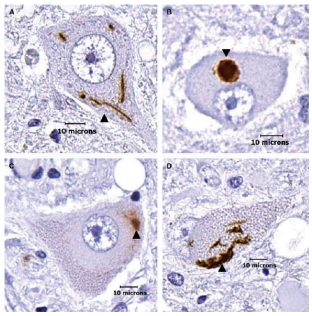
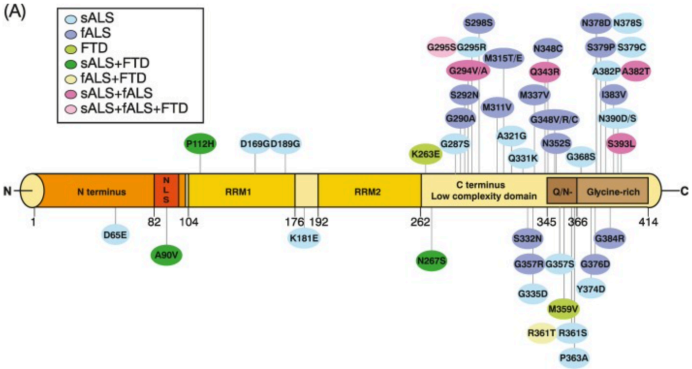
Lecture plan

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

ALS: genetics

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

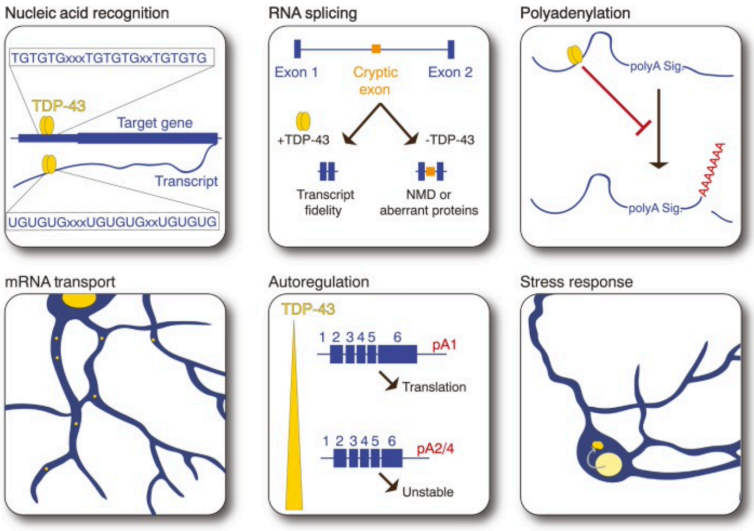




- ≥ 38 mutations
- All but one mutation (Y374X) lead to autosomal dominant inheritance
- Classical ALS phenotype, some mutations associated with frontotemporal lobar degeneration (FTLD)
- Originally identified as a transcriptional repressor
- Two RNA recognition motifs (RRM)
- Normal nuclear distribution → **cytosolic inclusions**
- **Widespread distribution of TDP-43+ inclusions in ALS**

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

Disease-relevant TDP-43 functions



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

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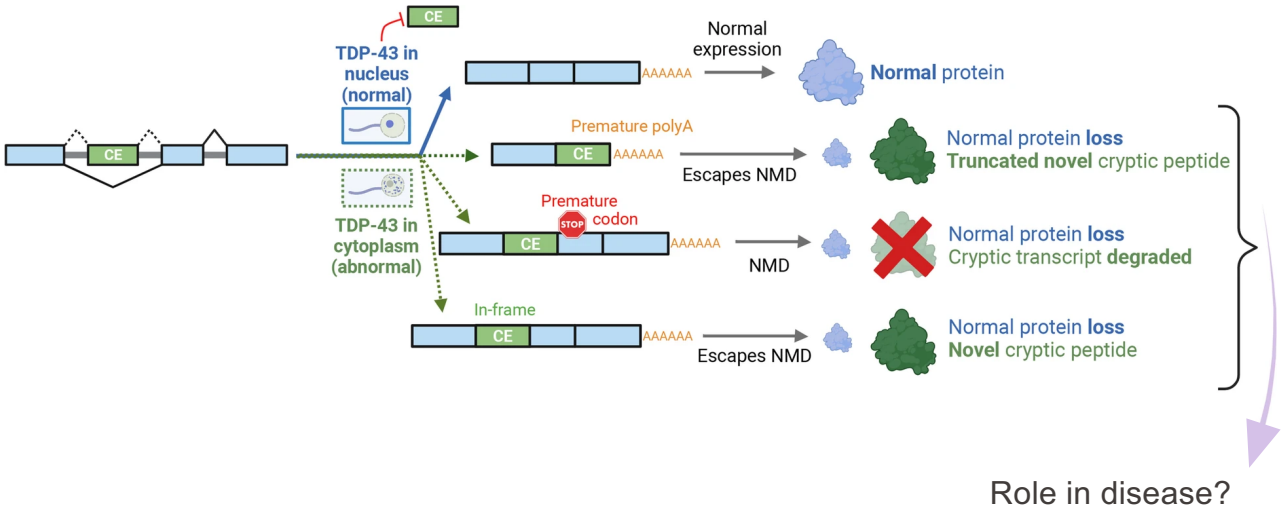
TDP-43-mediated incorporation of cryptic exons: implications for ALS-FTD



Role in disease?

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

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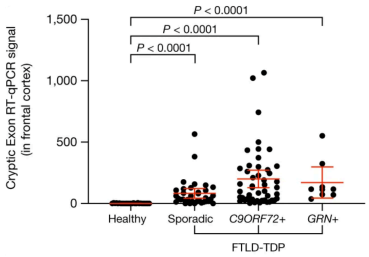
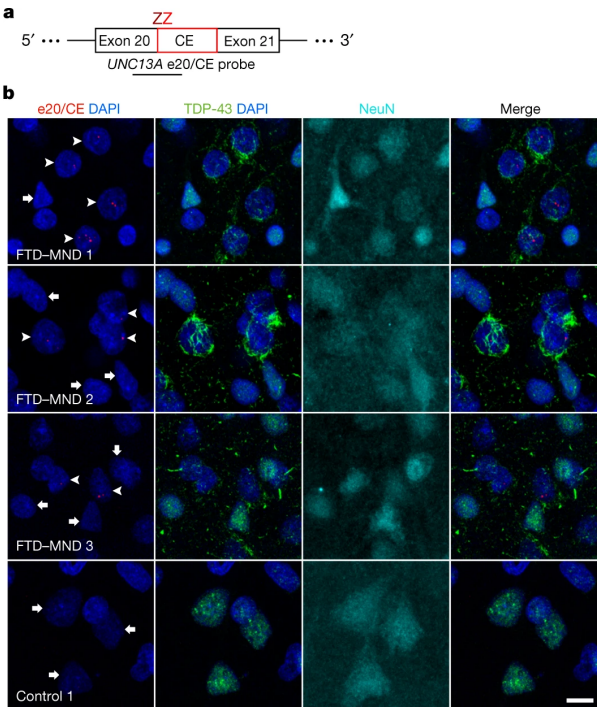
Loss of nuclear TDP-43 is associated with UNC13A cryptic splicing in patients with FTD and motor neuron disease





- *Nature volume 603, pages 124–130 (2022)*

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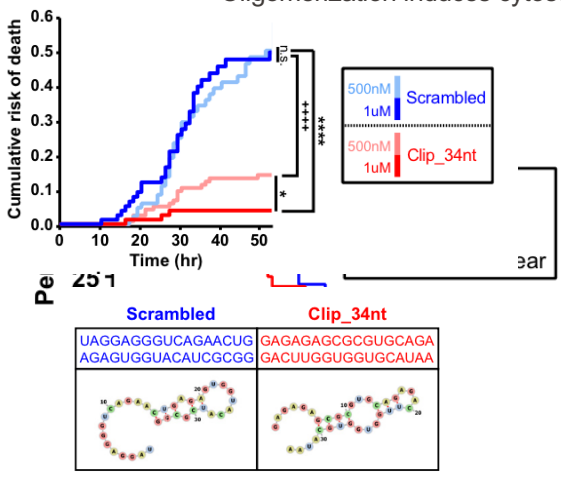
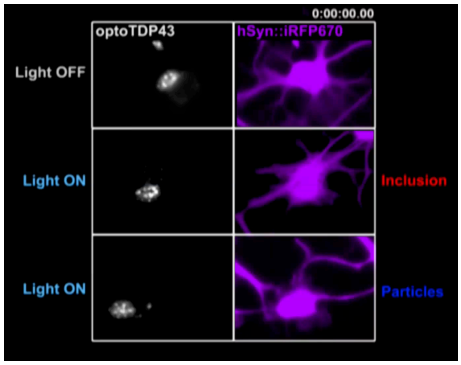
ALS: genetics

OptoTDP-43: light-inducible oligomerization and cytosolic relocalization

Oligomerization induces cytosolic localization of TDP-43





- RNA binding can antagonize TDP-43 mislocalization and aggregation

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

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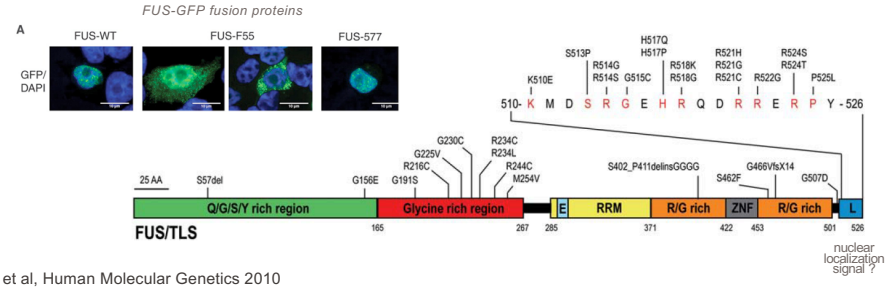
ALS: genetics

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

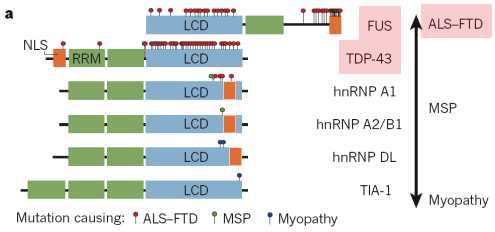
- ≥ 30 mutations
- Dominant inheritance except one recessive mutation (H517Q)
- Classical ALS without cognitive defects
- Some rare patients with FTLD
- One RNA recognition motif (RRM)
- Mainly nuclear protein
- **Abnormal cytosolic inclusions of FUS/TLS in neurons and glia of ALS patients (protein redistribution)**

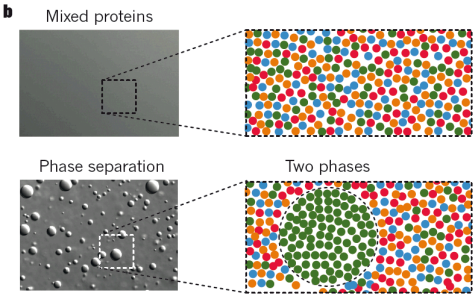


- Lagier-Tourenne C. et al, Human Molecular Genetics 2010
  Kwiatkowski TJ Jr, et al Science 323(5918):1205-8, 2009

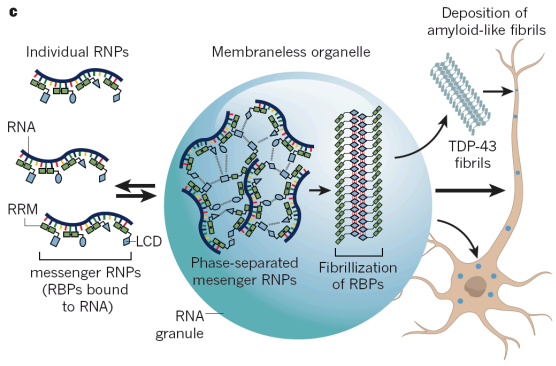
ALS: genetics

A class of proteins which contain low-complexity domains and form membrane-less organelles in association with RNA





RNP: ribonucleoprotein (protein/RNA)

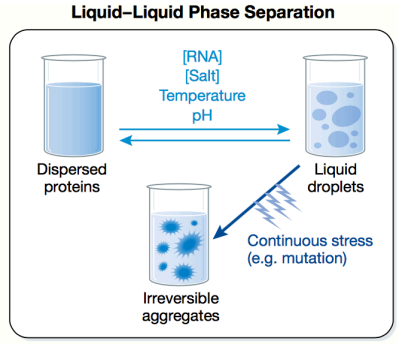


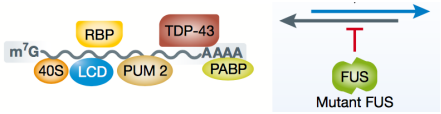
- Taylor JP et al, Nature 2016

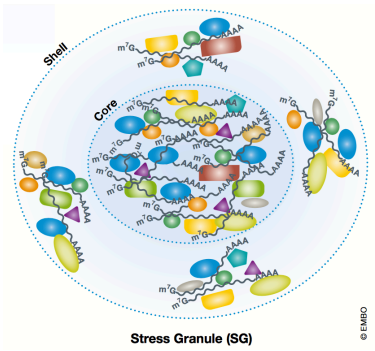
ALS: genetics

FUS/TDP43: regulate the formation of stress granules

- 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.





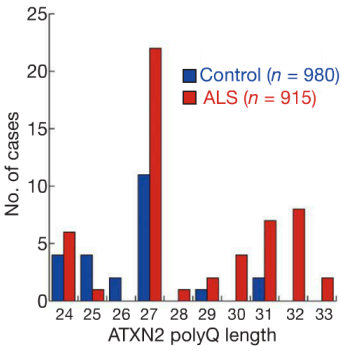


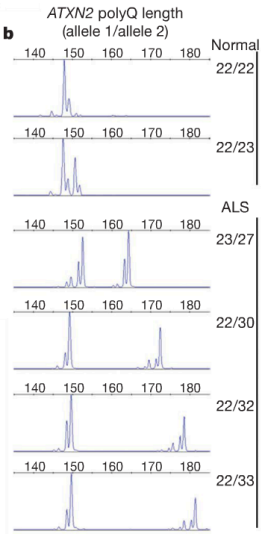
- Gao FB et al, EMBO J 2017
  Protter DSW et al, Trends in Cell Biol 2016

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



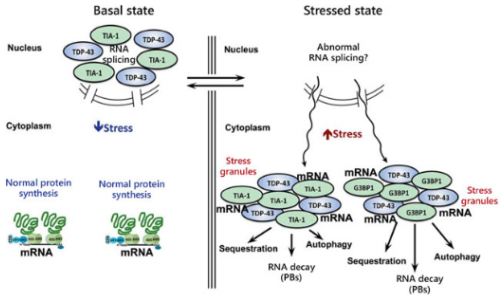


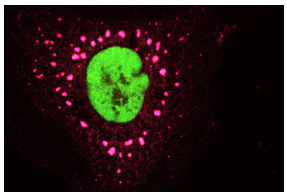
PolyQ lengths ≥27 are significantly enriched in ALS vs. controls

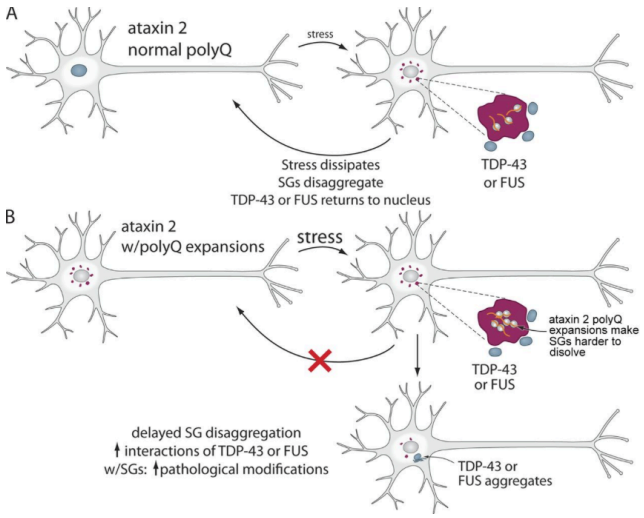
- Elden AC, et al Nature 2010

ALS: genetics

A role for Ataxin 2 in stress granule dynamics







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Motor neuron diseases: question 8

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

Normal:	nucleus	⟷	cytoplasm	⟷	stress granule	
ALS:	nucleus		cytoplasm		stress granule	⟷ aggregate

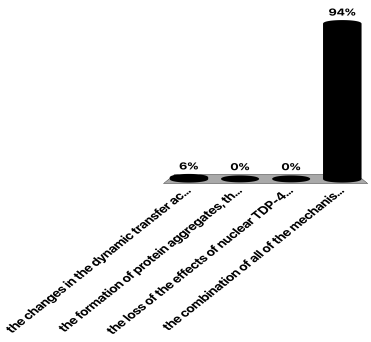
Based on these observations, would you think that neurodegeneration is caused by…

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.



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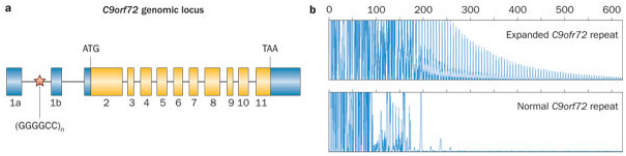
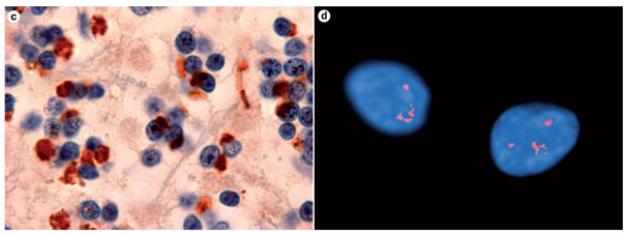
ALS: genetics

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



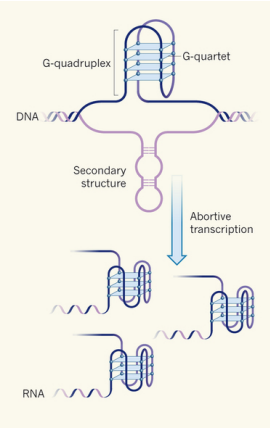
RNA foci (GGCCCC probe)

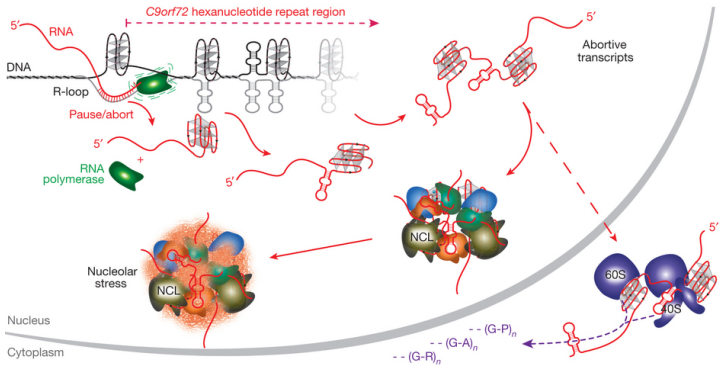
- Rademakers R. et al., Nat Rev Neurol 2012

ALS: genetics

C9orf72

- Hexanucleotide repeat expansion $[GGGGCC]_n$ generates a **G-quadruplex structure** (DNA and RNA)
- Abortive transcription (reduced C9orf72 expression)
- Nucleolar stress
- Aberrant dipeptide expression (RAN translation – *RAN = Repeat-associated non-ATG*)

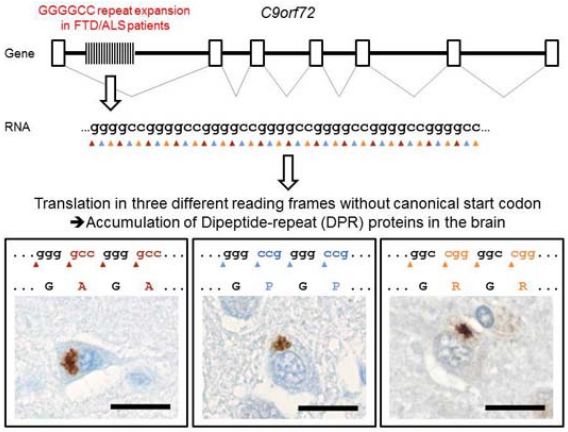




- Haeusler AR et al, Nature 2014

C9orf72

- Accumulation of the $[GGGGCC]_n$ repeats correlates with neuronal accumulation of **aggregating dipeptide-repeat proteins** (glycine-alanine, glycine-proline or glycine-arginine)
- Repeats also lead to **reduced expression of C9orf72** (unknown function, possibly related to endosomal processing and autophagy)



- Mori K et al, Science 2013

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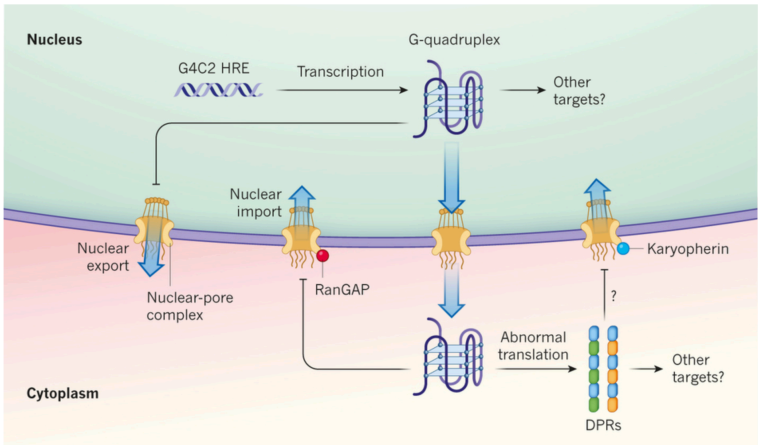
C9orf72 hexanucleotide expansion

Blockade of the **nucleocytoplasmic transport**



- Freibaum BD et al, Nature 2015
  Fox BW et al, Nature 2015

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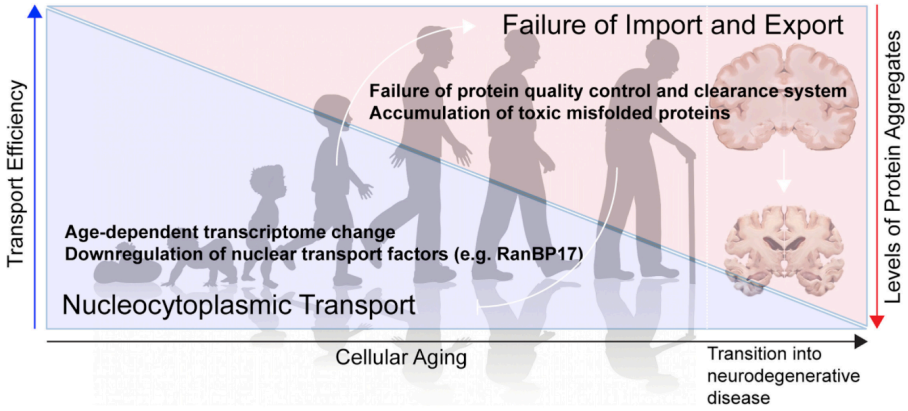
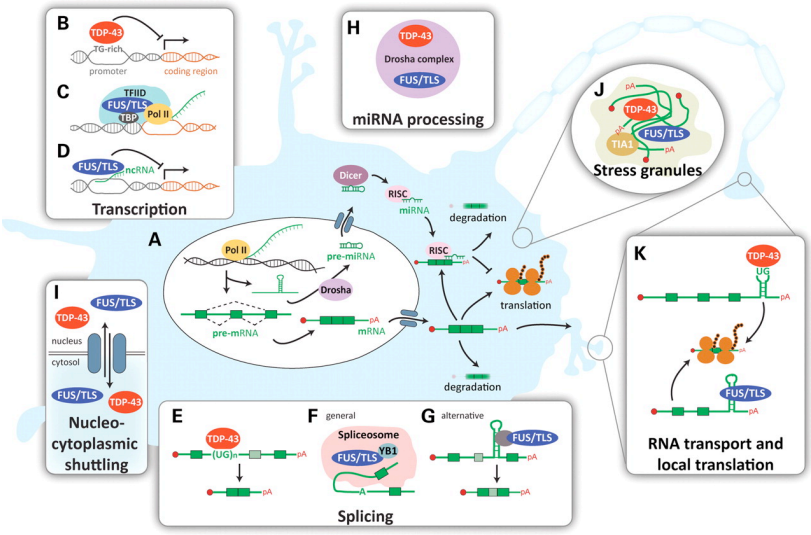
Cellular Aging, Disease, and Nucleocytoplasmic Transport



- Kim HJ, Taylor JP, Neuron 2017

An important role for RNA processing in ALS pathology ?



- TDP-43 & FUS/TLS
- Angiogenin: regulator of ribosomal RNA transcription
- Senataxin: helicase (DNA repair, RNA production)

- Lagier-Tourenne C. et al, Human Molecular Genetics 2010

Motor neuron diseases: question 9

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.

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