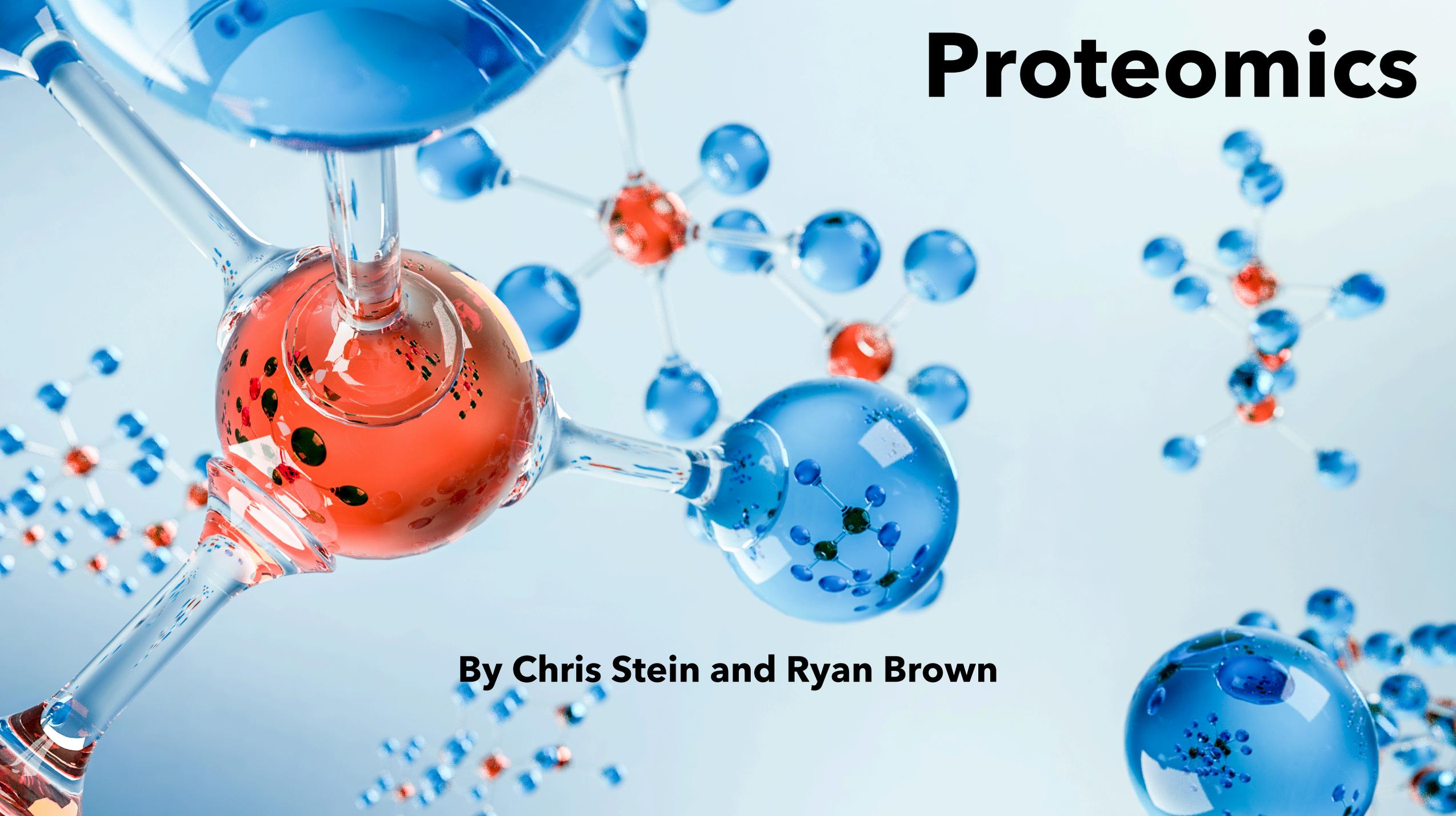
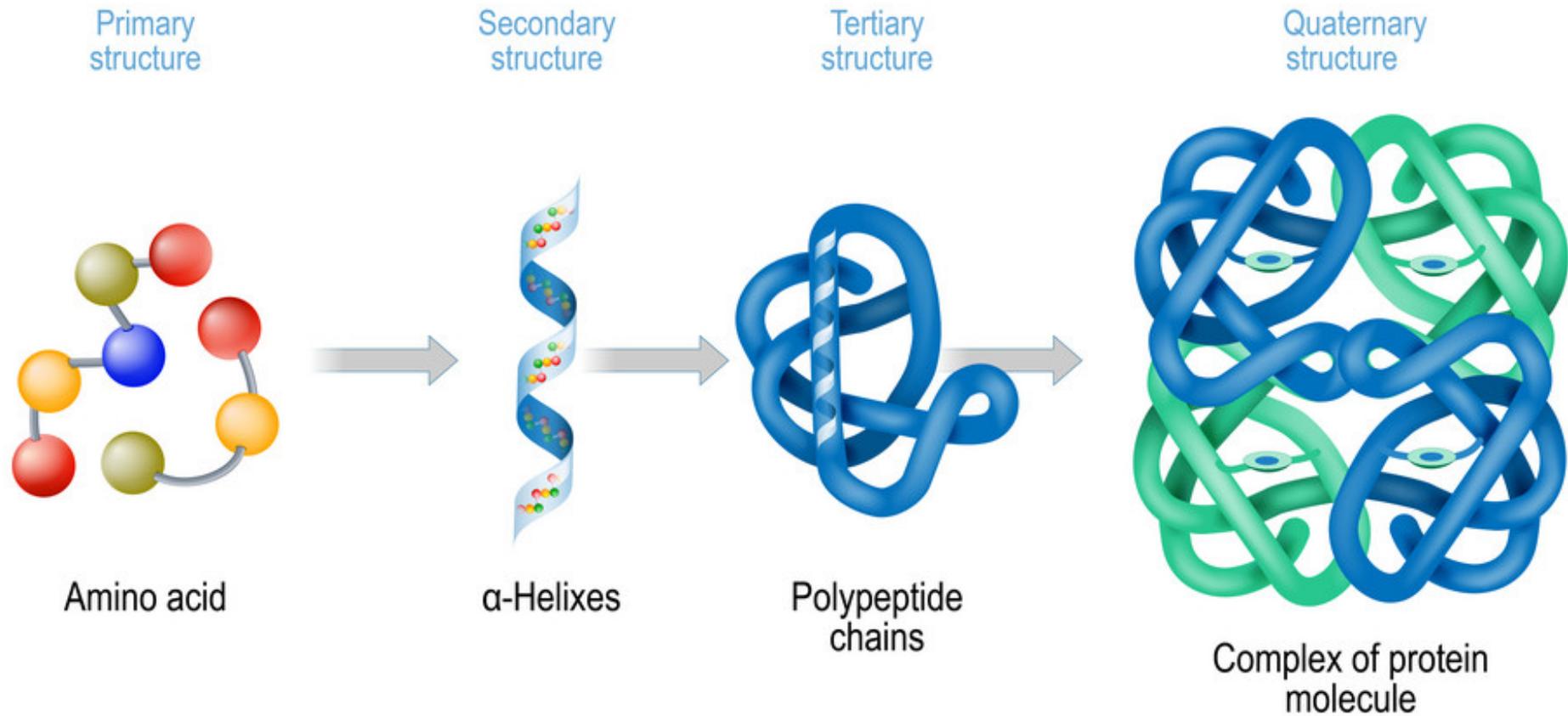


Proteomics

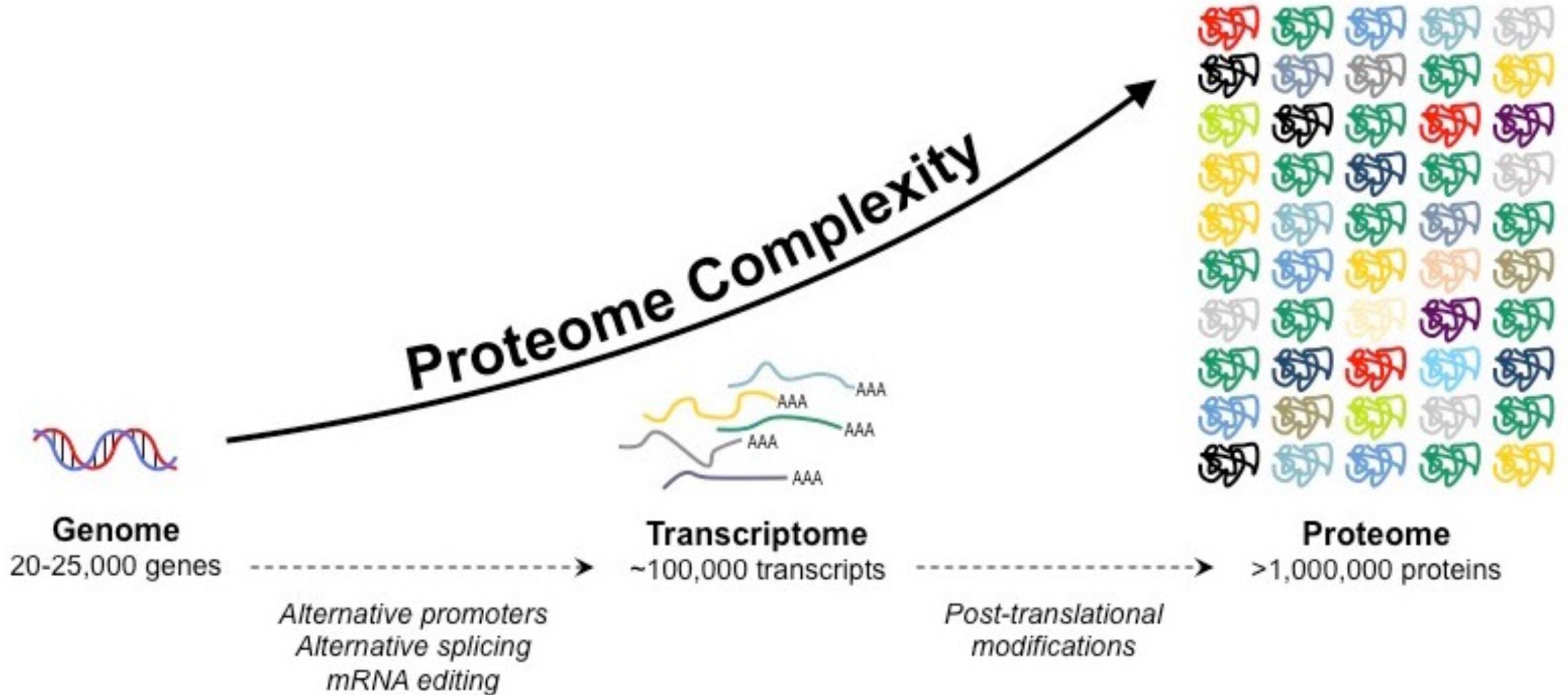
A 3D rendered image of a laboratory flask containing orange liquid, surrounded by blue and red molecular models. The flask is positioned in the center-left, and the molecular models are scattered around it, creating a scientific and technological atmosphere. The background is a light blue gradient.

By Chris Stein and Ryan Brown

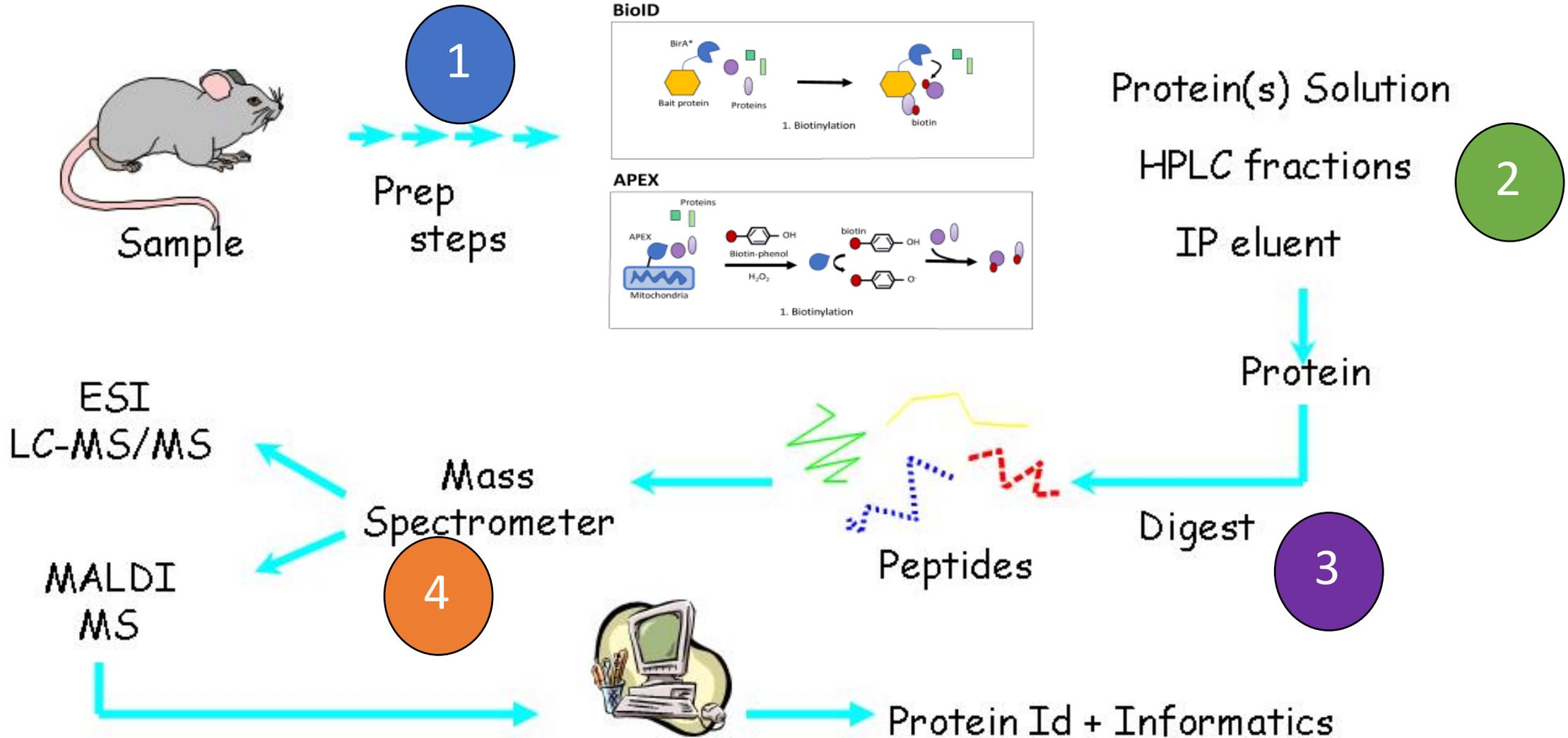
What are proteins?



What is proteomics?

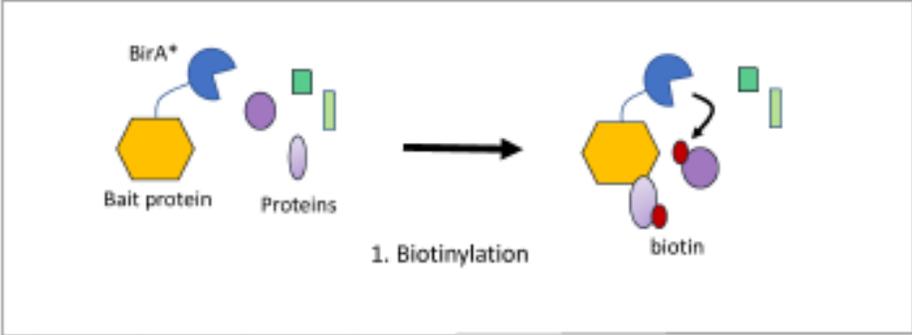


What steps are involved in proteomics?

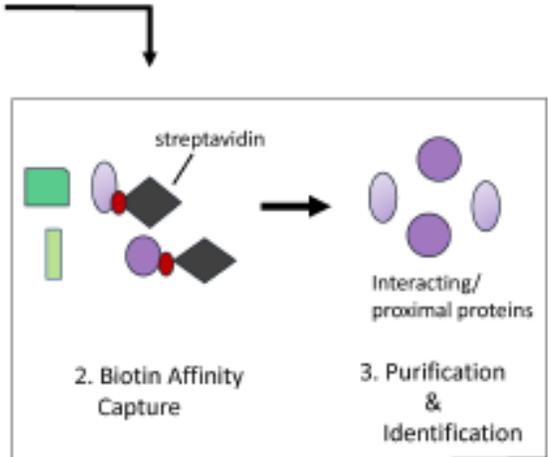
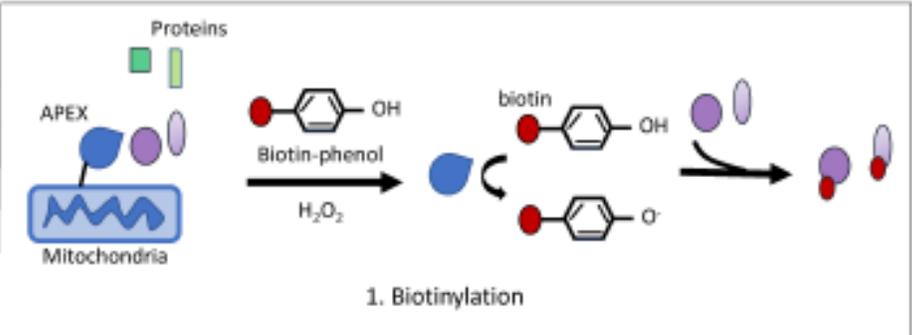


1) What are 3 main ways proteins can be isolated from a cell/tissue?

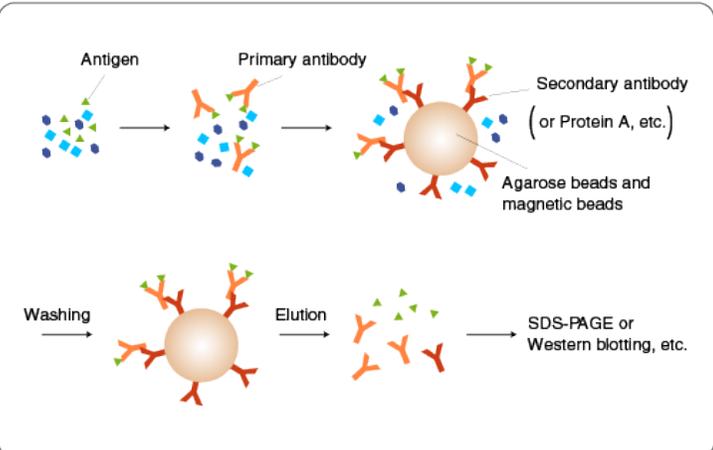
BioID



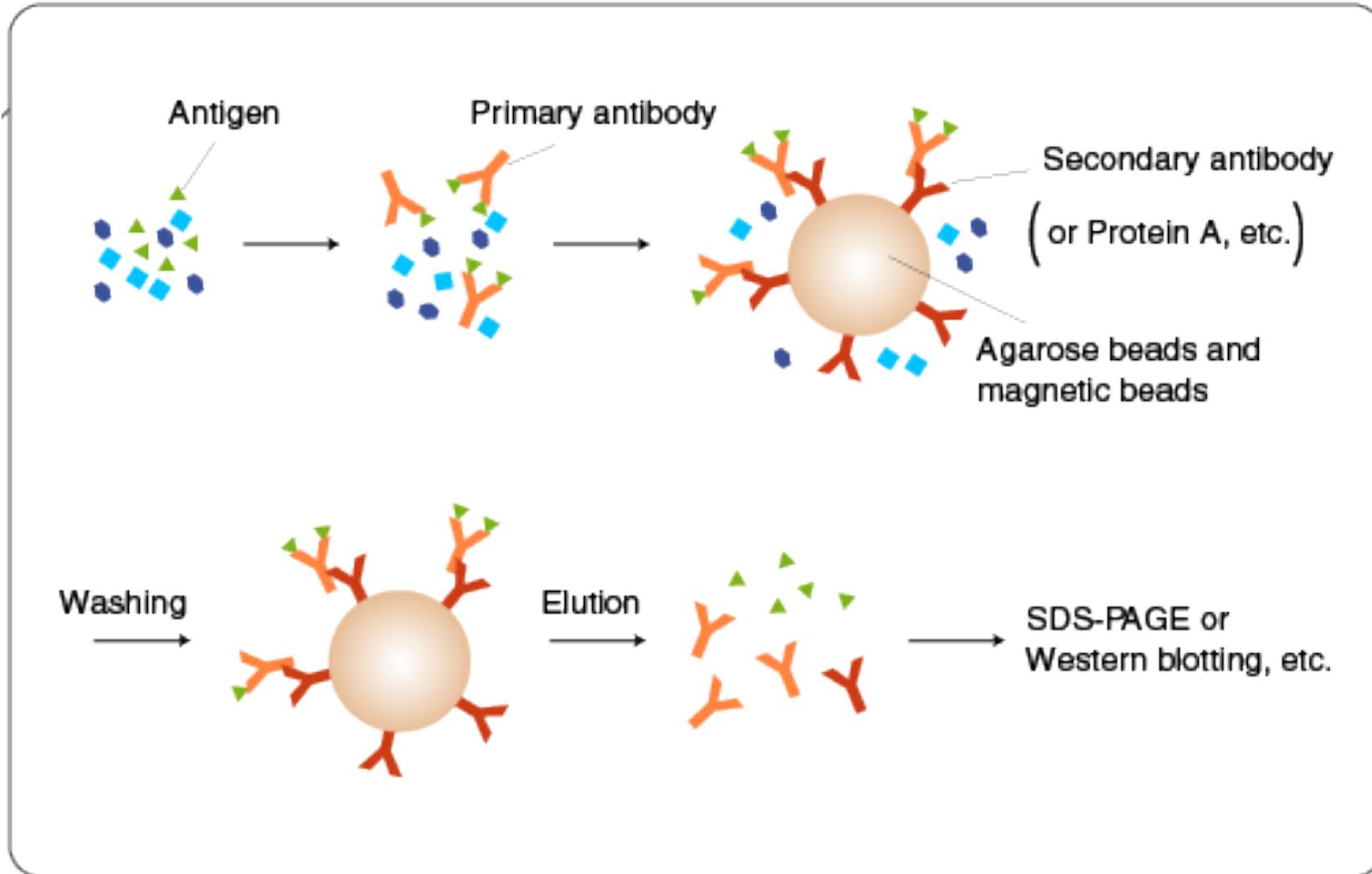
APEX



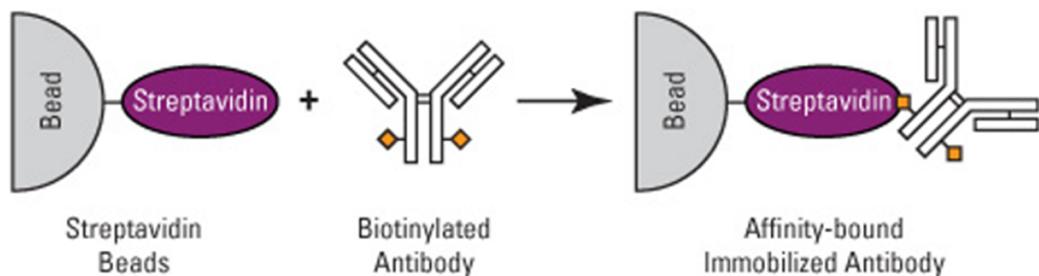
Immunoprecipitation



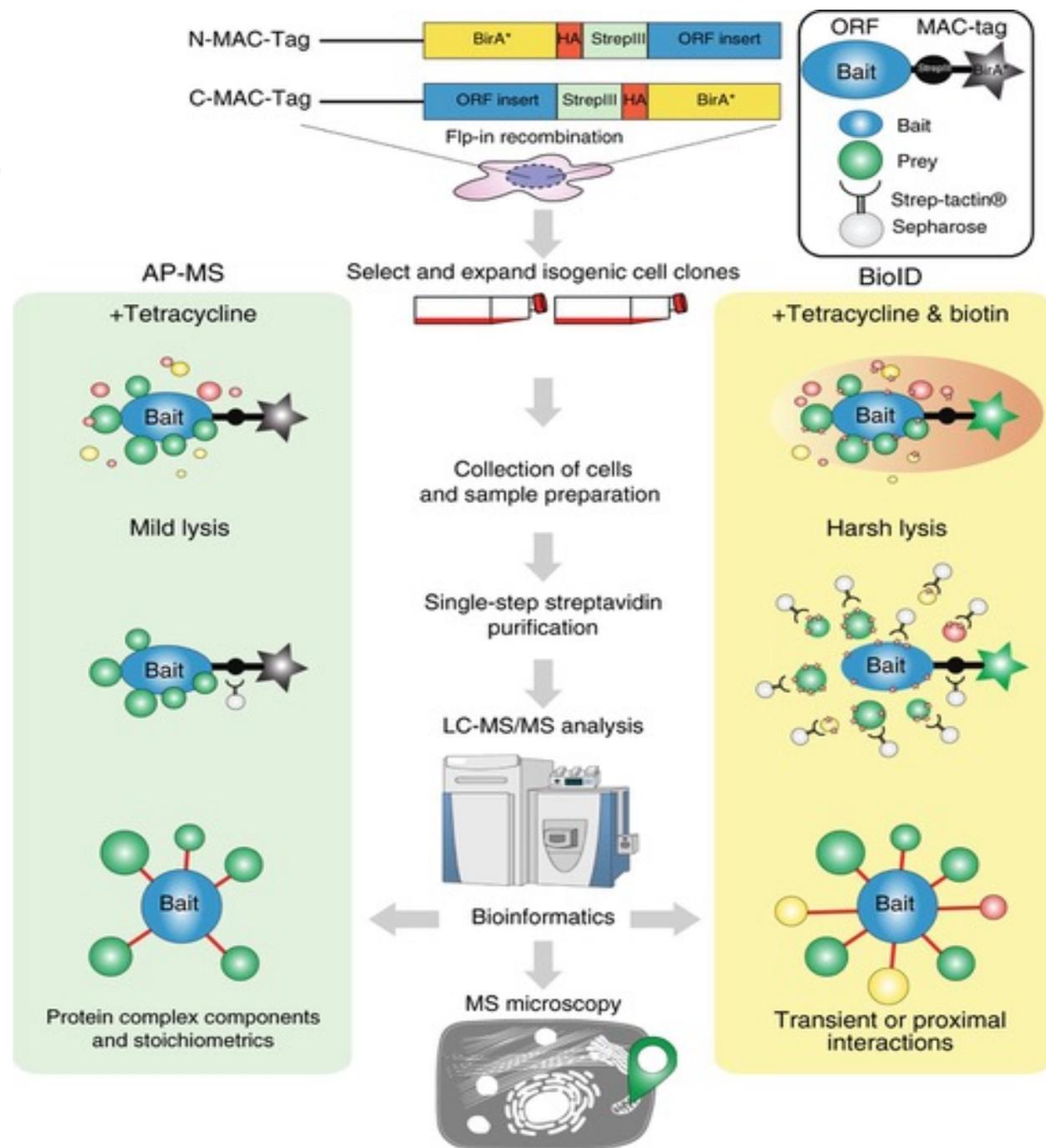
How are single proteins isolated?



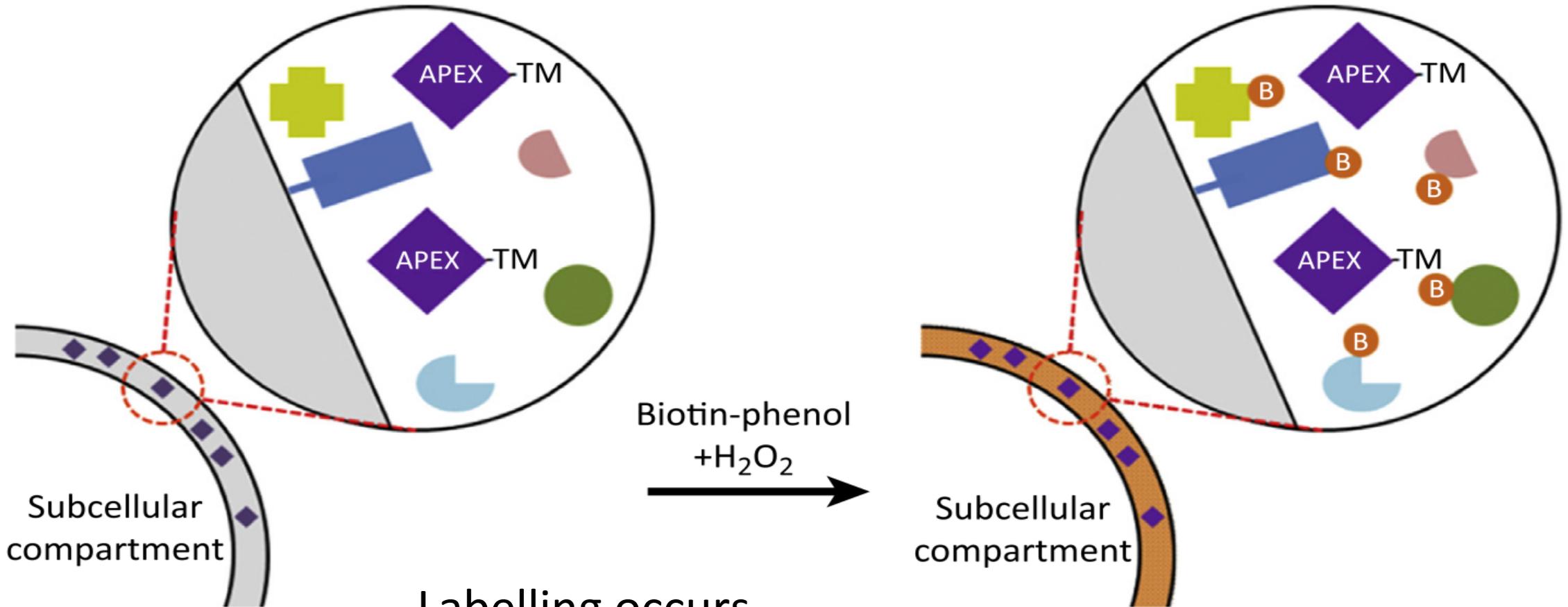
How are protein complexes isolated?



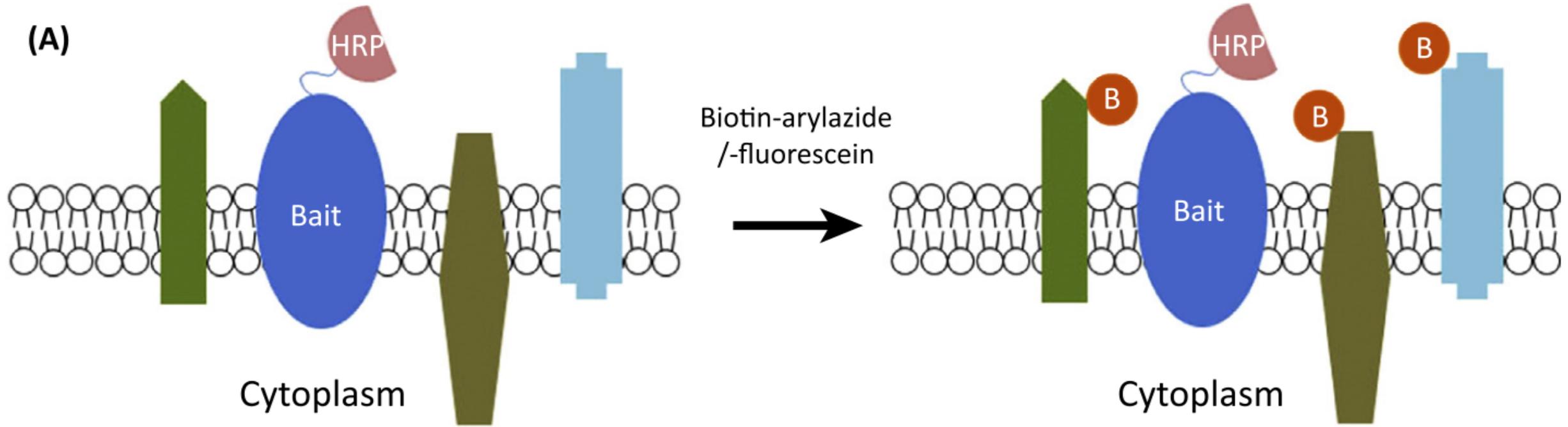
Labelling occurs in 15-18 hours



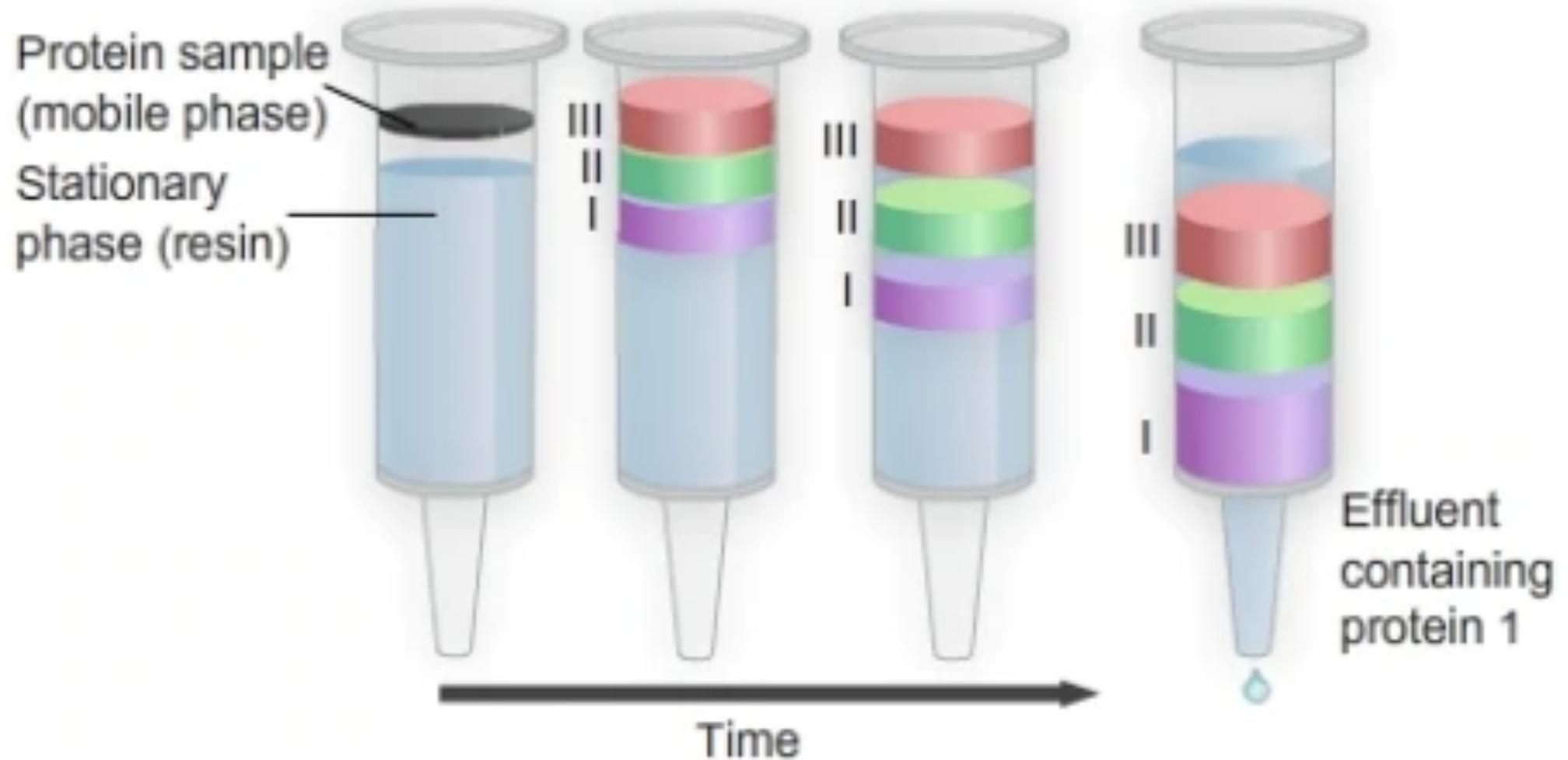
How are subcellular protein complexes isolated?



How are membrane proteins isolated?

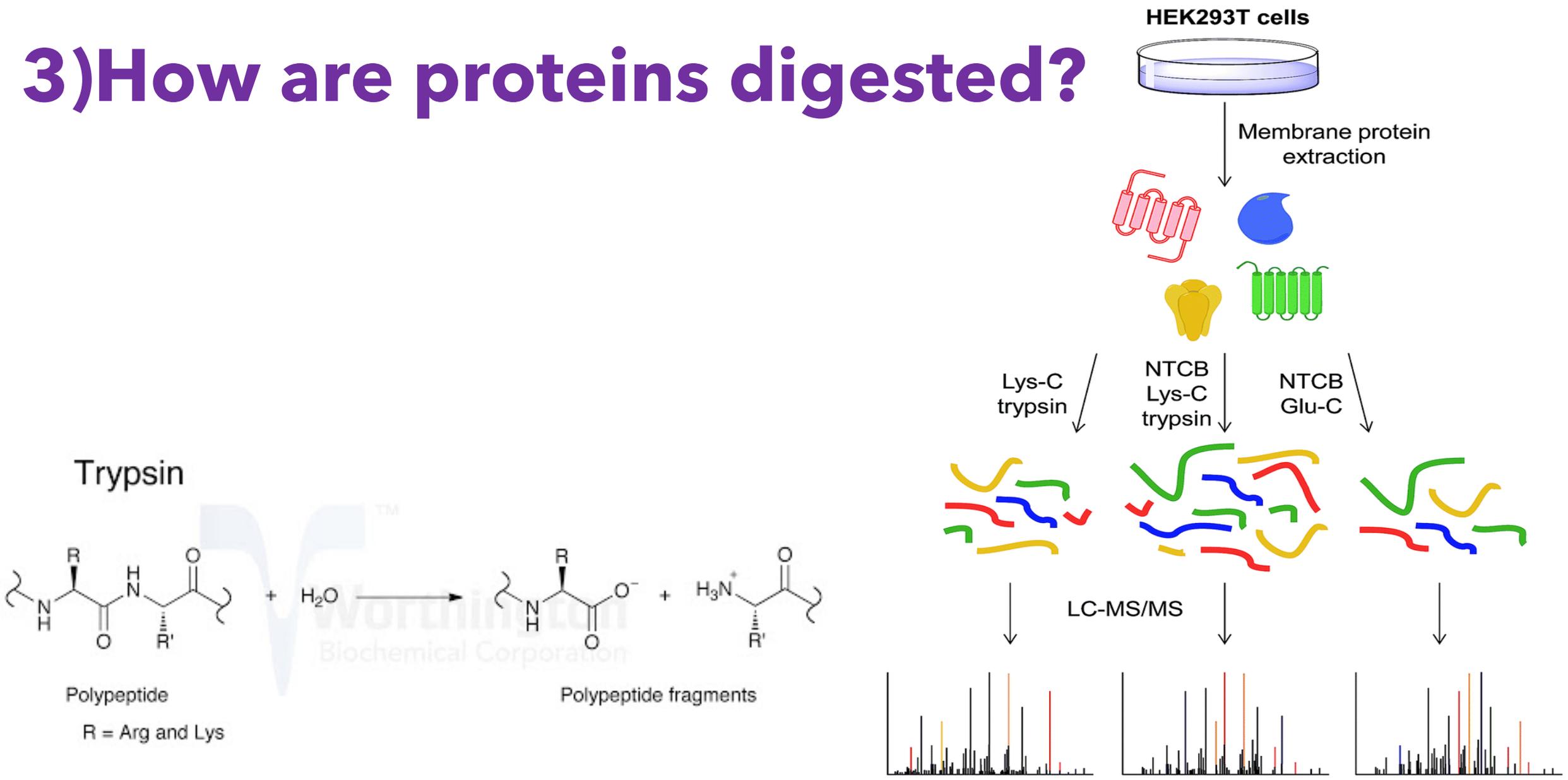


2) How are proteins separated?



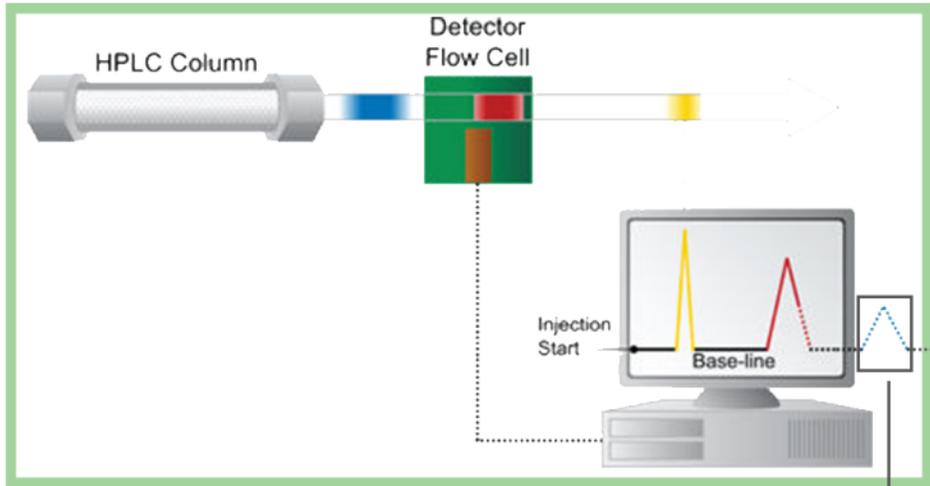
After IP/BioID/APEX/HRP, the specific proteins are separated with HPLC.

3) How are proteins digested?



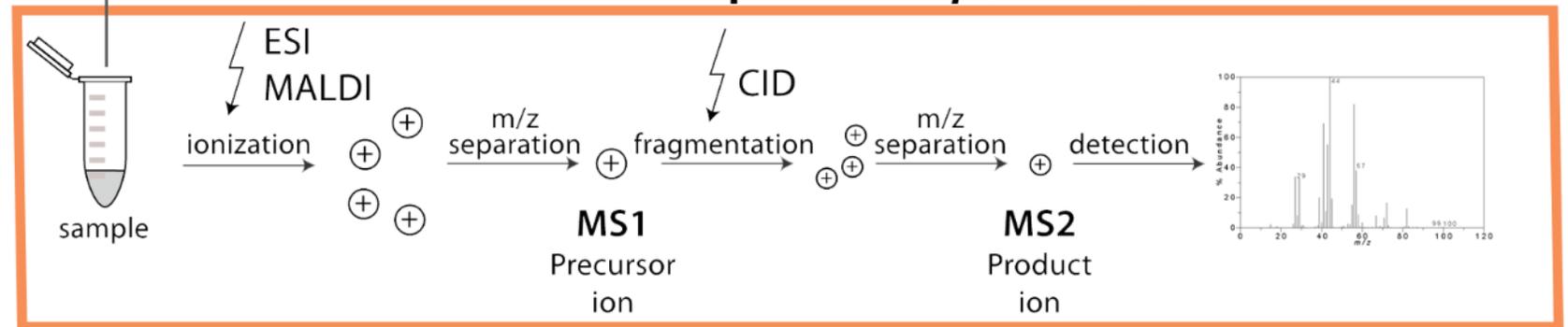
Proteases are commonly used to shorten proteins before MS.

4) How are proteins sequenced?



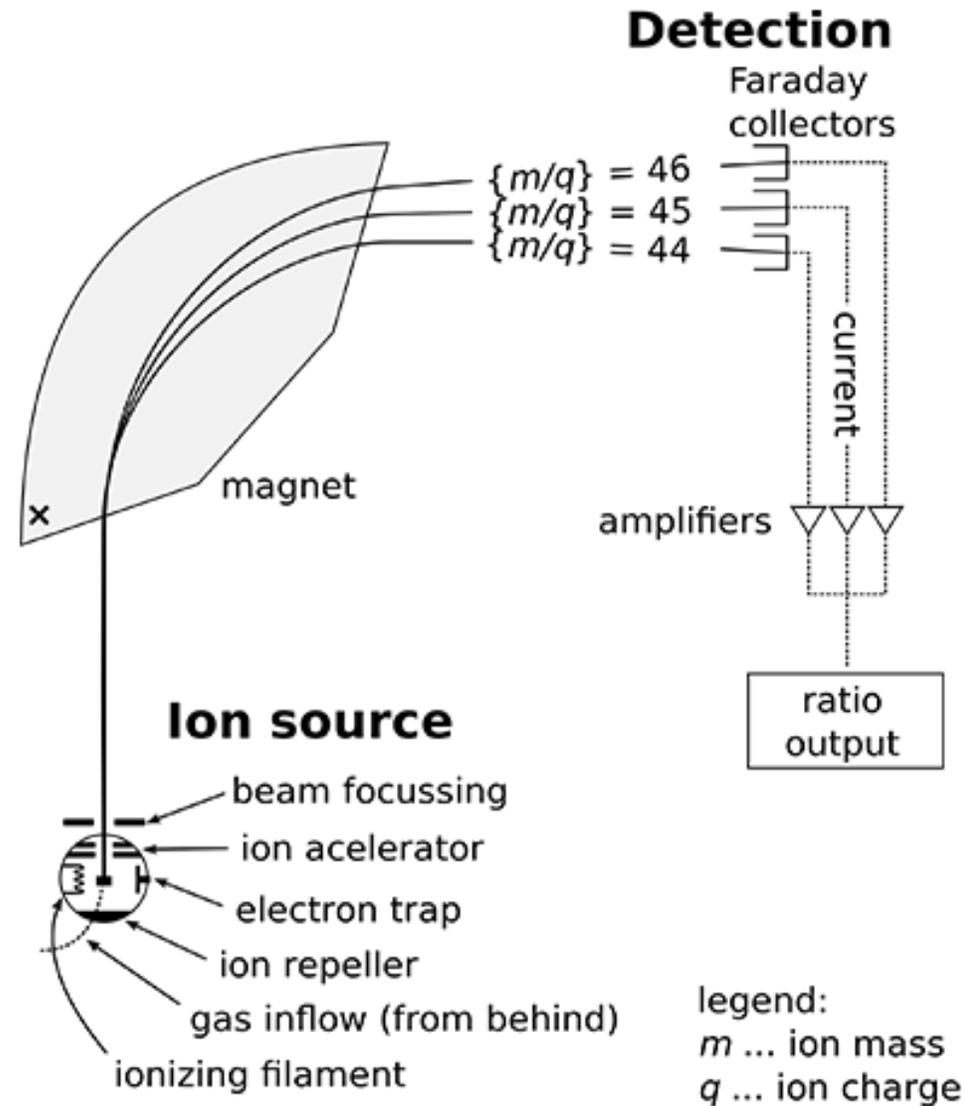
Liquid Chromatography (LC)

Tandem Spectrometry (MS/MS)

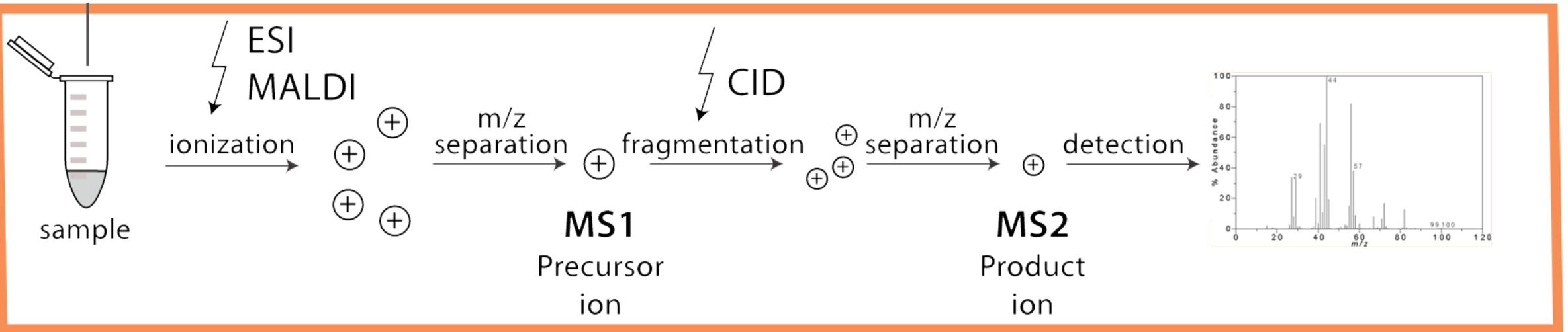


Protein fragments are sequenced with **tandem mass spectrometry**

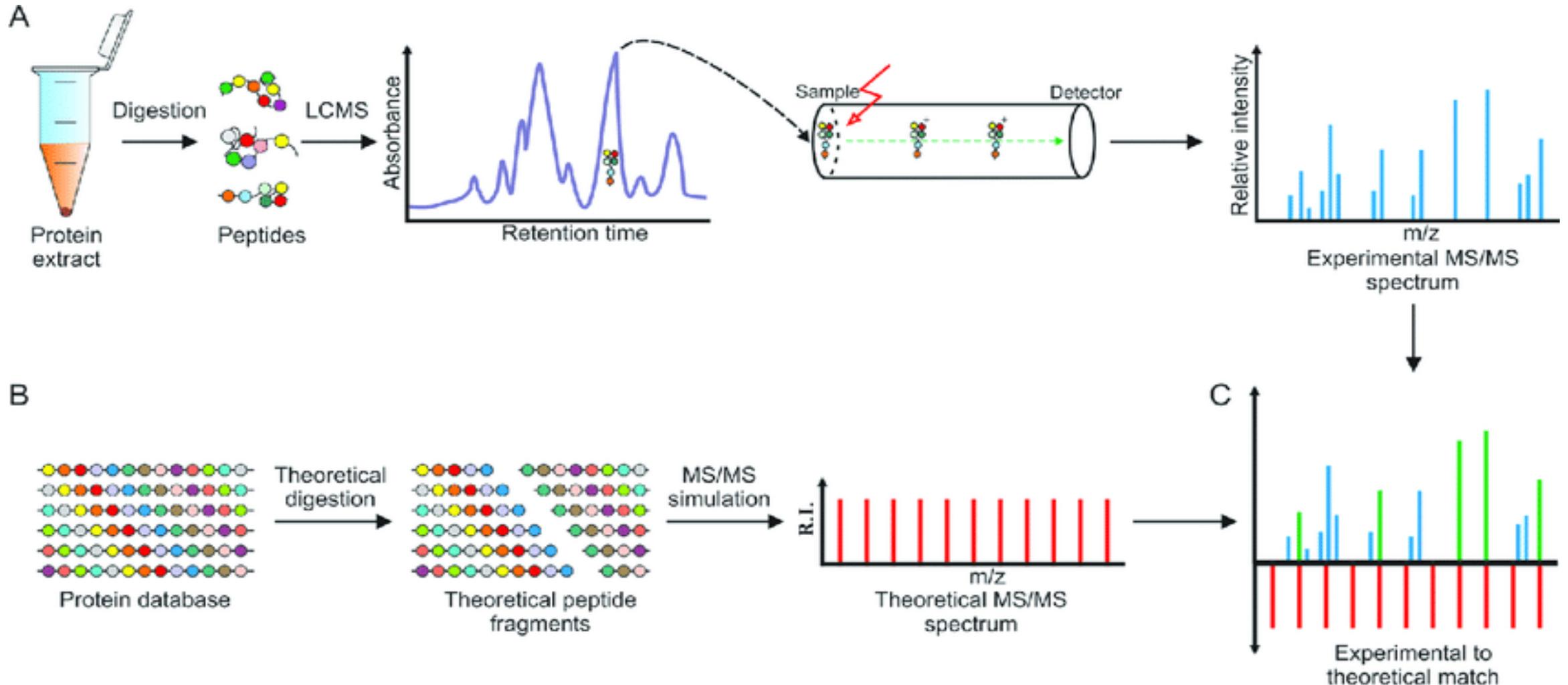
How does Mass Spectrometry (MS) work?



How is tandem MS different?



How are protein sequences determined?

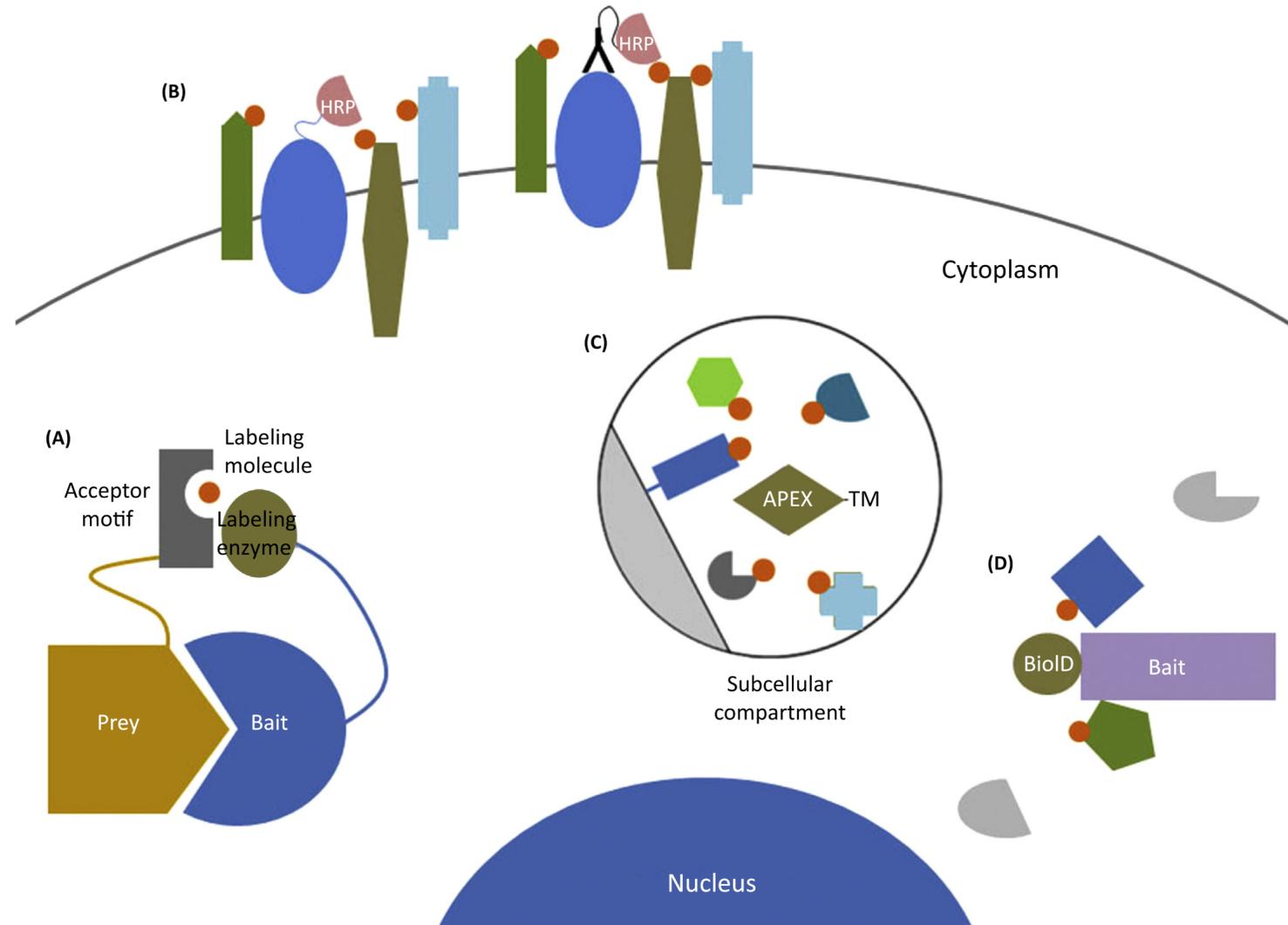


Experimental and theoretical MS are compared to determine proteins

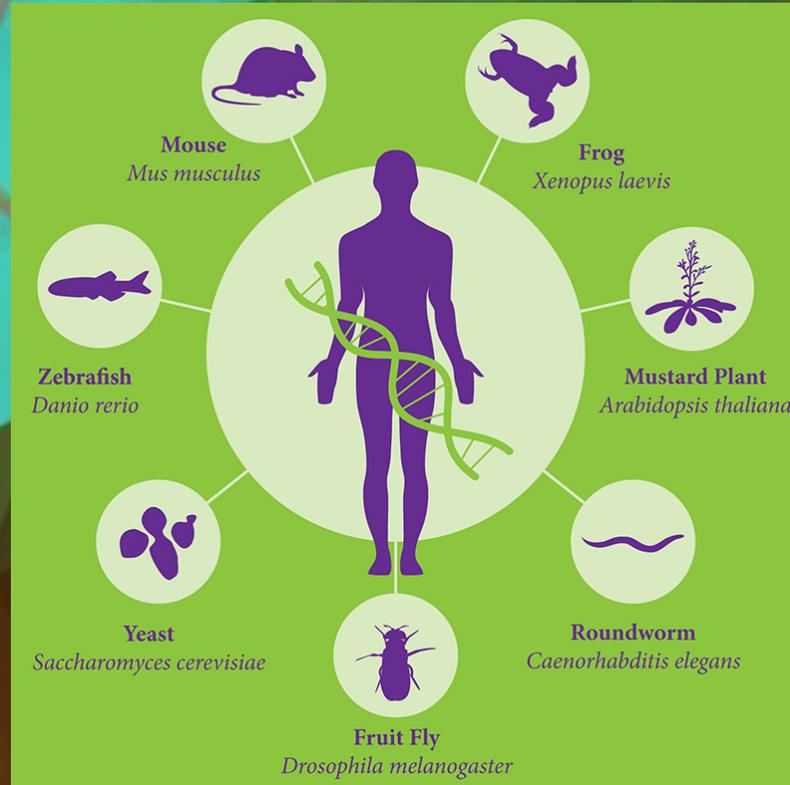
What is sequence coverage?

1	MKWVTFISLL	LLFSSAYSRG	VFRRDTHKSE	IAHR FKDLGE	EHFKGLVLIA
51	FSQYLQQCPF	DEHVKLVNEL	TEFAKTCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRE TYGD	MADCCEKQEP	ERNECFLSHK	DDSPDLPKLK	PDPNTLCDEF
151	KADEK KFWGK	LYE IARRHP	YFYAPELLYY	ANKY NGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPK AE
251	FVEVTKLVTD	LTKVHKE CCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEKDA	IPENLPPLTA	DFAEDKDVCK	NYQEAKDAFL
351	GSFLYEYSRR	HPEYAVSVLL	RLAKEYEATL	EECCA KDDPH	ACYSTVFDKL
401	KHLVDEPQNL	IKQNC DQFEK	LGEYGFQNAL	IVRYTRKVPQ	VSTPTLVEVS
451	RSLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRL CVLHEKT	PVSEKVT KCC
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIKKQT
551	ALVELLKHKP	KATEEQLKTV	MENFVAFVDK	CCAADDKEAC	FAVEGPKLVV
601	STQTALA				

What technique could you use for your aims?



What model organism should you use?



Enzyme	BioID	HRP	APEX
Organisms	Mammalian cells, xenograft tumors in mice, <i>Trypanosoma brucei</i> , <i>Toxoplasma gondii</i> , <i>Dictyostelium discoideum</i> , <i>Plasmodium berghei</i>	Mammalian cells	Mammalian cells, <i>Schizosaccharomyces pombe</i> , <i>Saccharomyces cerevisiae</i> , <i>Drosophila melanogaster</i>

Summary

- 1)** Proteomics is a powerful method to determine protein sequences and interactions.
- 2)** BioID and APEX are two methods based on IP that will label nearby proteins.
- 3)** APEX only gives a snapshot of protein activity; whereas, BioID gives a history of protein associations.

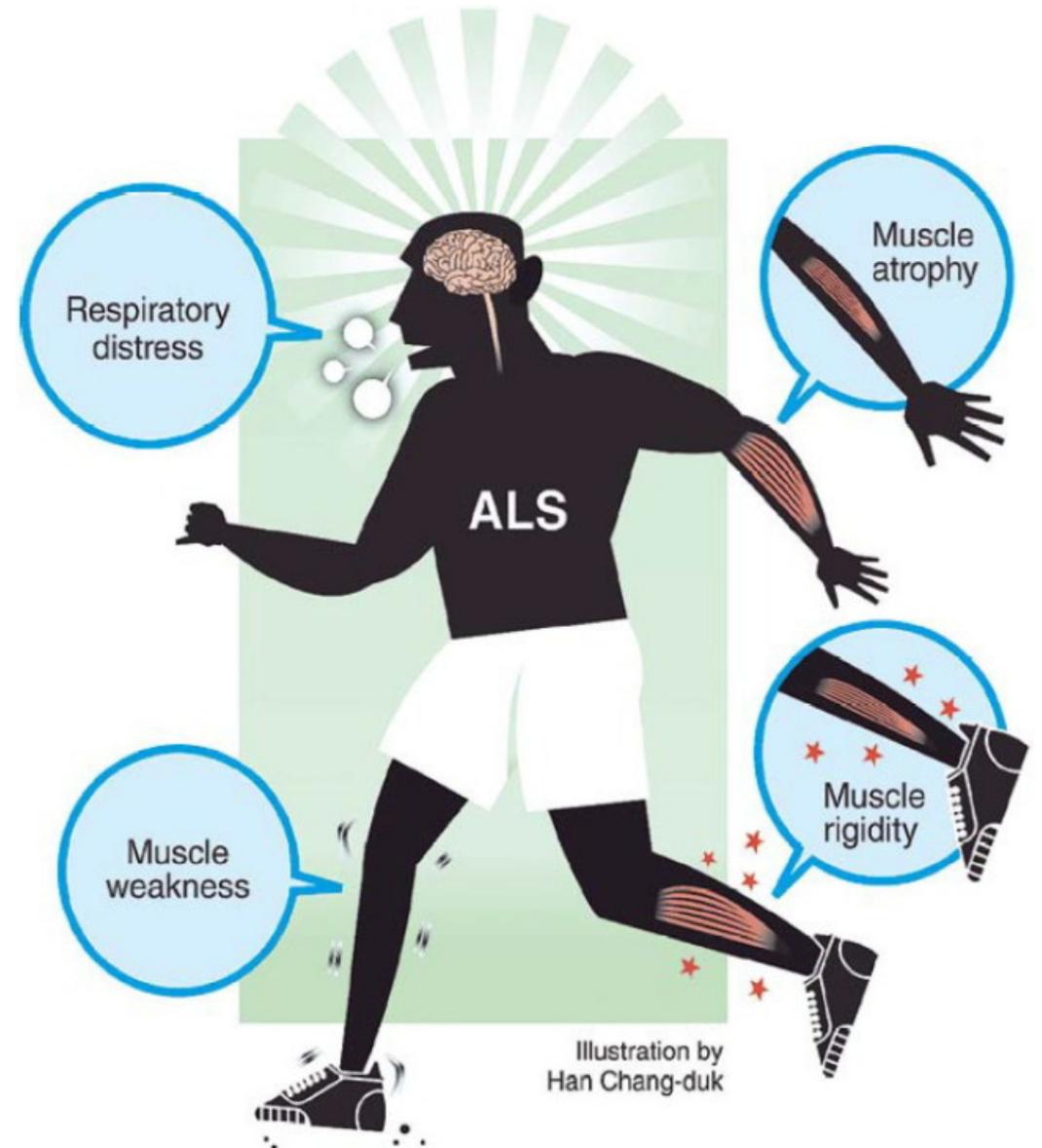
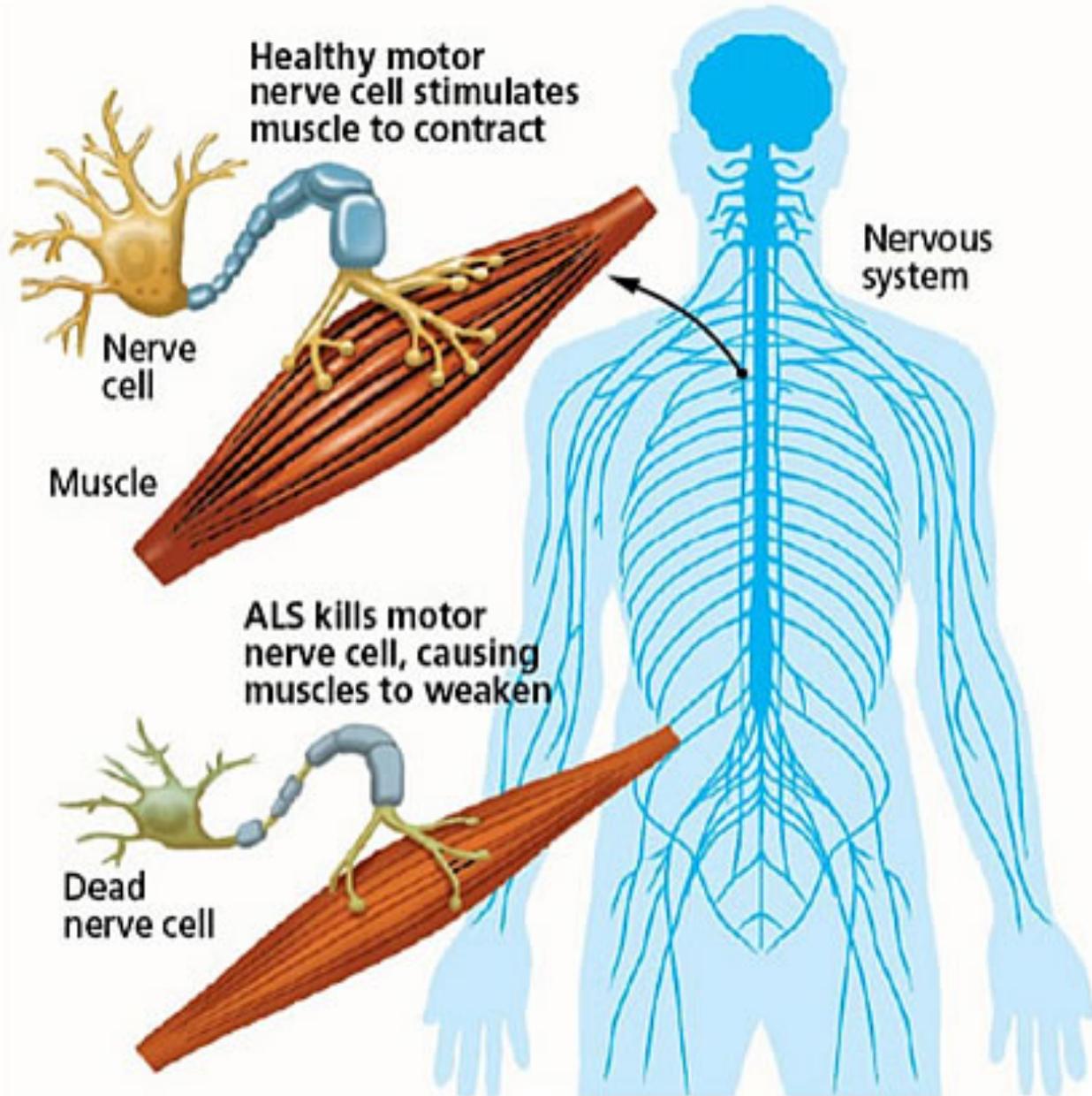
TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD

Ching-Chieh Chou et al.

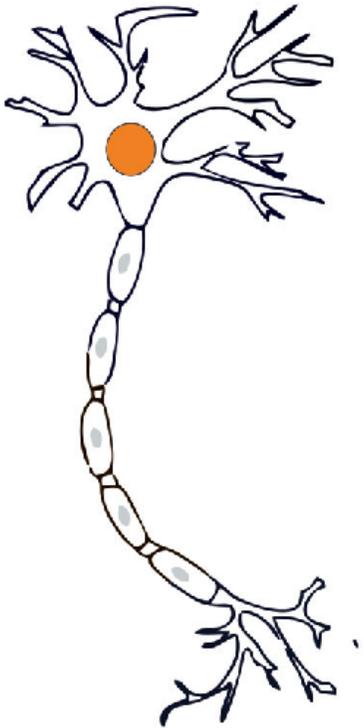
Nature Neuroscience

2018

What is Amyotrophic Lateral sclerosis (ALS)?

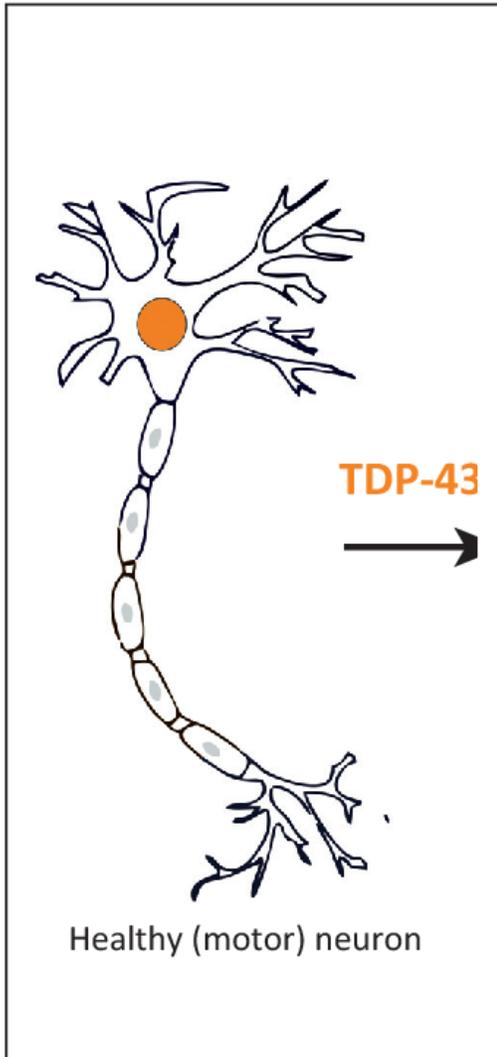


What **gene** is often mutated in ALS?

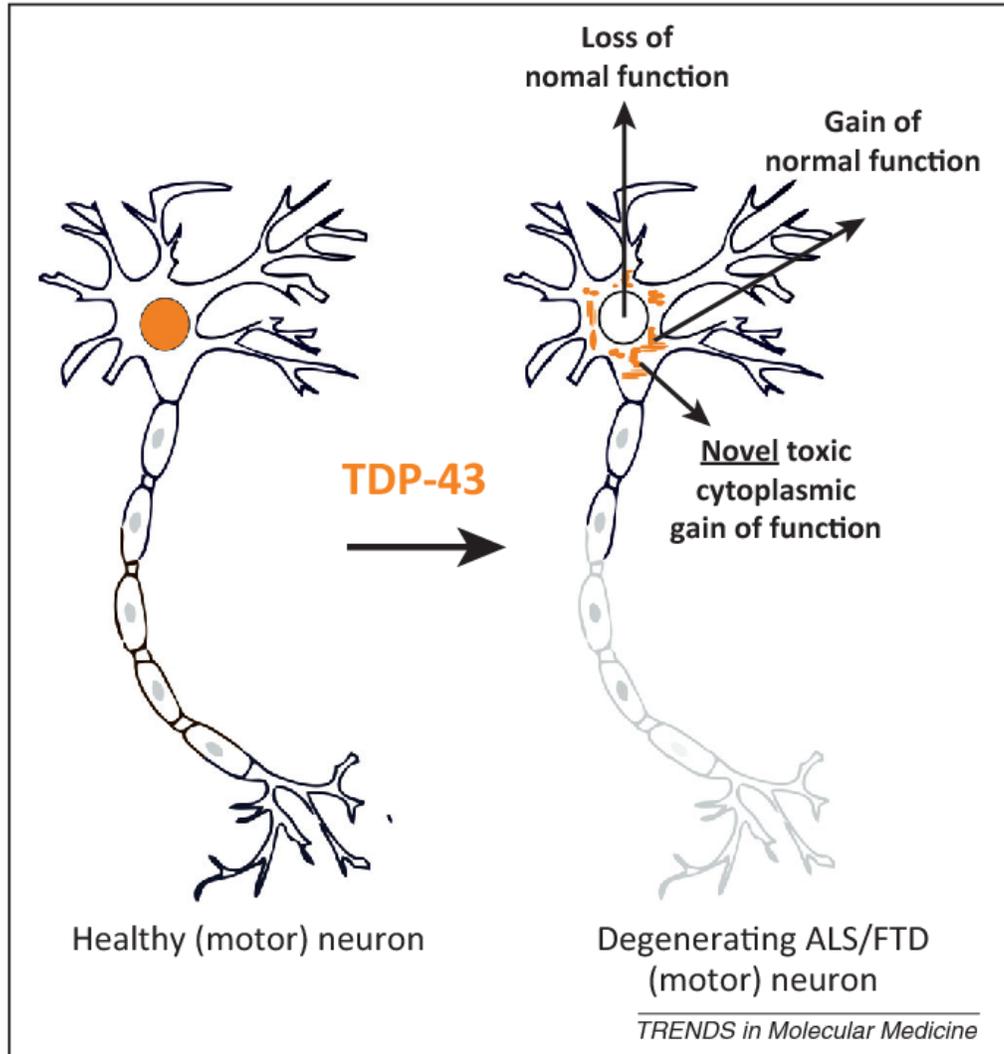


Healthy (motor) neuron

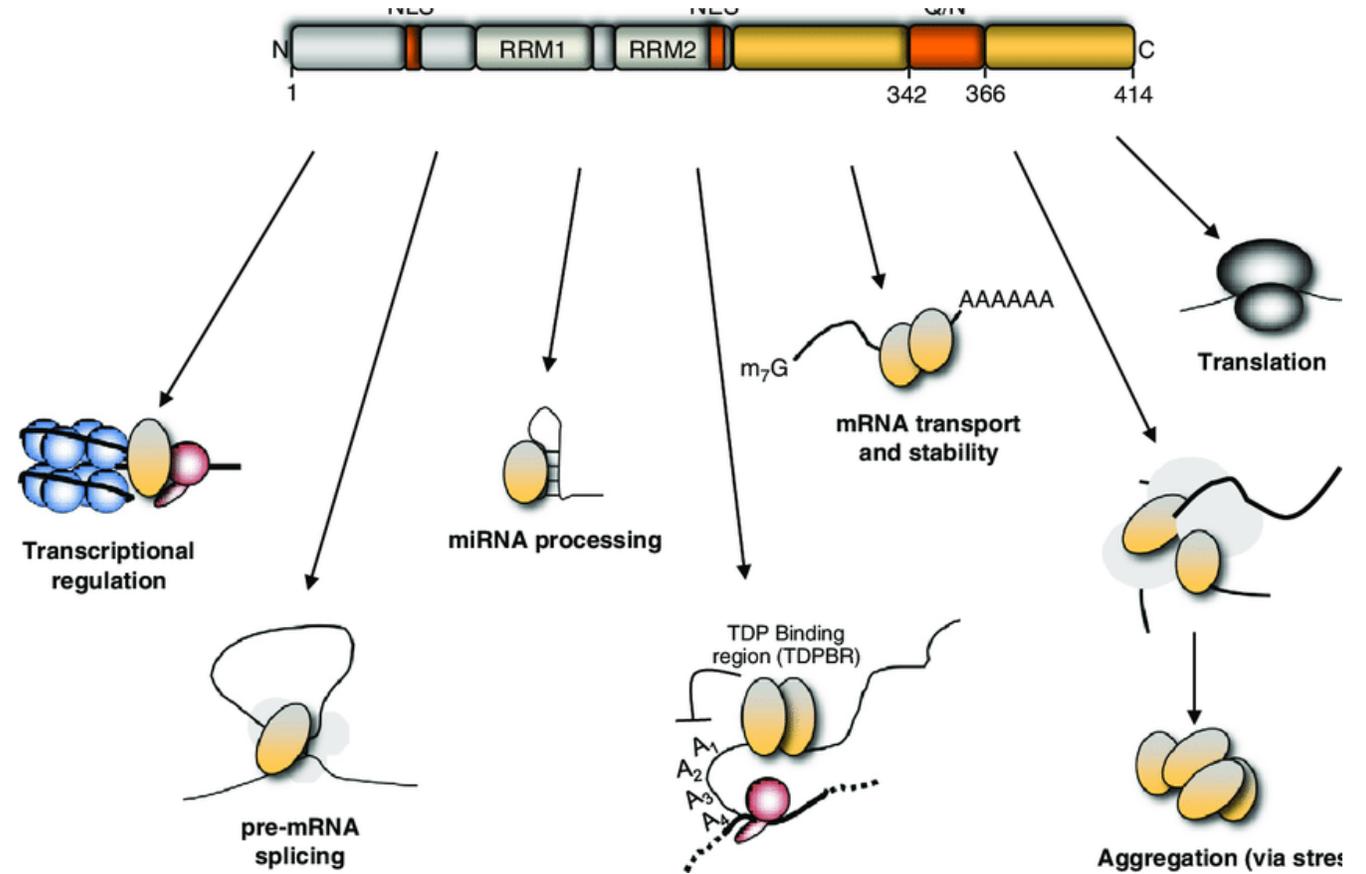
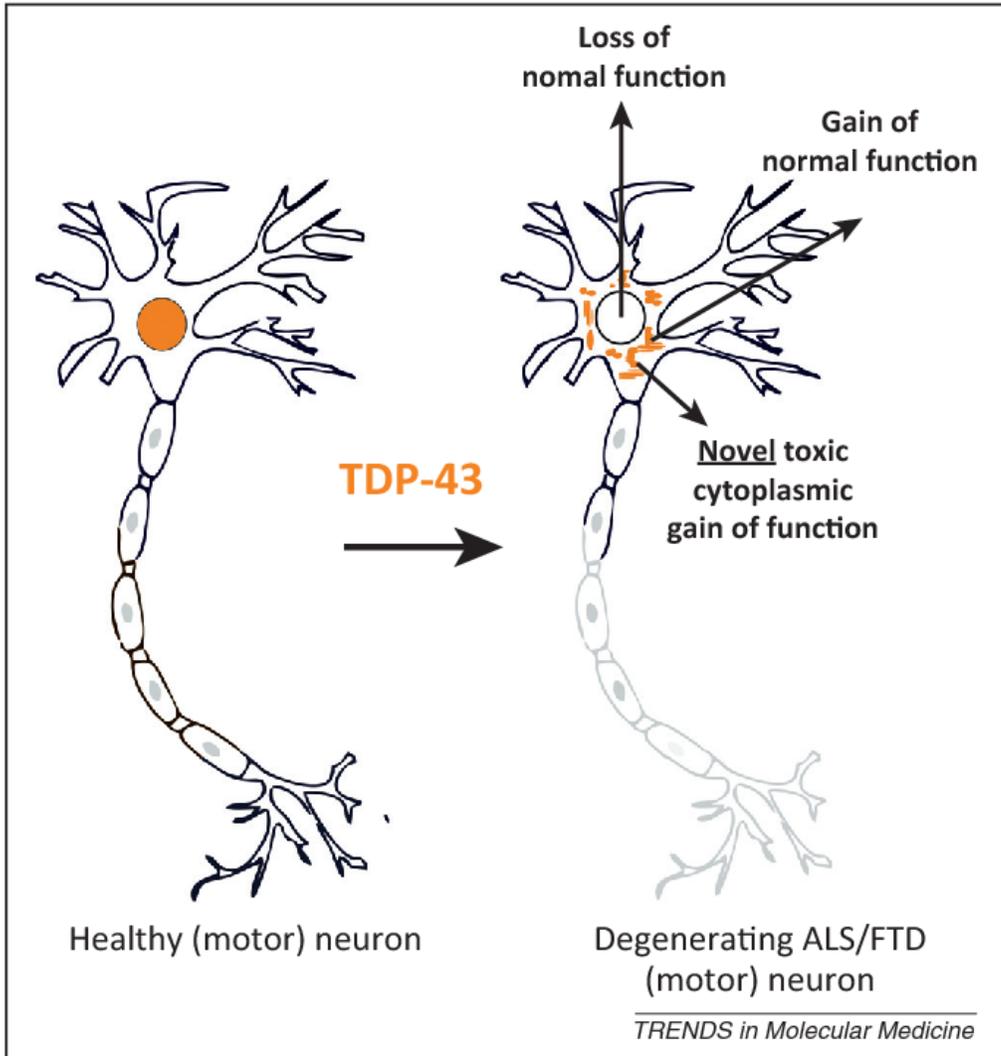
What **gene** is often mutated in ALS?



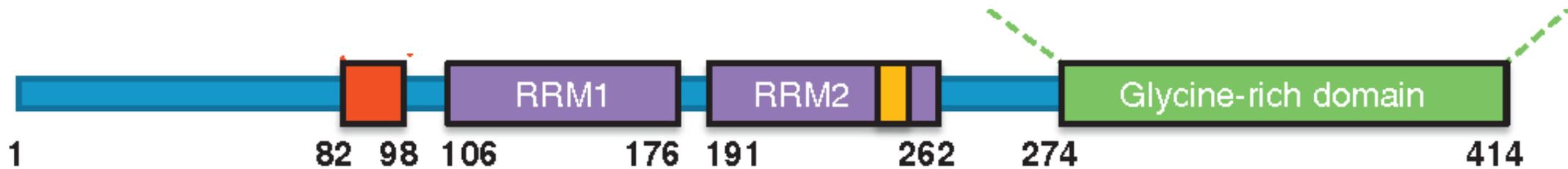
What **gene** is often mutated in ALS?



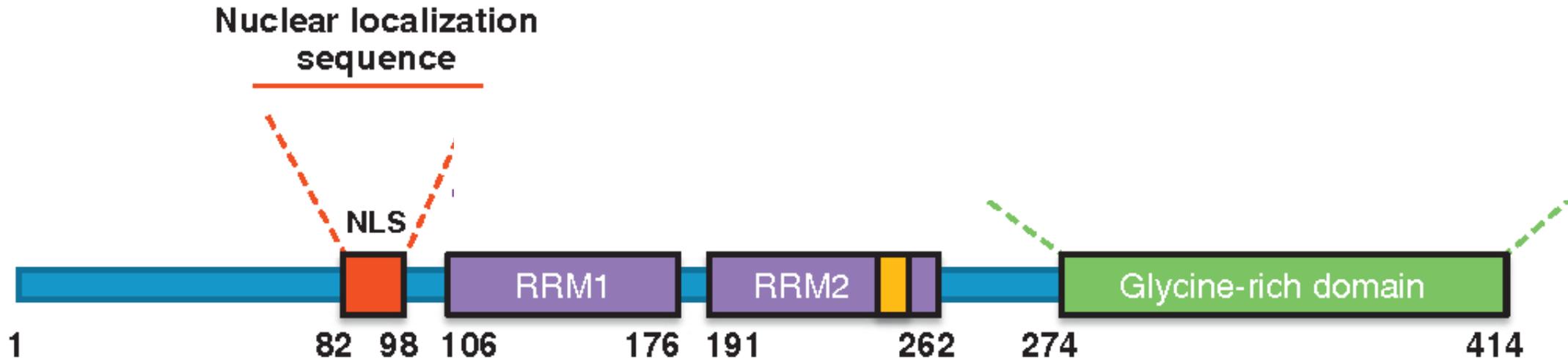
What **gene** is often mutated in ALS?



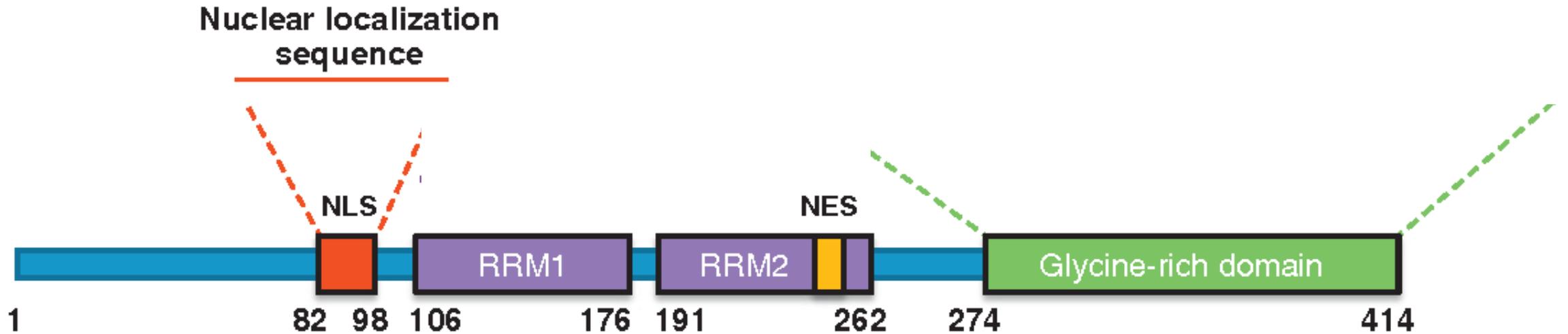
What domains make up the TDP-43 gene?



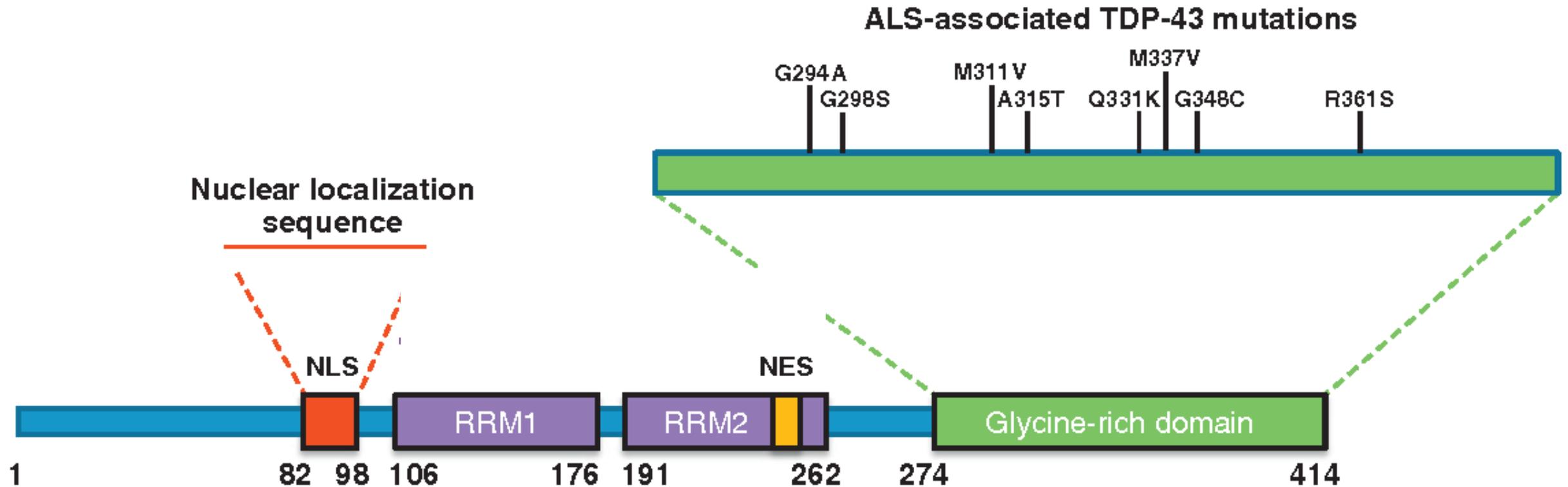
What domains make up the TDP-43 gene?



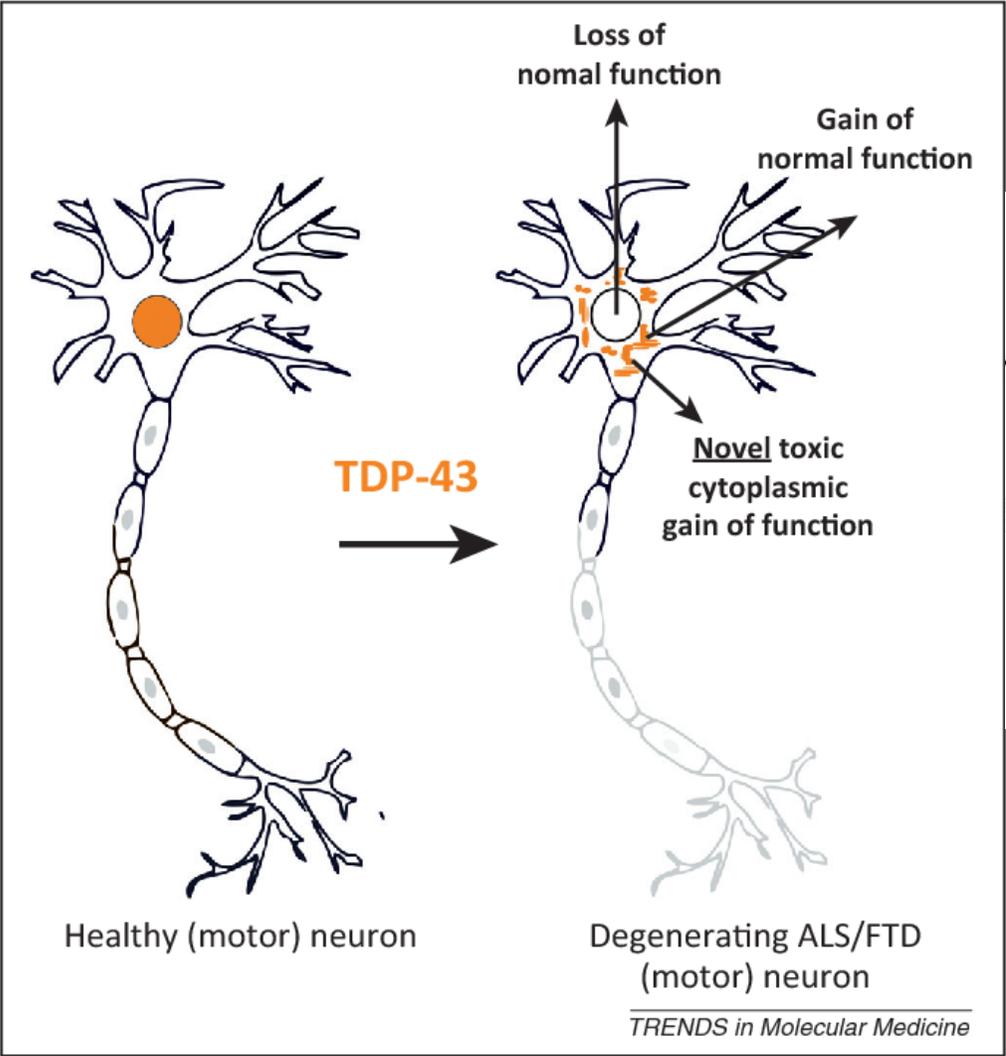
What domains make up the TDP-43 gene?



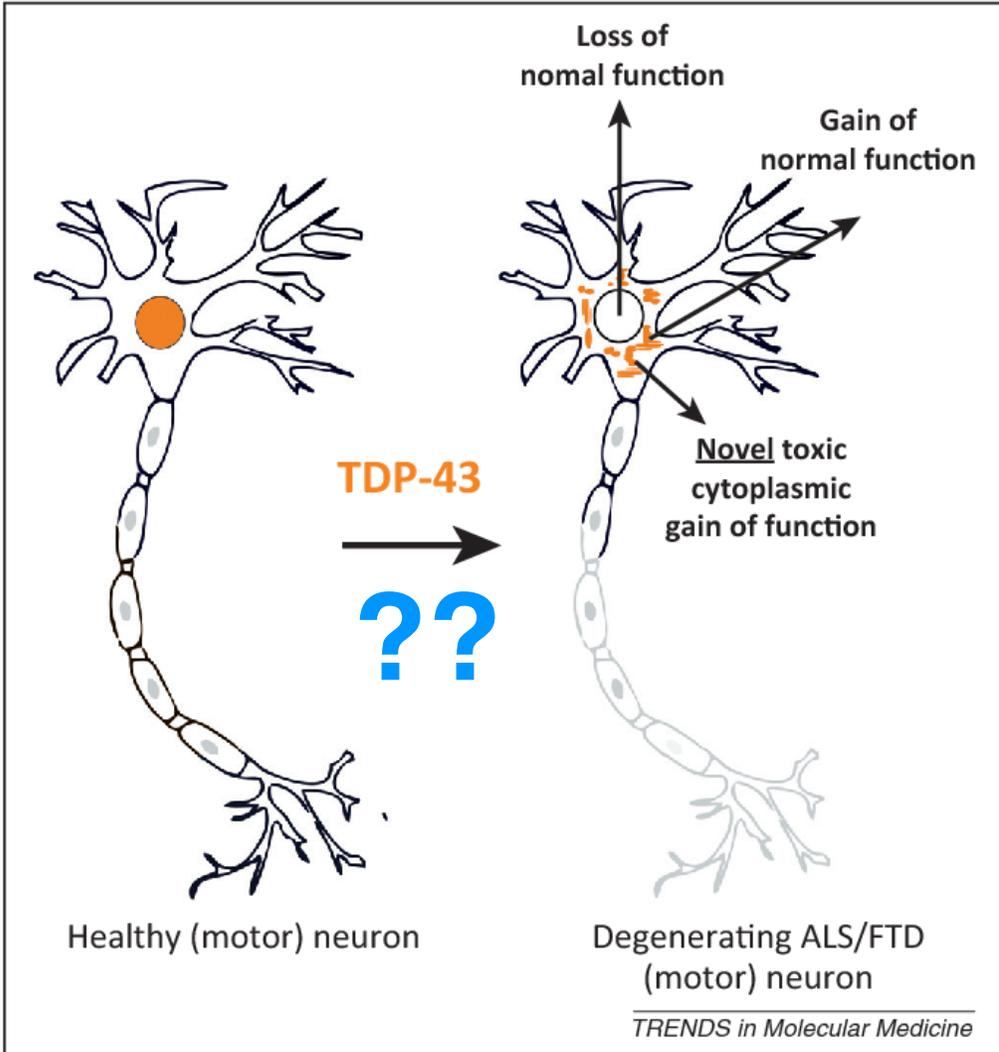
What domains make up the TDP-43 gene?



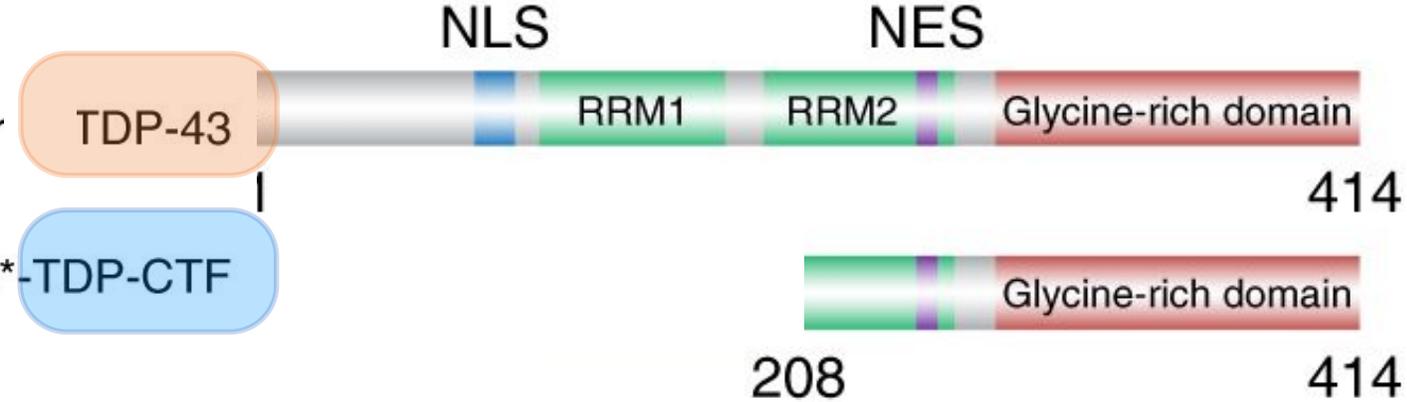
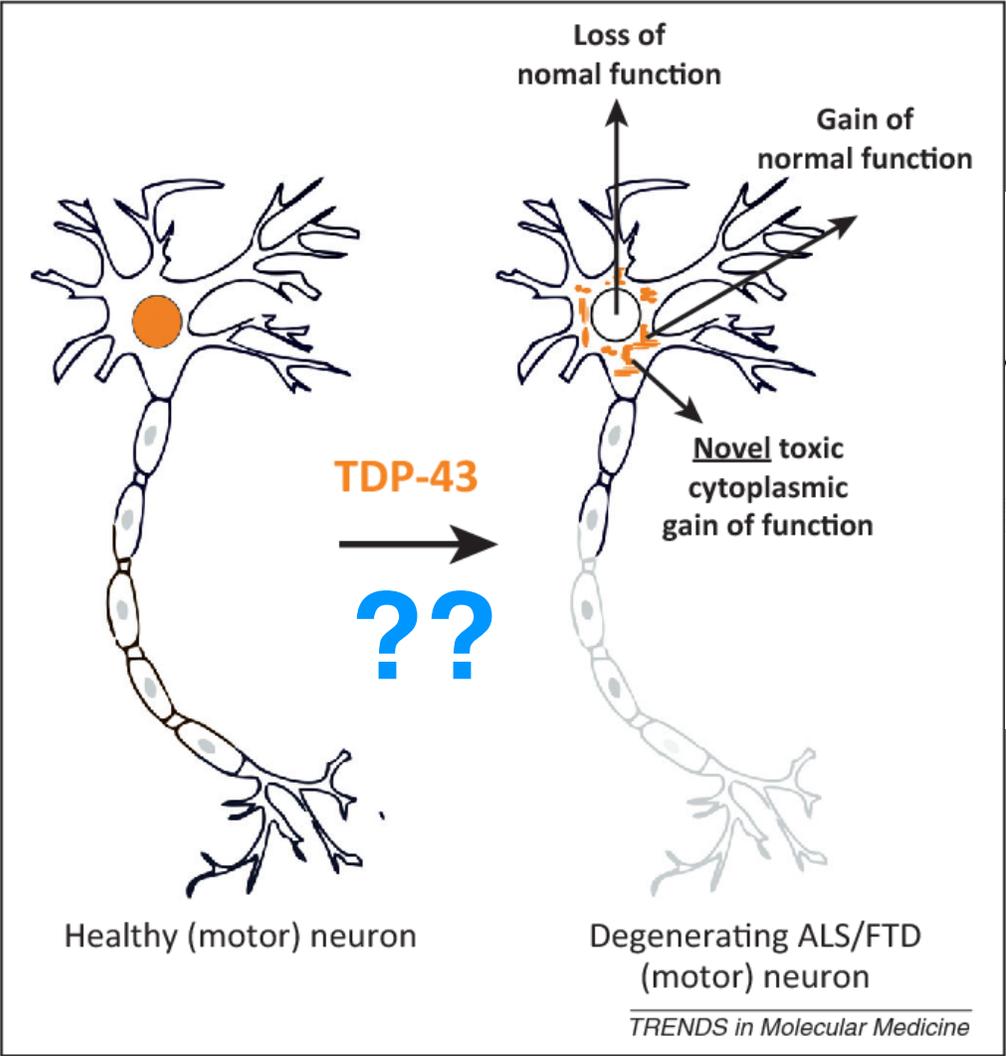
GOAL: How does the **pathological version of TDP-32 (TDP-43-CTF)** lead to neurodegeneration?



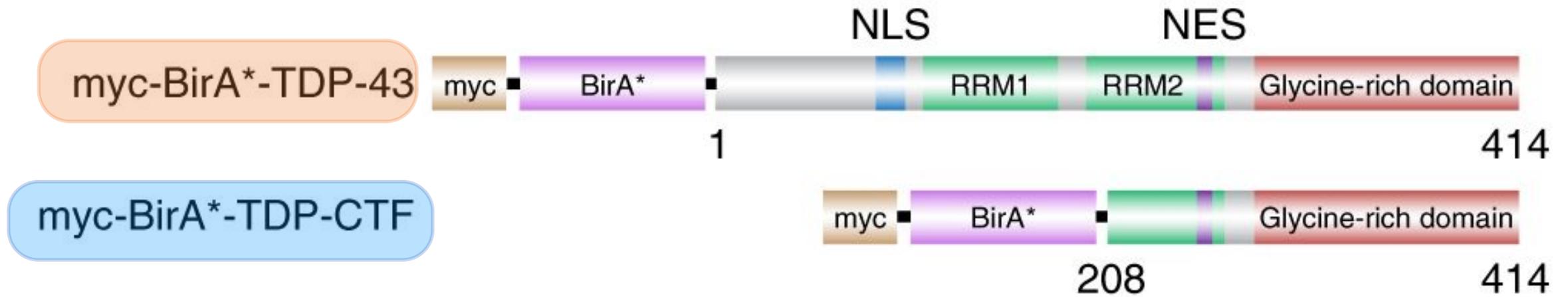
GOAL: How does the **pathological version of TDP-32 (TDP-43-CTF)** lead to neurodegeneration?



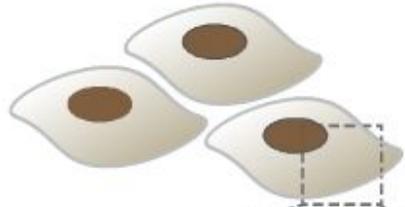
GOAL: How does the **pathological version of TDP-32 (TDP-43-CTF)** lead to neurodegeneration?



How was **pathological TDP-43-CTF** constructed?



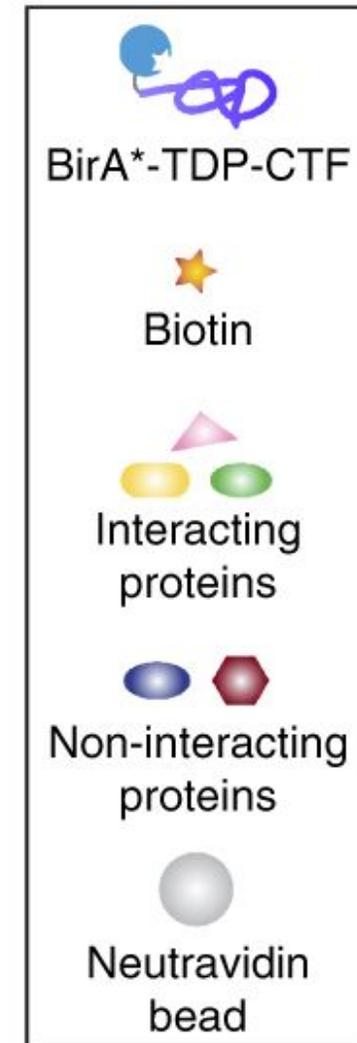
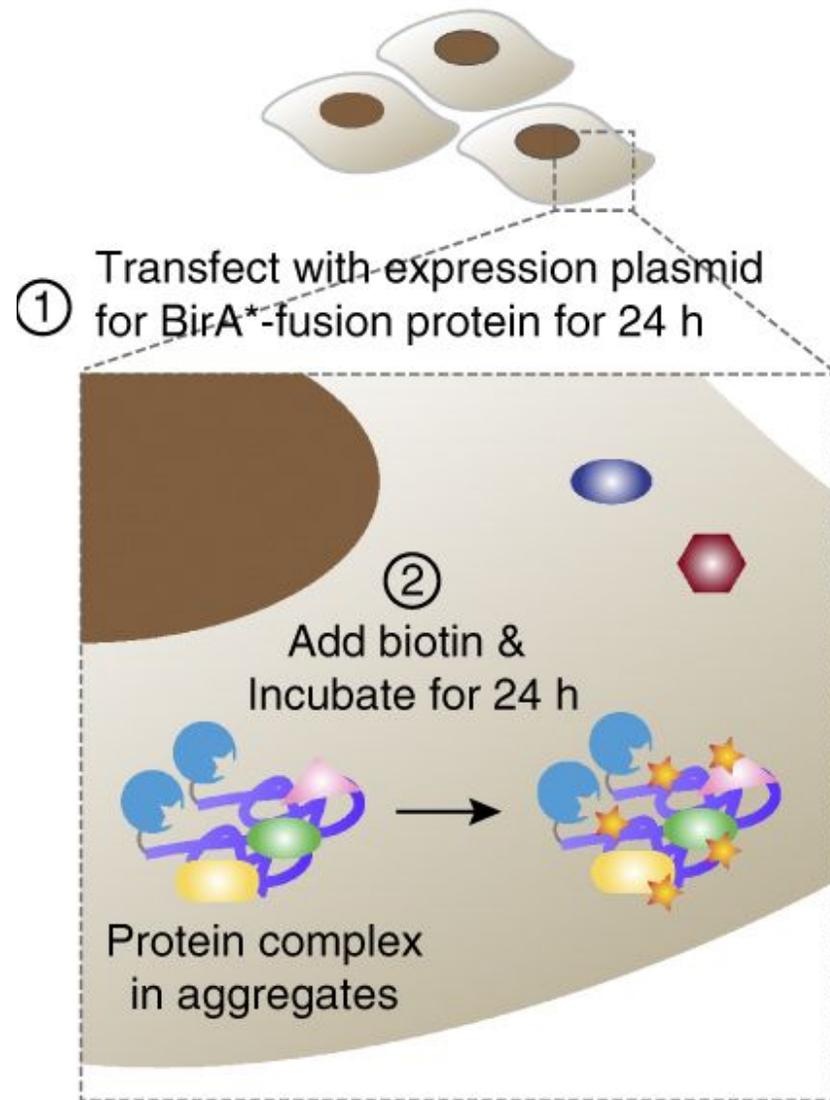
How did they identify TDP-43 protein interactions?



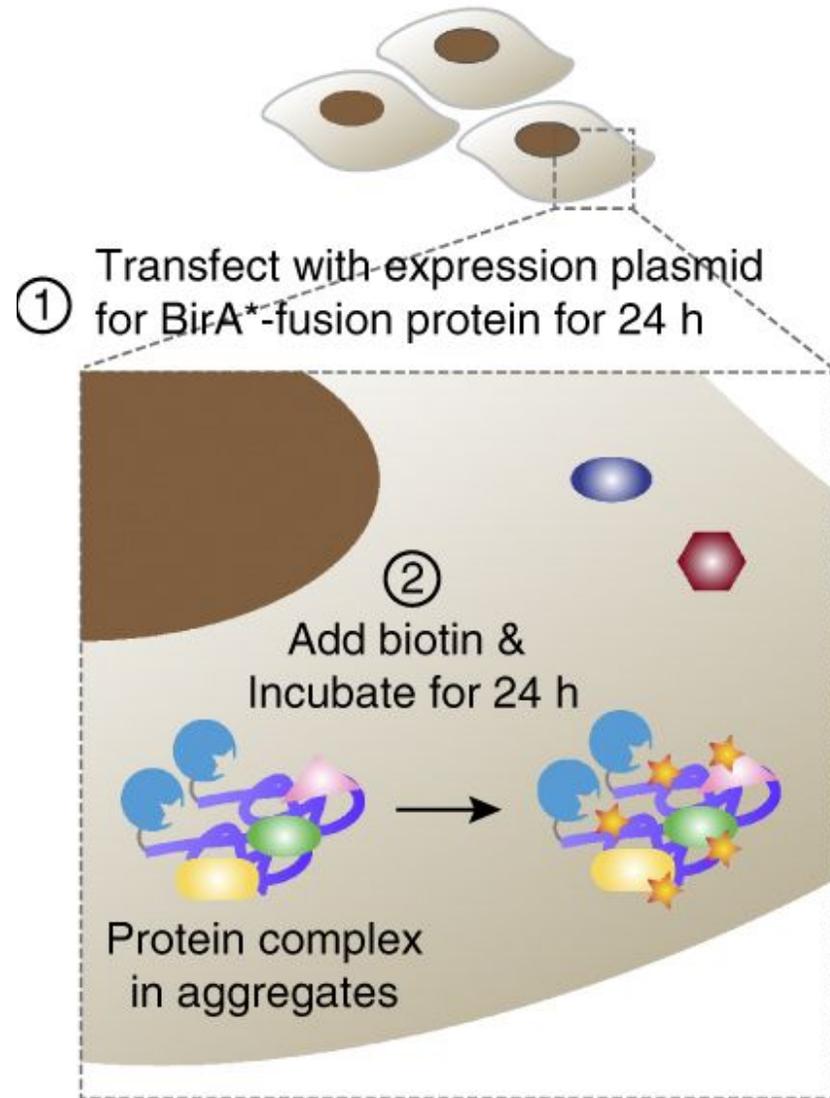
(Neuro-2A cells)

- ① Transfect with expression plasmid for BirA*⁻-fusion protein for 24 h

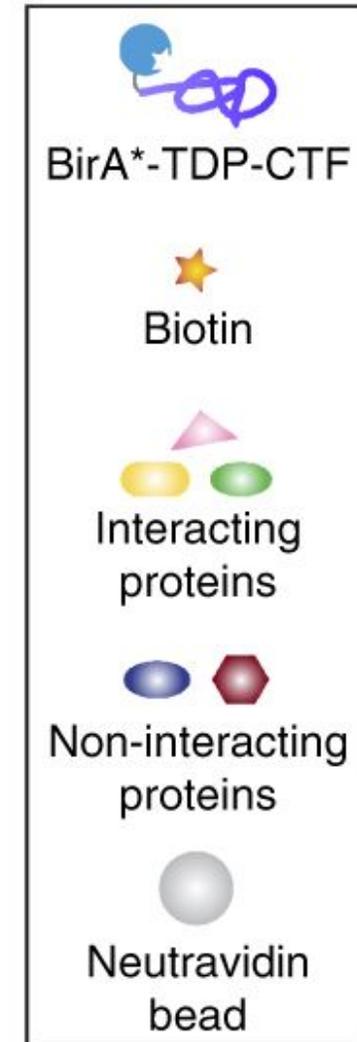
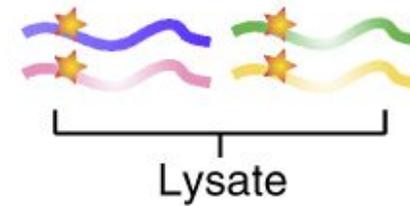
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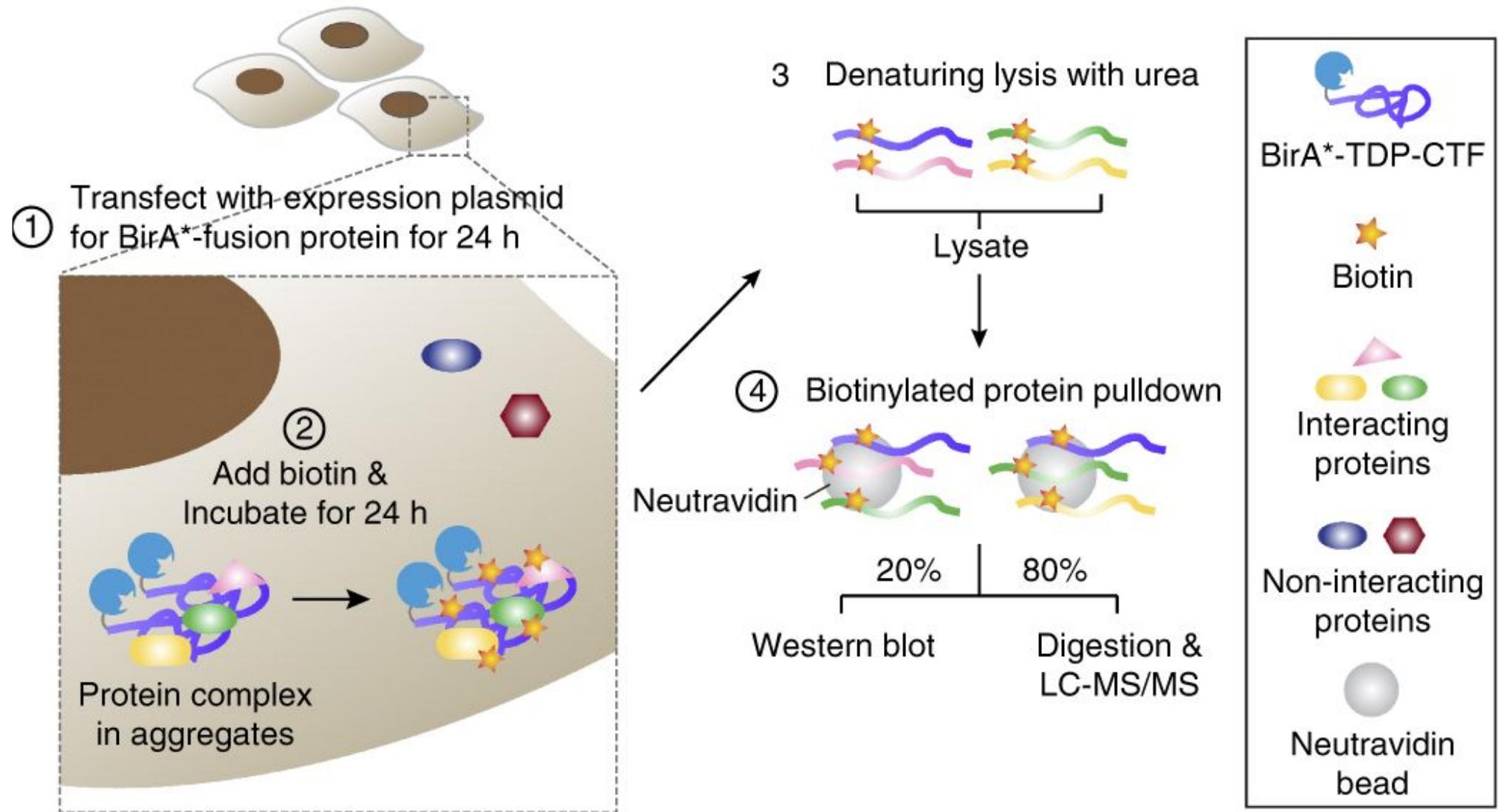
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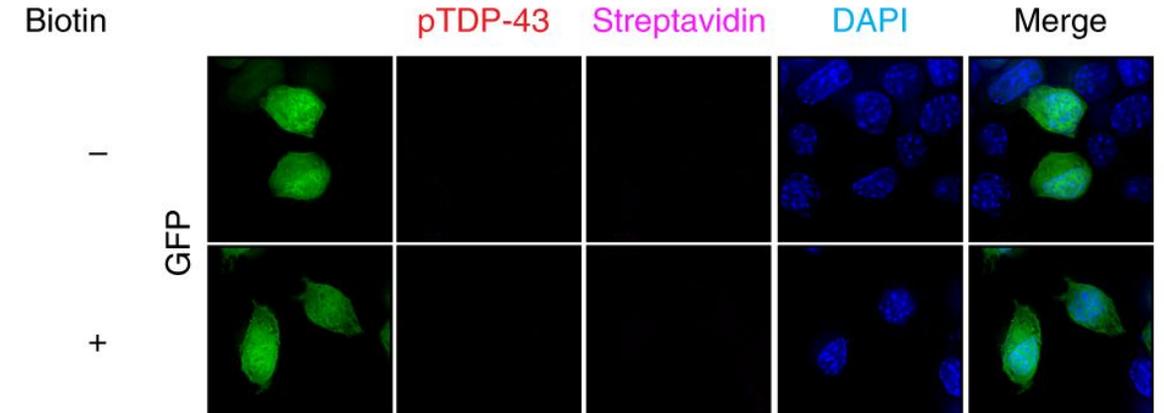
3 Denaturing lysis with urea



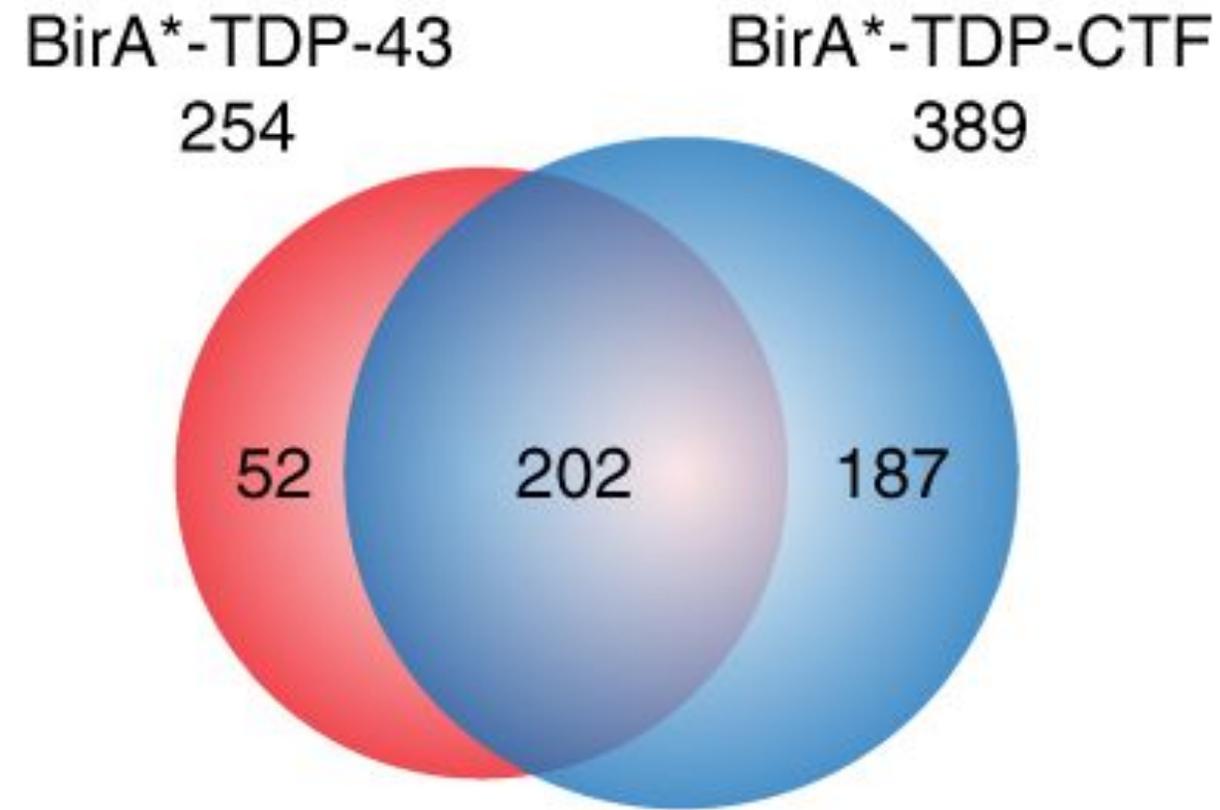
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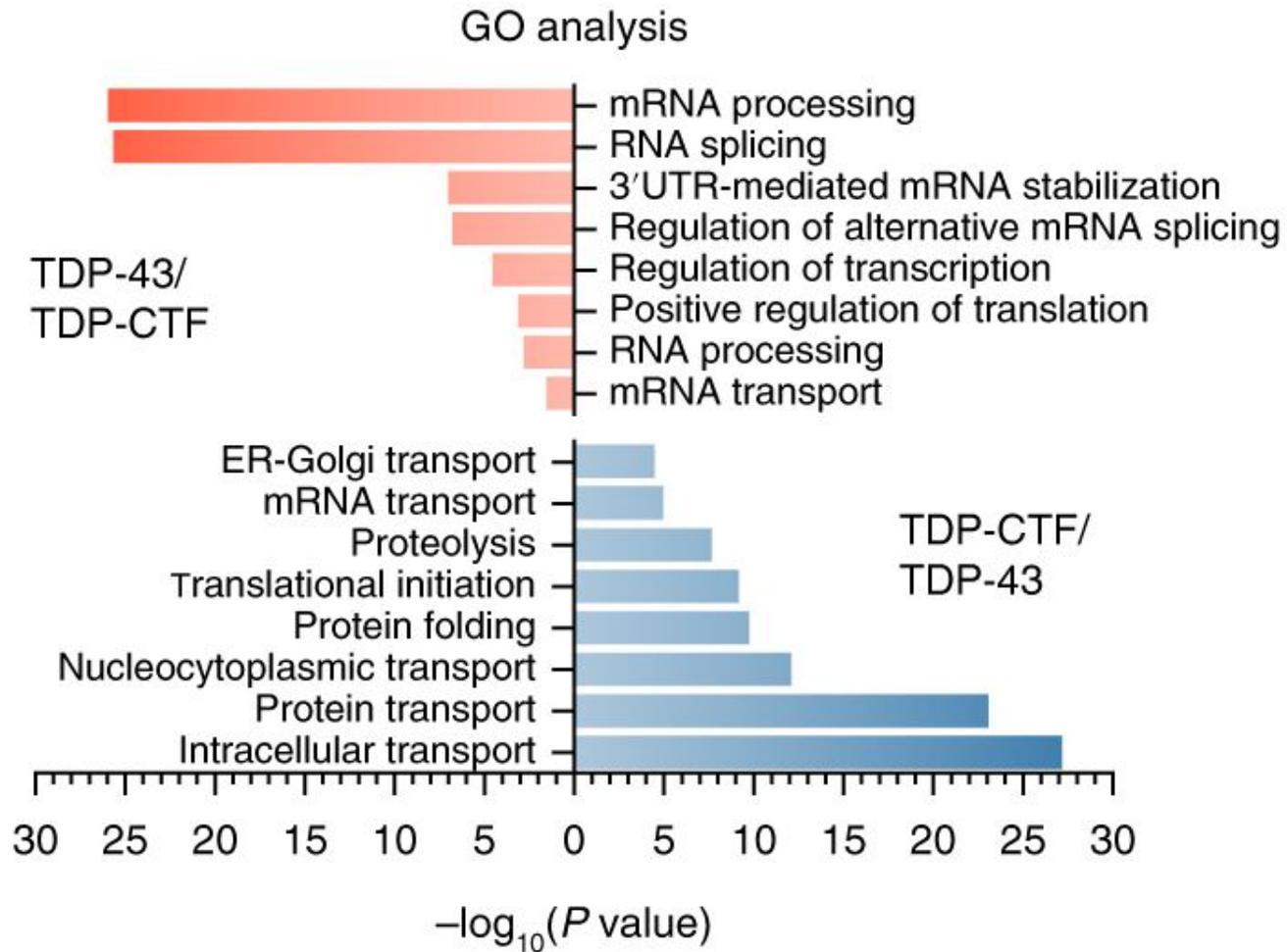
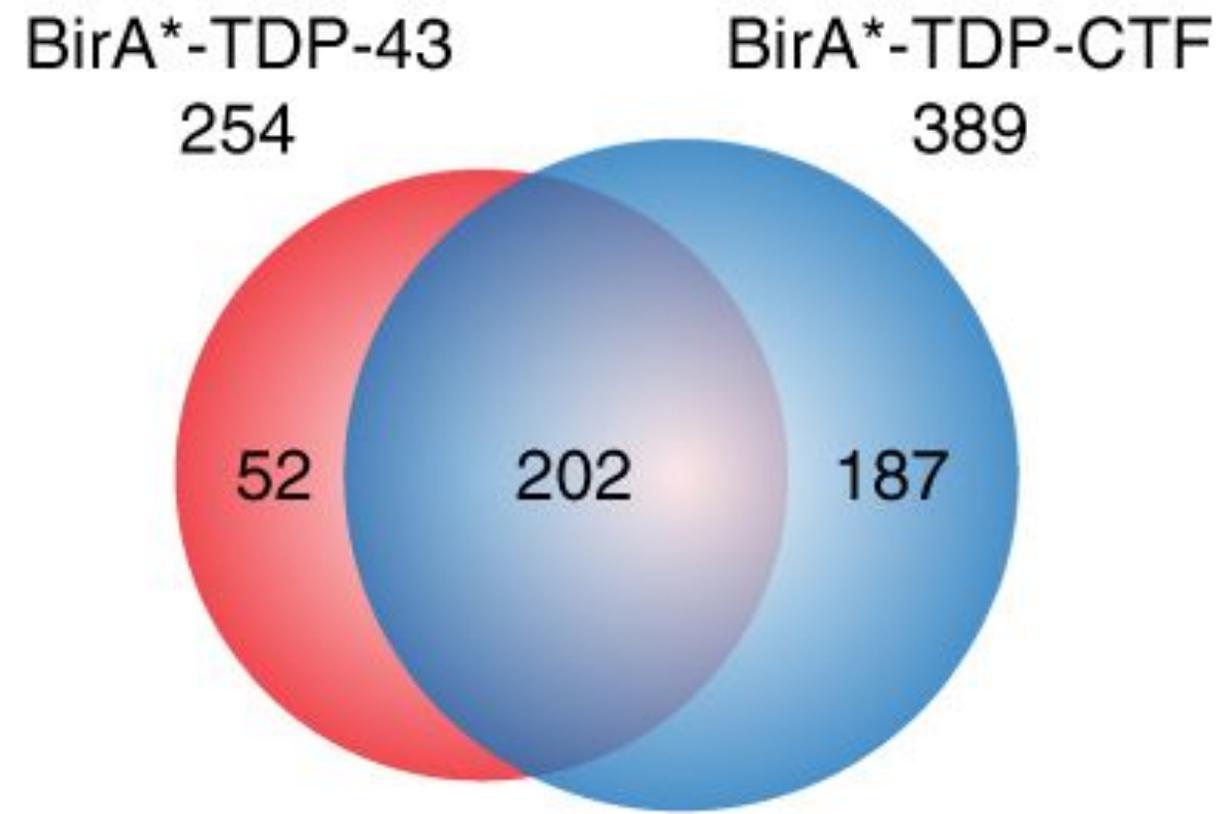
Where do **normal** and **aggregate** TDP-43 constructs localize?



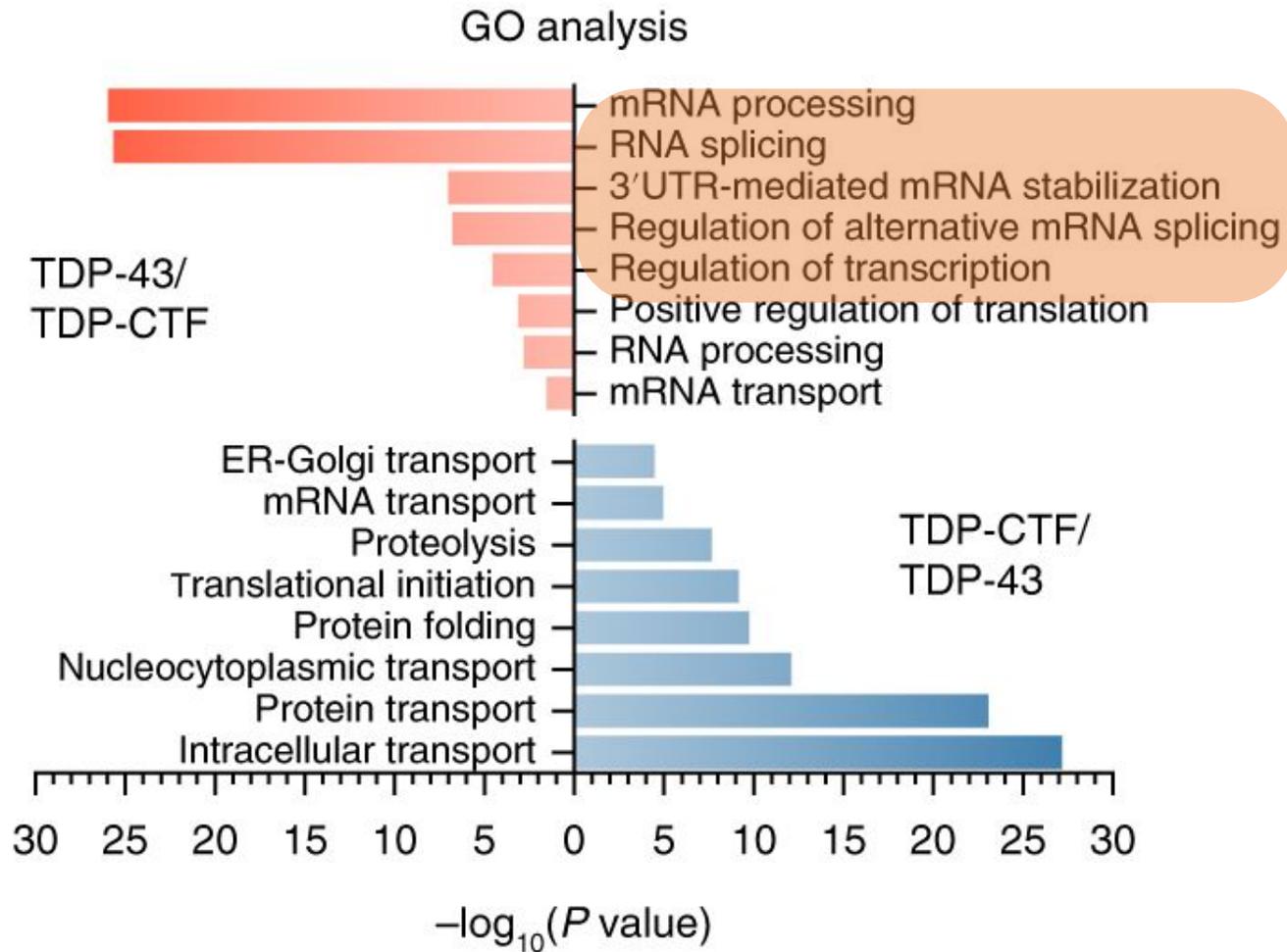
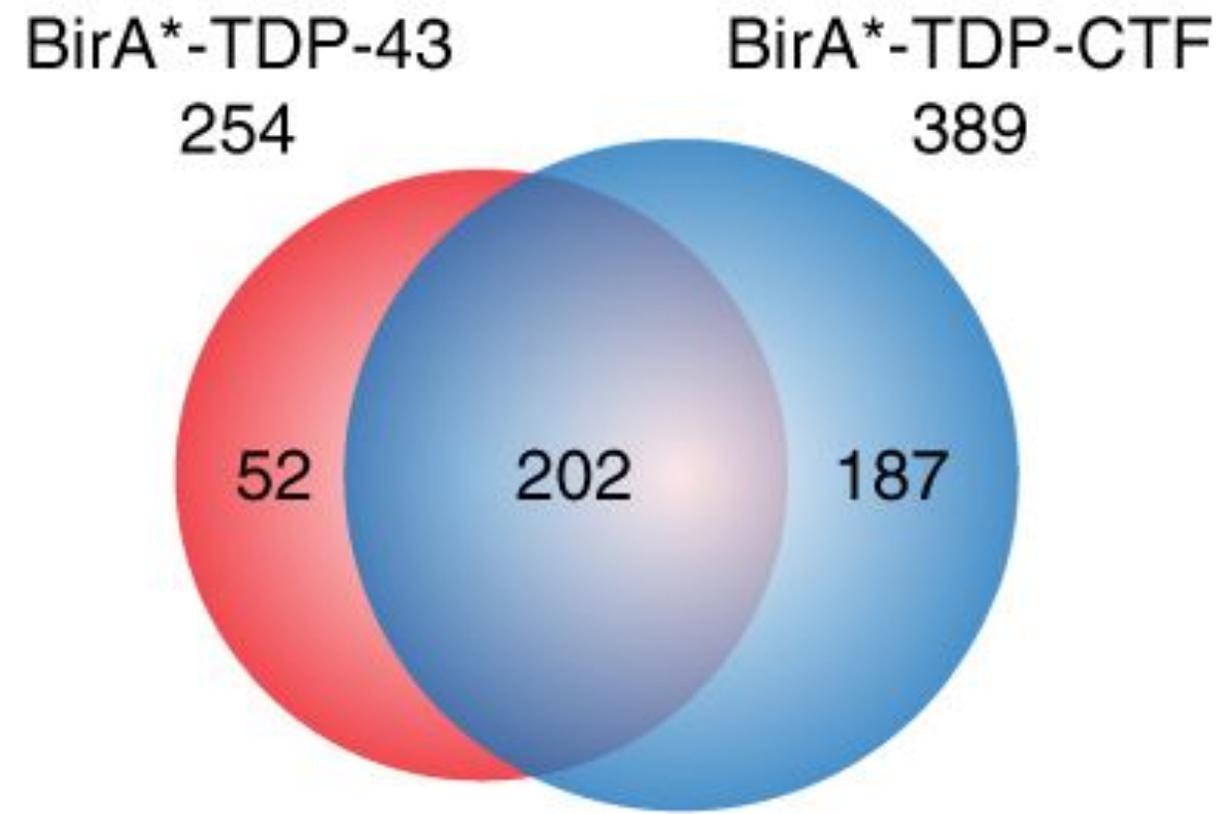
What is the molecular function of TDP-43 & CTF protein interactions?



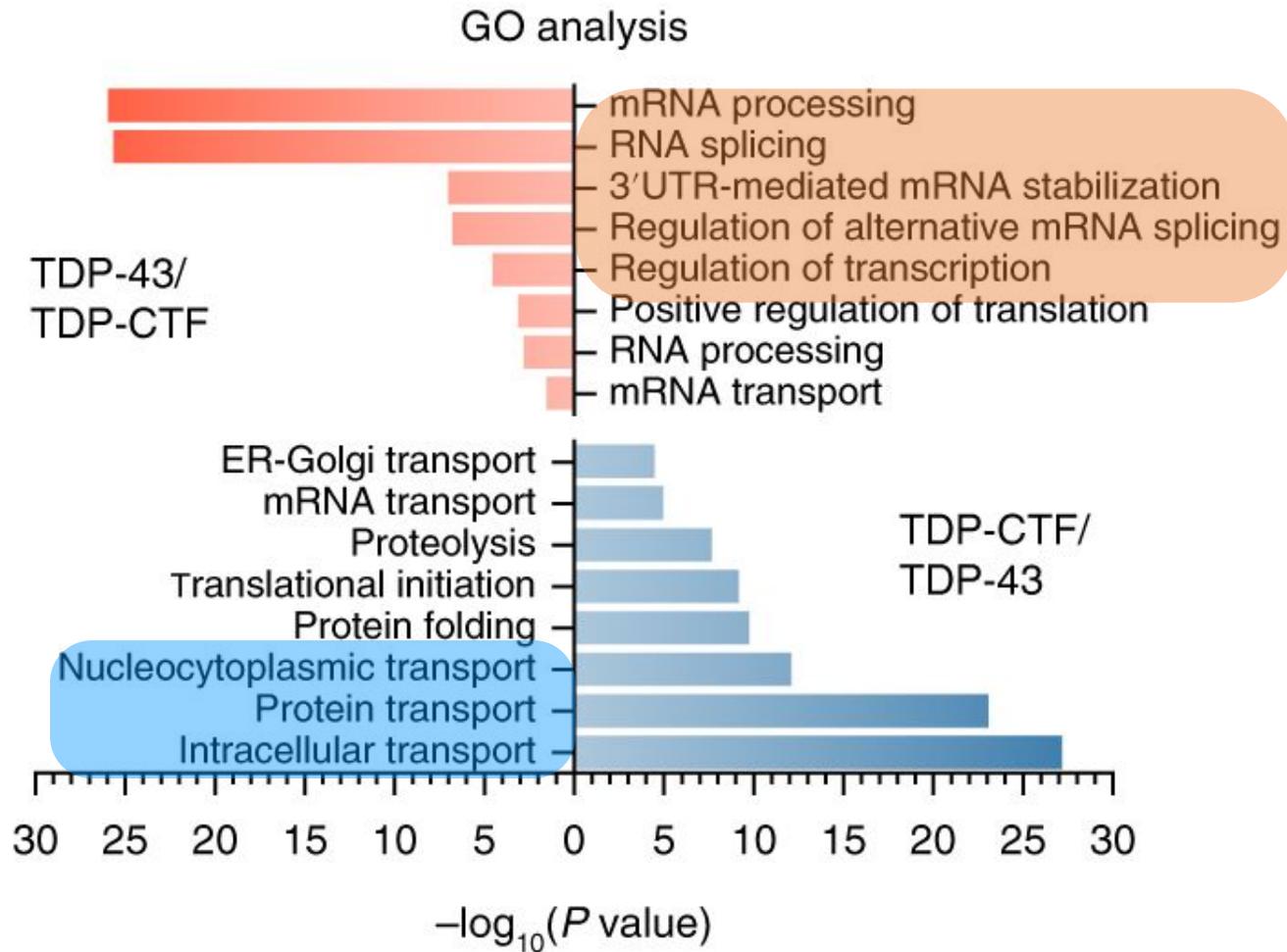
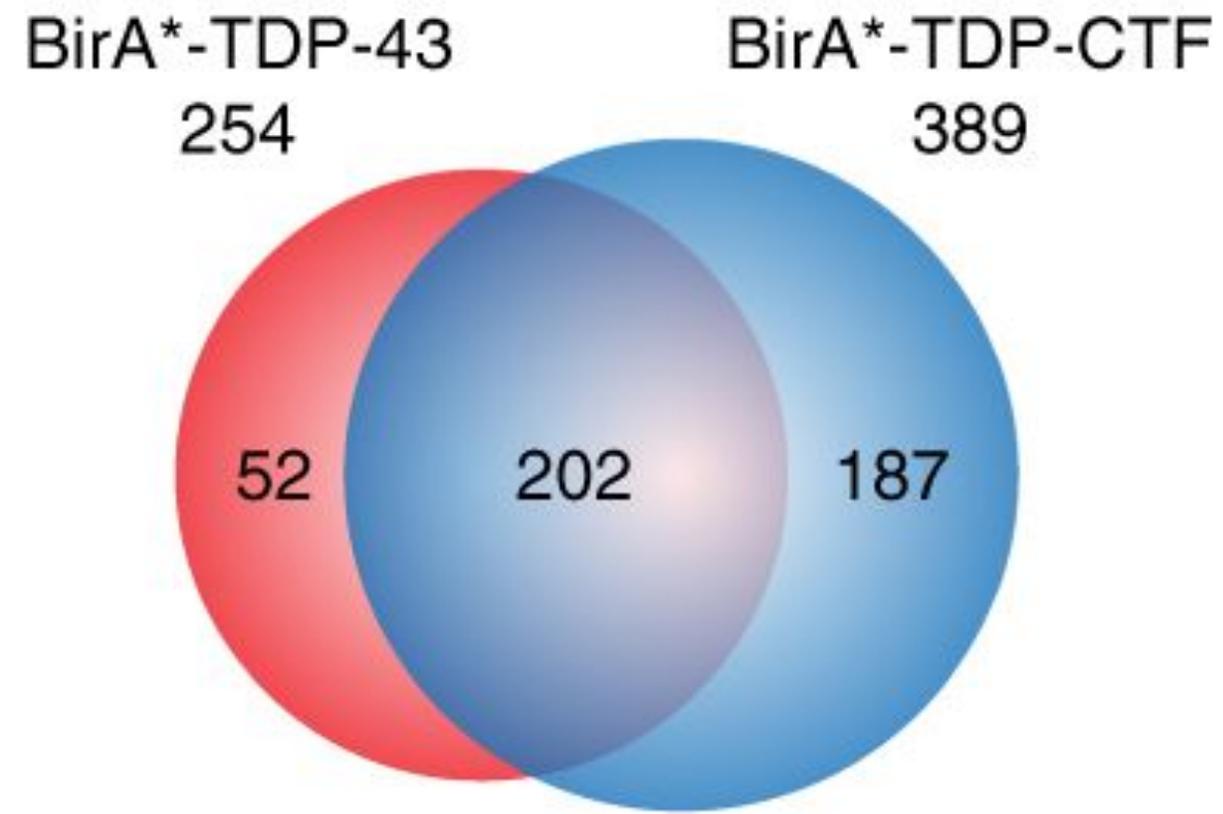
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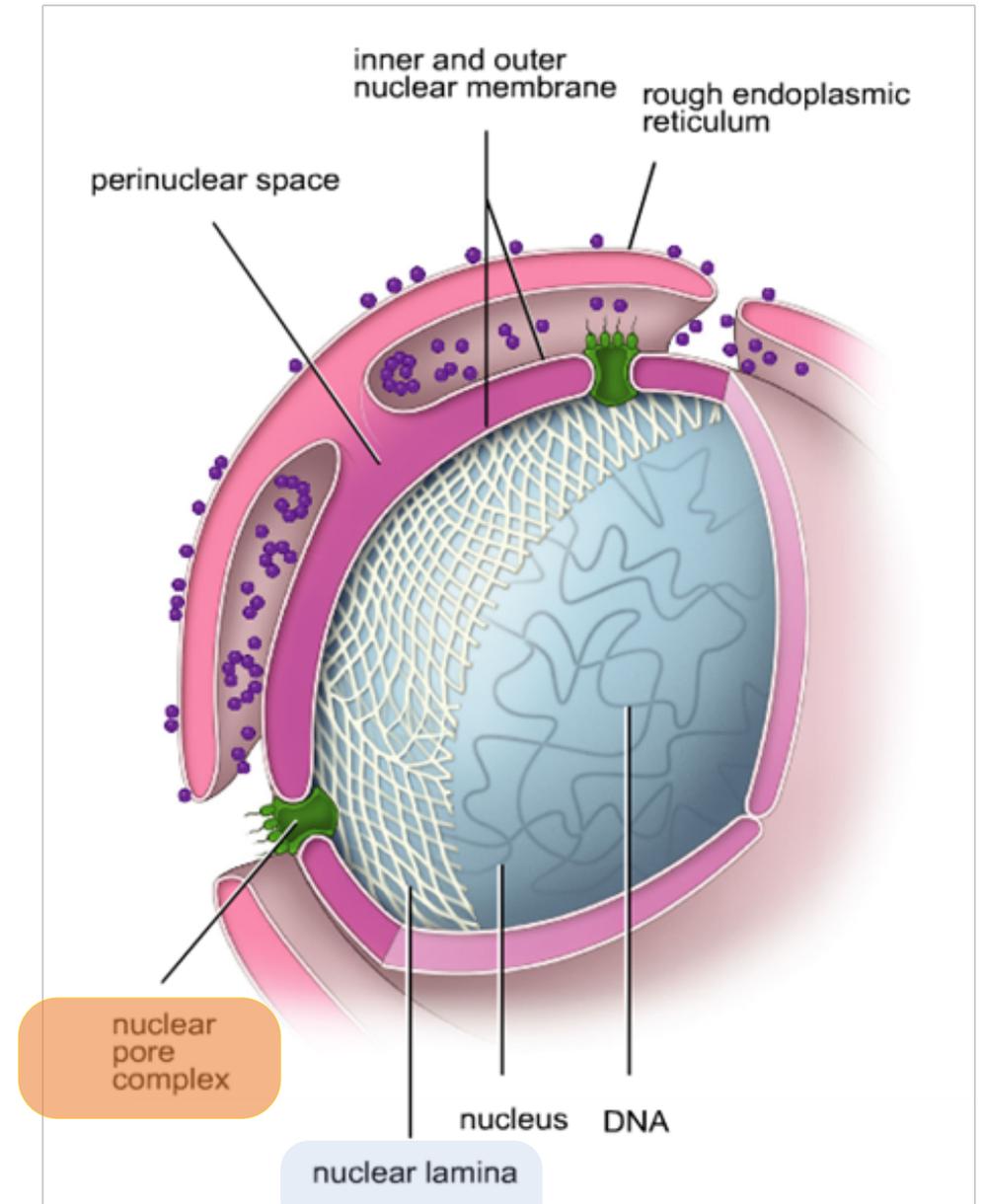
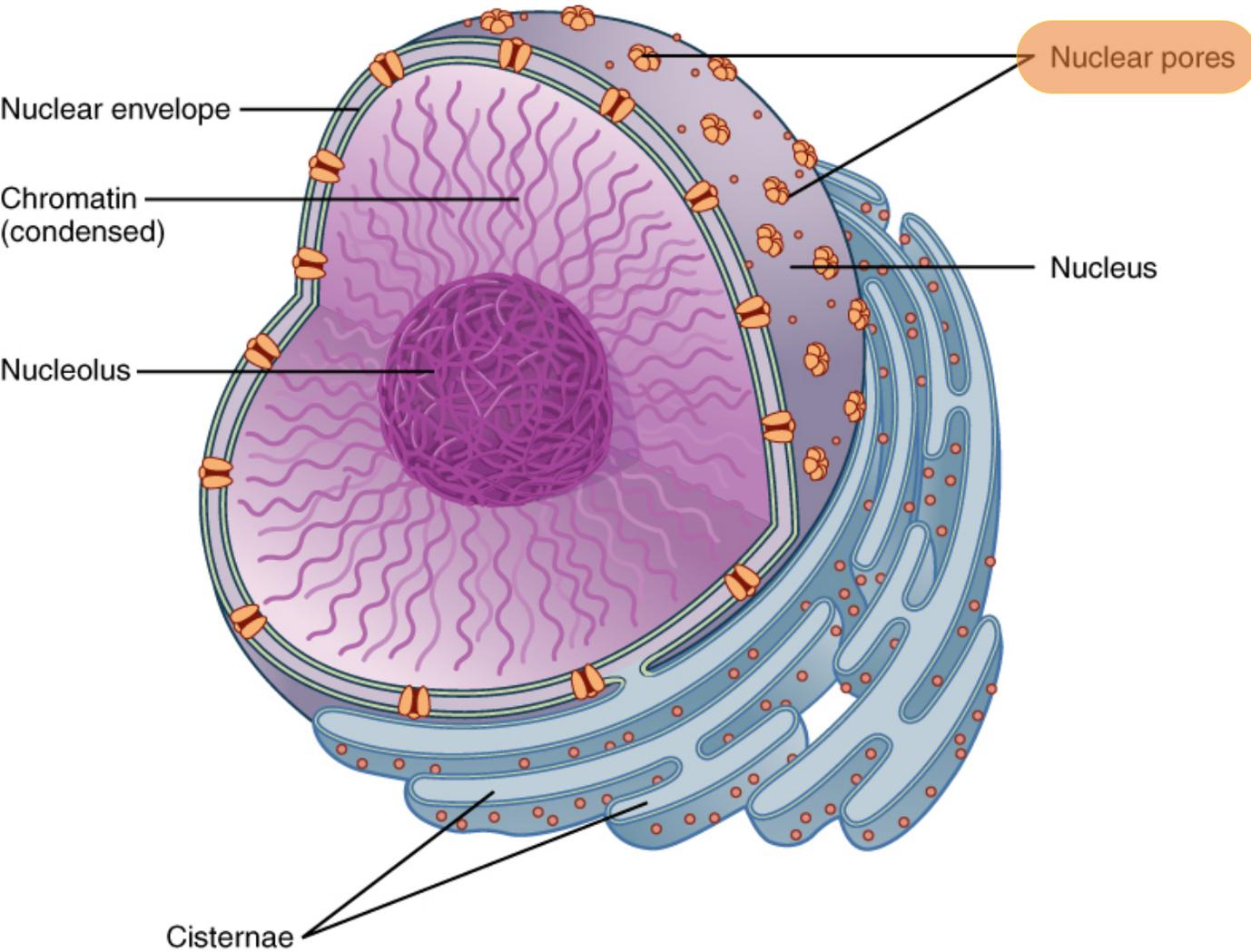
What is the molecular function of TDP-43 & CTF protein interactions?



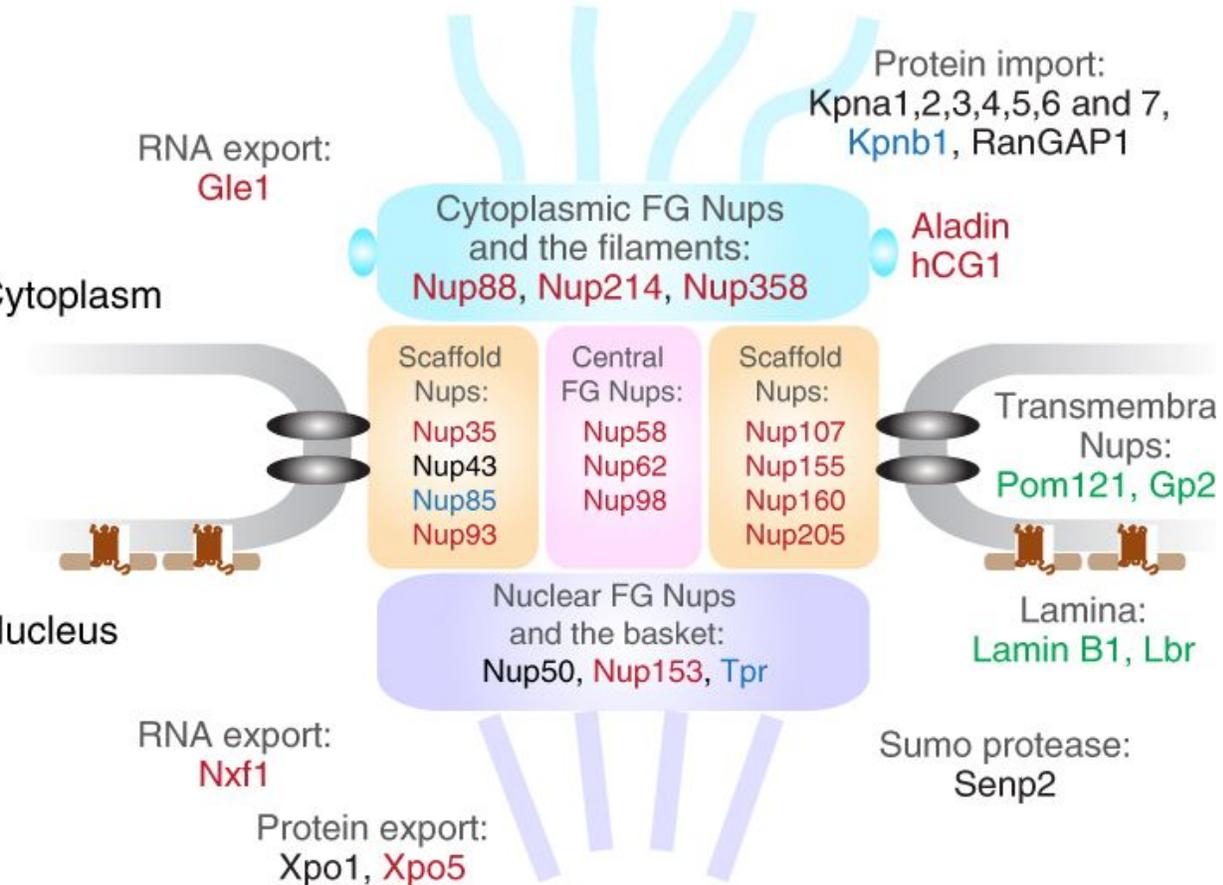
What is the molecular function of TDP-43 & CTF protein interactions?



What are **nucleoporins** and **nuclear lamina**?



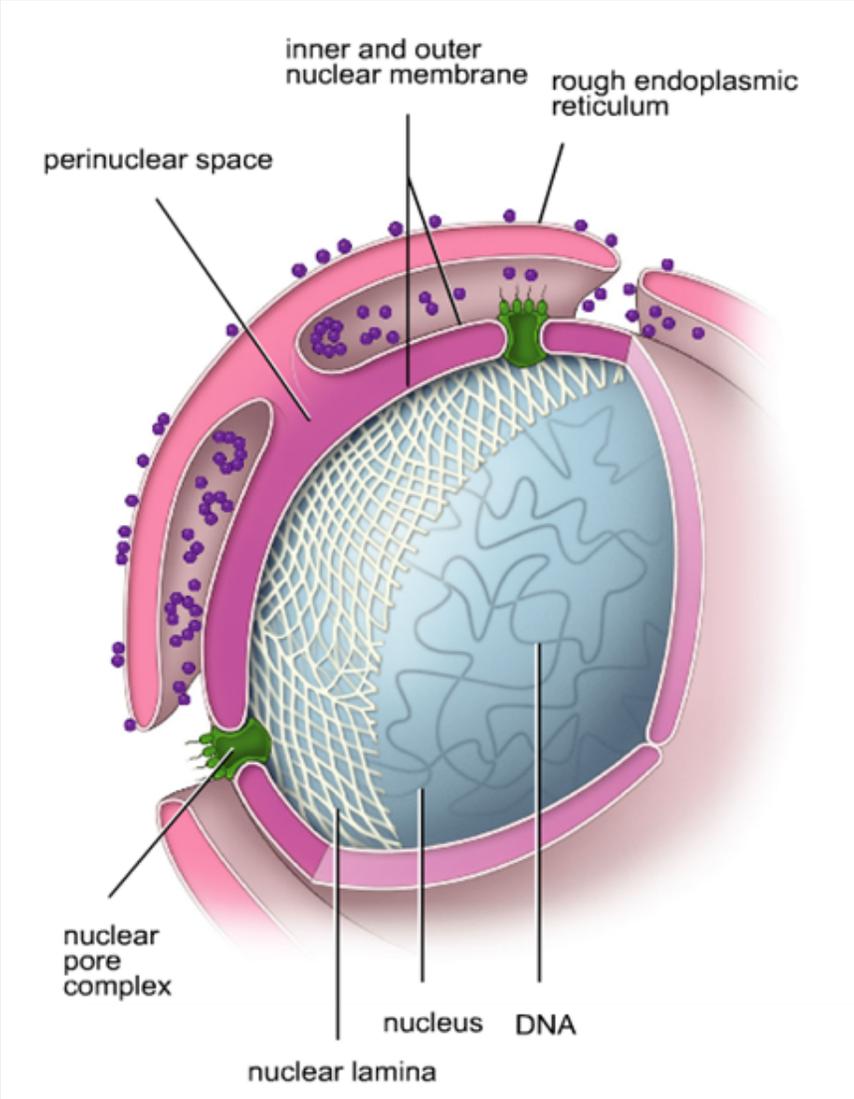
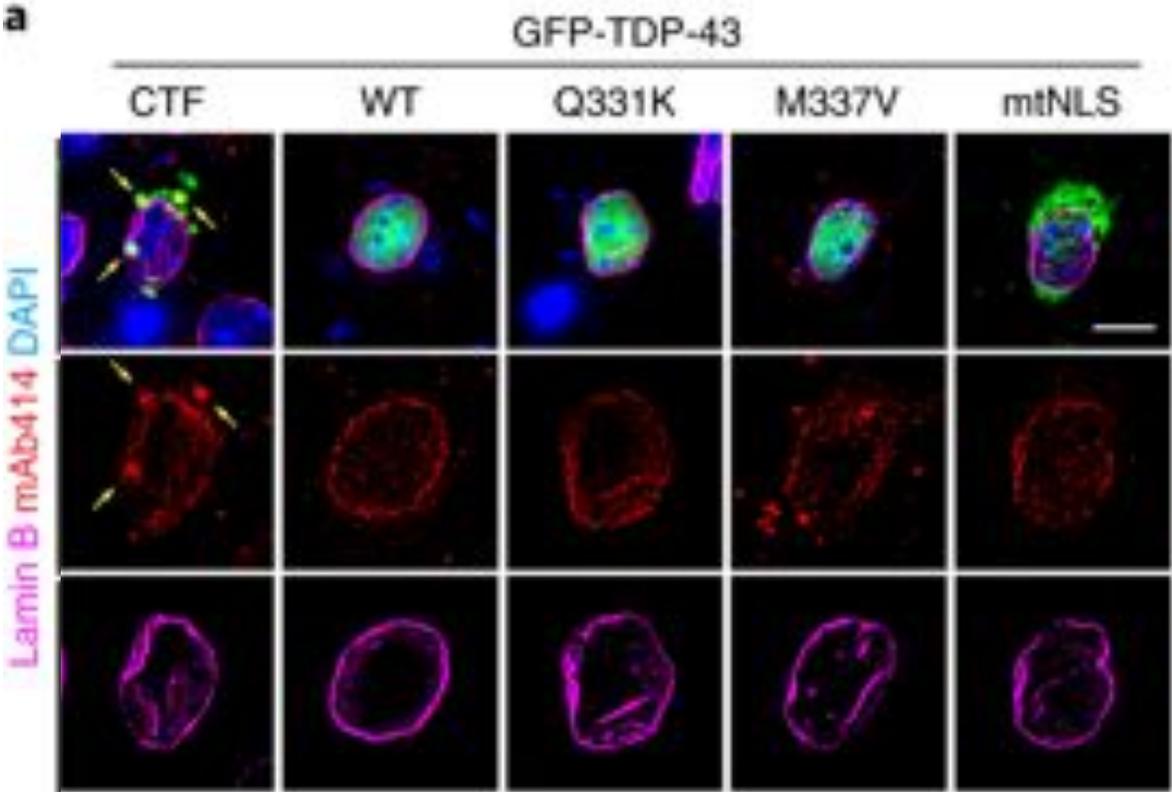
How is lamina localization affected in CTF?



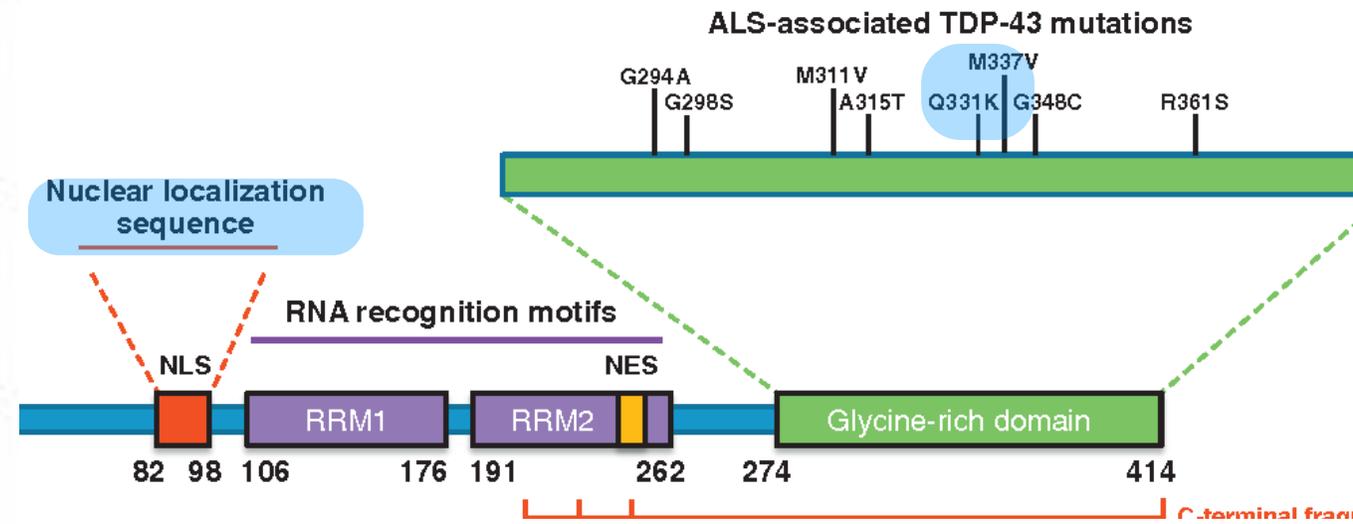
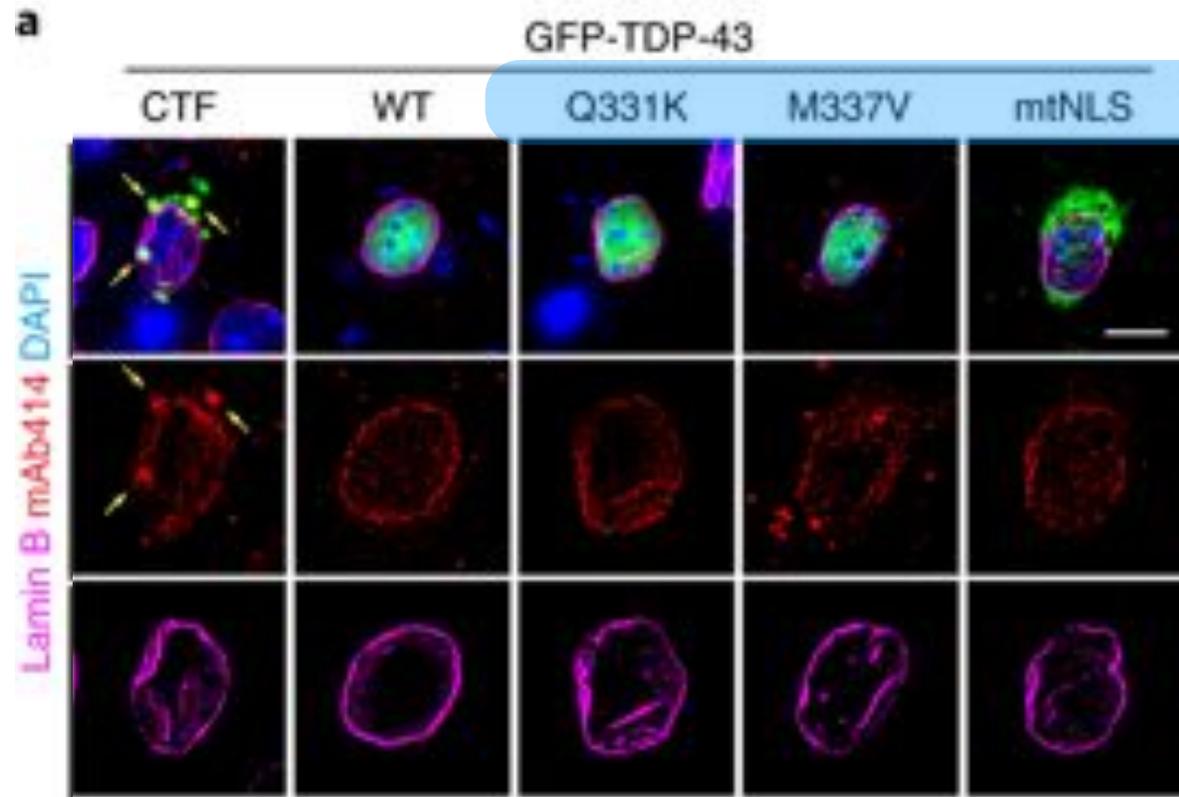
Interaction patterns

Coaggregation Mislocalization Disaggregation Not affected

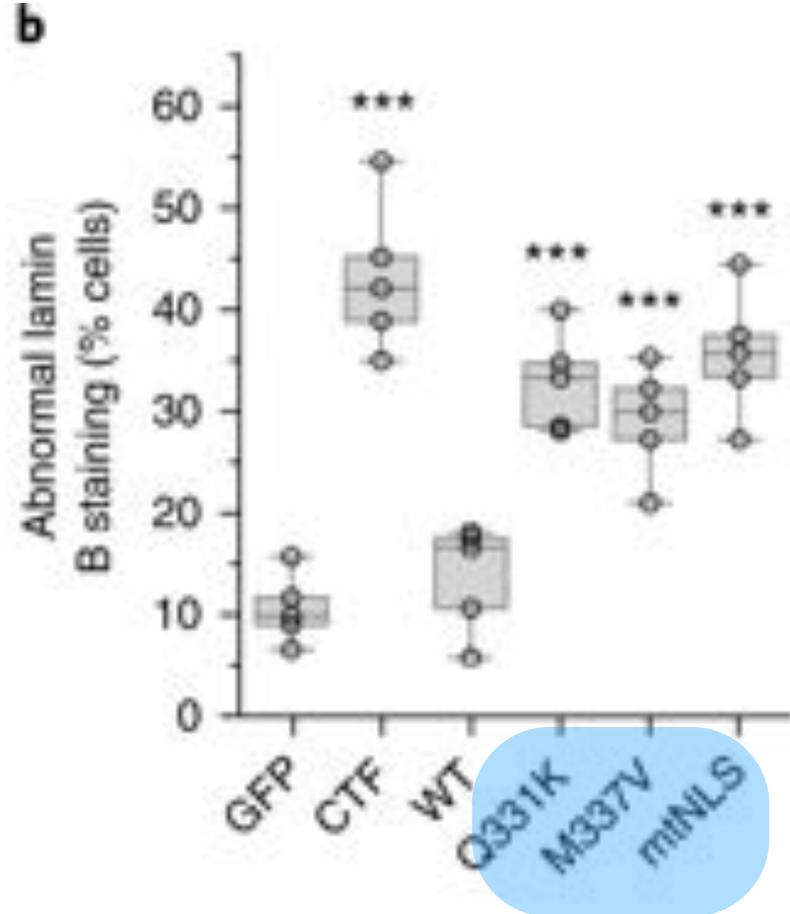
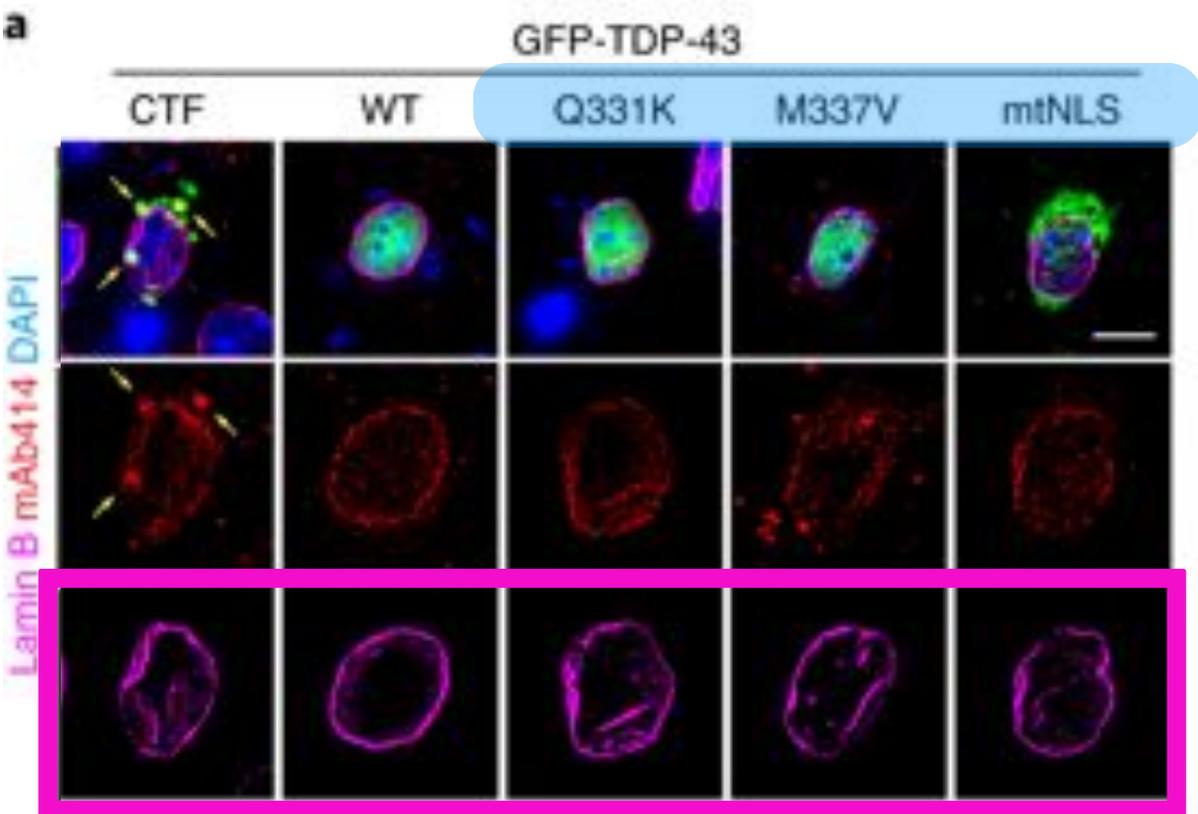
Do **TDP-43** mutants lead to defects in **nuclear lamina**?



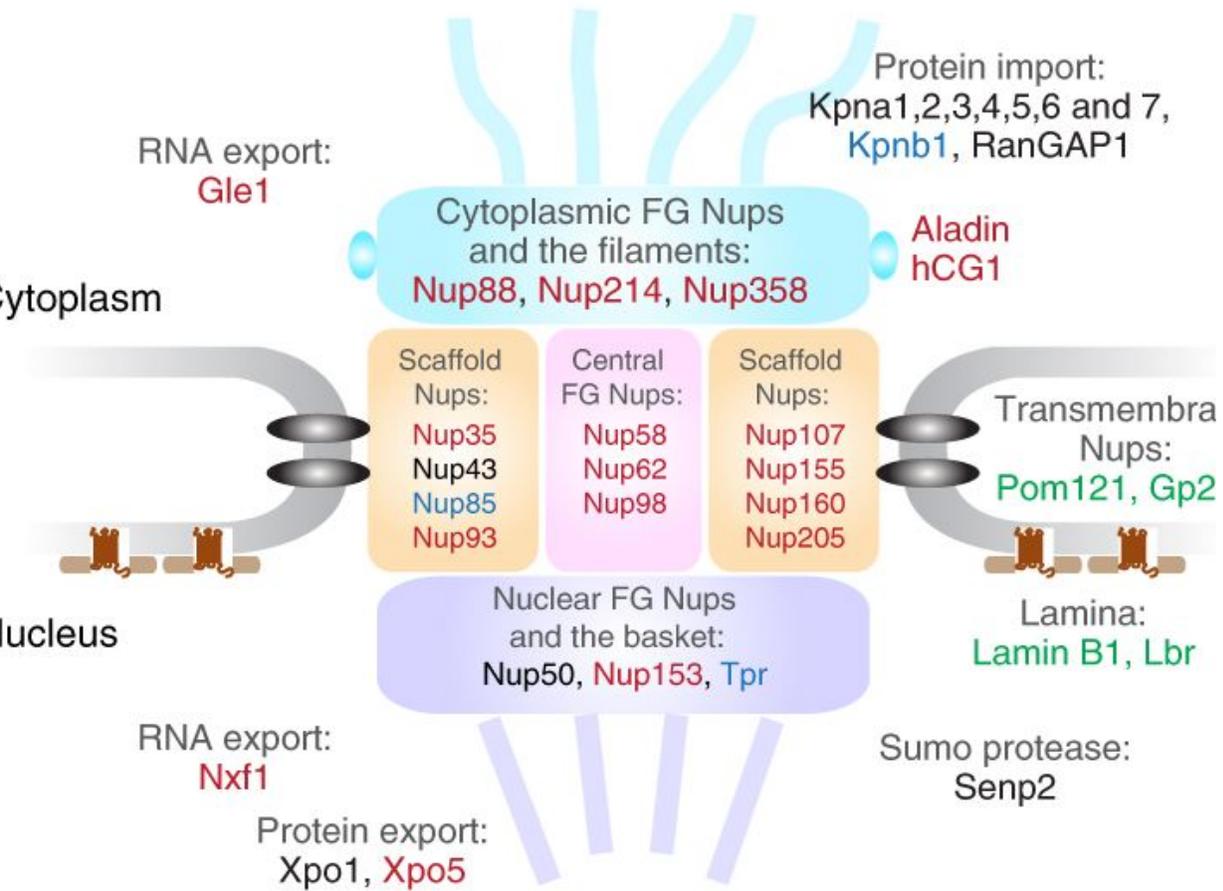
Do **TDP-43** mutants lead to defects in **nuclear lamina**?



Do **TDP-43** mutants lead to defects in **nuclear lamina**?



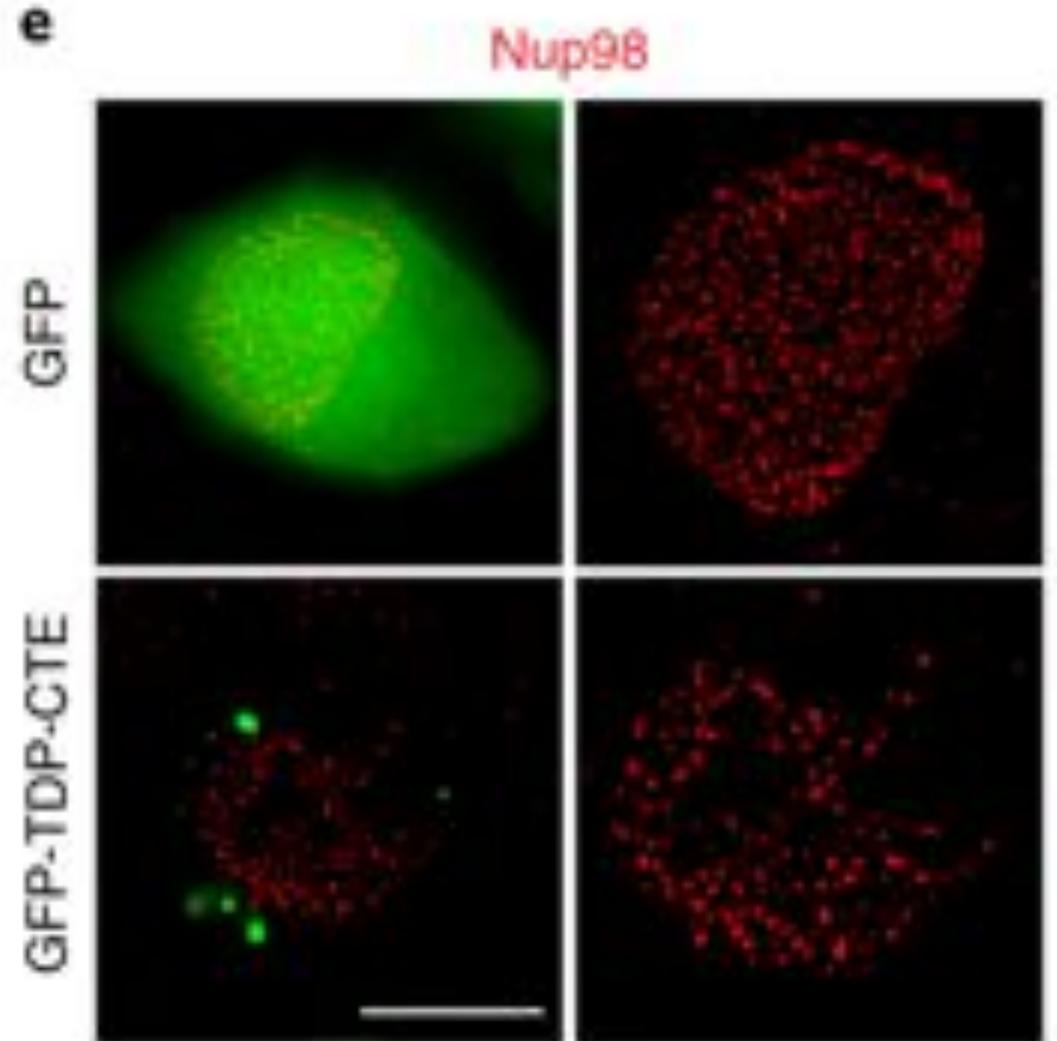
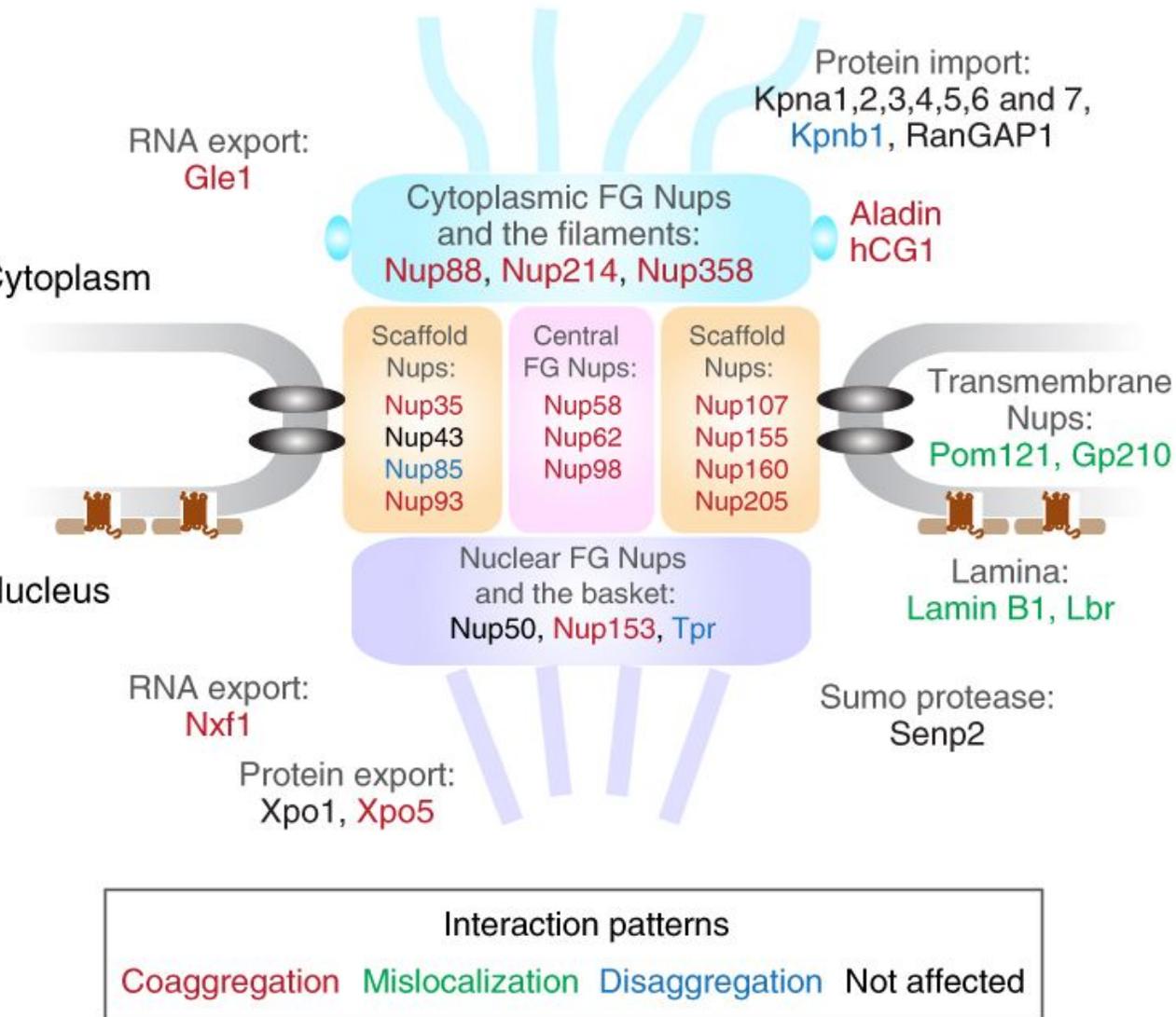
How are **nucleoporin (Nups)** localization affected?



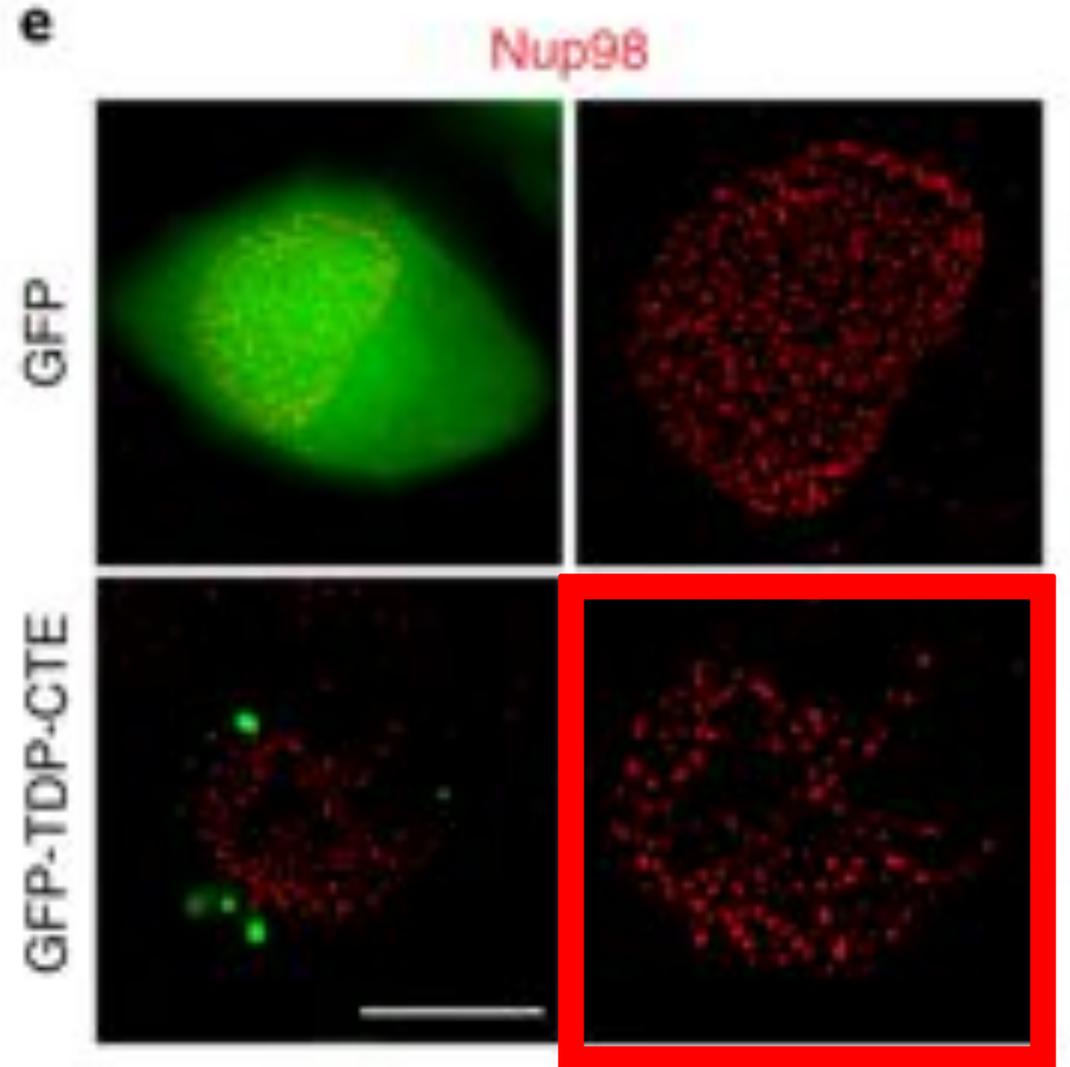
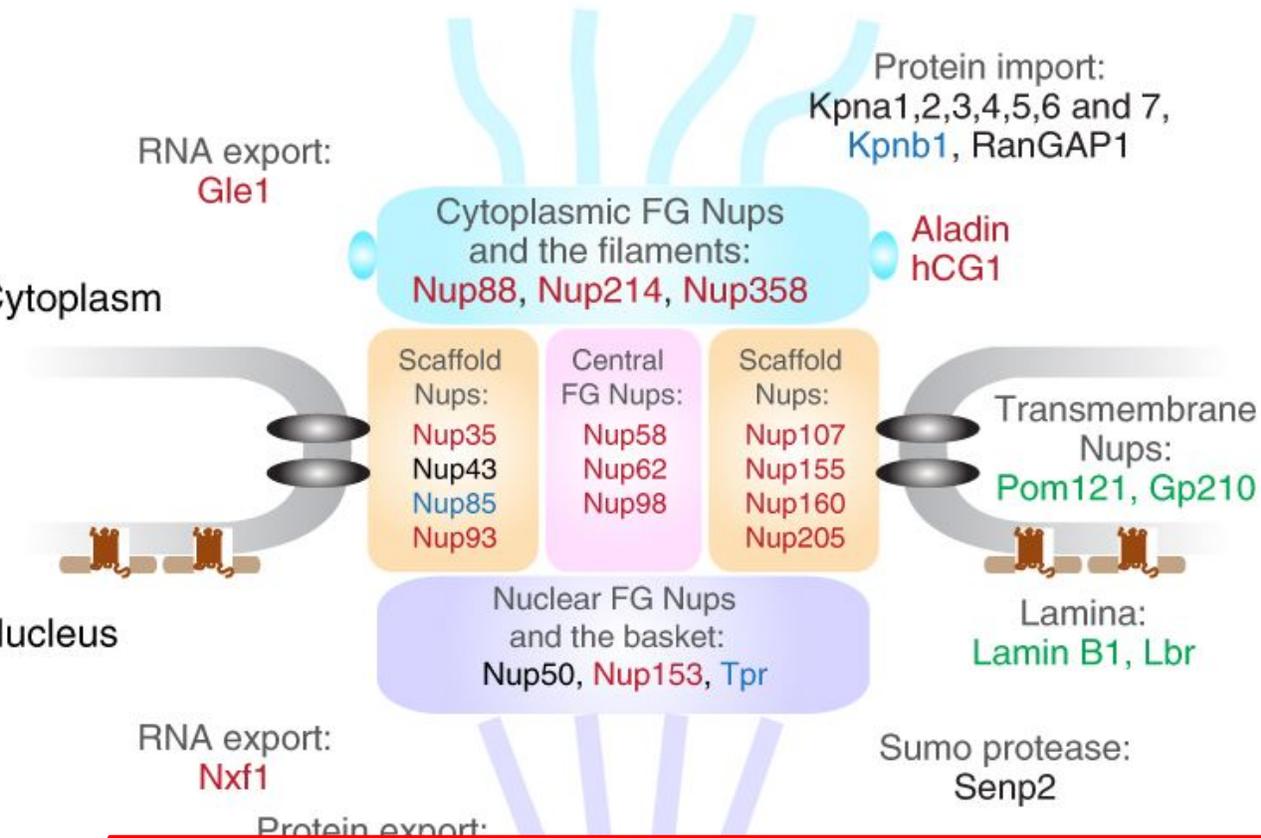
Interaction patterns

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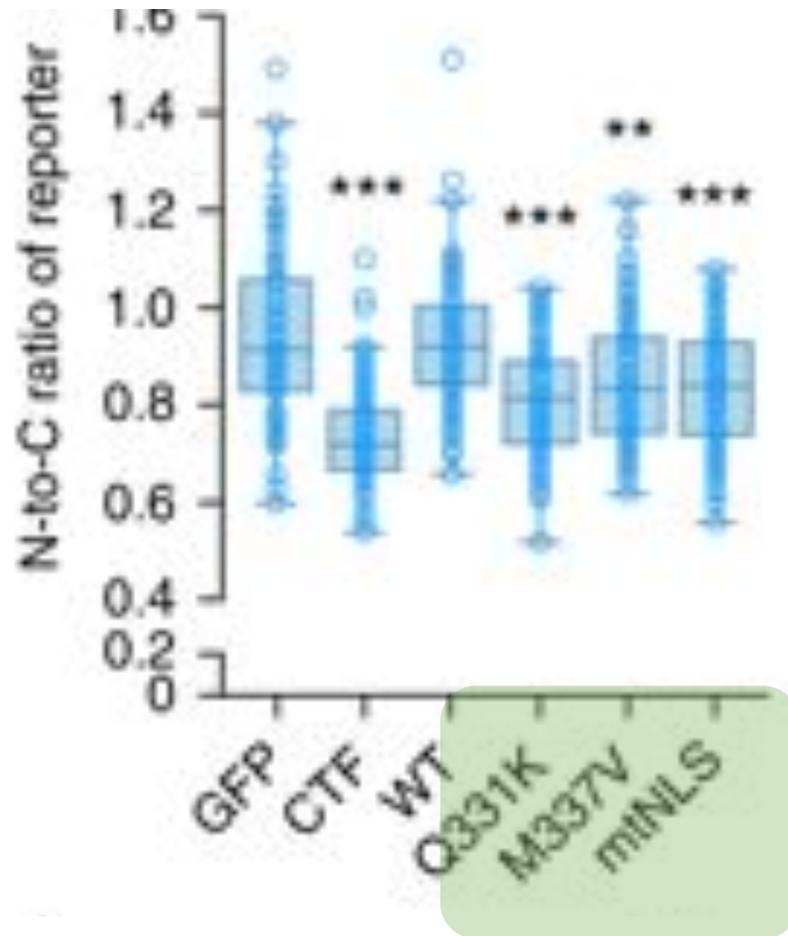
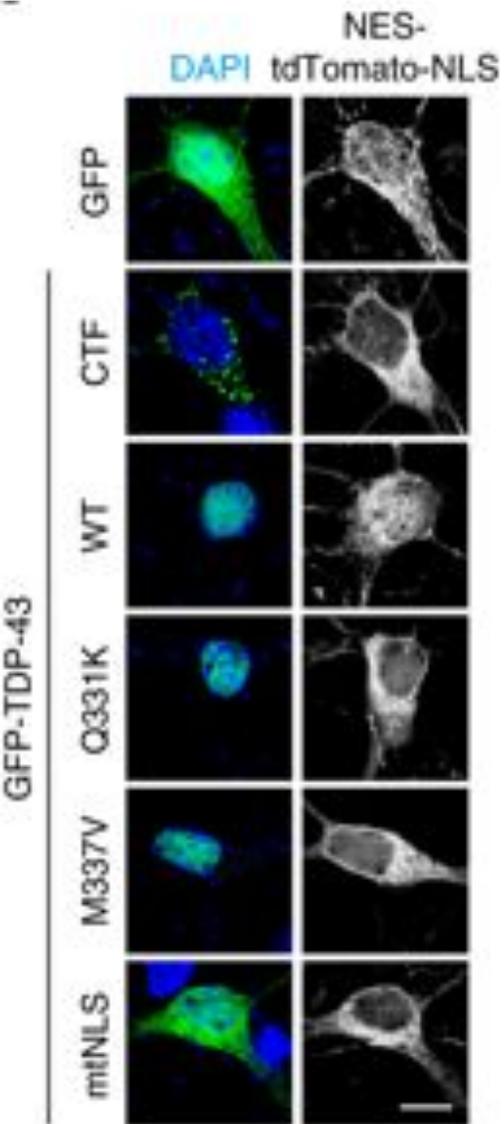


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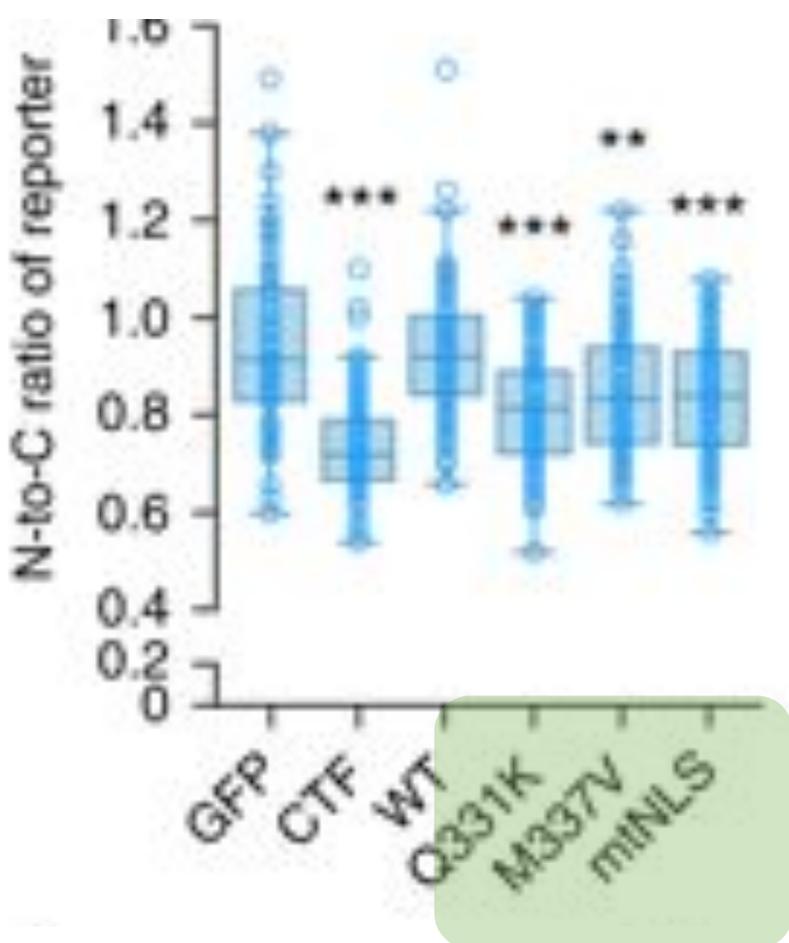
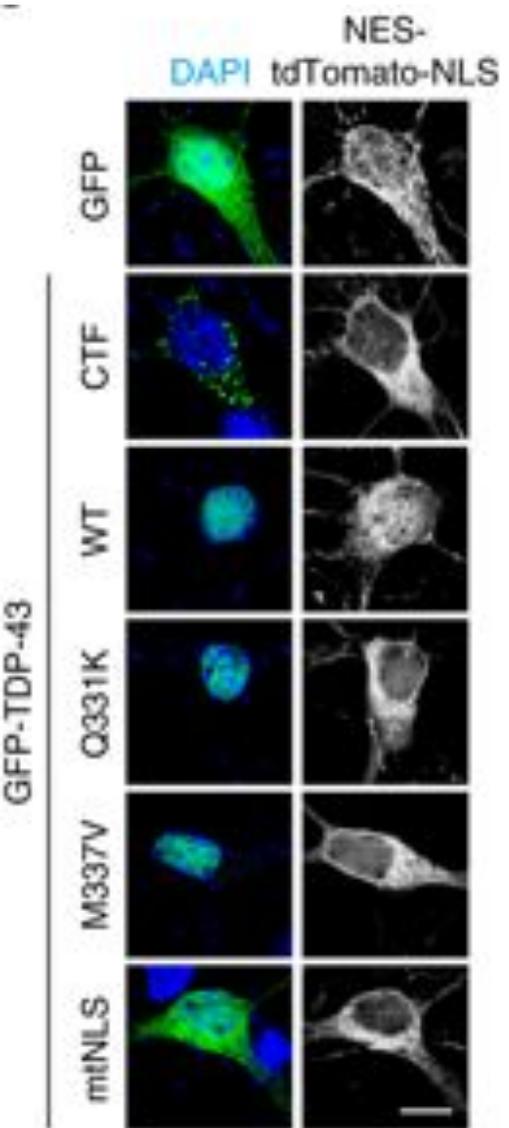
Nucleoporin Clustering

How is Nup import of **proteins** affected in **mutant TDP**?

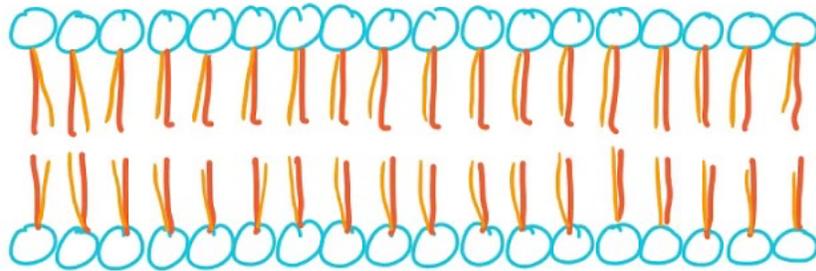


Lamins A/C interact with several transcription factors (c-fos, Rb, etc.) and regulate several important signalling pathways such as differentiating pathways Rb/E proliferating pathways AP-1 and TGF β for example. Via their interactions with SUN1/2 and Nesprins, A-type lamins form nucleocytoskeletal networks with actin and cytoplasmic intermediate filaments, providing mechanical resistance to the cell. AP-1 = activator protein 1; BAF = barrier to auto-integrative factor; INM = inner nuclear membrane; LAP = lamin associated protein; ONM = outer nuclear membrane; Rb = retinoblastoma protein; TGF β = transforming growth factor β .

How is Nup import of **proteins** affected in **mutant TDP**?

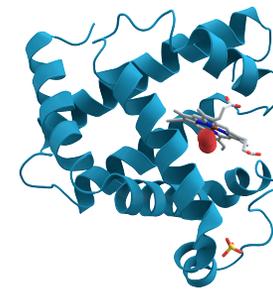
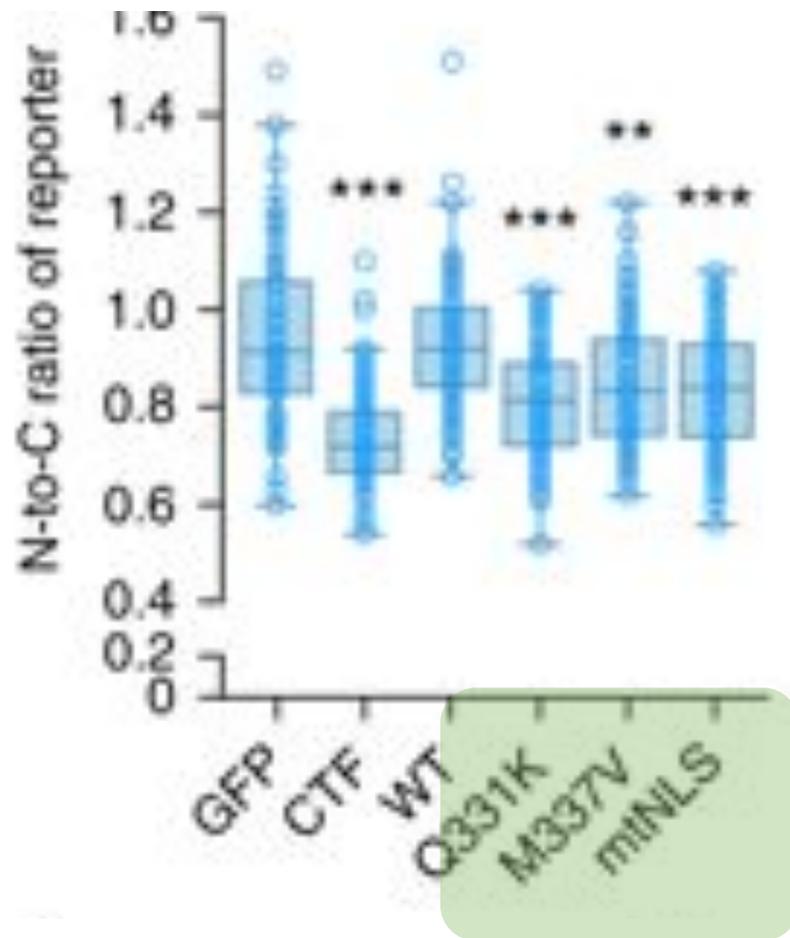
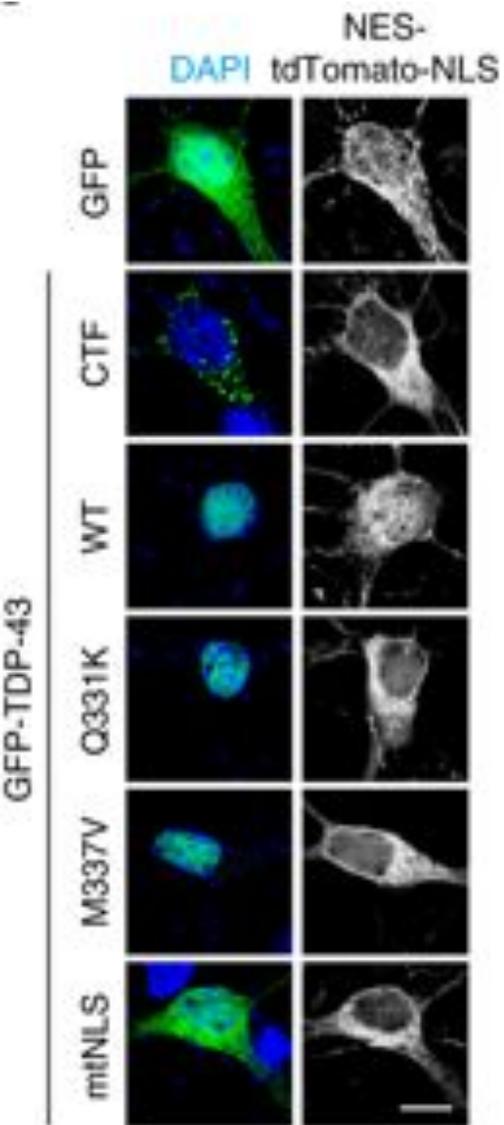


Cytoplasm

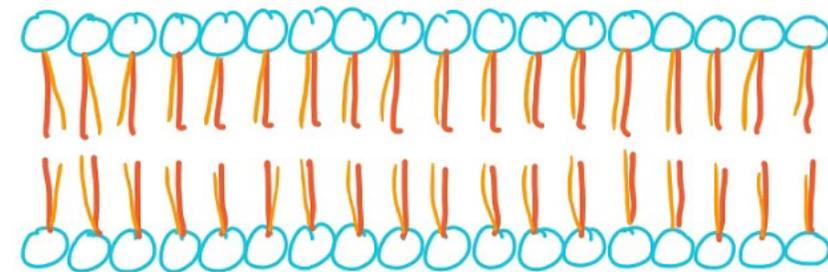


Nucleus

How is Nup import of **proteins** affected in **mutant TDP**?

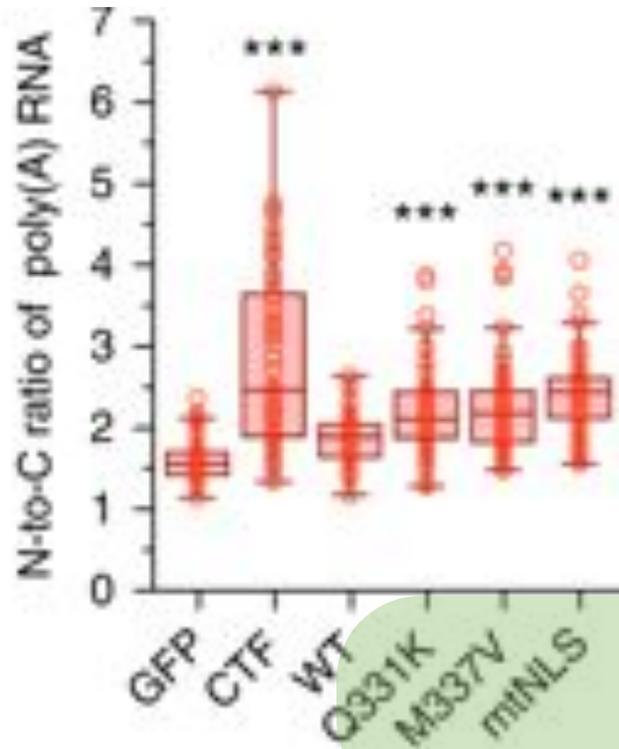
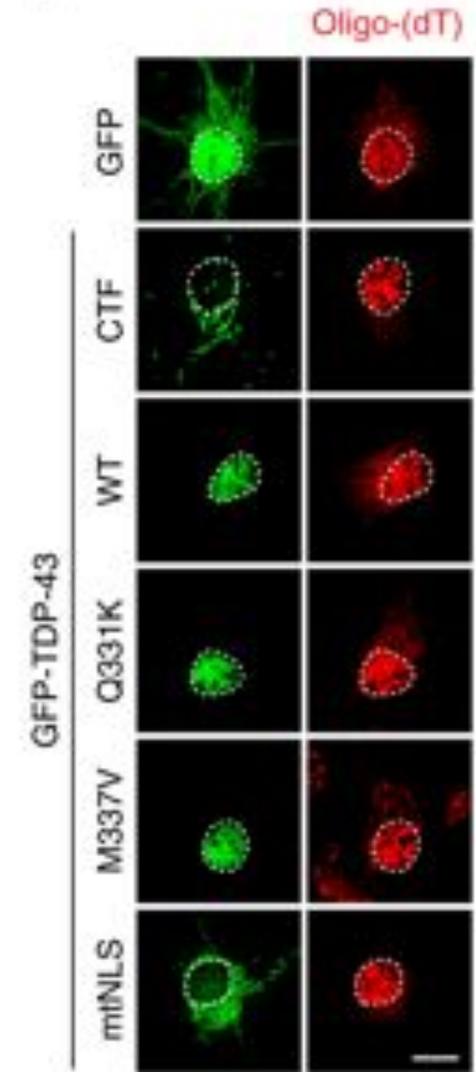


Cytoplasm



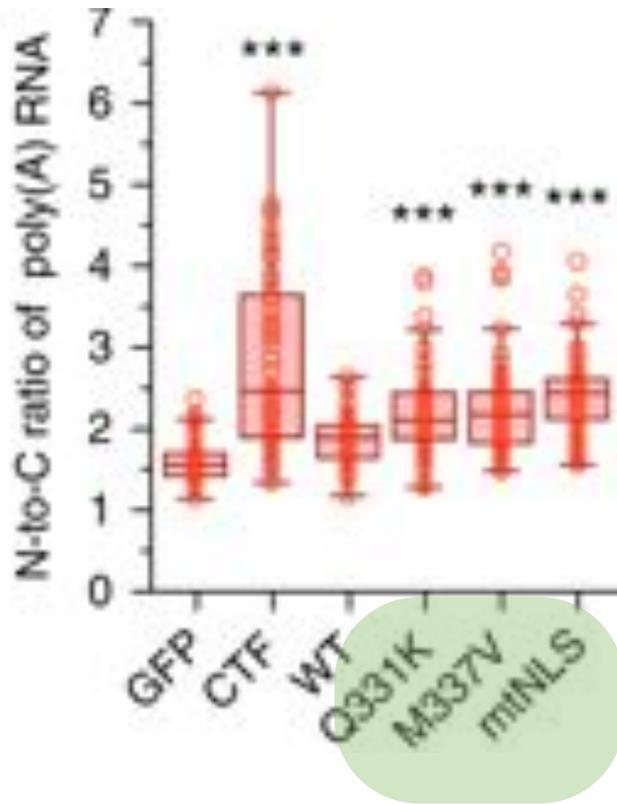
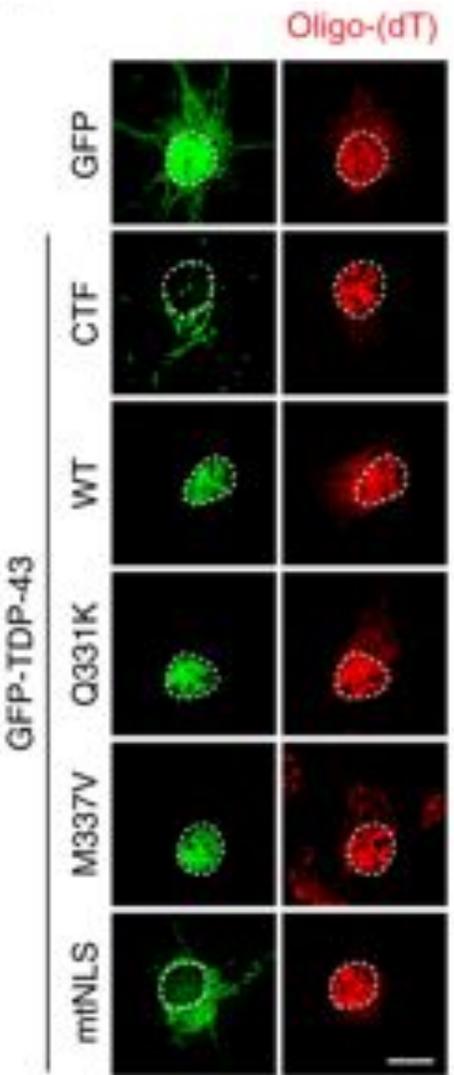
Nucleus

How is Nup export of mRNA affected in mutant TDP?

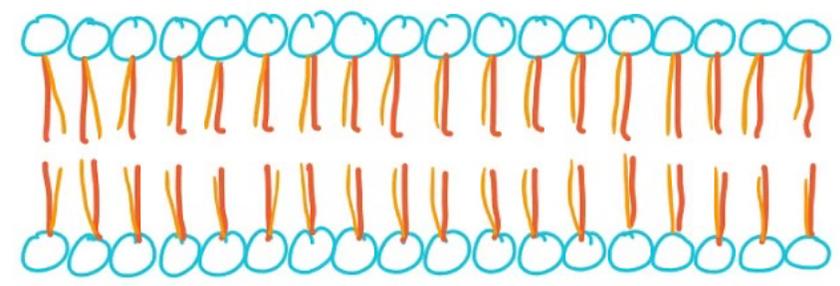


Lamins A/C interact with several transcription factors (c-fos, Rb, etc.) and regulate several important signalling pathways such as differentiating pathways Rb/E proliferating pathways AP-1 and TGF β for example. Via their interactions with SUN1/2 and Nesprins, A-type lamins form nucleocytoskeletal networks with actin and cytoplasmic intermediate filaments, providing mechanical resistance to the cell. AP-1 = activator protein 1; BAF = barrier to auto-integrative factor; INM = inner nuclear membrane; LAP = lamin associated protein; ONM = outer nuclear membrane; Rb = retinoblastoma protein; TGF β = transforming growth factor β .

How is Nup export of mRNA affected in mutant TDP?

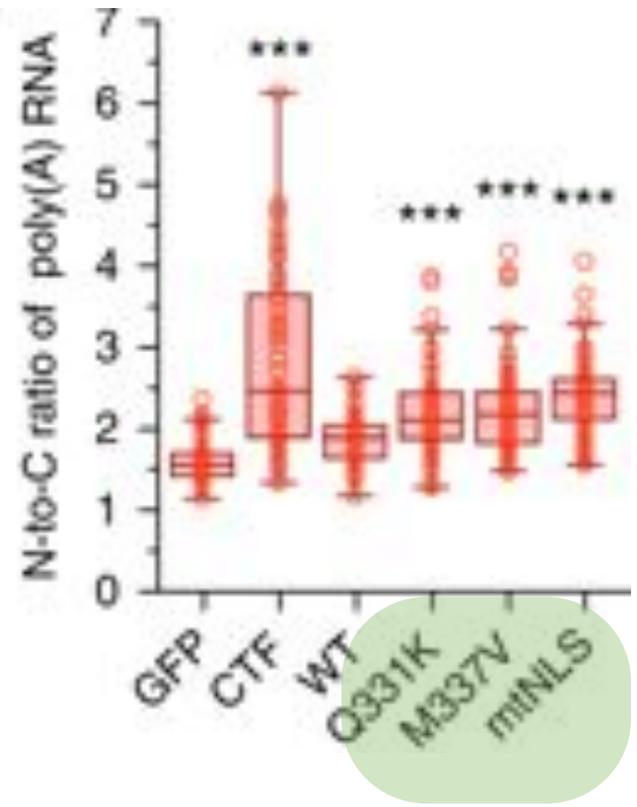
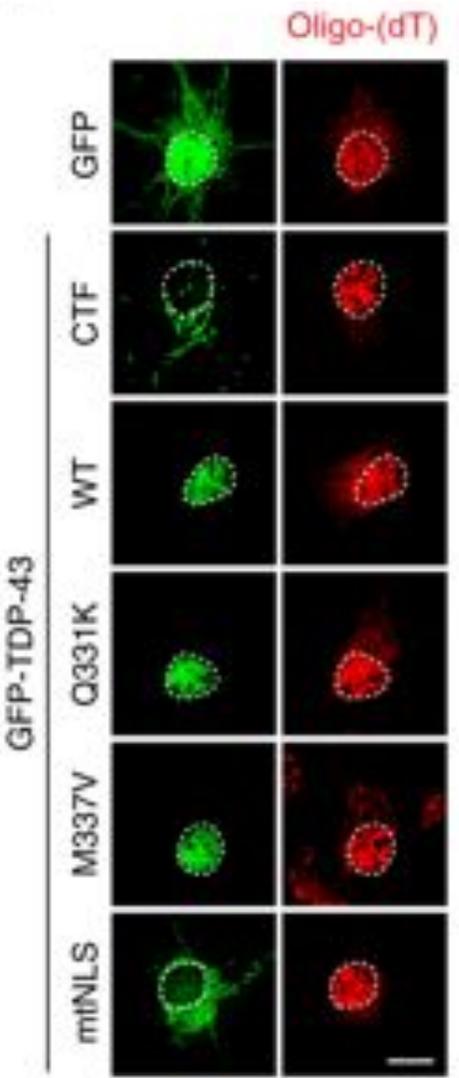


Cytoplasm

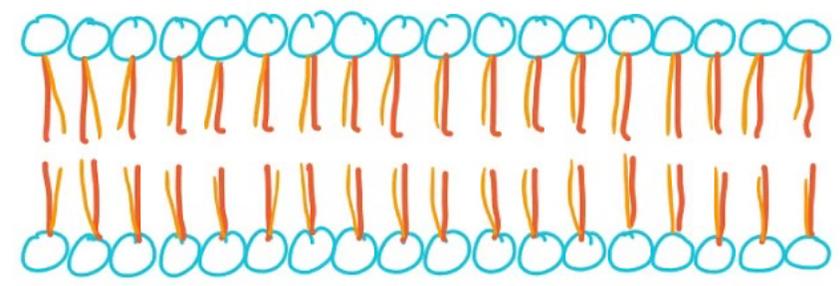


Nucleus

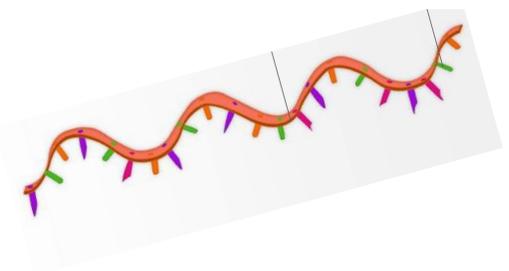
How is Nup export of mRNA affected in mutant TDP?



Cytoplasm



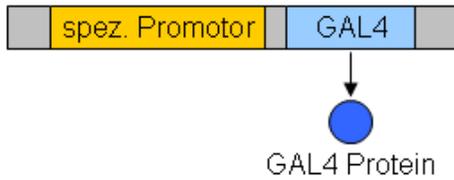
Nucleus



How can TDP and Nup interaction be modeled in organisms?

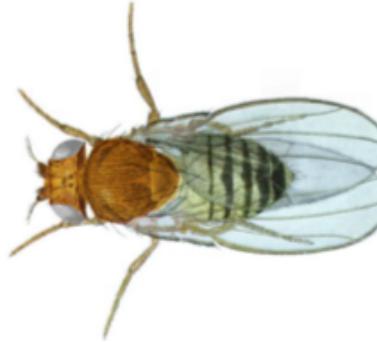
How can TDP and Nup interaction be modeled in organisms?

GAL4-Treiberline



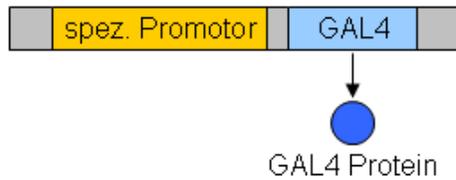
X

UAS-Line



How can TDP and Nup interaction be modeled in organisms?

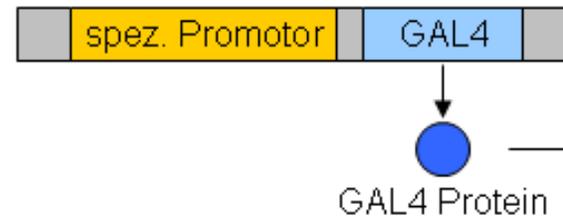
GAL4-Treiberline



UAS-Line

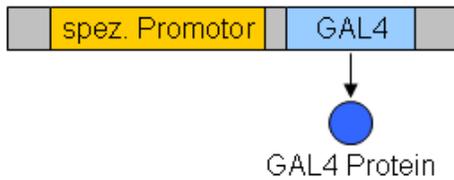


GAL4/UAS-Line

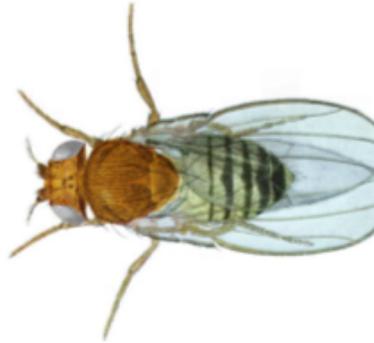


How can TDP and Nup interaction be modeled in organisms?

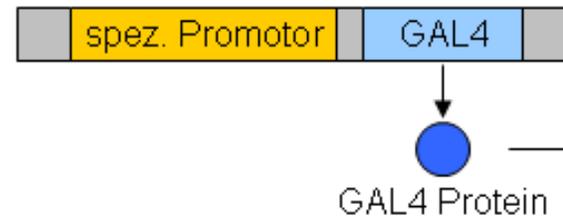
GAL4-Treiberline



UAS-Line

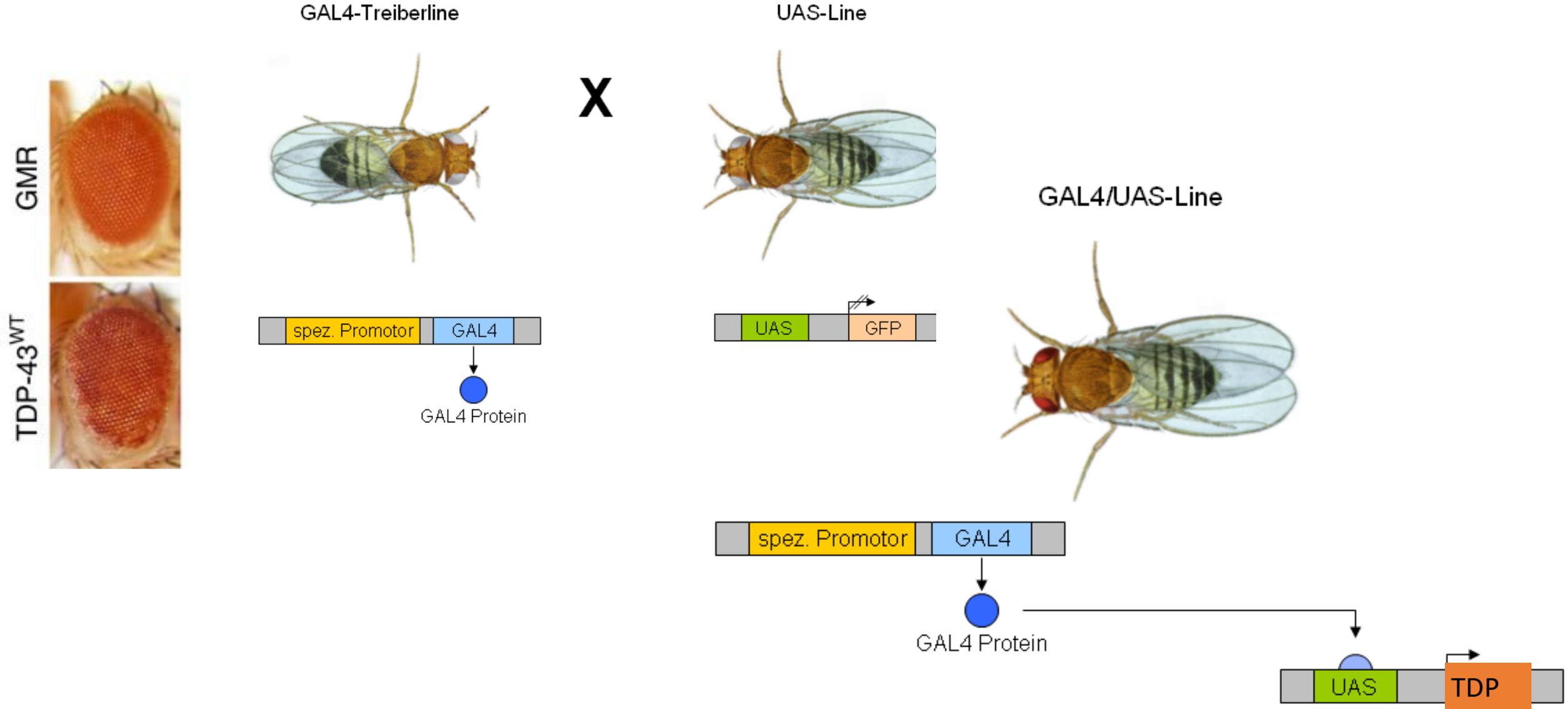


GAL4/UAS-Line



X

How can TDP and Nup interaction be modeled in organisms?



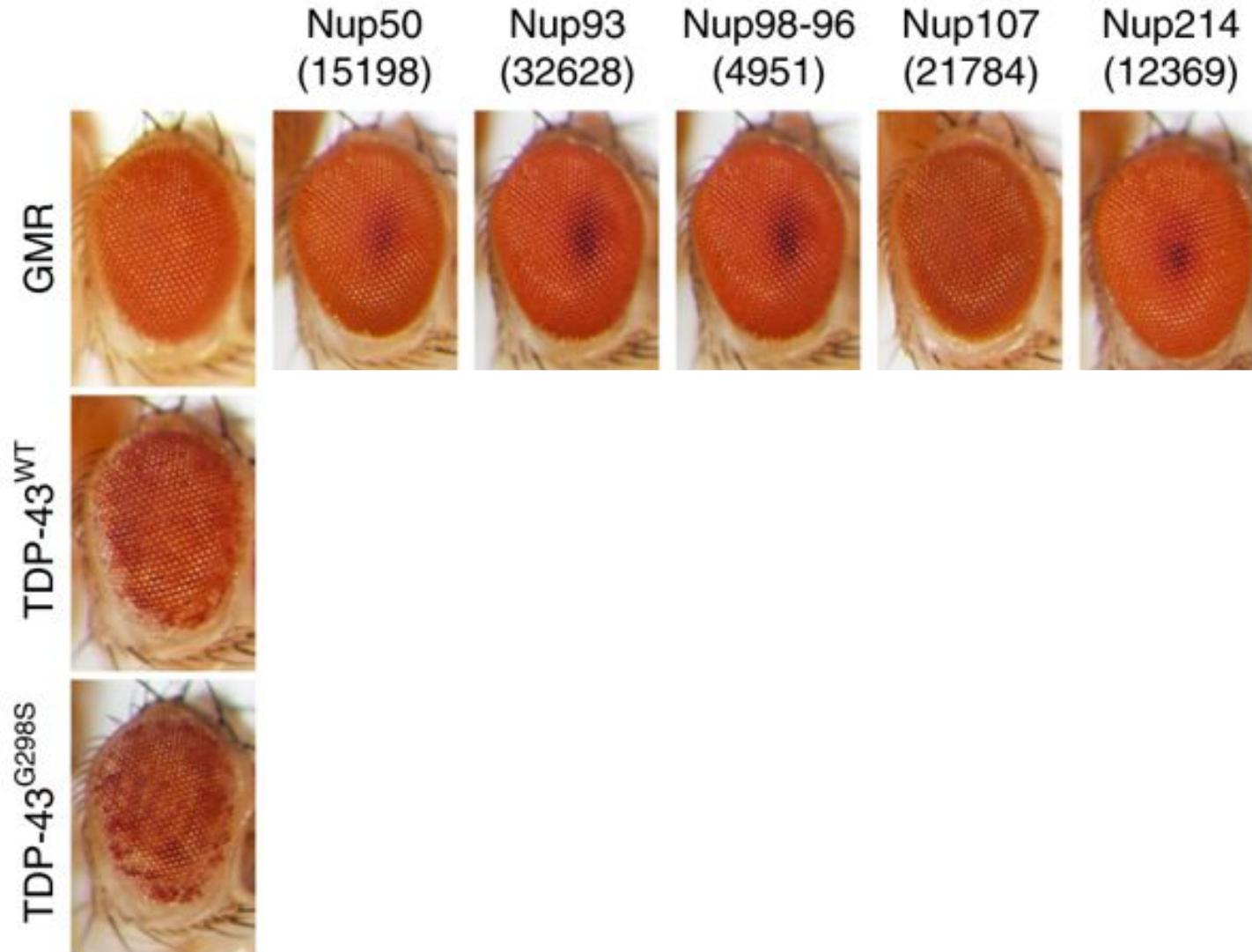
What happens in TDP fly mutants?

a



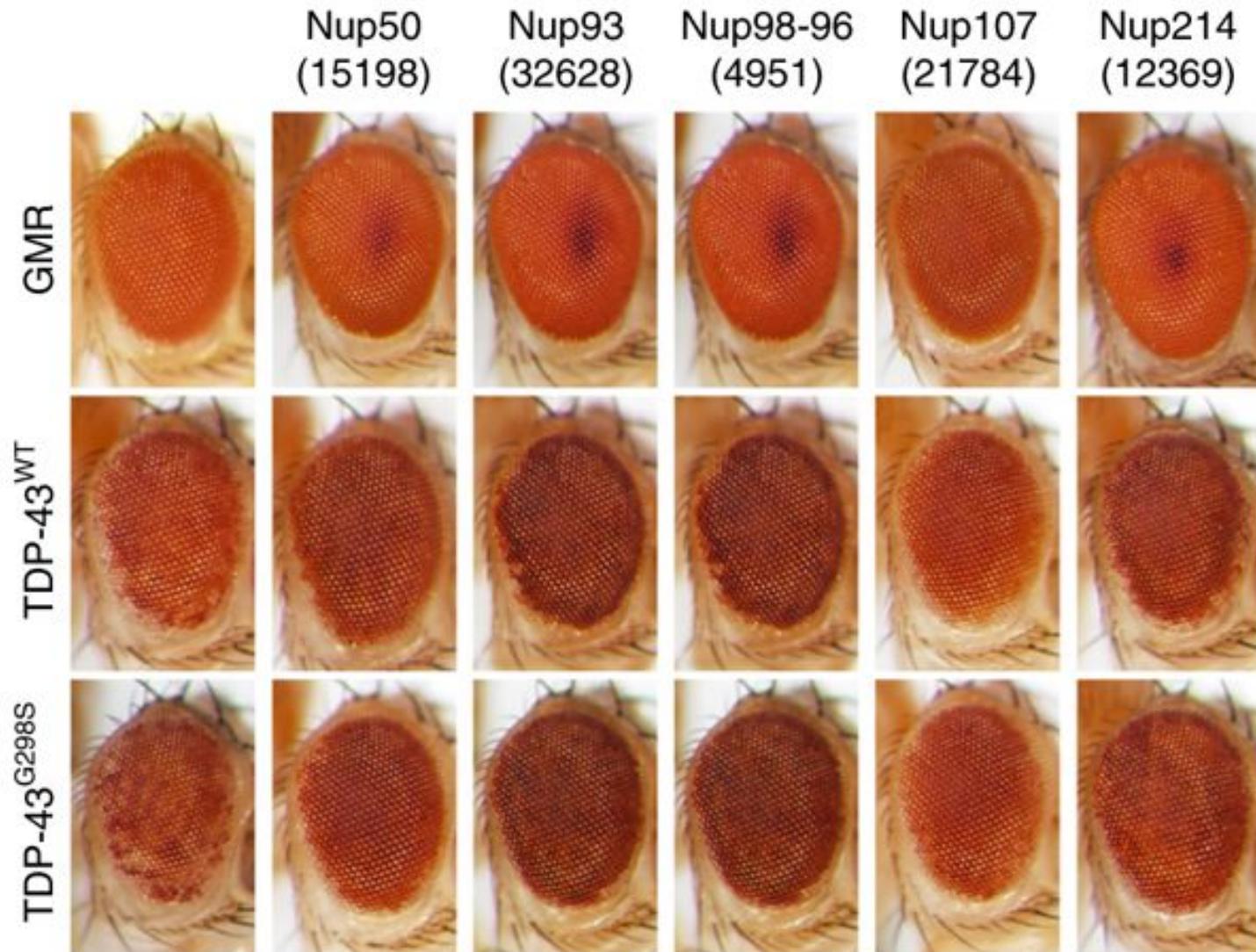
What happens in nucleoporin mutants?

a

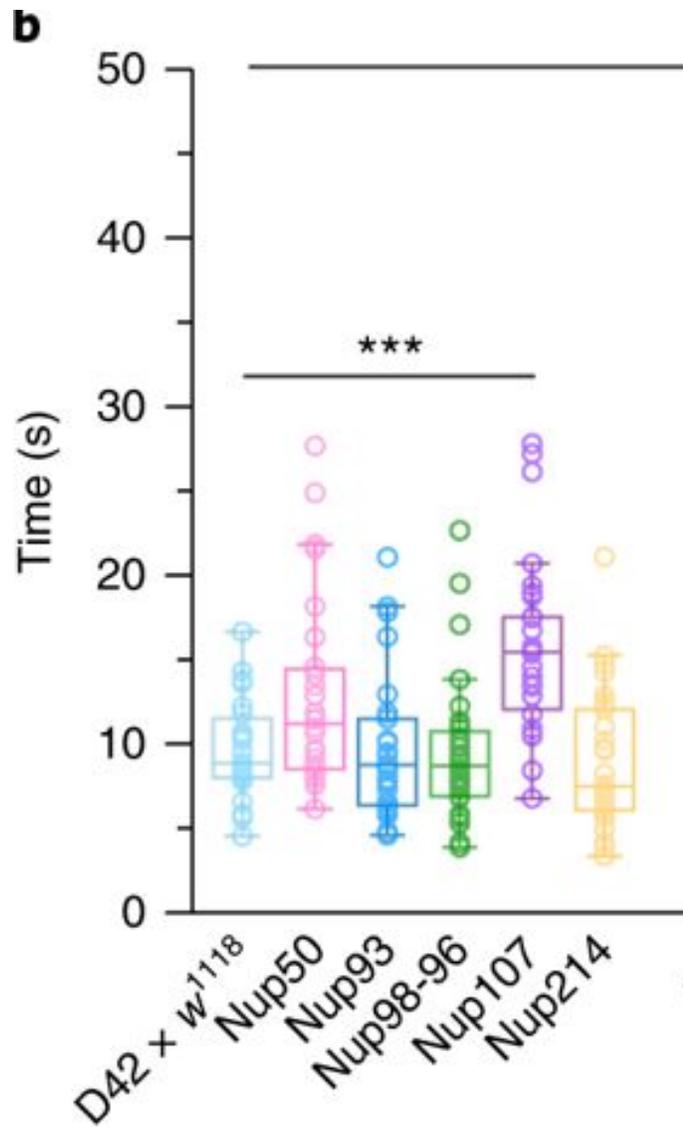


How do TDP and nucleoporin mutants interact?

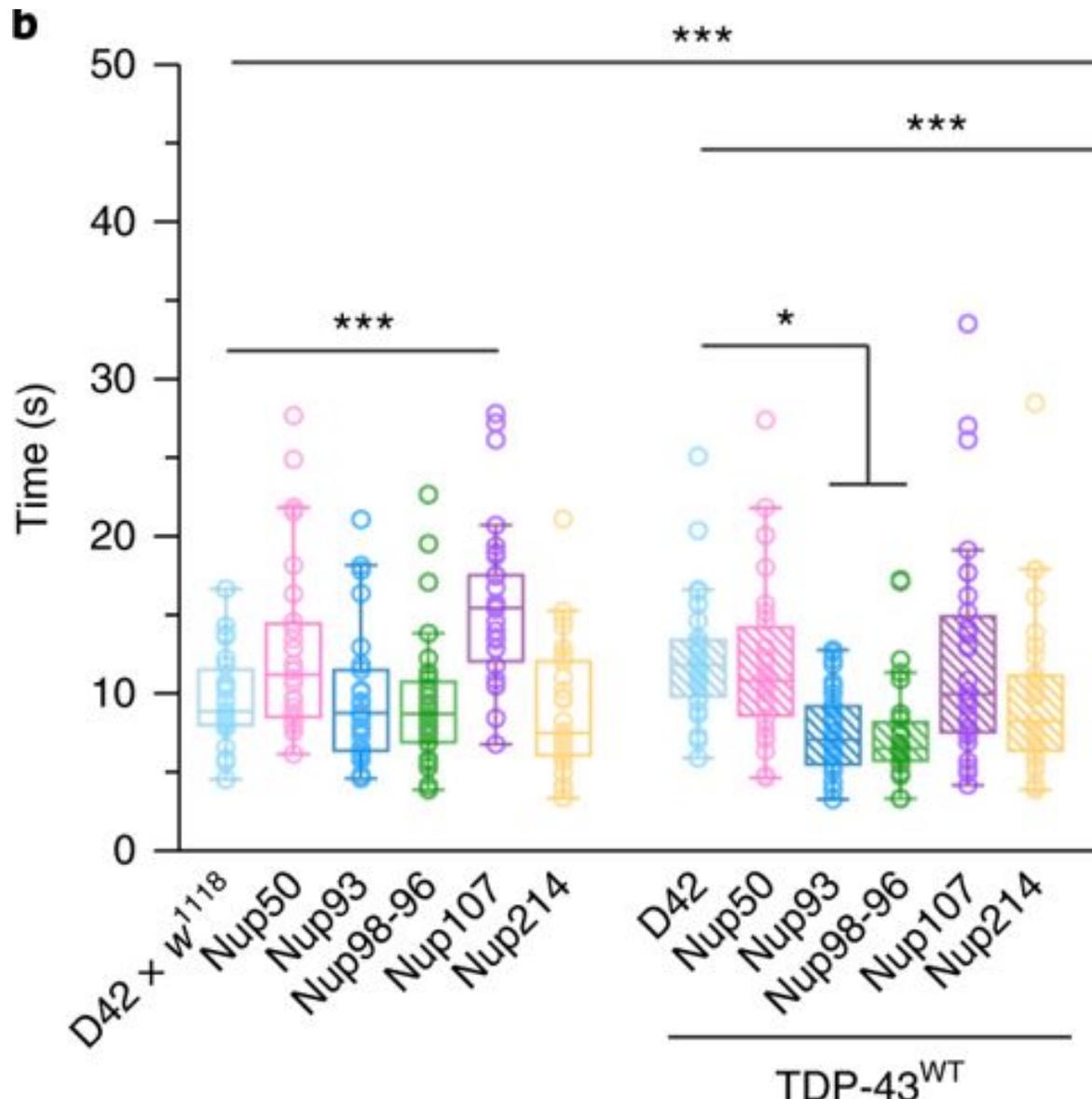
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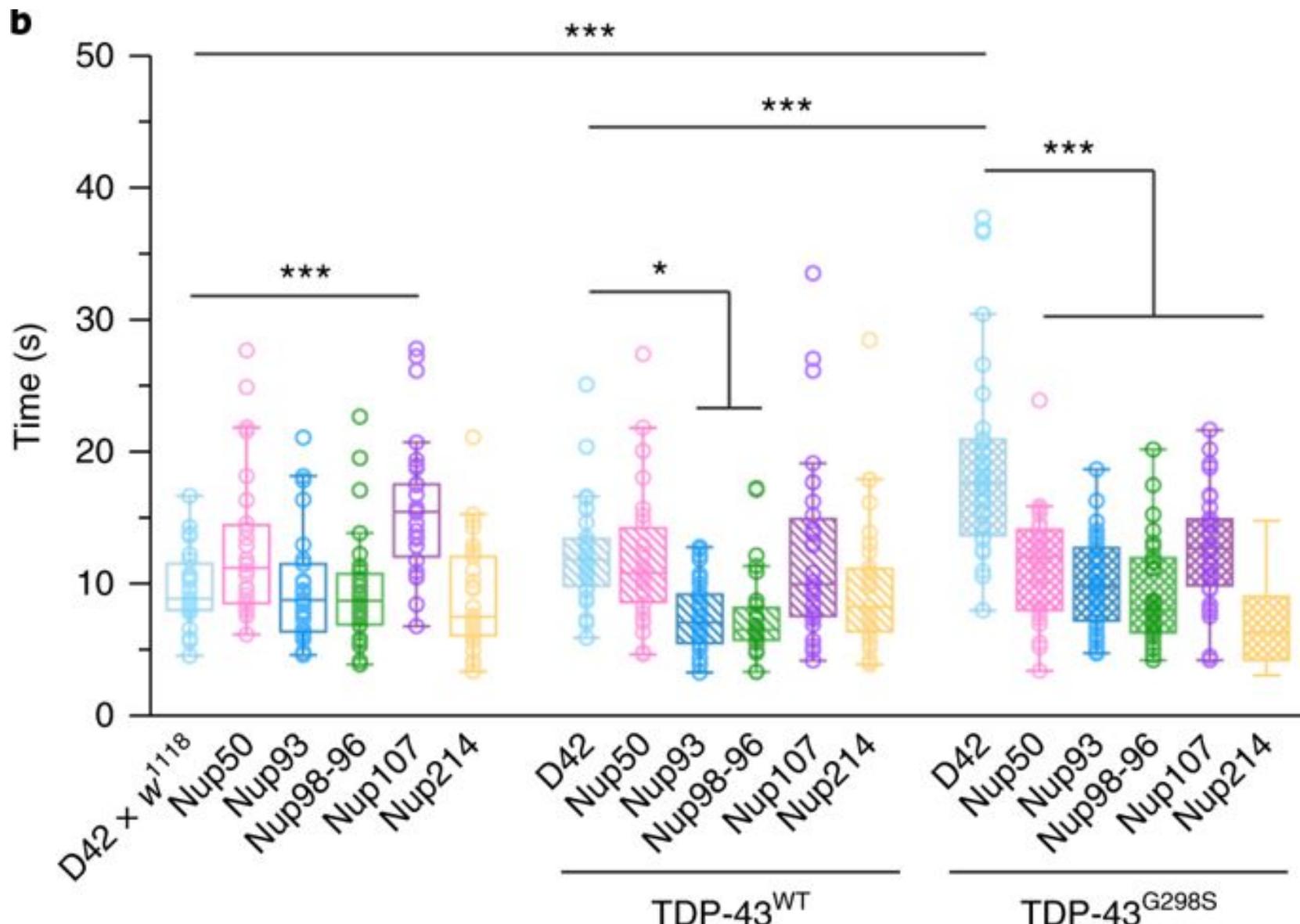
How was Fly motor function affected by Nup mutants?



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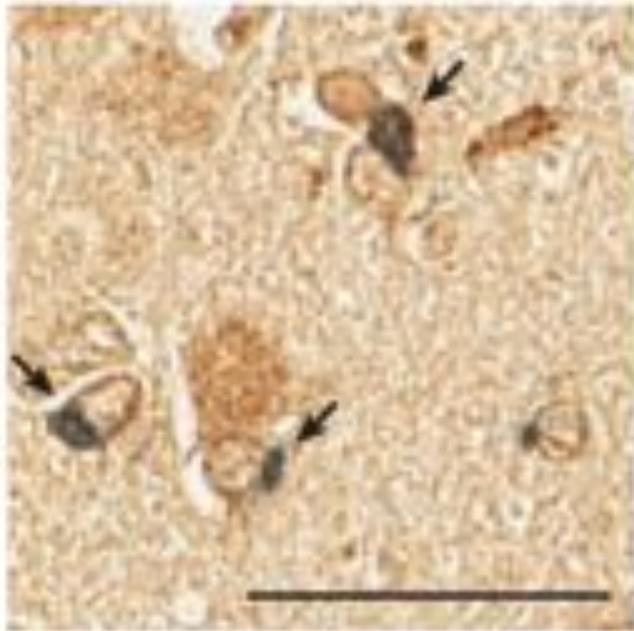


How do **TDP** and **Nups** interact in human ALS neurons?

b

TDP-ALS

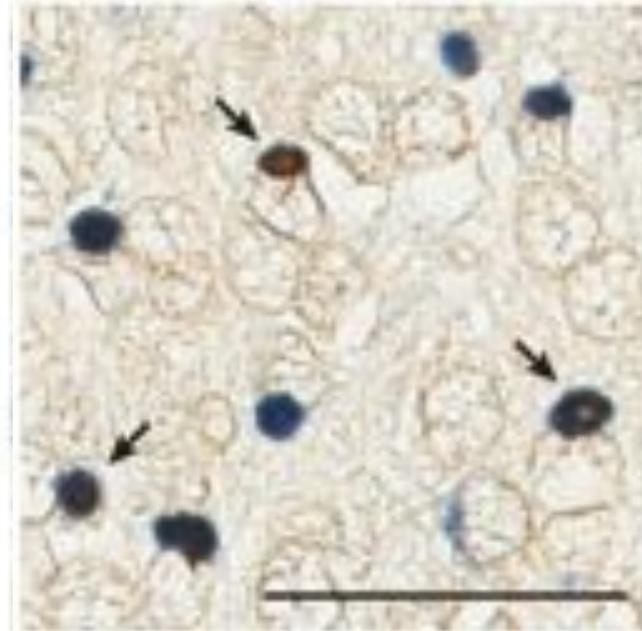
Motor cortex



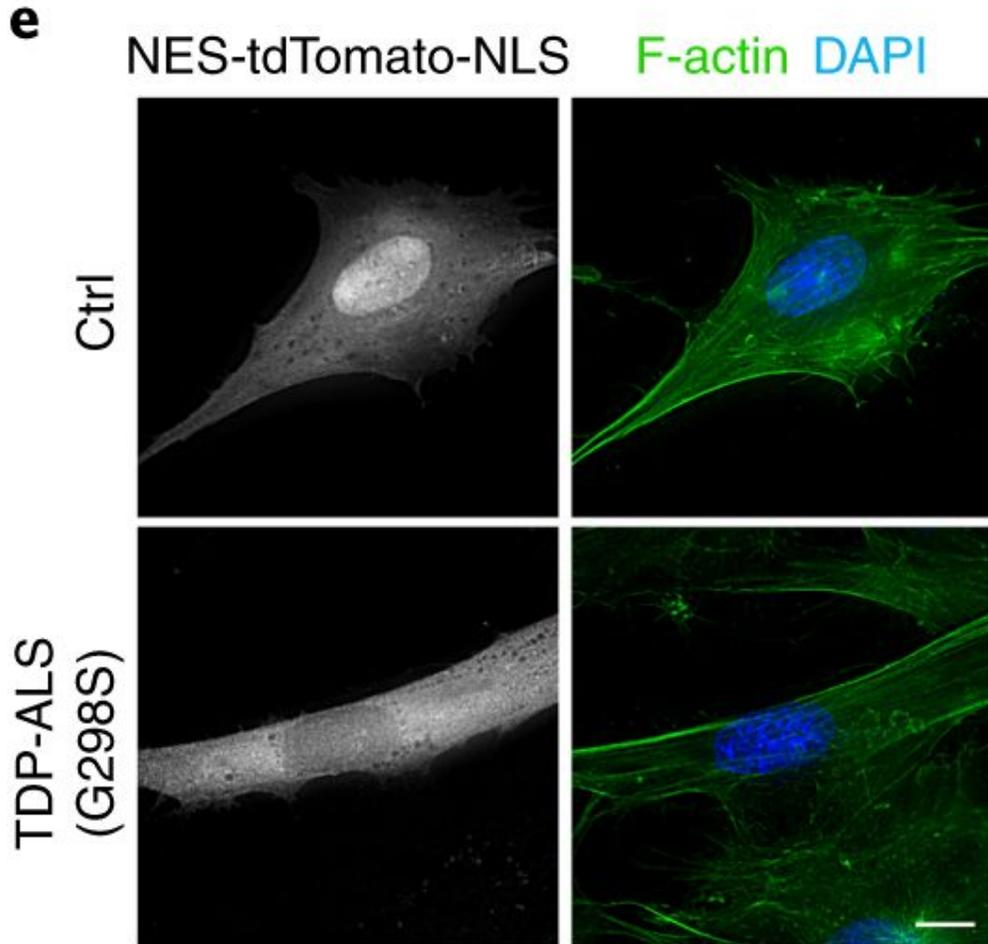
c

ALS

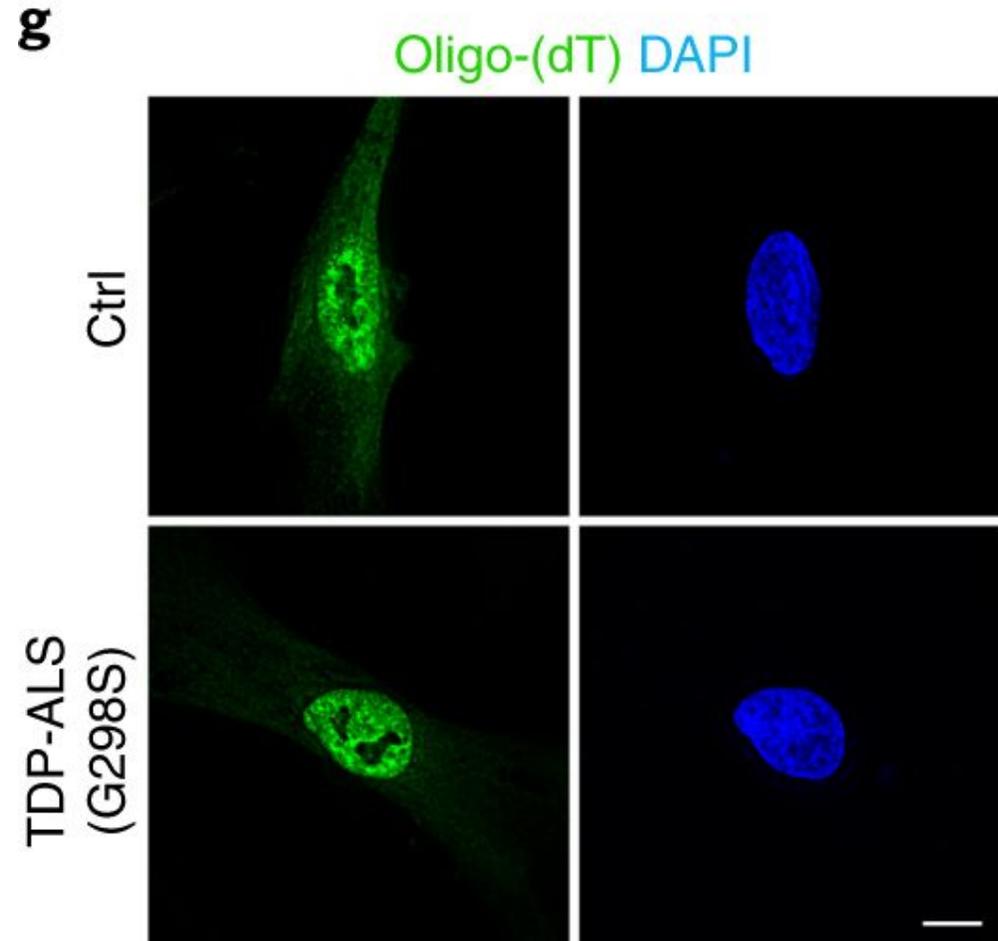
Hippocampus



How is **protein** and **mRNA** transport affected in TDP-43 ALS patients?

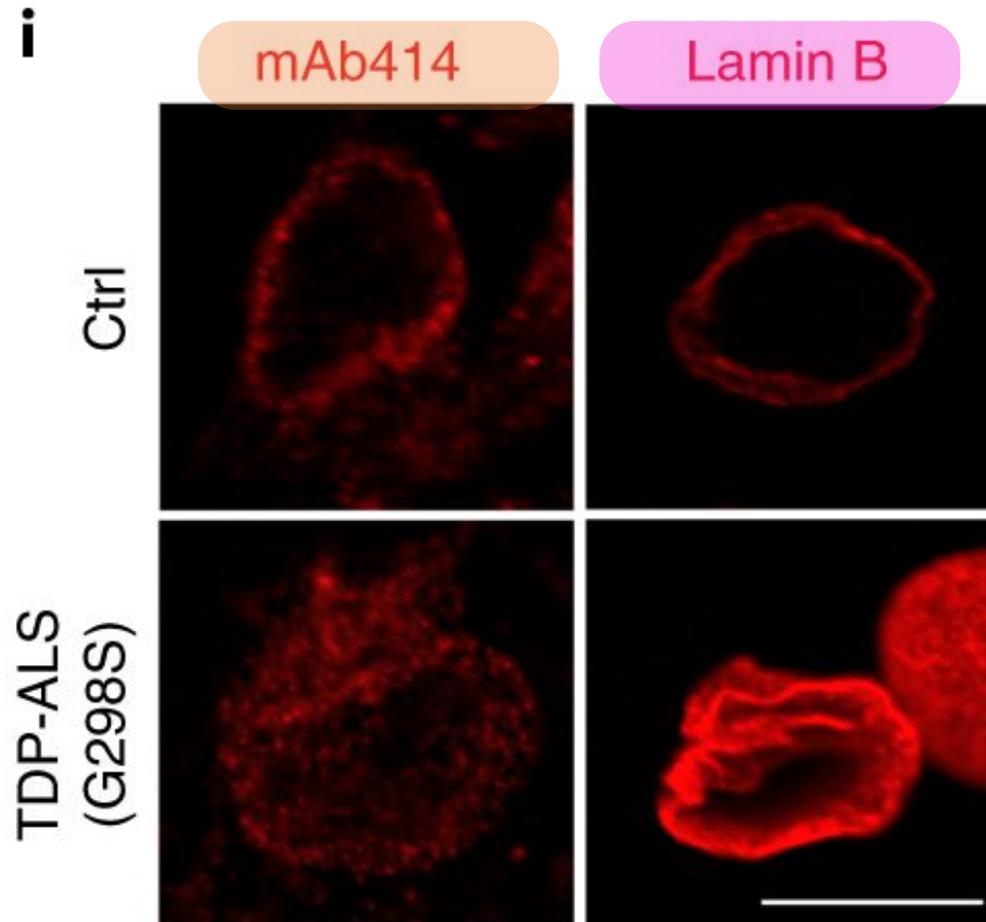


Protein transport



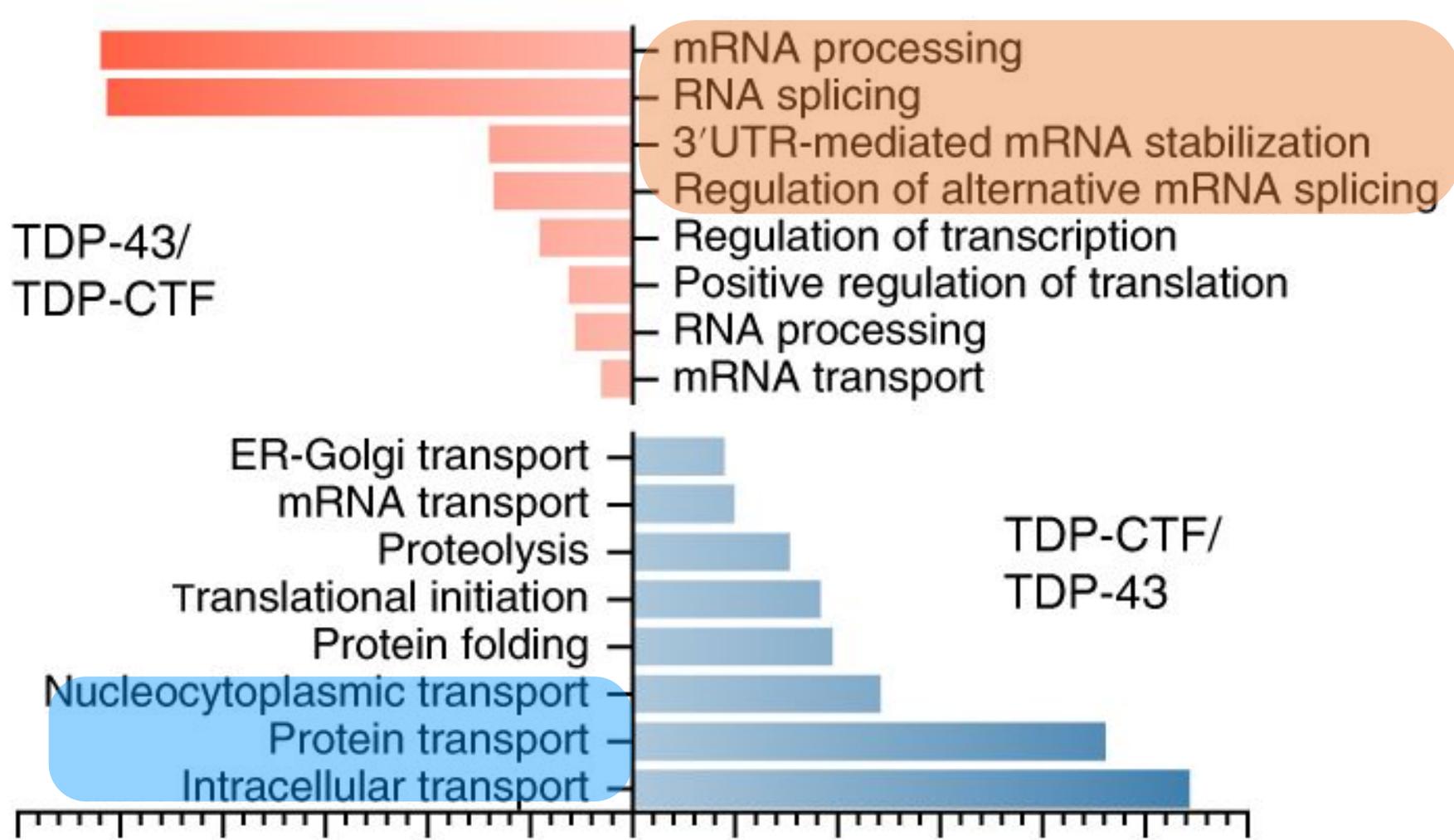
mRNA

How does ALS TDP affect **nucleoporin** and **lamina** localization?



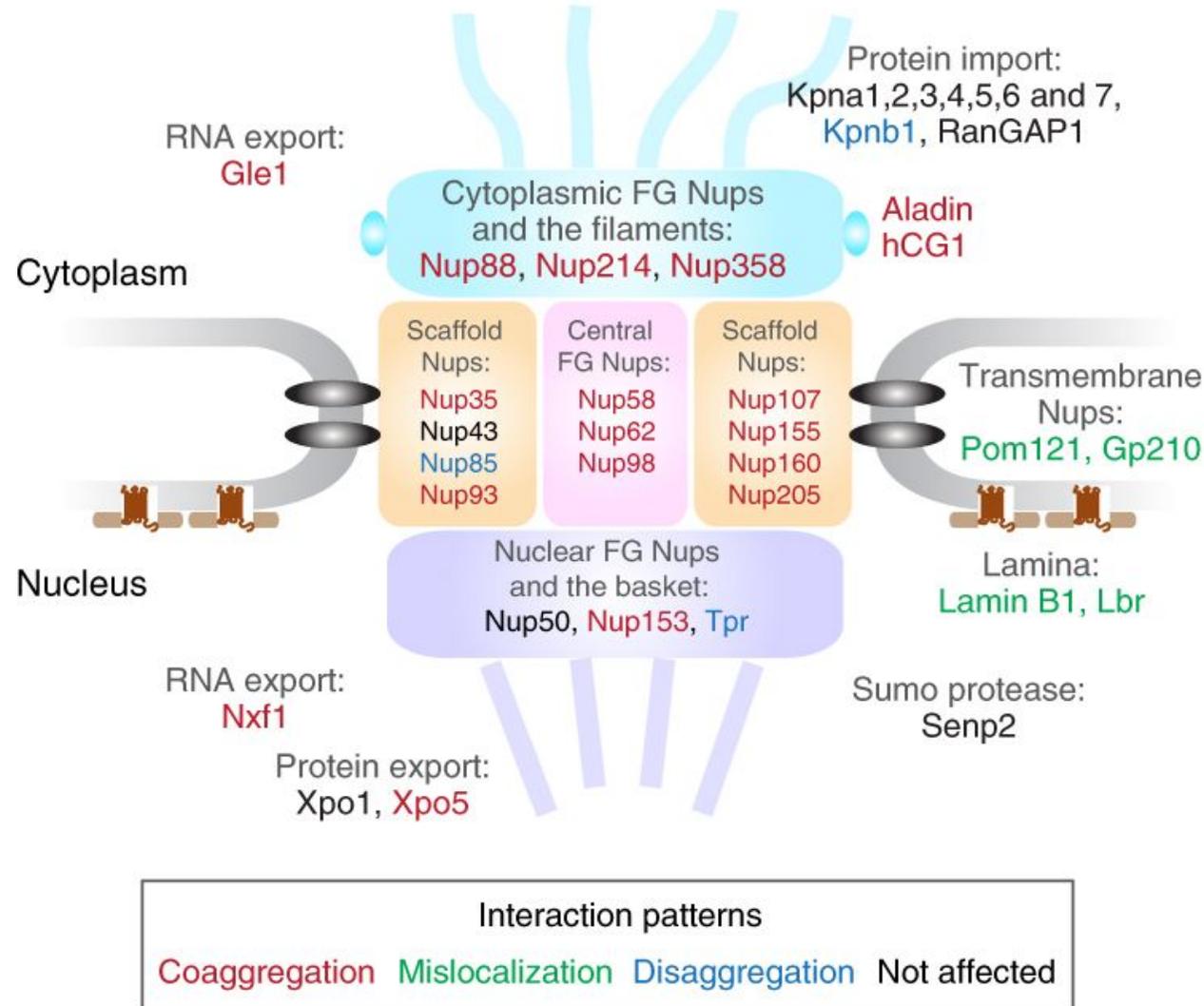
Summary

1: BioID based proteomic approaches were powerful way to identify new proteins involved in ALS



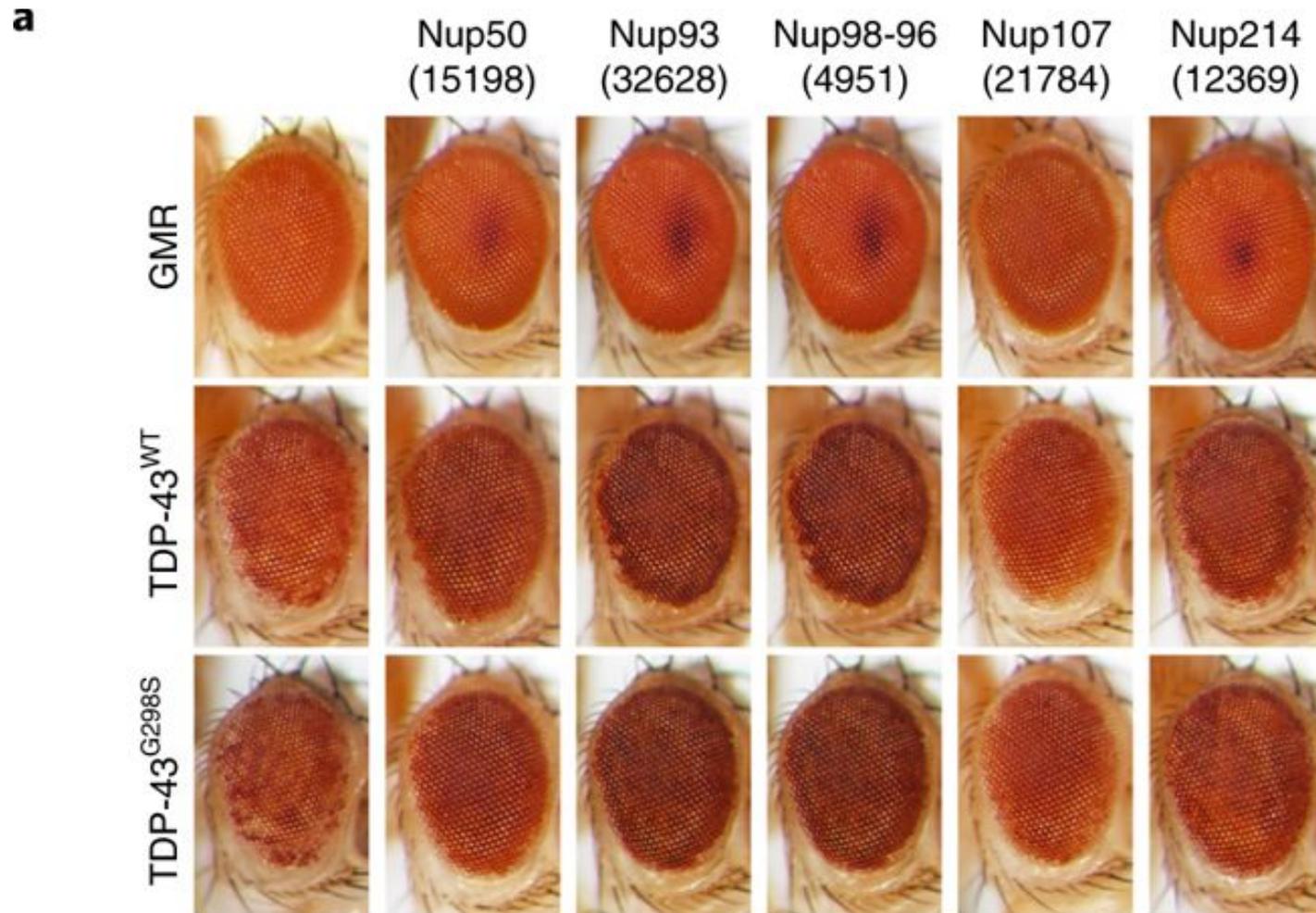
Summary

2. **Nucleoporins** and **nuclear lamina** proteins were identified as major targets for TDP43



Summary

3. Modeling nucleoporin defects in *Drosophila* revealed nup knockout can suppress mutant TDP-43 pathology.



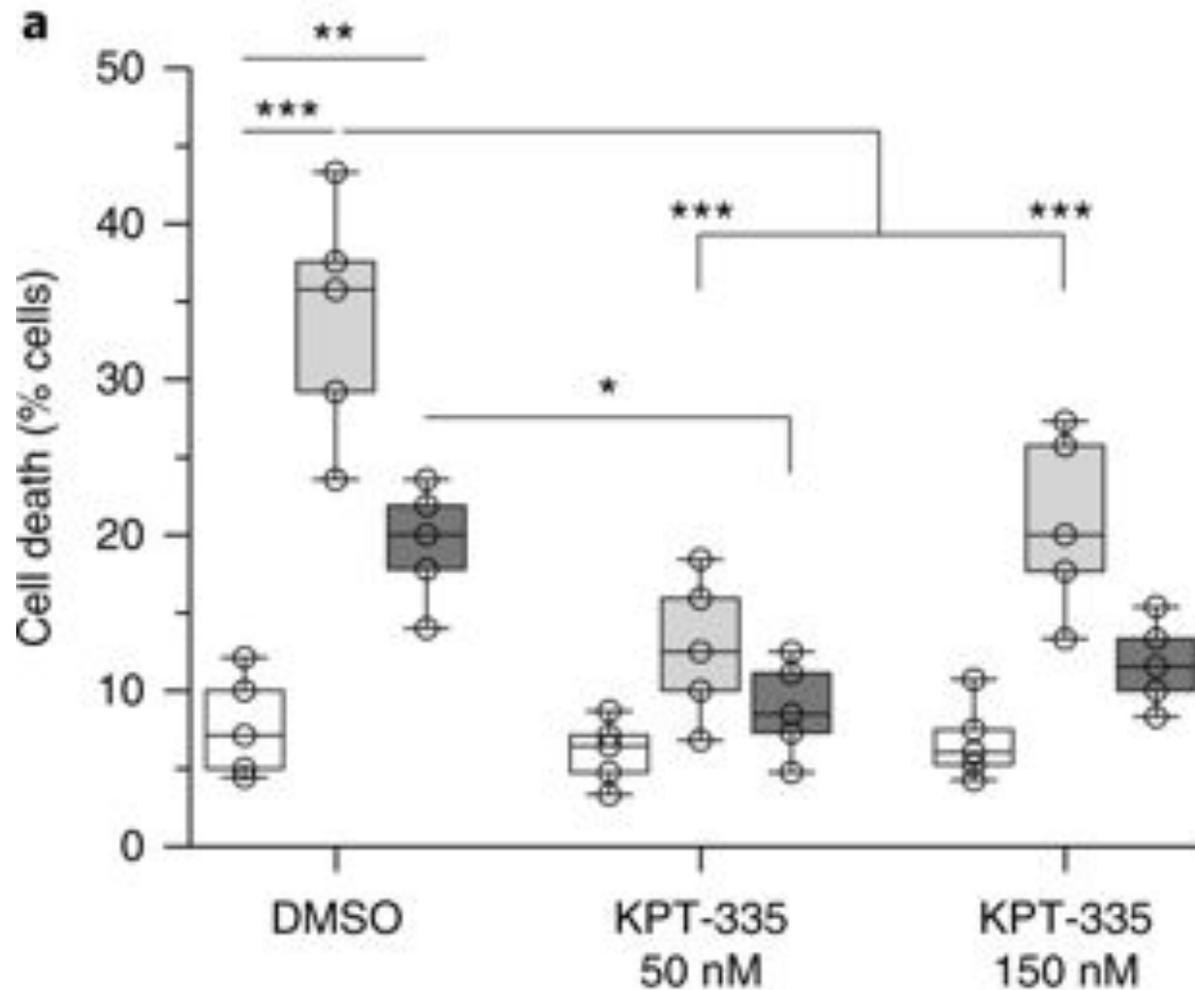
Summary

1: BioID based proteomic approaches were powerful way to identify new proteins involved in ALS

2. Nucleoporins and nuclear lamina proteins were identified as major targets for TDP43

3. Modeling nucleoporin defects in Drosophila revealed nup knockout can suppress TDP-43 pathology accurately.

How does a selective inhibitor of TDP-43 toxicity effect **cell death**?



How does a selective inhibitor of TDP-43 toxicity effect **morphology**?

