the interaction between proteins: one RBP (the "bait"), fused to an MBD (Microtubule Binding Domain) and a fluorescent tag is confined on the microtubule network and the second RBP (the "prey") with another fluorescent tag is overexpressed [1]



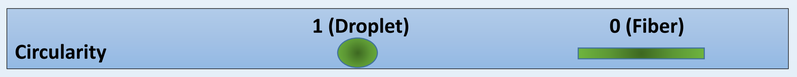
b To reveal the mixing or compartmentalization of RBP couples when confined on MT, both are overexpressed with MBD



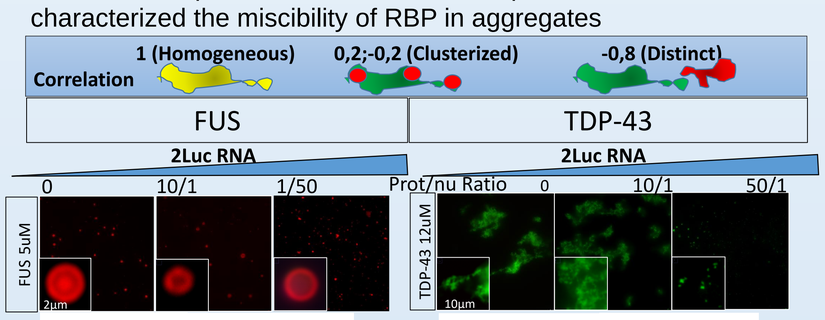
2,2 Structural analysis of RBP assemblies *in vitro*

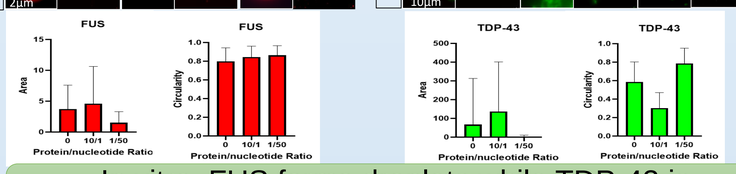
Mixed protein samples are incubated then fixed and glass slides before being marked with antibodies.

Circularity and **Area** have been used to describe shape of the aggregates



A **correlation** parameter has been developed in order to characterized the miscibility of RBP in aggregates

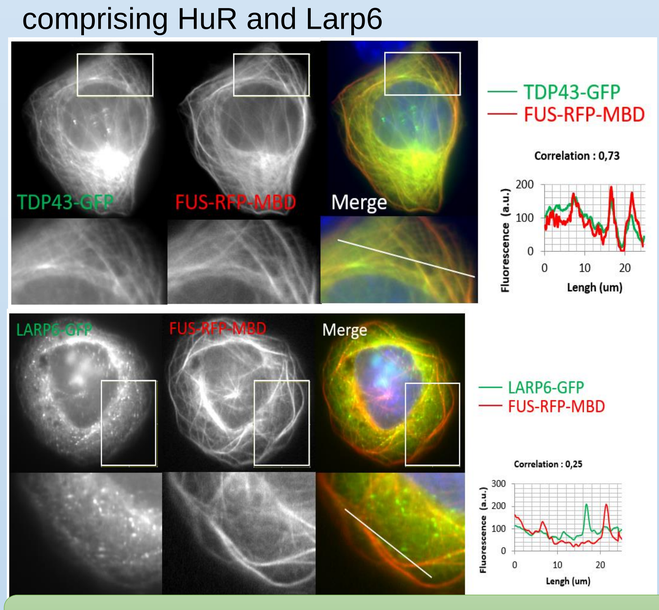


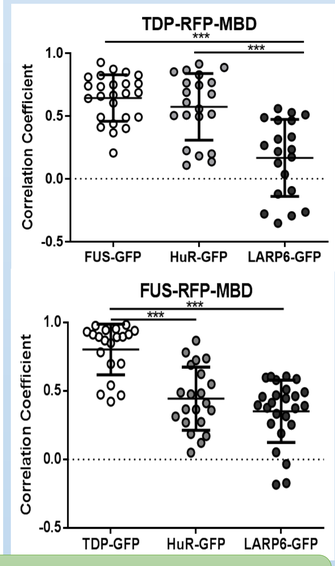


In vitro, FUS forms droplets while TDP-43 is aggregated. RNA shows buffering effect at high ratio

3 TDP-43 and FUS interaction with various RBPs

Bait/pray system has been used in order to detected protein-protein interaction in cellular context. Several RBPs have been tested comprising HuR and Larp6

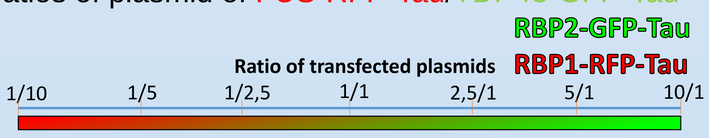


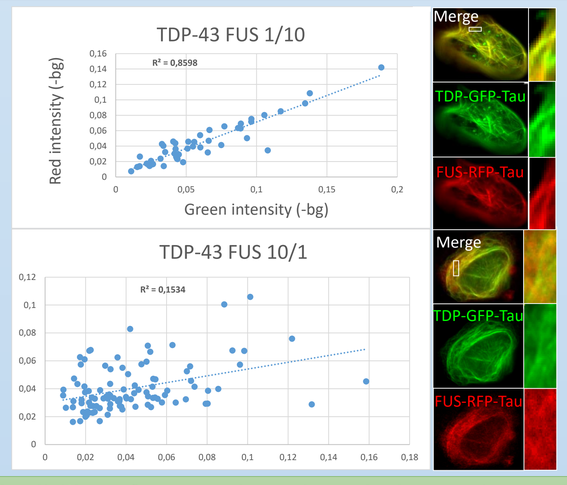


TDP-43 and FUS interaction is higher than other RBPs

4 Mixing of TDP-43 and FUS in cellular context

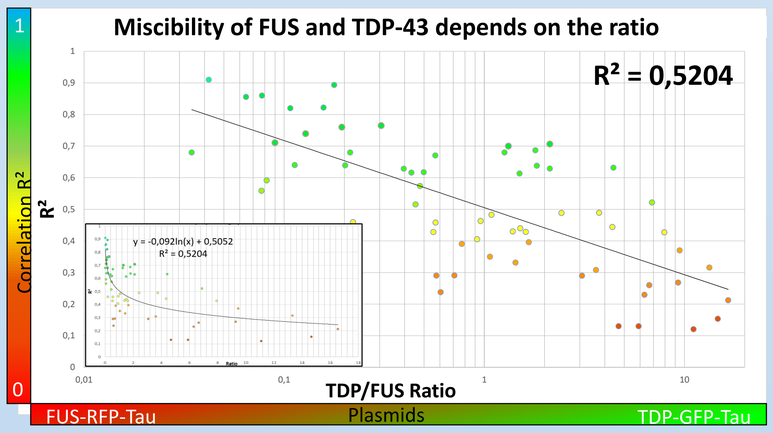
a U2OS cells has been transfected with several ratios of plasmid of FUS-RFP-Tau/TDP43-GFP-Tau





b Determination of the correlation coefficient according to the expression level of TDP-43 and FUS

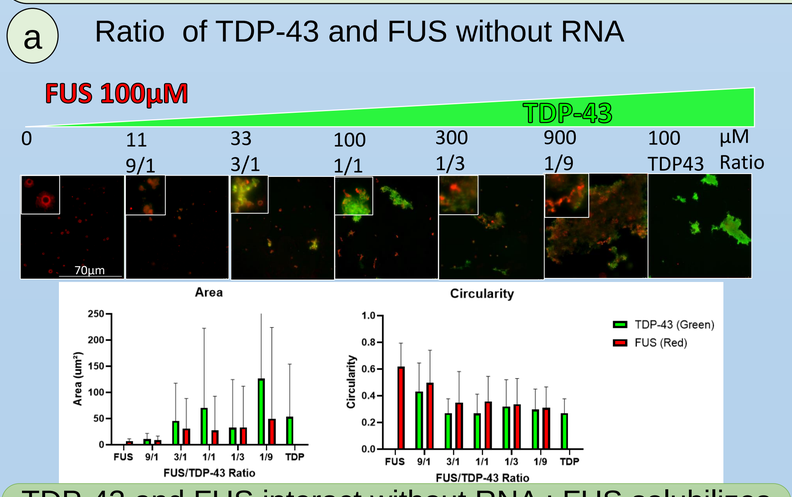
Correlation has been measured automatically (Cell profiler) by analyzing repartition of fluorescence intensity along microtubule



Miscibility of TDP-43 and FUS depends on the expression level, FUS solubilizes TDP-43, while TDP-43 prefers to interact with itself

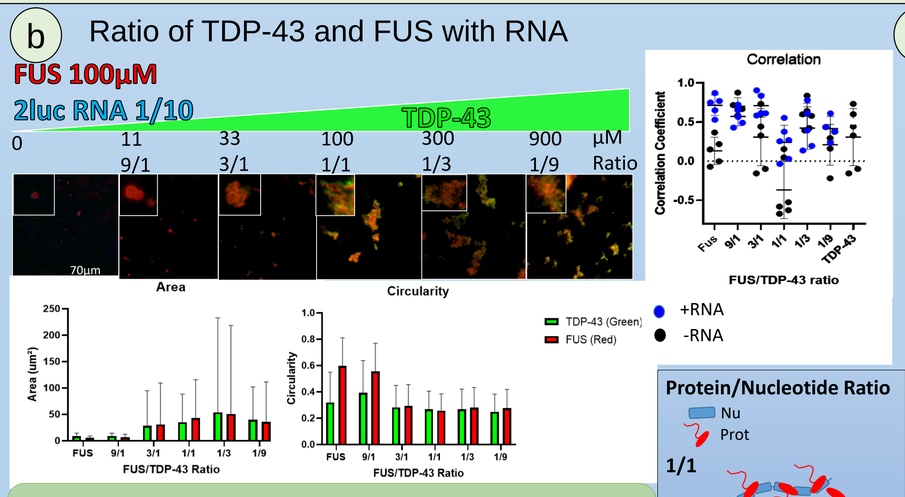
5 Ultrastructural analysis of FUS and TDP-43 assemblies

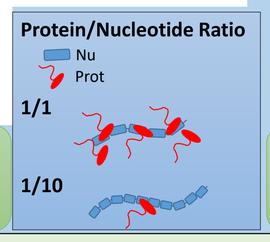
a Ratio of TDP-43 and FUS without RNA



TDP-43 and FUS interact without RNA : FUS solubilizes TDP-43 in its phase at a high ratio, while FUS forms clusters in TDP-43 aggregates at a low ratio

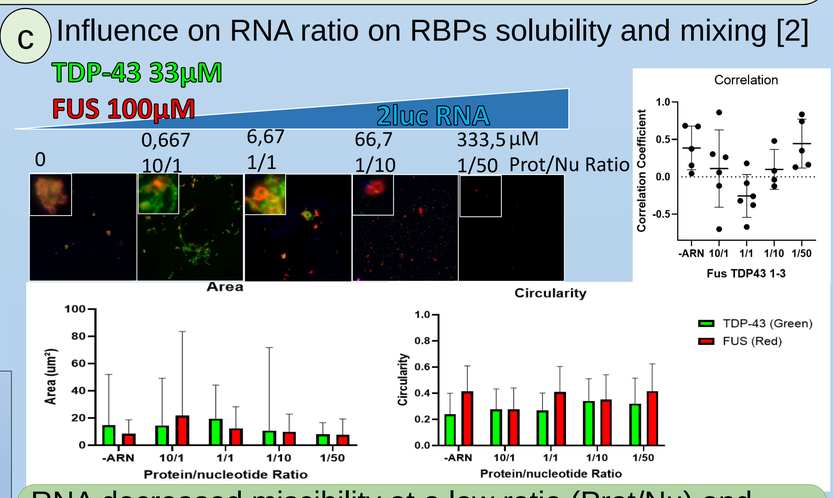
b Ratio of TDP-43 and FUS with RNA





Miscibility of TDP-43 and FUS is improved by RNA but still depend on the protein ratio

c Influence on RNA ratio on RBPs solubility and mixing [2]



RNA decreased miscibility at a low ratio (Prot/Nu) and improved miscibility at a high ratio while preventing aggregation

Conclusion

Using an original method, we demonstrated that TDP-43 and FUS interact in cellular context and In vitro. However, this interplay depends on the expression level of proteins, it appears that FUS is able to accept TDP43 in its environment while TDP43 forms a dense and rich phase where FUS is hardly inserted. Such differences could be related to the structure of the RBP assemblies. FUS forms droplet that could solubilize TDP43 upon quite elevated ratio while TDP43 has the propensity to produce higher order assemblies with a filamentous structure in which FUS clusterized. Moreover, RNA, through its buffering effect, is important for protein solubility and seems to increase the interplay between these 2 RBPs. Regarding their involvement in neurodegenerative diseases as Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Lobar Degeneration (FTLD) [3] and the more recently described Limbic-predominant age-related TDP-43 Encephalopathy (LATE), it seems important to better characterized the comportment of these RBPs. Indeed, these proteins are aggregated in neurons of patients under different forms (ubiquitinylated, mutated, phosphorylated…) and could coexist in the same assemblies. The overlap of these neurodegenerative diseases suggests a common mechanism of degeneration, where loss of function in RNA metabolism is suspected. Better understanding the aggregation process and how to prevent it remains essential.

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[3] Dobra, I., Pankivskyi, S., Samsonova, A., Pastre,D., Hamon L. (2018) Relation between stress granules and cytoplasmic protein aggregates linked to neurodegenerative diseases. Current neurology and neuroscience reports vol 18, p107.