RNA Binding Proteins:

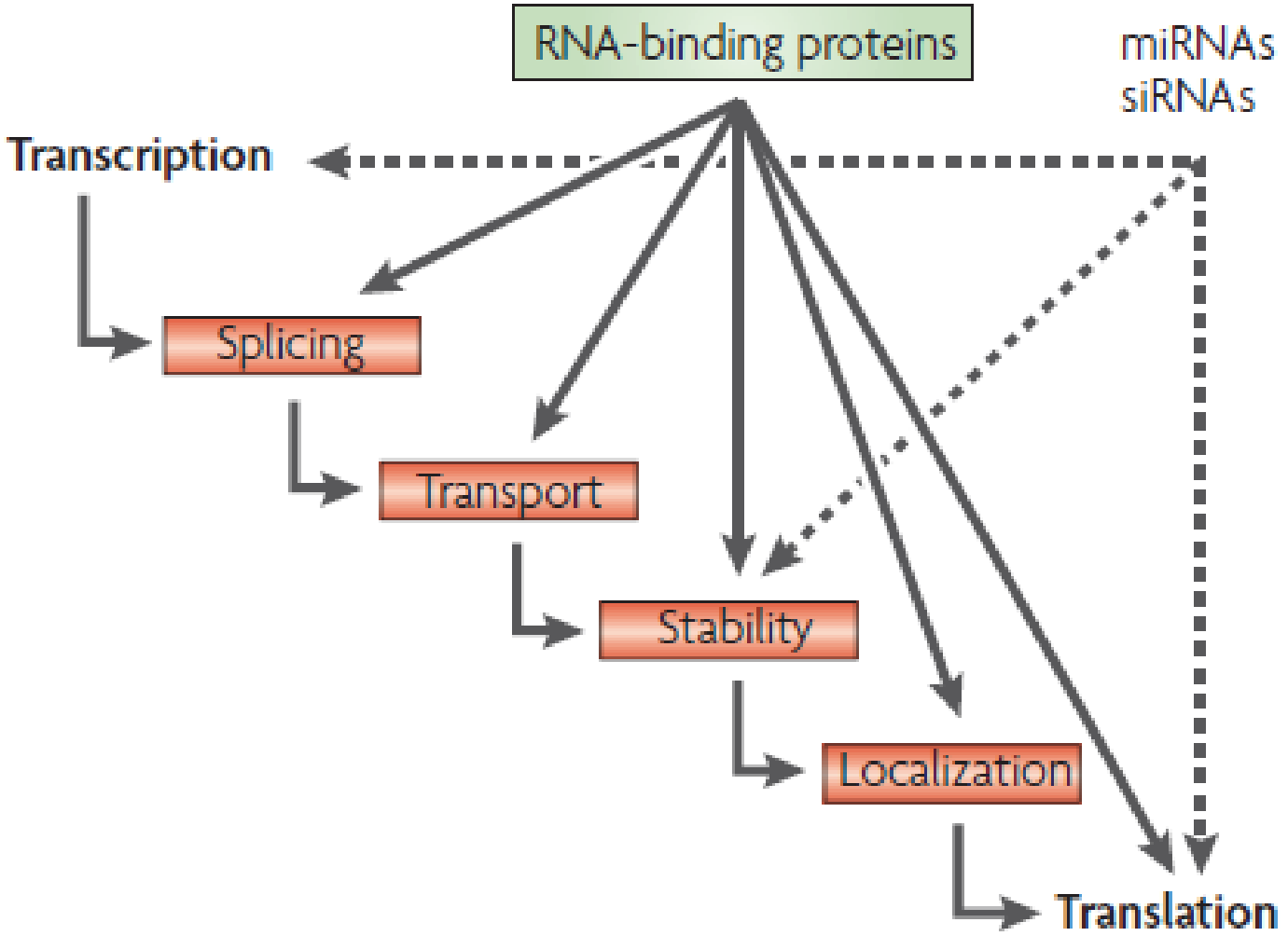
from physiology to pathology

An update

Stefano Sellitto

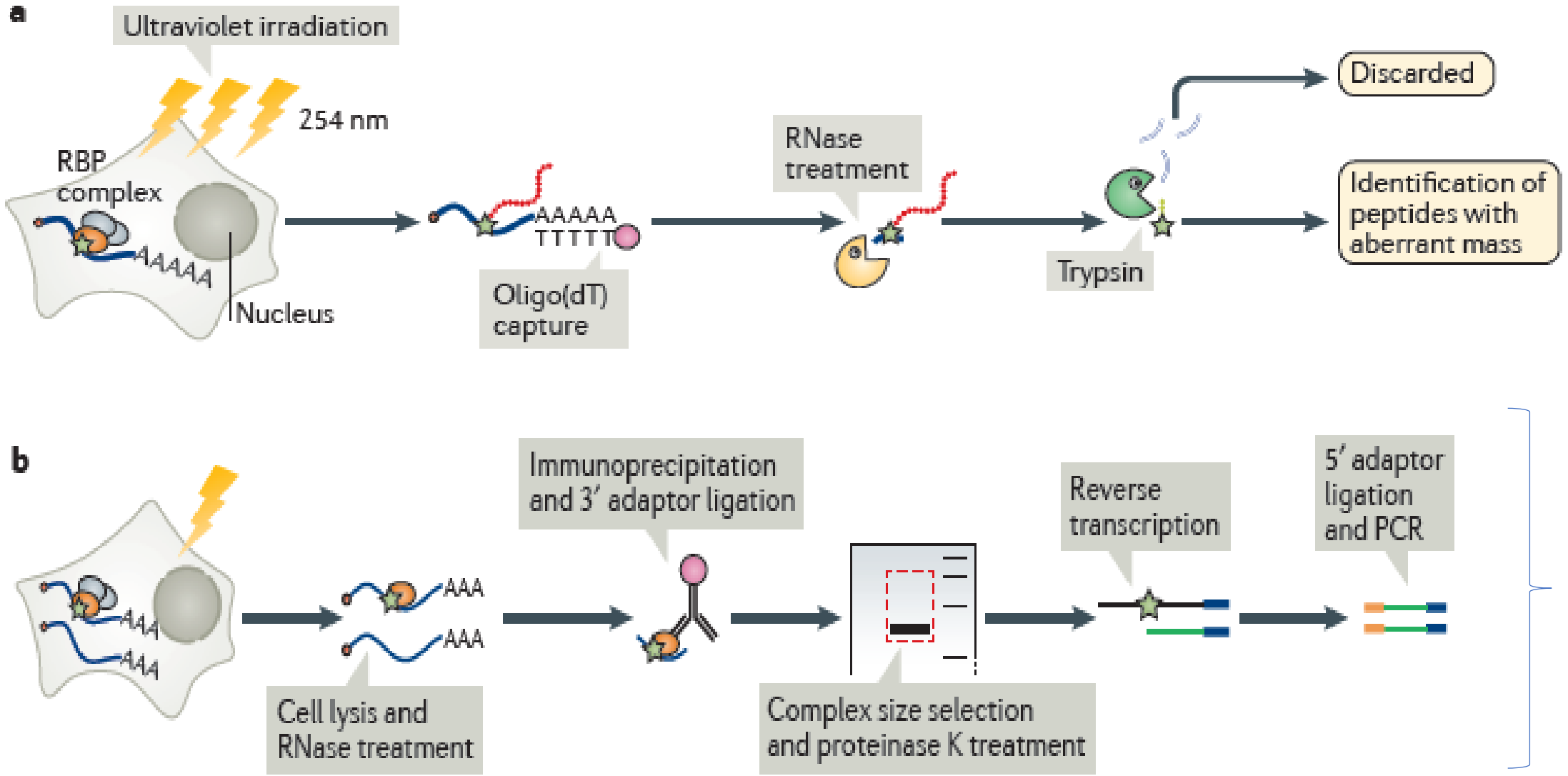
RBPs regulate the RNA metabolism

A ‘conventional’ RNA-Binding Protein (RBP) participates in the formation of ribonucleoprotein (RNP) complexes that are principally involved in gene expression.



Keene, 2007

High-Throughuput sequencing to study the RBPs world



RIP-Seq

(i)CLIP

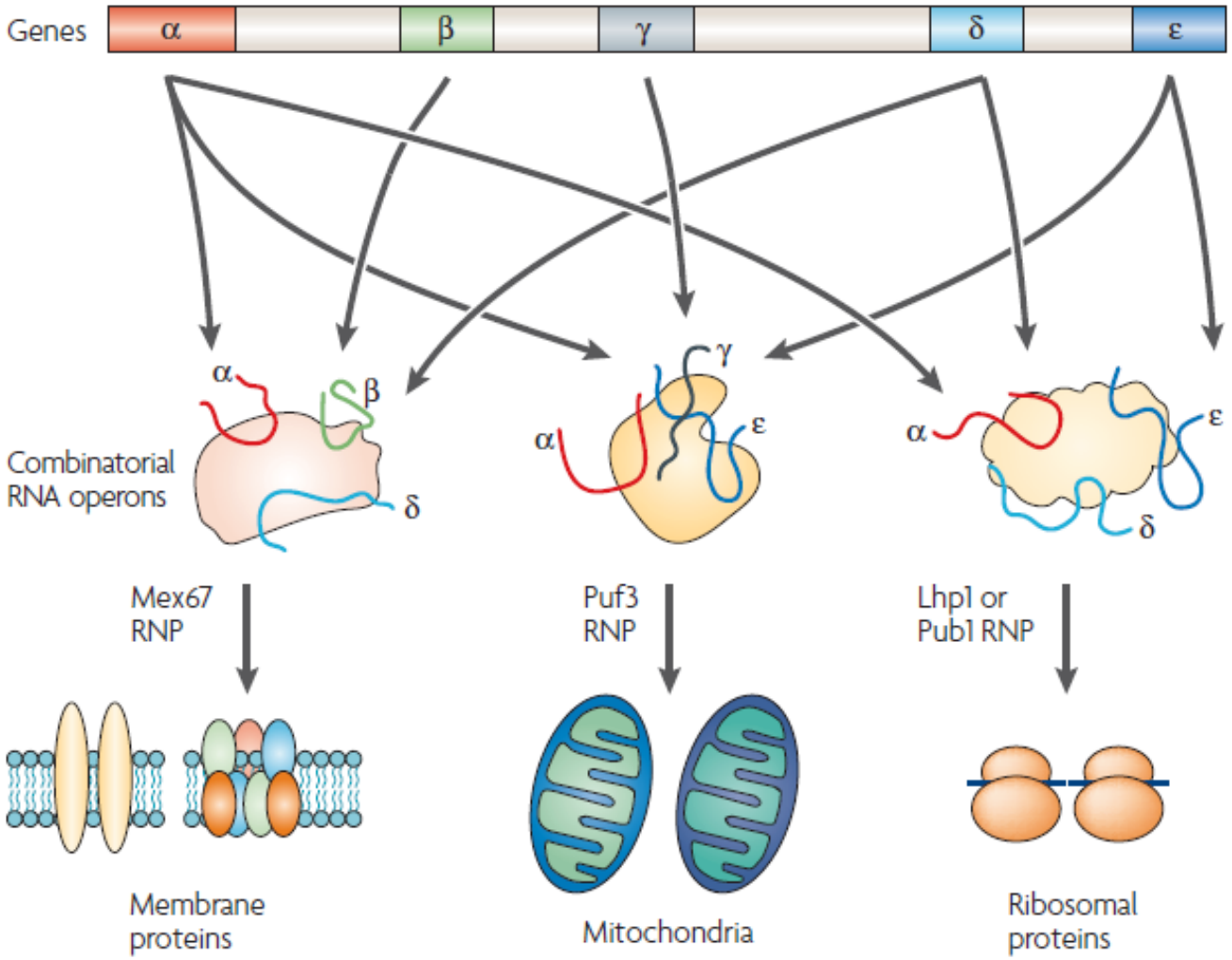
PAR-CLIP

Hentze et al., 2018

The concept of RNA operon

The RBPs profile is highly dynamic

The combinatorial association of many RBPs acting in trans on RNA molecules results in the metabolic regulation of a distinct RNA subpopulations.



Keene, 2007

The molecular features of protein-RNA interactions

"Classical" RNA-Binding Domains

Table 1 | **Common RNA-binding domains and their properties**

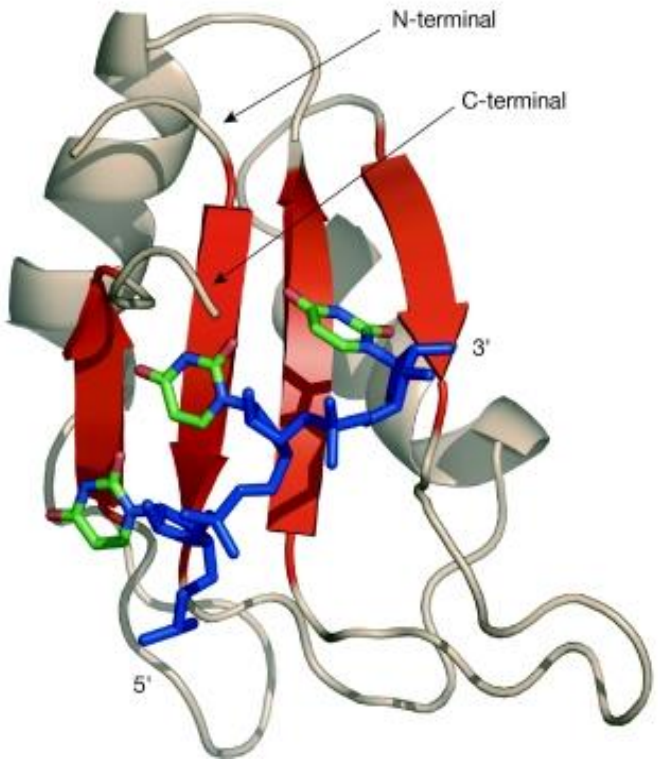
Domain	Topology	RNA-recognition surface	Protein–RNA interactions	Representative structures (PDB ID)
RRM	αβ	Surface of β-sheet	Interacts with about four nucleotides of ssRNA through stacking, electrostatics and hydrogen bonding	U1A N-terminal RRM[14] (1URN)
KH (type I and type II)	αβ	Hydrophobic cleft formed by variable loop between β2, β3 and GXXG loop. Type II: same as type I, except variable loop is between α2 and β2	Recognizes about four nucleotides of ssRNA through hydrophobic interactions between non-aromatic residues and the bases; sugar-phosphate backbone contacts from the GXXG loop, and hydrogen bonding to bases	Nova-1 KH3 (type I)[41] (1EC6), NusA (type II)[42] (2ASB)
dsRBD	αβ	Helix α1, N-terminal portion of helix α2, and loop between β1 and β2	Shape-specific recognition of the minor–major–minor groove pattern of dsRNA through contacts to the sugar-phosphate backbone; specific contacts from the N-terminal α-helix to RNA in some proteins	dsRBD3 from Staufen[51] (1EKZ)
ZnF-CCHH	αβ	Primarily residues in α-helices	Protein side chain contacts to bulged bases in loops and through electrostatic interactions between side chains and the RNA backbone	Fingers 4–6 of TFIIIA[56] (1UN6)
ZnF-CCCH	Little regular secondary structure	Aromatic side chains form hydrophobic binding pockets for bases that make direct hydrogen bonds to protein backbone	Stacking interactions between aromatic residues and bases create a kink in RNA that allows for the direct recognition of Watson–Crick edges of the bases by the protein backbone	Fingers 1 and 2 of TIS11d[57] (1RGO)
S1	β	Core formed by two β-strands with contributions from surrounding loops	Stacking interactions between bases and aromatic residues and hydrogen bonding to the bases	Ribonuclease II[121] (2IX1), exosome[99] (2NN6)
PAZ	αβ	Hydrophobic pocket formed by OB-like β-barrel and small αβ motif	Recognizes single-stranded 3′ overhangs of siRNA through stacking interactions and hydrogen bonds	PAZ[73] (1SI3), Argonaute[75] (1U04), Dicer[72] (2FFL)
PIWI	αβ	Highly conserved pocket, including a metal ion that is bound to the exposed C-terminal carboxylate	Recognizes the defining 5′ phosphate group in the siRNA guide strand with a highly conserved binding pocket that includes a metal ion	PIWI[75] (1YTU), Argonaute (1U04)[75]
TRAP	β	Edges of β-sheets between each of the 11 subunits that form the entire protein structure	Recognizes the GAG triplet through stacking interactions and hydrogen bonding to bases; limited contacts to the backbone	TRAP[122] (1C9S)
Pumilio	α	Two repeats combine to form binding pocket for individual bases; helix α2 provides specificity-determining residues	Binding pockets for bases provided by stacking interactions; specificity dictated by hydrogen bonds to the Watson–Crick face of a base by two amino acids in helix α2	Pumilio[84] (1M8Y)
SAM	α	Hydrophobic cavity between three helices surrounded by an electropositive region	Shape-dependent recognition of RNA stem–loop, mainly through interactions with the sugar-phosphate backbone and a single base in the loop	Vts1[123] (2ESE)

dsRBD, double-stranded RNA-binding domain; KH, K-homology; OB-like, oligonucleotide/oligosaccharide binding-like; PDB ID, Protein Data Bank identification; RRM, RNA-recognition motif; siRNA, small interfering RNA; ssRNA, single-stranded RNA; ZnF, zinc finger.

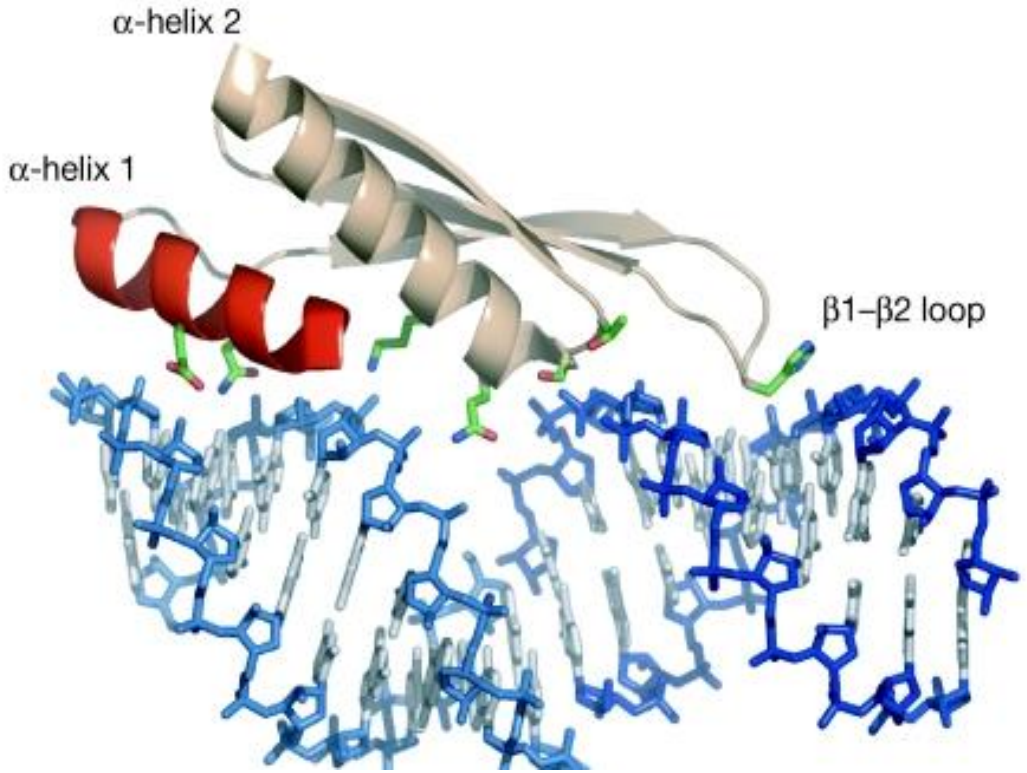
Lunde et al., 2007

"Classical" RNA-Binding Domains

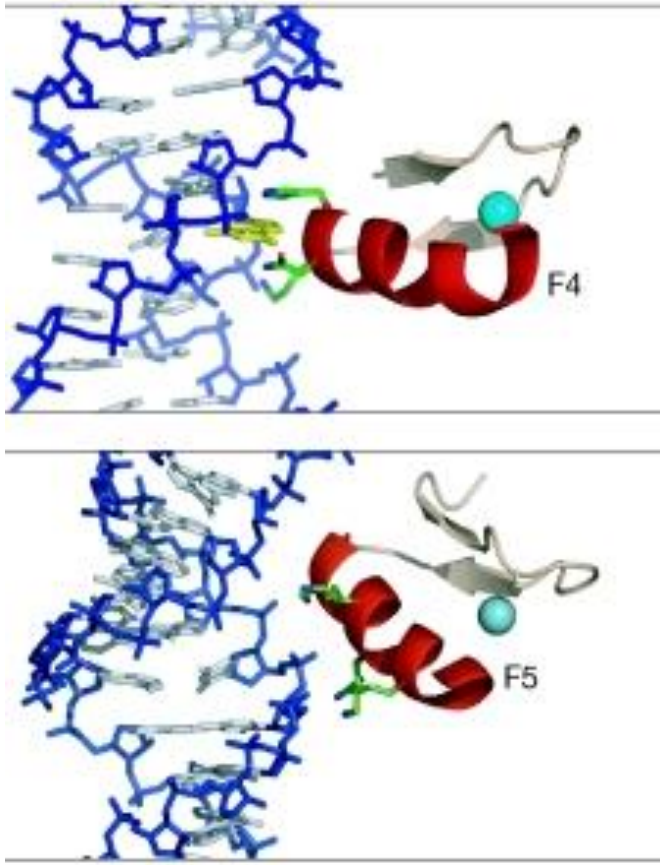
RNA-recognition motif (RRM)



double-stranded RNA-binding motif (dsRBM)



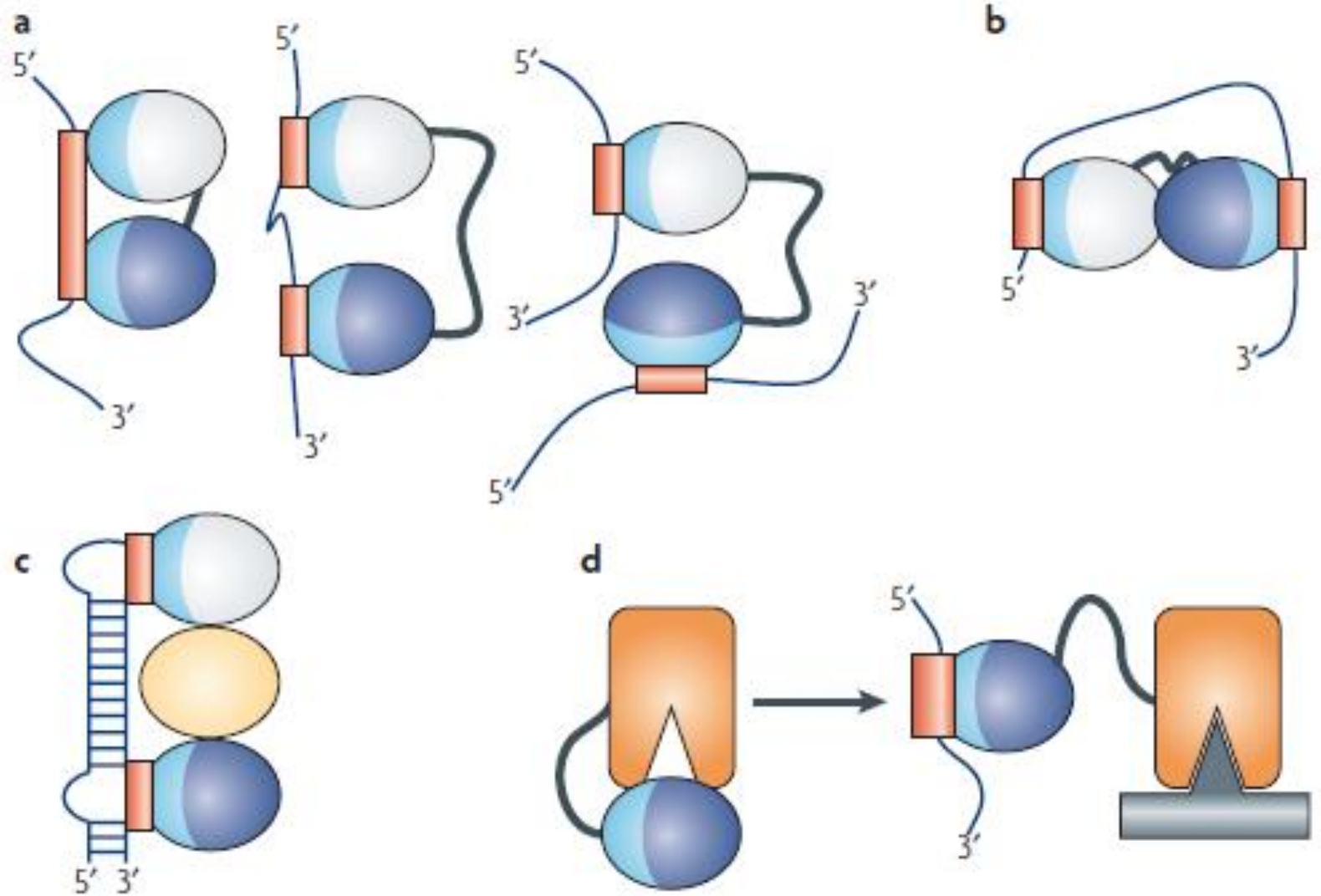
Zinc-finger motif



Stefl et al., 2005

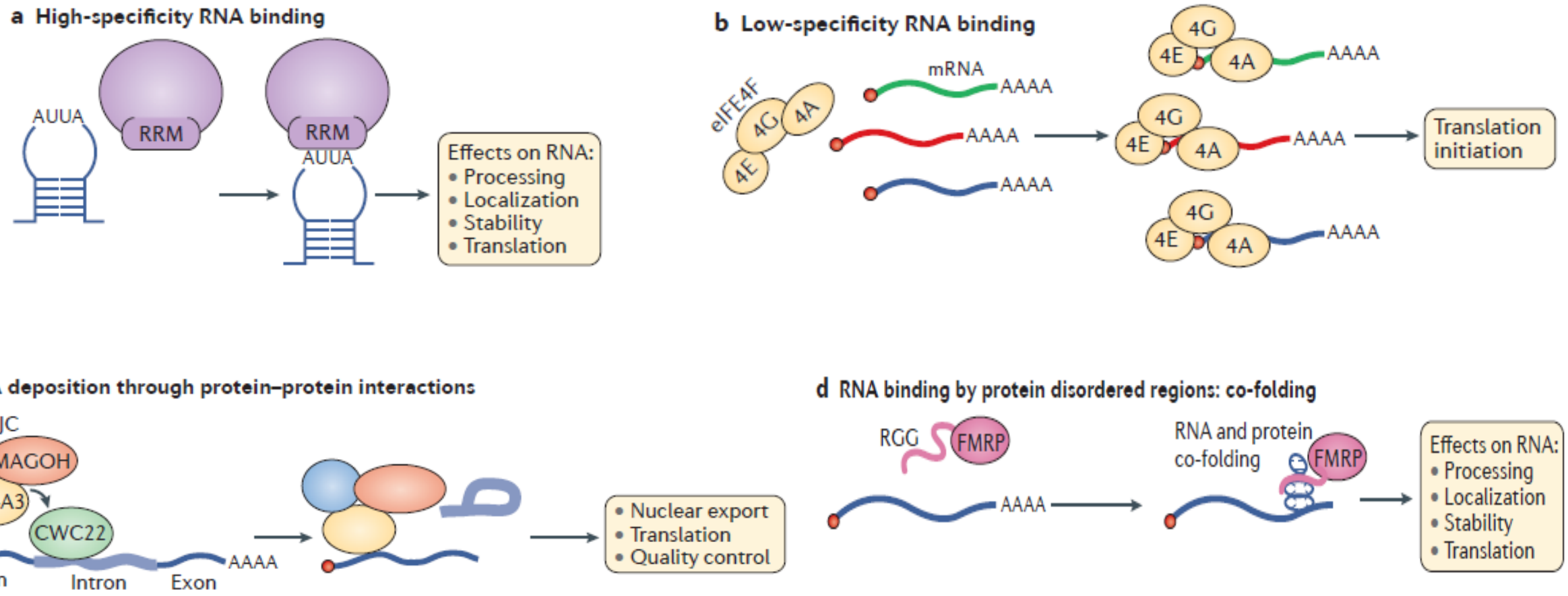
Modularity of RBPs

RBPs are usually composed by several repeated domains



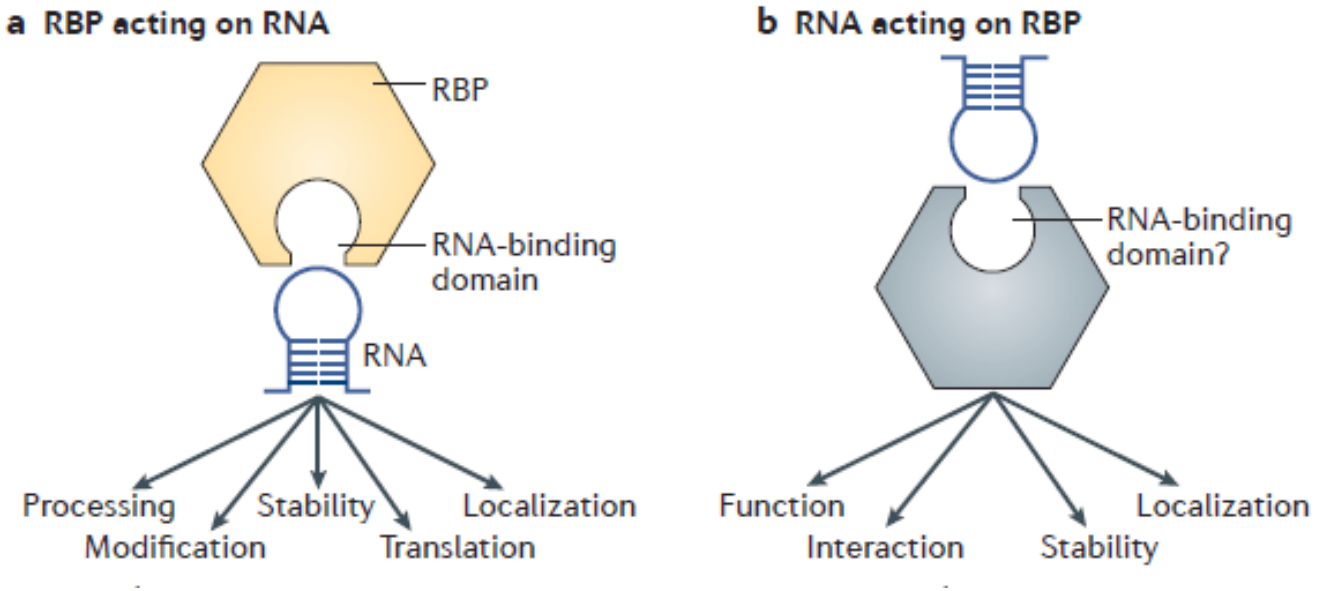
Lunde et al., 2007

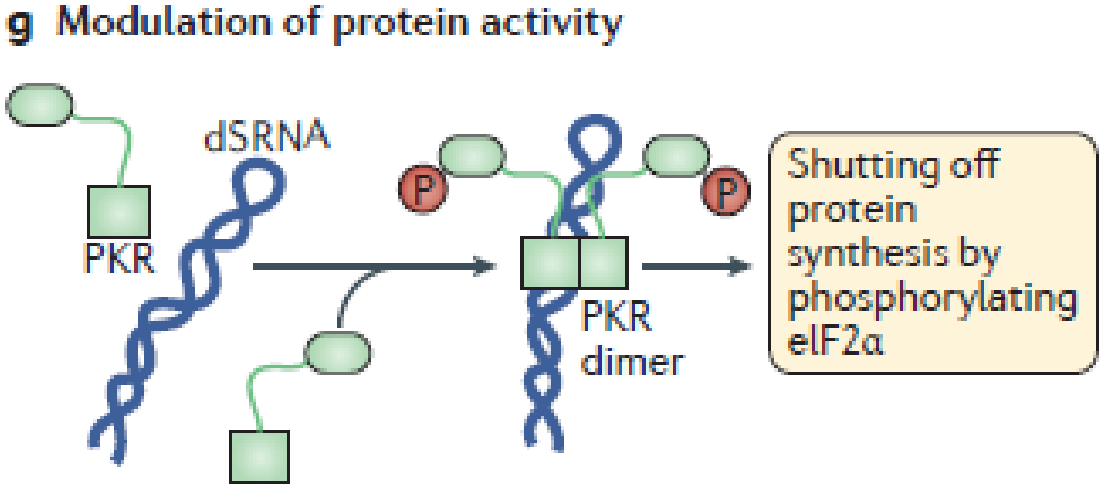
Expanding the concept of the RNA Binding



Hentze et al., 2018

Novel types of RNA binding

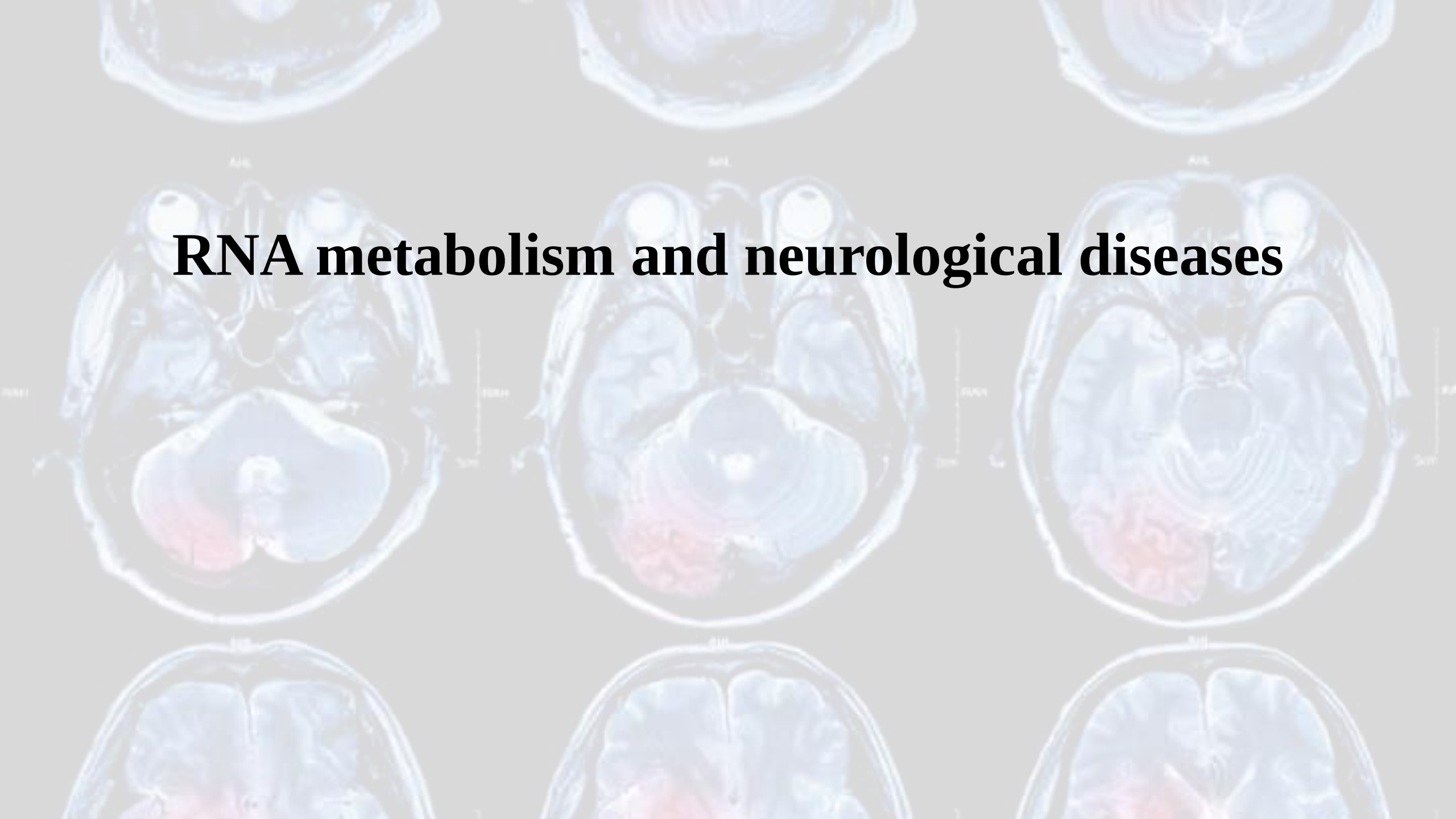




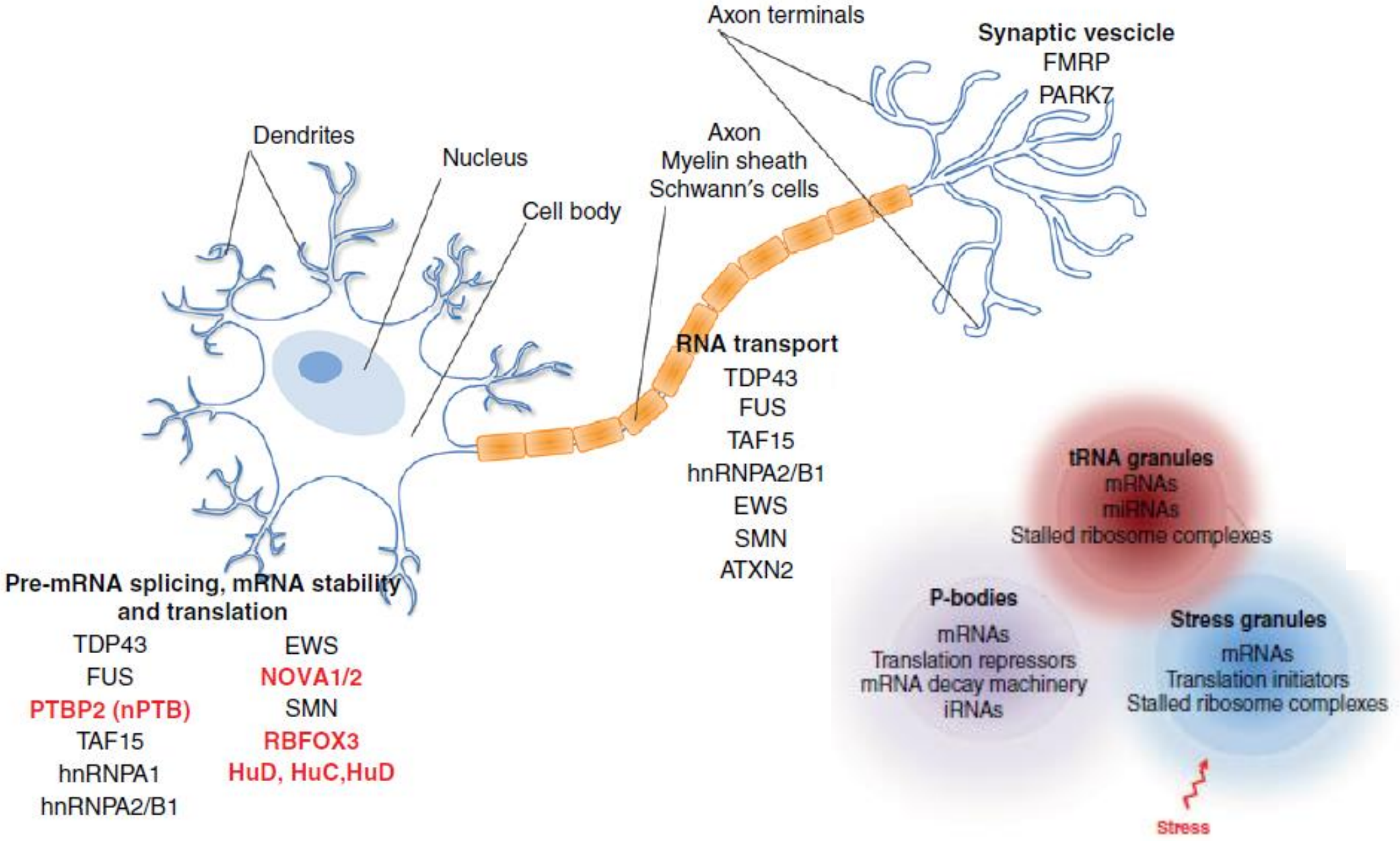
Hentze et al., 2018

Lunde et al., 2007

RNA metabolism and neurological diseases

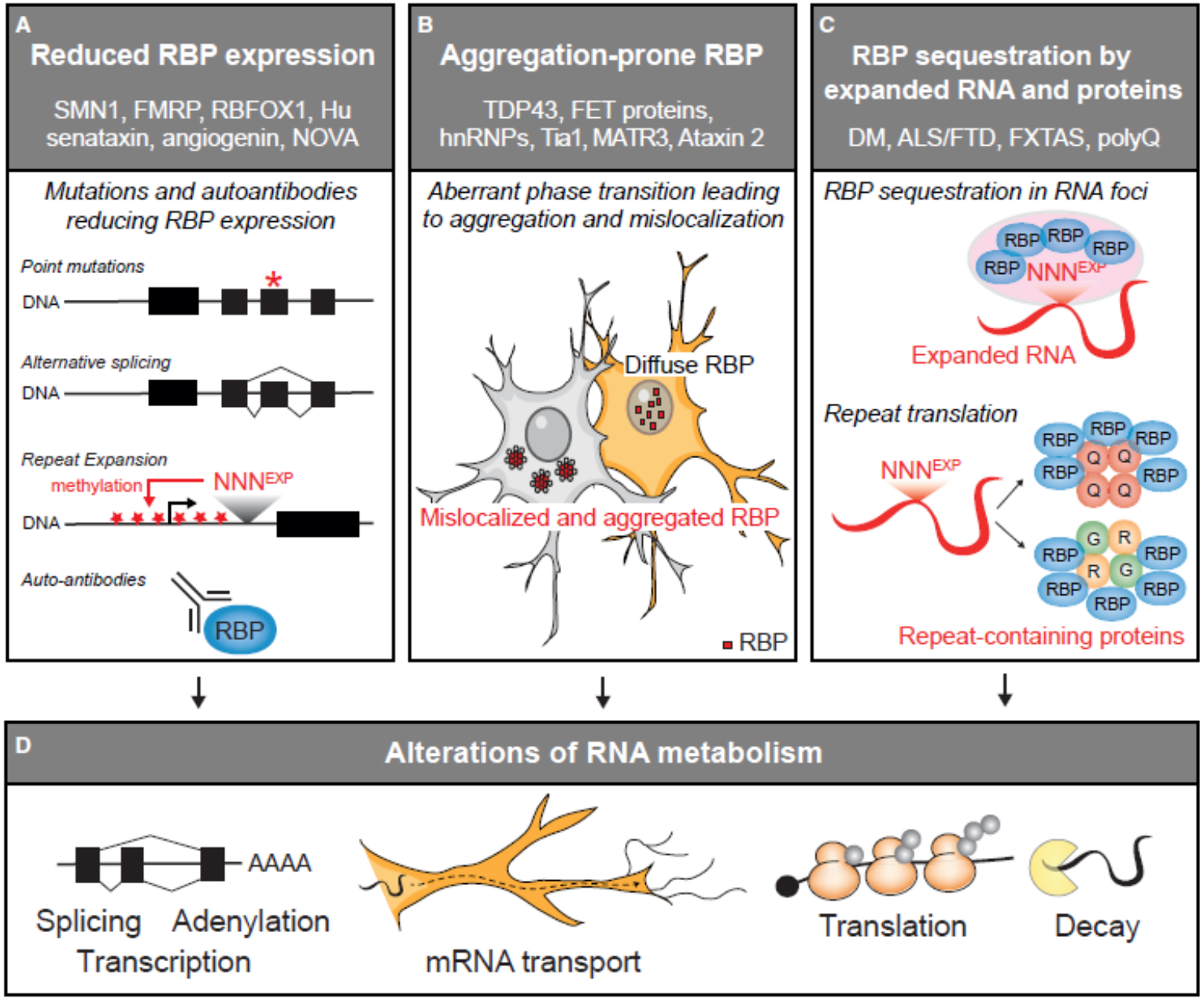


RNA binding proteins preserves neuronal integrity



De Conti et al., 2017

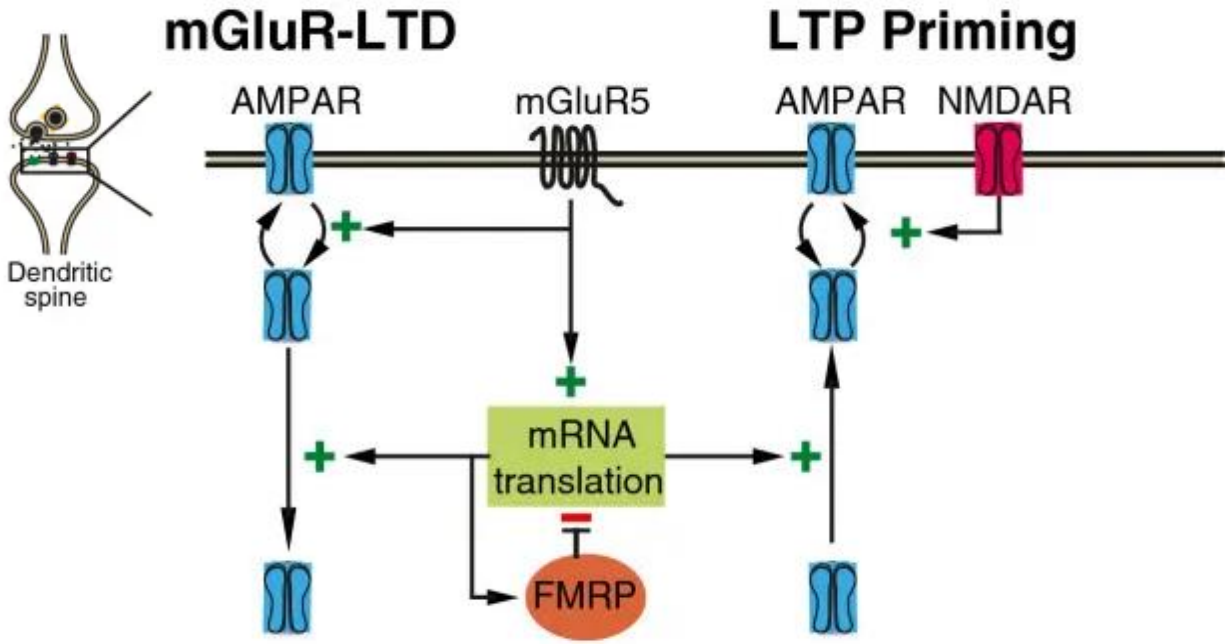
Alterations of RBPs in neurological disorders

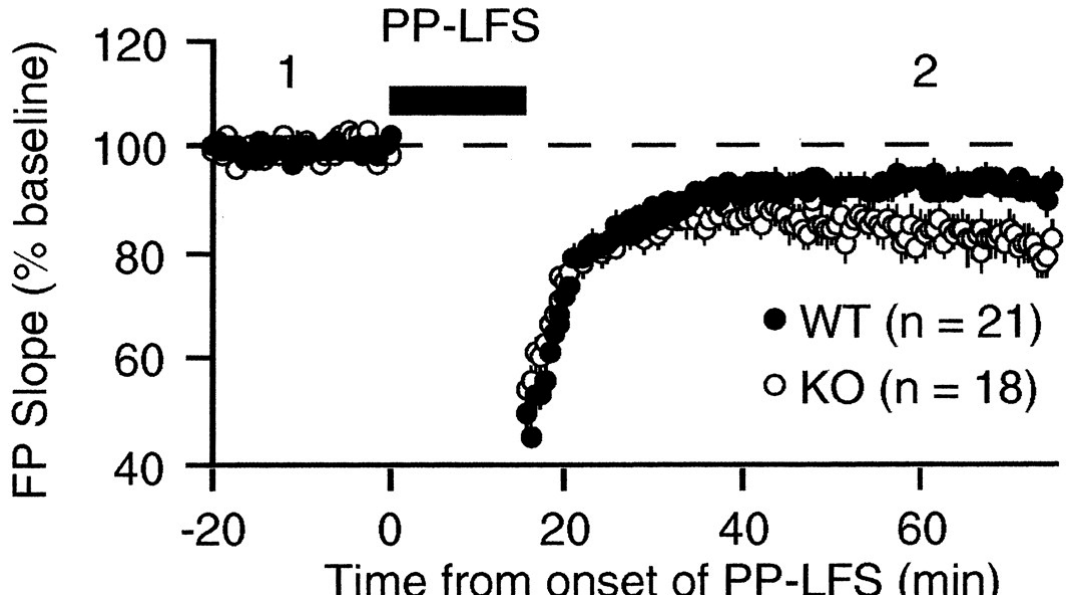


Nussbacher et al., 2019

Microsatellite expansion in FXS: reduced FMR1 transcription

The reduction of FMRP levels induces an overexpressed LTD activity



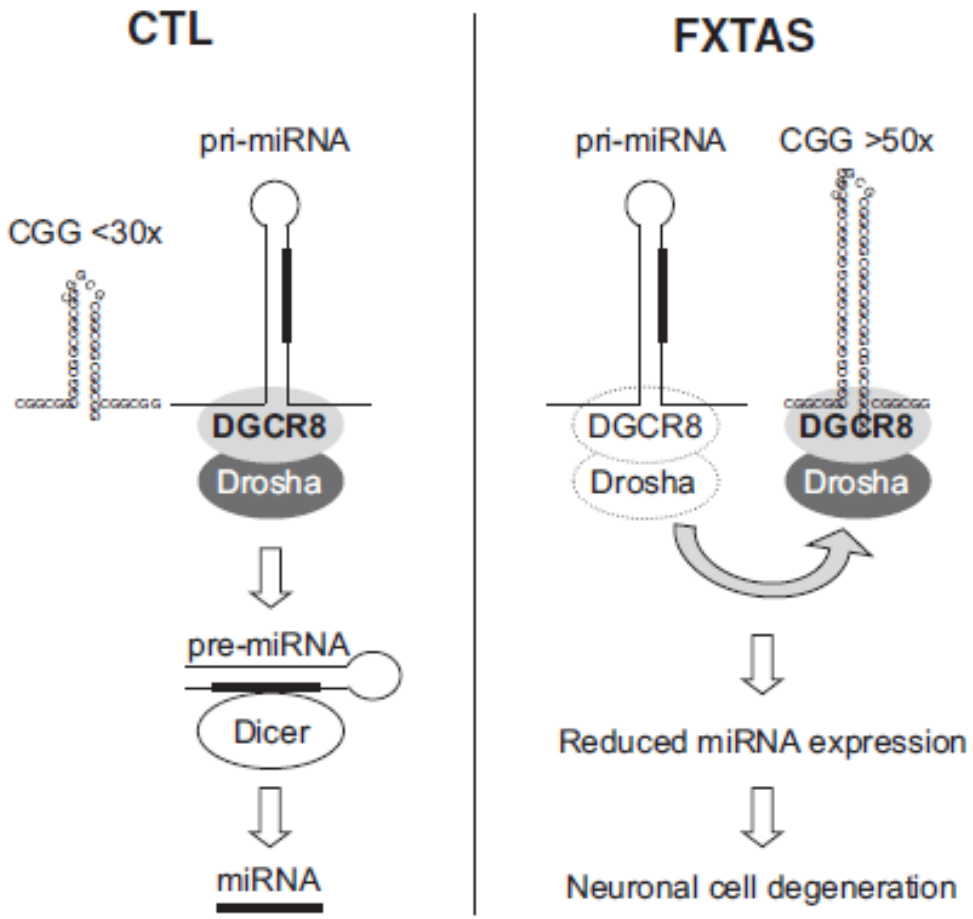


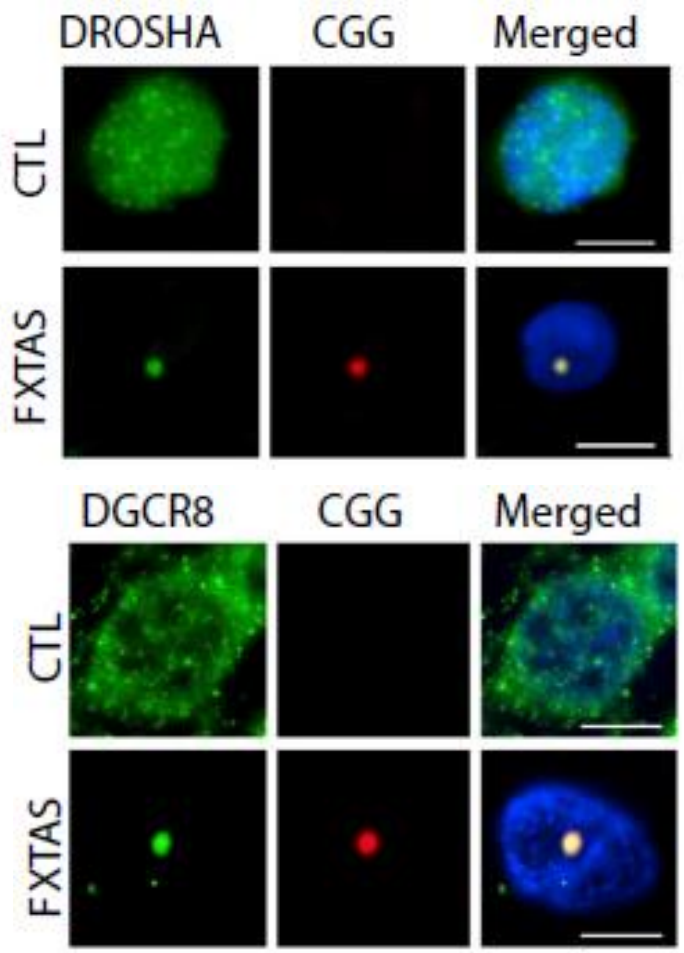
Bassell & Warren, 2008

Huber et al., 2002

Microsatellite expansion in FXTAS: FMR1 RNA-meidated toxicity

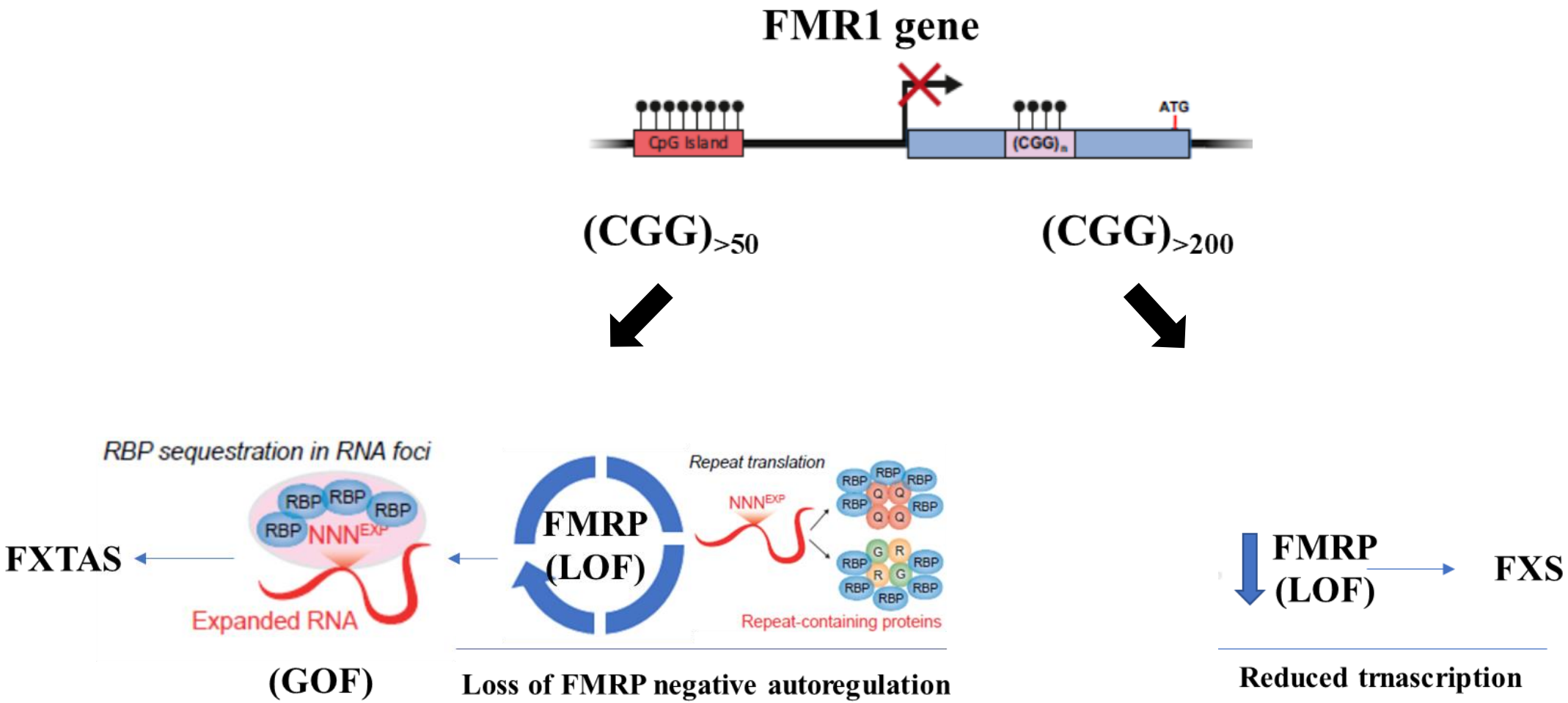
FMR1 expanded RNA impairs the miRNA processing





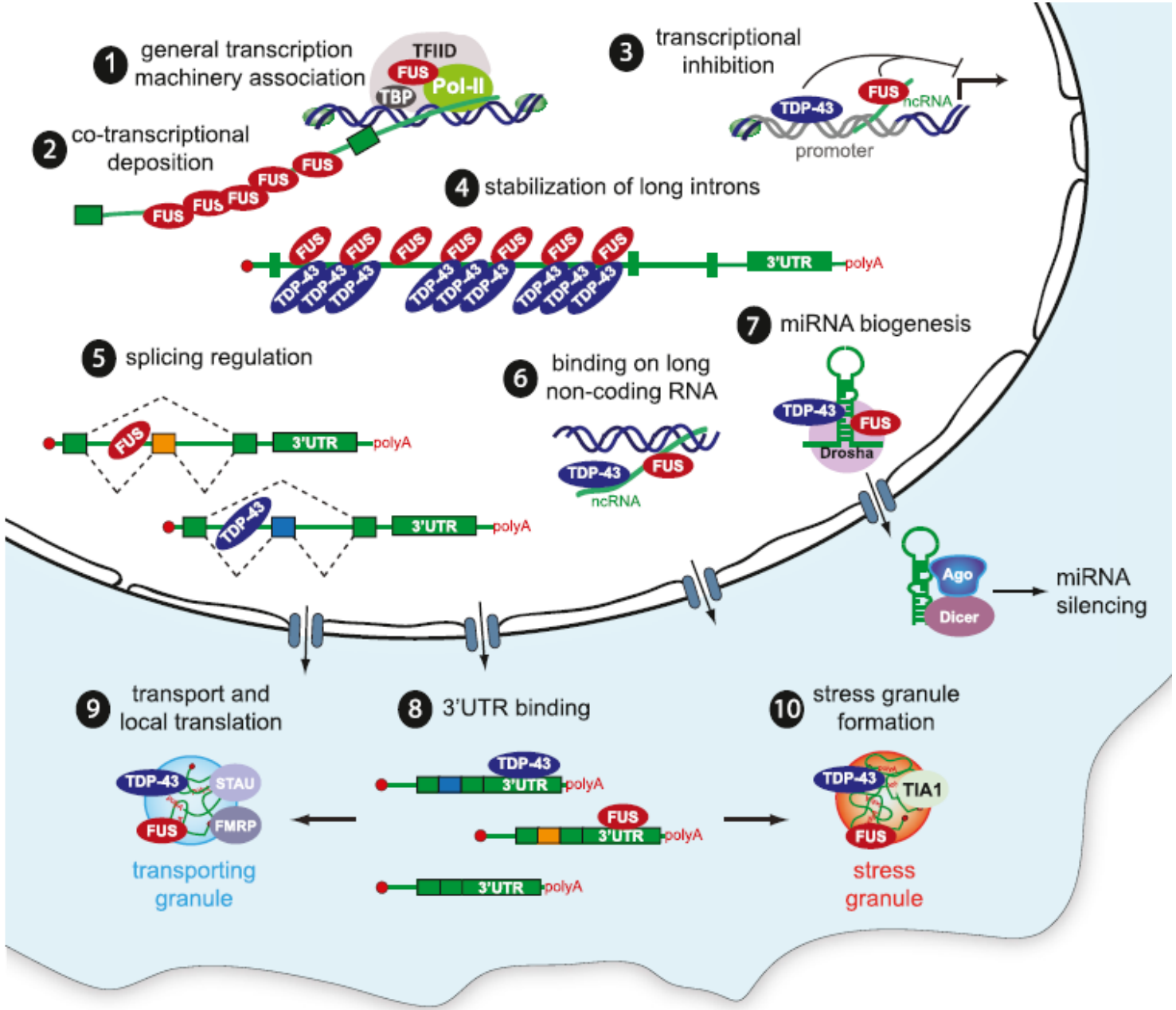
Sellier et al., 2013

Loss-of-function VS Gain-of-function



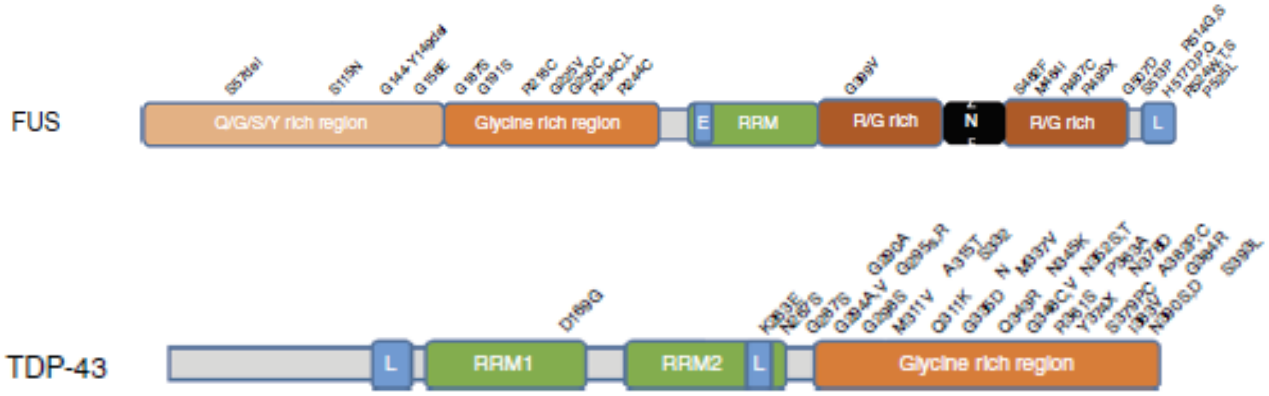
Park et al., 2015

TDP-43 and FUS/TLS

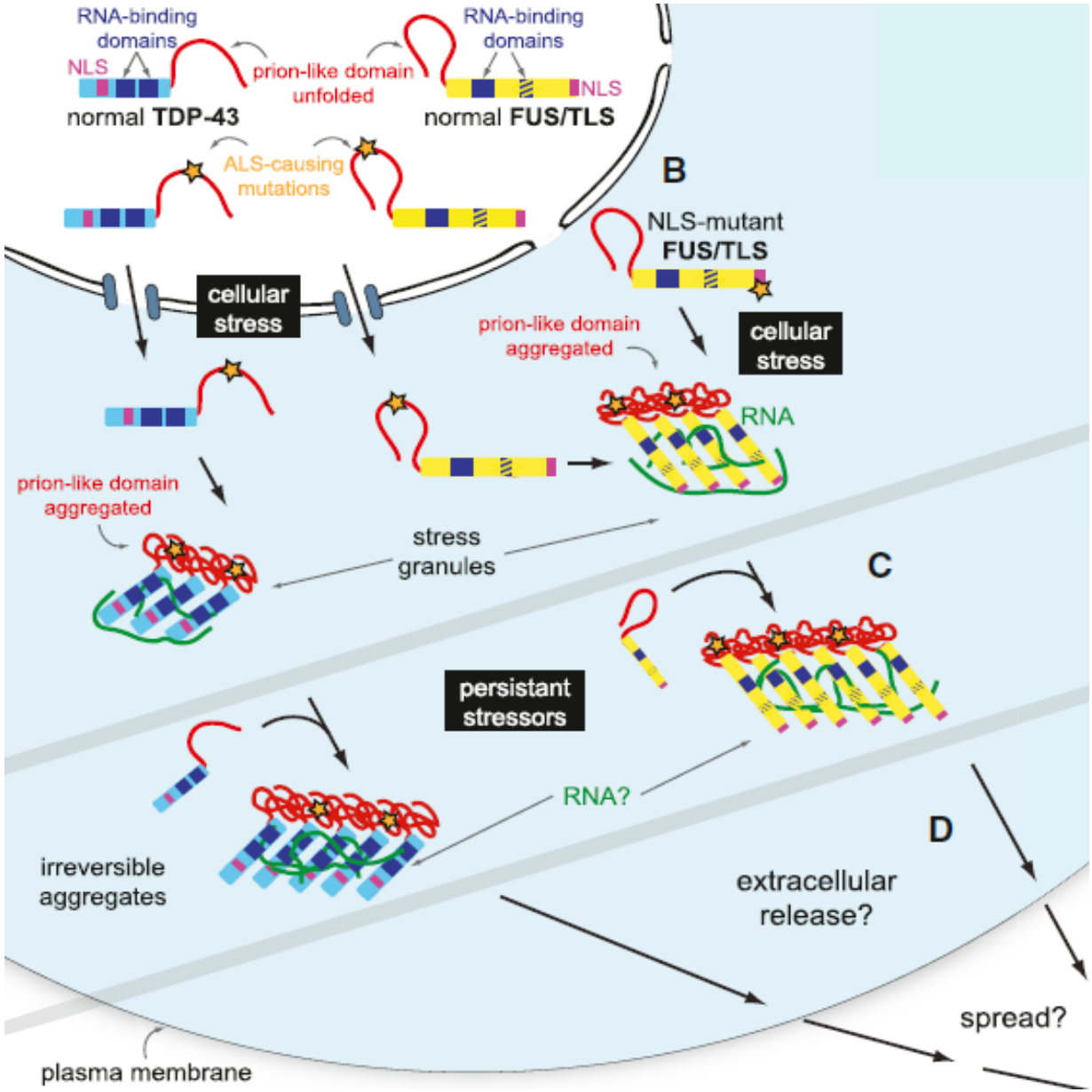


Ling et al., 2013

TDP-43 and FUS/TLS in ALS and FTD



Cookson, 2017



Ling et al., 2013

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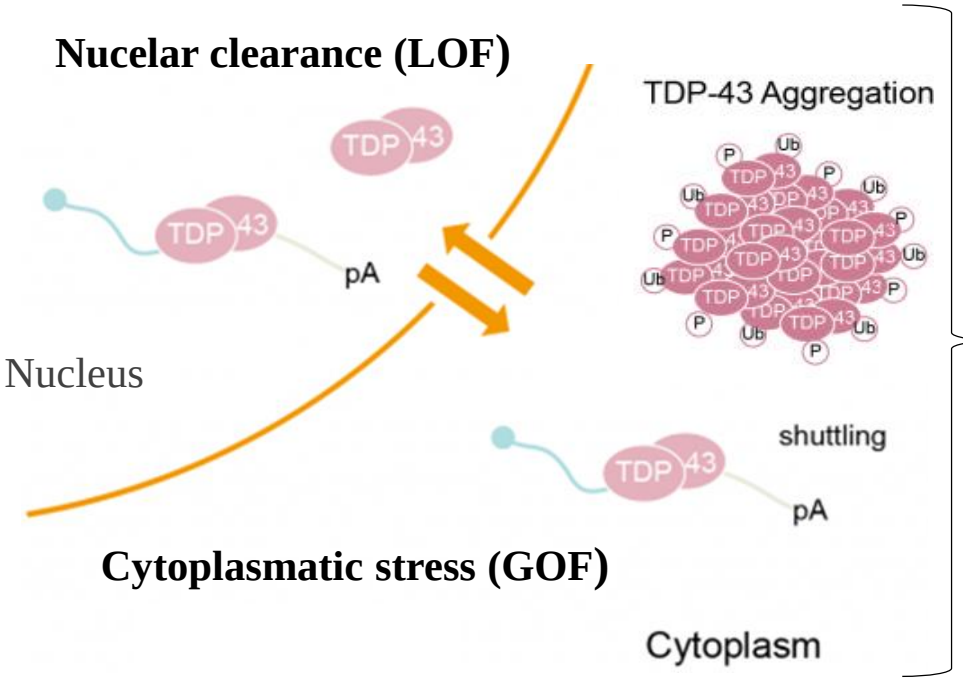

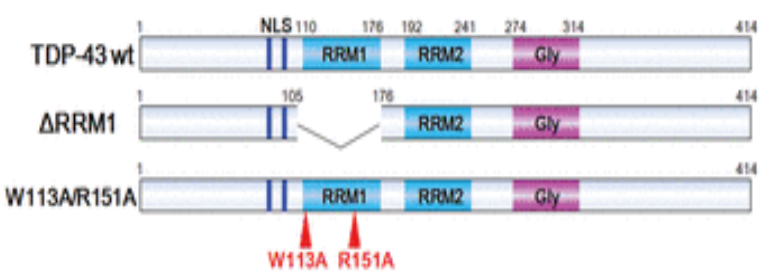

TDP-43

Loss of TDP-43 negative autoregulation

Nuclear TDP-43 (LOF)

Abolishing RNA binding ability mitigates mutant TDP-43 toxicity

Prevent mutant TDP-43 toxic activity on RNAs (GOF)

Ihara et al., 2013

There is much to learn about the biology of RBPs

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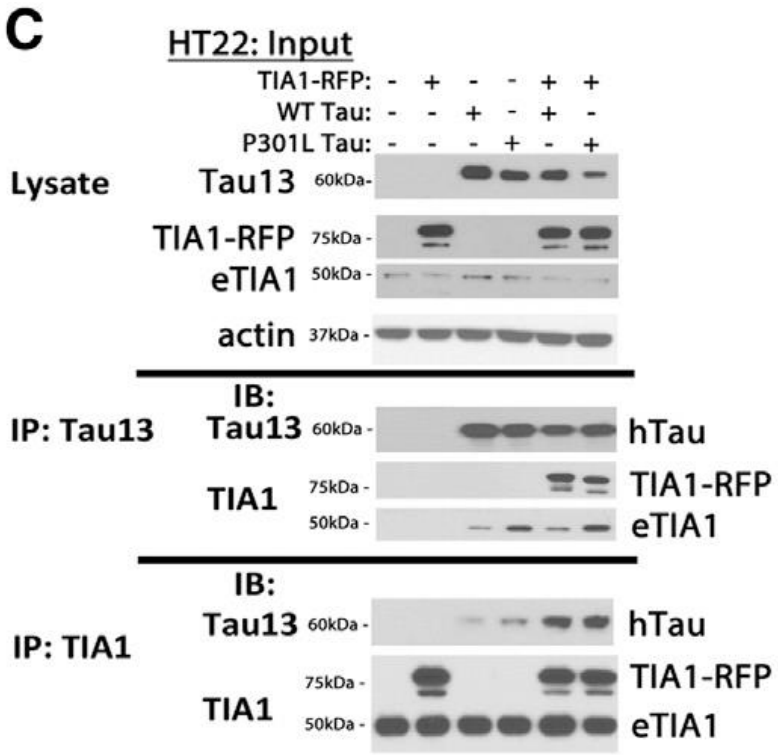
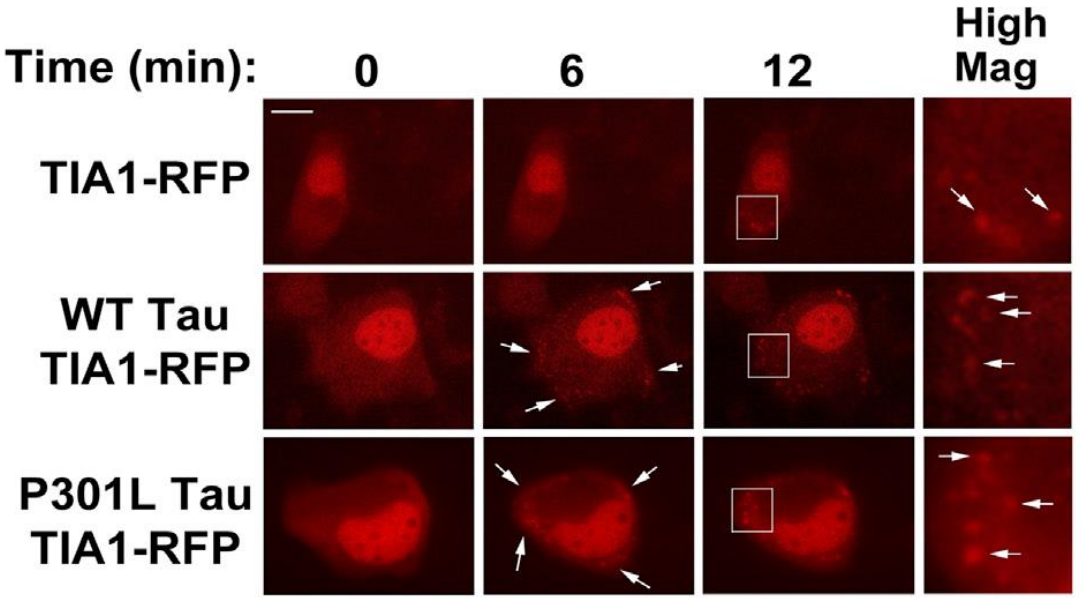
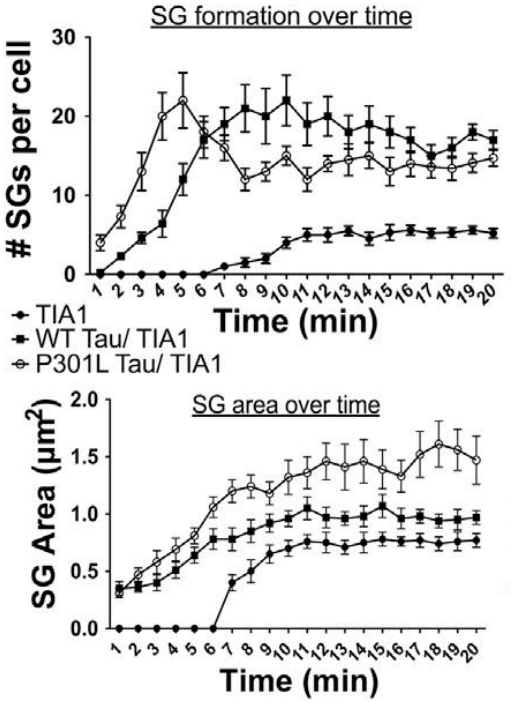
- RBPs are master regulators of neuronal physiology and are consequently also involved in neuronal alterations.
- The number and the complexity of the pathways in which RBPs are involved do not allow for an easy dissection of specific activities, mechanisms of toxicity can be described partially.
- New findings are pointing towards functional interactions between RBPs and other proteins implicated in neurodegenerative diseases.

RBPs are involved also in AD

Tau promotes TIA1 stress granules formation upon a stress stimulus

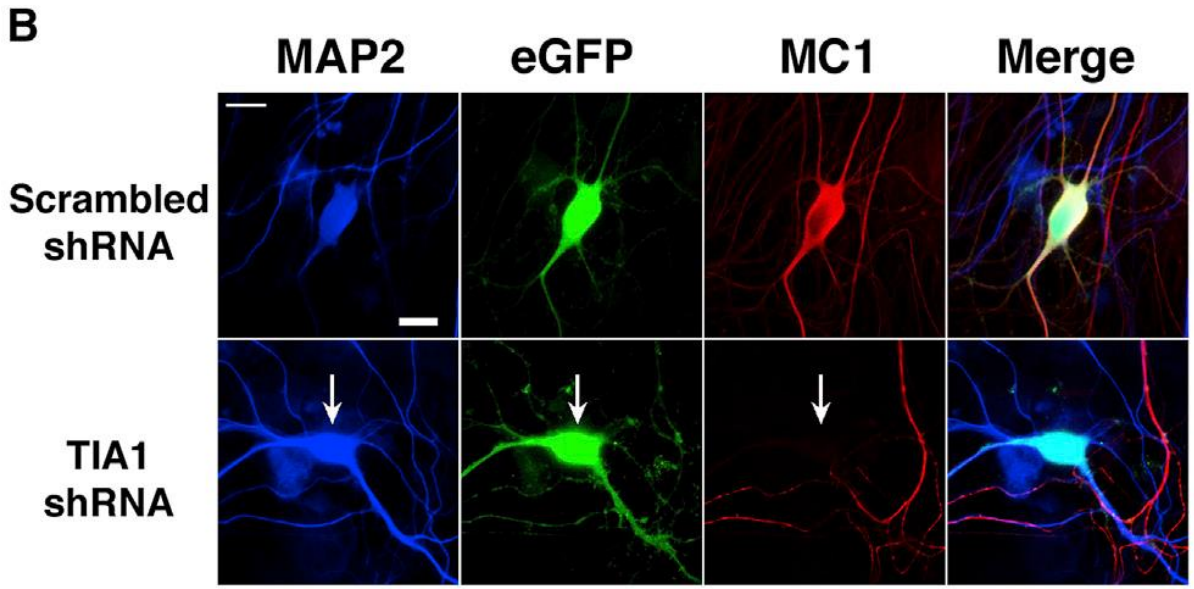






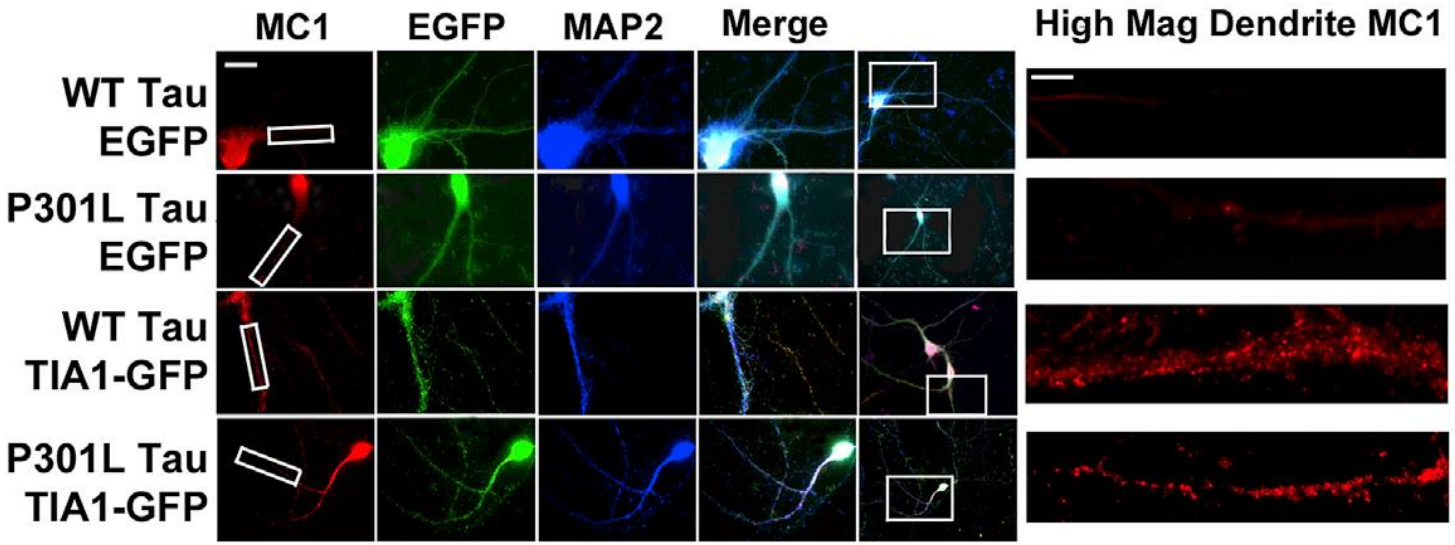
Vanderweyde et al., 2016

RBPs are involved also in AD



TIA1 KD reduces Tau protein misfolding

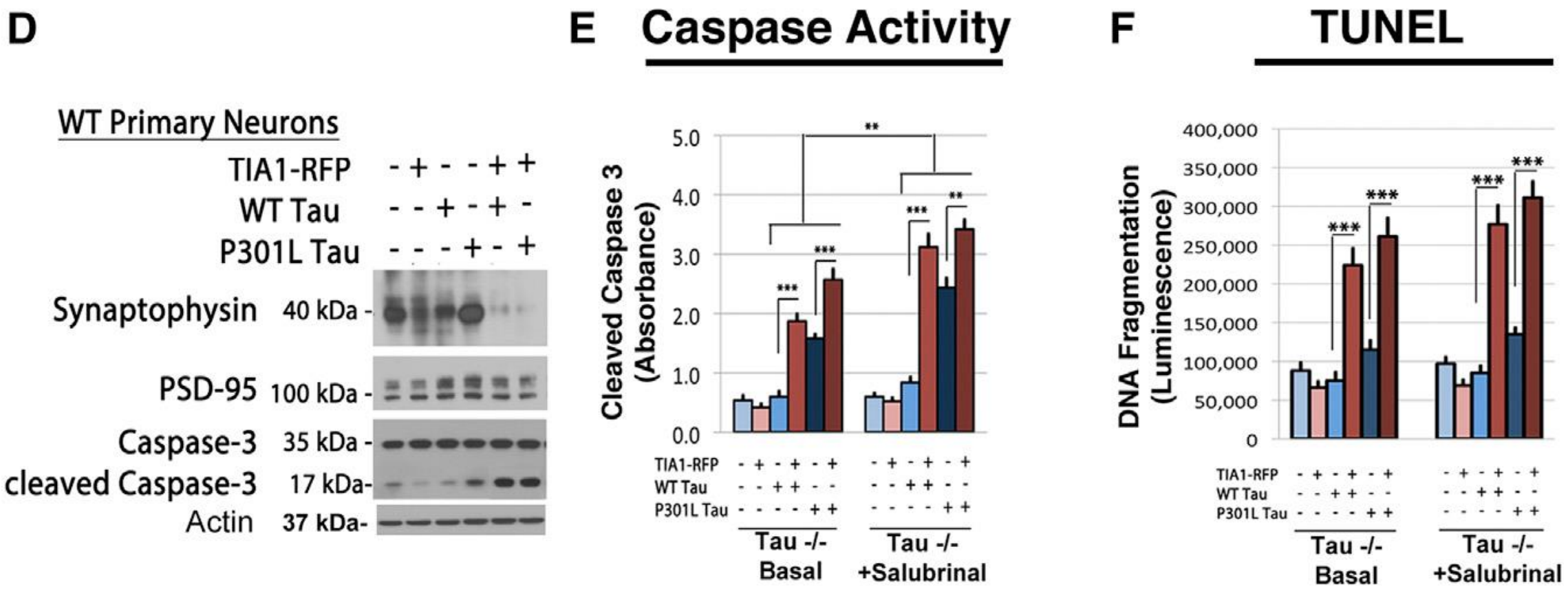
TIA1 overexpression induces Tau aggregation



Vanderweyde et al., 2016

RBPs are involved also in AD

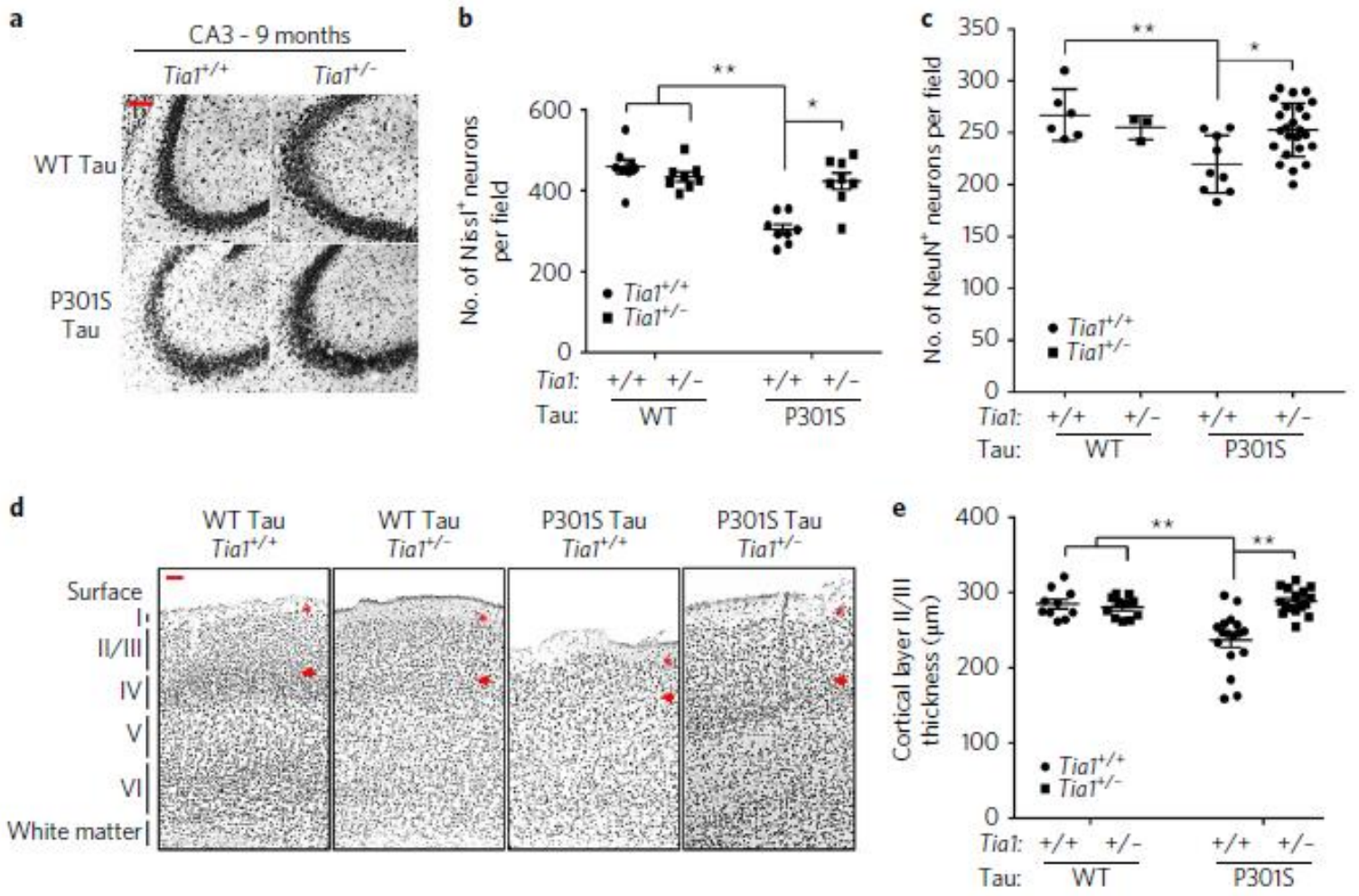
TIA1 overexpression promotes Tau-dependent neurotoxicity

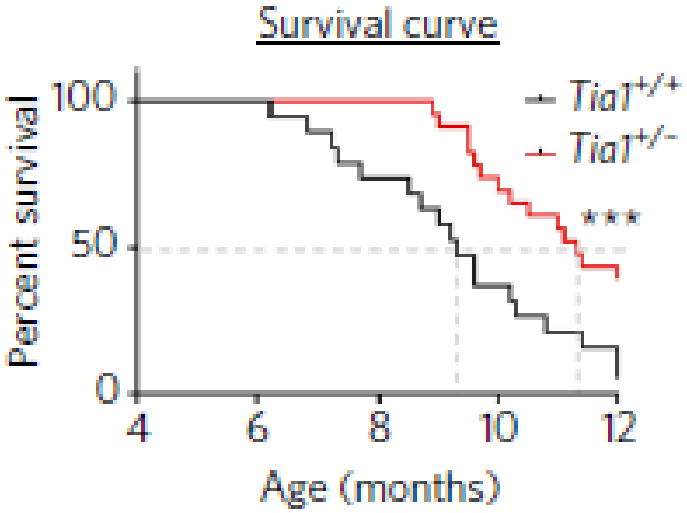


Vanderweyde et al., 2016

RBPs are involved also in AD

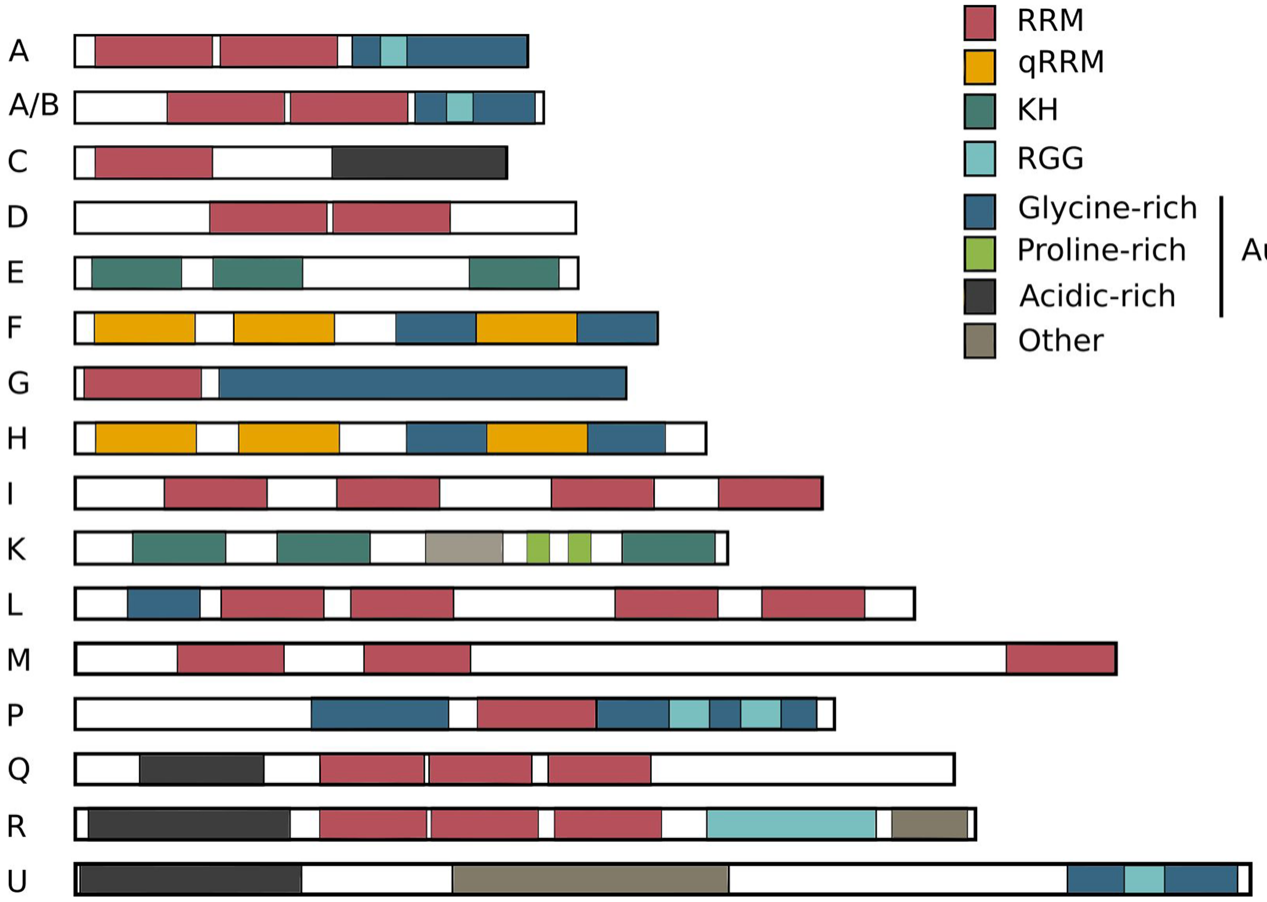
Reducing TIA1 in vivo protects against neurodegeneration and prolongs survival in transgenic P301S Tau mice



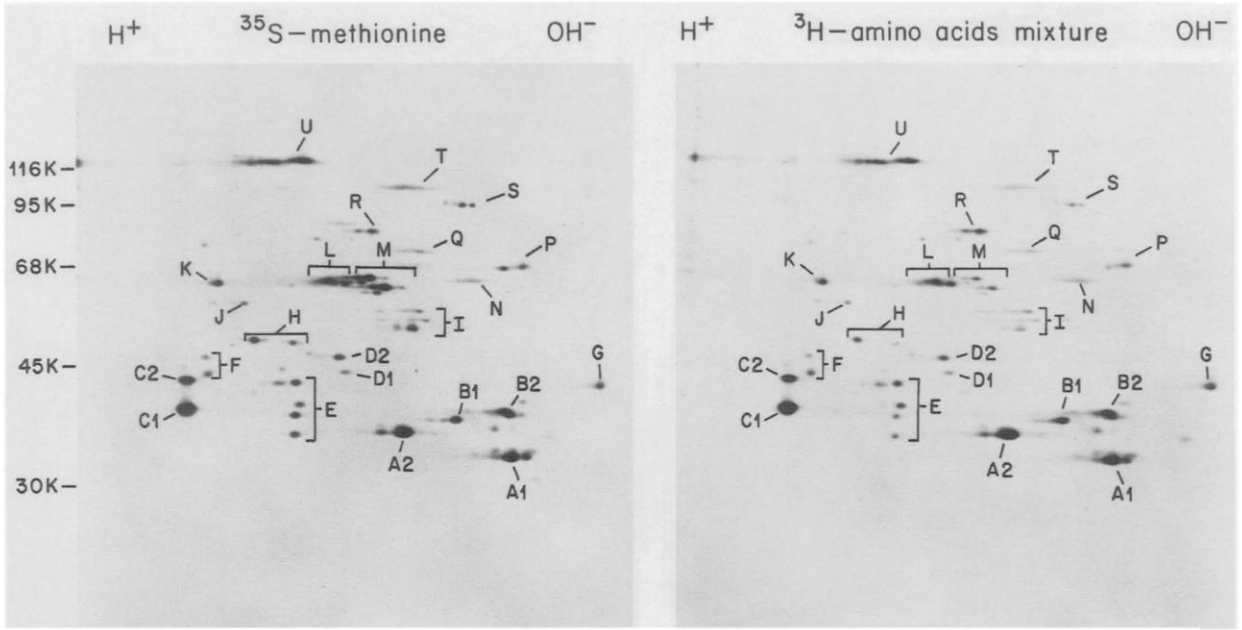


Apicco et al., 2018

hnRNP: a subset of RBPs

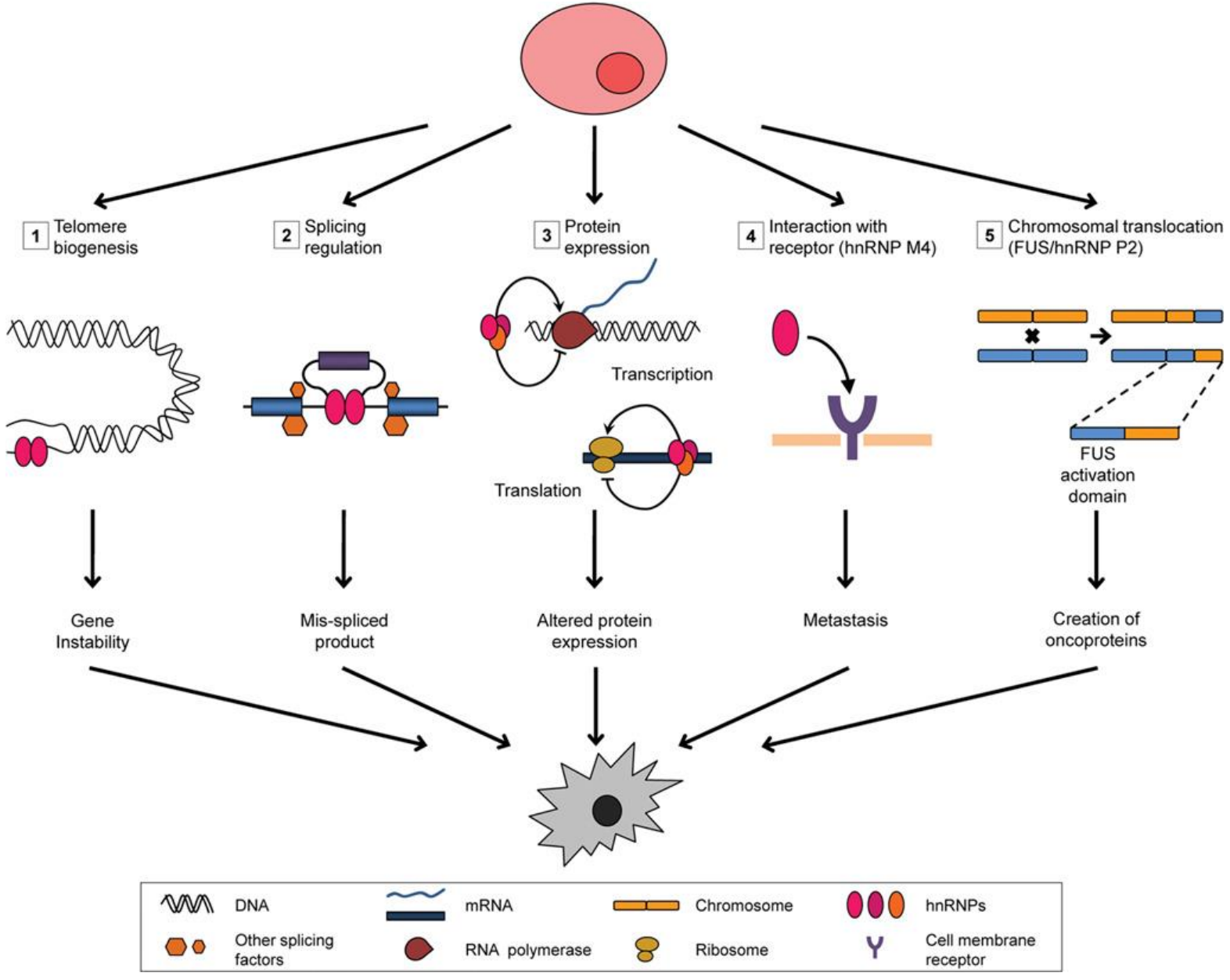


Geuens et al., 2016



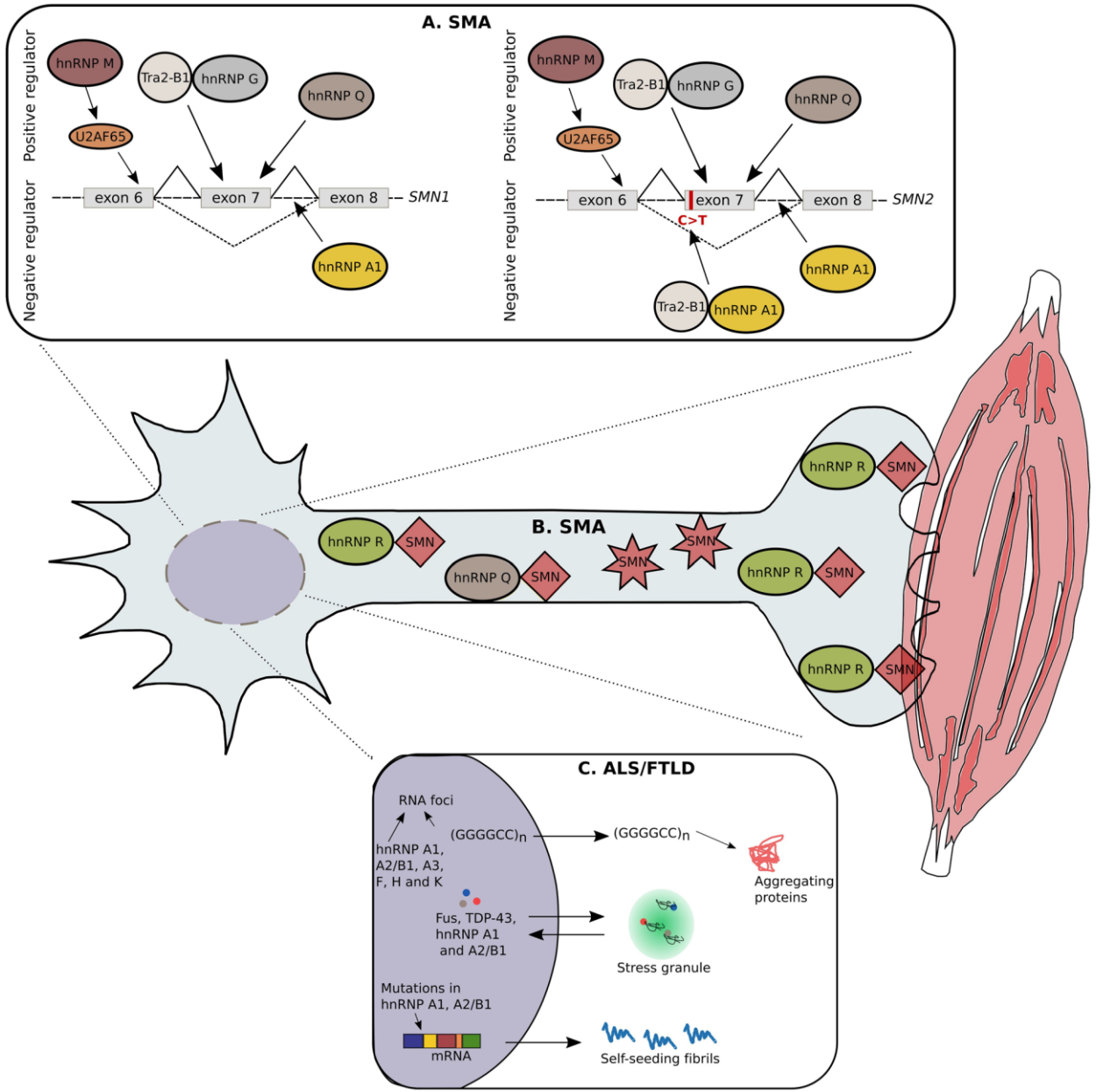
Piñol-Roma et al., 1988

hnRNPs in cancer…



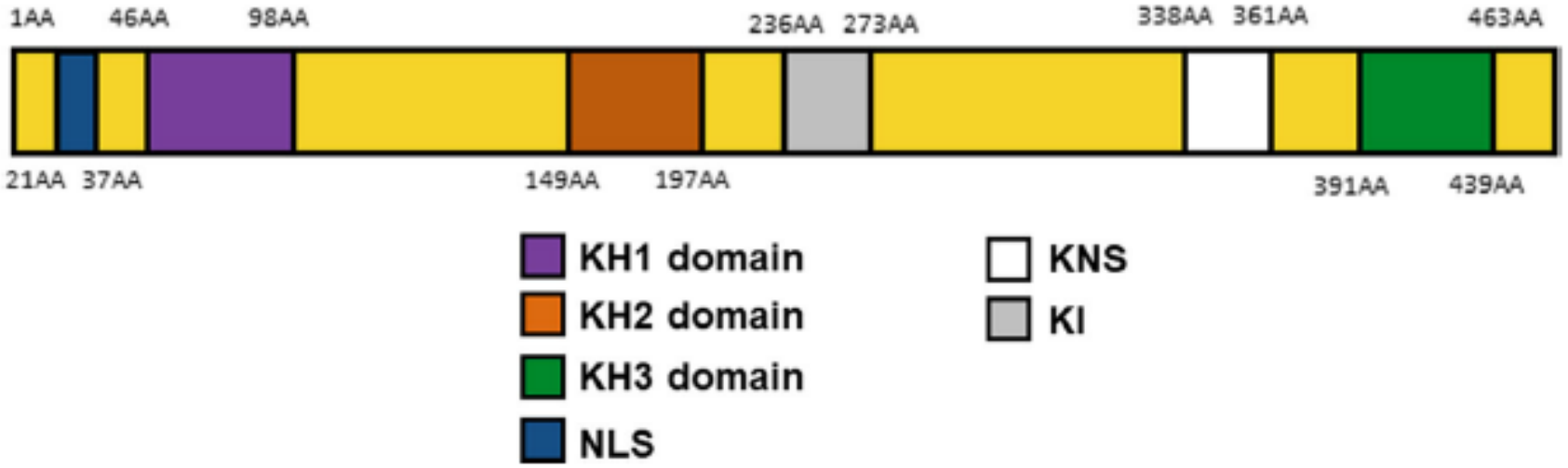
Han et al., 2010

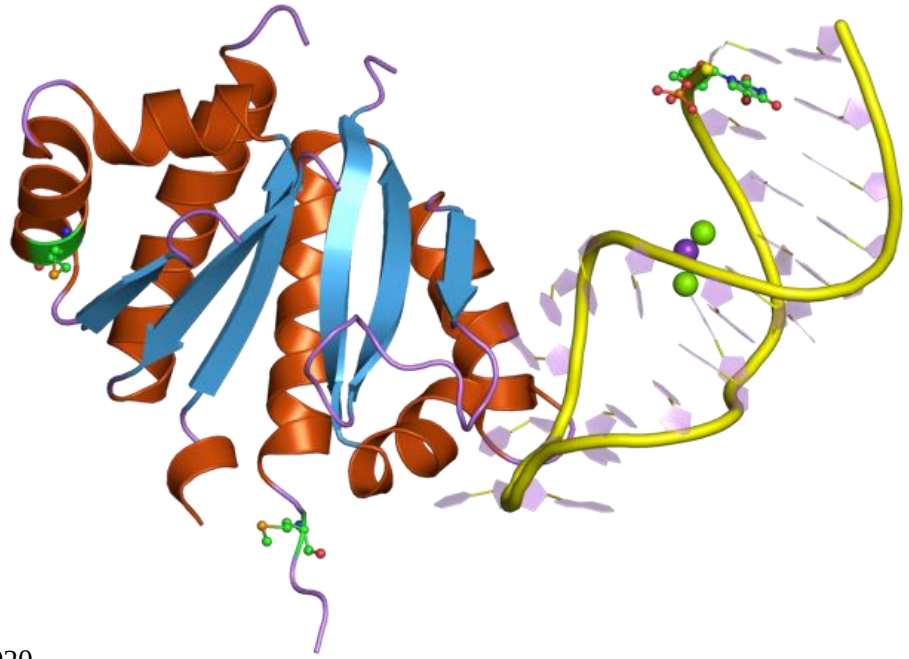
…and neurodegeneration

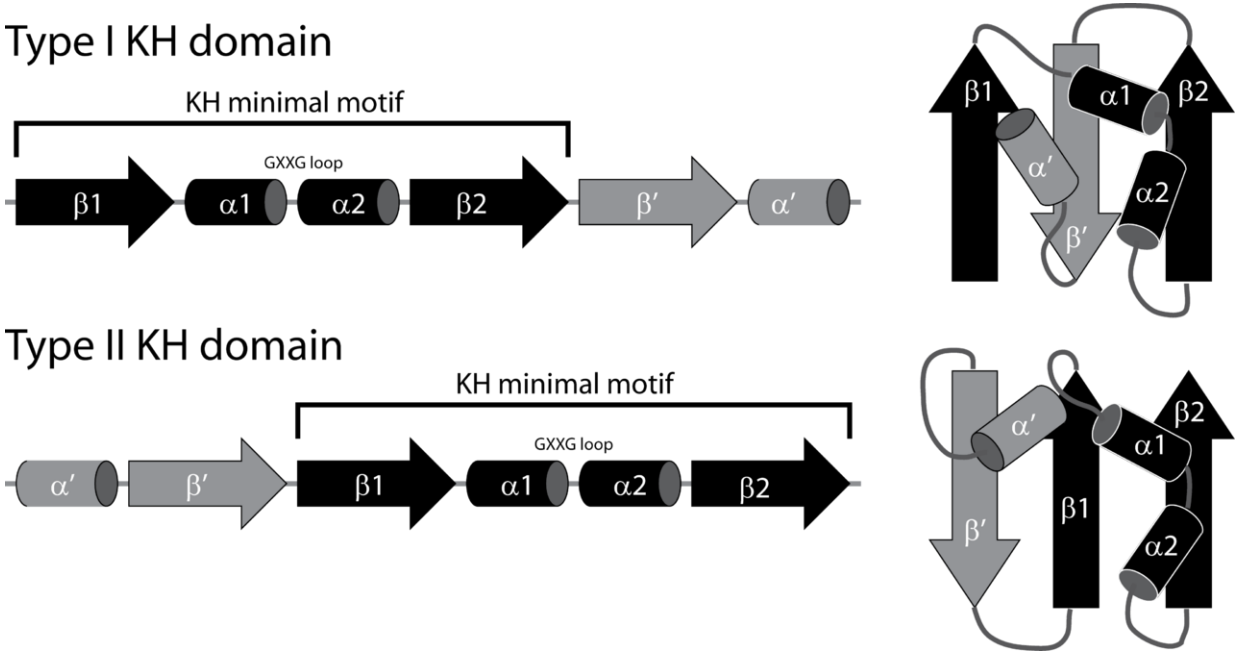


Geuens et al., 2016

hnRNP K has a peculiar structural composition

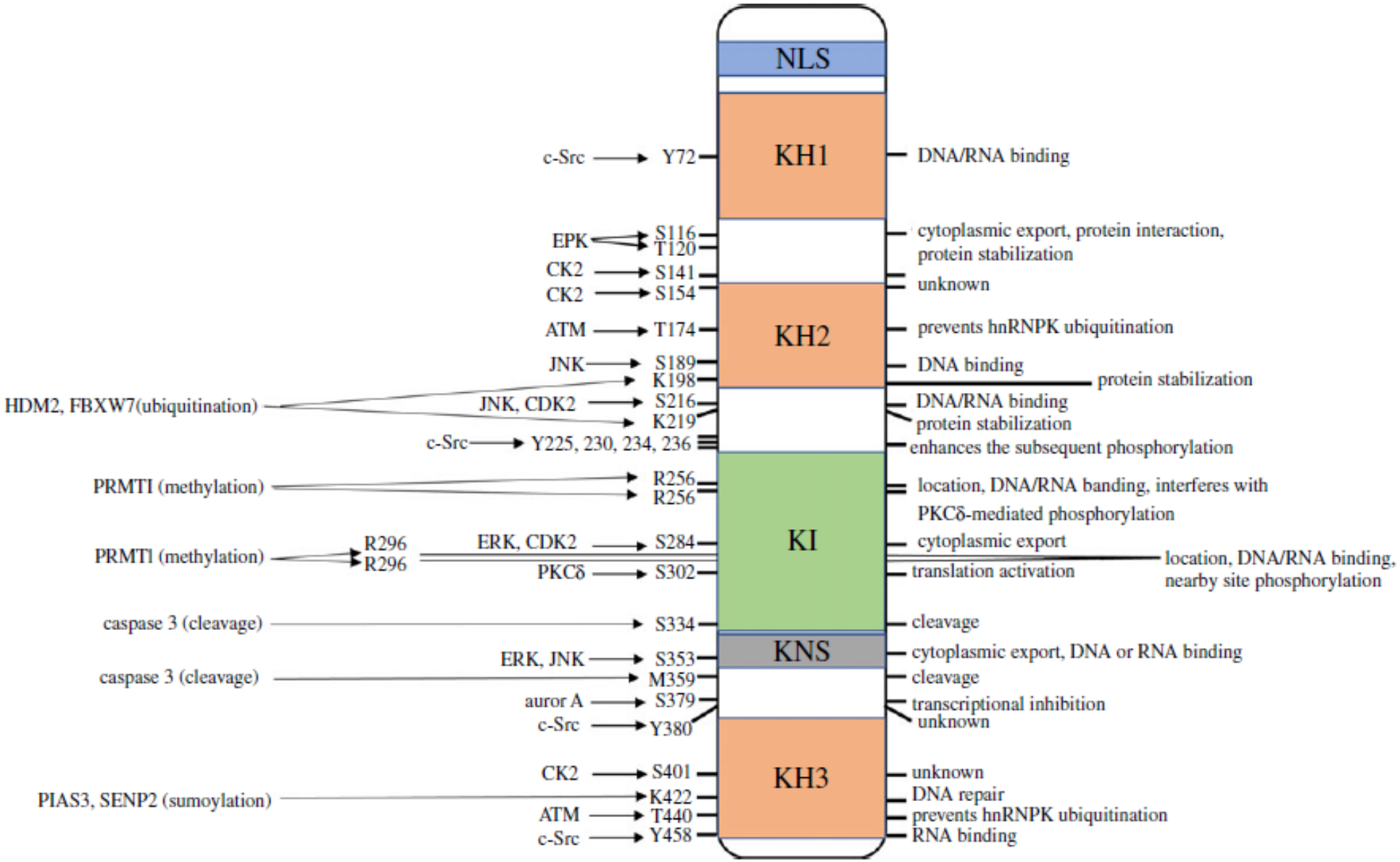






Wang et al., 2020

hnRNPK has several PTMs



Xu et al., 2019

hnRNPK regulates multiple functions in the cell

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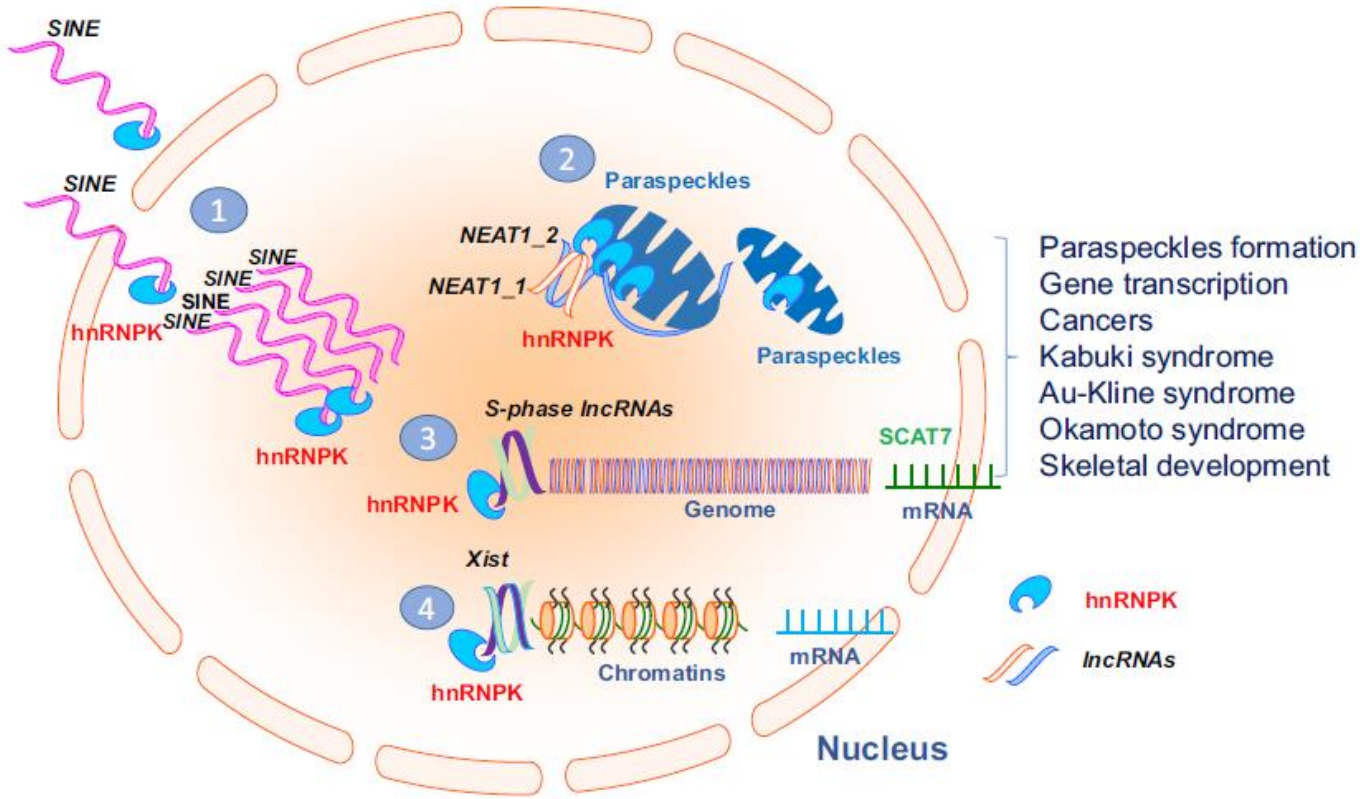
- Regulation of RNAs nuclear-cytoplasmatic shuttling and nuclear retention (Xu et al., 2019a; Zhu et al., 2019)

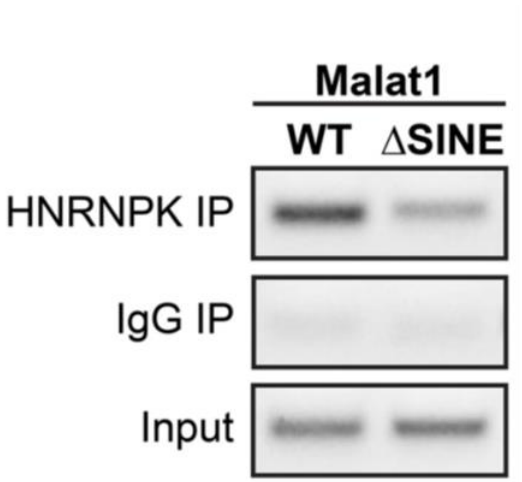
hnRNPK binds and regulates many different lncRNAs

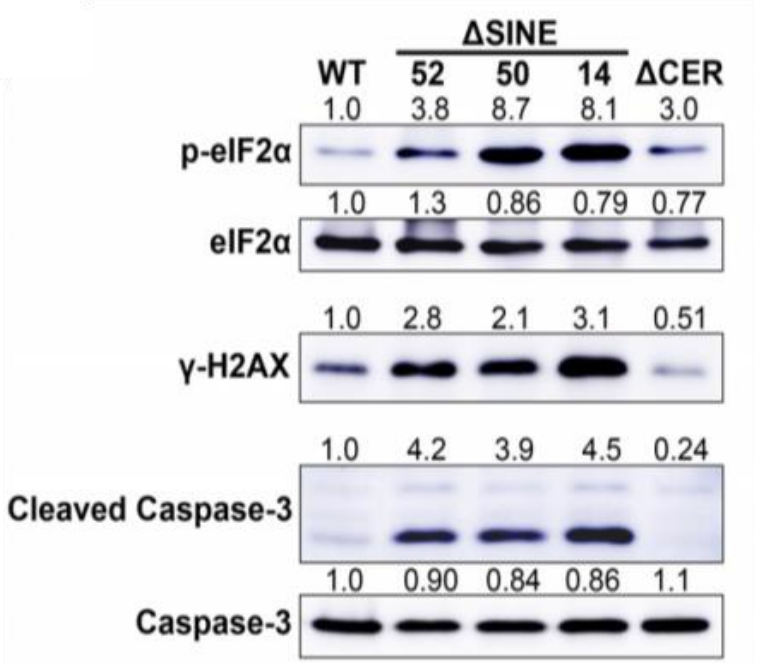
lncRNAs	Location	Dysregulation	Function	Mechanism
LincRNA-p21	Nucleus	Upregulated	Promote mouse MEFs proliferation, p53-mediated apoptosis, regulate somatic reprogramming	Transcriptional regulation
TUNA	Nucleus	Upregulated	ESC pluripotency and neural differentiation	activate the pluripotency genes
Lncenc1	Nucleus	Upregulated	Maintain the self-renewal of nESCs	Regulate the transcription of glycolytic genes
pancEts-1	Nucleus	Upregulated	Promote the growth, invasion, and metastasis of NB cells	Activate β-catenin
CASC11	Nucleus	Upregulated	Promote CRC cell proliferation and metastasis	Activate Wnt/β-catenin pathway
lncRNA 91H	Exosom	Upregulated	Promote colorectal cancer development and metastasis.	Regulate hnRNPK expression
EWSAT1	Nucleus	Upregulated	Facilitates the development of Ewing sarcoma	Repress the expression of a subset of target genes in the context of Ewing sarcoma
SLINKY	Nucleus	Upregulated	Regulate cancer cell proliferation	Transcriptional regulation
MYCLo-2	Nucleus	Upregulated	Colon cancer transformation and tumorigenesis	Repress *p21* transcription
LncRNA-OG	Nucleus	Upregulated	Promote BM-MSC osteogenic differentiation	Promoting H3K27 acetylation of the lncRNA-OG promoter
LncRNA-LBCS	Nucleus	Downregulated	Inhibits self-renewal, chemoresistance and tumor initiation of BCSCs	Repress SOX2 transcription via mediating H3K27me3
SCAT7	Nucleus	Upregulated	Promote cell proliferation and tumor development	Activate FGF/FGFR transcription and its downstream PI3K/AKT and MAPK pathways
MYU	Cytoplasm	Upregulated	Promote proliferation and tumorigenicity	Stabilize CDK6 expression
linc00460	Cytoplasm	Upregulated	Lung cancer development	Translocate hnRNPK to the cytoplasm
PTOV1-AS1	Cytoplasm	Upregulated	Promote proliferation and metastasis of various cancer	Modulate HMOX1 expression
treRNA	Cytoplasm	Upregulated	Promote tumorigenesis	Suppress translation of E-cadherin
Xist	Nucleus	Upregulated	Inactive X-chromosome	Modify underlying chromatin
NEAT1	Nucleus	-	Modulates the alternative *NEAT1* 3′-end processing	HnRNPK competed with CPSF6 for binding to NUDT21

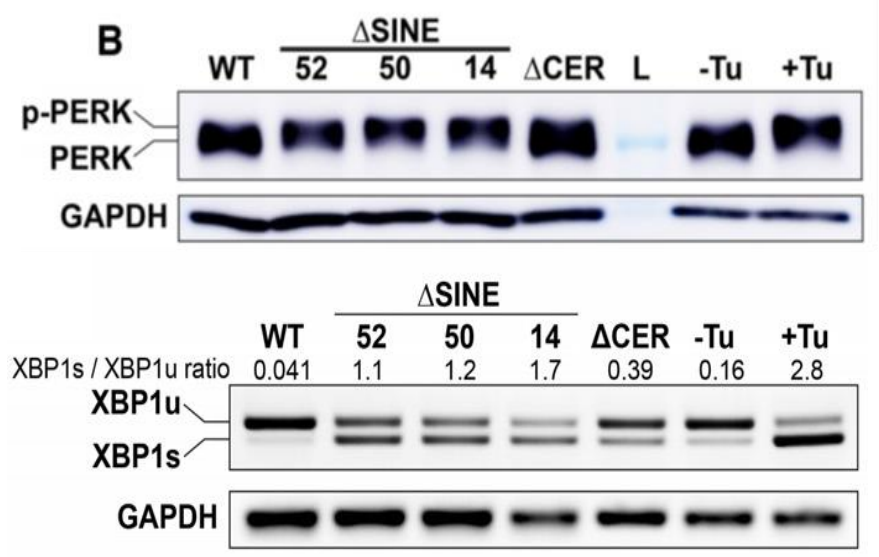


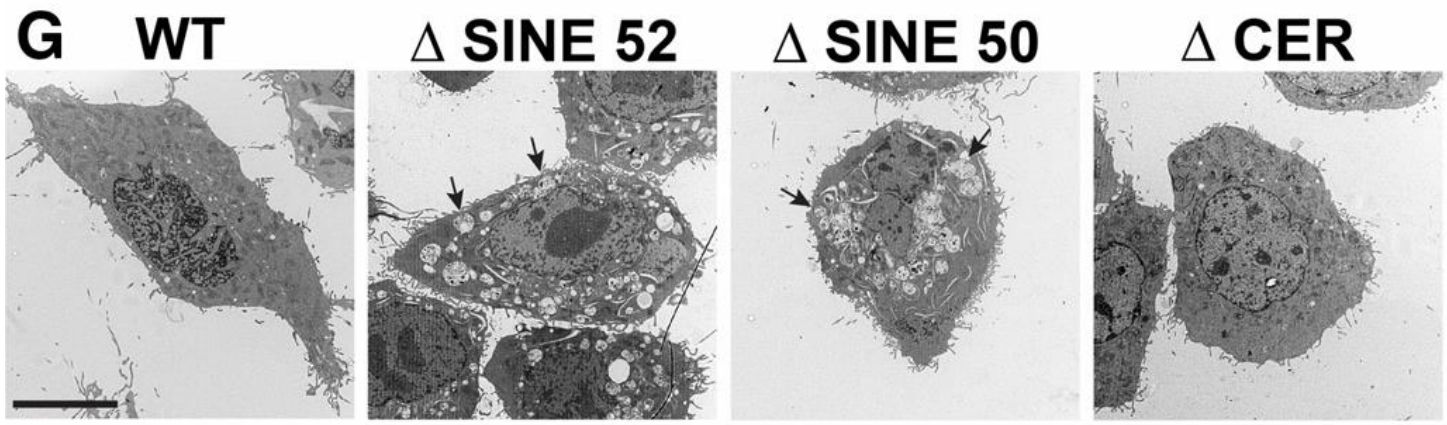
Xu et al., 2019a Zhu et al., 2019

SINE-dependent *Malat1* mislocalization activates ISR and UPR









Nguyen et al., 2020

hnRNPK and neurodegeneration

Neurobiology of Disease

FOXO3a Is Broadly Neuroprotective *In Vitro* and *In Vivo* against Insults Implicated in Motor Neuron Diseases

Jelena Mojsilovic-Petrovic,[1] Natalia Nedelsky,[2] Marco Boccitto,[1] Itzhak Mano,[3] Savvas N. Georgiades,[4] Weiguo Zhou,[1] Yuhong Liu,[5] Rachael L. Neve,[6] J. Paul Taylor,[2] Monica Driscoll,[3] Jon Clardy,[4] Diane Merry,[5] and Robert G. Kalb[1,2]

marine drugs

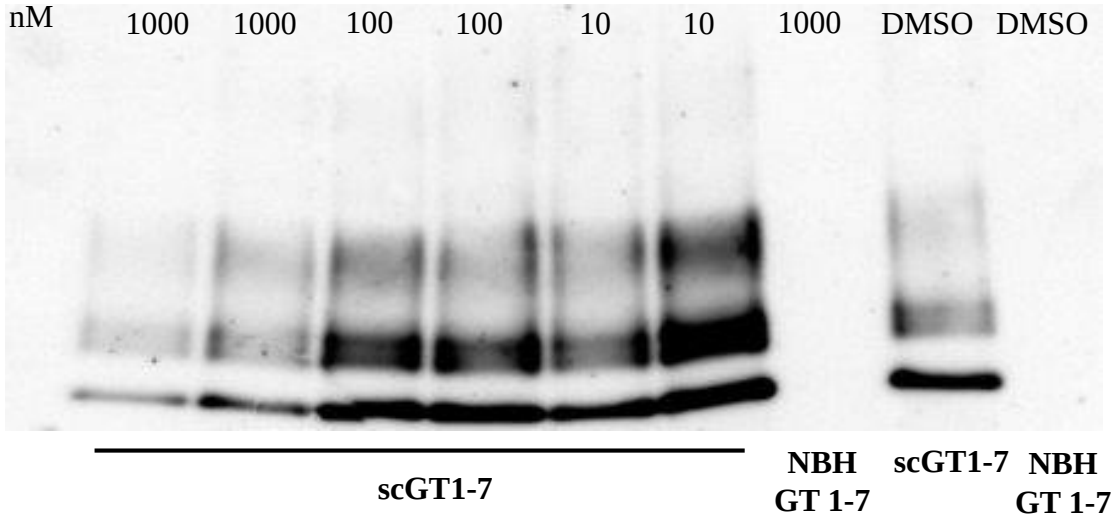
MDPI

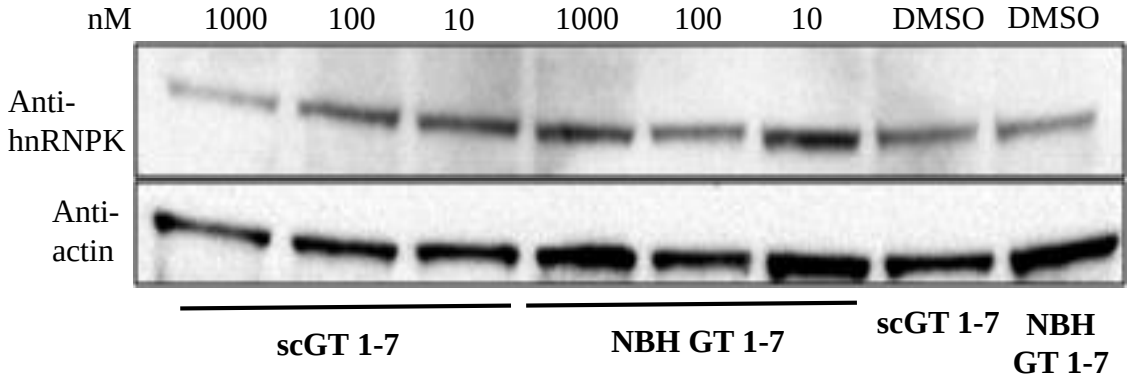
Article

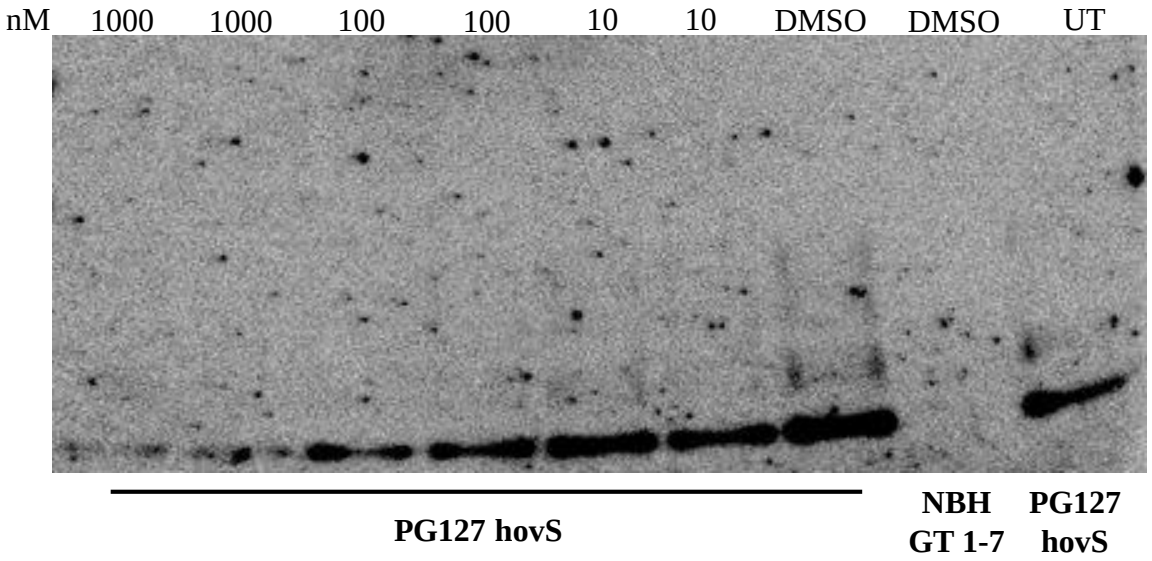
The Neuroprotective Marine Compound Psammaplysene A Binds the RNA-Binding Protein HNRNPK

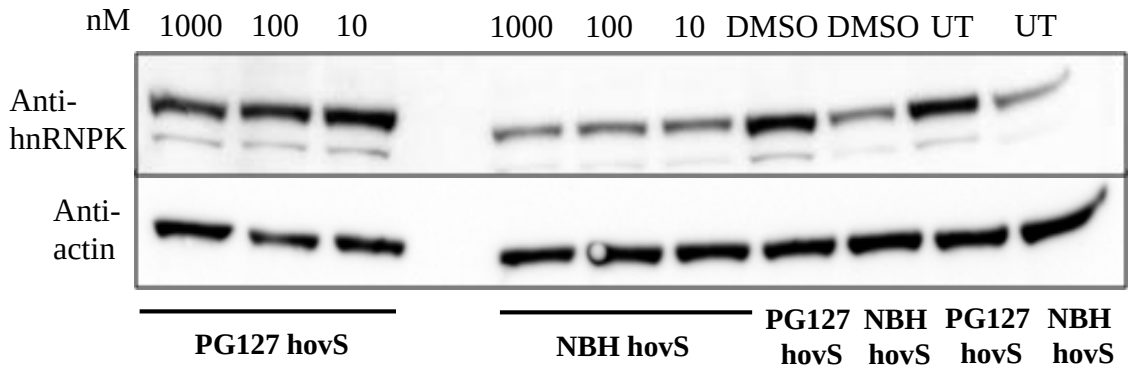
Marco Boccitto [1,3], Nayoung Lee [4], Satoshi Sakamoto [5], Lynn A. Spruce [7], Hiroshi Handa [6], Jon Clardy [4], Steven H. Seeholzer [7] and Robert G. Kalb [1,2,3,*]

hnRNPK and prion diseases









Conclusion and future perspectives

- The complexity introduced by the roles of RBPs in neurological diseases is providing new clues for further investigation in the field.

Conclusion and future perspectives

- The complexity introduced by the roles of RBPs in neurological diseases is providing new clues for further investigation in the field.

- Exploring the mechanistics of hnRNPK in prion propagation could reveal new topics in prion and similar neurodegenerative diseases.

Thank you for the attention

Bibliography

Apicco, D. J. *et al.* (2018) 'Reducing the RNA binding protein TIA1 protects against tau-mediated neurodegeneration in vivo', *Nature Neuroscience*, 21(1), pp. 72–82. doi: 10.1038/s41593-017-0022-z.

Bassell, G. J. and Warren, S. T. (2008) 'Fragile X Syndrome: Loss of Local mRNA Regulation Alters Synaptic Development and Function', *Neuron*. Elsevier Inc., 60(2), pp. 201–214. doi: 10.1016/j.neuron.2008.10.004.

Boccitto, Marco, Nayoung Lee, Satoshi Sakamoto, Lynn A. Spruce, Hiroshi Handa, Jon Clardy, Steven H. Seeholzer, and Robert G. Kalb. 2017. "The Neuroprotective Marine Compound Psammaplysene A Binds the RNA-Binding Protein HNRNPK." Marine Drugs 15(8).

De Conti, L., Baralle, M. and Buratti, E. (2017) 'Neurodegeneration and RNA-binding proteins', *Wiley Interdisciplinary Reviews: RNA*, 8(2), pp. 1–12. doi: 10.1002/wrna.1394.

Cookson, M. R. (2017) 'RNA-binding proteins implicated in neurodegenerative diseases', *Wiley Interdisciplinary Reviews: RNA*, 8(1), pp. 1–13. doi: 10.1002/wrna.1397.

Folci, A. *et al.* (2014) 'Loss of hnRNP K impairs synaptic plasticity in hippocampal neurons', *Journal of Neuroscience*, 34(27), pp. 9088–9095. doi: 10.1523/JNEUROSCI.0303-14.2014.

Fukuda, T. *et al.* (2009) 'hnRNP K interacts with RNA binding motif protein 42 and functions in the maintenance of cellular ATP level during stress conditions', *Genes to Cells*, 14(2), pp. 113–128. doi: 10.1111/j.1365-2443.2008.01256.x.

Geuens, T., Bouhy, D. and Timmerman, V. (2016) 'The hnRNP family: insights into their role in health and disease', *Human Genetics*. Springer Berlin Heidelberg, 135(8), pp. 851–867. doi: 10.1007/s00439-016-1683-5.

Han, S. P., Tang, Y. H. and Smith, R. (2010) 'Functional diversity of the hnRNPs: Past, present and perspectives', *Biochemical Journal*, 430(3), pp. 379–392. doi: 10.1042/BJ20100396.

Hentze, M. W. *et al.* (2018) 'A brave new world of RNA-binding proteins', *Nature Reviews Molecular Cell Biology*. Nature Publishing Group, 19(5), pp. 327–341. doi: 10.1038/nrm.2017.130.

Huber, K. M. *et al.* (2002) 'Altered synaptic plasticity in a mouse model of fragile X mental retardation', *Proceedings of the National Academy of Sciences of the United States of America*, 99(11), pp. 7746–7750. doi: 10.1073/pnas.122205699.

Hutchins, E. J. and Szaro, B. G. (2013) 'C-jun N-terminal kinase phosphorylation of heterogeneous nuclear ribonucleoprotein K regulates vertebrate axon outgrowth via a posttranscriptional mechanism', *Journal of Neuroscience*, 33(37), pp. 14666–14680. doi: 10.1523/JNEUROSCI.4821-12.2013.

Ihara, R. *et al.* (2013) 'RNA binding mediates neurotoxicity in the transgenic Drosophila model of TDP-43 proteinopathy', *Human Molecular Genetics*, 22(22), pp. 4474–4484. doi: 10.1093/hmg/ddt296.

Keene, J. D. (2007) 'RNA regulons: Coordination of post-transcriptional events', *Nature Reviews Genetics*, 8(7), pp. 533–543. doi: 10.1038/nrg2111.

Leal, G. *et al.* (2017) 'The RNA-binding protein hnRNP K mediates the effect of BDNF on dendritic mRNA metabolism and regulates synaptic NMDA receptors in hippocampal neurons', *eNeuro*, 4(6). doi: 10.1523/ENEURO.0268-17.2017.

Ling, S. C., Polymenidou, M. and Cleveland, D. W. (2013) 'Converging mechanisms in ALS and FTD: Disrupted RNA and protein homeostasis', *Neuron*. Elsevier Inc., 79(3), pp. 416–438. doi: 10.1016/j.neuron.2013.07.033.

Lubelsky, Y. and Ulitsky, I. (2018) ‘Sequences enriched in Alu repeats drive nuclear localization of long RNAs in human cells’, *Nature*. Nature Publishing Group, 555(7694), pp. 107–111. doi: 10.1038/nature25757.

Lunde, B. M., Moore, C. and Varani, G. (2007) ‘RNA-binding proteins: Modular design for efficient function’, *Nature Reviews Molecular Cell Biology*, 8(6), pp. 479–490. doi: 10.1038/nrm2178.

Mojsilovic-Petrovic, Jelena, Natalia Nedelsky, Marco Boccitto, Itzhak Mano, Savvas N. Georgiades, Weiguo Zhou, Yuhong Liu, Rachael L. Neve, J. Paul Taylor, Monica Driscoll, Jon Clardy, Diane Merry, and Robert G. Kalb. 2009. “FOXO3a Is Broadly Neuroprotective in Vitro and in Vivo against Insults Implicated in Motor Neuron Diseases.” Journal of Neuroscience 29(25):8236–47.

Nguyen, T. M. *et al.* (2020) ‘The SINEB1 element in the long non-coding RNA Malat1 is necessary for TDP-43 proteostasis’, *Nucleic Acids Research*, 48(5), pp. 2621–2642. doi: 10.1093/nar/gkz1176.

Nussbacher, J. K. *et al.* (2019) ‘Disruption of RNA Metabolism in Neurological Diseases and Emerging Therapeutic Interventions’, *Neuron*. Elsevier Inc., 102(2), pp. 294–320. doi: 10.1016/j.neuron.2019.03.014.

Park, C. Y. *et al.* (2015) ‘Reversion of FMR1 Methylation and Silencing by Editing the Triplet Repeats in Fragile X iPSC-Derived Neurons’, *Cell Reports*. Elsevier, 13(2), pp. 234–241. doi: 10.1016/j.celrep.2015.08.084.

Piñol-Roma, S. *et al.* (1988) ‘Immunopurification of heterogeneous nuclear ribonucleoprotein particles reveals an assortment of RNA-binding proteins.’, *Genes & development*, 2(2), pp. 215–227. doi: 10.1101/gad.2.2.215.

Sellier, C. *et al.* (2013) ‘Sequestration of DROSHA and DGCR8 by expanded CGG RNA Repeats Alters microRNA processing in fragile X-associated tremor/ataxia syndrome’, *Cell Reports*. The Authors, 3(3), pp. 869–880. doi: 10.1016/j.celrep.2013.02.004.

Stefl, R., Skrisovska, L. and Allain, F. H. T. (2005) ‘RNA sequence- and shape-dependent recognition by proteins in the ribonucleoprotein particle’, *EMBO Reports*, 6(1), pp. 33–38. doi: 10.1038/sj.embor.7400325.

Vanderweyde, T. *et al.* (2016) ‘Interaction of tau with the RNA-Binding Protein TIA1 Regulates tau Pathophysiology and Toxicity’, *Cell Reports*, 15(7), pp. 1455–1466. doi: 10.1016/j.celrep.2016.04.045.

Wang, Z. *et al.* (2020) ‘The emerging roles of hnRNPK’, *Journal of Cellular Physiology*, 235(3), pp. 1995–2008. doi: 10.1002/jcp.29186.

Xu, Y. *et al.* (2019a) ‘New Insights into the Interplay between Non-Coding RNAs and RNA-Binding Protein HnRNPK in Regulating Cellular Functions’, *Cells*, 8(1), p. 62. doi: 10.3390/cells8010062.

Xu, Y. *et al.* (2019b) ‘Post-translational modification control of RNA-binding protein hnRNPK function’, *Open Biology*, 9(3). doi: 10.1098/rsob.180239.

Zhu, S., Wang, Z. and Xu, J. (2019) ‘Connecting Versatile lncRNAs with Heterogeneous Nuclear Ribonucleoprotein K and Pathogenic Disorders’, *Trends in Biochemical Sciences*. Elsevier Ltd, 44(9), pp. 733–736. doi: 10.1016/j.tibs.2019.06.001.