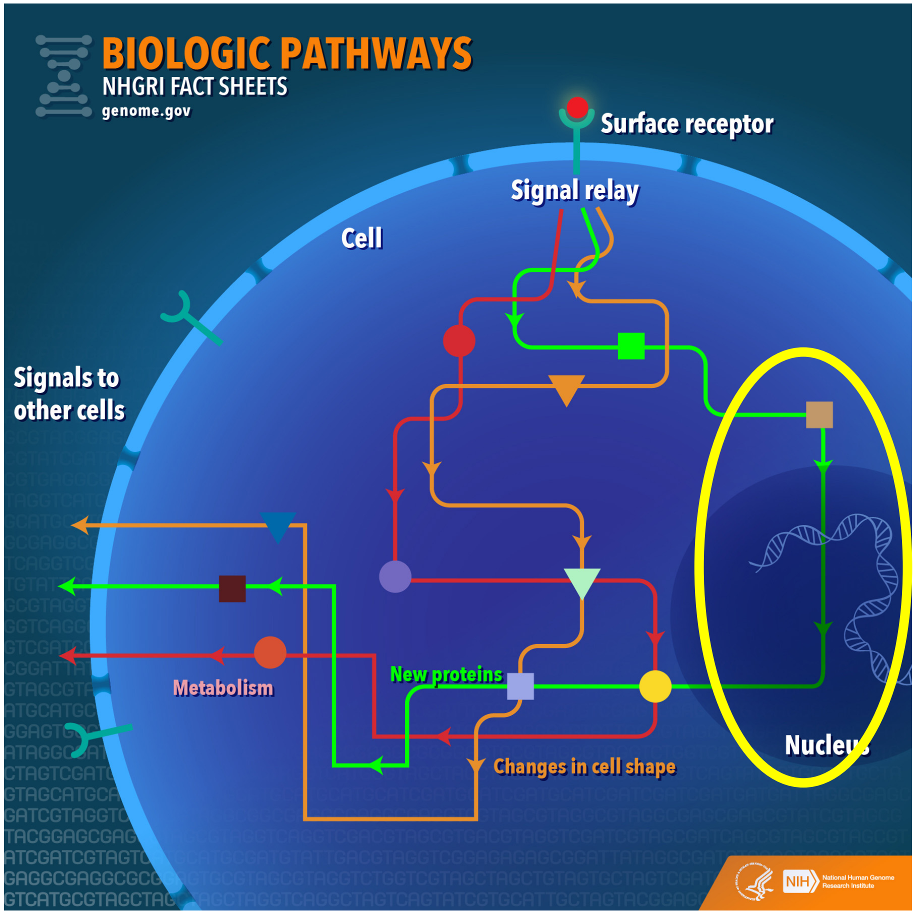
RNA Binding Proteins

…are proteins that bind RNA

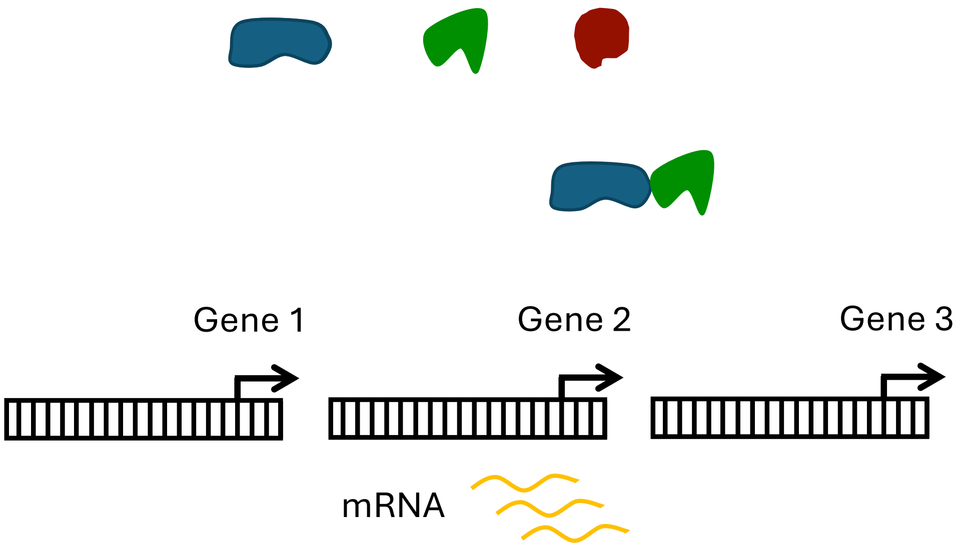
How do cells maintain their identity and respond to their environment?

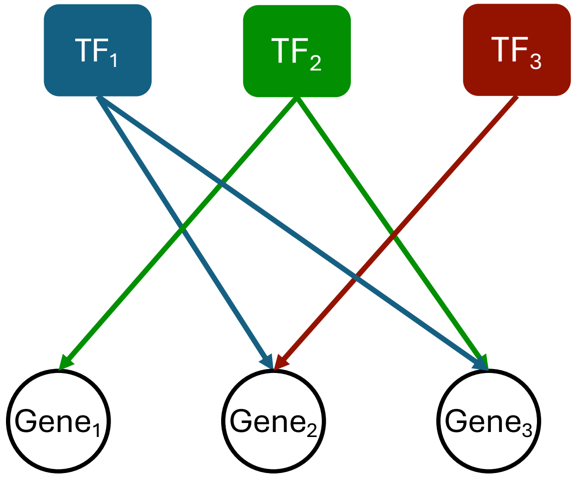


How do cells maintain their identity and respond to their environment?

https://www.genome.gov/genetics-glossary/Central-Dogma

How do cells maintain their identity and respond to their environment?





These TF → gene edges depend on the cell's environment and state

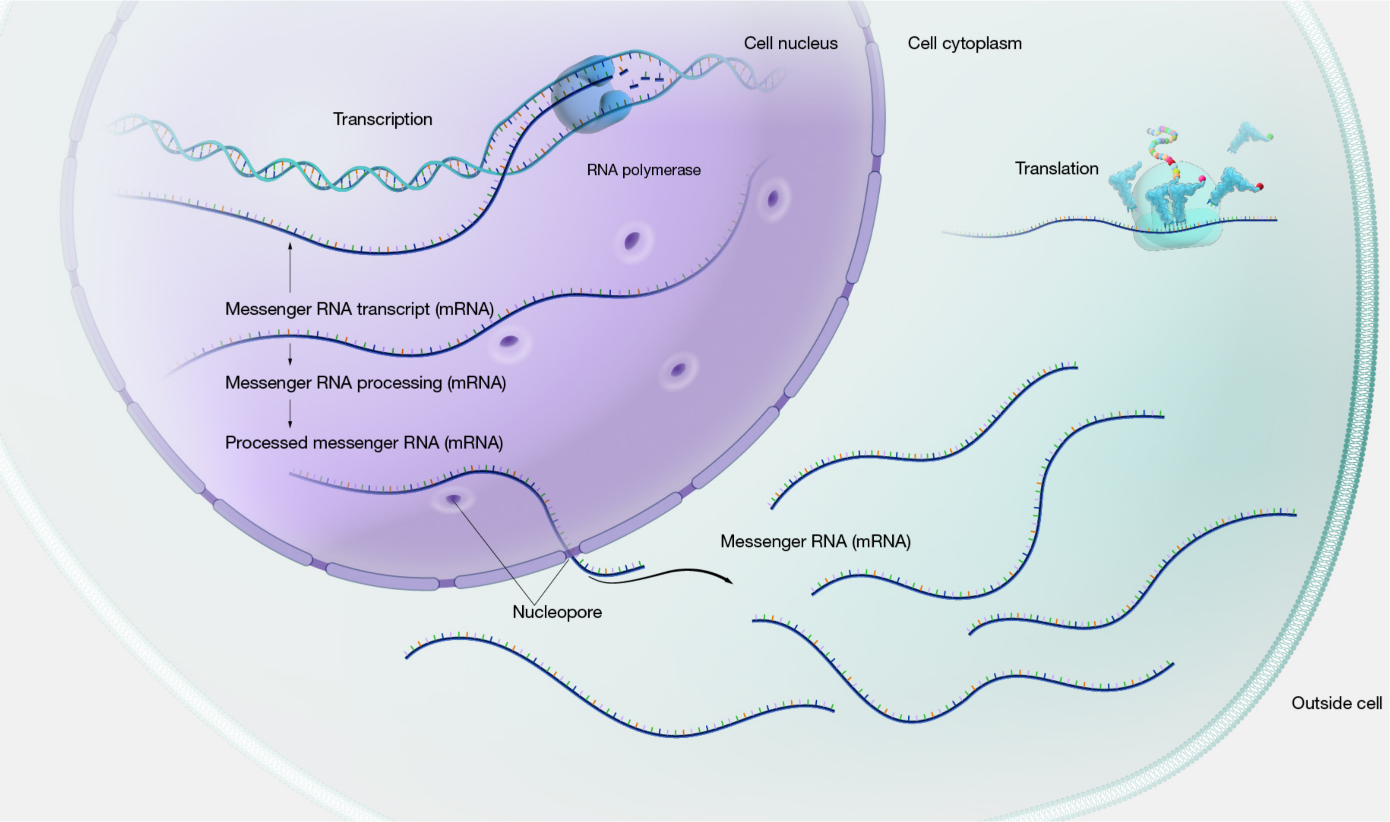
Sidebar: Most RNA is not mRNA!

Relative abundance of different RNAs

- rRNA: 80%
- tRNA: 15%
- mRNA: ~3%
- miRNA: <1%
- eRNA: <1%
- lncRNA: <1%
- Other RNA: <1%

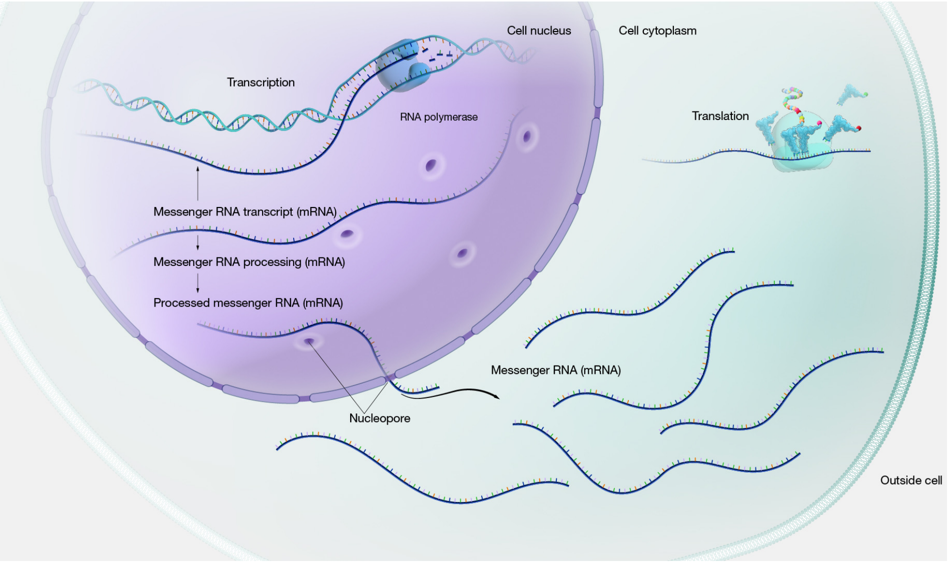
We have traditionally focused on mRNA because these specify the proteome

The life of an mRNA



https://www.genome.gov/genetics-glossary/Gene-Expression

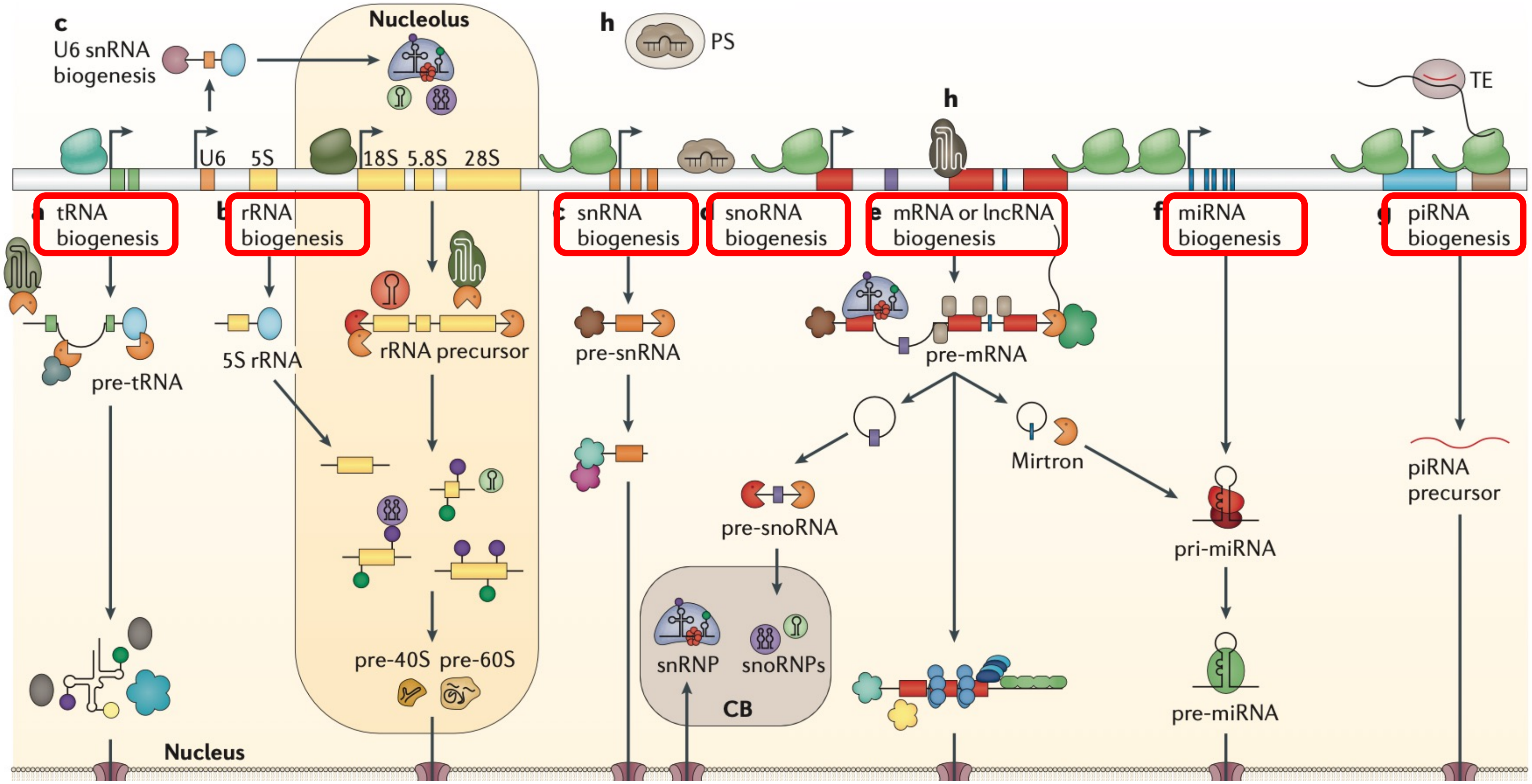
The life of an mRNA



- Processing:
    - 5’ capping
    - Intron splicing
    - Addition of 3’ poly-A tail
- Export
- Translation
- Decay

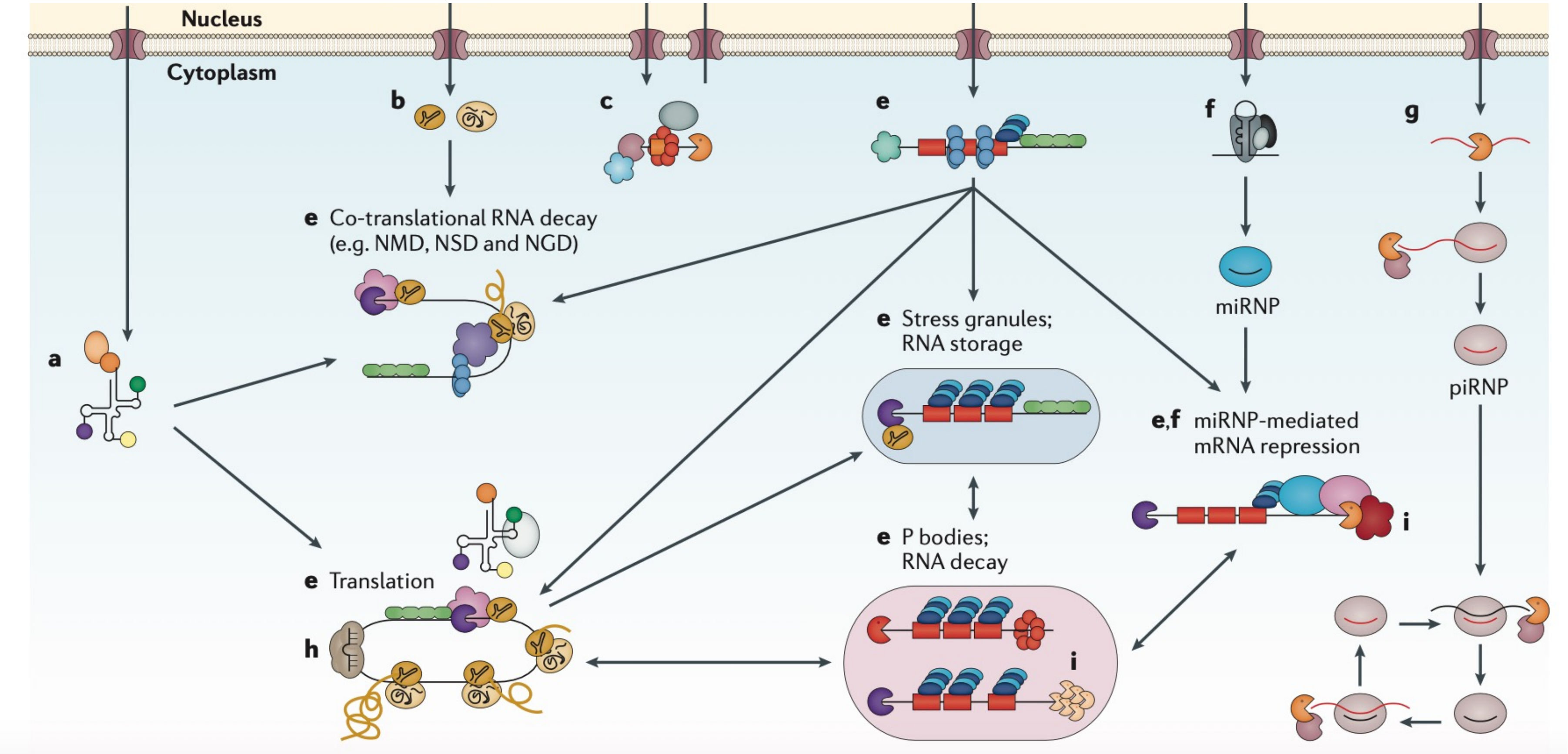
https://www.genome.gov/genetics-glossary/Gene-Expression

RBPs shepherd RNA through their life cycle



Gerstberger 2014

RBPs shepherd RNA through their life cycle



Gerstberger 2014

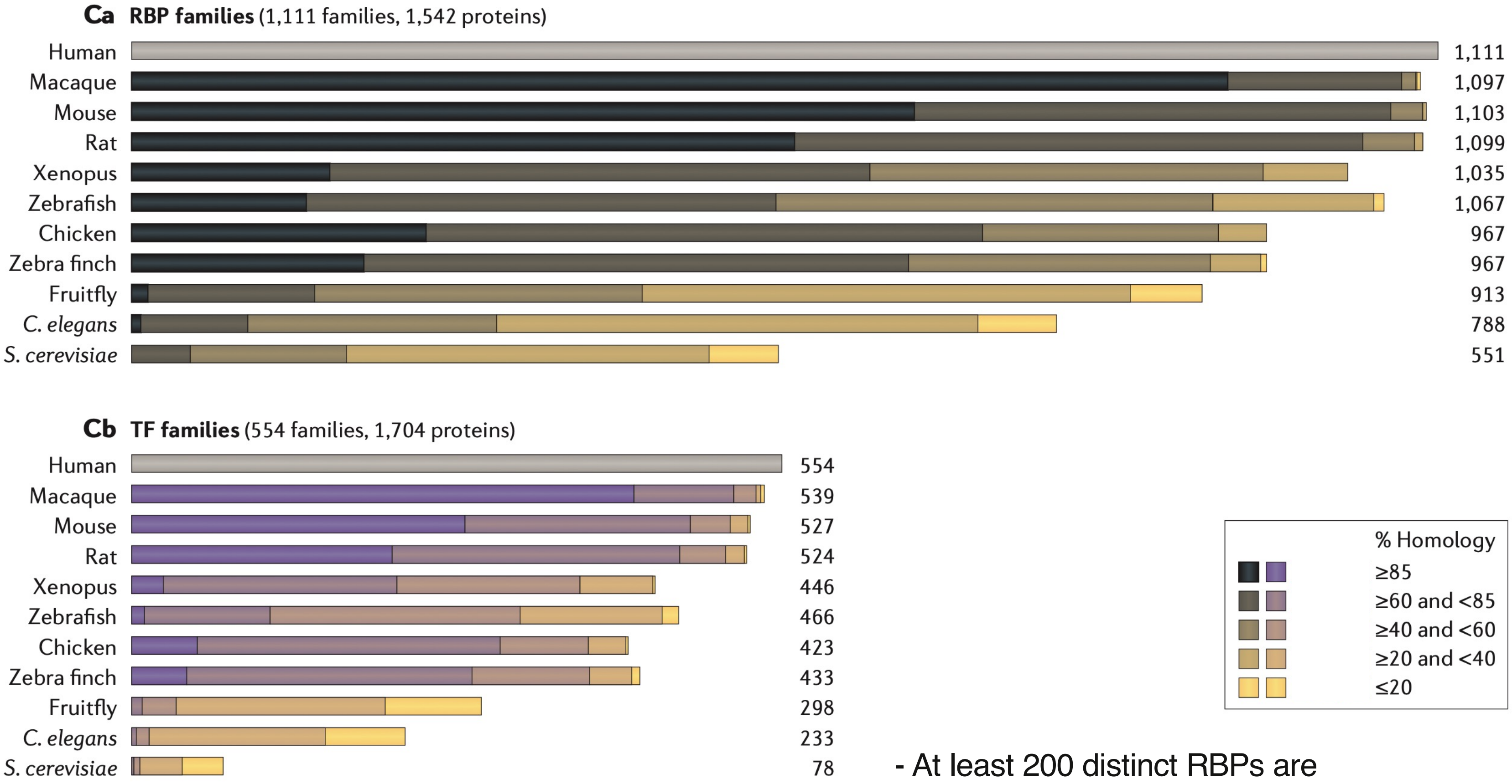
What counts as an RBP?

- Two major papers in the literature (currently):

1. Gerstberger et. al. (2014). Nature Reviews Genetics.
   - “Here, we present a census of 1,542 manually curated RBPs that we have analysed for their interactions with different classes of RNA, their evolutionary conservation, their abundance and their tissue-specific expression.”
2. Hentze et. al. (2018). Nature Reviews Molecular Cell Biology
   - “Recent proteome-wide studies have more than doubled the number of proteins implicated in RNA binding and uncovered hundreds of additional RBPs lacking conventional RBDs (RNA binding domains). In this Review, we discuss these new RBPs and the emerging understanding of their unexpected modes of RNA binding”

The plot thickens:
“RNA-binding proteins that lack canonical RNA-binding domains are rarely sequence-specific.” Ray et. al., Scientific Reports 2023.

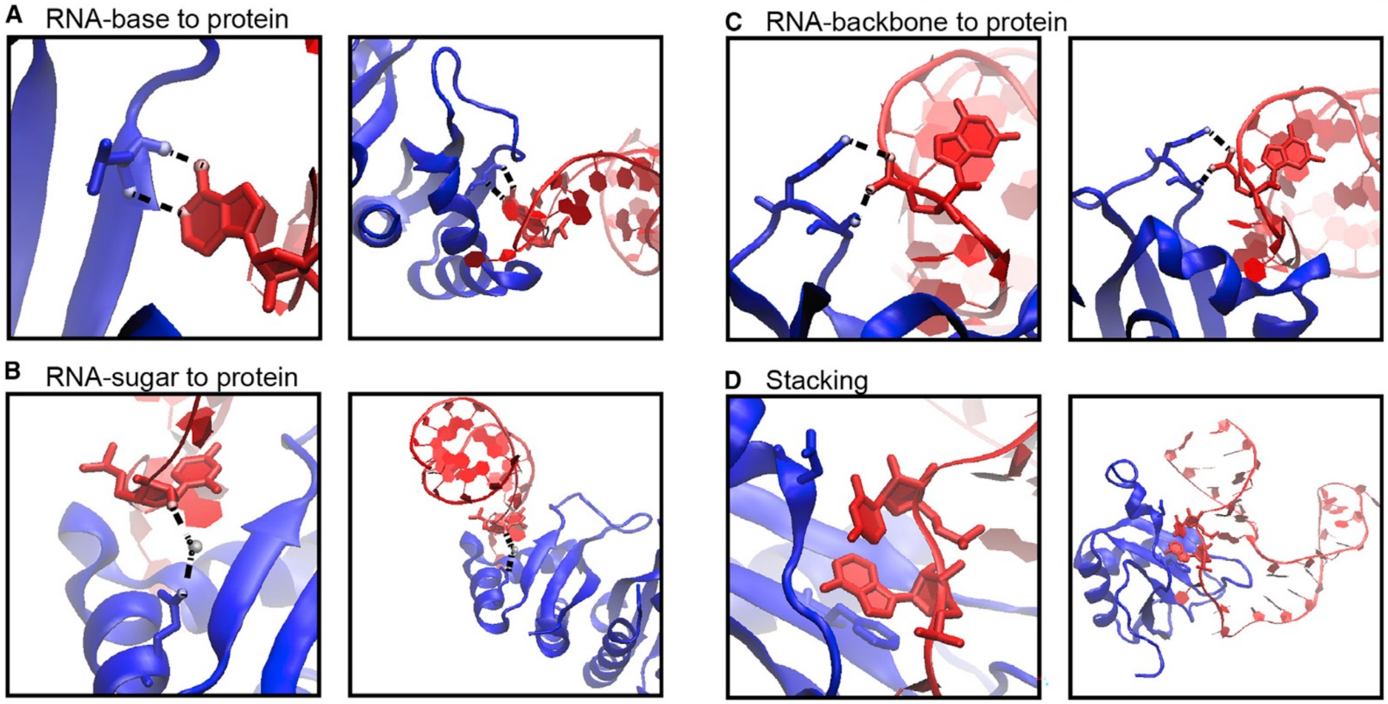
RBPs are more conserved than TFs



- At least 200 distinct RBPs are present in the lowest common ancestor of animals
- only 14% of the human TF families are found in S. cerevisiae

Gerstberger, 2014

How do RBPs bind RNA?



Corley 2020. Molecular Cell Review

Sidebar: There's some evidence that TFs bind RNA!
DOI: 10.1016/j.molcel.2023.06.012

RRM: RNA Recognition Motif

- Most common domain in RBPs
- Average 90 amino acids
- 2-8 nt interaction with single stranded RNA
- Repeated RRMs increases binding affinity
- Protein Families containing domain

Protein families containing domain:
- hnRNPs, splicing factors

Example: RNA sequence motif for RRM in RBFOX (CISBP-RNA)

KH: K homology domain

- Second most common domain
- Smaller than the RRM (70 AA)
- Repeated domains increase binding

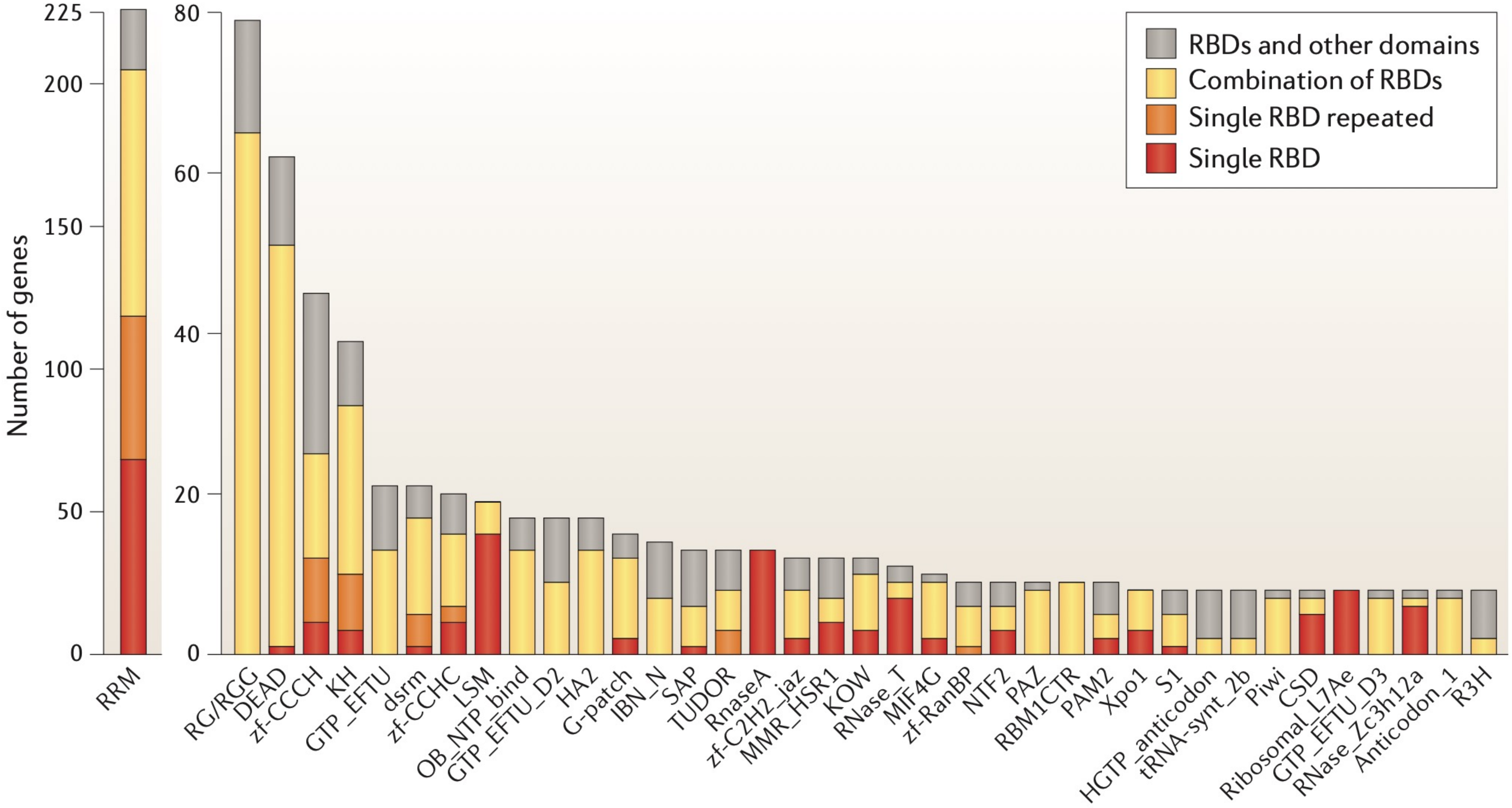
Example: RNA sequence motif for HNRNPK (CISBP-RNA)

“AU-rich element RNA-binding protein KSRP, which has 4 KH domains, KH domains 3 and 4 behave as independent binding modules to interact with different regions of the AU-rich RNA targets.”
- InterPro

Protein families containing domain:

- hnRNPs, translation regulation proteins

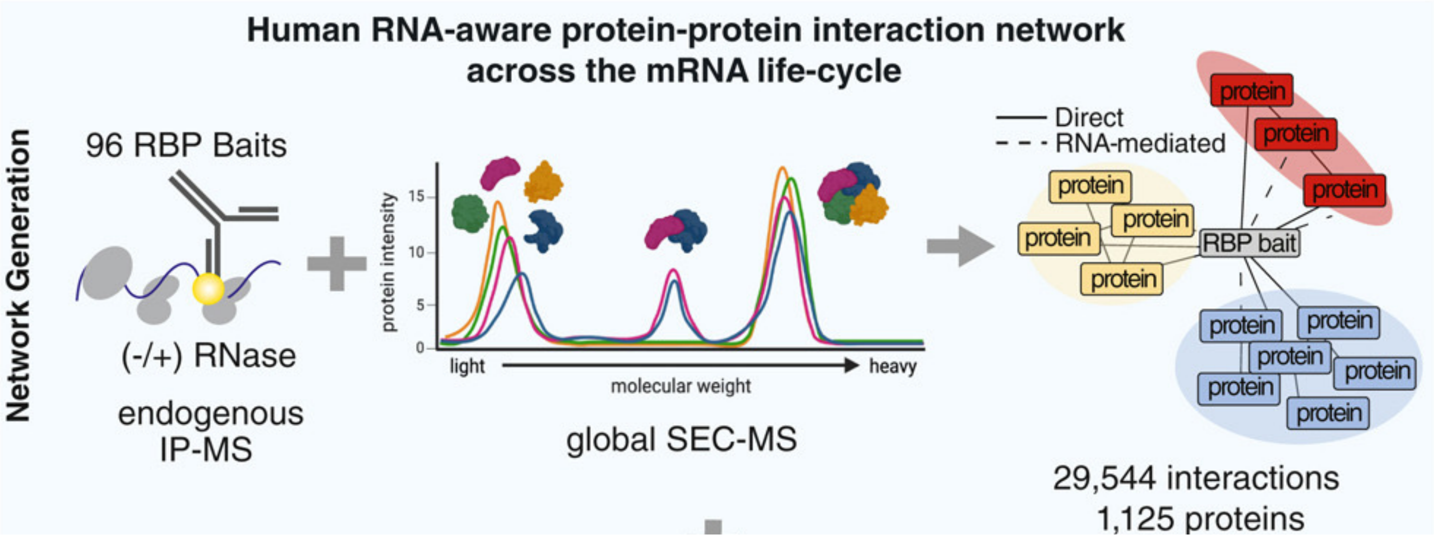
Better together: RBPs use many domains



Gerstberger 2014

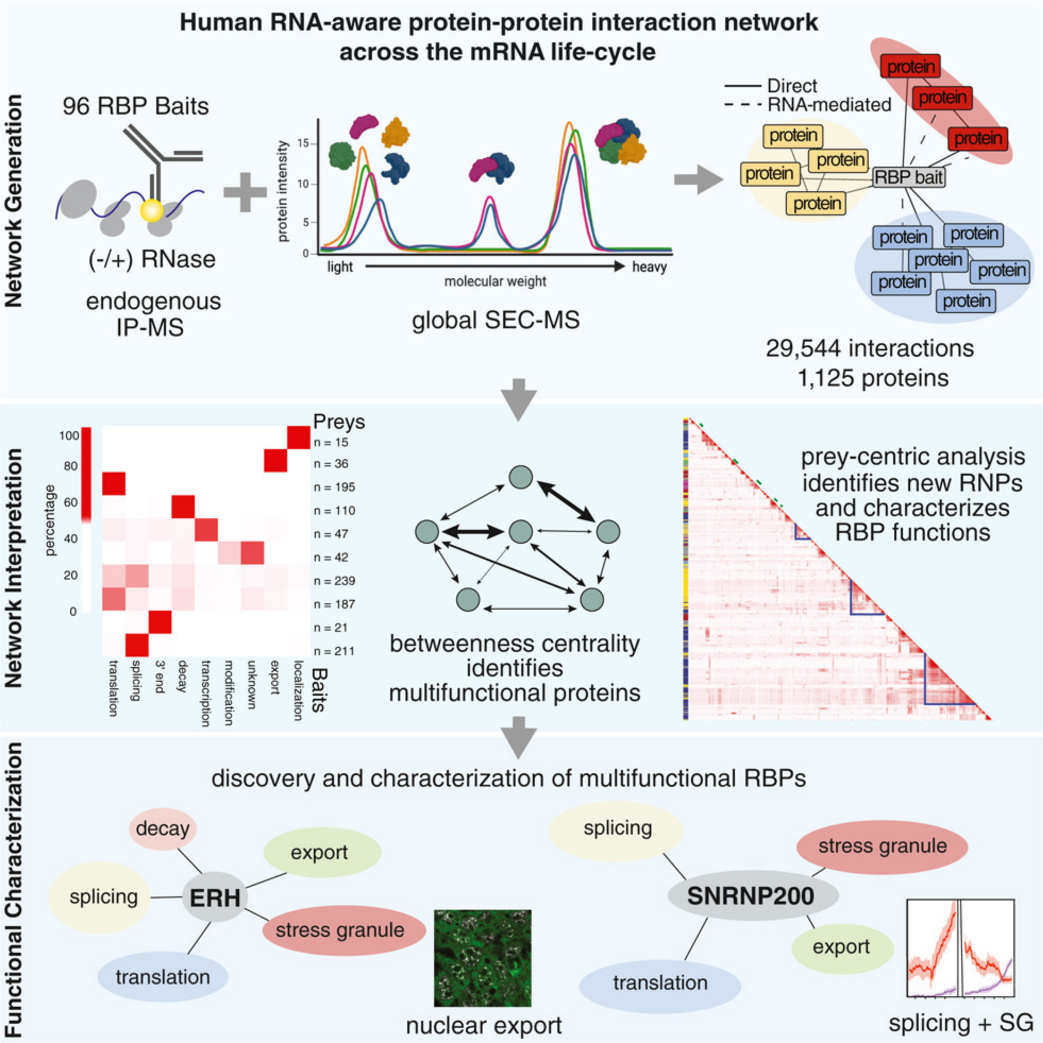
RBP-RBP interactions

RBPs will bind with each other to carry out context-specific functions or different roles in the RNA life cycle



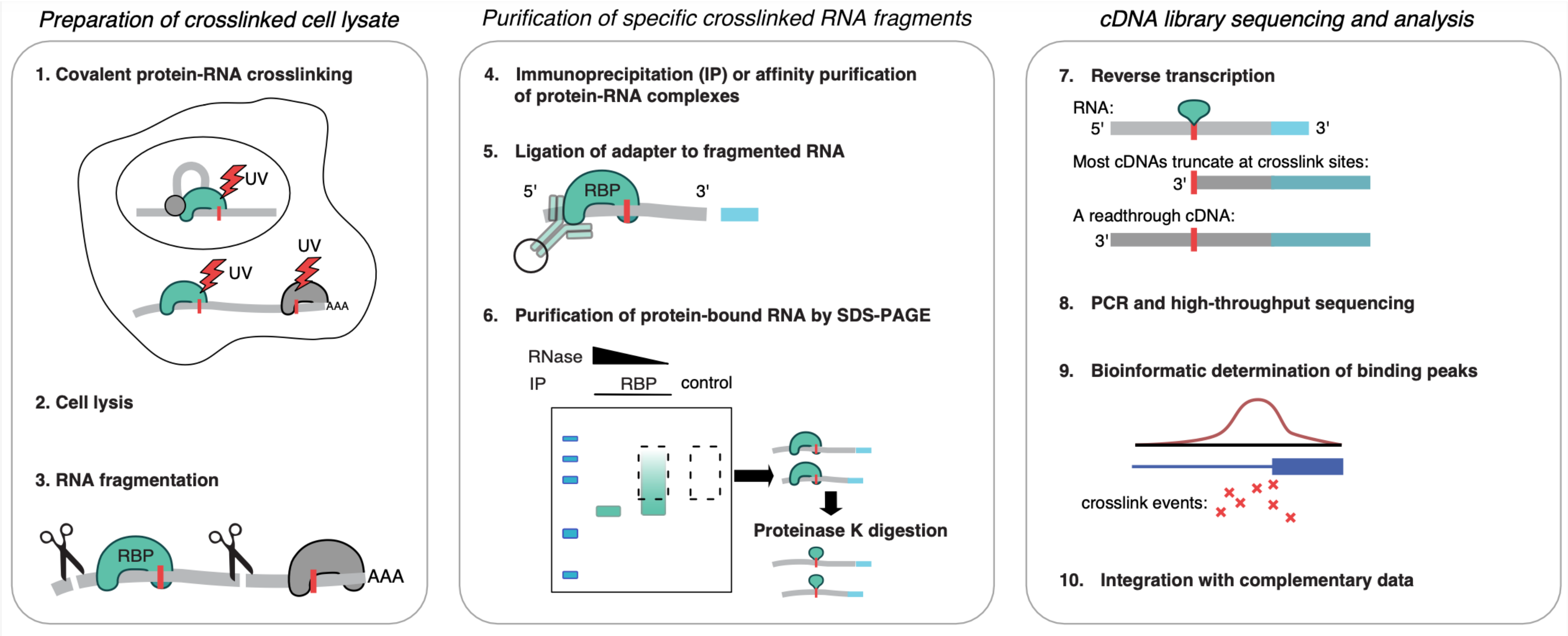
Street et. al., Mol. Cell 2024

RBP-RBP interactions



Street et. al., Mol. Cell 2024

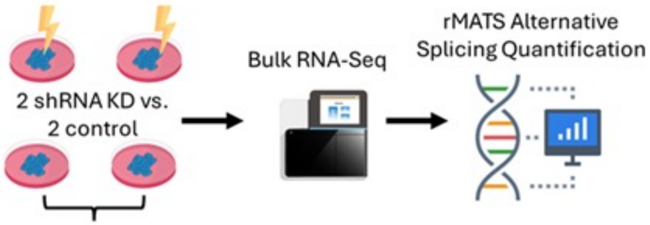
How do we assay RBP-RNA binding?

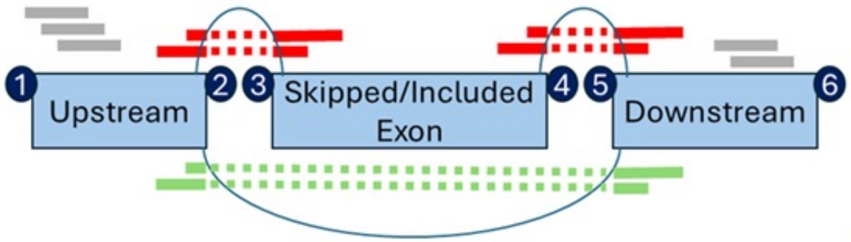


Hafner, Nature Reviews Methods Primers, 2021.

How do we assay function?

Example: alternative splicing





Percent Spliced In (PSI) = Inclusion (🟥) / (Inclusion (🟥) + Skipping (🟩)) = ★ 4/6 (0.66)

Courtesy of Y. Raghav

Integrated RBP data (ENCORE)

- eCLIP for 150 RBPs: 120 in K562, 103 in HepG2, 73 in both
- RNA-seq following knockdown (KD—RNA-seq) for 263 RBPs, also in K562 and HepG2
- eCLIP + matched KD RNA-seq for 105 (HepG2), 139 (K562) RBPs

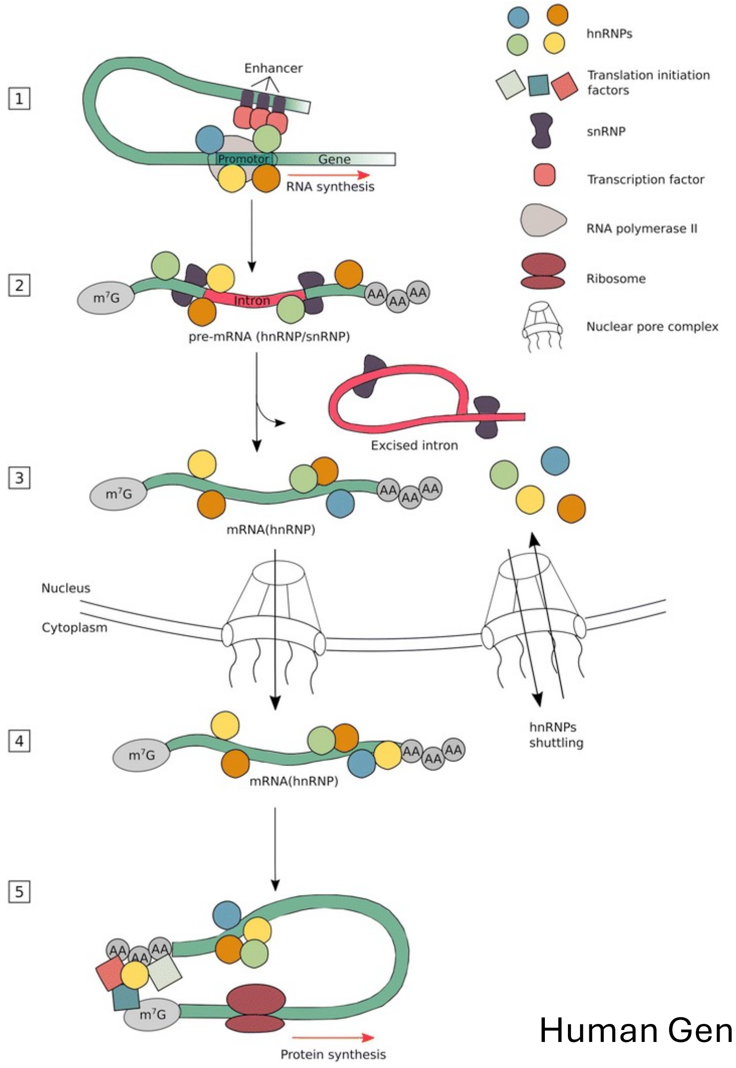
RBP families you should know

RNA binding proteins: hnRNPs

Heteronuclear RNA nucleoproteins (hnRNPs) help package the pre-mRNA into functional complexes. Abundant in cells and tissues.

- ~20 major types of hnRNPs
- Control mRNA export
- Regulate alternative splicing and polyadenylation (hnRNPA2/B1)
- Some hnRNPs shuttle between nucleus and cytoplasm
- Involved in mRNA translation

Role of hnRNPs in RNA metabolism



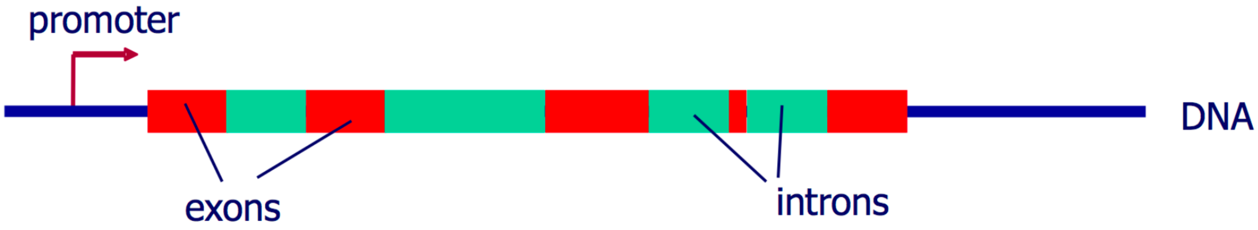
Human Genetics 2016 Geuens et al.

RNA binding proteins: SR proteins

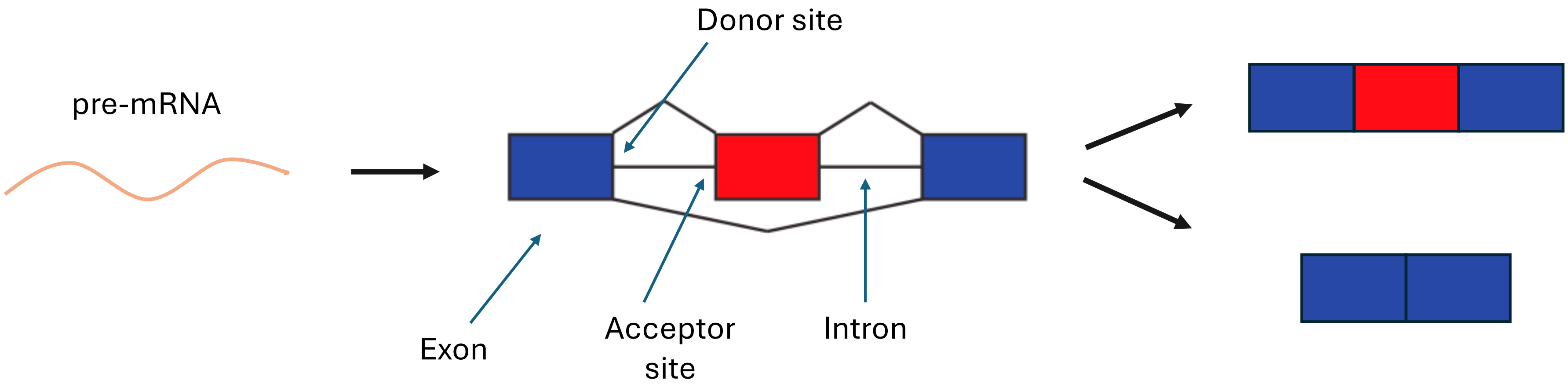
- Nuclear but shuttle between nucleus and cytoplasm
- Can bind RNA through RRM domain and other proteins through a characteristic SR domain (Arginine:Serine dipepetide)
- Control splicing and alternative splicing
- Involved in splice site selection and exon inclusion
- Regulate transcriptional elongation (RNA polII CTD interactions and R-loop formation)
- Involved mRNA translation and microRNA processing
- Cancer

Part II: RBP Function

mRNA processing



Splicing (usually) removes introns and joins exons



pre-mRNA

Donor site

Exon

Acceptor site

Intron

From Park, et. al, AJHG 2018

Splicing (usually) removes introns and joins exons

From Park, et. al, AJHG
2018

How does the cell decide which exons to keep?

…through recruitment of the core splicing machinery (the spliceosome)

- these include RNA binding proteins (RBPs)

The Spliceosome:

- Catalyzes pre-mRNA splicing in the nucleus
- Composed of five small nuclear RNAs (snRNAs) and associated proteins (snRNPs) : Snurps

snRNAs: U1, U2, U4/U6 and U5

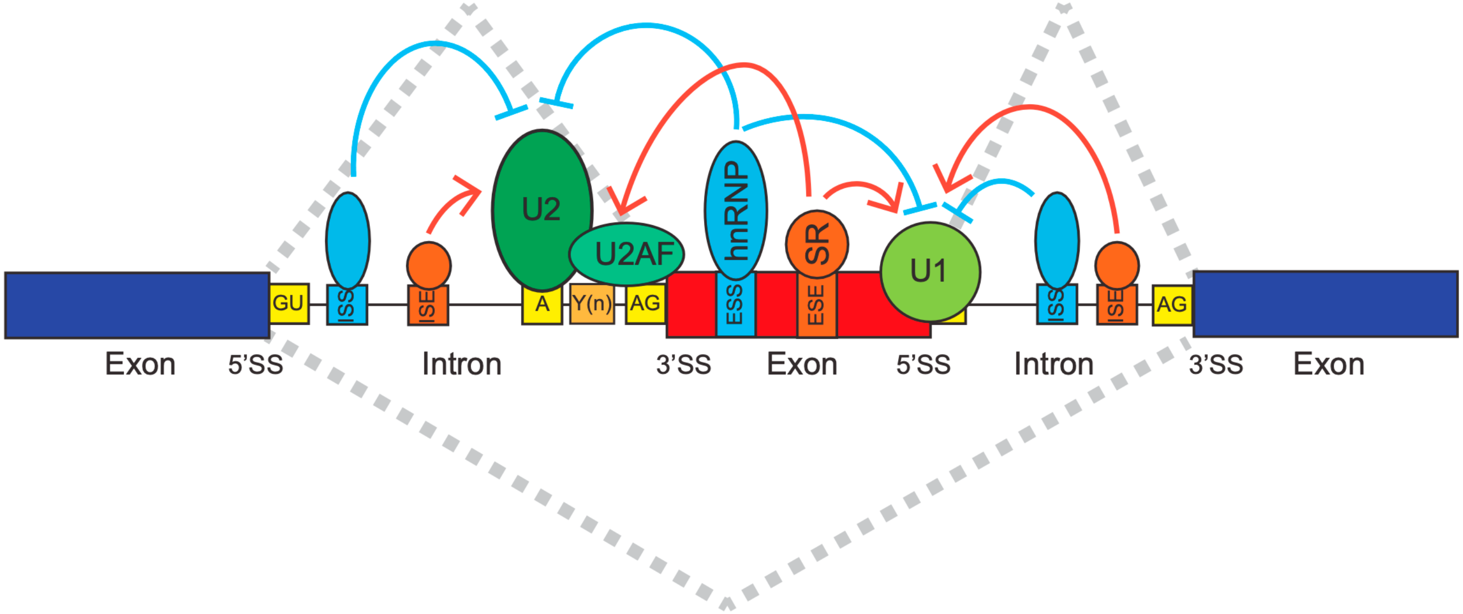
Proteins >100 yeast, >300 in human

- Splicing reaction is catalyzed by RNA

How does the cell decide which exons to keep?

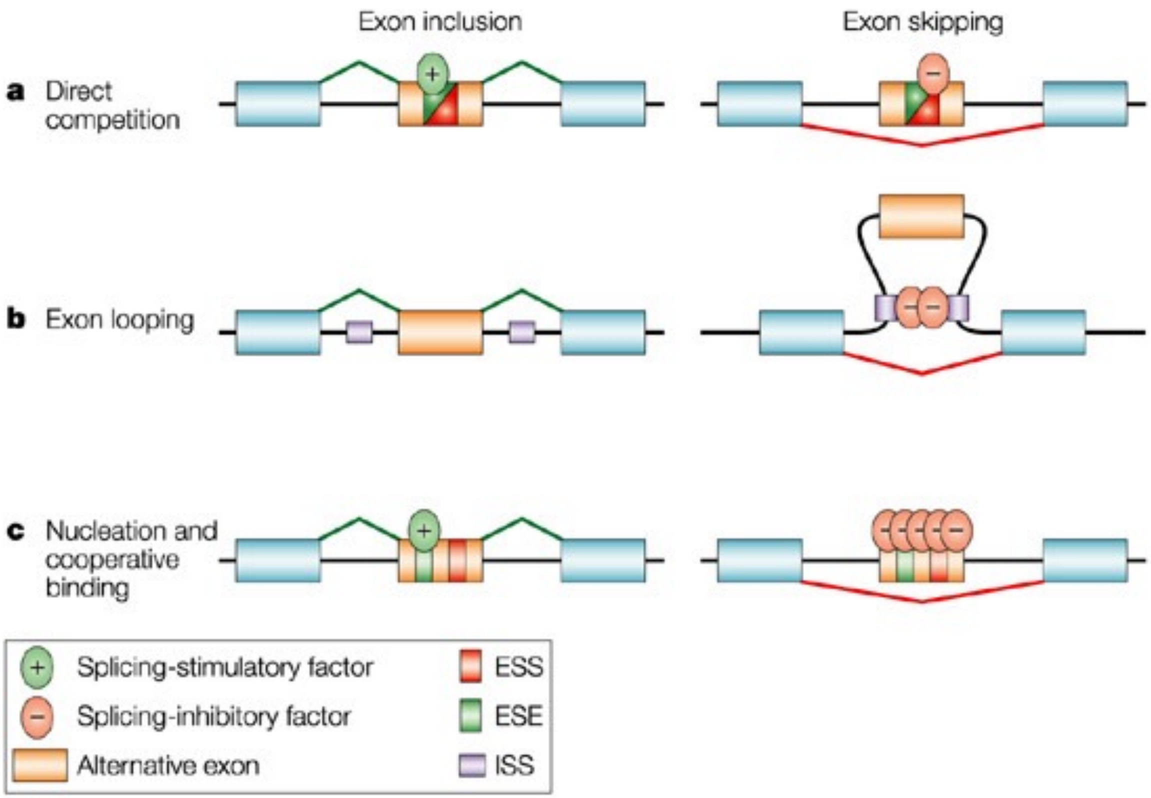
...through recruitment of the core splicing machinery (the spliceosome)

- these include RNA binding proteins (RBPs)



From Park, et. al, AJHG 2018

Alternative splicing is regulated by RNA binding proteins



ESS: Exonic splicing silencer element
ESE: Exonic splicing enhancer element
ISS: Intronic splicing silencer element

Nature Reviews | Genetics

Courtesy M. Kuyumcu-Martinez

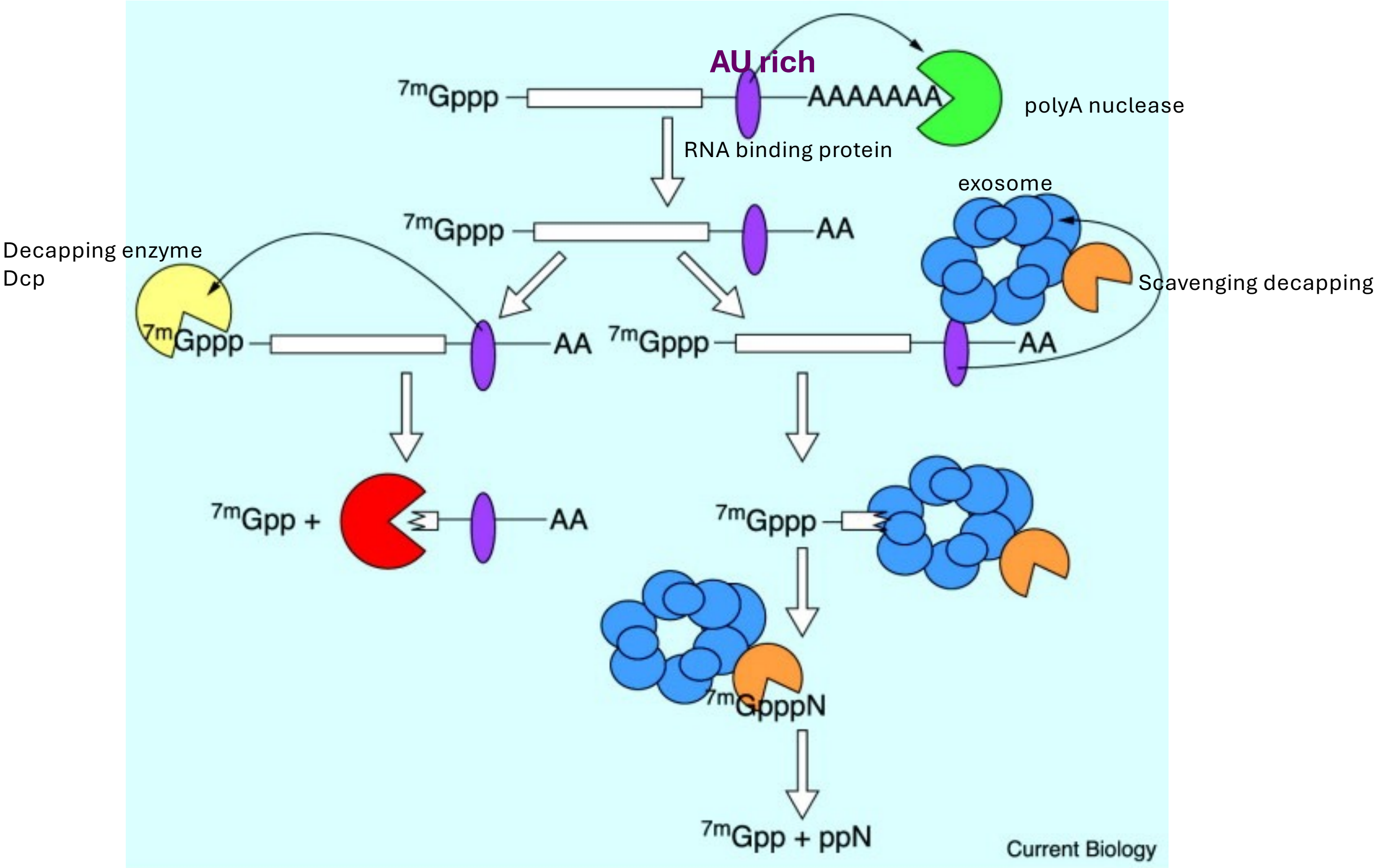
RNA binding proteins and mRNA decay

- Examples of short lived mRNAs
  - Cytokines, TNFa
  - Cell Cycle Genes
    - S-Phase, c-Fos, c-Jun

- Sequences in the 3'UTR (untranslated region)
  - Multiple copies (sometimes overlapping) of AUUUA, AU-rich elements (ARE)
  - **UGUUUGUUUGU**- GU- rich elements (GRE)
  - RNA binding proteins bind and regulate mRNA stability and degradation

Courtesy M. Kuyumcu-Martinez

RNA binding proteins that bind to AU-rich elements (ARE) regulate mRNA decay



Courtesy M. Kuyumcu-Martinez

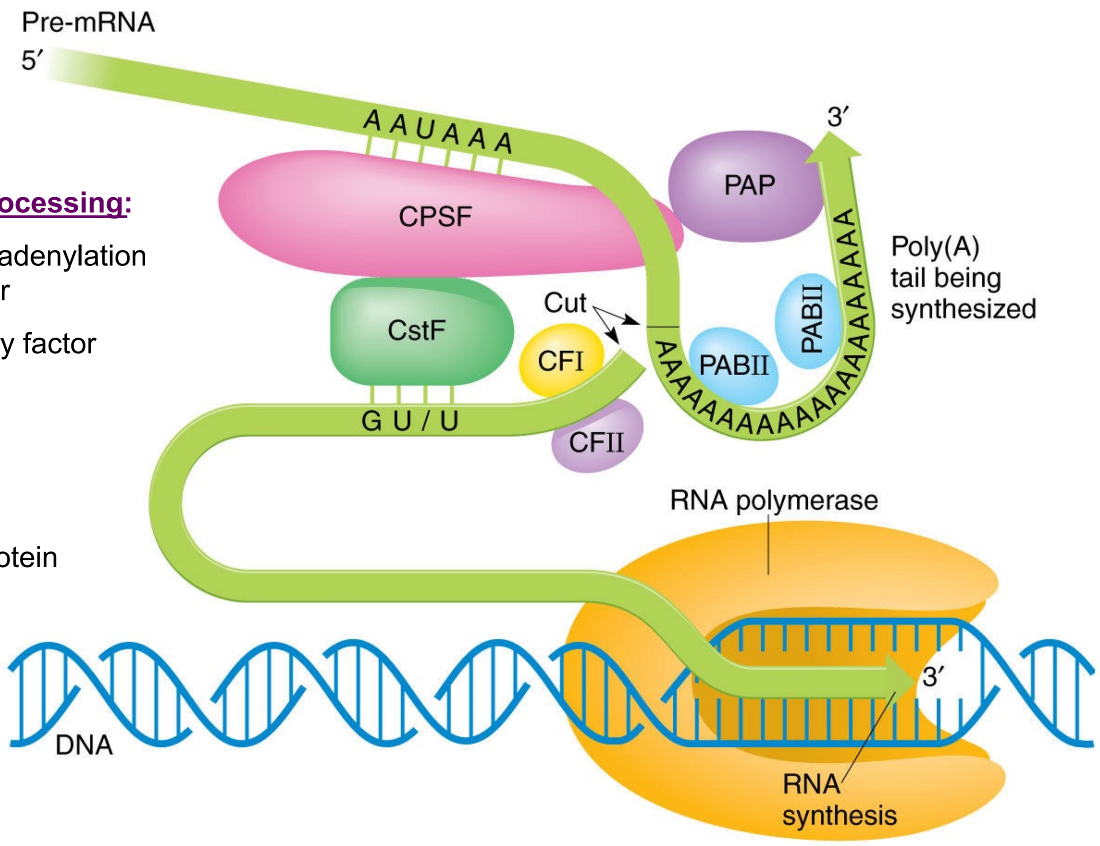
RNA binding proteins and 3’ cleavage and polyadenylation

- RNA polymerase II does not usually terminate at distinct site
- Pre-mRNA is cleaved ~10-35 nucleotides downstream of polyadenylation signal (AAUAAA)
- ~200 Adenosine monophosphates (AMP) are then added to the 3´ end of pre-mRNA.
- Almost all mRNAs have poly(A) tail

Courtesy M. Kuyumcu-Martinez

Determinants of 3' mRNA processing

b) Addition of the poly(A) tail



__Proteins involved in 3' processing:__

CPSF – cleavage and polyadenylation specificity factor

CstF – cleavage stimulatory factor

CFI – cleavage factor I

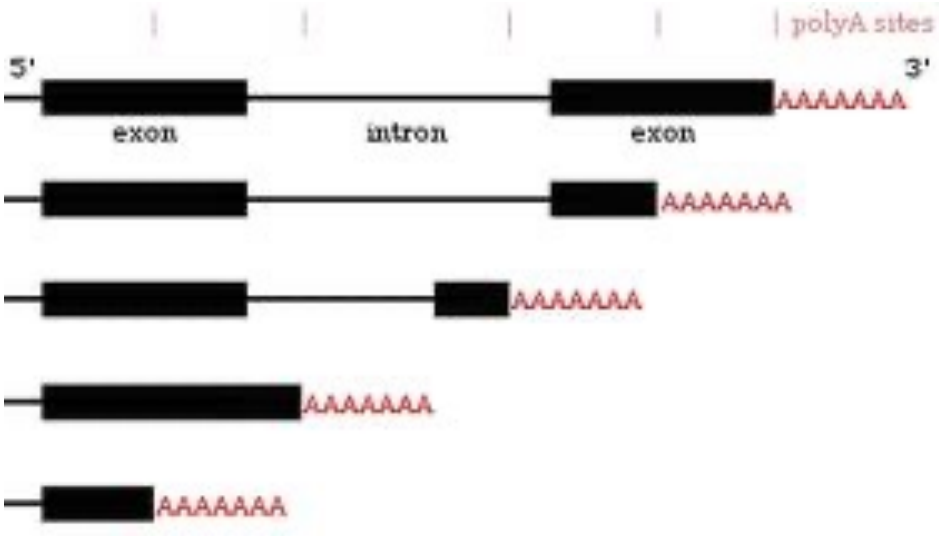
CFII – cleavage factor II

PAP – polyA polymerase

PABP II – polyA binding protein

Courtesy M. Kuyumcu-Martinez

Regulation of polyadenylation sites



- Alternative splicing affects poly(A) sites
- Shortening of 3'UTR by alternative polyadenylation
  - -cancer cells (reduced microRNA regulation) (Cell 2009)
  - -oncogenes (Cell 2009)

Courtesy M. Kuyumcu-Martinez

mRNA export occurs through the nuclear pores

29 November 2007 www.nature.com/nature $10

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

GEROSCIENCE
Can old age be 'healthy'?

DIABETES
Resveratrol-like drugs show potential

CORE-MANTLE INTERACTION
Earth, the remix

GATEKEEPER FOR THE NUCLEUS

Architecture of the nuclear pore complex

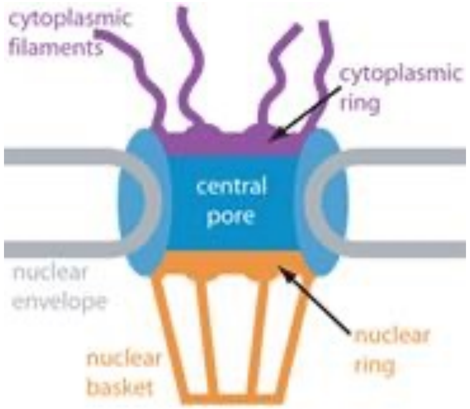
NATUREJOBS
Life science collaborations

Nuclear pore: "Gatekeeper" for the nucleus

- Composed of protein complexes and cytoplasmic filaments to move the cargo in and out

- Import and export of proteins occur through the nuclear pore

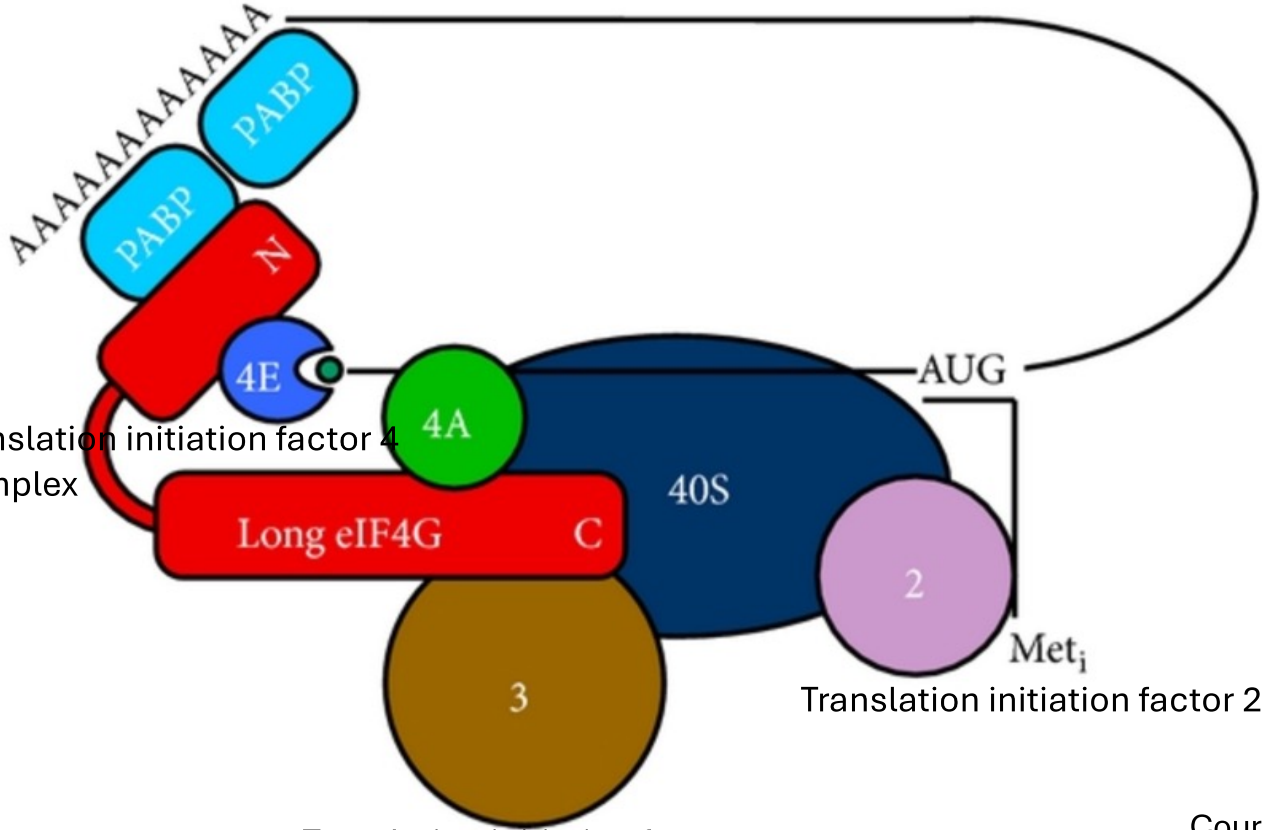
- Export of mRNAs to the cytoplasm for protein synthesis in the cytoplasm



Courtesy M. Kuyumcu-Martinez

RBPs and mRNA translation

Translation factors that circularize mRNAs and recruit ribosomes are RBPs.



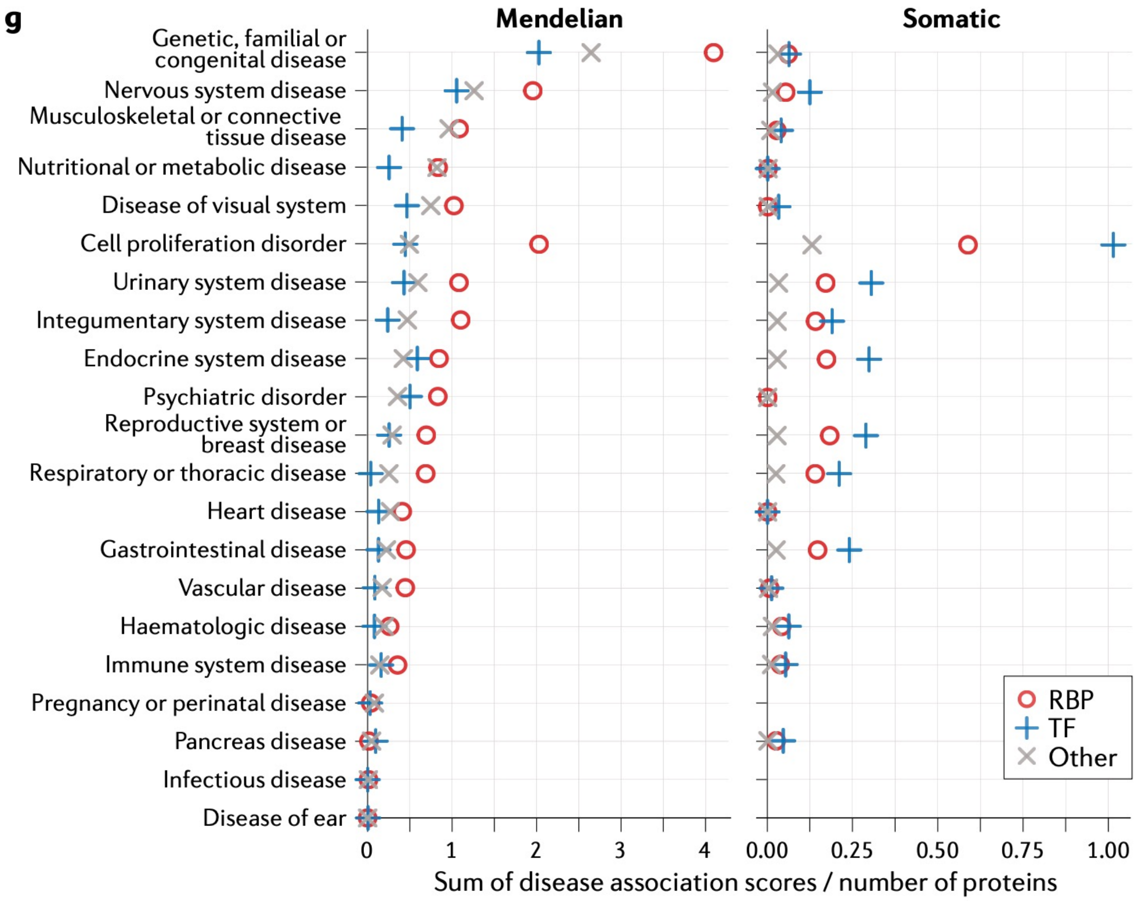
Translation initiation factor 4 complex

Translation initiation factor 2

Translation initiation factor 3

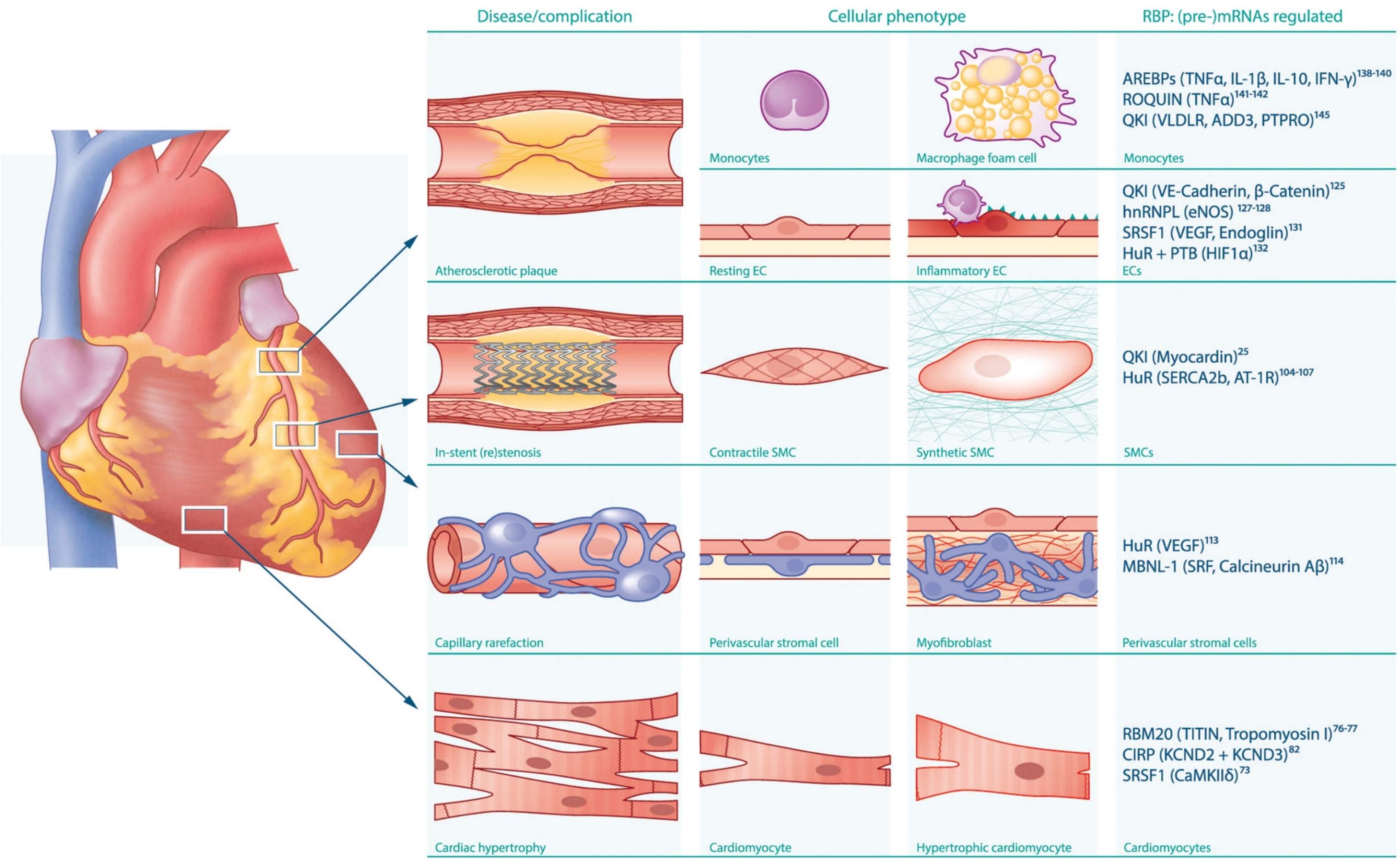
Courtesy M. Kuyumcu-Martinez

RBPs and Disease



“Therapeutic areas of disease-associated RBPs. Disease mutations with an association score >0.2 from Open Targets.” Gebauer et. al., Nat. Rev. Gen. 2020.

RBPs involved in cardiovascular disease pathogenesis

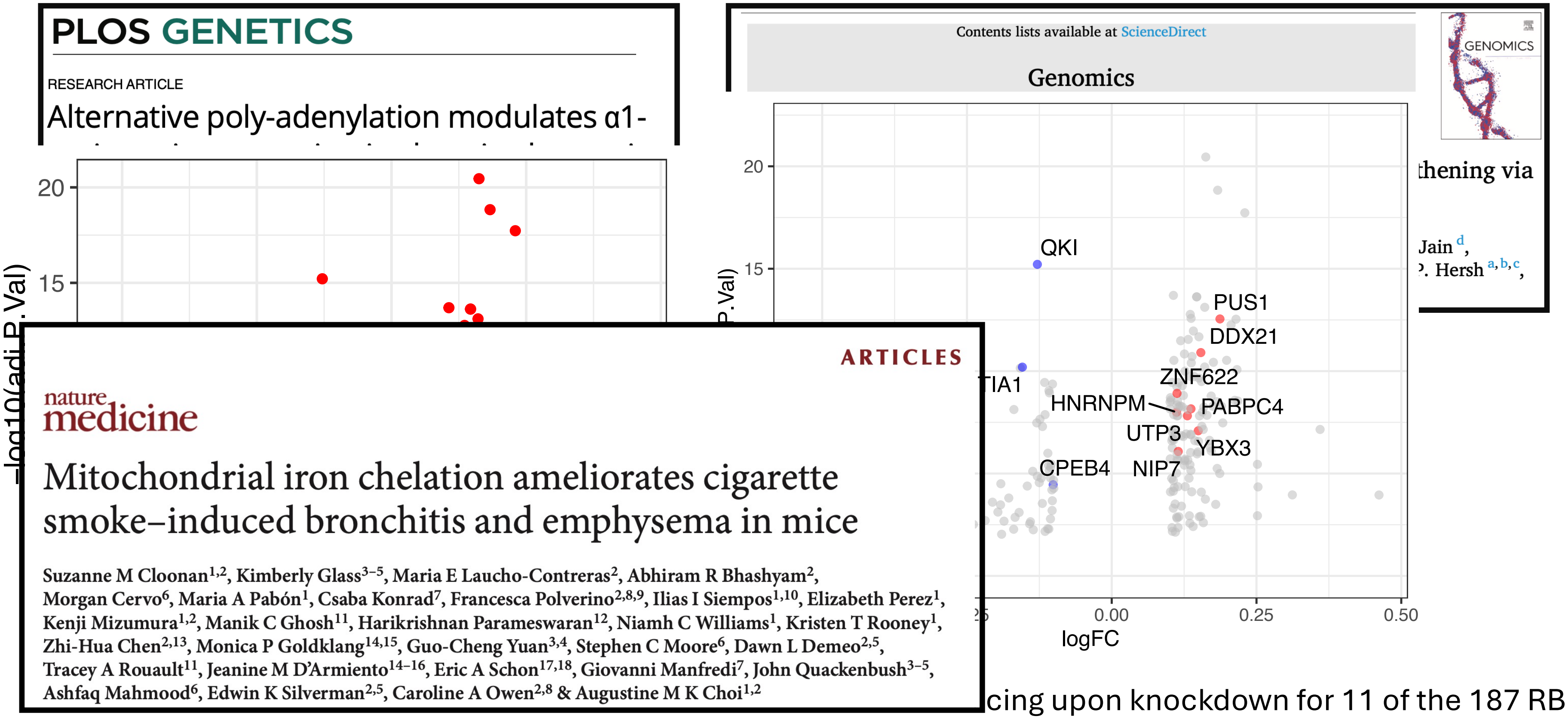


Disease/complication	Cellular phenotype		RBP: (pre-)mRNAs regulated
Atherosclerotic plaque	Monocytes	Macrophage foam cell	AREBPs (TNFα, IL-1β, IL-10, IFN-γ)[138-140] ROQUIN (TNFα)[141-142] QKI (VLDLR, ADD3, PTPRO)[145] Monocytes
	Resting EC	Inflammatory EC	QKI (VE-Cadherin, β-Catenin)[125] hnRNPL (eNOS) [127-128] SRSF1 (VEGF, Endoglin)[131] HuR + PTB (HIF1α)[132] ECs
In-stent (re)stenosis	Contractile SMC	Synthetic SMC	QKI (Myocardin)[25] HuR (SERCA2b, AT-1R)[104-107] SMCs
Capillary rarefaction	Perivascular stromal cell	Myofibroblast	HuR (VEGF)[113] MBNL-1 (SRF, Calcineurin Aβ)[114] Perivascular stromal cells
Cardiac hypertrophy	Cardiomyocyte	Hypertrophic cardiomyocyte	RBM20 (TITIN, Tropomyosin I)[76-77] CIRP (KCND2 + KCND3)[82] SRSF1 (CaMKIIδ)[73] Cardiomyocytes

Diabetic cardiomyopathy Rbfox2
Hypoplastic left heart syndrome

Courtesy M. Kuyumcu-Martinez

RBPs in COPD



PLOS GENETICS

RESEARCH ARTICLE

Alternative poly-adenylation modulates α1-

Contents lists available at ScienceDirect

Genomics

thening via

Jain [d],

?. Hersh [a,b,c],

ARTICLES

nature medicine

Mitochondrial iron chelation ameliorates cigarette smoke–induced bronchitis and emphysema in mice

Suzanne M Cloonan[1,2], Kimberly Glass[3–5], Maria E Laucho-Contreras[2], Abhiram R Bhashyam[2], Morgan Cervo[6], Maria A Pabón[1], Csaba Konrad[7], Francesca Polverino[2,8,9], Ilias I Siempos[1,10], Elizabeth Perez[1], Kenji Mizumura[1,2], Manik C Ghosh[11], Harikrishnan Parameswaran[12], Niamh C Williams[1], Kristen T Rooney[1], Zhi-Hua Chen[2,13], Monica P Goldklang[14,15], Guo-Cheng Yuan[3,4], Stephen C Moore[6], Dawn L Demeo[2,5], Tracey A Rouault[11], Jeanine M D'Armiento[14–16], Eric A Schon[17,18], Giovanni Manfredi[7], John Quackenbush[3–5], Ashfaq Mahmood[6], Edwin K Silverman[2,5], Caroline A Owen[2,8] & Augustine M K Choi[1,2]

cing upon knockdown for 11 of the 187 RBPs

(adj.P < 0.01 and log2FC magnitude > 0.1)

Differential isoform usage between COPD cases and controls