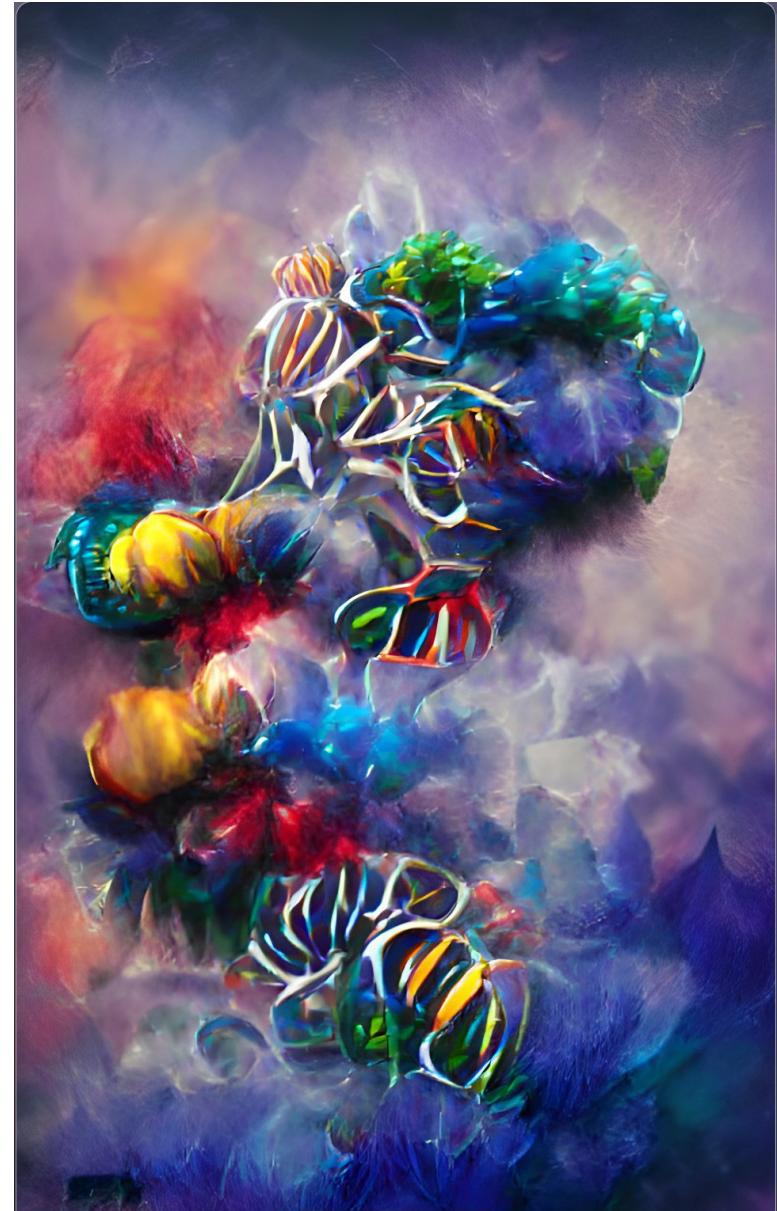


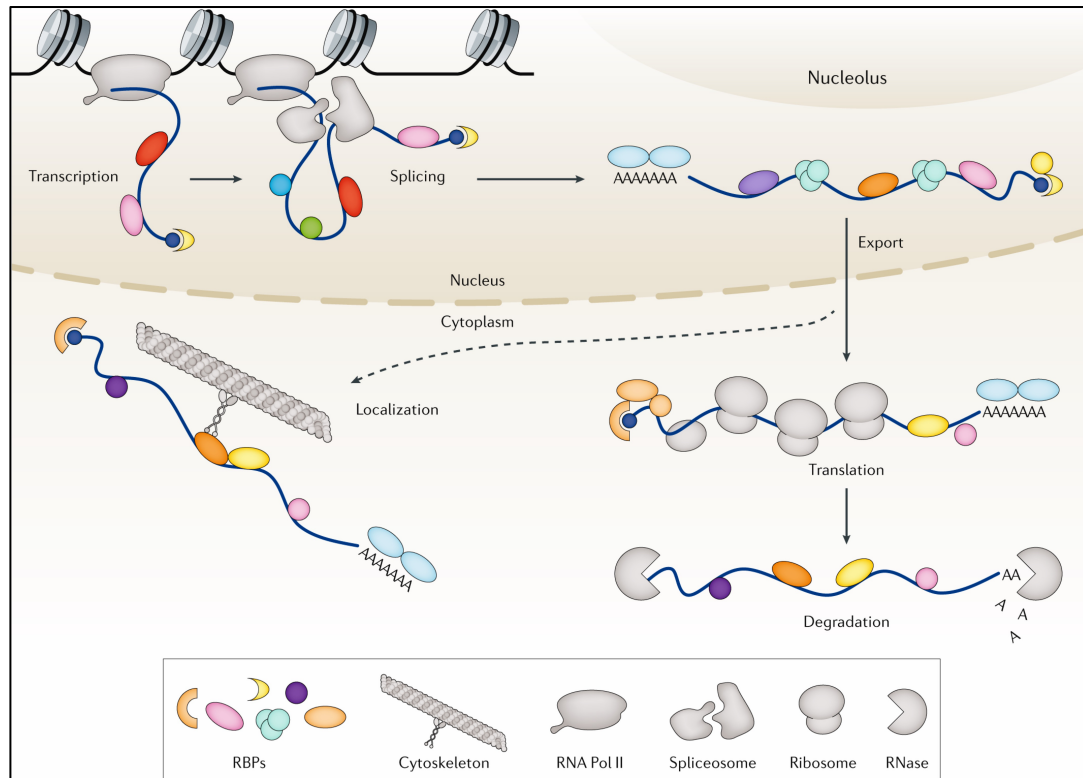
# RNA-binding protein interactions: methodology for detection and examples in disease

Evgenia Ntini  
evgenia.ntini@imbb.forth.gr  
RNA & Gene Regulation  
IMBB-FoRTH



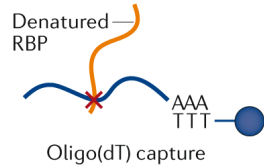
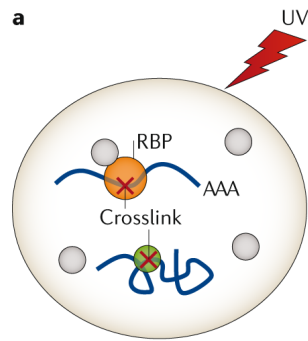
## RNA-binding proteins in gene expression:

- Fundamental co-transcriptional and post-transcriptional roles
- RNA processing (capping, splicing, polyadenylation)
- Stability (Turnover rates)
- Localization/Nuclear export



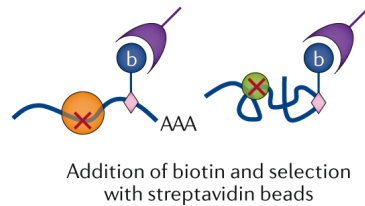
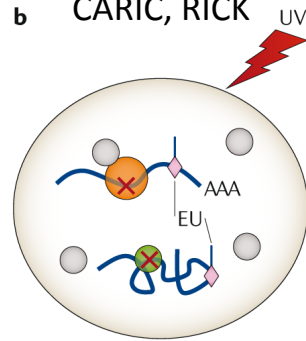
RNA-binding protein interactions: Deregulation in several diseases  
 Classic RBPs: characterized RNA-binding domains (RBDs) (defined 3D structures)

RNA-interactome capture (RIC)  
 eRIC  
 seRIC



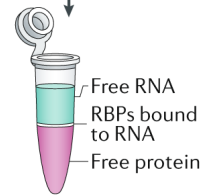
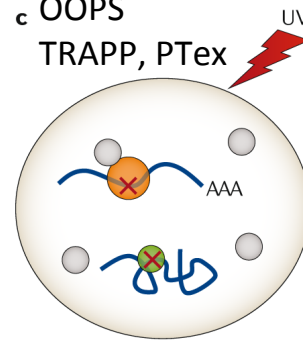
MS identification

Click chemistry  
 CARIC, RICK

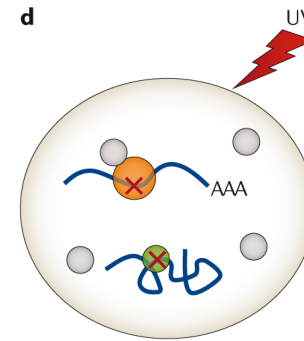


MS identification

~differential solubility  
 OOPS  
 TRAPP, PTex



MS identification



Protease digestion and SEC enrichment

RNase treatment and C18 enrichment

MS identification (specialized workflow)

To identify proteins bound on a given transcript:

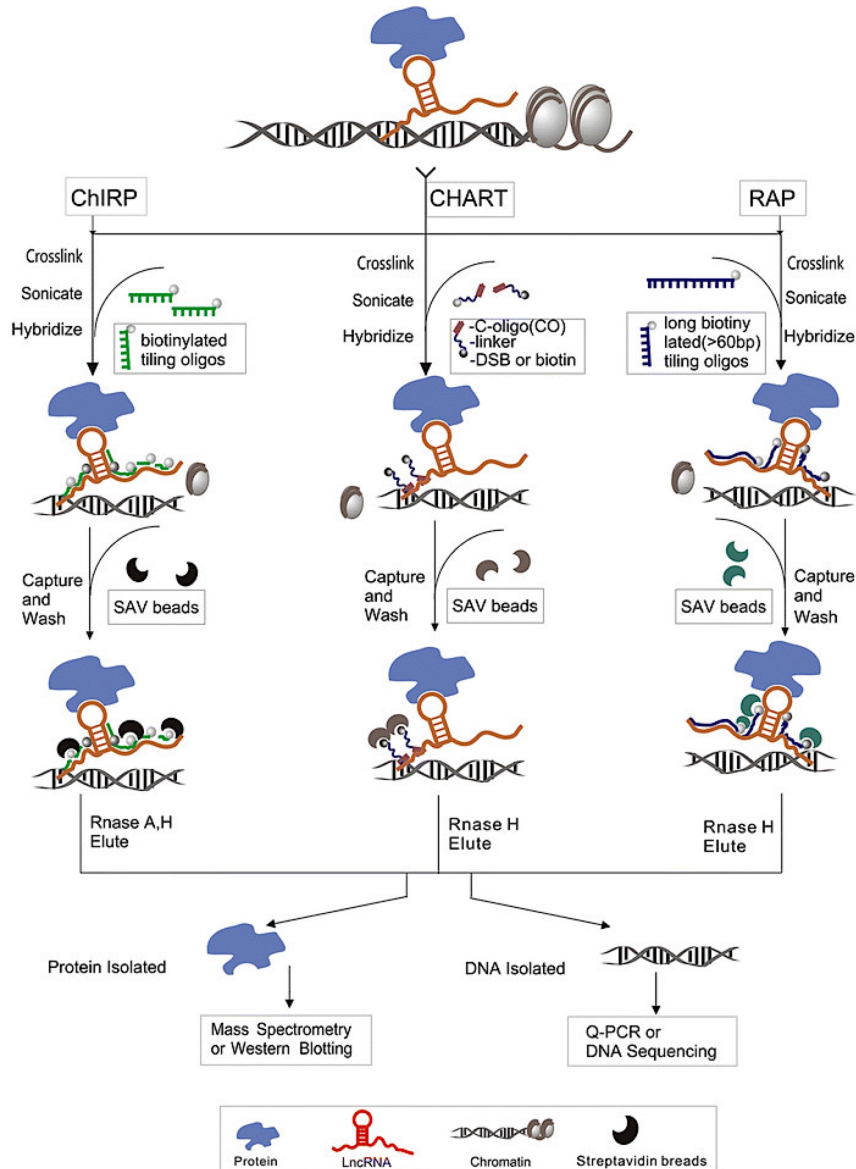
Methods like:

ChART  
ChIRP

Transcript-specific

Protein-specific methods:

RIP-seq  
PAR-clip  
eCLIP



Unveiling the hidden function of long non-coding RNA by identifying its major partner-protein  
Yang et al., Cell & Bioscience 5:59 (2015)  
Doi:10.1186/s13578-015-0050-x



To identify proteins bound on a given transcript:

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ChART  
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Protein-specific methods:

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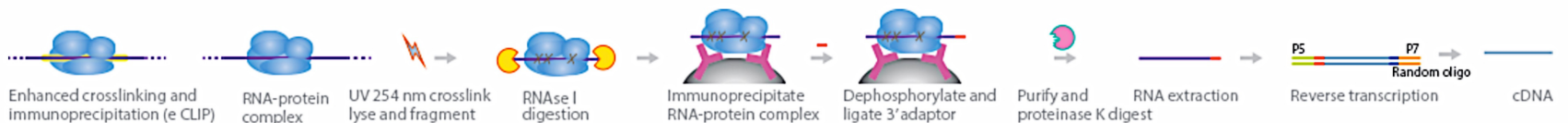
# RIP-Seq

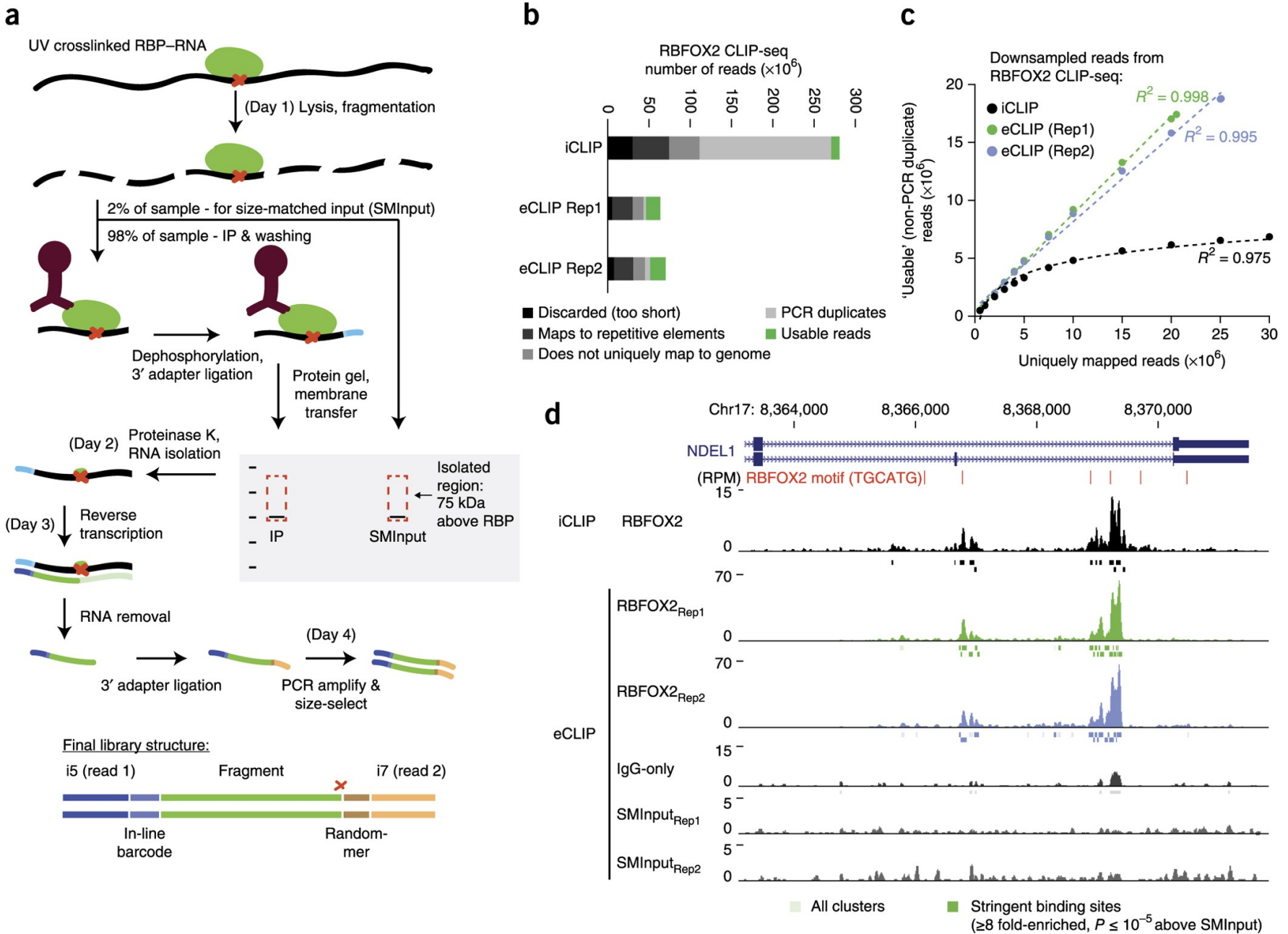


# Par-CLIP



# eCLIP

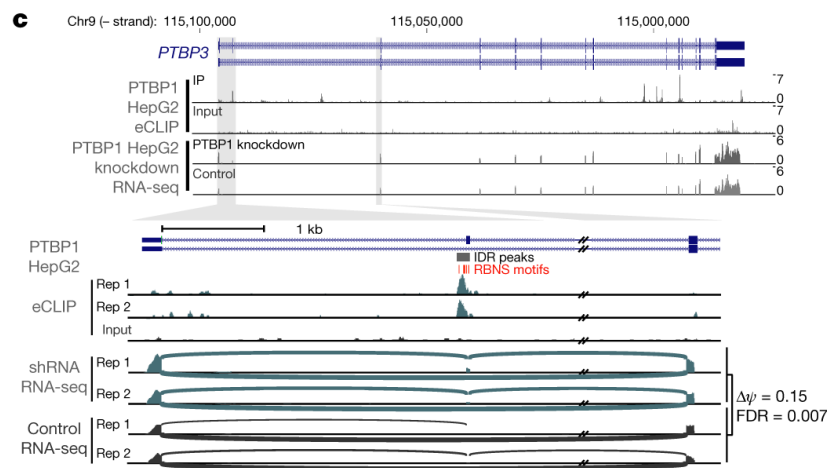
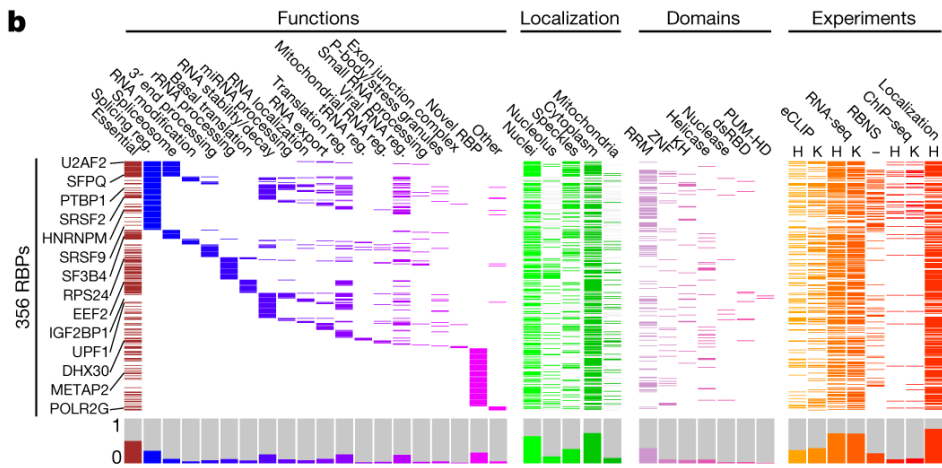
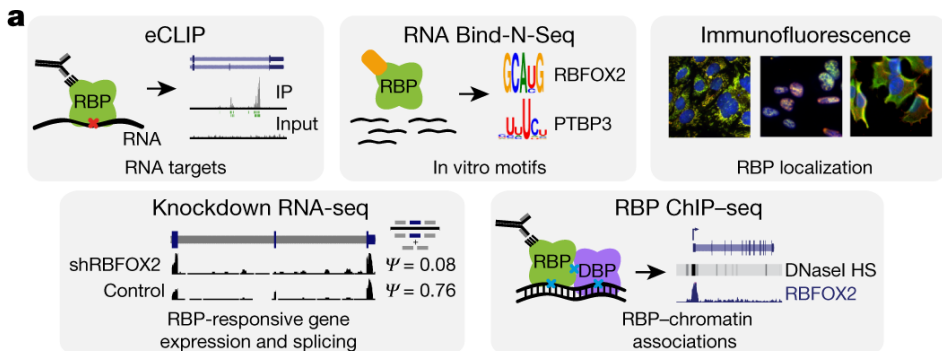




From: Robust transcriptome-wide discovery of RNA-binding protein binding sites with enhanced CLIP (eCLIP).  
 Van Nostrand et al., Nature Methods 2016

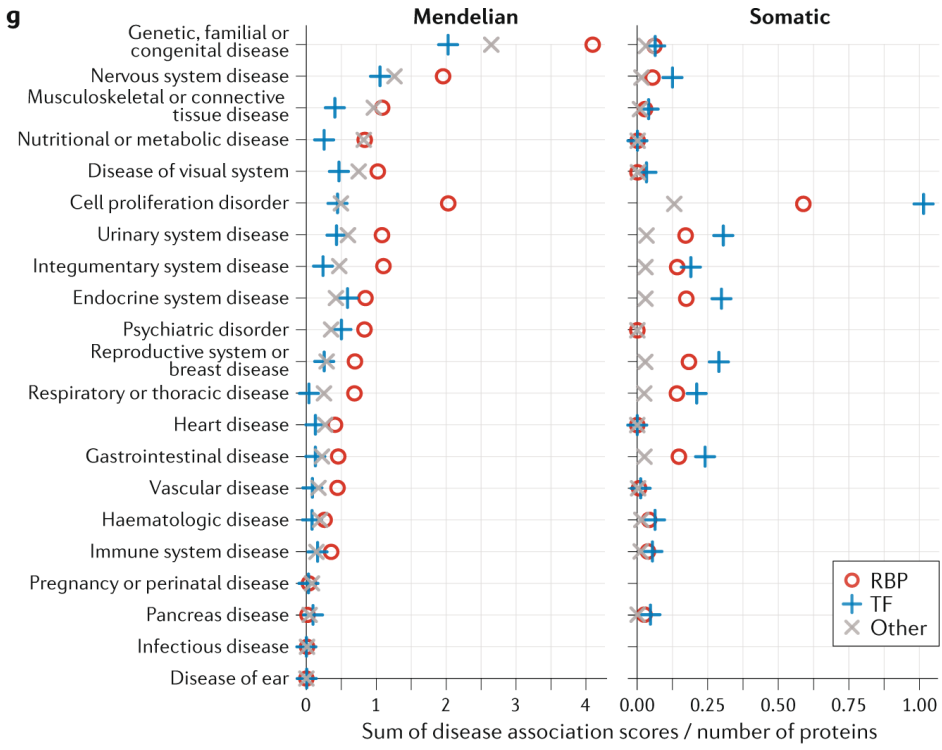
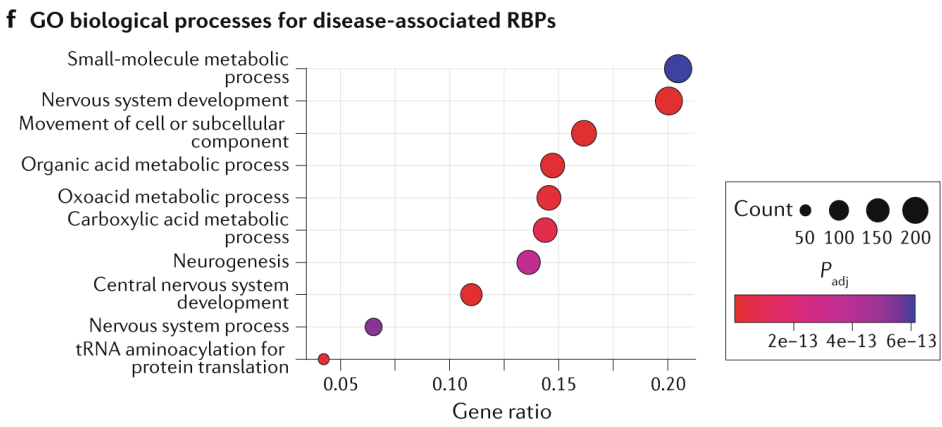
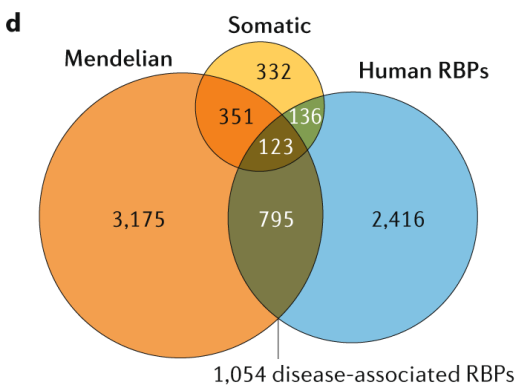
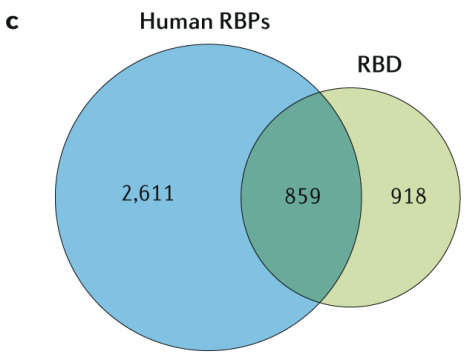
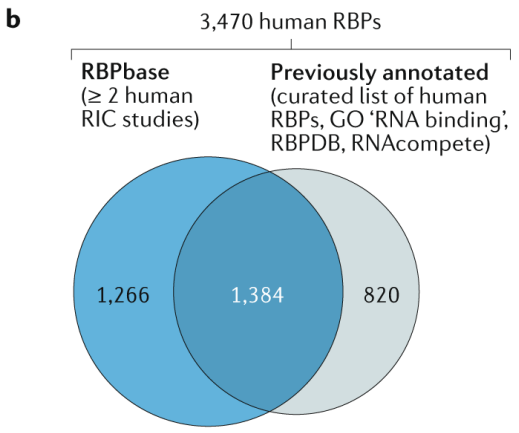
• Van Nostrand et al., Nature 2020. A large-scale binding and functional map of human RNA-binding proteins  
 PMID: 32728246

• Van Nostrand EL, et al. Genome Biol. 2020. Principles of RNA processing from analysis of enhanced CLIP maps for 150 RNA binding proteins.  
 PMID: 32252787

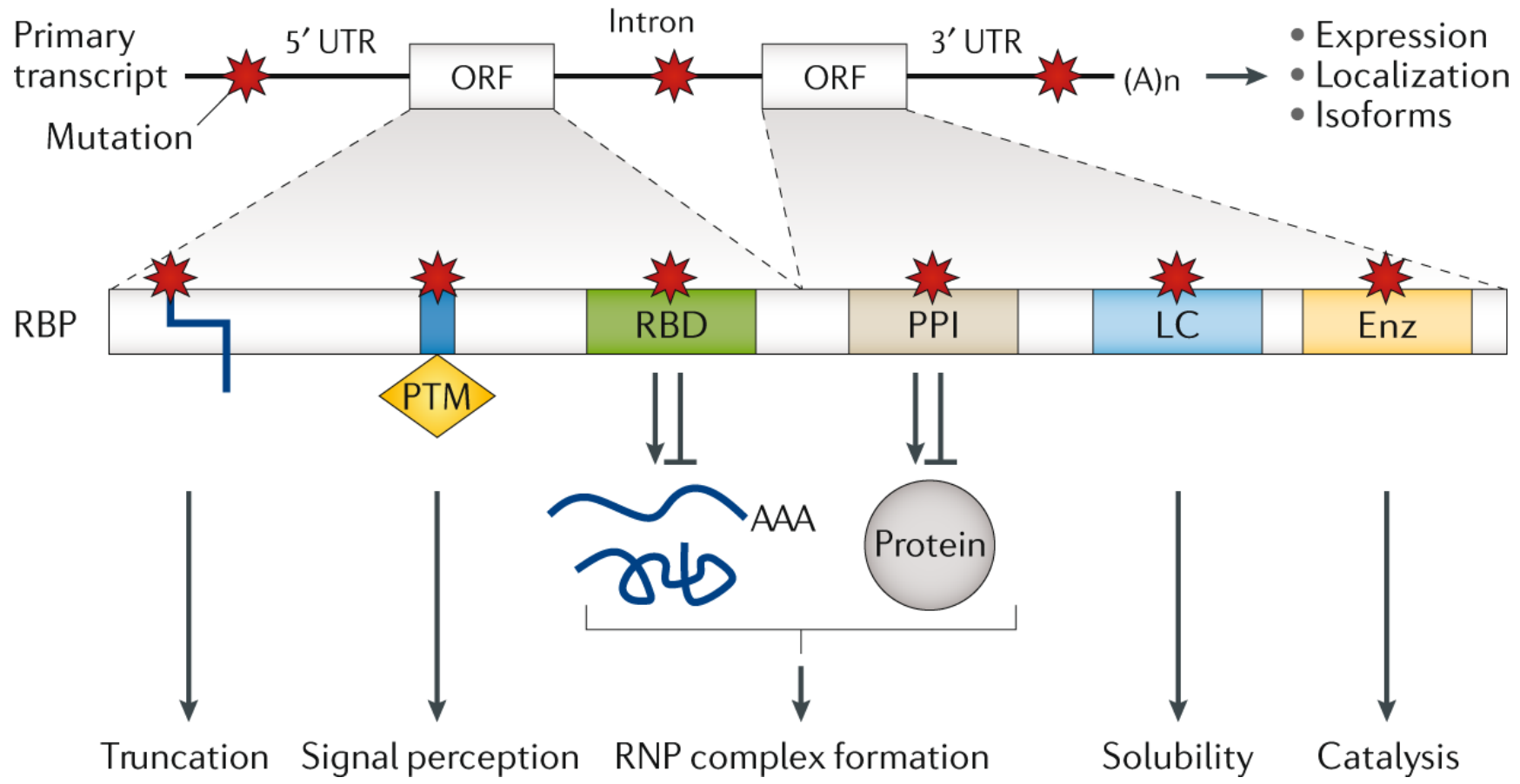


From: A large-scale binding and functional map of human RNA-binding proteins

# Disease-associated RBPs



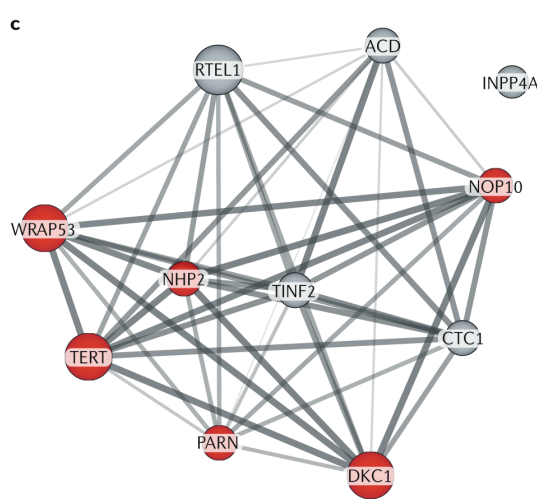
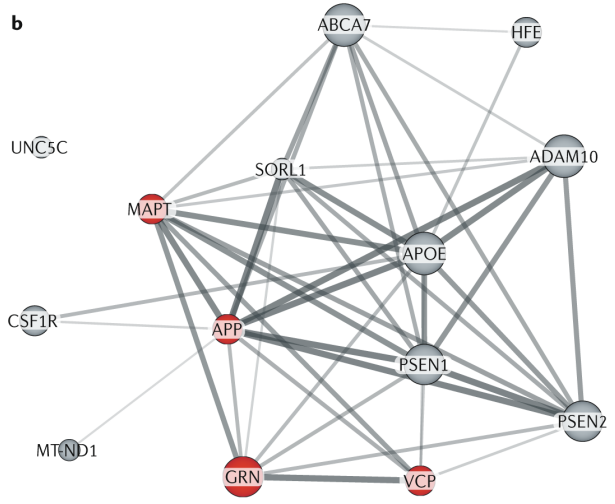
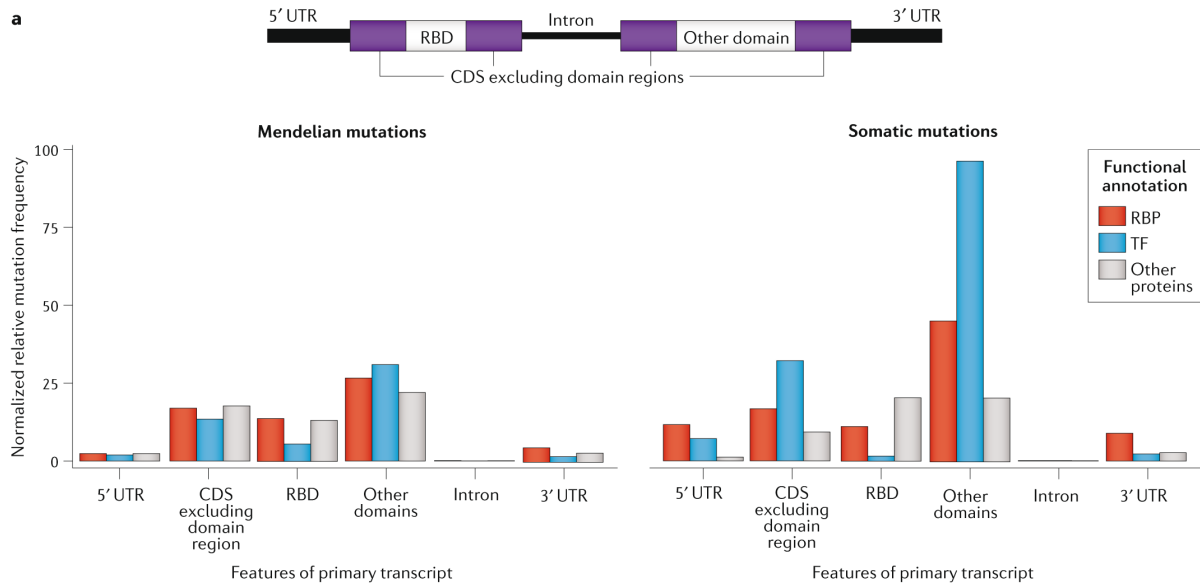
# Mutations in RBP genes and potential effects



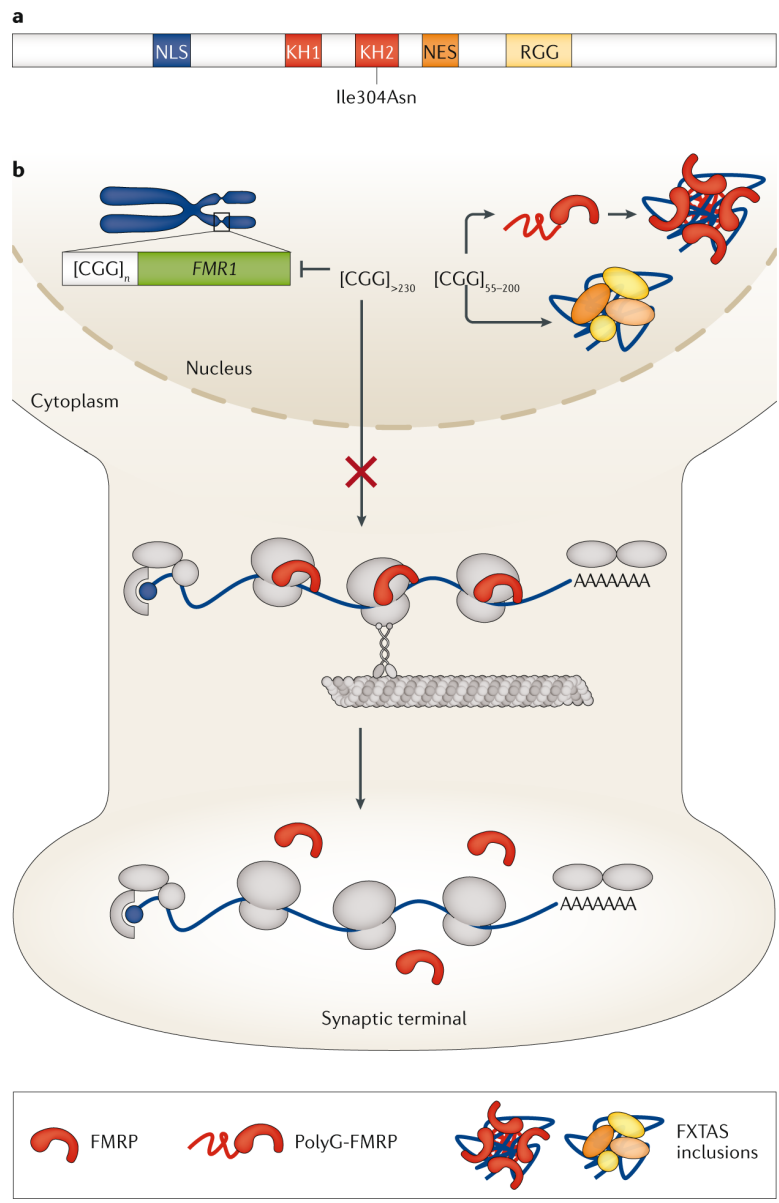
Gebauer et al., Nature Reviews Genetics 22, p. 185–198 (2021)  
 PMID: 33235359



# Protein-protein networks of disease-associated mutations

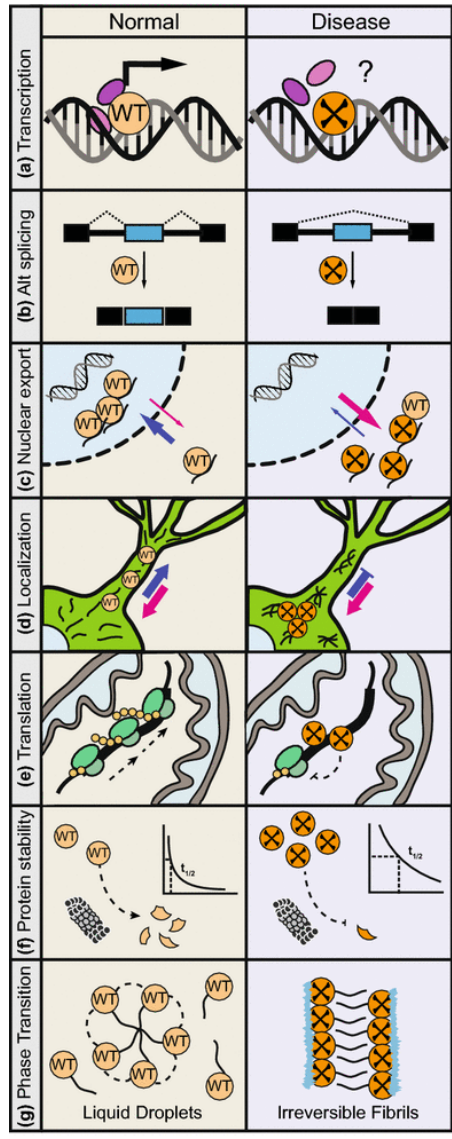
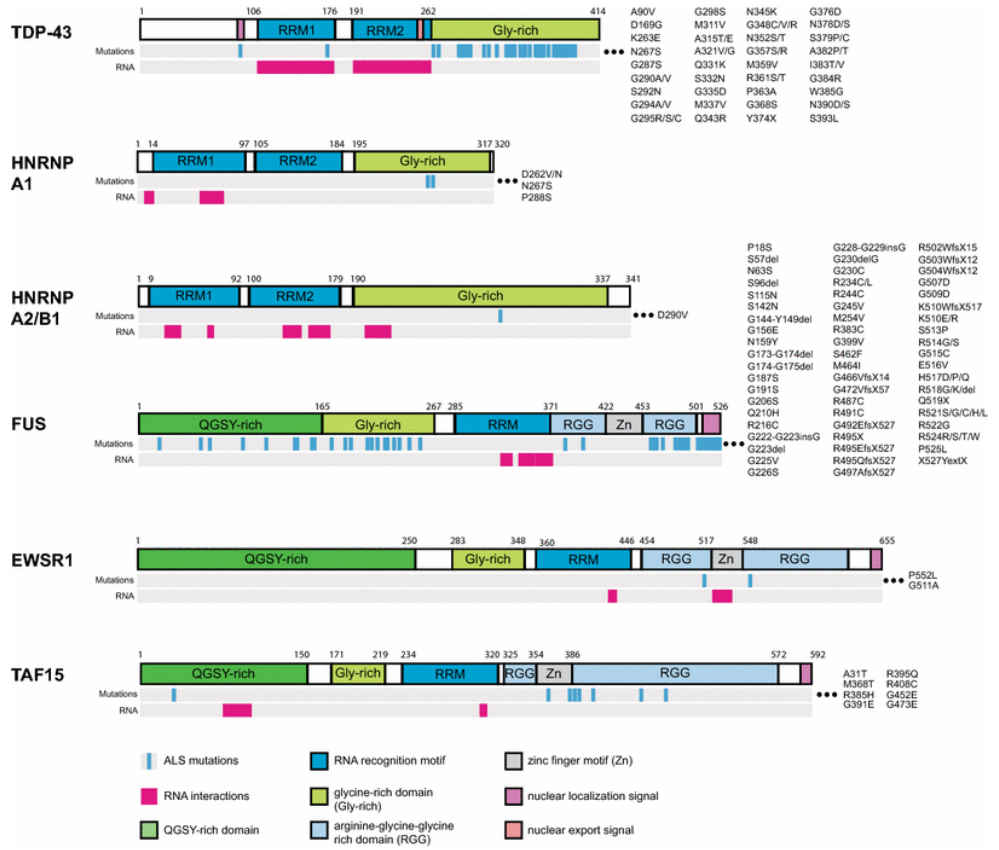


# Example 1. fragile X-associated tremor ataxia syndrome (FXTAS) / Fragile X syndrome (FXS)



Example 2. Somatic mutations in RNA processing factors (involved in splicing):  
 Like RBM10 in numerous cancers, or PRP8 (core spliceosome component)

Example 3. Mutated RBPs in ALS

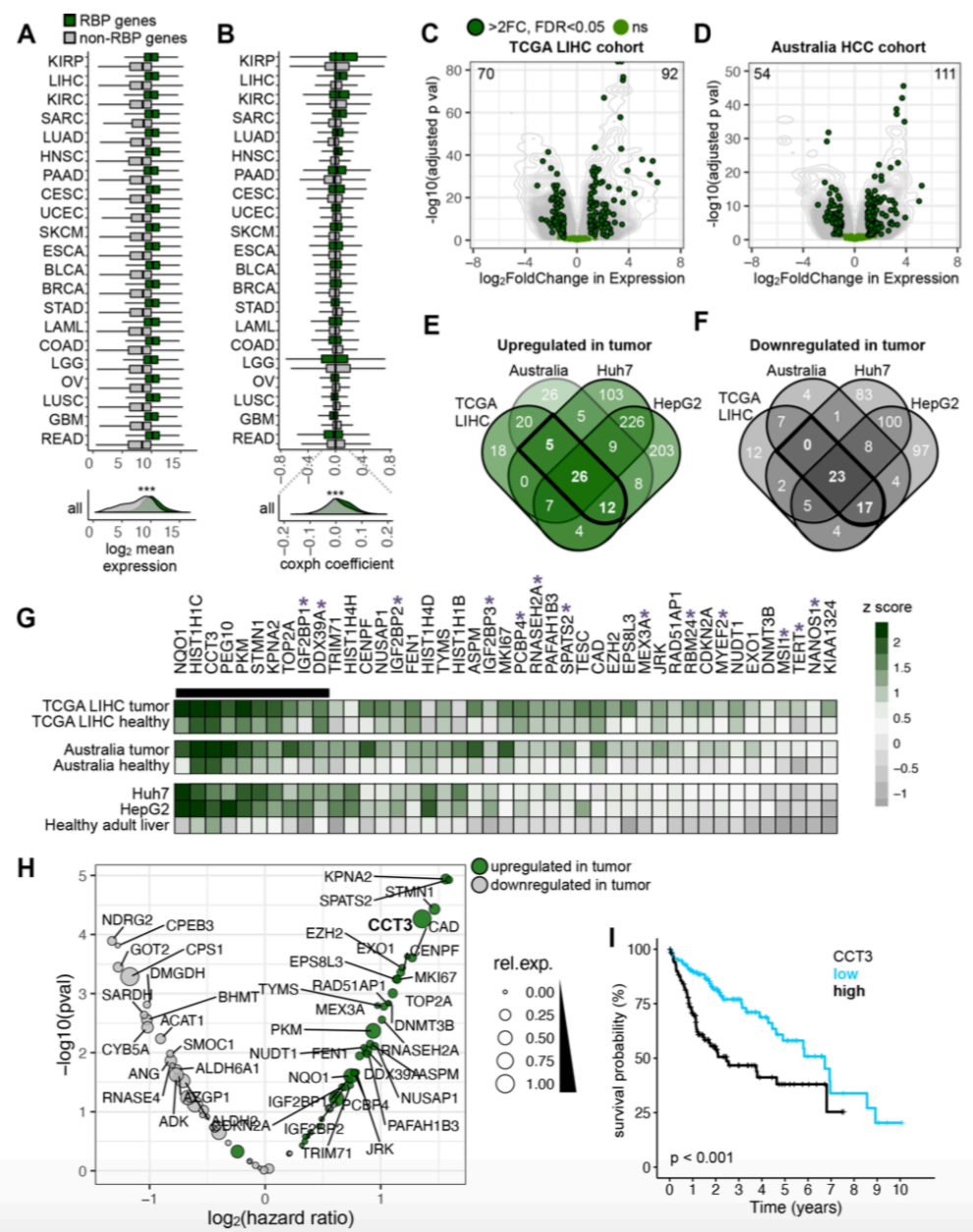


WT Wild-type RBP  
 X Mutant RBP

Example 4. RBP-IncRNA interaction networks and their importance in disease

The *CCT3-LINC000326* interaction regulates lipid metabolism in HCC

Nørskov Søndergaard et al., Gut 2022;0:1–12. doi:10.1136/gutjnl-2021-325109



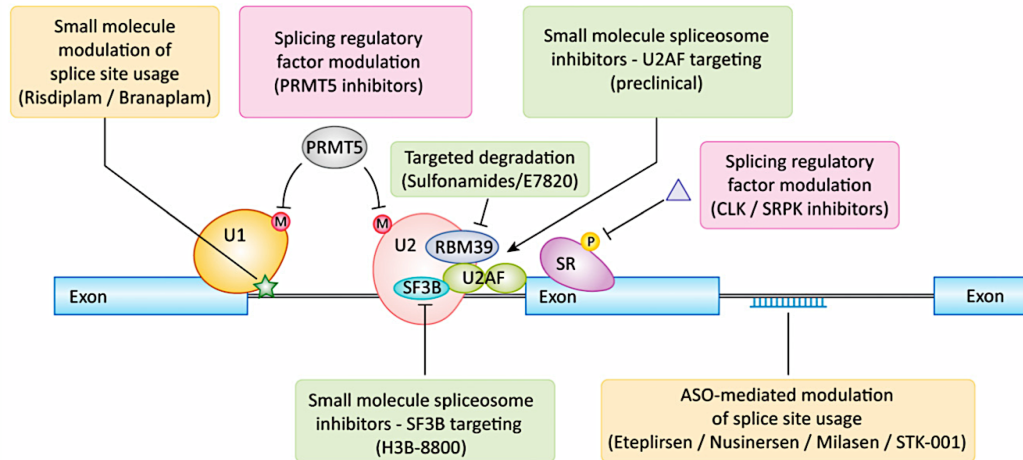
# Are RBPs druggable?

RNA-based or RNA-targeted therapeutics

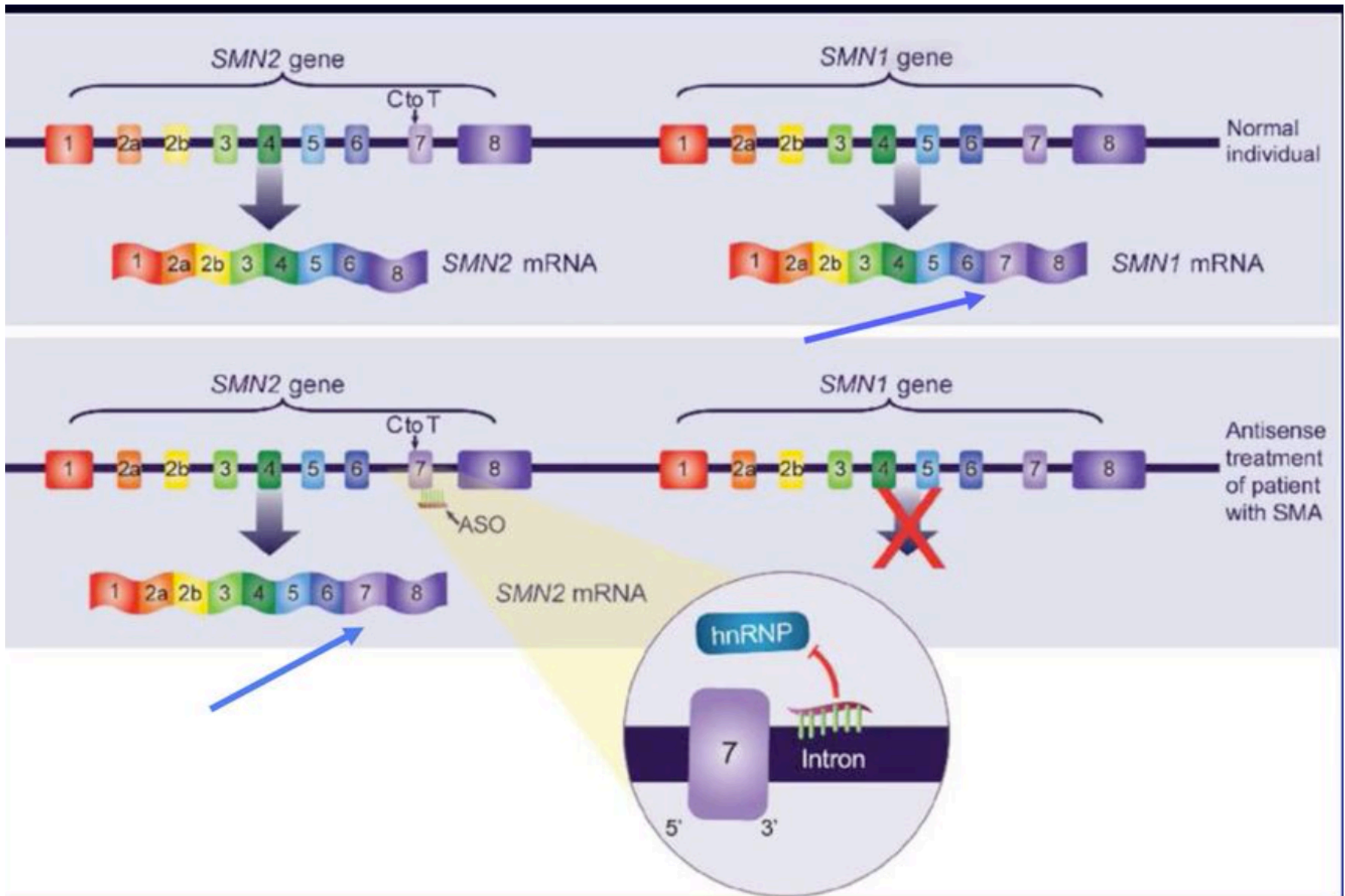
**Targeting RNA-protein interactions** (or RNA elements designated for/stabilized by protein binding), like with the FDA-approved *branaplam* small molecules: Recognition of a target-specific RNA element in conjunction with a (general) RBP component (of cellular metabolism or regulatory networks) may help achieve both **specificity** (via the sequence-specific RNA targeted component) and **affinity** (due the high information content of a protein surface).

## Reprogramming RNA processing: an emerging therapeutic landscape

Neil et al., 2021; doi.org/10.1016/j.tips.2022.02.011





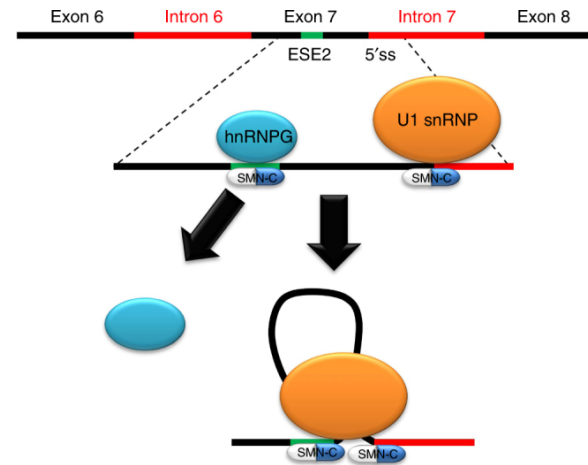
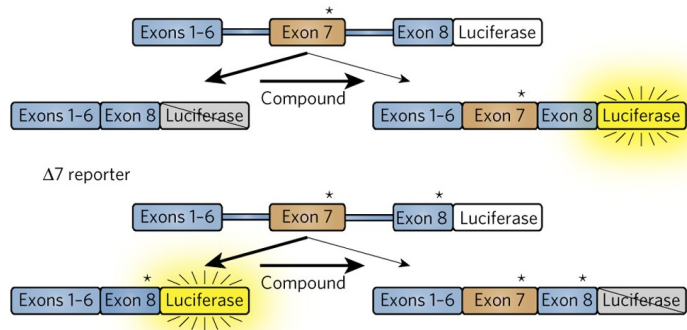


Spiranza (antisense) – FDA approved

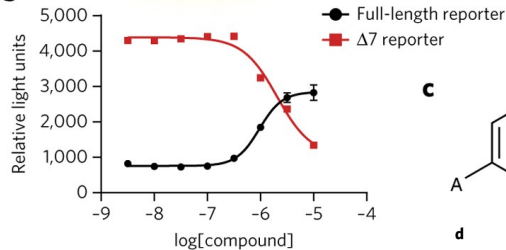
## Small molecules –splicing modifiers (~ branaplam FDA approved)

- Palacino et al., SMN2 splice modulators enhance U1–pre-mRNA association and rescue SMA mice. *Nature Chemical Biology* 11, p 511–517 (2015)
- Sivaramakrishnan et al., *Nature Communications* 2017 8(1):1476. Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers. PMID: 29133793

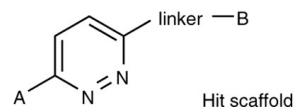
**a** Full-length reporter



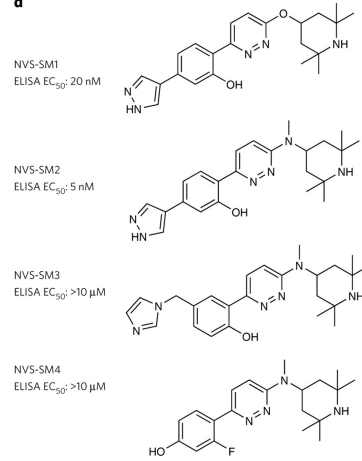
**b**



**c**



**d**



More references:

Gerstberger, S., Hafner, M. & Tuschl, T. A census of human RNA-binding proteins. *Nat. Rev. Genet.* 15, 829–845 (2014).

Hentze, M. W., Castello, A., Schwarzl, T. & Preiss, T. A brave new world of RNA-binding proteins. *Nat. Rev. Mol. Cell Biol.* 19, 327–341 (2018).

Gerstberger, S., Hafner, M. & Tuschl, T. A census of human RNA-binding proteins. *Nat. Rev. Genet.* 15, 829–845 (2014).

Castello, A. et al. Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* 149, 1393–1406 (2012).

Baltz, A. G. et al. The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. *Mol. Cell* 46, 674–690 (2012).

Castello, A. et al. Comprehensive identification of RNA-binding domains in human cells. *Mol. Cell* 63, 696–710 (2016). RBDmap: a method for the high-throughput identification of RBDs within RBPs. Peptides adjacent to the actual RNA-binding residues are identified.

Noncoding RNA therapeutics — challenges and potential solutions

Winkle et al. *Nature Reviews Drug Discovery* volume 20, pages 629–651 (2021)

Advances in oligonucleotide drug delivery

Thomas C. Roberts, Robert Langer & Matthew J. A. Wood

*Nature Reviews Drug Discovery* volume 19, pages 673–694 (2020) (incl. Table with FDA approved ASO, and ASO-based platforms)