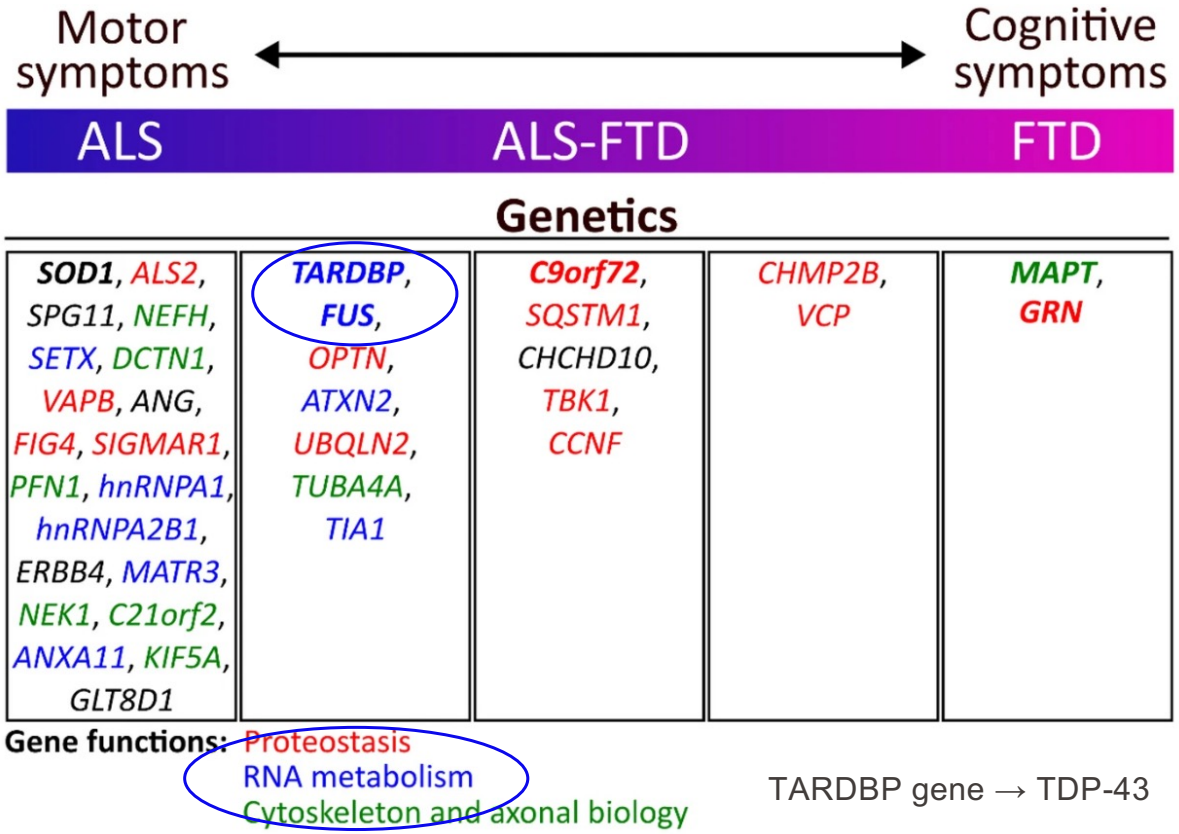
Lecture plan

1. **Motor system**
   - Overview
2. **Motor Neuron Diseases**
   - Clinical presentation
   - Molecular pathology
3. **Amyotrophic Lateral Sclerosis**
   - Clinical presentation, epidemiology, etiology
   - Non-cell autonomous mechanisms
   - Molecular pathology: RNA metabolism

Why is RNA metabolism a focus for research on disease mechanisms in ALS?

RNA metabolism is a prevalent mechanistic pathway in genes linked to ALS



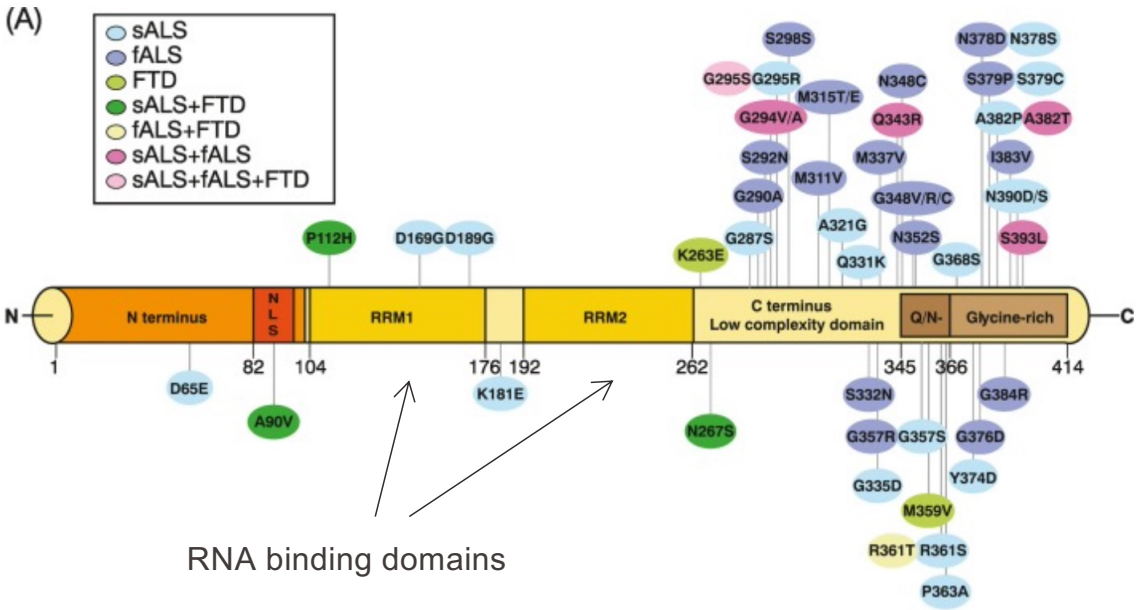
TARDBP gene → TDP-43

Mol Neurodegeneration 20, 64 (2025). https://doi.org/10.1186/s13024-025-00851-y

ALS: genetics

TDP-43 (43 kDa trans-active response DNA-binding protein)

- ≥ 38 mutations, <2% of ALS cases
- All but one mutation (Y374X) lead to autosomal dominant inheritance
- Classical ALS phenotype, some mutations associated with frontotemporal lobar degeneration (FTLD)
- Originally identified as a transcriptional repressor
- Two RNA recognition motifs (RRM)
- Normal nuclear distribution → **cytosolic inclusions**

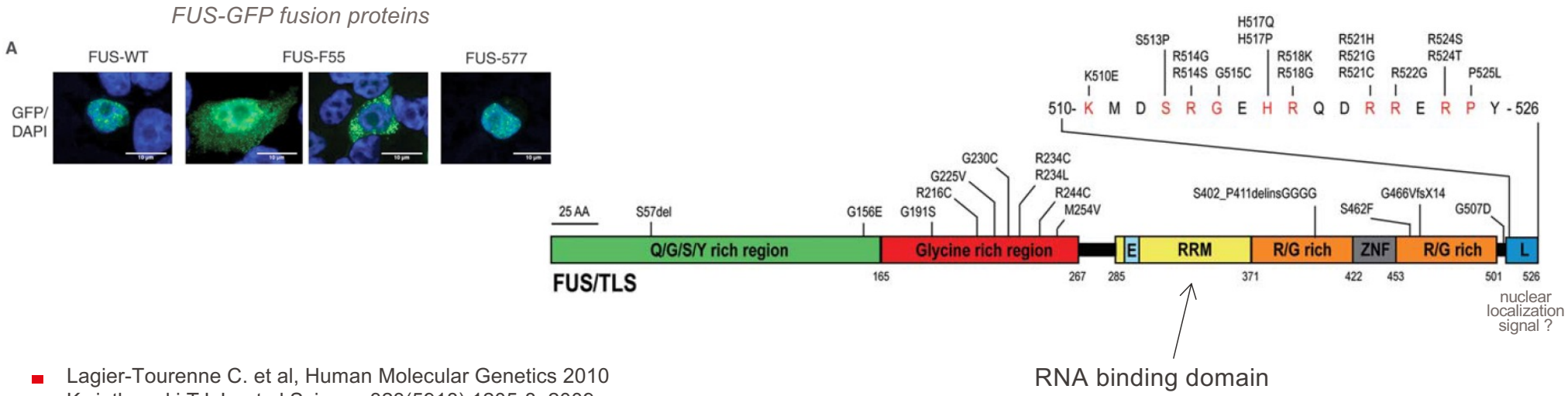


Bodanski A. et al, Amyotrophic Lateral Sclerosis 2010
Lagier-Tourenne C. et al, Human Molecular Genetics 2010
https://doi.org/10.1016/j.tins.2021.02.008

ALS: genetics

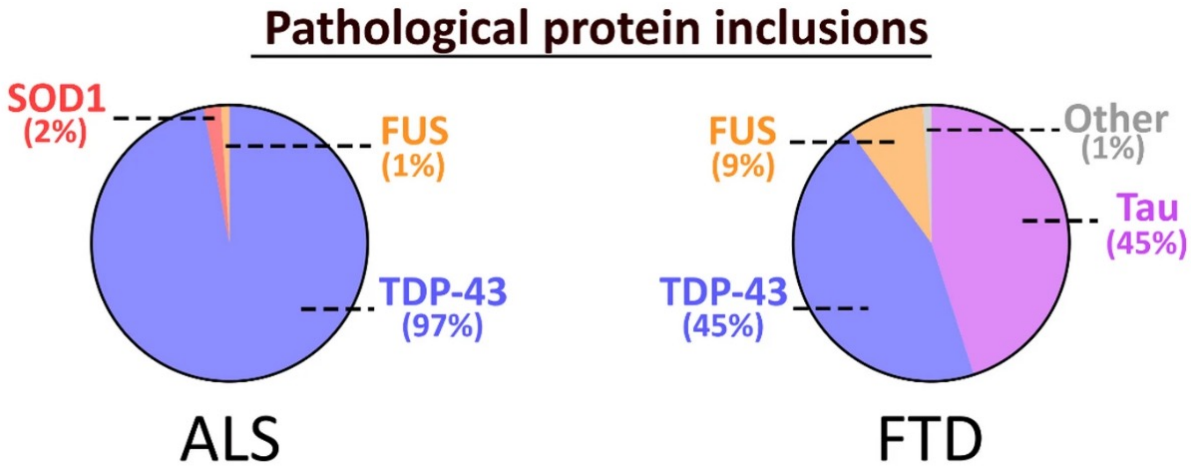
FUS/TLS (fused in sarcoma/translocated in liposarcoma)

- ≥ 30 mutations, <2% of ALS cases
- Dominant inheritance except one recessive mutation (H517Q)
- Familial ALS, sometimes with early onset (<30 yrs) and rapid progression
- Some rare patients with FTLD
- One RNA recognition motif (RRM)
- Mainly nuclear protein → **cytosolic in stress/pathologic conditions**

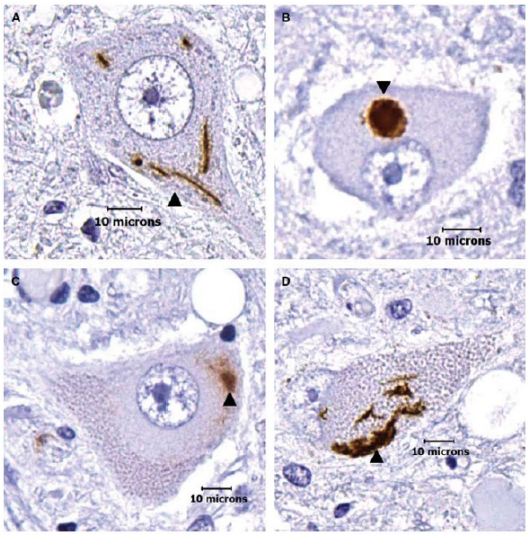


- Lagier-Tourenne C. et al, Human Molecular Genetics 2010
  Kwiatkowski TJ Jr, et al Science 323(5918):1205-8, 2009

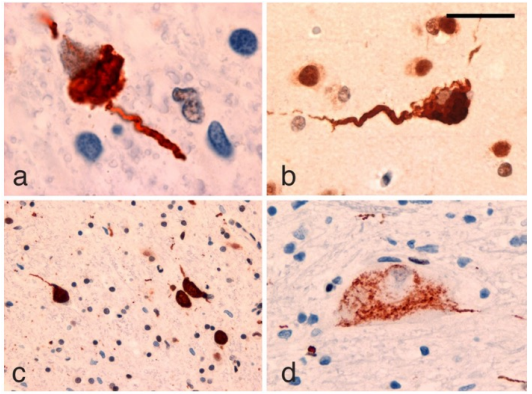
Pathology: TDP-43 and FUS are mislocalized and form aggregates in ALS



TDP-43 inclusions (in **97%** of ALS cases)



FUS inclusions



⇒ **TDP-43 pathology is considered a common feature in ALS**
⇒ Central mechanism?

TDP-43 pathology: mechanisms

TDP-43: loss of function or gain of a toxic activity ?

Gain of a toxic function

- Presence of TDP-43 aggregates
- Accumulation of the protein in the cytosol
- Autosomal dominant inheritance
- Experimental evidence for toxic aggregation activities

?

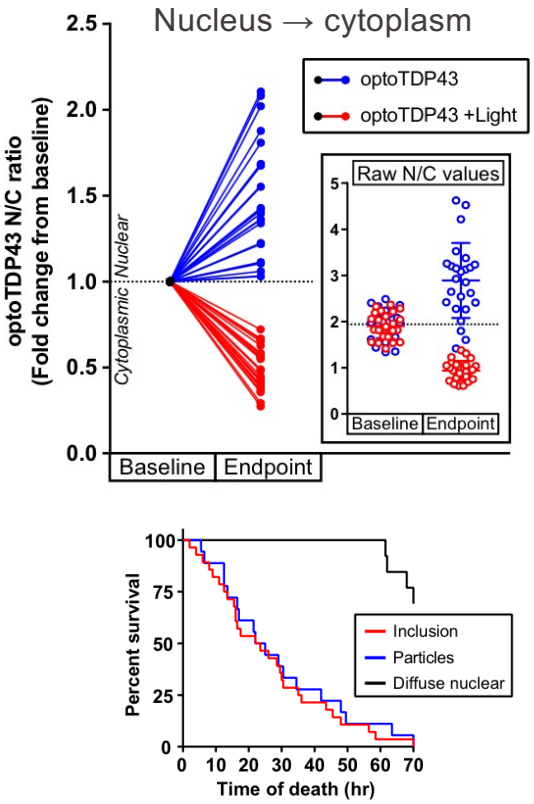
Loss-of-function

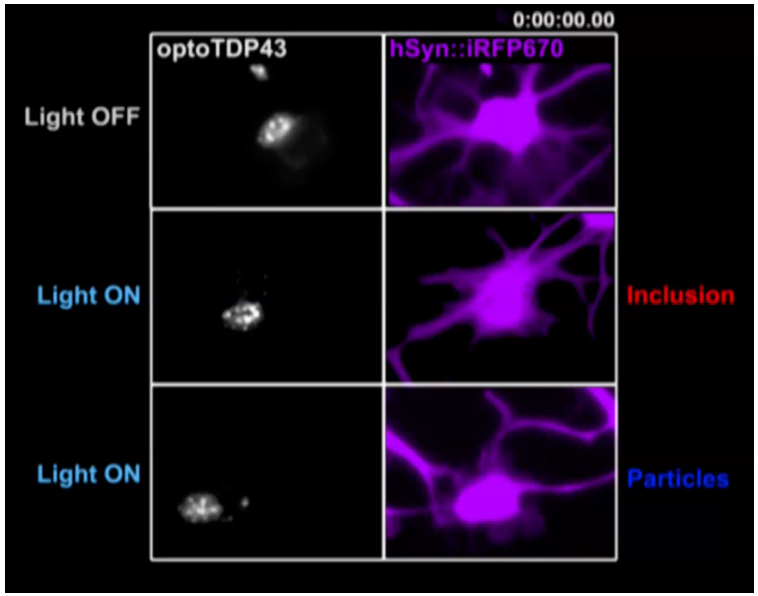
- Loss of nuclear TDP-43 (even partial loss has consequences)
- Knock-out of TDP-43 is embryonic lethal
- Very important function in RNA metabolism, mRNA transport, response to stress
- Experimental evidence for loss-of-function toxicity

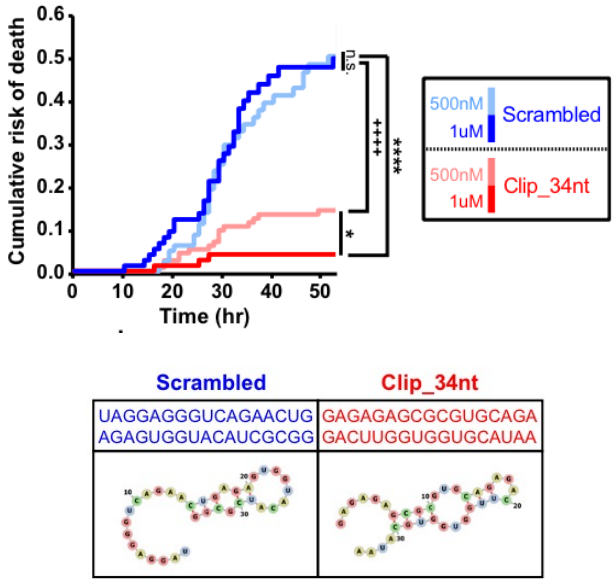
TDP-43: evidence for the gain of toxic activity

OptoTDP-43: light-inducible oligomerization

Oligomerization induces cytosolic localization of TDP-43 ⇒ neuronal toxicity opposed by RNA binding







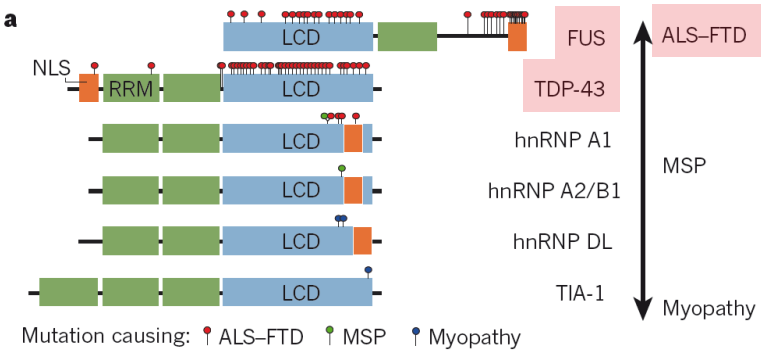
- RNA binding antagonizes TDP-43 toxicity

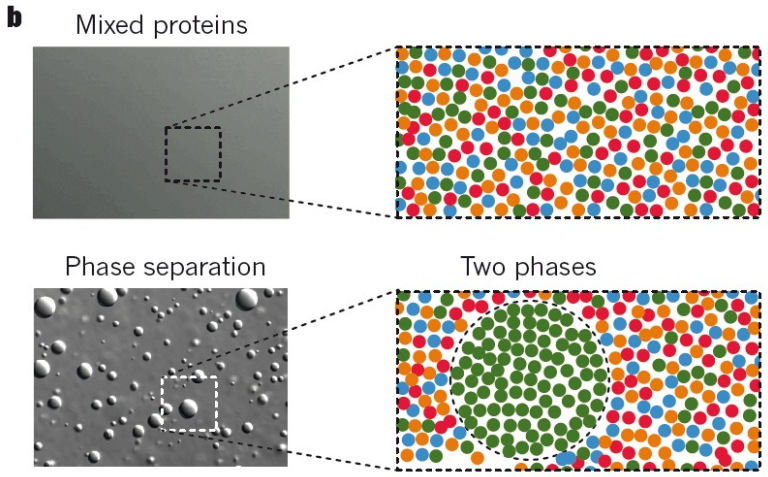
- Mann JR, Neuron 2019,102(2):321-338.e8

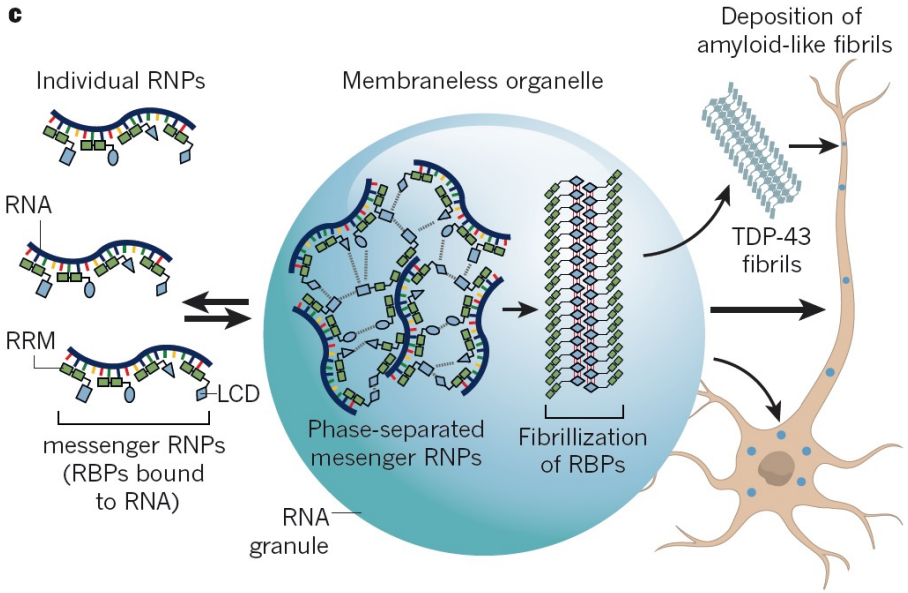
TDP-43: evidence for the gain of toxic activity

A class of proteins with low-complexity domains (LCD) that form membrane-less organelles

protein ↕ RNA — TDP-43 — protein ↕ protein → **Fibril formation**





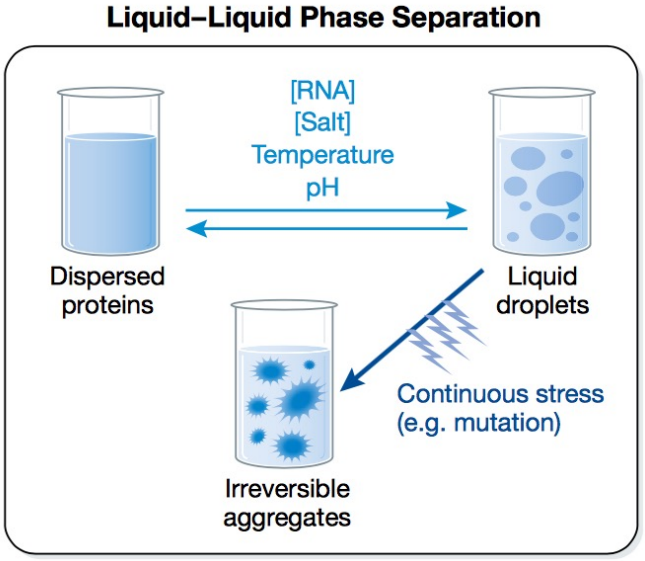


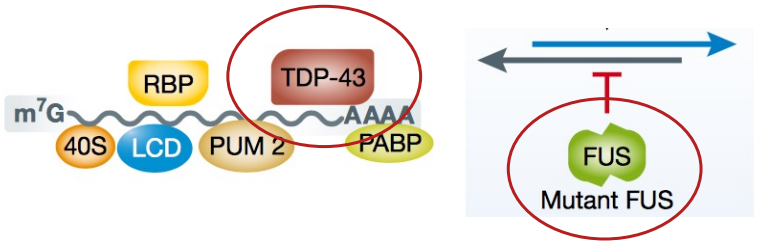
Taylor JP et al, Nature 2016

TDP-43: evidence for the gain of toxic activity

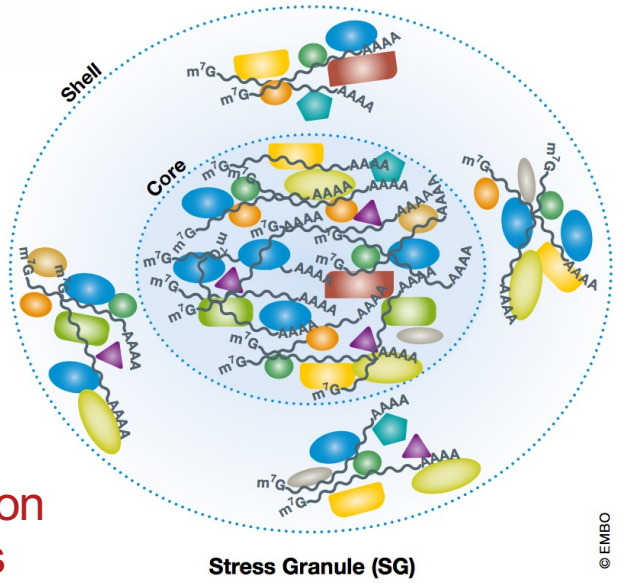
Stress granules: cytoplasmic hubs for mRNA triage and cellular stress adaptation

- RNP granules concentrating specific cellular components is a **conserved strategy** across multiple organisms and in different cellular compartments. They are formed in response to **stress** or drugs.
- Stress granules represent **assemblies of mRNPs stalled in translation initiation**.
- Stress granules are **dynamic structures**, controlled by protein chaperones, RNA helicases, and post-translational modifications.
- Mutations that alter stress granule formation contribute to some **neurodegenerative diseases** and cancers.





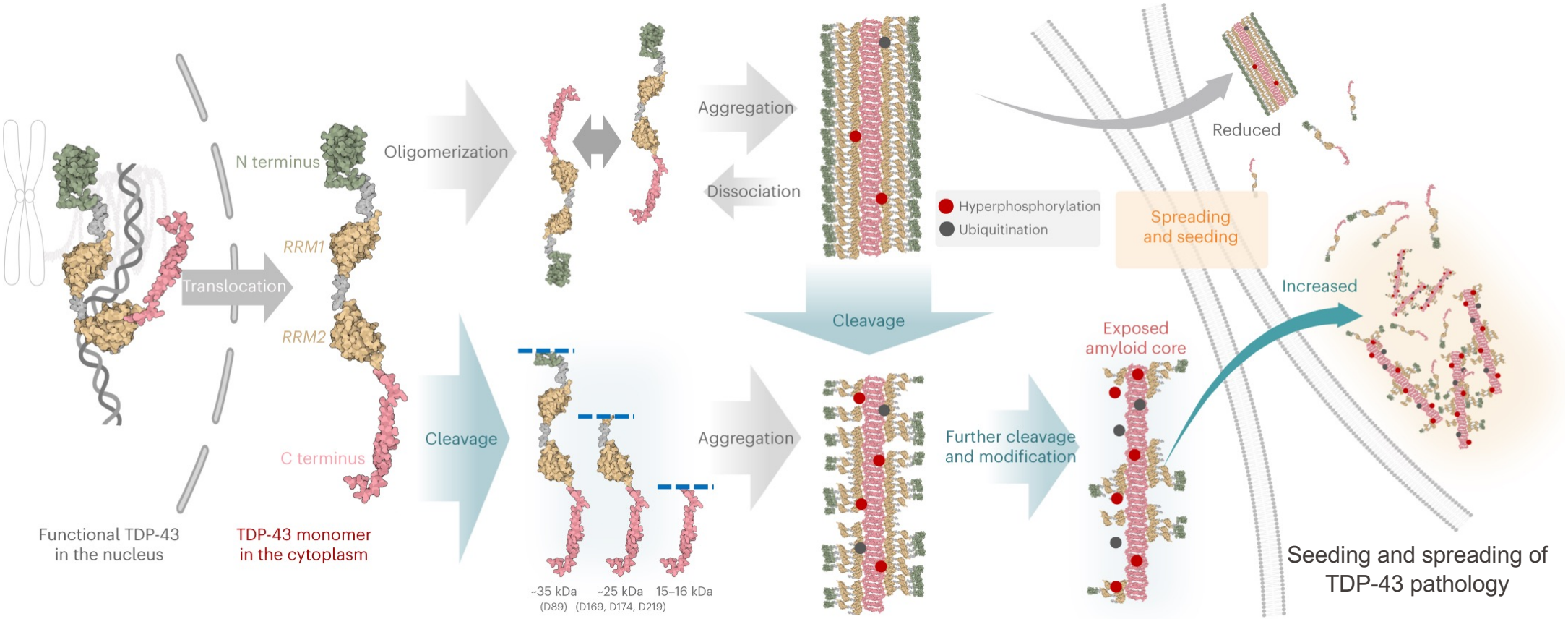
FUS and TDP43 regulate the formation and disassembly of stress granules



- Gao FB et al, EMBO J 2017
  Protter DSW et al, Trends in Cell Biol 2016

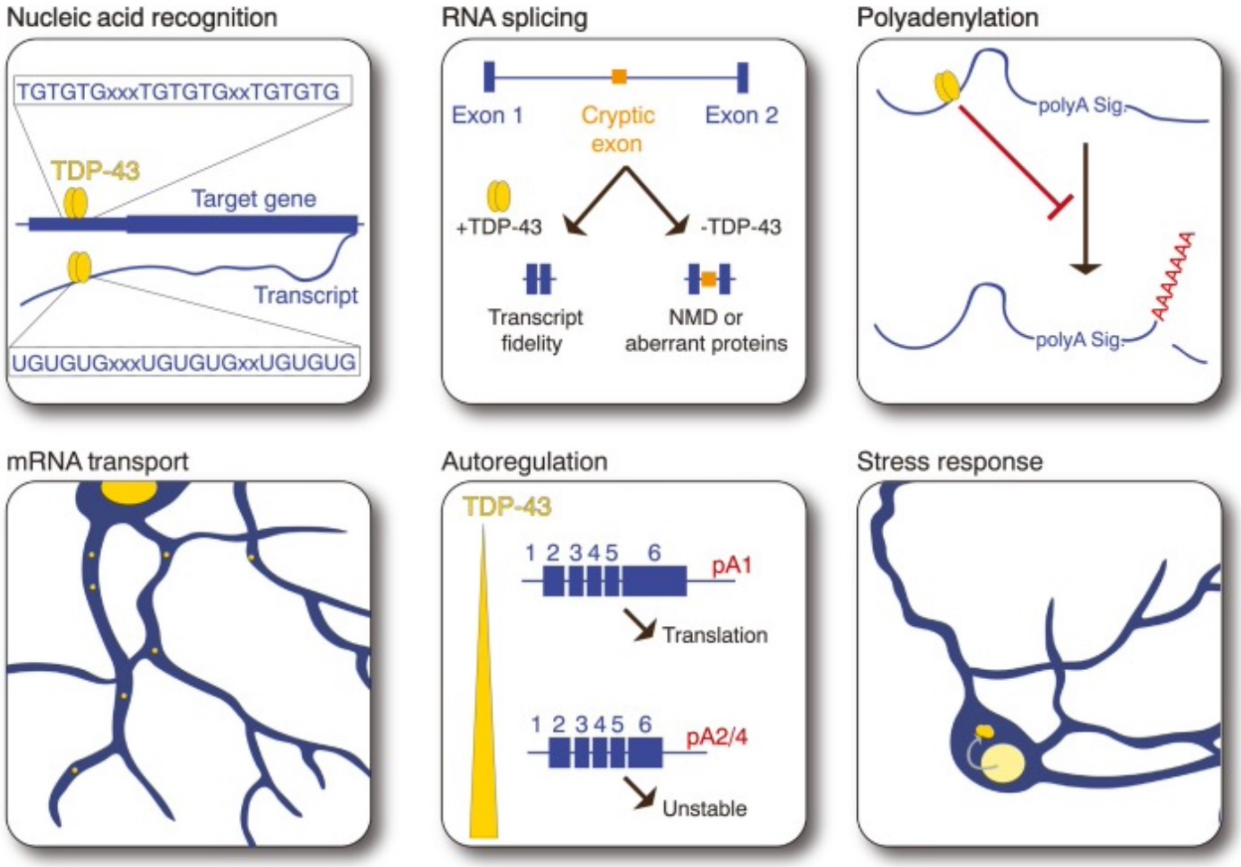
TDP-43: evidence for the gain of toxic activity

The path towards aggregation: mechanisms of TDP-43 filament formation



- *Nature Neuroscience volume 26, pages 983–996 (2023)*

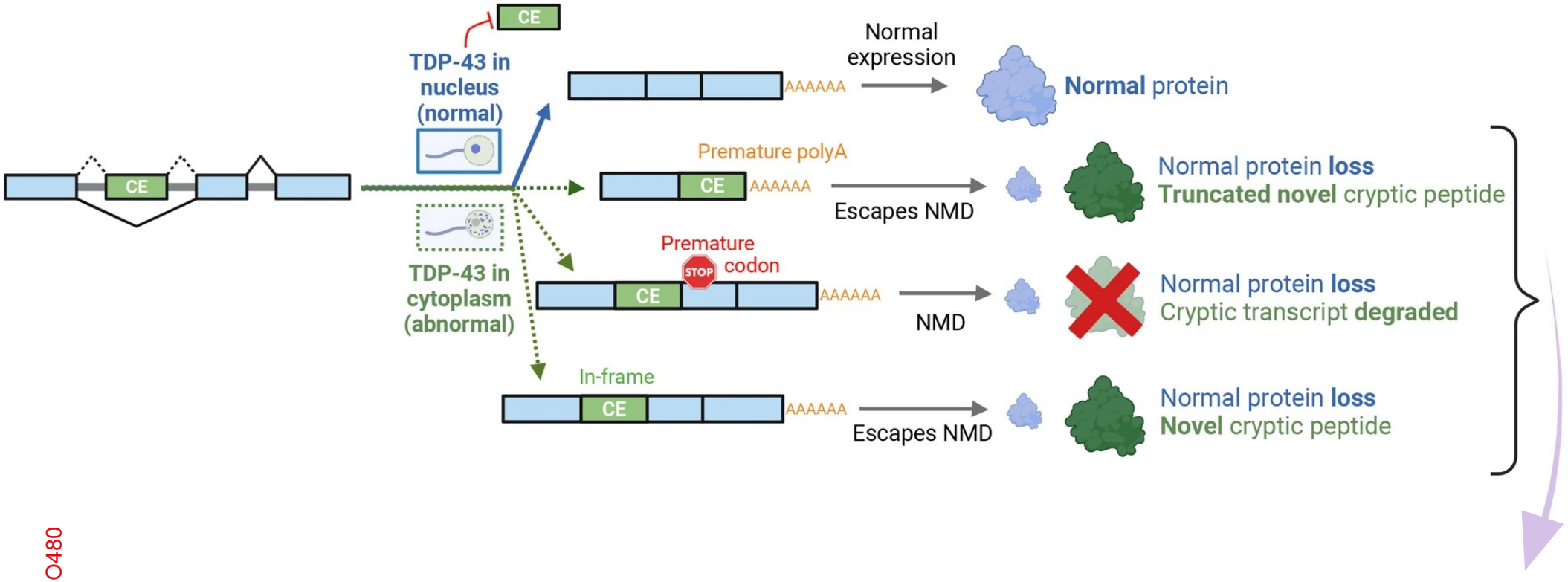
TDP-43: evidence for the loss of function toxicity



- https://doi.org/10.1016/j.tins.2021.02.008

TDP-43: evidence for the loss of function toxicity

TDP-43-mediated incorporation of cryptic exons: implications for ALS-FTD

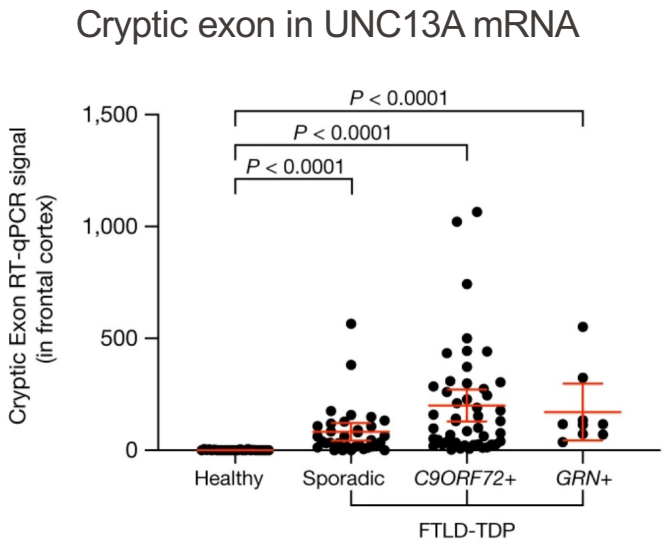


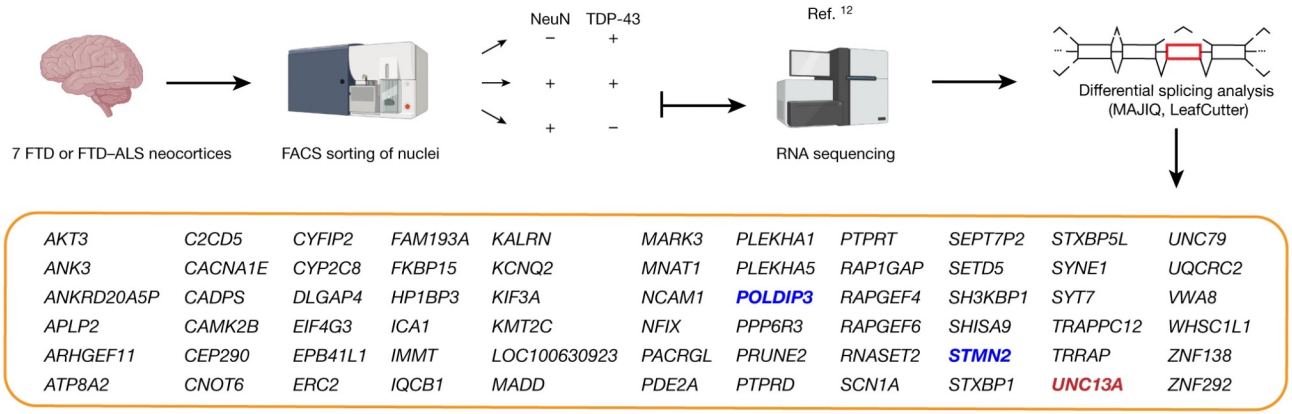
Role in disease?

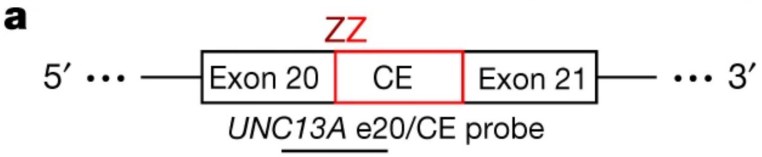
Mol Neurodegeneration 18, 16 (2023). https://doi.org/10.1186/s13024-023-00608-5

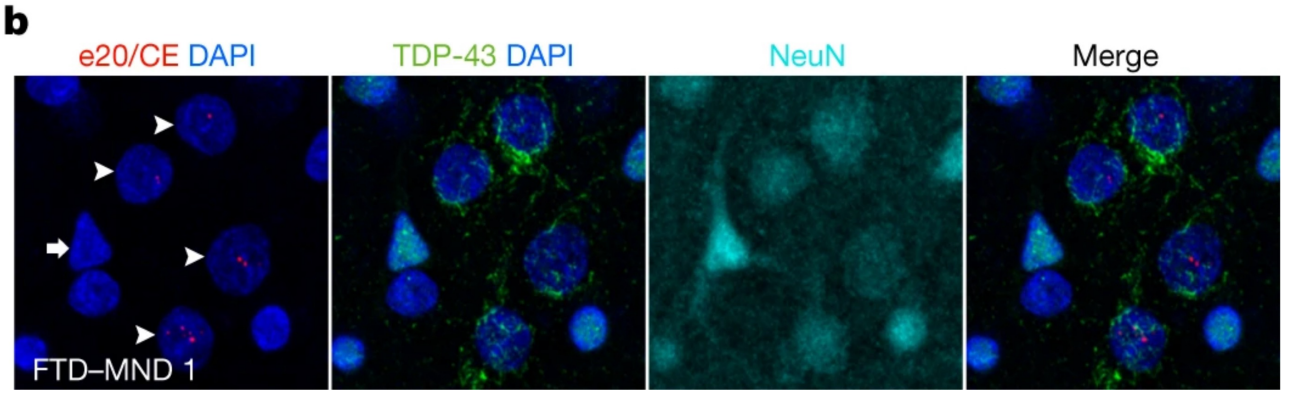
TDP-43: evidence for the loss of function toxicity

Loss of nuclear TDP-43 is associated with cryptic exon splicing in patients with FTD and MND







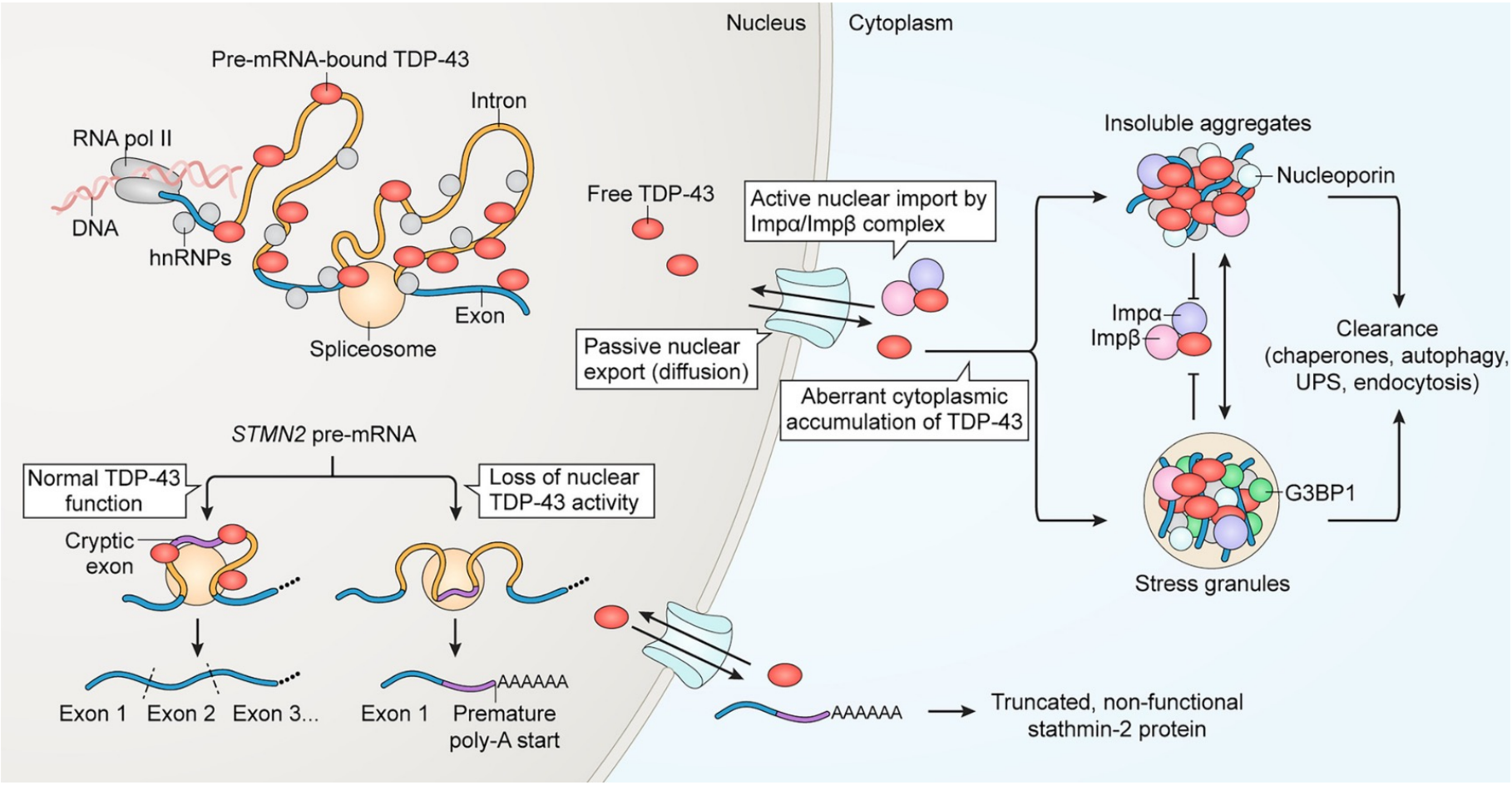


Splicing alterations in 100s → 1000s of genes related to TDP-43!

Nature volume 603, pages 124–130 (2022)

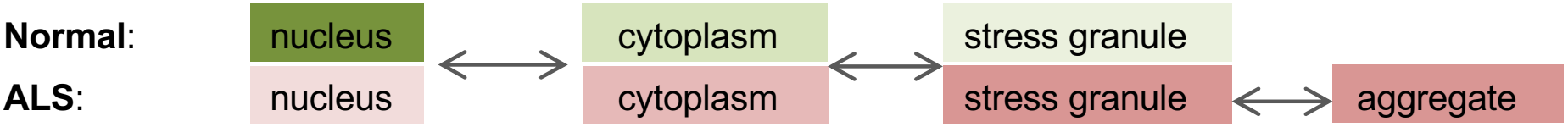
TDP-43: evidence for the loss of function toxicity

Role of TDP-43 in RNA metabolism



Motor neuron diseases: question 10

TDP-43 biology in normal and diseased conditions shows that pathology affects the protein distribution across 4 different compartments:



Based on these observations, would you think that neurodegeneration is caused by…

A. the changes in the dynamic transfer across these compartments.

B. the formation of protein aggregates, the main pathological hallmark.

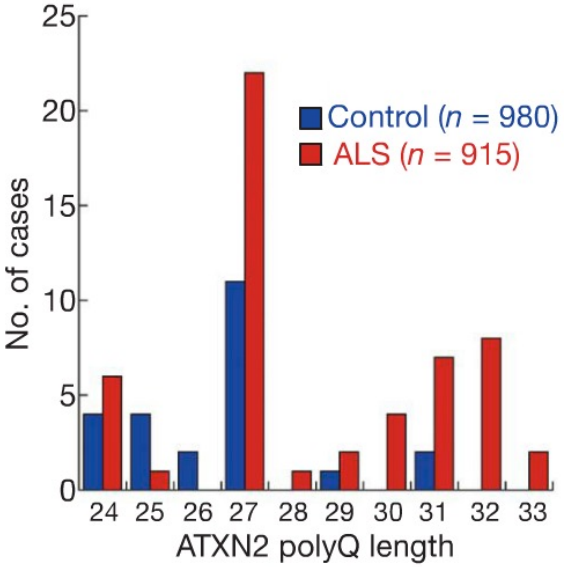
C. the loss of the effects of nuclear TDP-43 on RNA and gene expression.

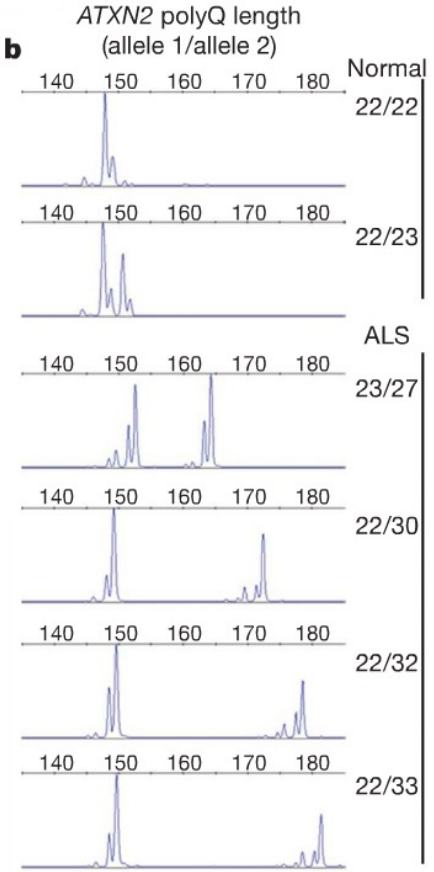
D. the combination of all of the mechanisms mentioned above.

ALS: genetics

Ataxin 2: CAG repeats as a risk factor for sporadic ALS

- >34 CAG repeats in ATXN2 → spinocerebellar ataxia type 2
- Ataxin 2 is part of a complex with TDP-43



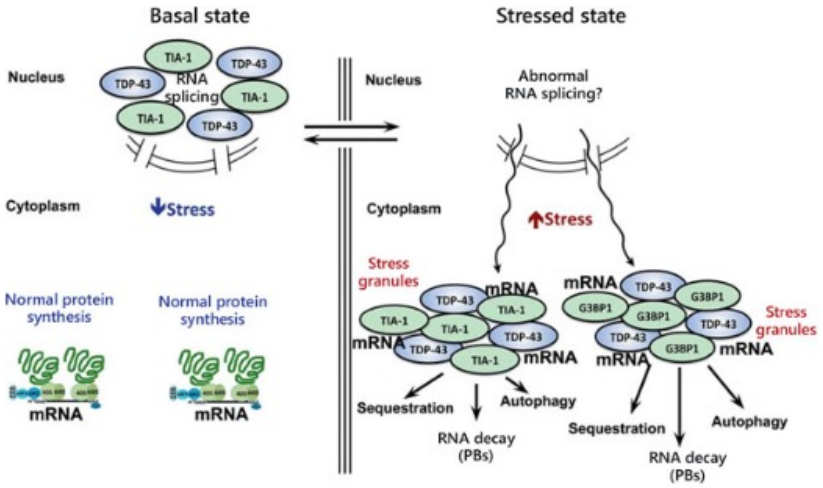


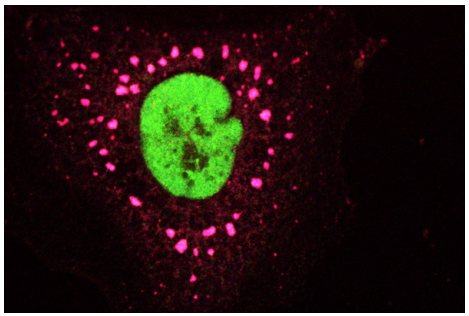
PolyQ lengths ≥27 are significantly enriched in ALS vs. controls

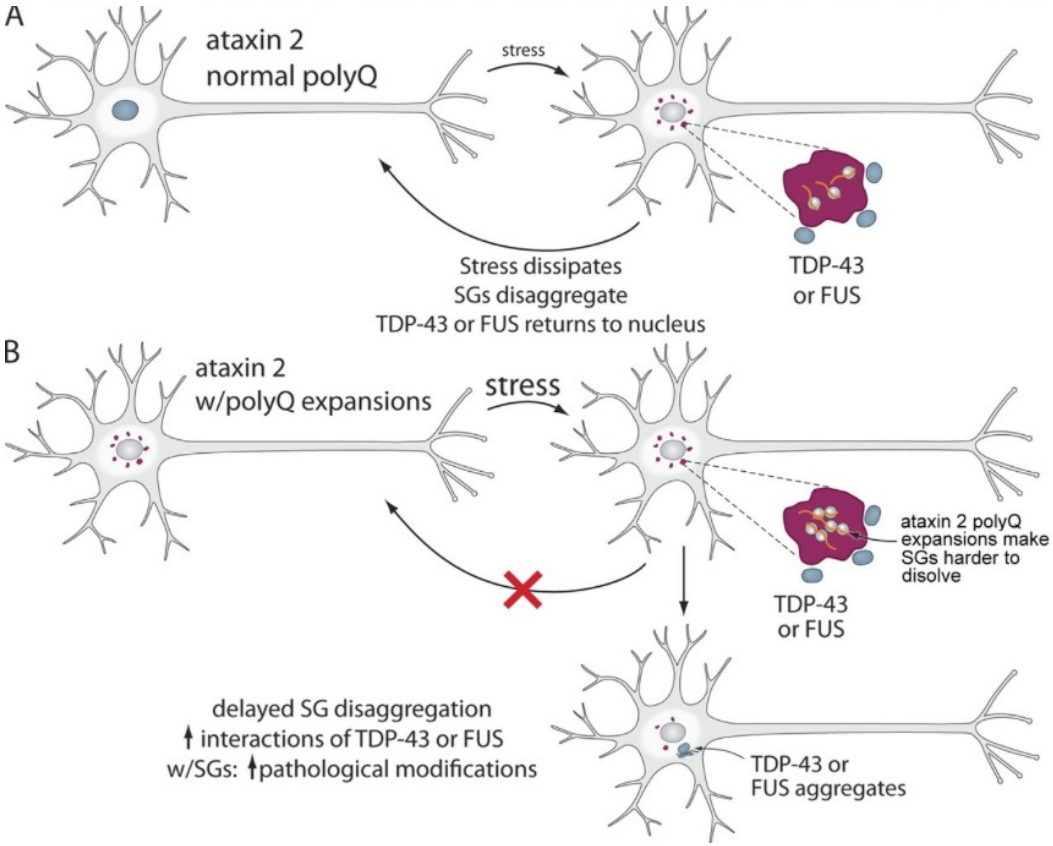
- Elden AC, et al Nature 2010

ALS: genetics

A role for Ataxin 2 in stress granule dynamics







Motor neuron diseases: question 11

You want to start a research program to explore why motoneurons are particularly vulnerable to perturbations of RNA metabolism in ALS.
What would be your work hypothesis (one possibility)?

A. Motoneurons have high gene expression levels in general, therefore they are sensitive to changes in RNA metabolism

B. Splicing is important in motoneurons because they need a broader variety of proteins

C. Expression of miRNA is more important in this cell type than in other cell types

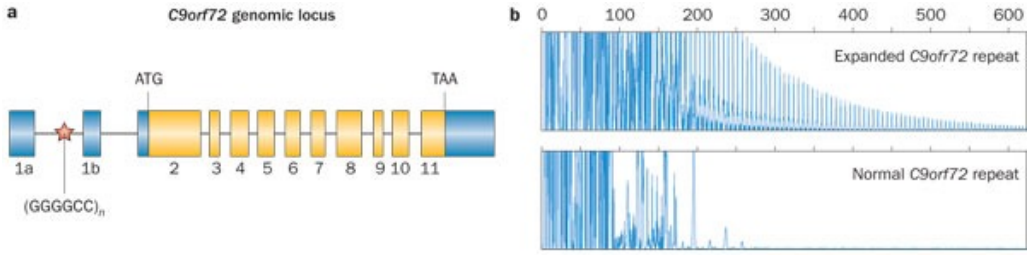
D. These cells are highly specialized and therefore the distribution of RNA in specific cell compartments is very important

E. There is one specific RNA species which is perturbed and causes disease. It needs to be found.

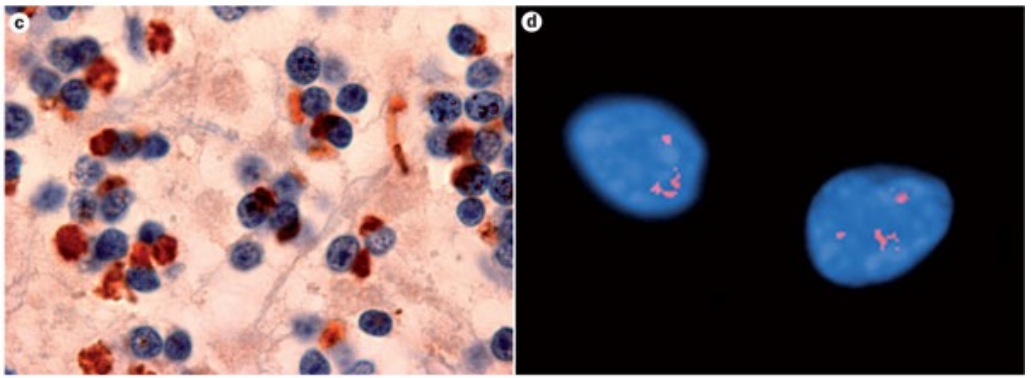
ALS: genetics

C9orf72

- Non-coding hexanucleotide repeat expansion $[GGGGCC]_n$
- Normal allele: 2-25 **intronic** repeats (majority <8)
- Tens to thousands of repeats: association with **ALS/frontotemporal dementia**
- 5% of sALS, 20-40% of fALS (founder effects?)
- Possible implication in Huntington's and Alzheimer's disease



Ubiquitin-positive aggregates



RNA foci (GGCCCC probe)

- Rademakers R. et al., Nat Rev Neurol 2012

Exercise 2