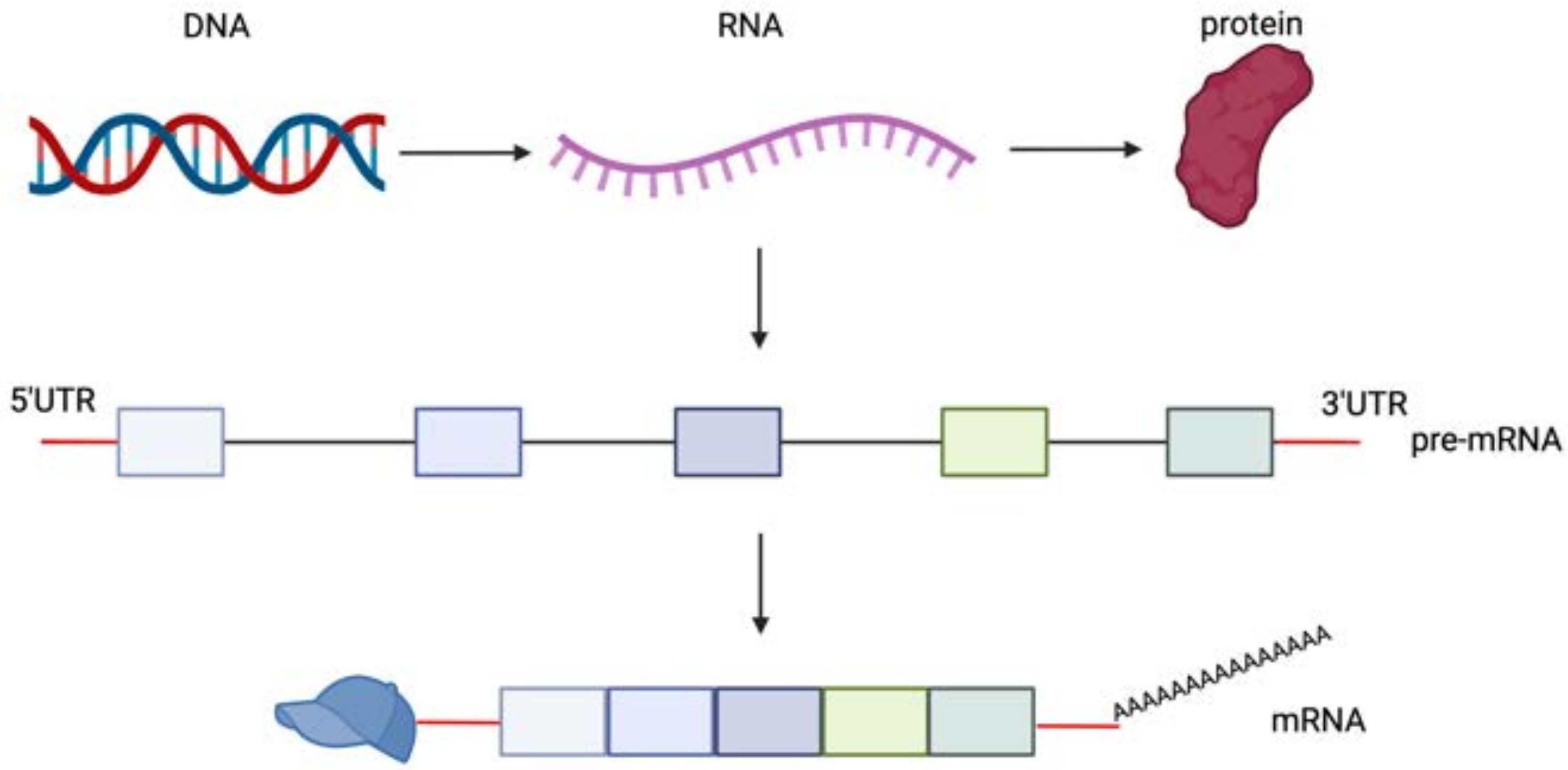
RNA life cycle

The Life of Eukaryotic mRNA

Genetic flow of information



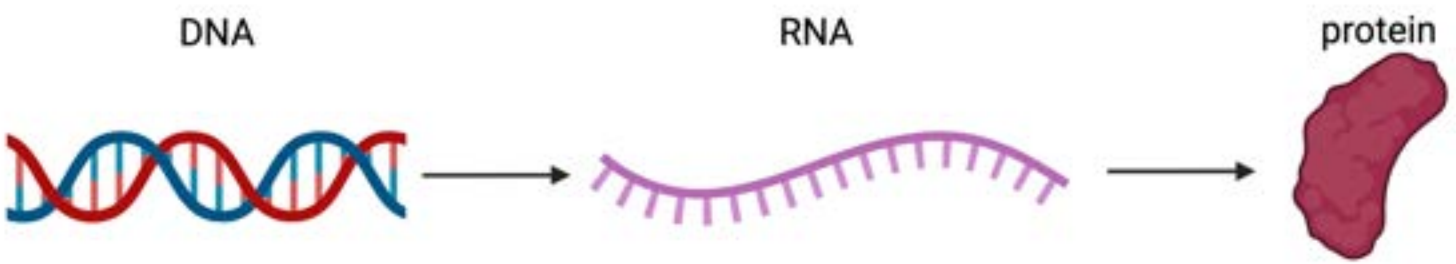
Gene expression

development

day/night

infection

nutrients



temperature

stress

WHERE?

WHEN?

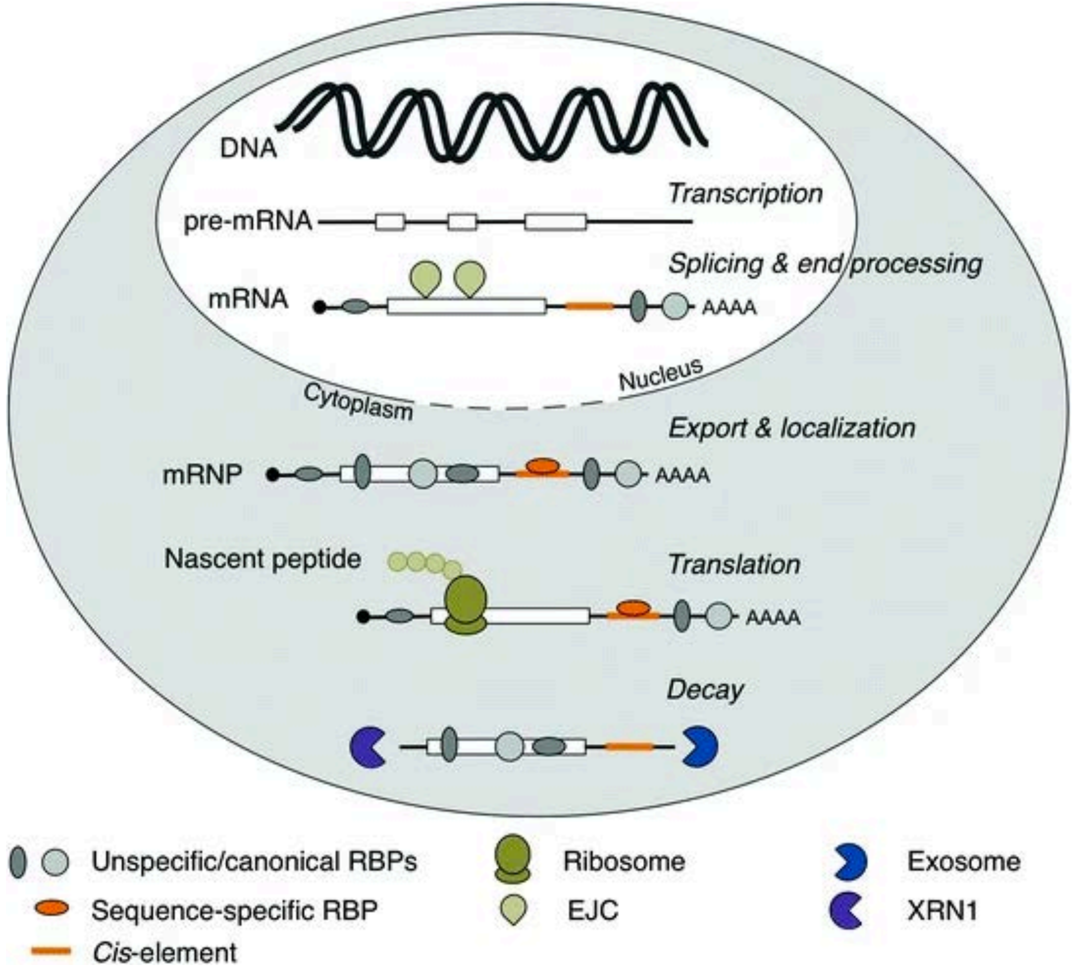
HOW MUCH?

DNA is not enough..

vs

LENGTH	~mm-cm	~1.75 m
# CELLS	~1000	~trillions
# GENES	**20000**	

The life of eukaryotic mRNA

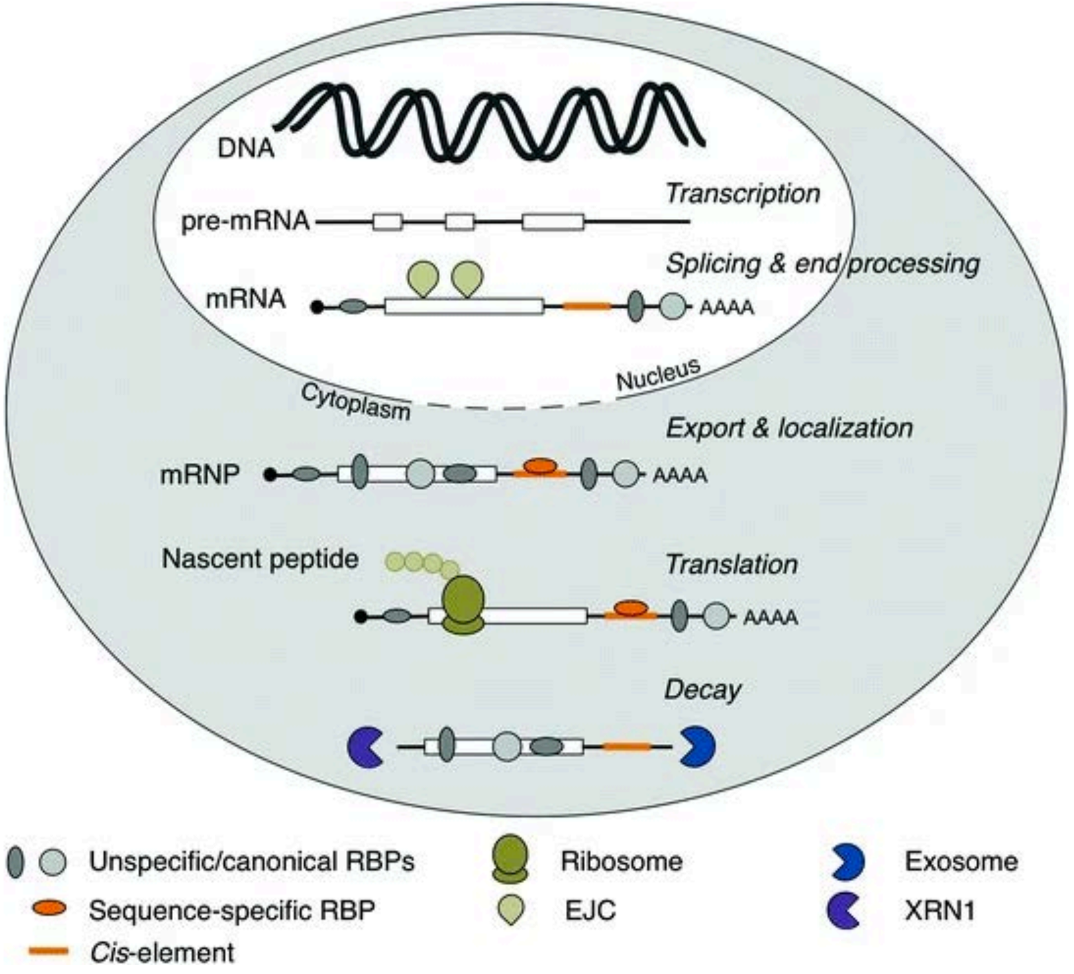


Nuclear fate of RNA

Cytoplasmic fate of RNA

RNA therapy

The life of eukaryotic mRNA



Nuclear fate of RNA

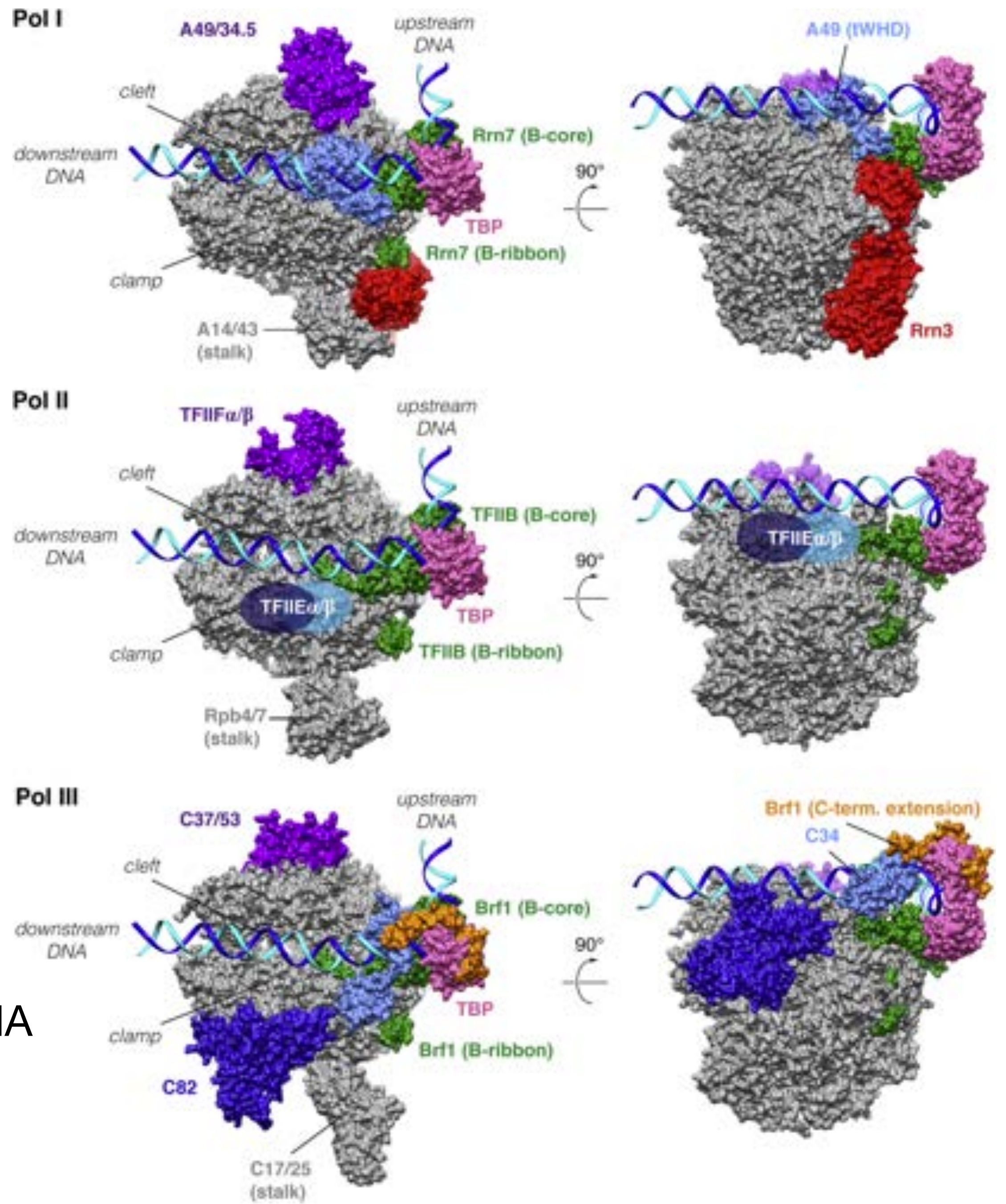
Cytoplasmic fate of RNA

Disclaimer:
- division is artificial

Nuclear fate of RNA

Birth of RNA - transcription

Eukaryotic polymerases



ribosomal RNA

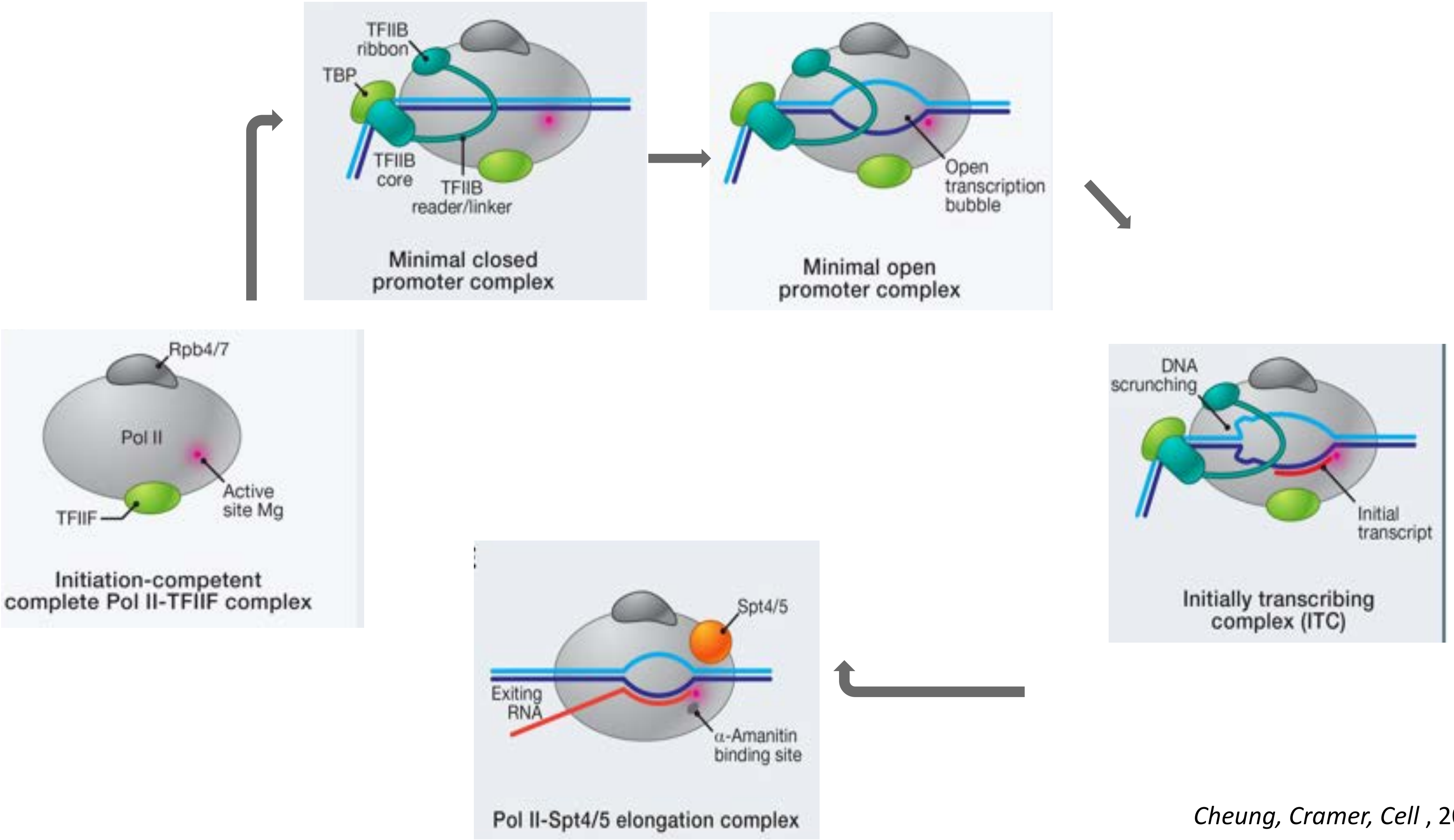
protein-coding transcripts
ncRNAs

mRNAs

transfer RNA (tRNA),
5S rRNA
other classes of small RNA

Vannini and Cramer, 2012, Mol Cell

Transcription cycle by PolII

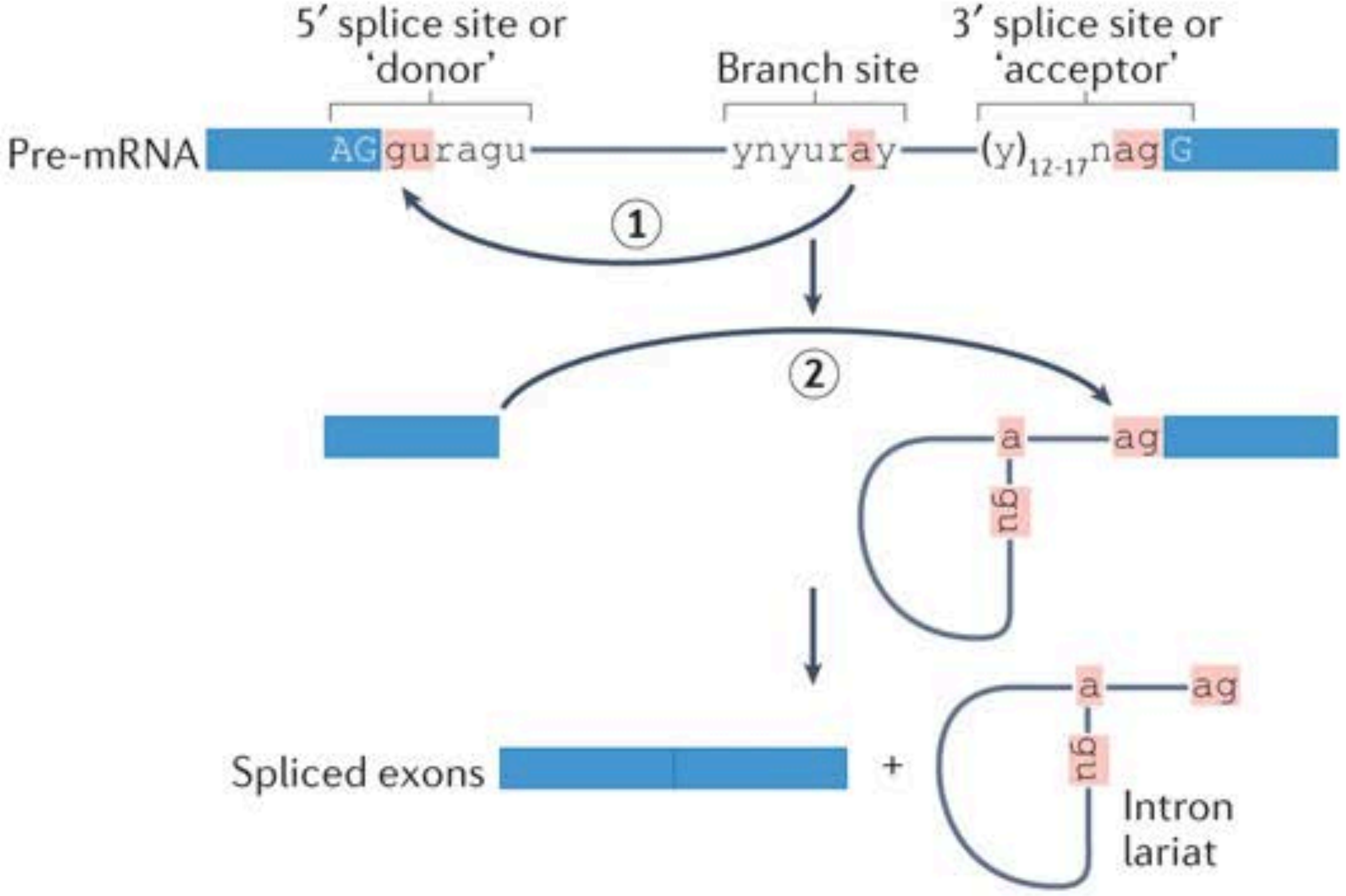


Cheung, Cramer, Cell , 2012

Nuclear fate of RNA

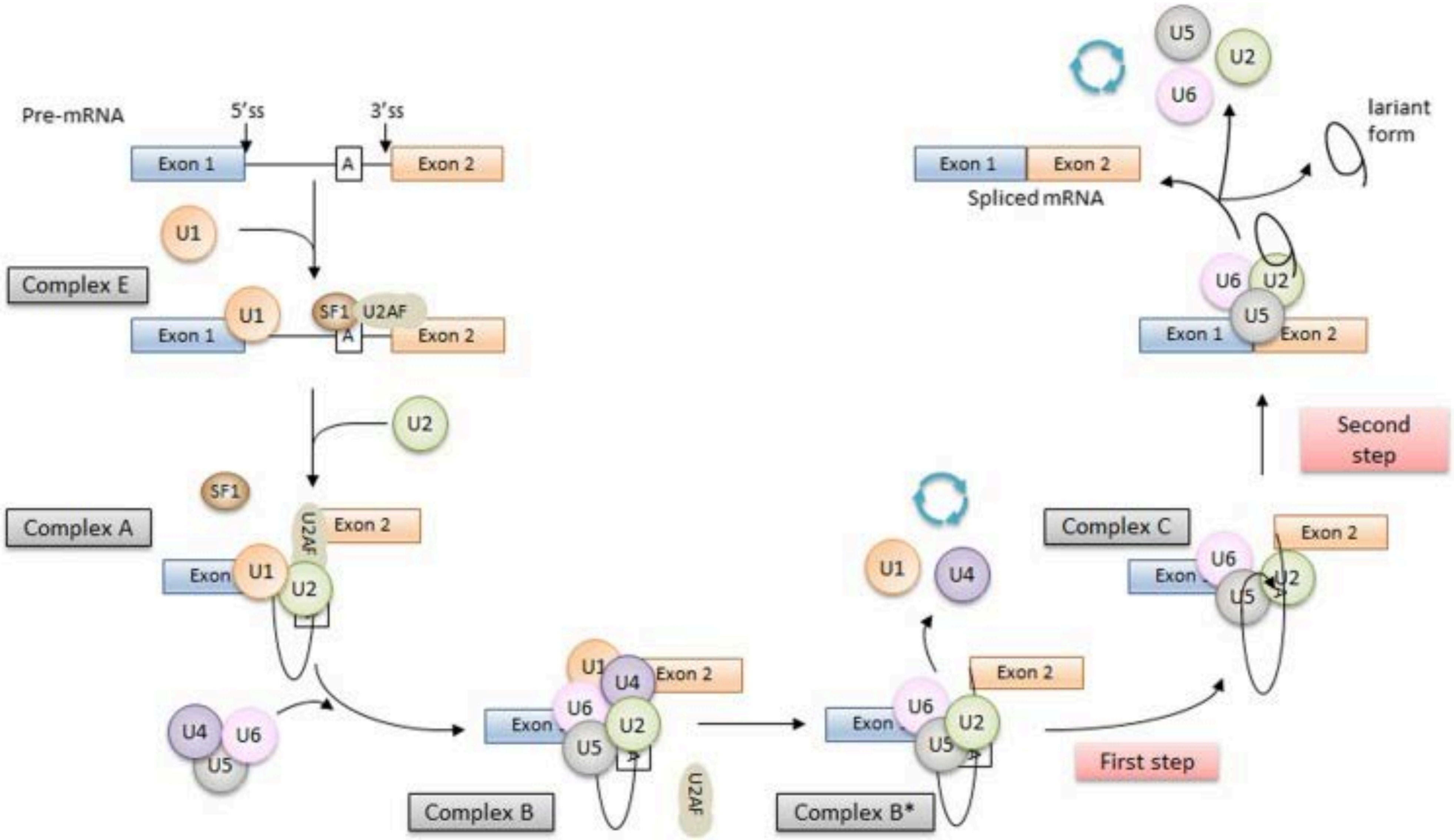
RNA matures - RNA processing

Two-step splicing reaction

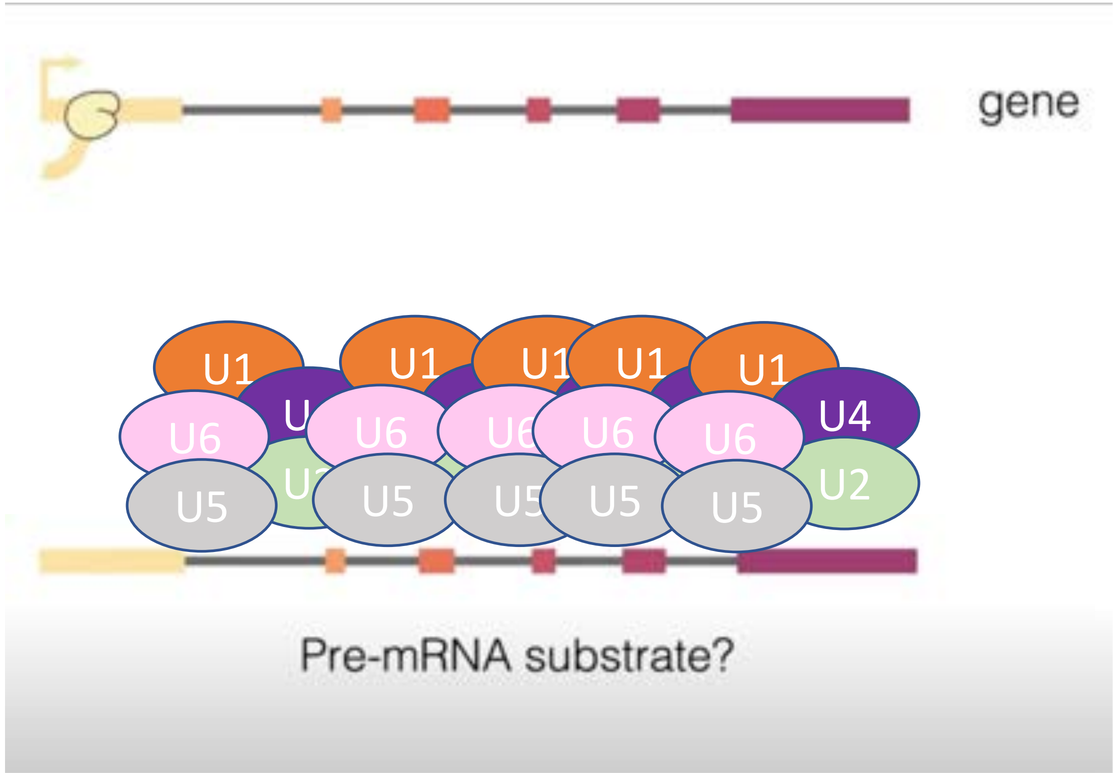


Luciano E. Marasco & Alberto R. Kornblihtt,
Nature Reviews, 2022

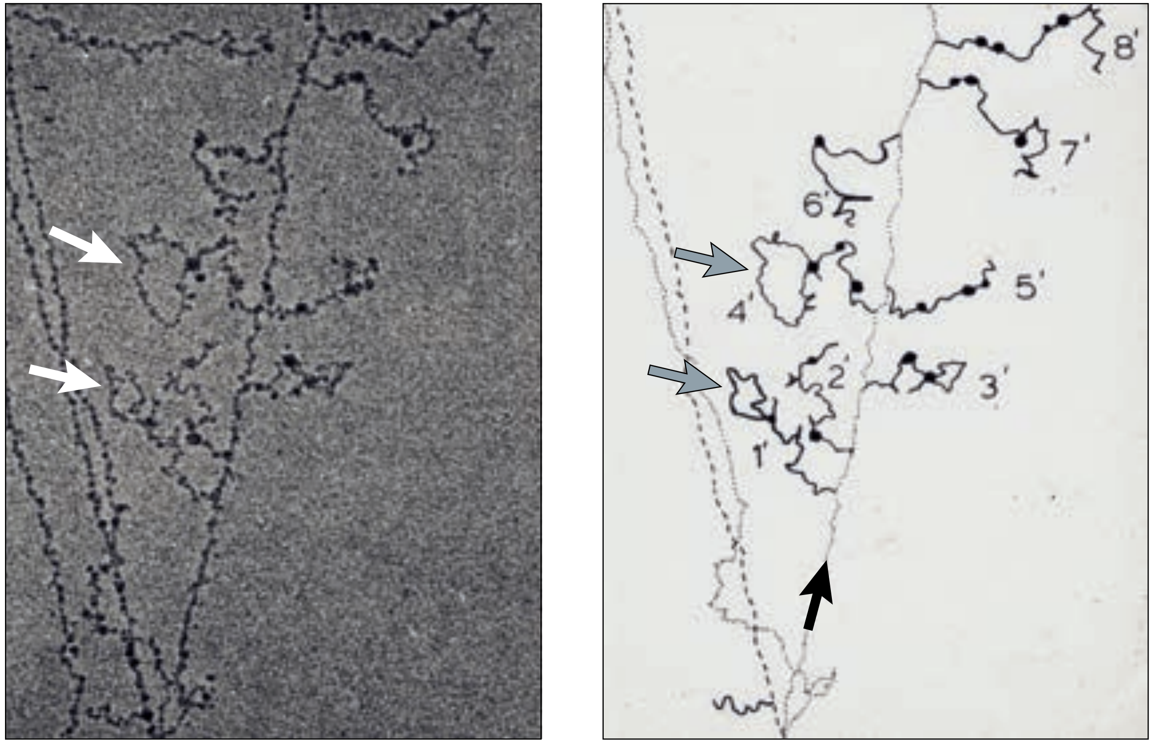
Splicing machinery



Eukaryotic gene architecture



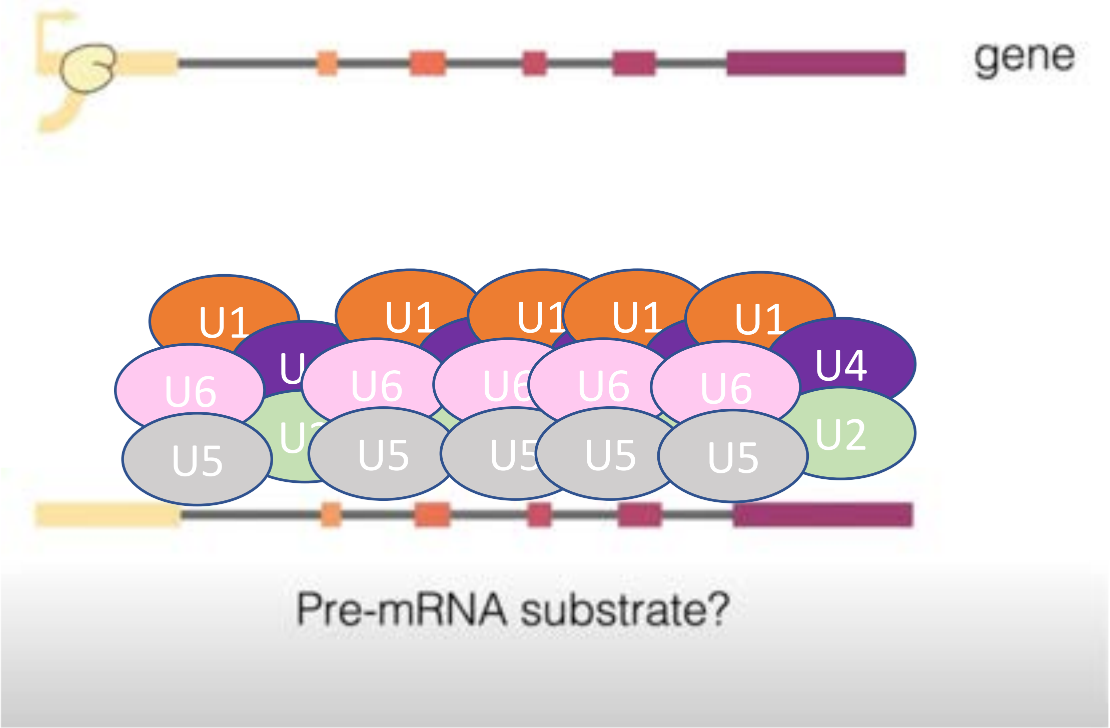
pre -mRNA maturation occurs co-transcriptionally



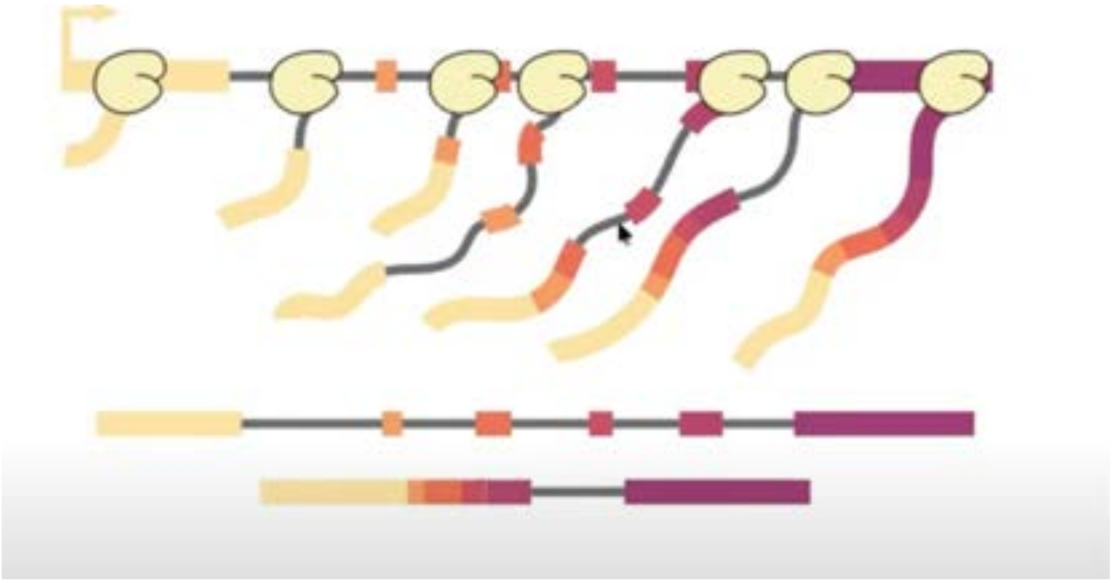
Beyer, A. L. & Osheim, Y. N. Genes Dev. 2, 754–765 (1988)

Co-transcriptional RNA splicing

Instagram

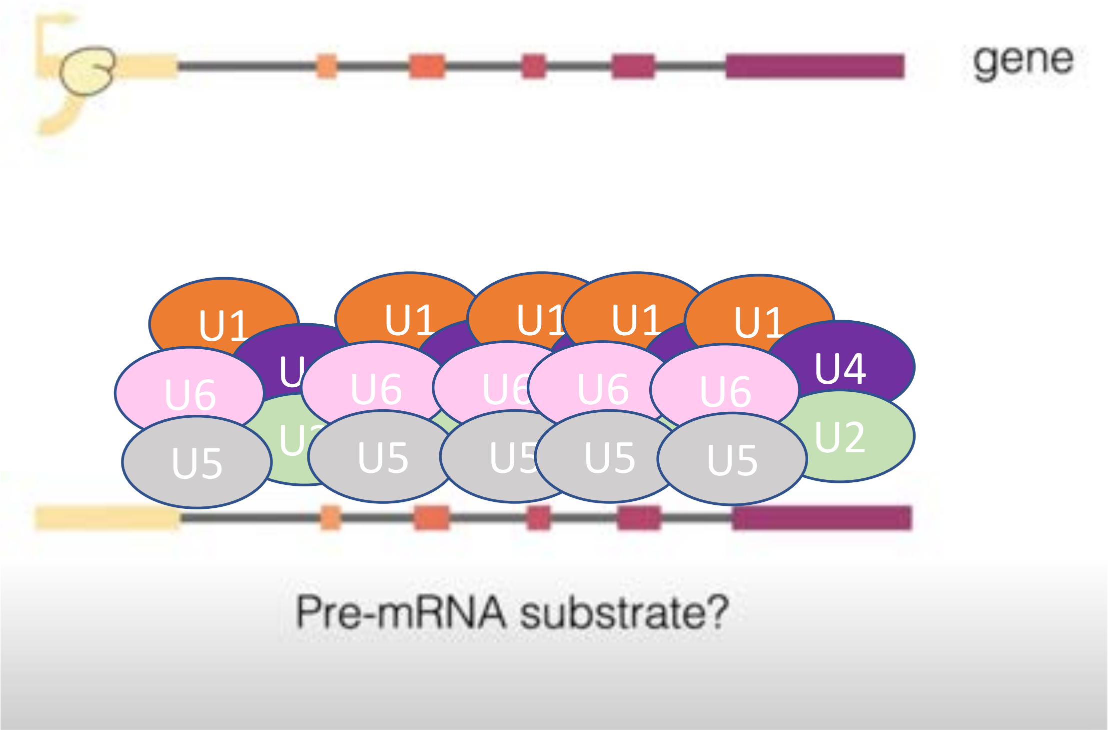


Reality

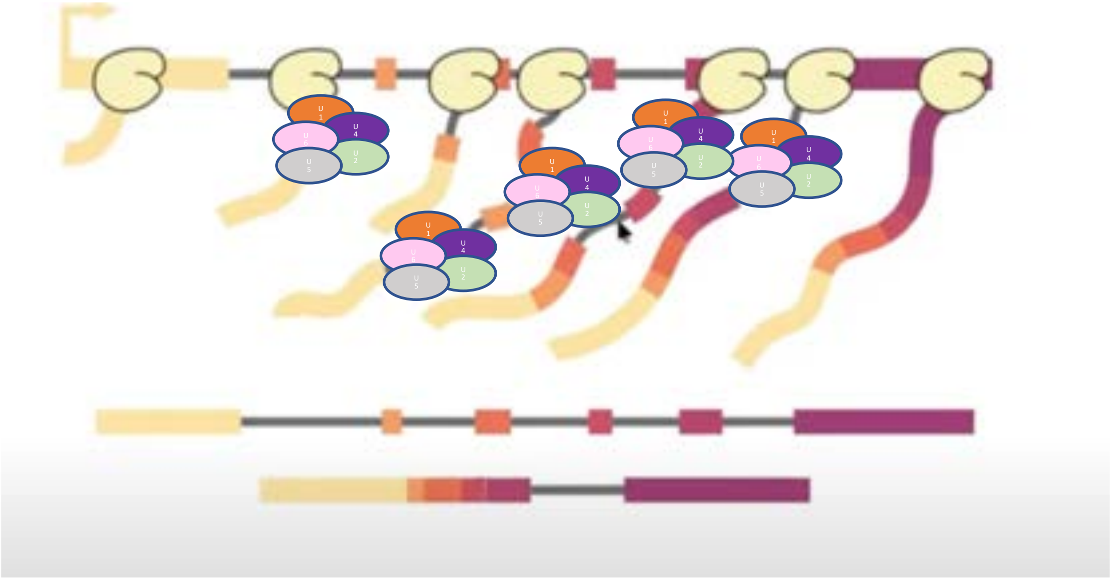


Co-transcriptional RNA splicing

Instagram

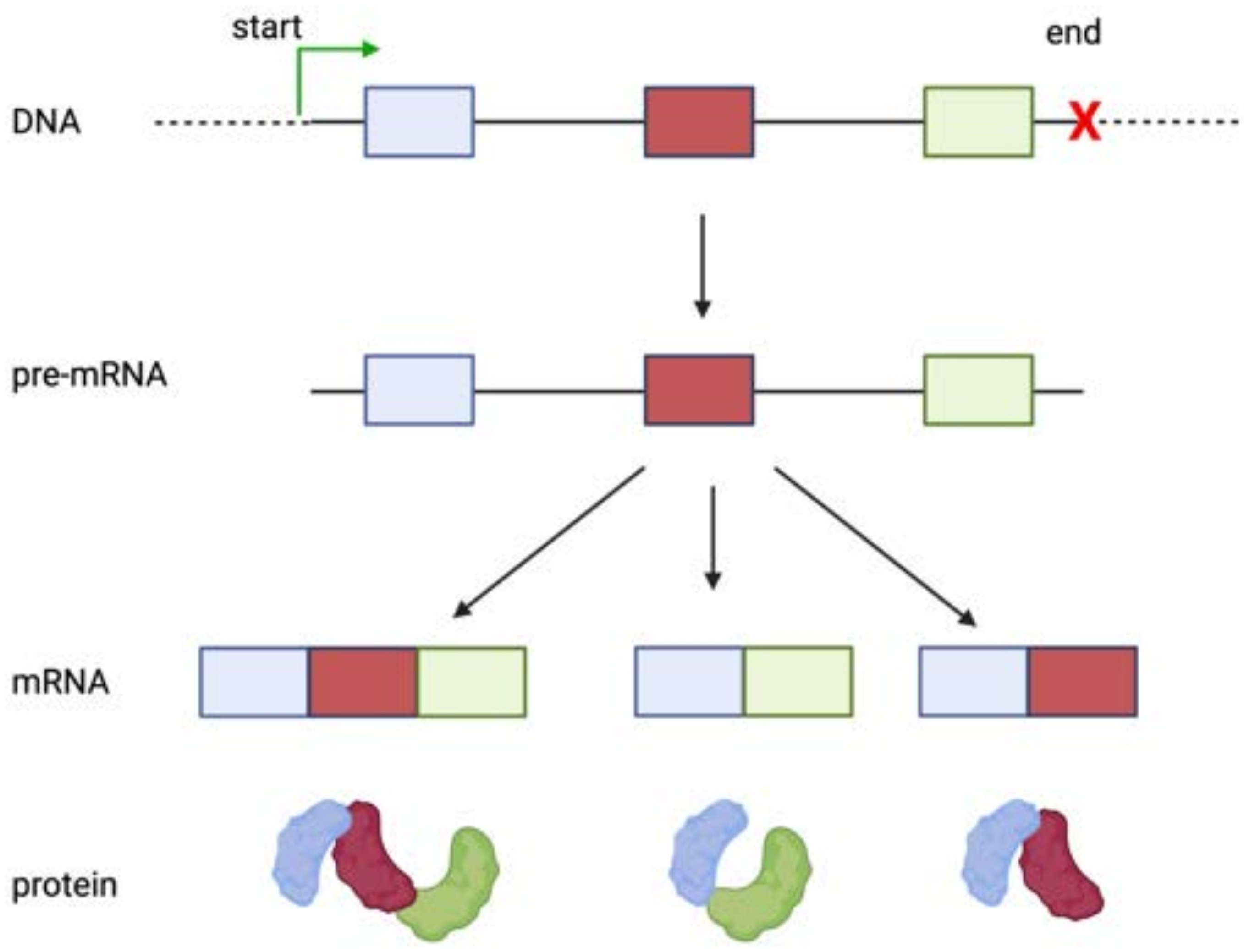


Reality

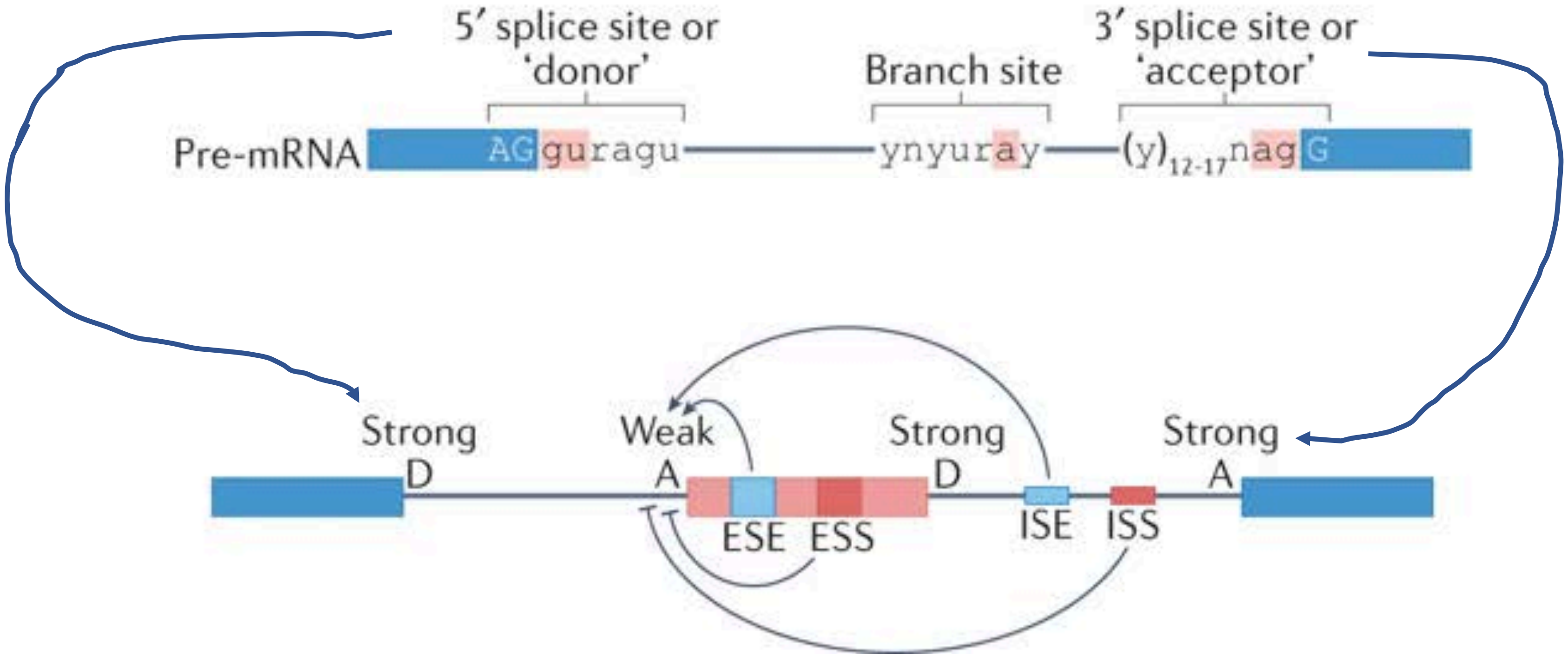


Cellular environment can modify RNA life cycle

Alternative splicing- when multiple distinct mRNAs are made from the same gene by splicing different combinations of the exons and introns



Cellular environment can modify RNA life cycle



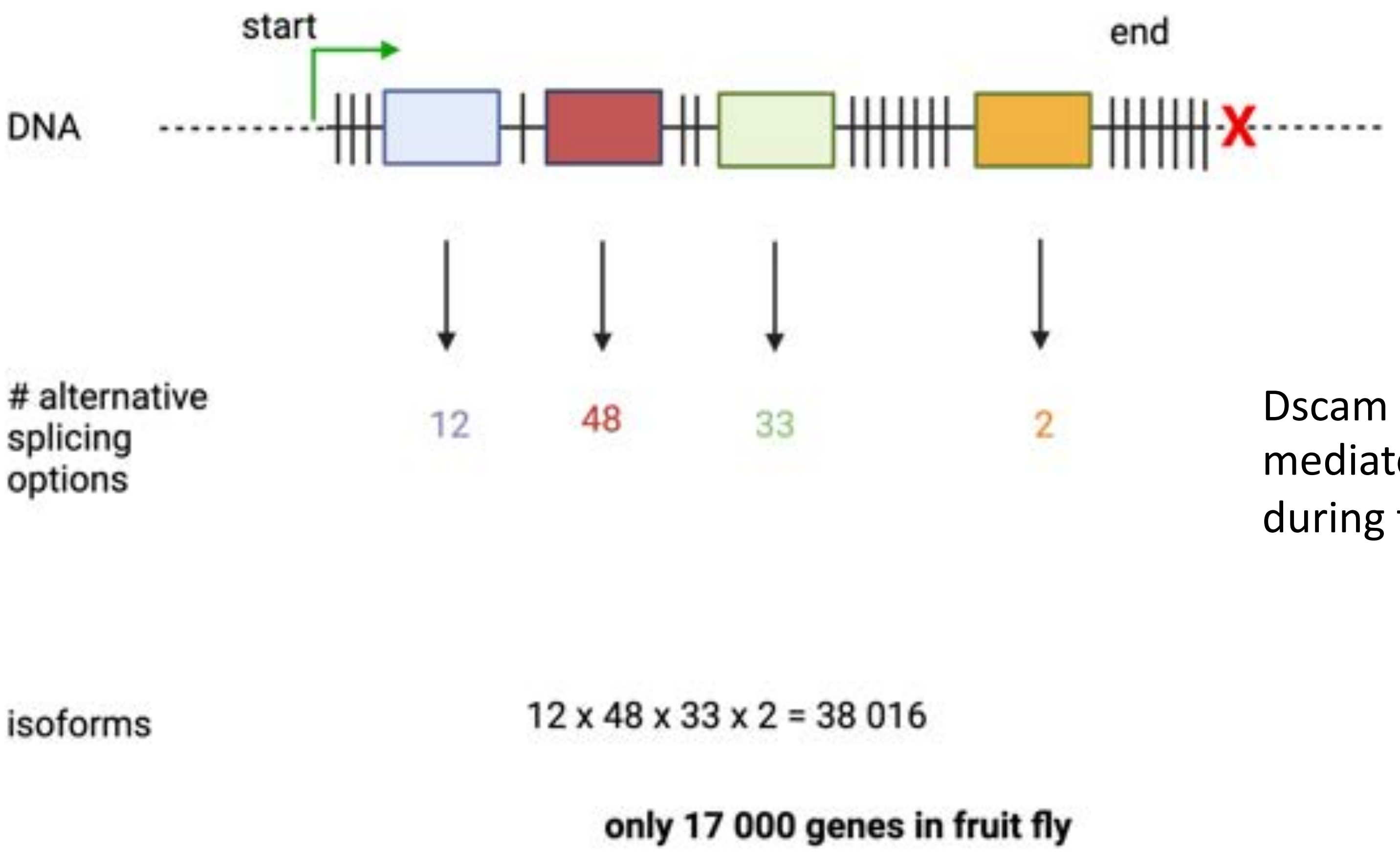
Constitutive splicing

Alternative splicing

Alternative Splicing   Alternative promoter   Alternative polyadenylation

What is the potential for the alternative splicing?

DSCAM



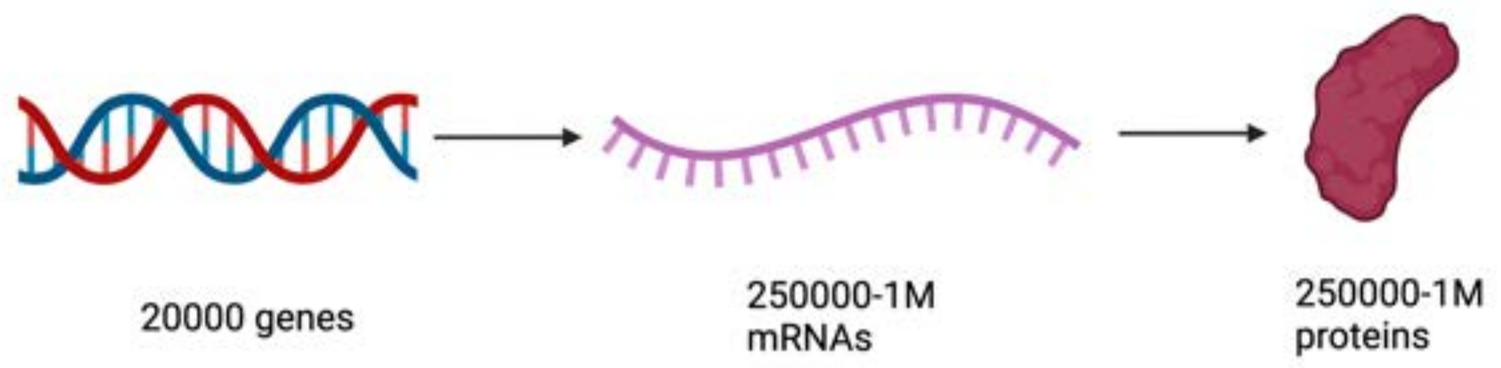
Dscam isoforms function to mediate cell recognition events during the wiring process.

Majority of human genes undergo alternative splicing

vs

LENGTH	~mm-cm		~1.75 m
# CELLS	~1000		~trillions
# GENES		**20000**	
# introns/gene	~5		~8
% of Alt Spliced genes	~ 10-25%		~ 70-95%

Alternative processing leads to increased proteome diversity

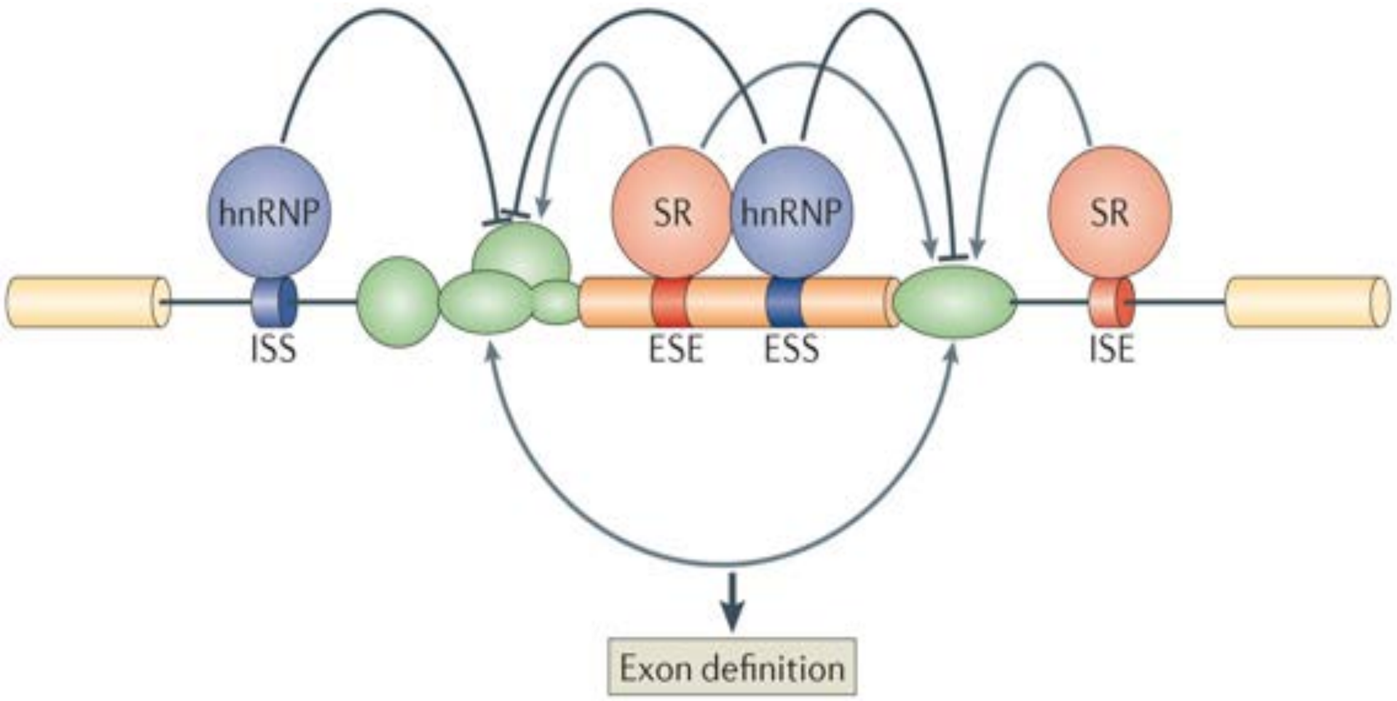


Nuclear fate of RNA

RNA processing – factors and co- transcriptional regulation

RNA processing regulation -splicing

Interaction of splicing factors with cis-sequences

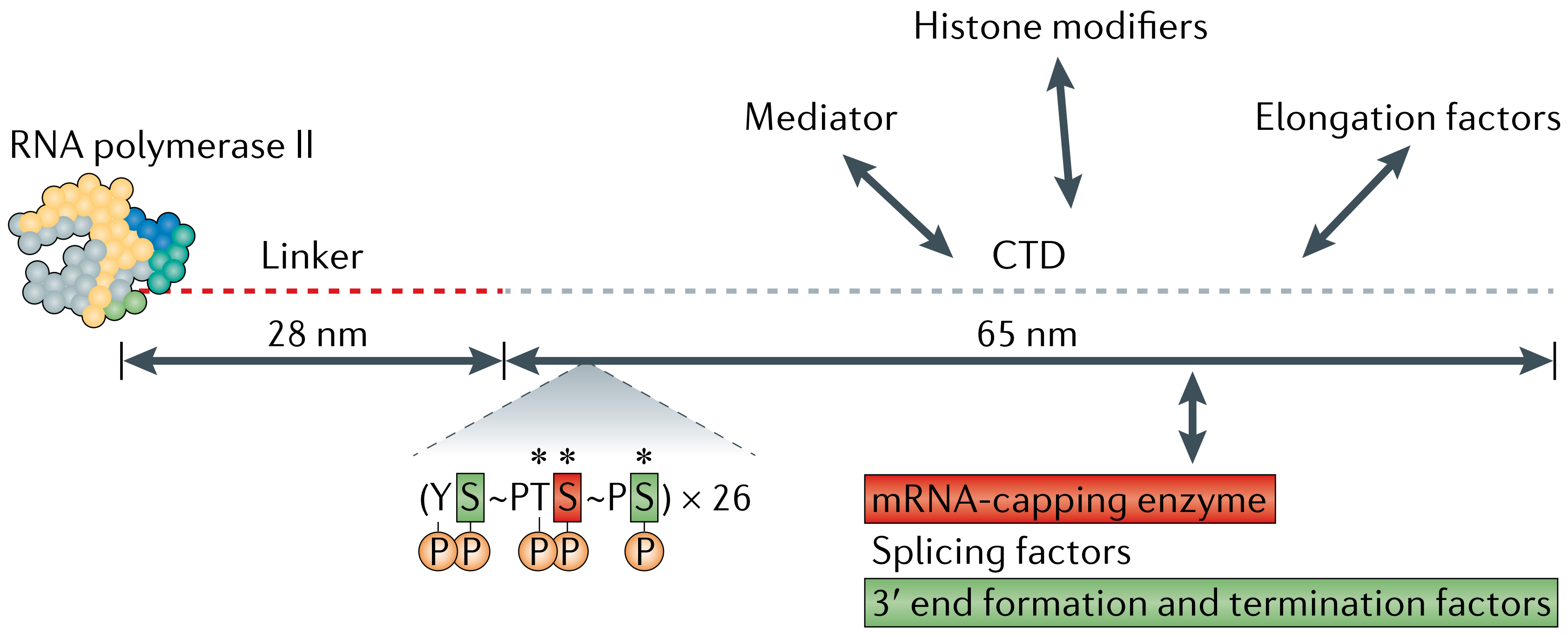


Nature Reviews | Molecular Cell Biology

Alberto Kornblihtt (University of Buenos Aires)

Pol II coordinates RNA maturation..

via CTD domain

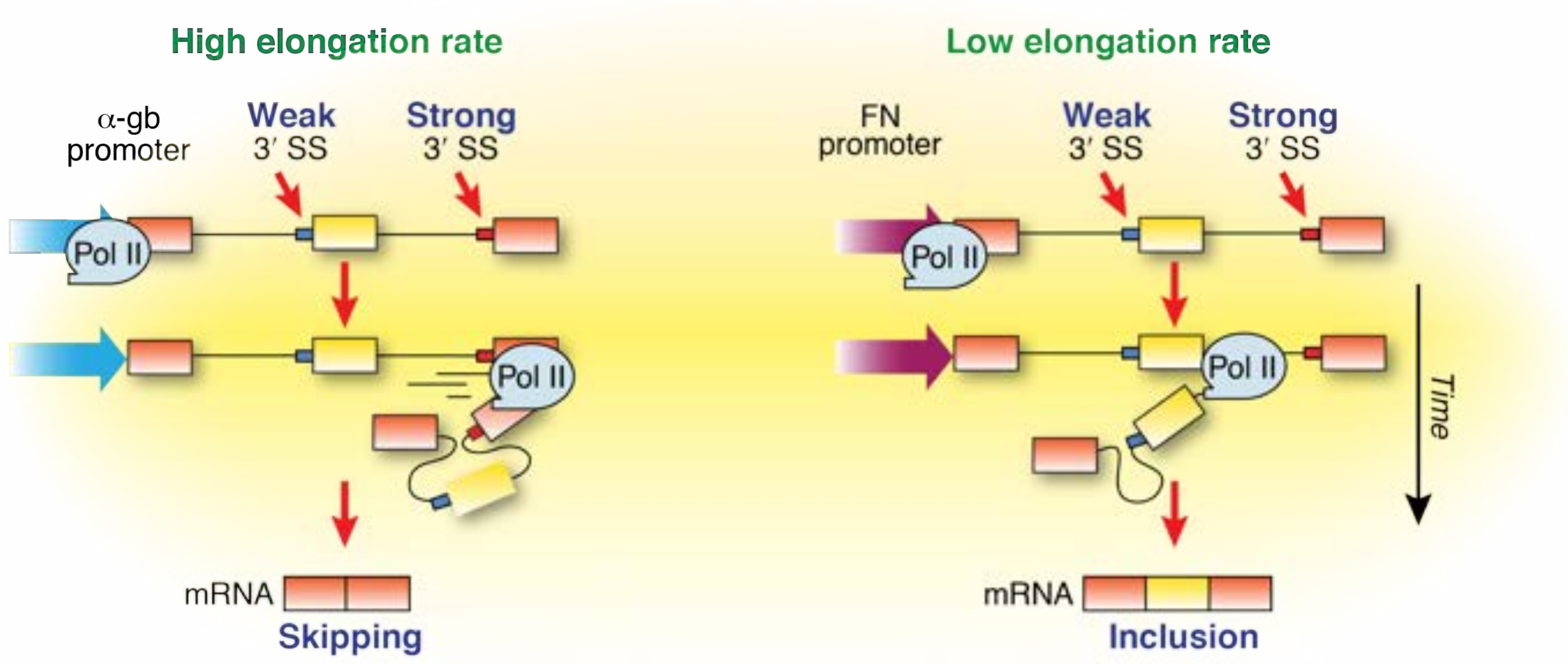


Bentley, Nature Reviews 2012

Pol II coordinates RNA maturation..

via elongation rate

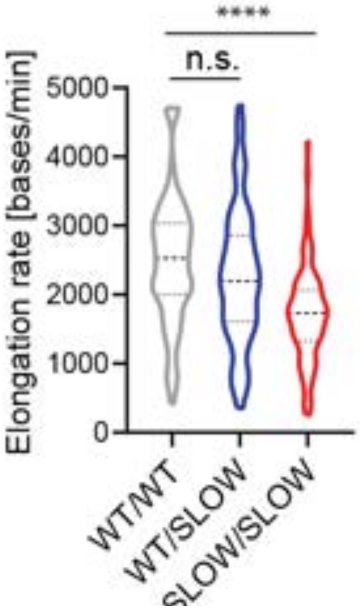
Kinetic model



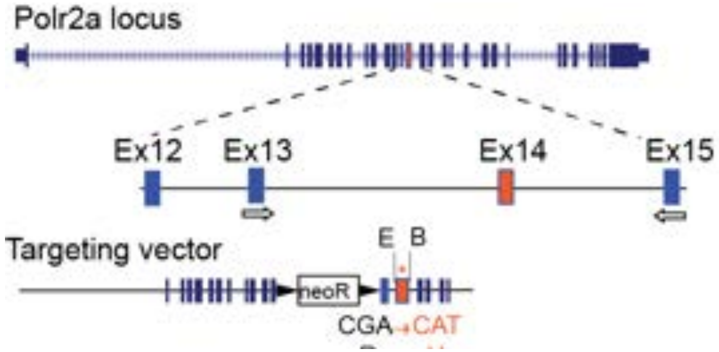
Kinetic regulation by Pol II is essential

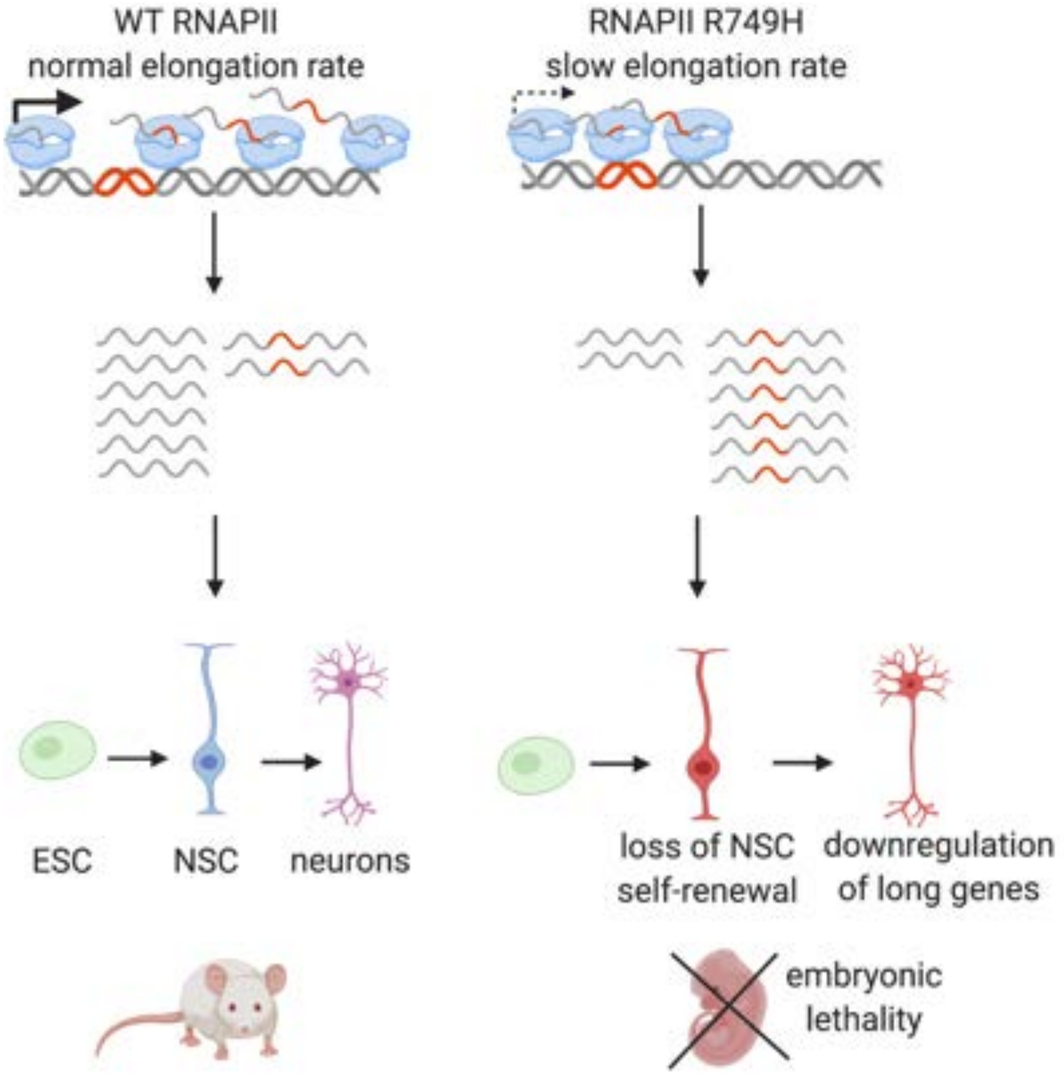
***Drosophila* C4: Rbp1 R741H**

Greenleaf et al. 1980;
Coulter and Greenleaf, 1985



Mammalian C4: Rbp1 R749H



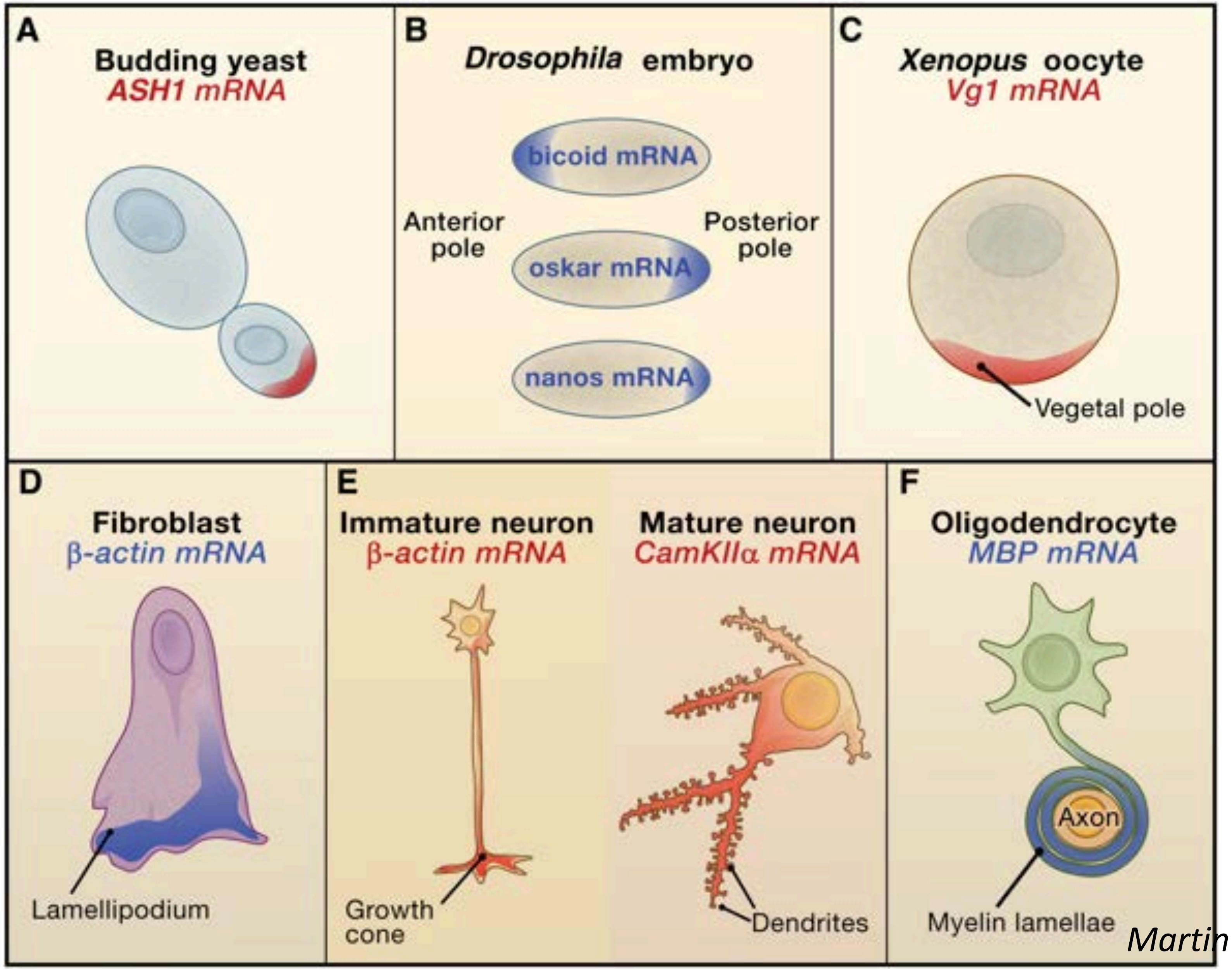


Maslon et al, 2019, EMBO J.

Cytoplasmic fate of mRNA

RNA moves

Classic Examples of Localized mRNAs



Martin and Ephrussi, Cell 2009

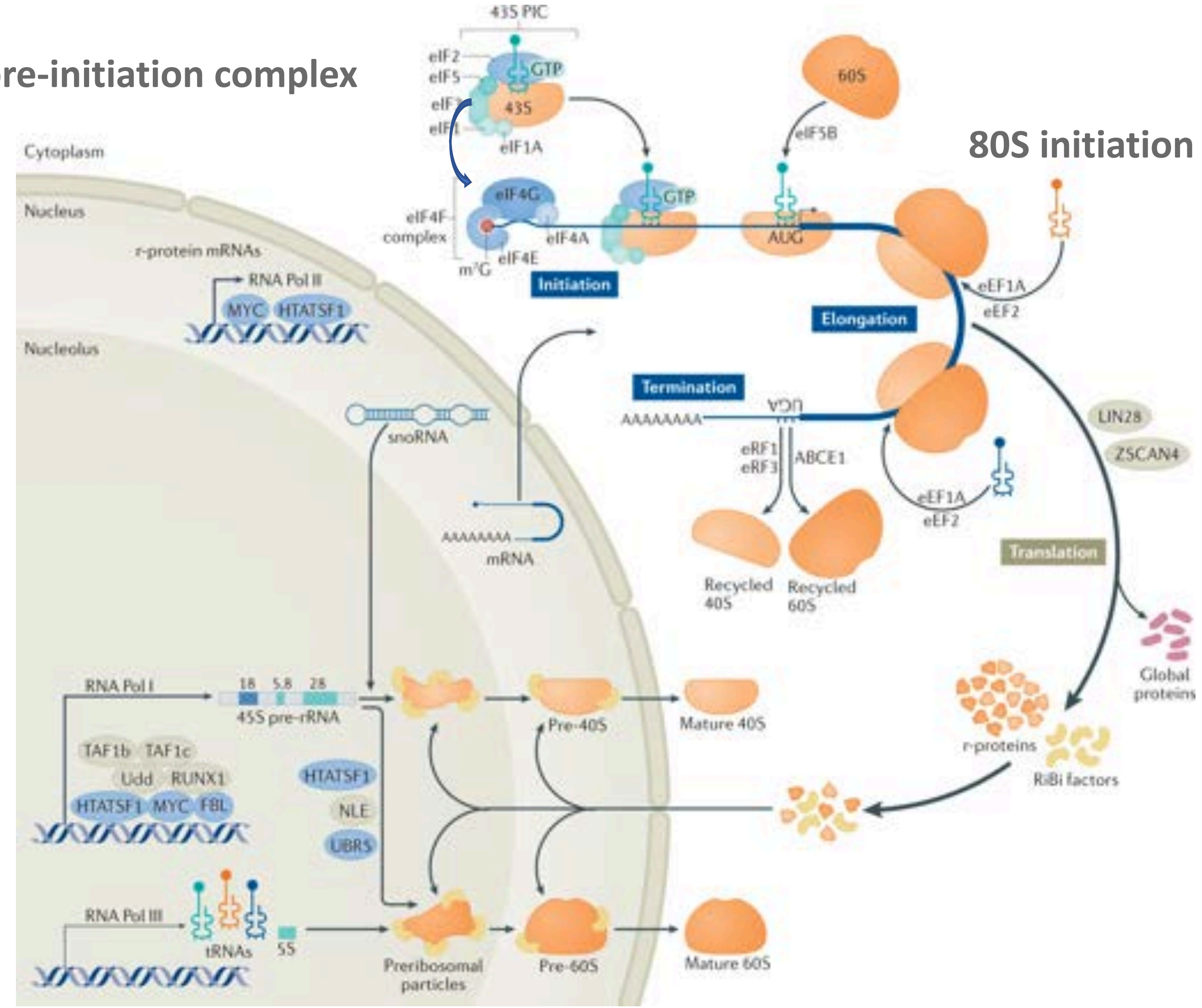
Cytoplasmic fate of mRNA

RNA plays its role - translation

Mechanisms of translation regulation

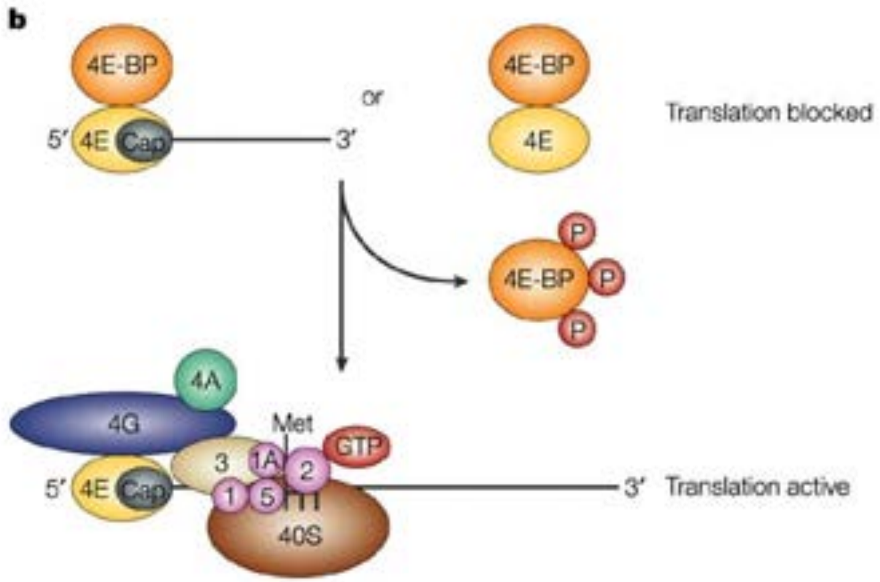
43S pre-initiation complex

80S initiation complex



Saba, Liakath-Ali, Green & Watt, Nature Reviews, 2021

Mechanisms of translation regulation

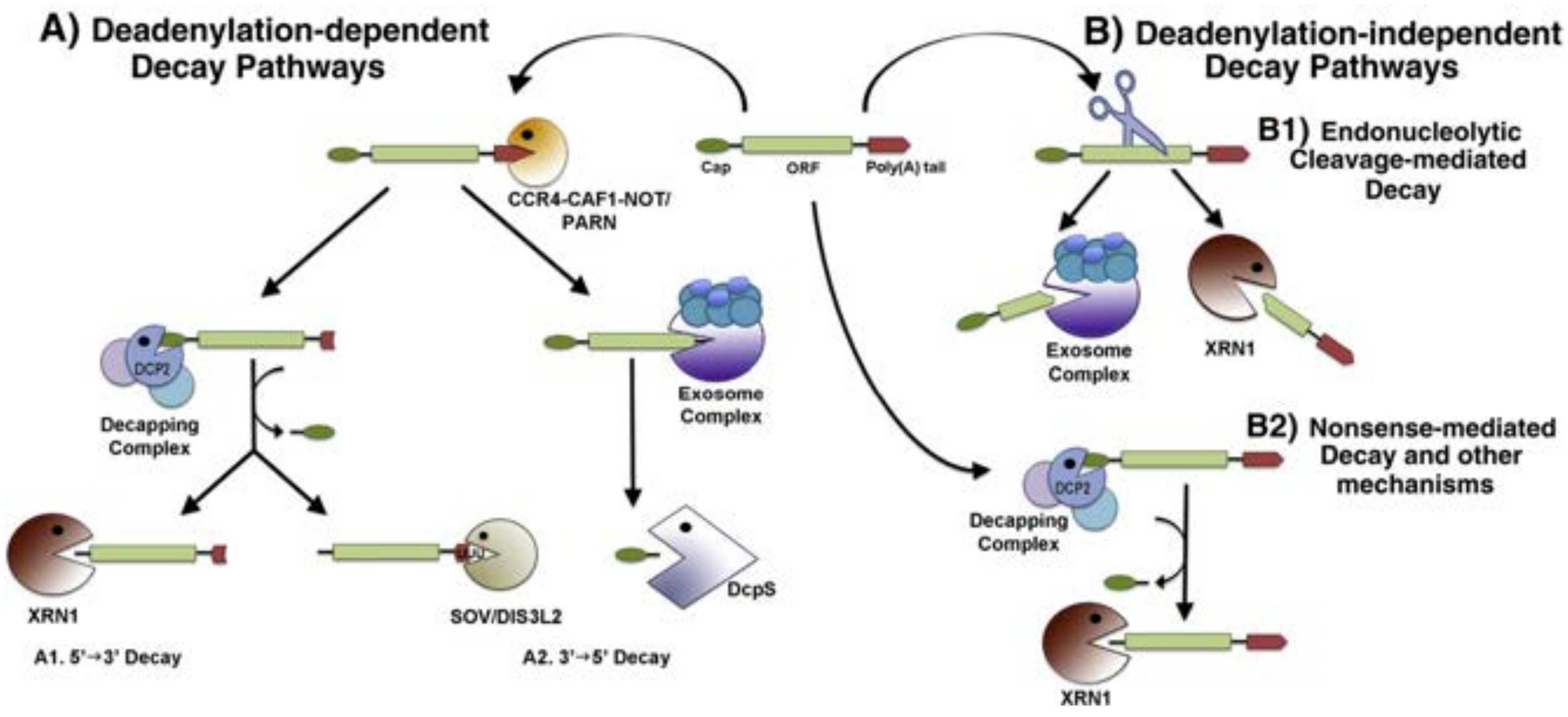


Gebauer & Hentze, Nature Reviews 2004

Cytoplasmic fate of mRNA

End of RNA- RNA degradation

Mechanisms of mRNA degradation in eukaryotes

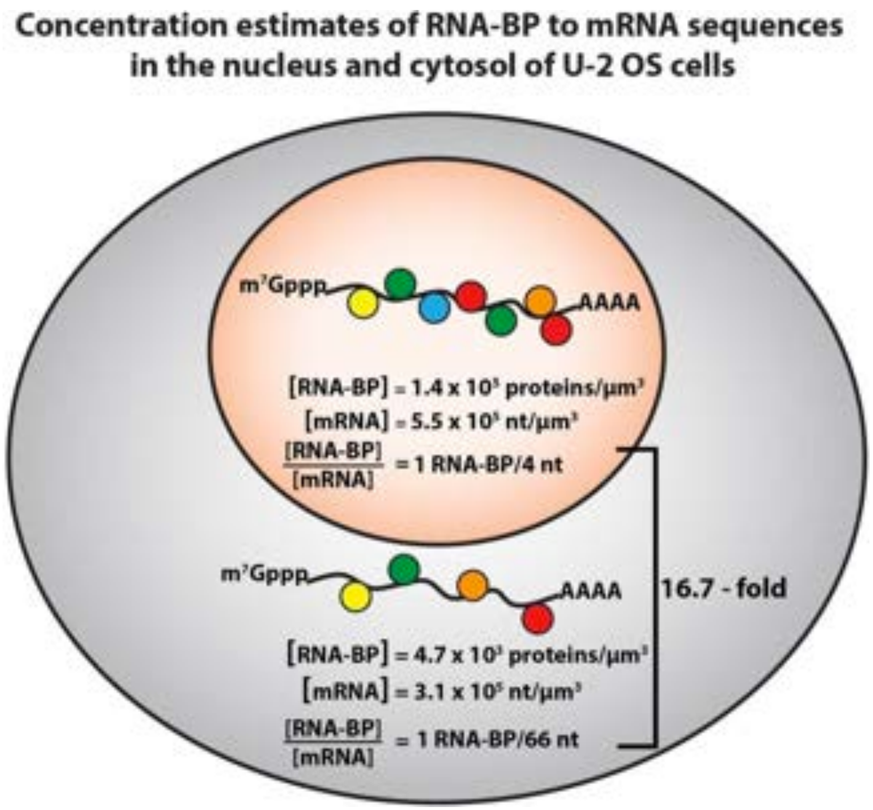


Nagarajan, Green, 2013

Fate of mRNA

RNA binding proteins

The life of eukaryotic mRNA - mRNPs



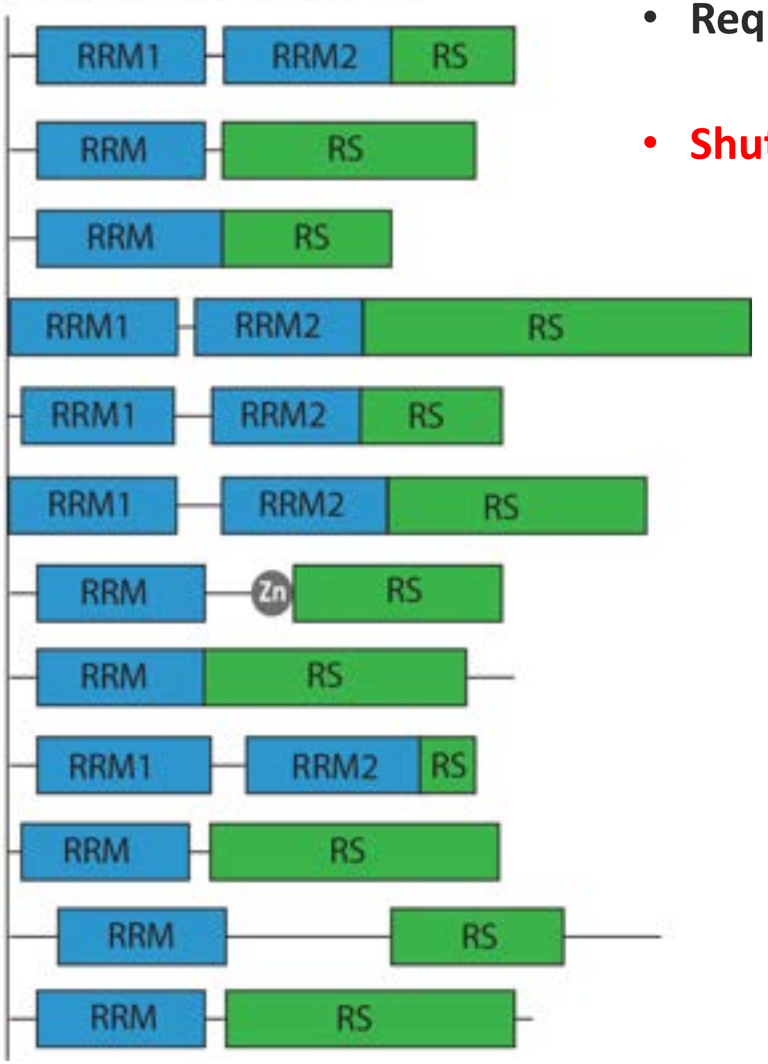
Khong and Parker, RNA 2020.

- **RNAs in cells are associated with RBPs**
- **The RBPs function is context-dependent/shapes development**
- **RBPs developmentally regulated through levels/localization.**

SR proteins control all steps of mRNA life cycle

Name	Aliases
SRSF1	ASF, SF2
SRSF2	SC35, PR264, SRp30
SRSF3	SRp20
SRSF4	SRp75
SRSF5	SRp40, HRS
SRSF6	SRp55, B52
SRSF7	9G8
SRSF8	SRp46
SRSF9	SRp30c
SRSF10	TASR1, SRp38, SRp40
SRSF11	p54, SRp54
SRSF12	SRp35

Domain Structure

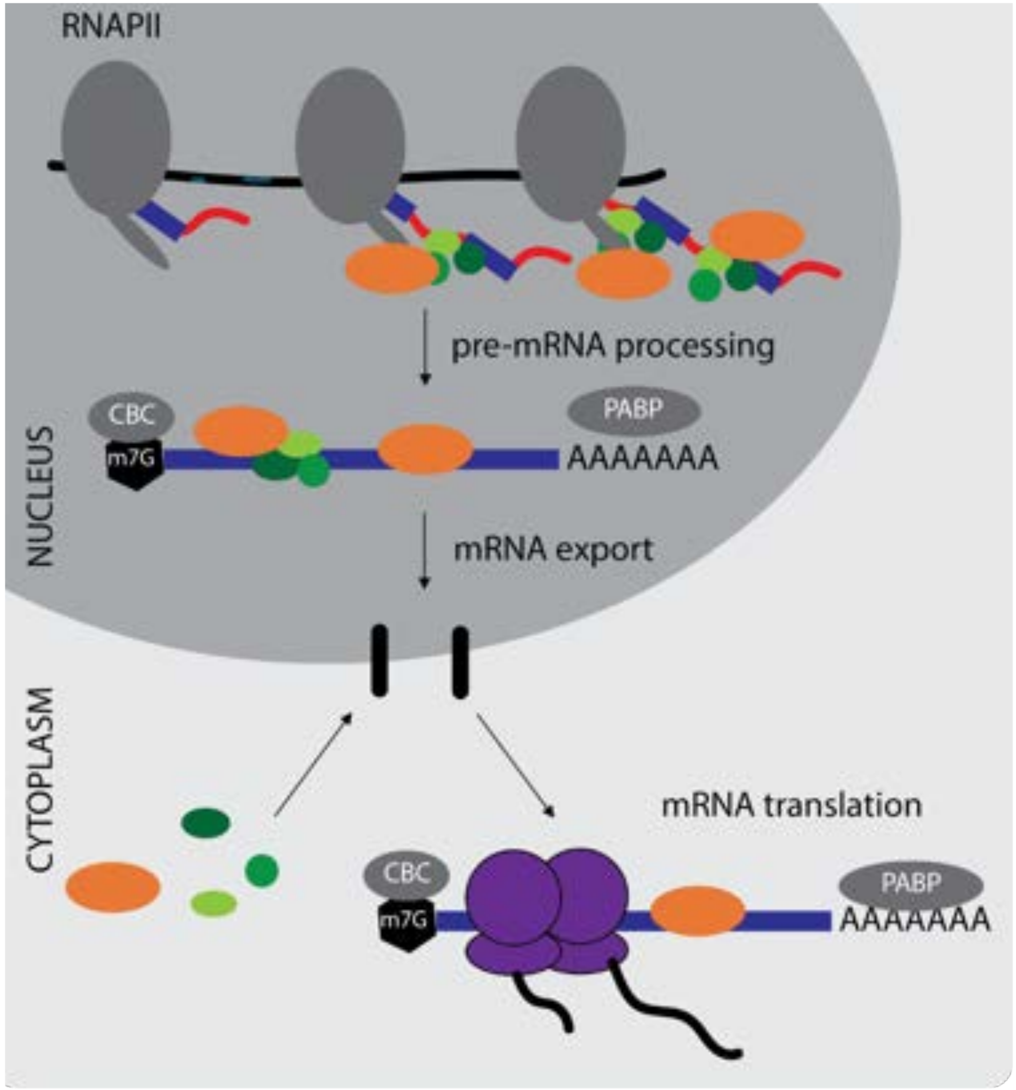


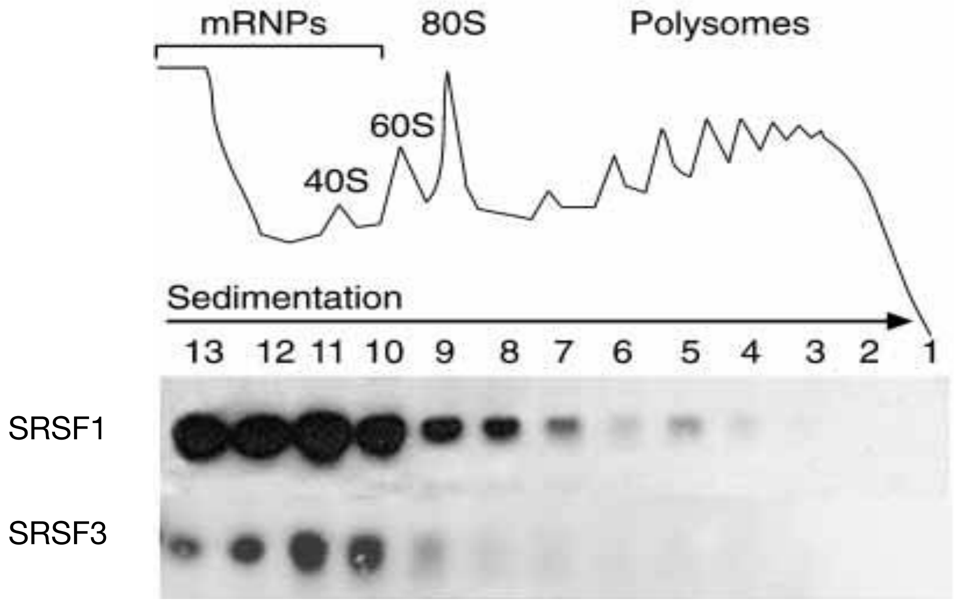
- **Required for constitutive and alternative splicing**
- **Shuttling SR proteins have numerous cytoplasmic functions**

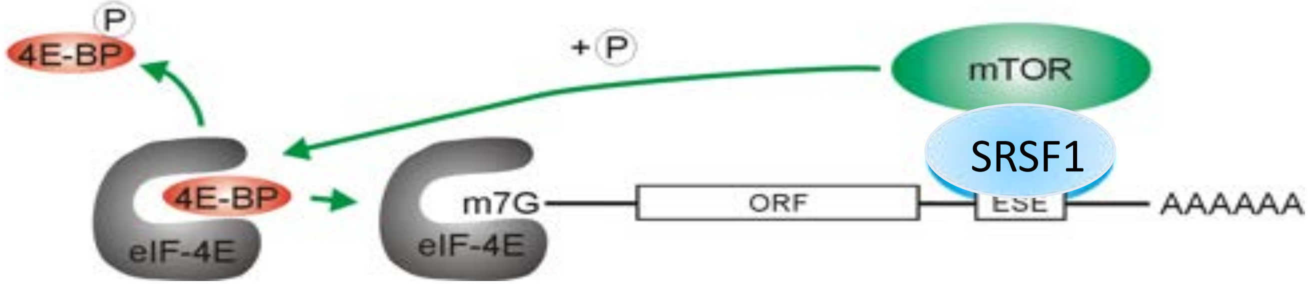
Manley and Krainer (2010); Twyffels et al (2011)]

SR proteins control all steps of mRNA life cycle

e.g. translation



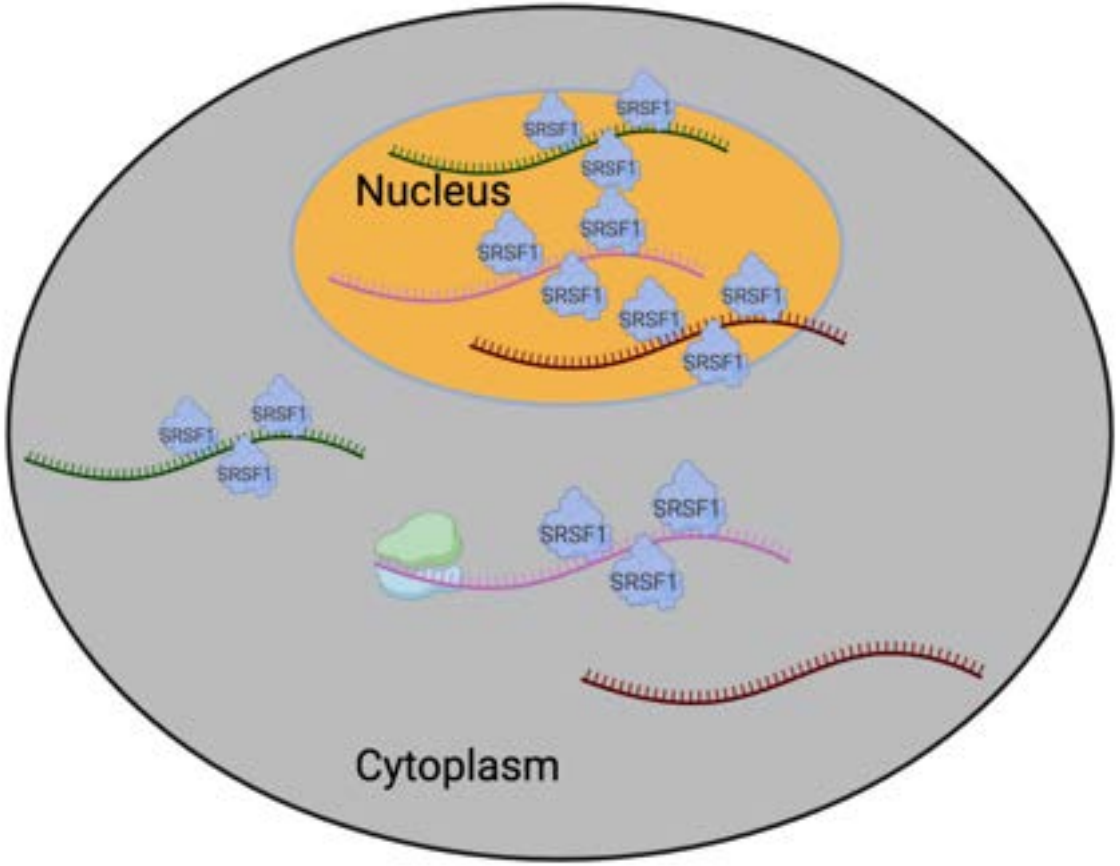


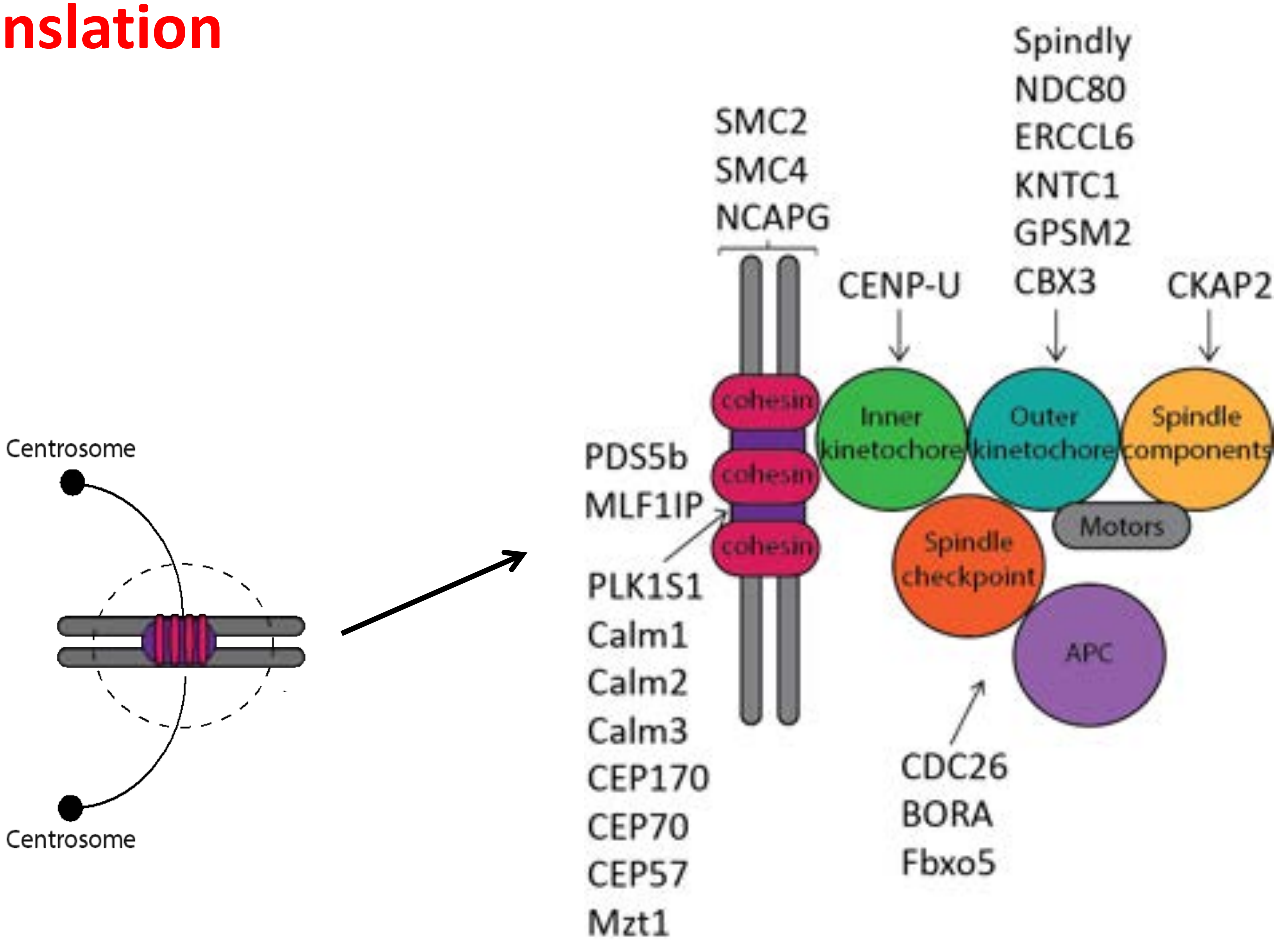


Michlewski et al. (2008) Mol Cell

SR proteins control all steps of mRNA life cycle

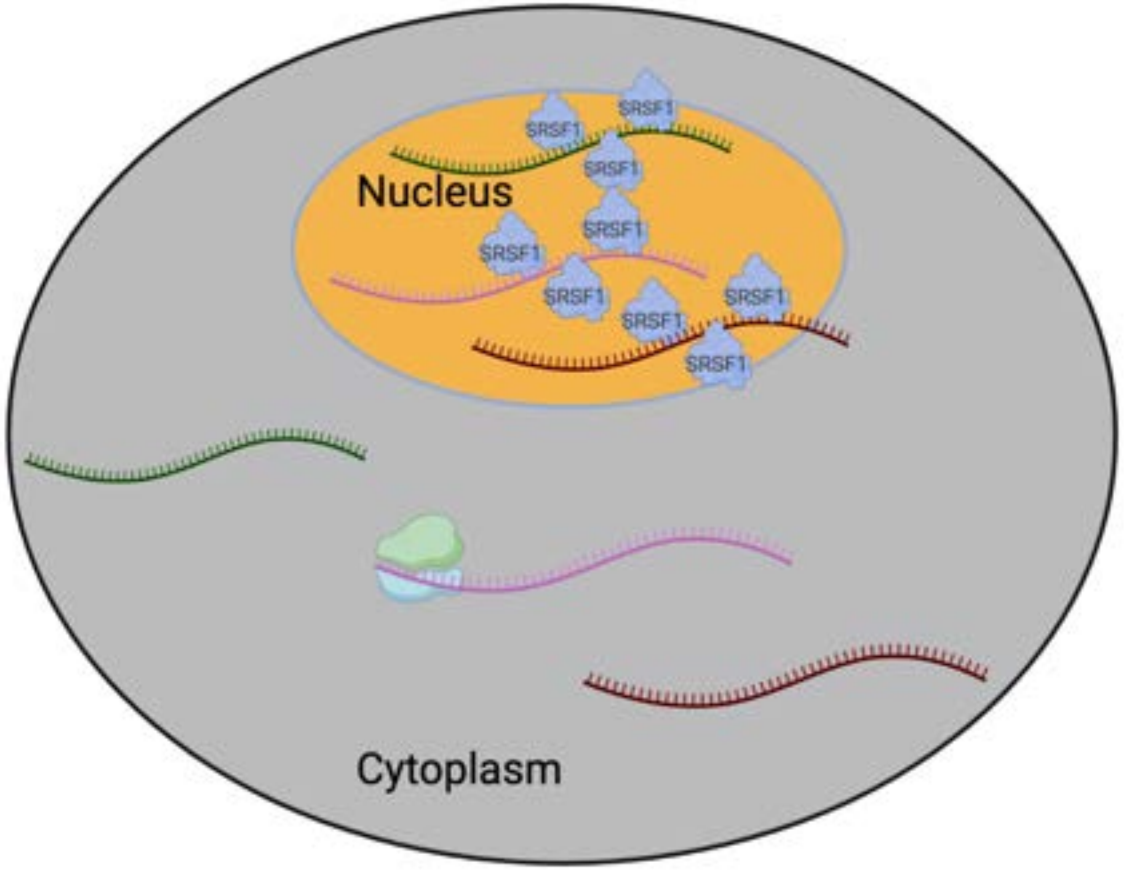
e.g. translation





Maslon and Heras et al, 2014, Elife

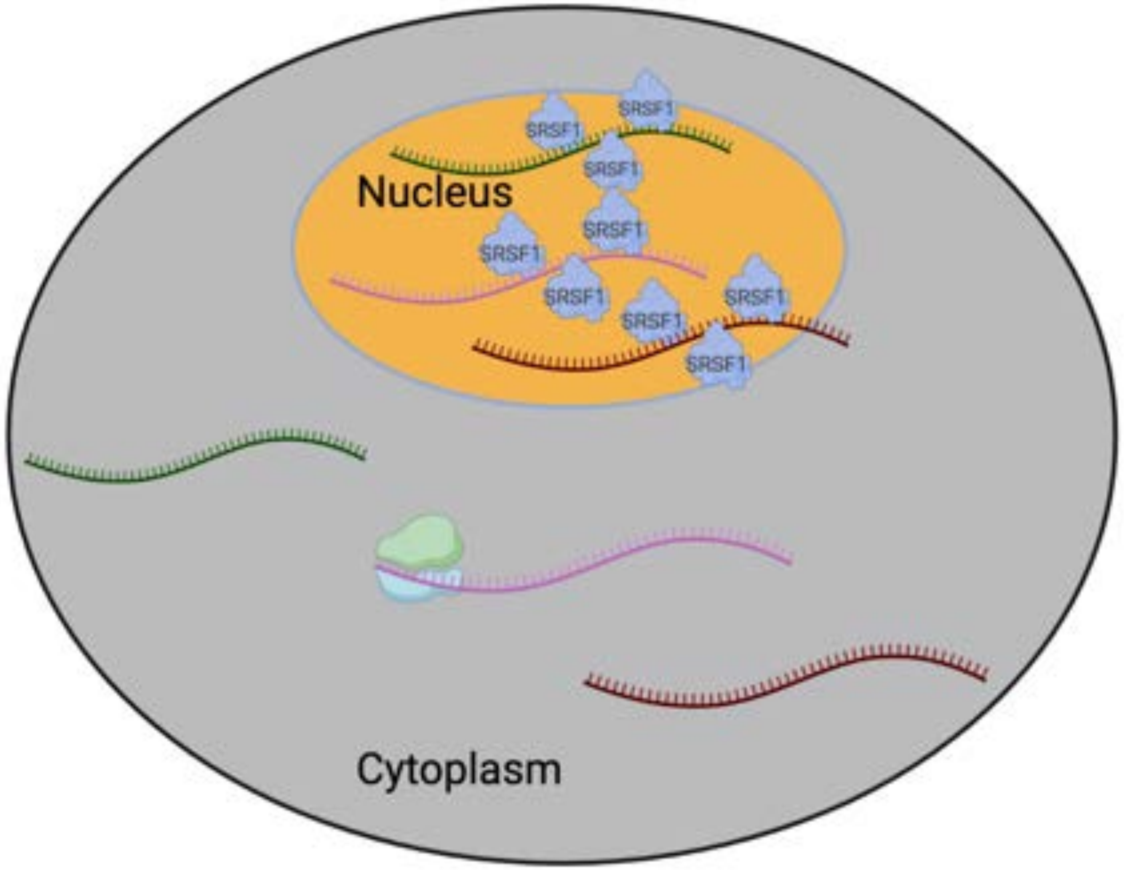
Consequences of lack of SRSF1 in cytoplasm

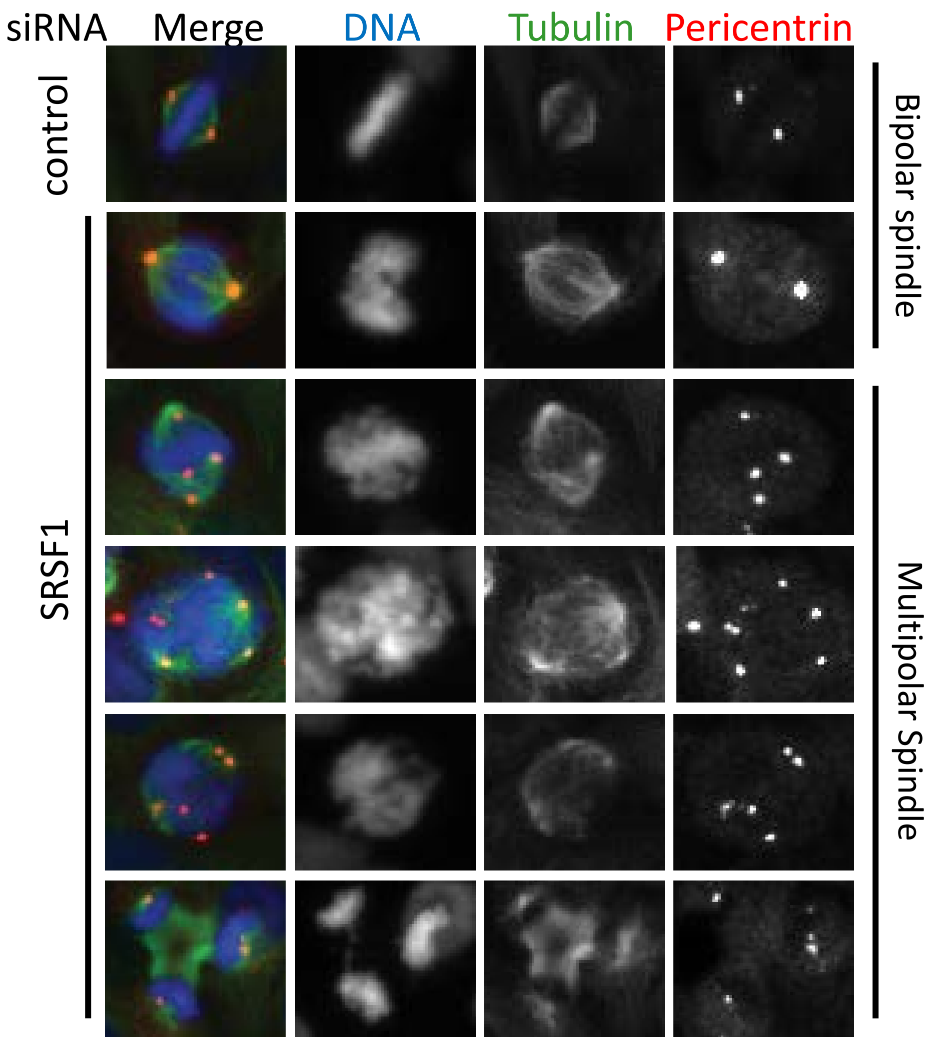


CELL

ORGANISM

SRSF1 translation ensures appropriate cell division



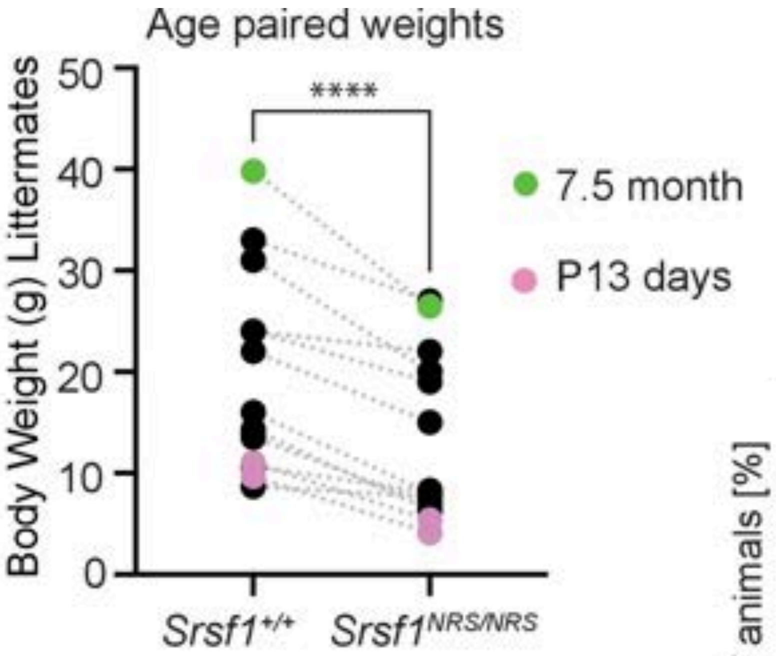


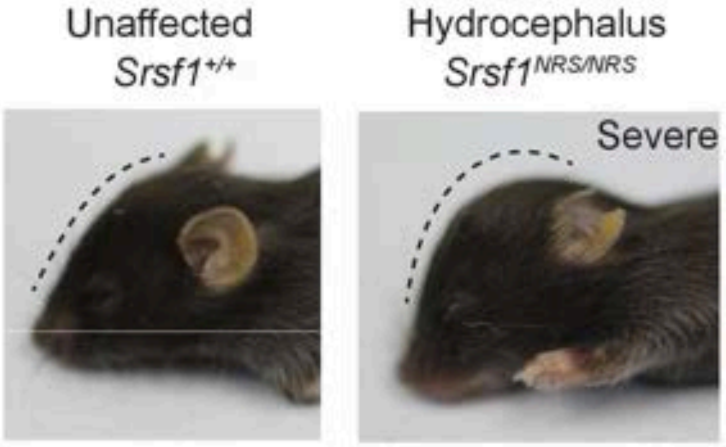
Maslon and Heras et al, 2014, Elife

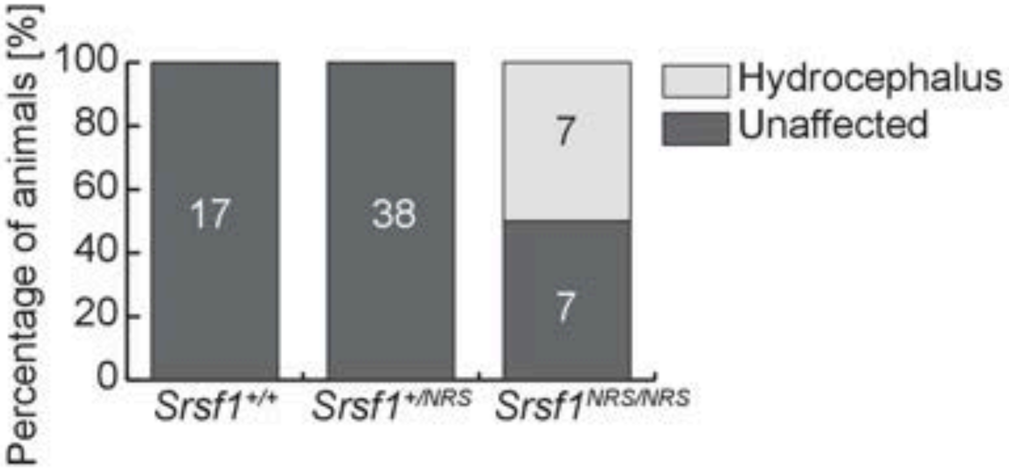
SRSF1 translation ensures appropriate development

Hydrocephaly

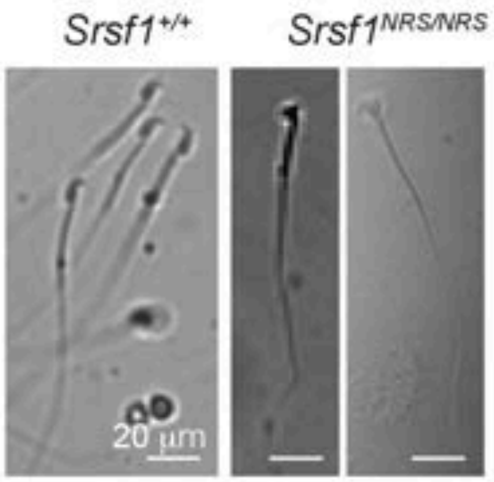
Growth restriction







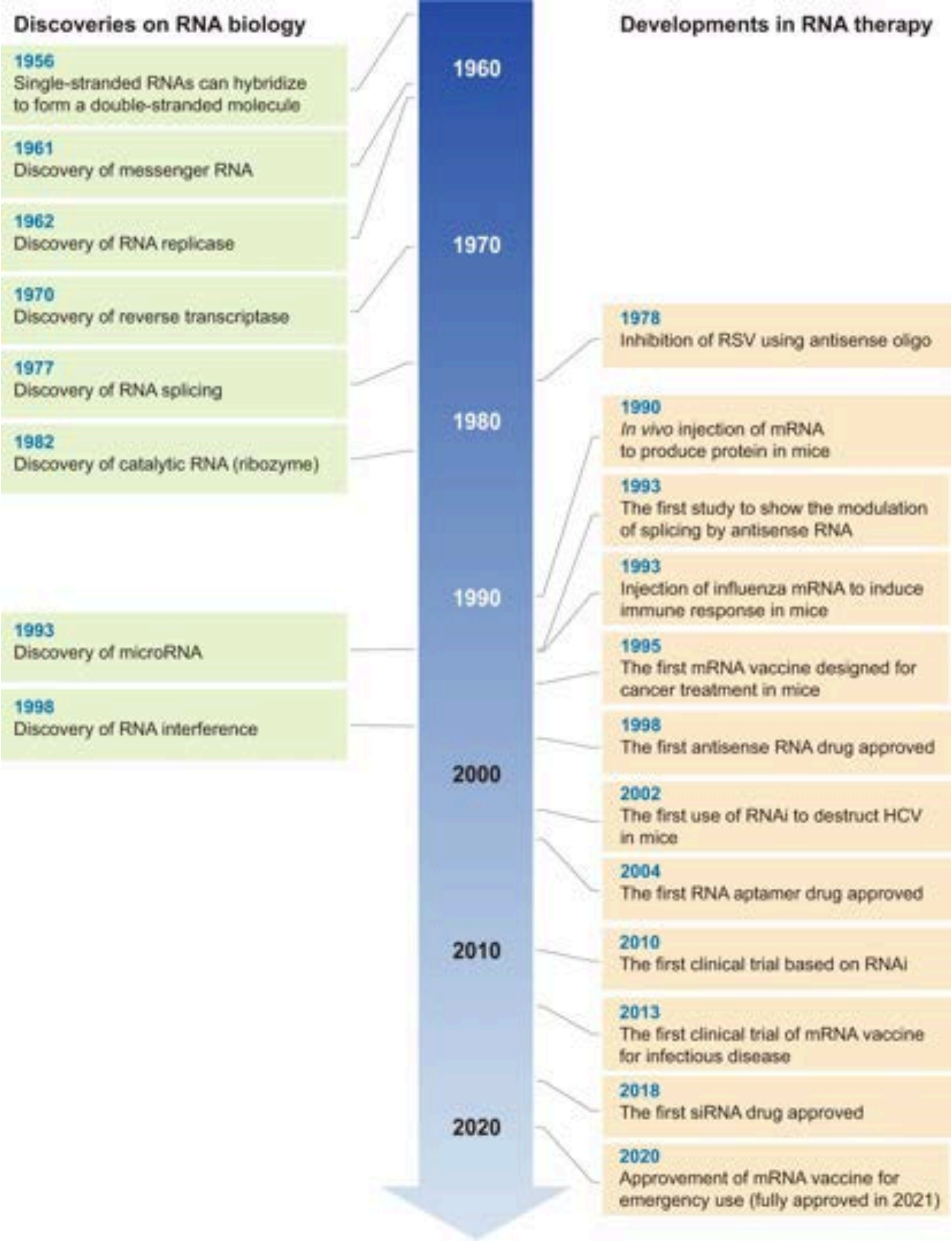
Immotile sperm

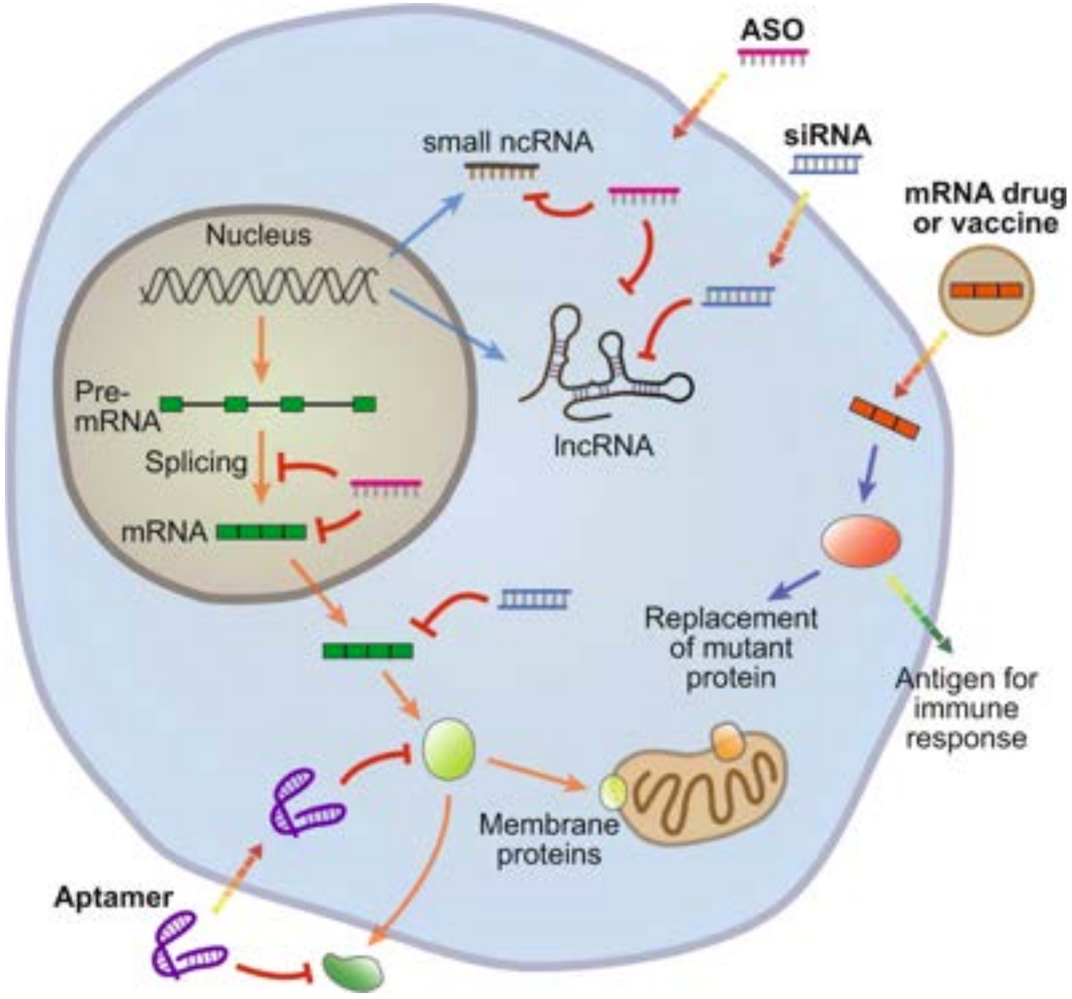


	Motile sperm (%)
$Srsf1^{+/+}$	82.0 ± 6.2
$Srsf1^{NRS/NRS}$	8.0 ± 5.3

Haward, Maslon*, Yeyati* et al., Elife 2021*

RNA based drugs





Splicing in disease

Cancer

Overexpression of SF

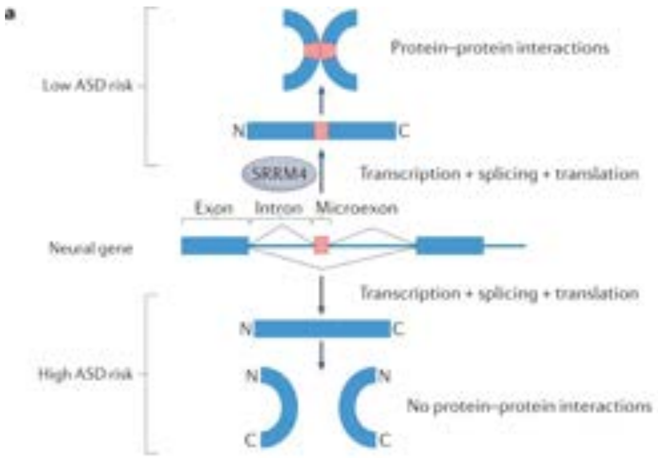
SRSF6

SRSF1

Mutations

SRSF2 Pro95
U2AF1

ASD



Schisophrenia

ALS

Early-onset Parkinson disease

Bipolar disorder

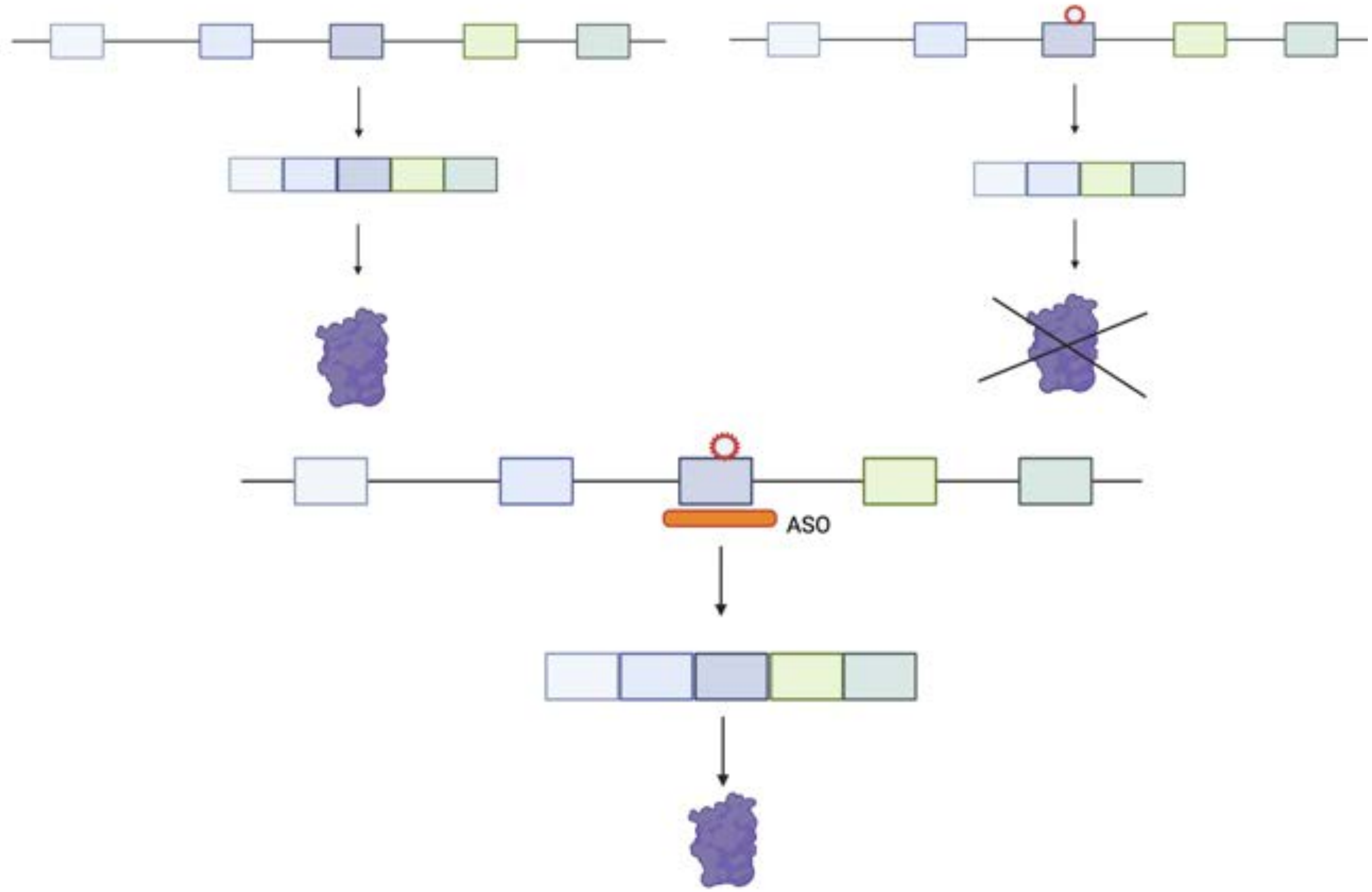
Multiple Sclerosis

Spinal muscular atrophy

Duchenne muscular dystrophy

Autoimmune disease

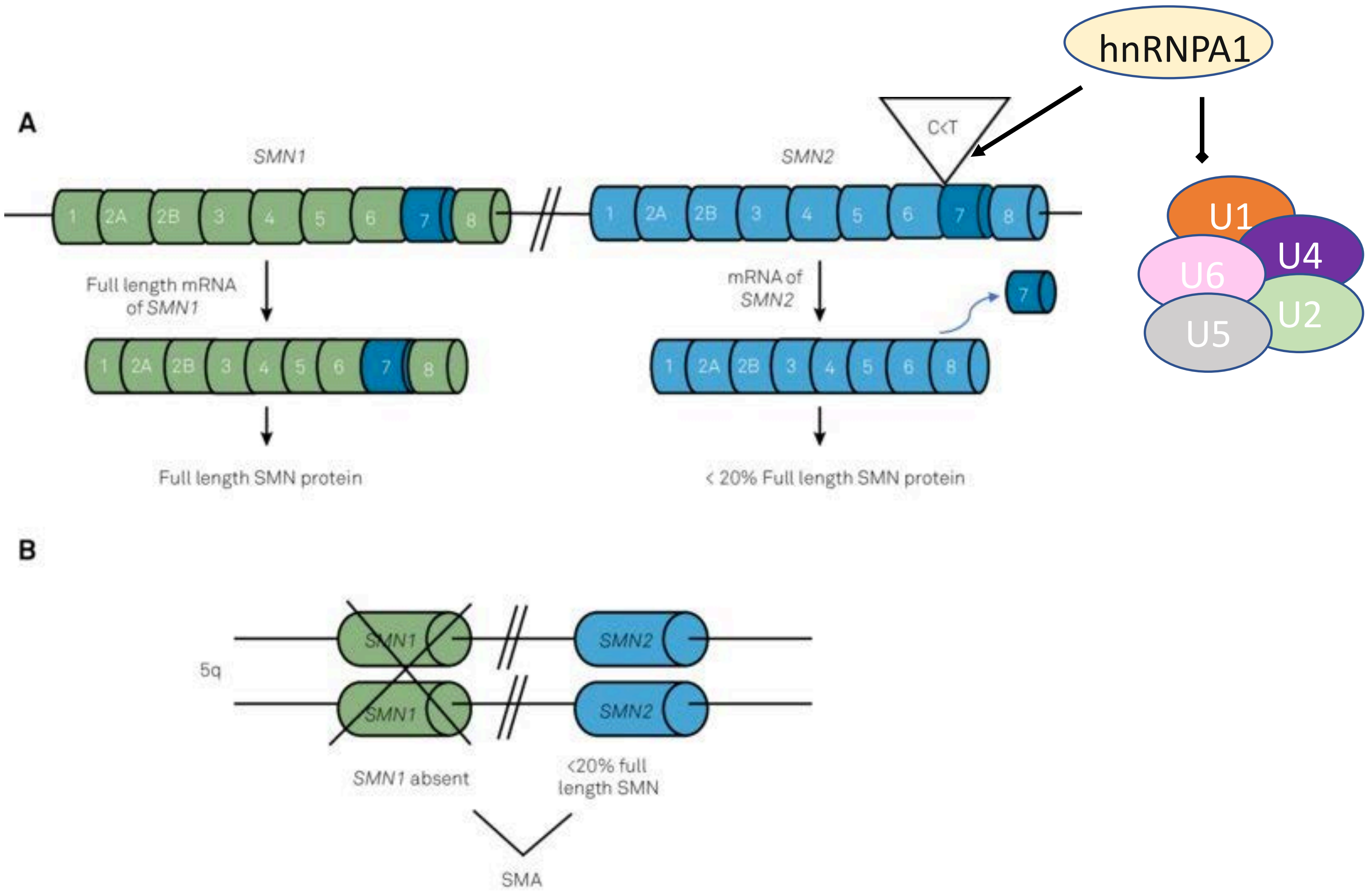
We can trick splicing machinery by using antisense oligonucleotide (ASO)



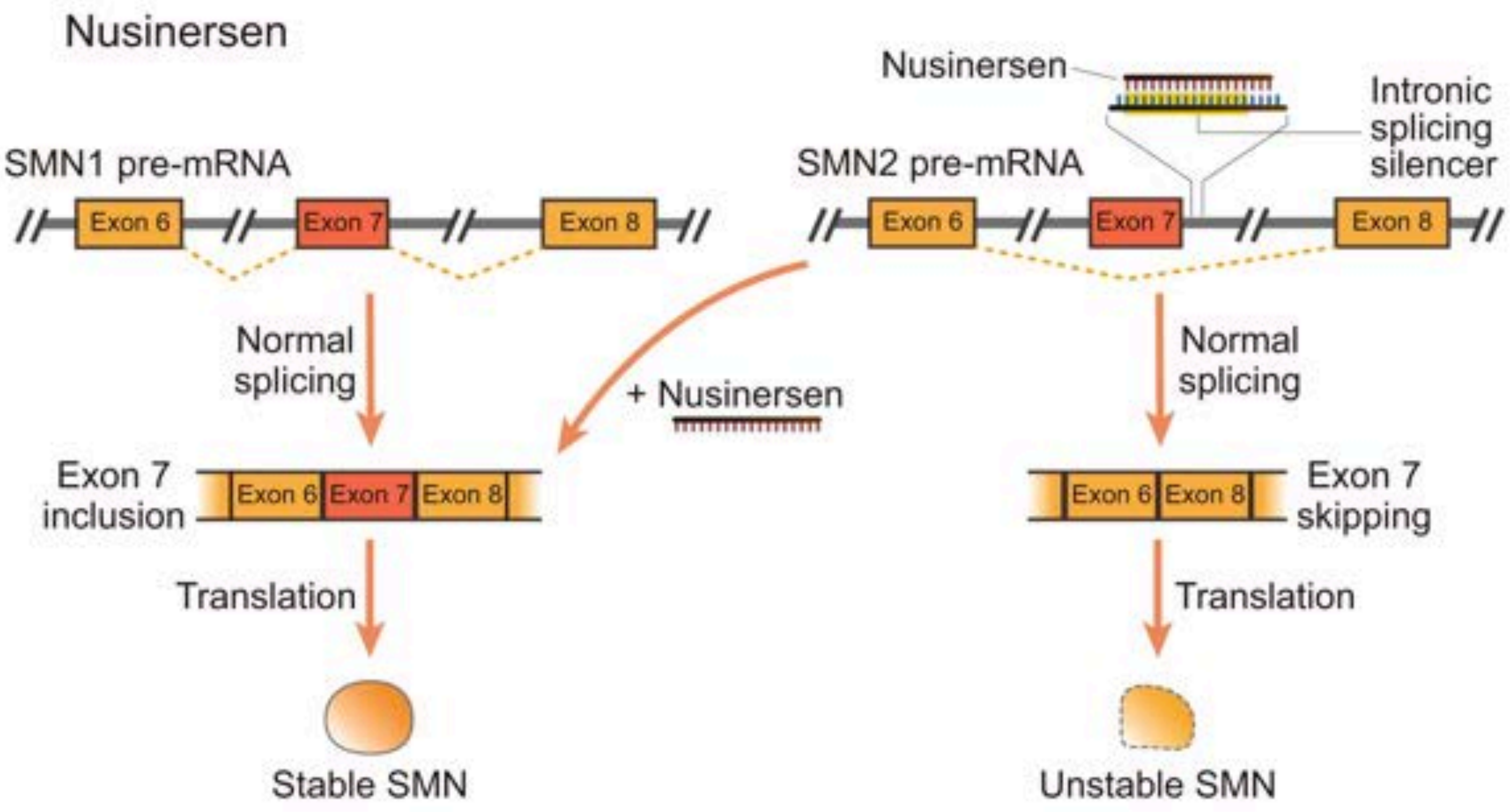
Pre-mRNA

Pre-mRNA with mutation

SMA is caused by recessive mutation in SMN1 gene



Therapeutic modulation of alternative splicing



Marasco & Kornblihtt, Nature Reviews 2022

Summary

- Most genes are able to produce multiple mRNA products
- mRNA life cycle is dependent on the coordinated effects of a large and intricate set of regulatory machinery
- RNA binding proteins are involved in all steps of RNA life cycle
- Errors of RNA biology are common and found in human disorders