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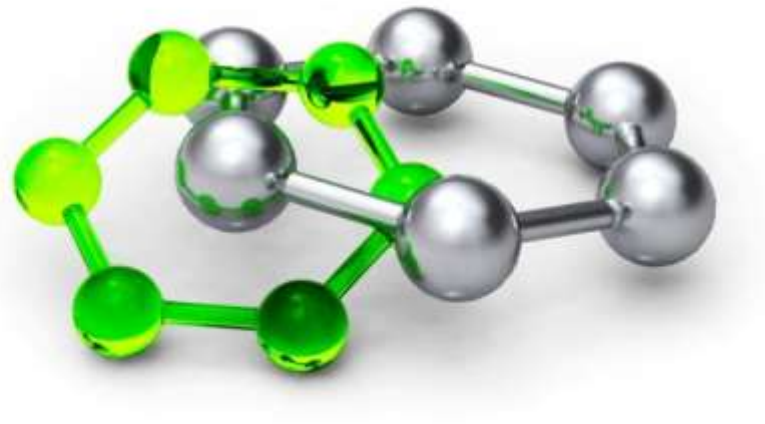
Regulation of gene expression in the cytoplasm

Peter Josef Lukavsky

Research Group Leader

Brno, 2019



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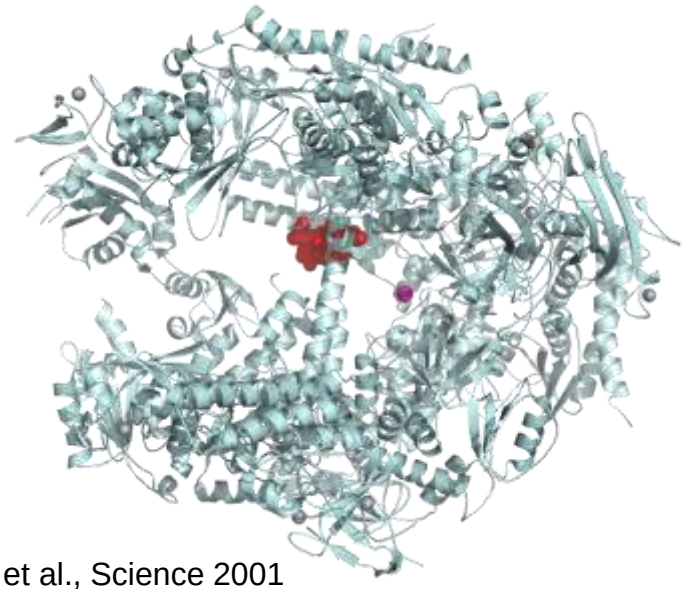
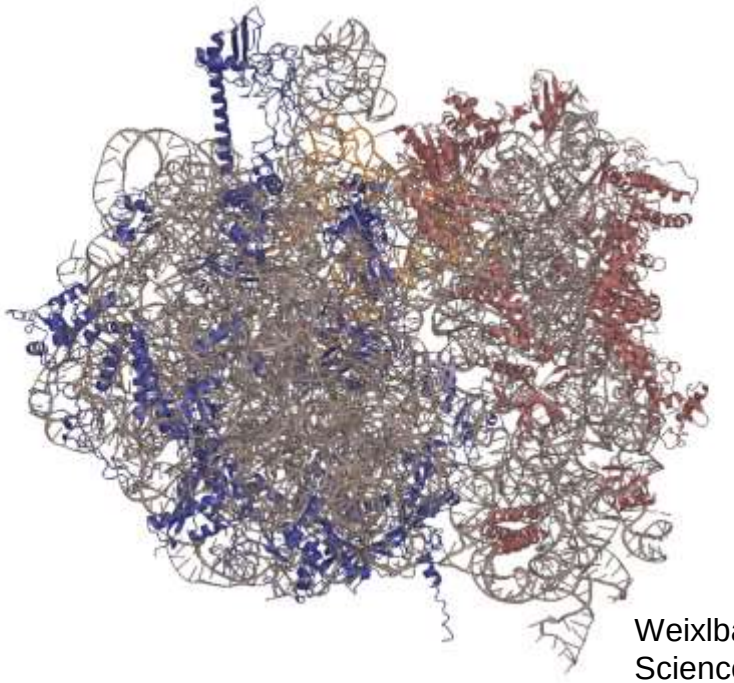
RNA plays a central role in biology

DNA → RNA → Protein



Cramer et al., Science 2001

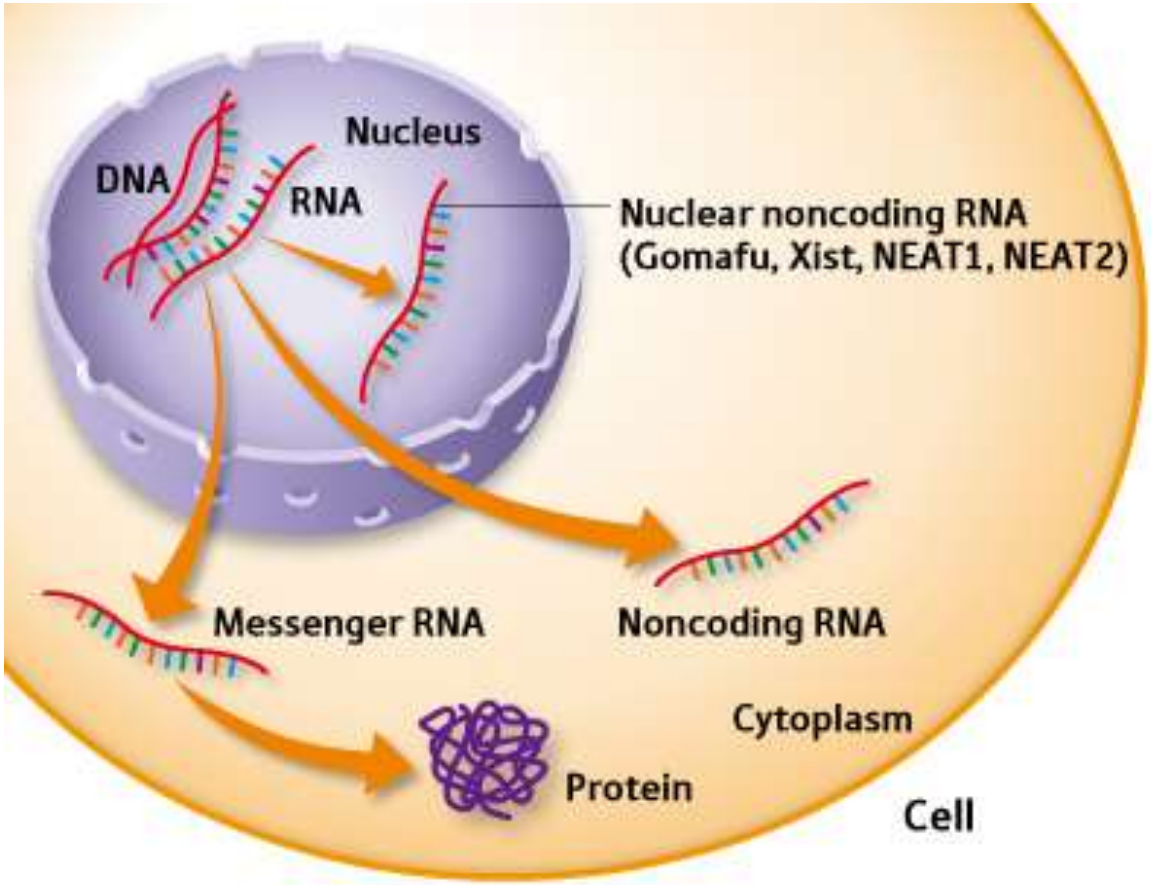
RNA polymerase II



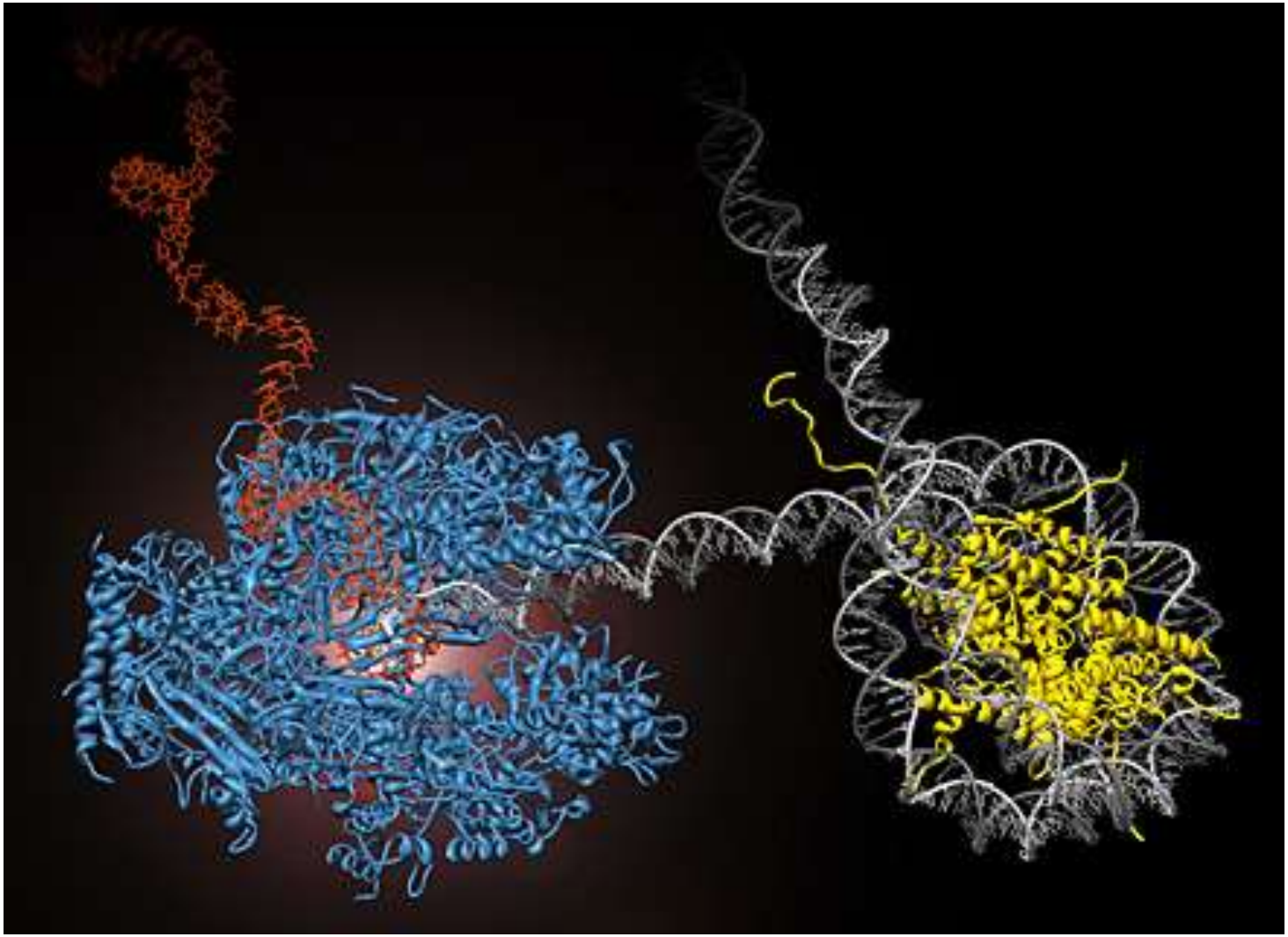
Weixlbaumer et al, Science 2008

70S ribosome

RNAs function both in the nucleus and cytoplasm



The life of an RNA starts with transcription in the nucleus…..



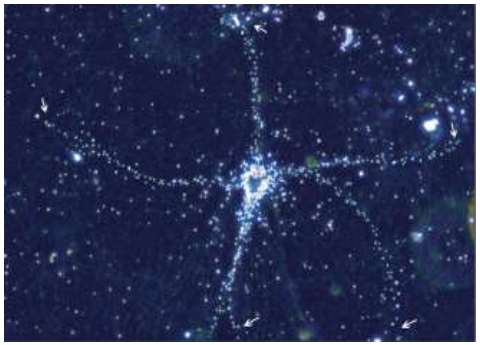
Post-transcriptional regulation of gene expression

- Alternative splicing of pre-mRNA
- mRNA processing (5'-/3'-end formation)
- Nuclear export
- cytoplasmic RNA transport
- Translational control
- mRNA decay

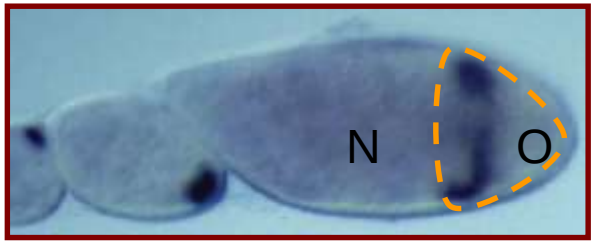
Regulated by multiple RNA signals in complex with Proteins

Why regulate gene expression post-transcriptionally?

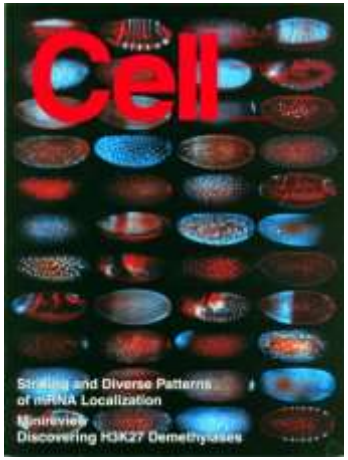
- Greater diversity of gene products
- Rapid response to stimuli
- Spatial and temporal control of protein synthesis
- Flexible control allows fine-tuning of protein synthesis



Dendritic mRNA transport for memory formation
(Sutton et al. Cell 2006)



Anterior-posterior and axis formation during development
(Martin et al. Cell 2009)



~70% of transcripts could be asymmetrically localized (Lecuyer et al., Cell 2007)

mRNPs regulate gene expression:

fertilization
development
cell cycle
stress response

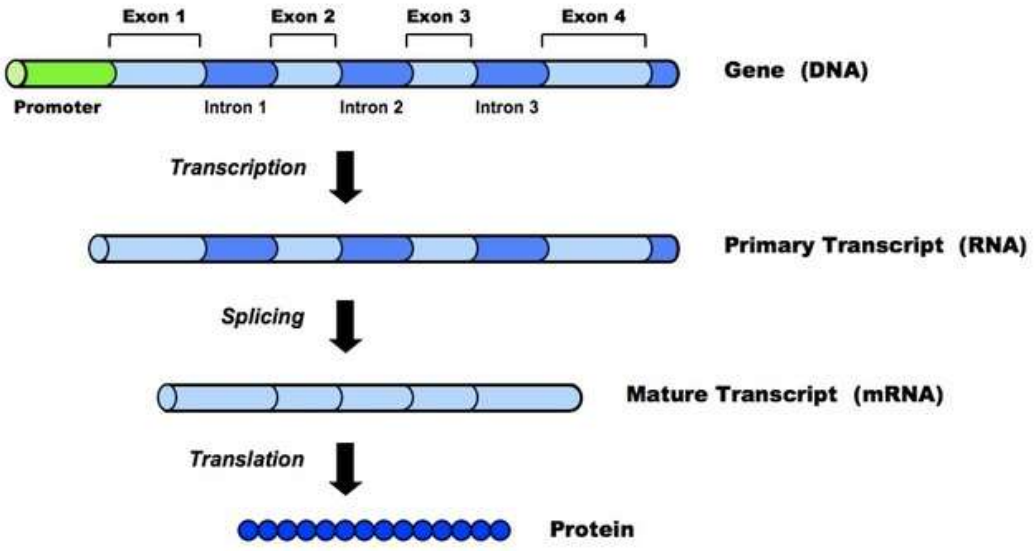
Deregulation leads to disease:

cancer
diabetes
neurological disorders

=> RNA-protein interactions are a valuable drug target!!

mRNAs are usually transcribed as precursors

- **Exon**: Any nucleotide sequence encoded by a gene that remains present within the final RNA product.
- **Intron**: Any nucleotide sequence encoded by a gene that is removed by RNA splicing from the final RNA product.



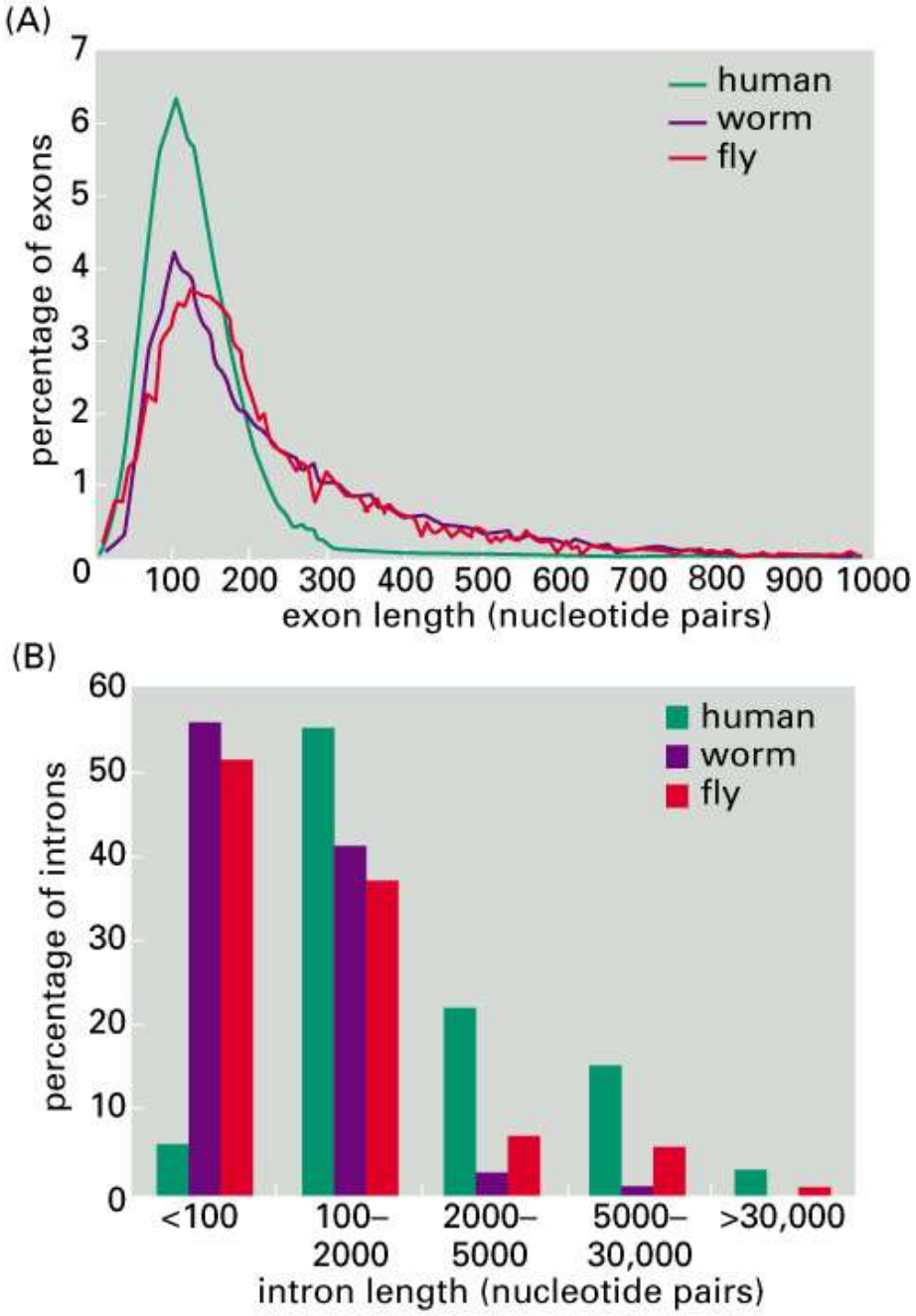
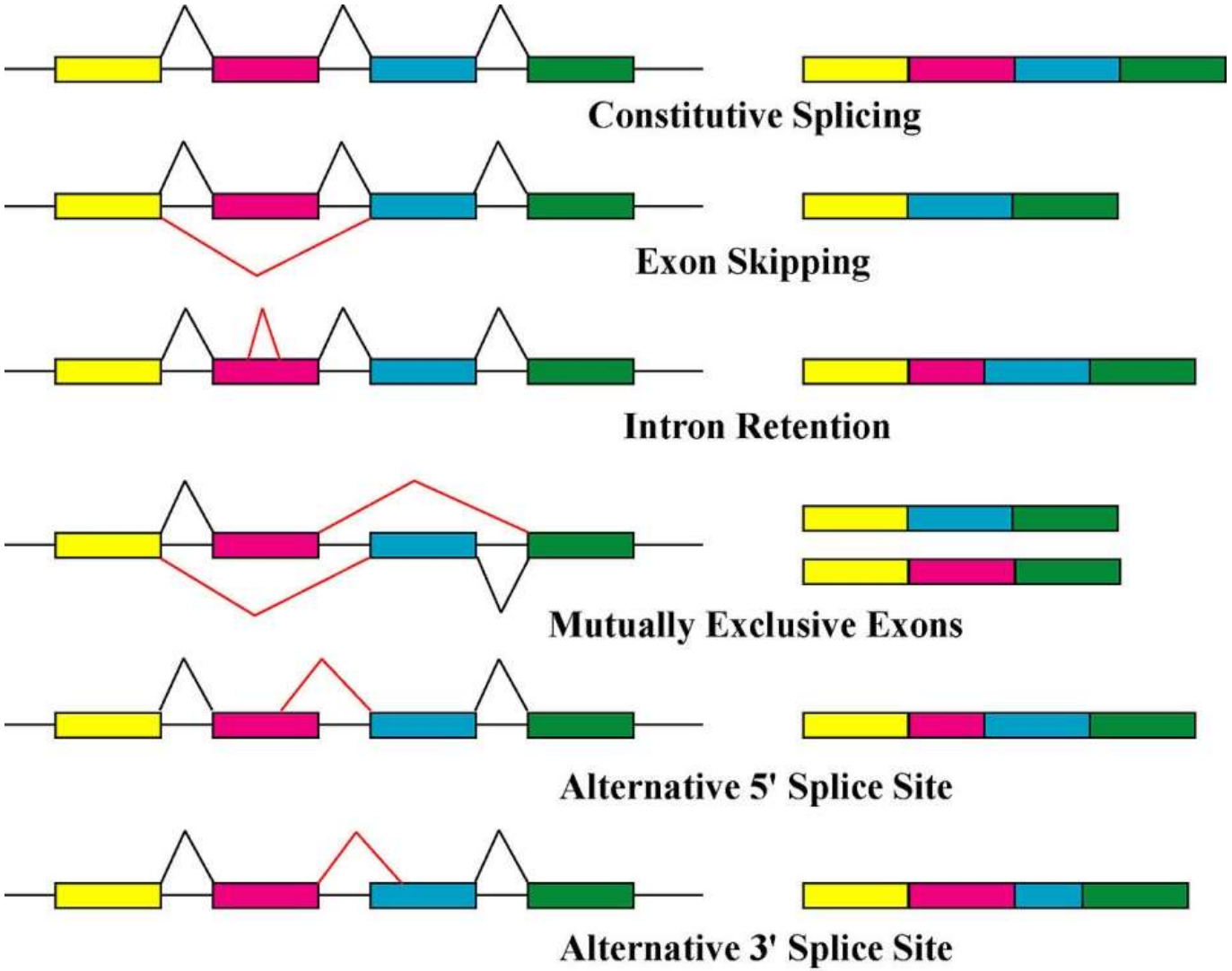


Figure 6–32. Molecular Biology of the Cell, 4th Edition.

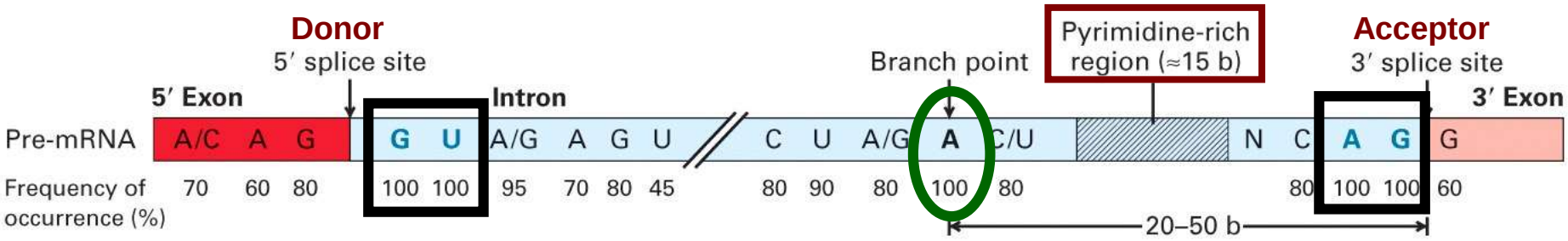
Exons are similar in size

Introns are highly variable in size

mRNA splicing diversifies gene expression



5’ and 3’ splice sites (SS) in vertebrate pre-mRNAs



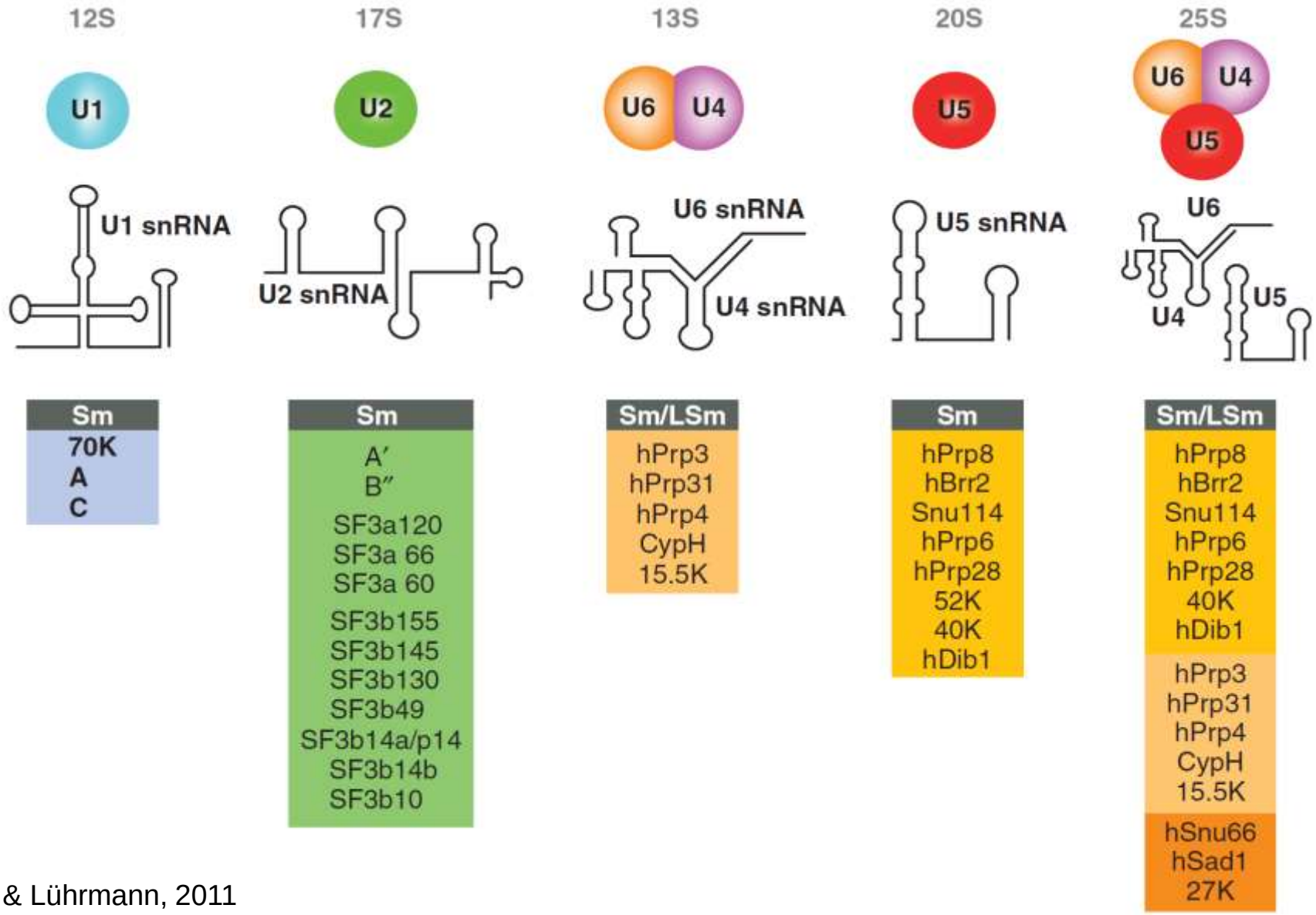
- Pyrimidine-rich region (~15 bases) is located upstream of 3’ SS

- Donor splice site: GU

- Acceptor splice site: AG

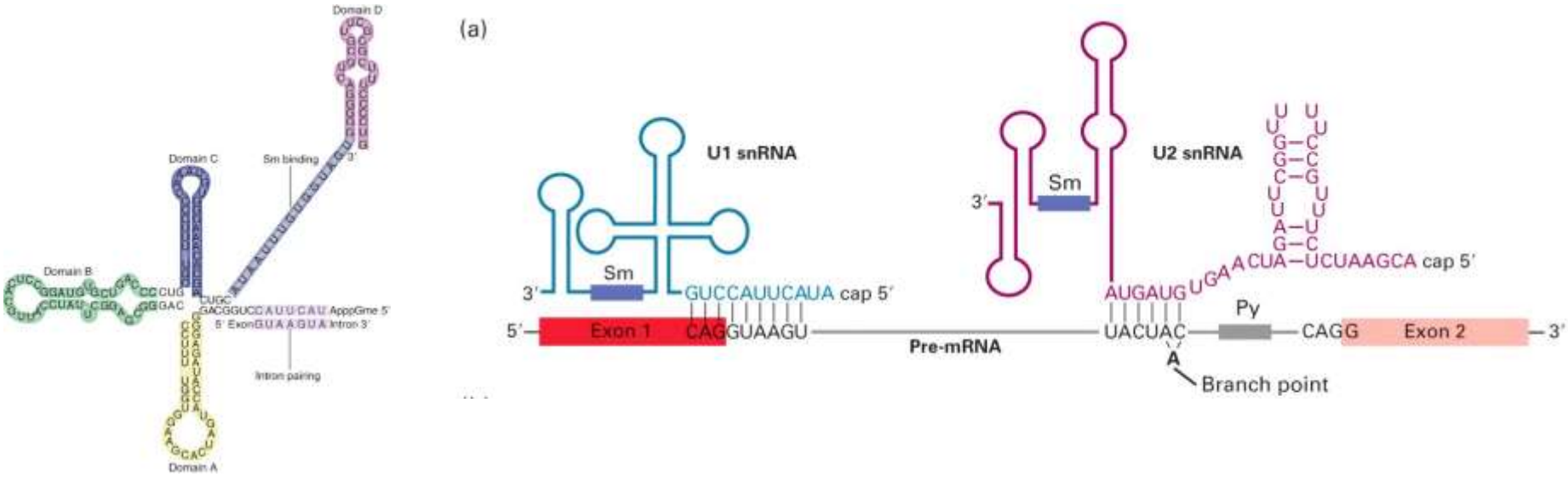
- Branch point: A

The human spliceosomal snRNPs



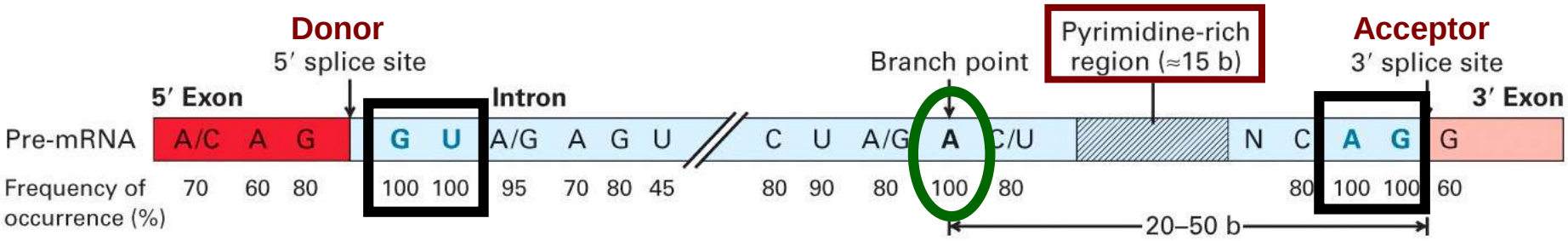
Will & Lührmann, 2011

snRNA base pairing with pre-mRNA

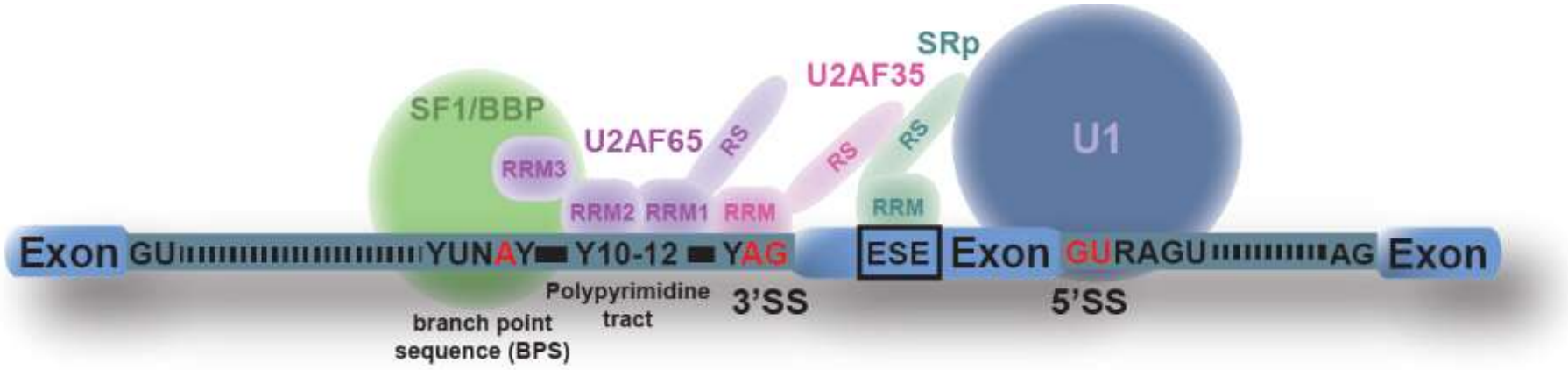


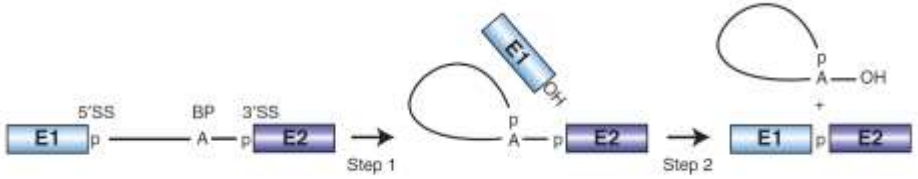
U1 snRNA

Definition of exon boundaries

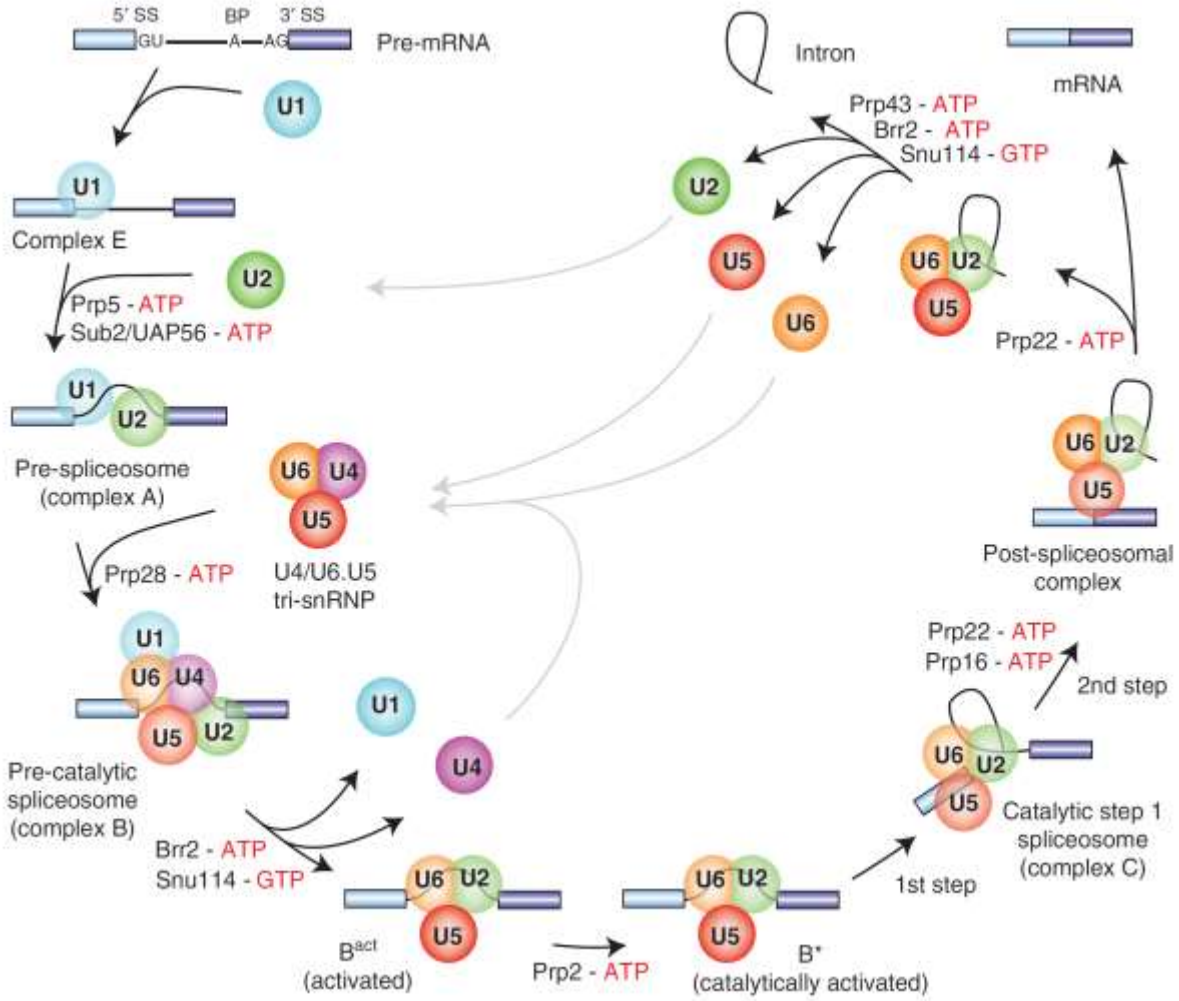


Spliceosomal E complex





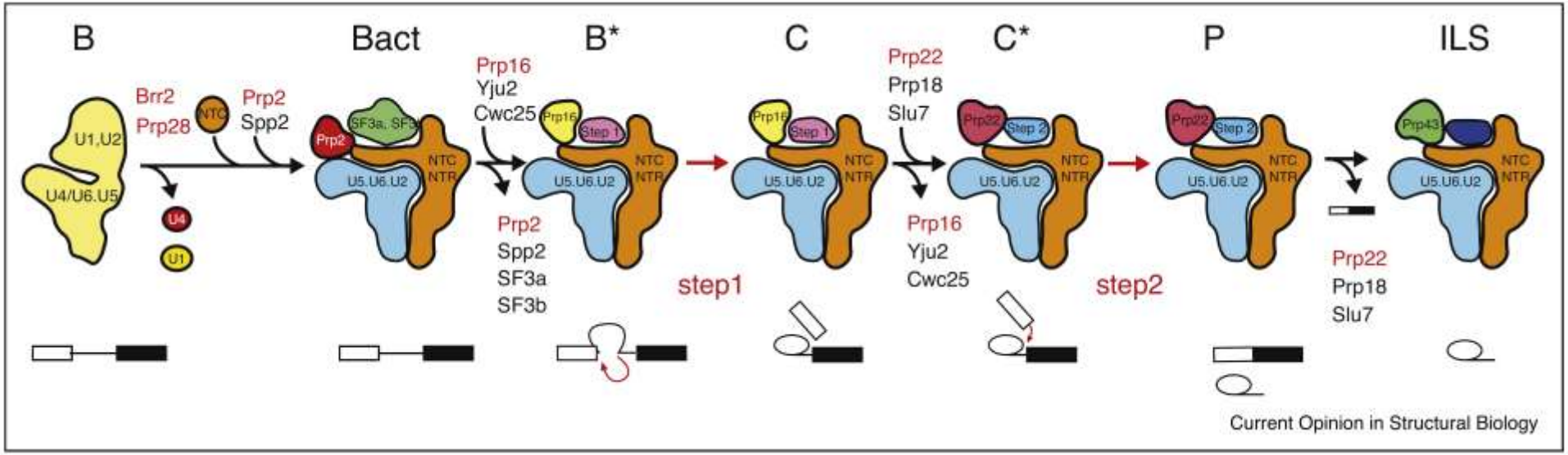
Introns are removed by two consecutive transesterification reactions



Spliceosomal assembly and disassembly pathway

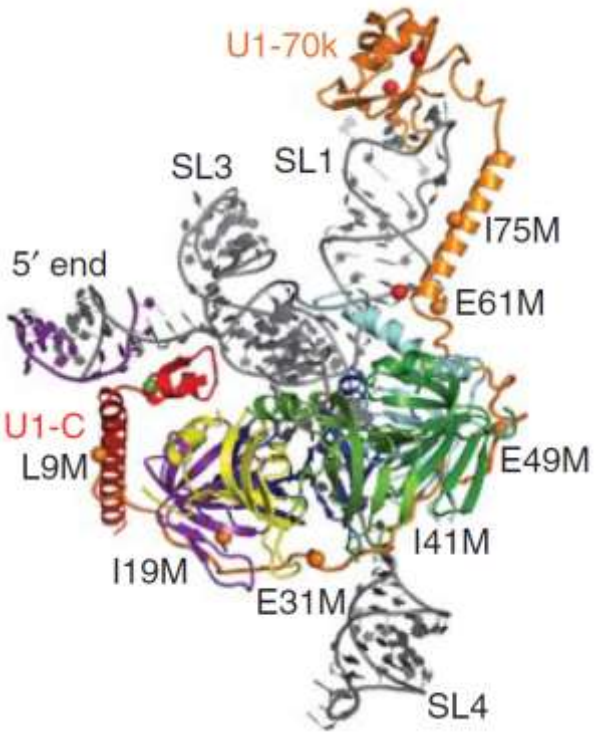
Will & Lührmann, 2011

Remodelling of the Spliceosome by RNA helicases and auxiliary proteins

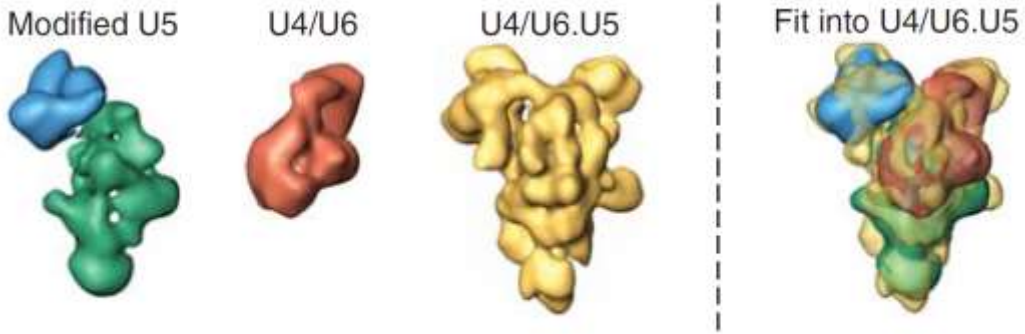


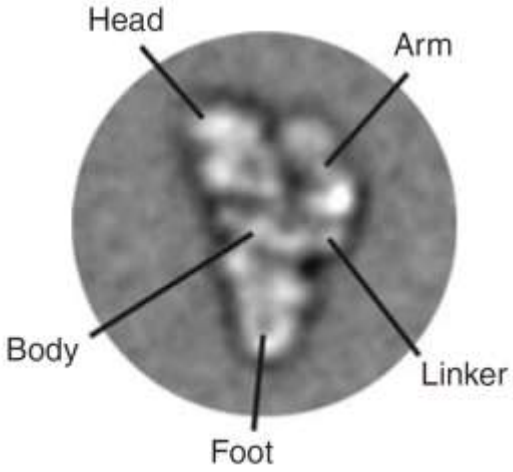
Brr2 unwinds U4/U6 snRNA duplex and displaces U4, Prp28 displaces U1
Series of conformational changes => catalytically active B* complex formed
Prp16 releases factors, Prp22 binds => catalytically active C* complex formed
Prp22 dissociates and ligated exons leave the spliceosome followed by disassembly

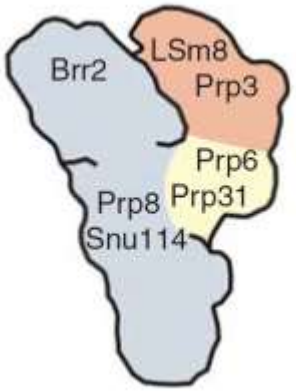
Structural views of the spliceosome



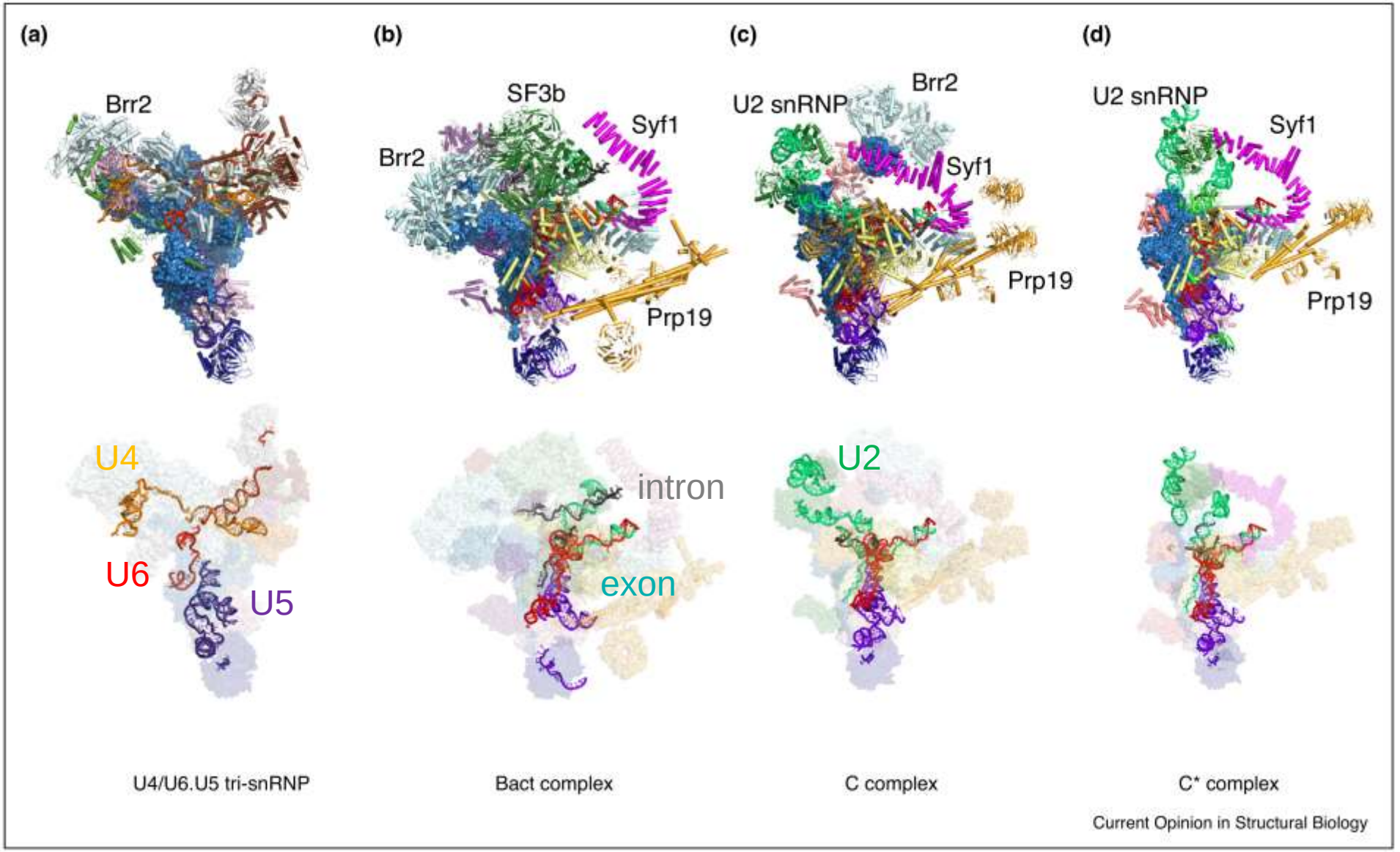
U1 snRNP



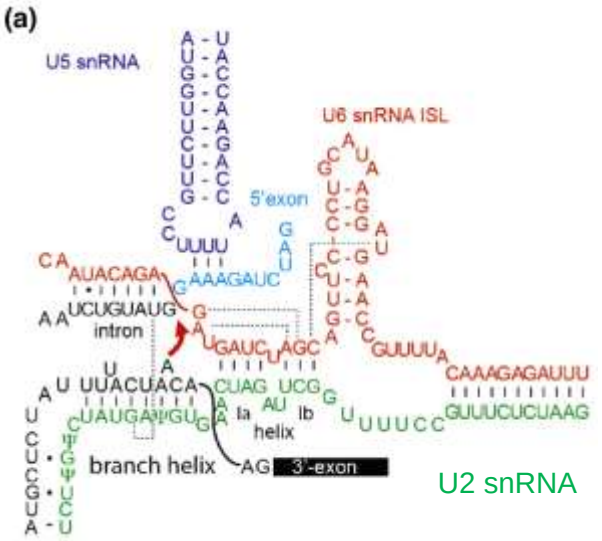


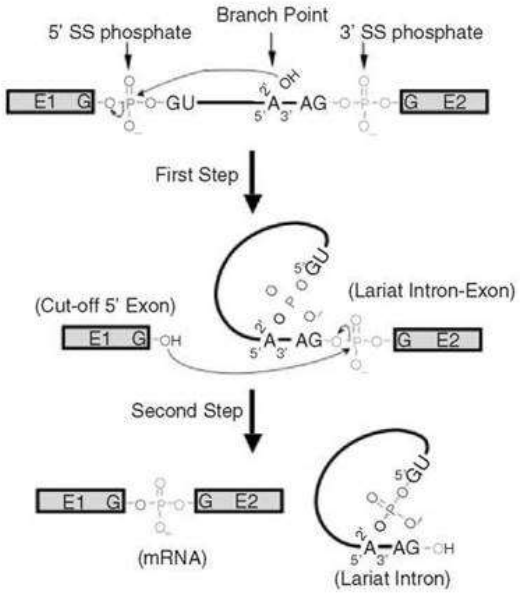


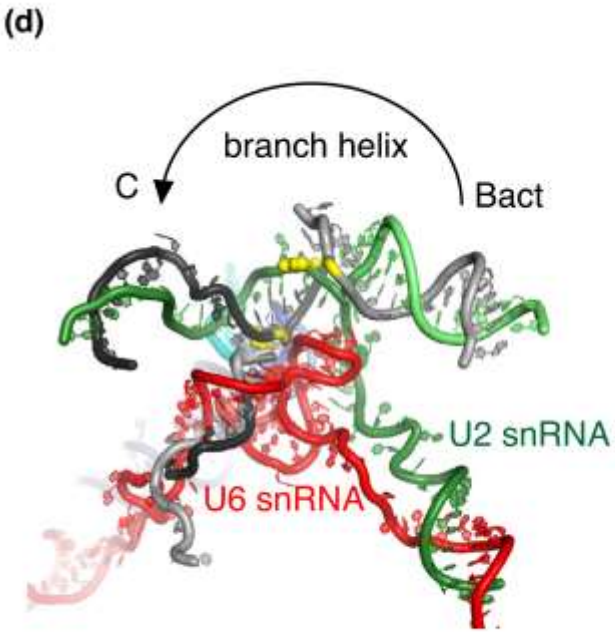
Extensive remodeling of the RNA



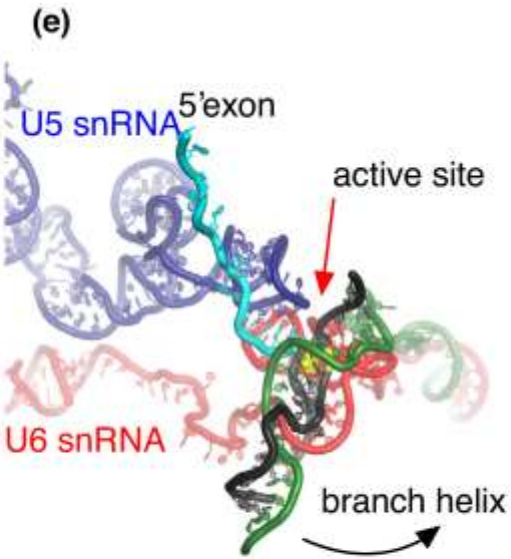
Major RNA remodeling required to form the active sites of the spliceosome



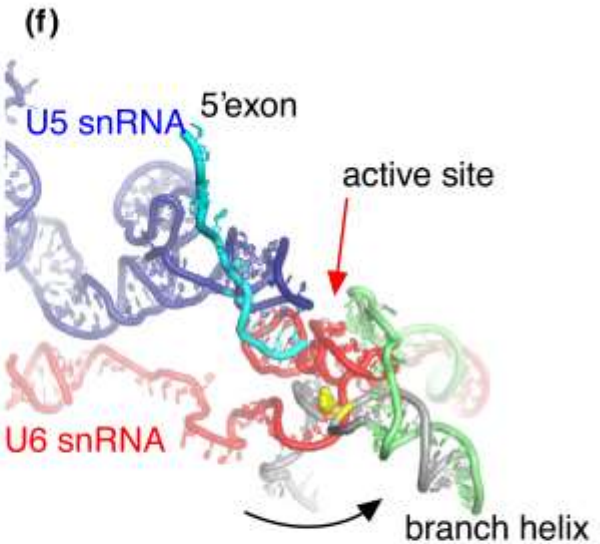




Bact to C

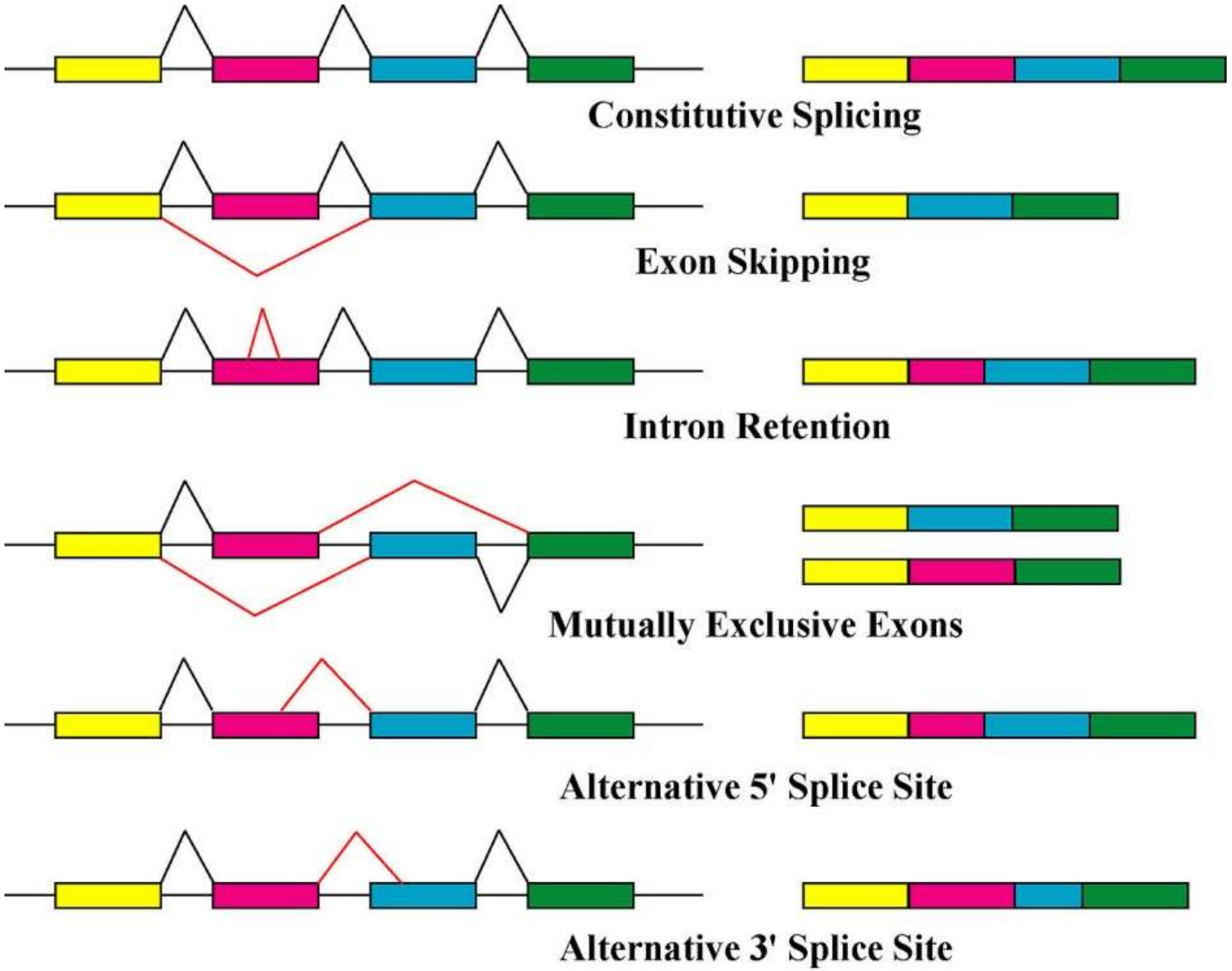


C complex

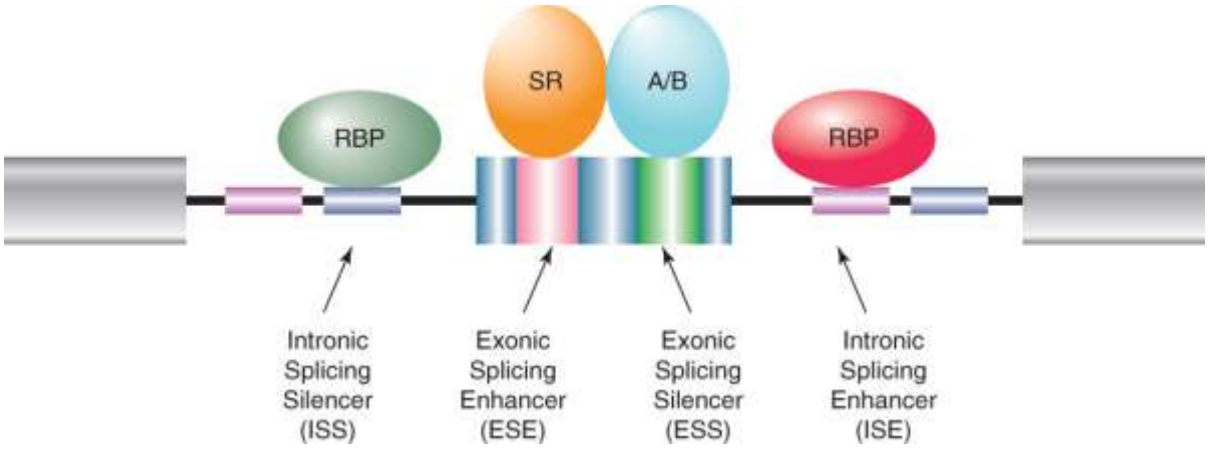


C* complex

mRNA splicing diversifies gene expression



Alternative splicing is regulated by exonic and intronic splicing enhancer and silencer sequences



- Alternative splicing is often associated with weak splice sites
- Sequences surrounding alternative exons are often more evolutionarily conserved than sequences flanking constitutive exons
- Specific exonic and intronic sequences can enhance or suppress splice site selection
- four *cis*-regulatory RNA elements which influence exon definition during splicing:
  - exonic splicing enhancers (ESE): SR protein family
  - exonic splicing silencers (ESS): hnRNP protein family
  - intronic splicing enhancers (ISE): hnRNP F/H, NOVA, or FOX proteins
  - intronic splicing silencers (ISS): hnRNP protein family
- SR protein-ESE interactions facilitate assembly of the E complex and recognition of frequently found weak 5'ss

RRM domain and interaction with RNA

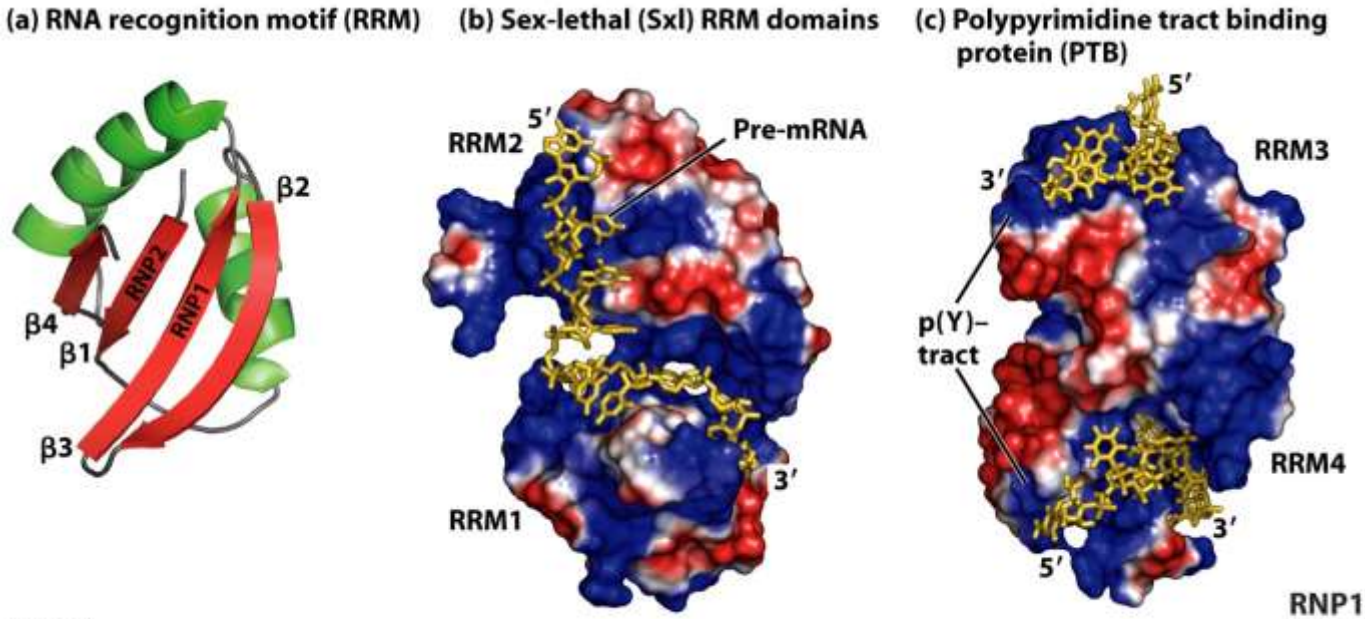
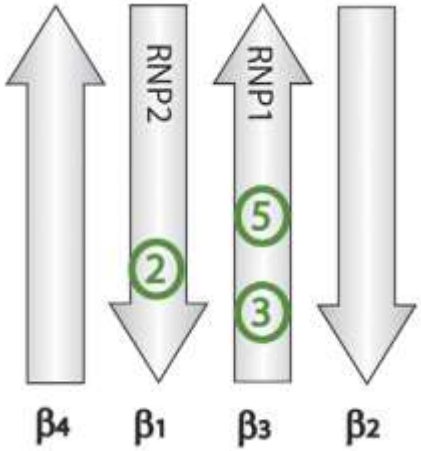


Figure 8-5
Molecular Cell Biology, Sixth Edition

RRM: RNA-binding domain containing about 80 amino acid residues

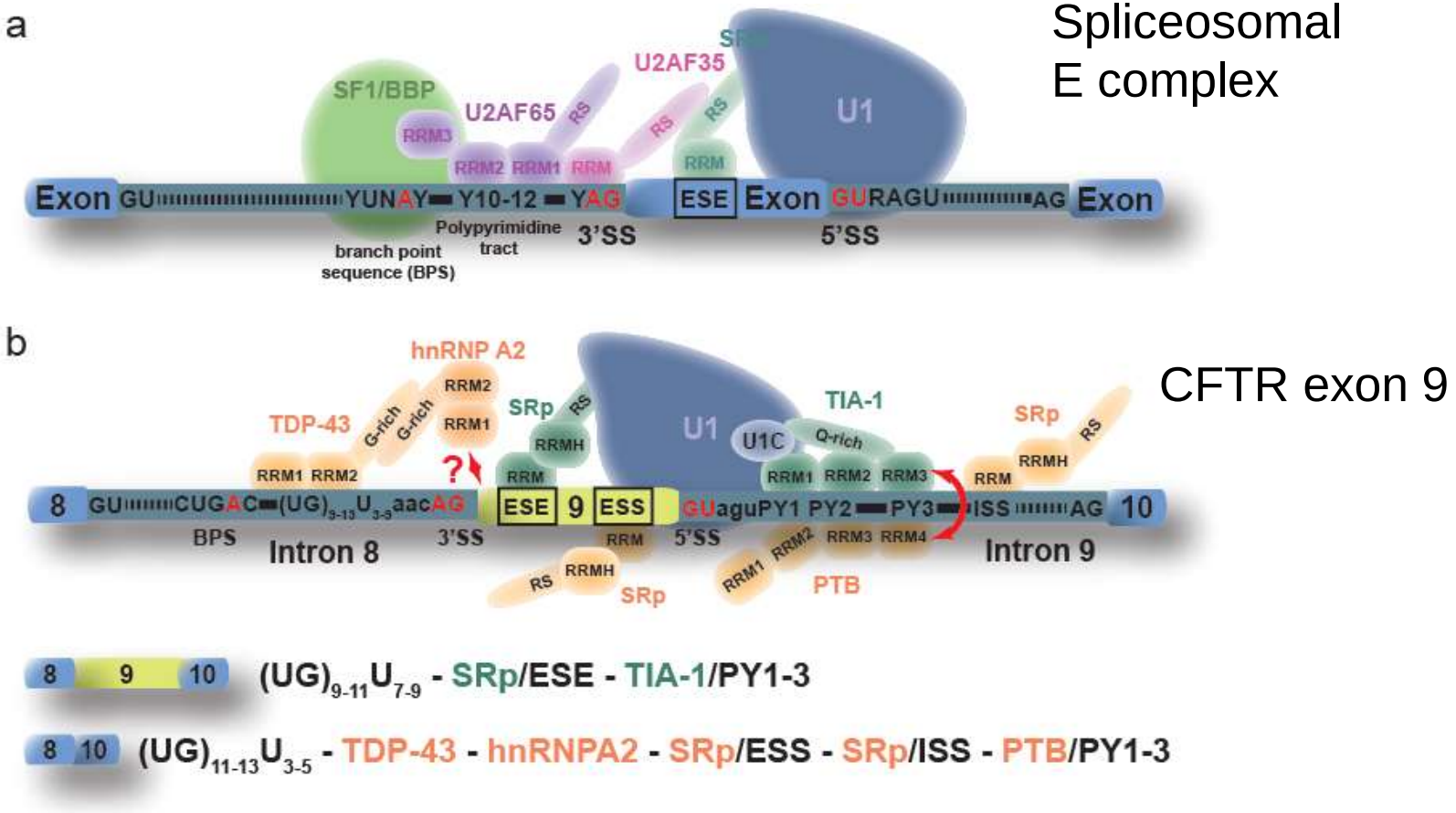
RNP1: [RK]-G-[FY]-[GA]-[FY]-[ILV]-X-[FY]
(3 5)

RNP2: [ILV]-[FY]-[ILV]-X-N-L
(2)



The RRM domain contains two highly conserved sequences, RNP1 (β3) and RNP2 (β1) mediating specific RNA recognition.

mRNA splicing regulated through multiple RNA-protein interactions



Cystic fibrosis (CF):

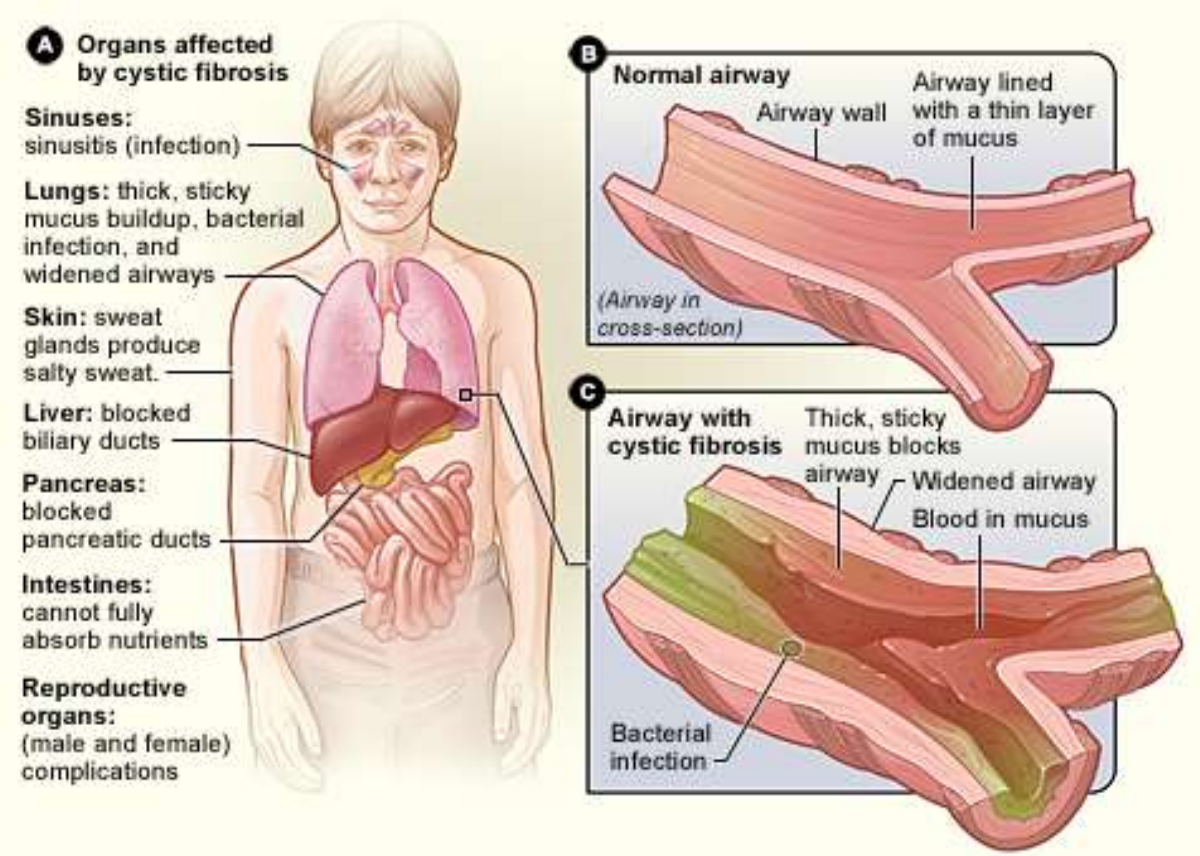
- most frequent genetic disease in newborns (1 in 2000-3000)

- 1 in 25 caucasians carries one allele with CF mutations

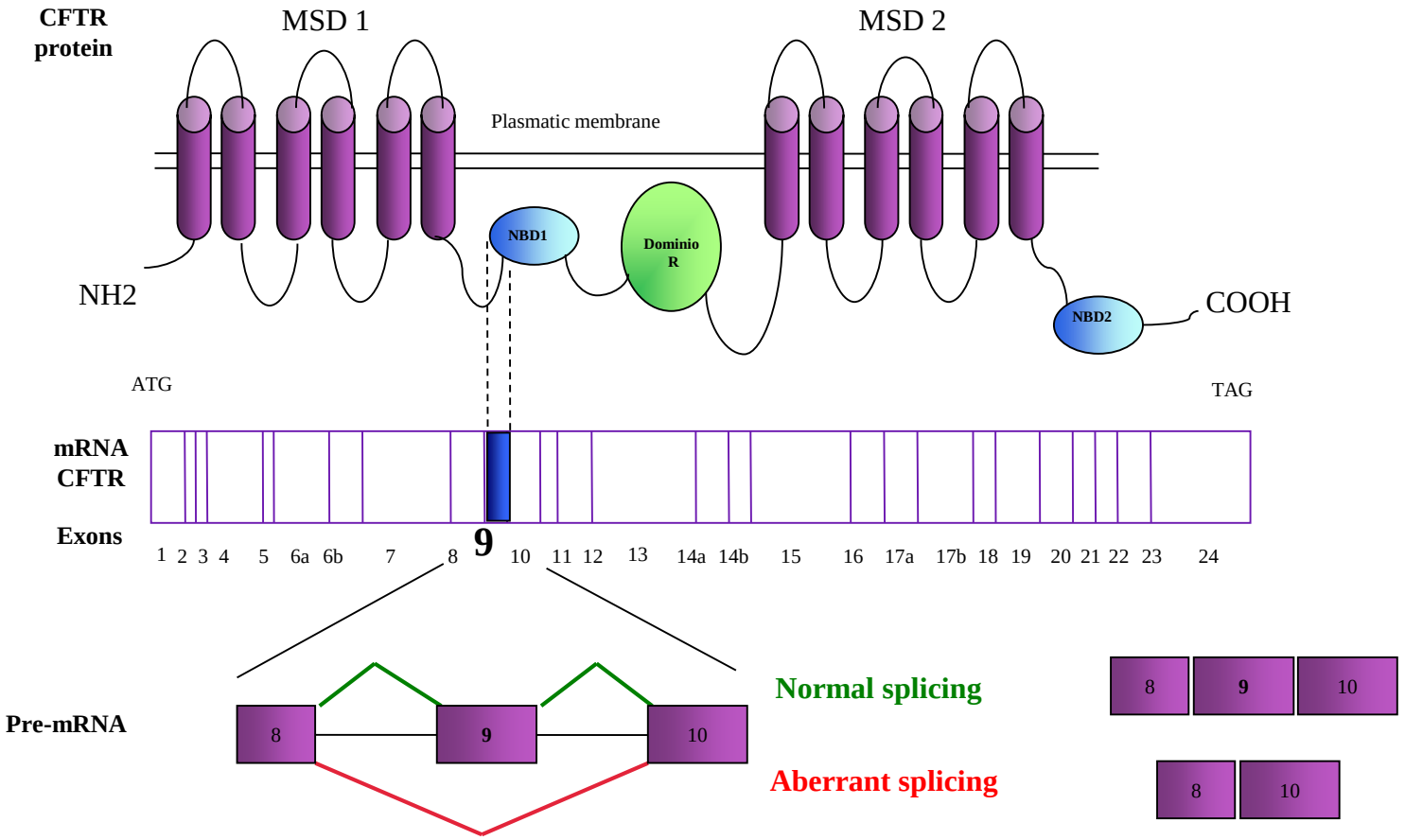
- Most frequent mutations in CFTR gene

- abnormal transport of chloride and sodium ions

- accumulation of mucus in several organs

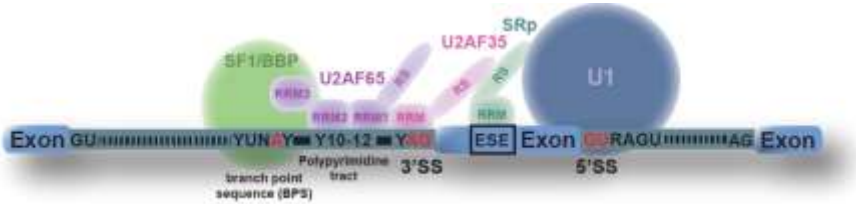


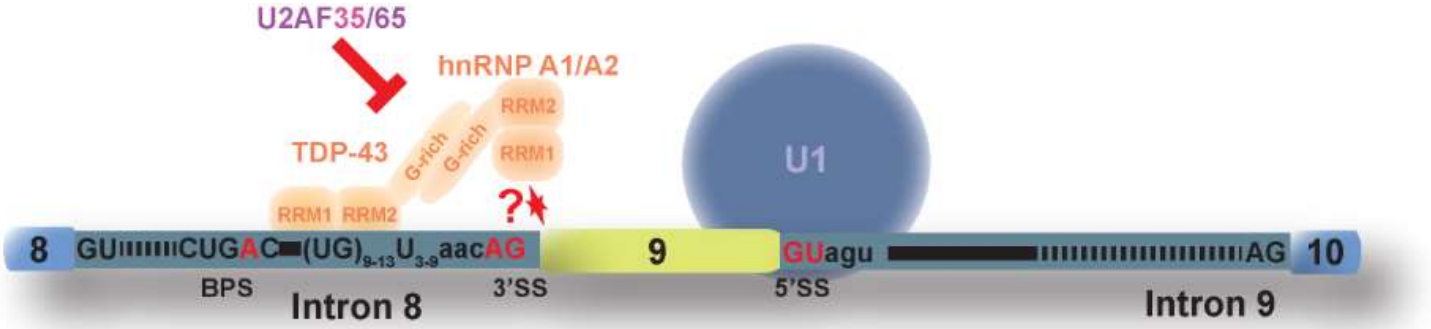
Aberrant splicing of cystic fibrosis transmembrane conductance regulator (CFTR) exon 9

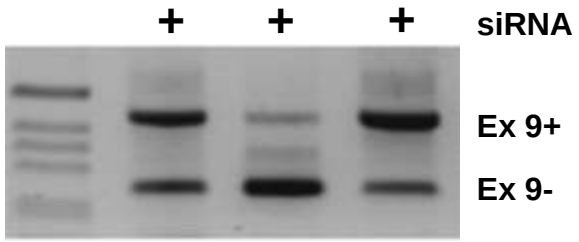


TDP-43 is a dominant inhibitor of CFTR exon 9 splicing

Spliceosomal E complex



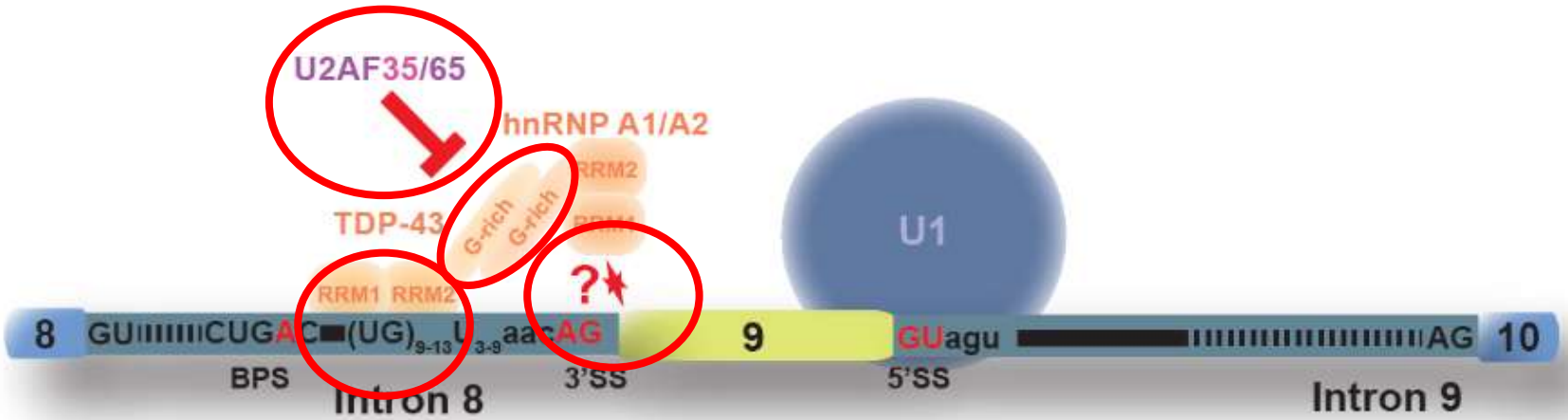




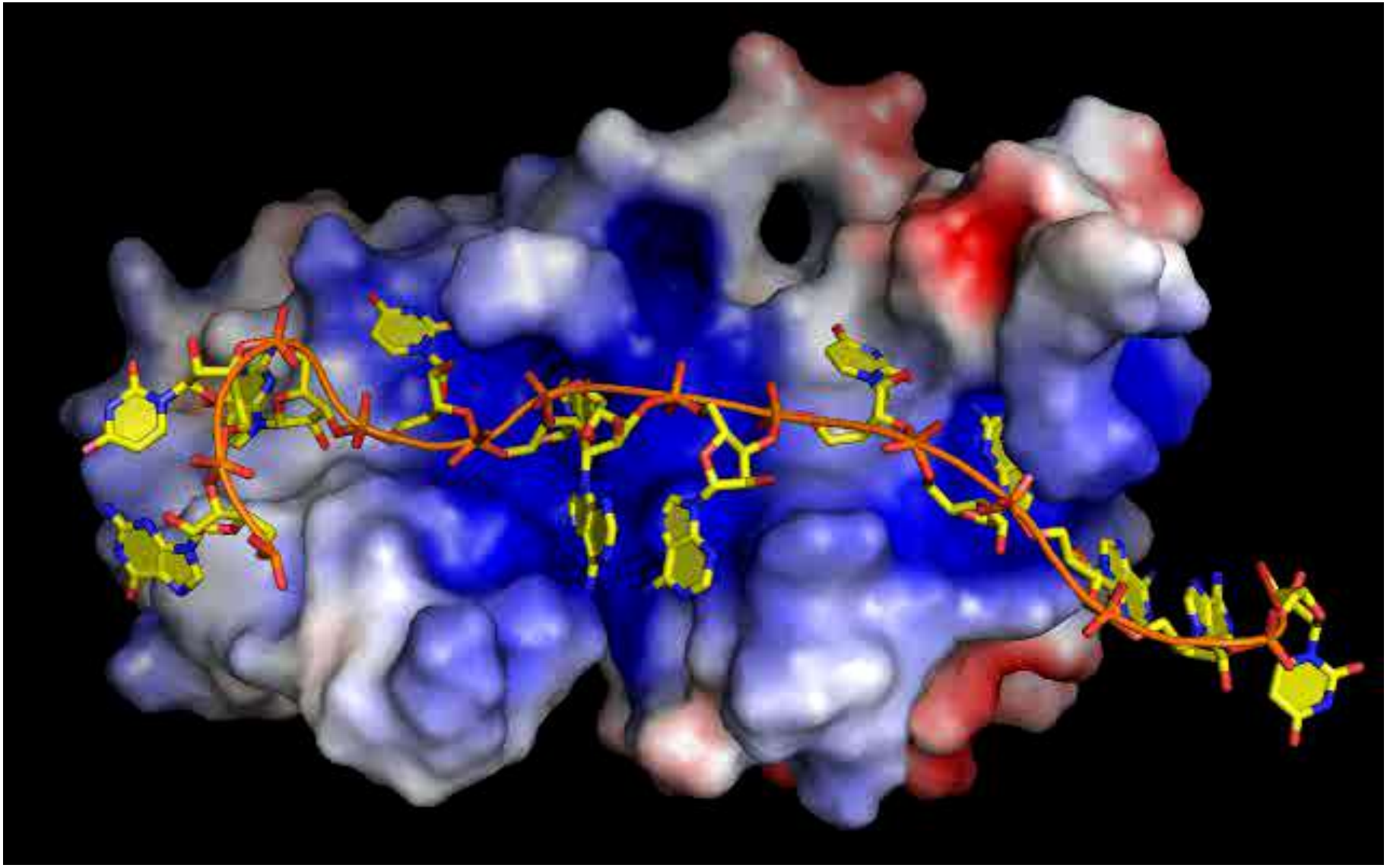
add back - **WT** **mut**

Buratti et al, *EMBO J* (2001)

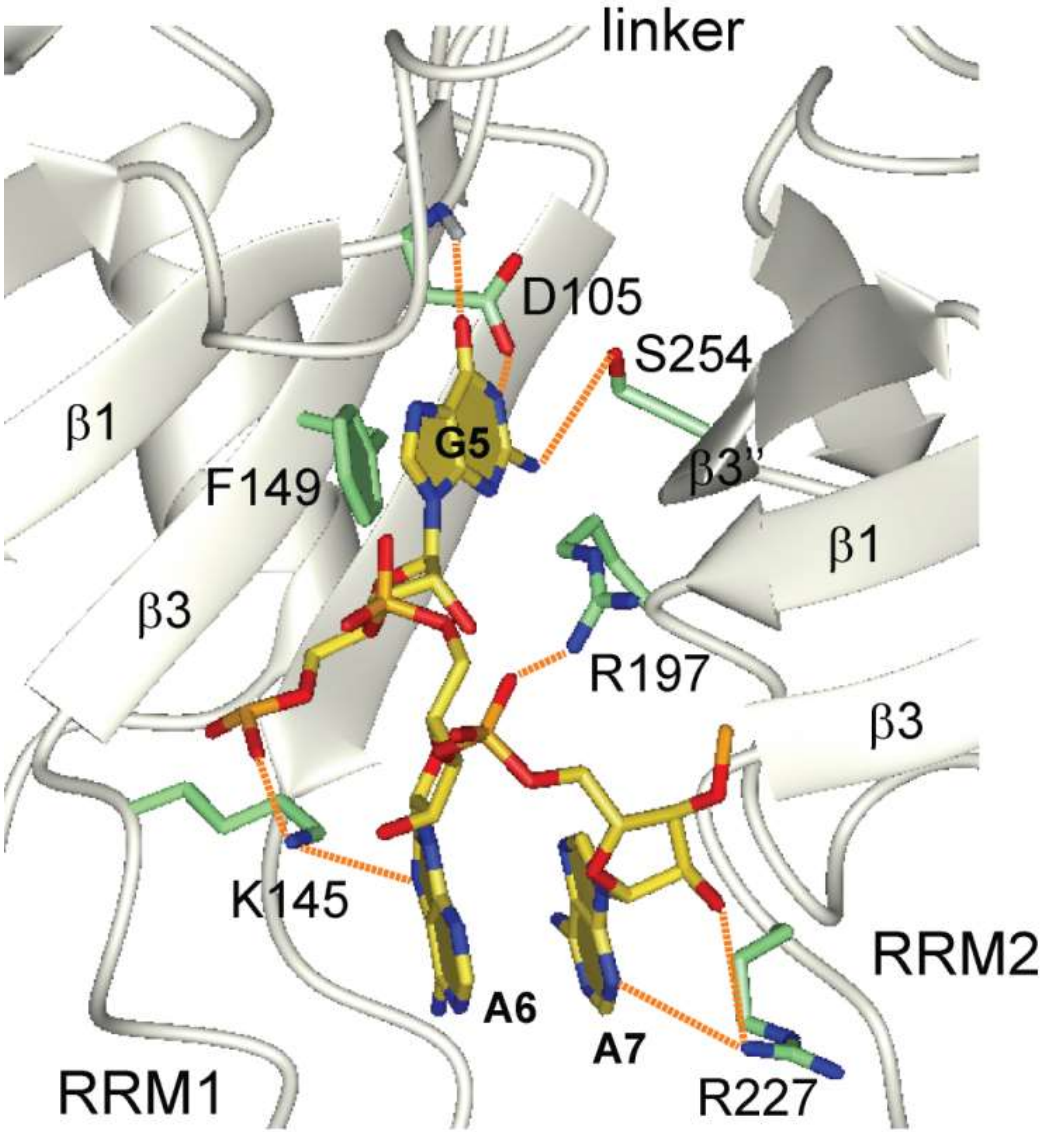
Questions…



protein	domain composition	binding sequence
TDP-43	RRM-1 (1–106) RRM-2 (177–259) Gly-rich (274–414)	UG-repeats
hnRNP A1	RRM-1 (1–88) RRM-2 (106–188) Gly-rich (195–320)	UAG(G)

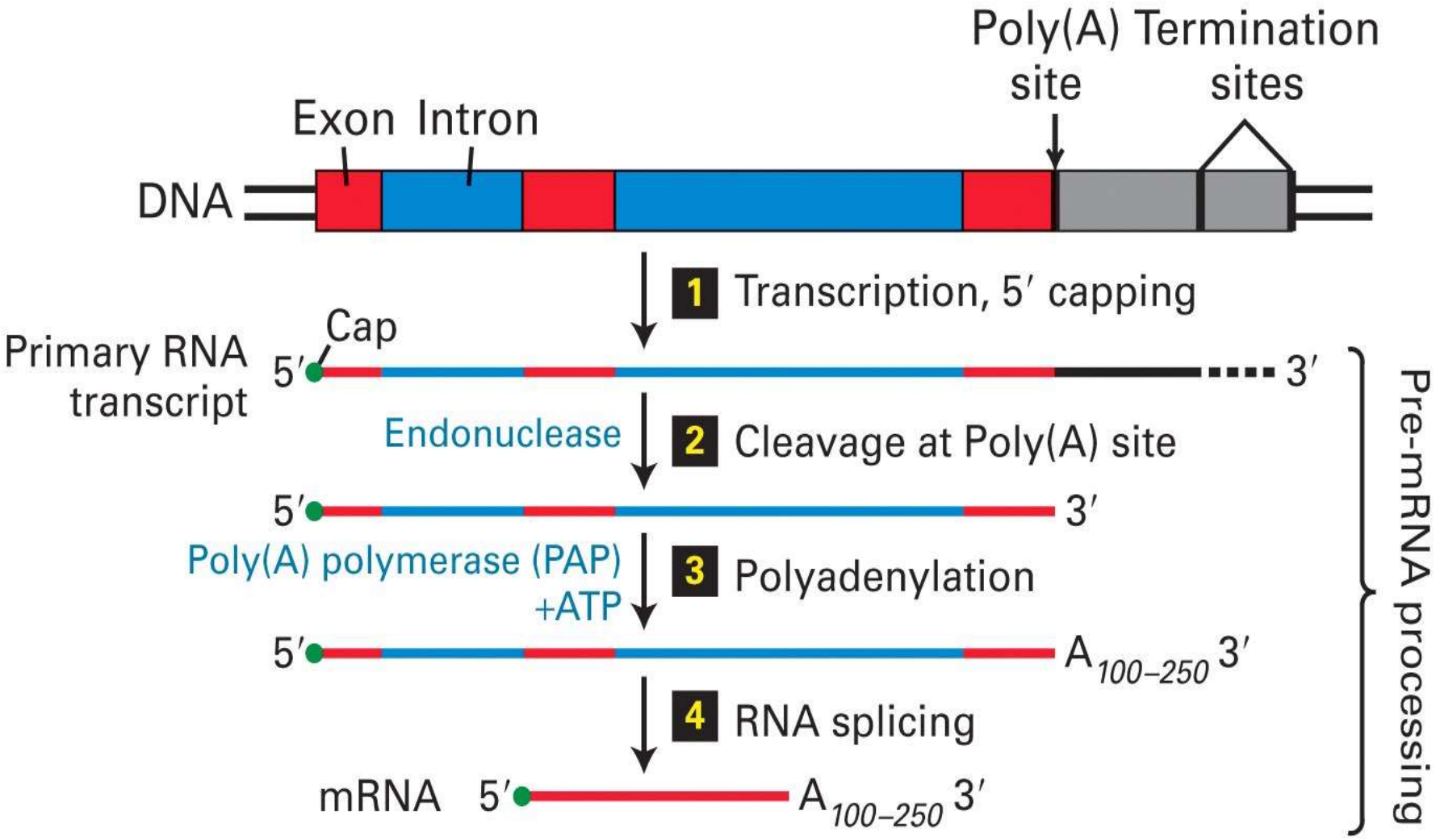


G5, A6, A7 interact at the interface of both RRMs



GUGUGAAUGAAU

More processing of eukaryotic pre-mRNA



Structure of the 5’ CAP

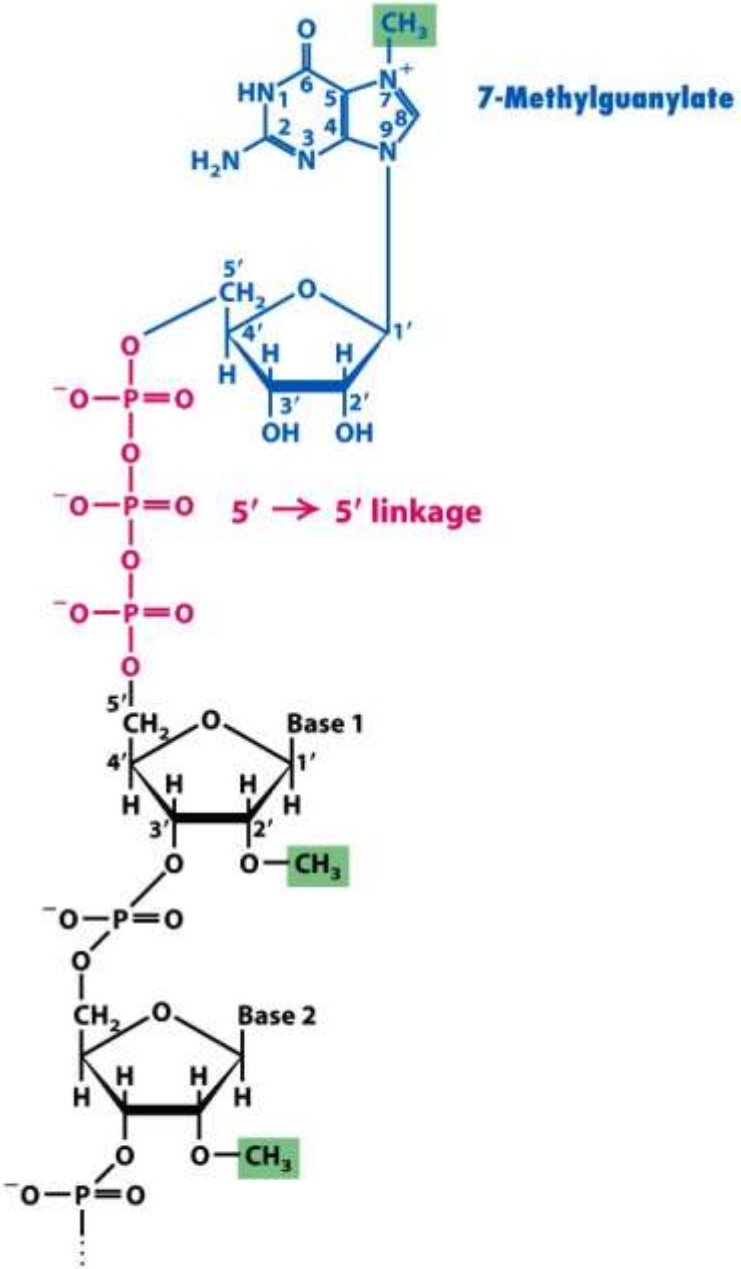
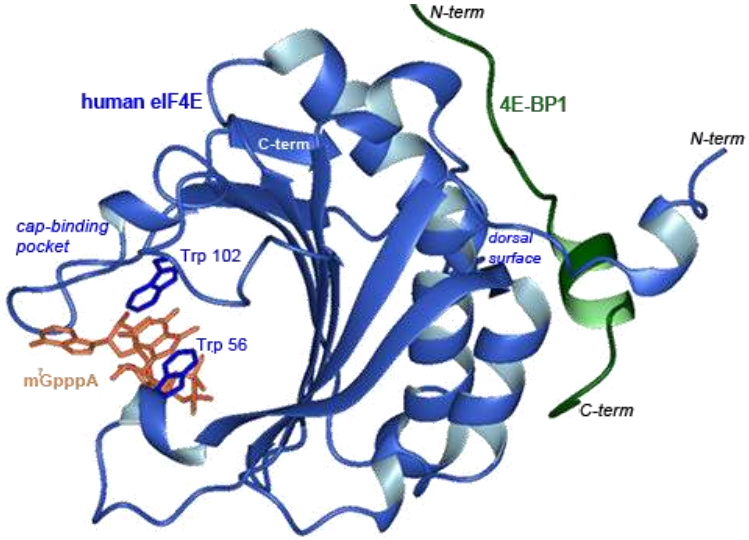


Figure 4-14
Molecular Cell Biology, Sixth Edition

A methyl group from S-adenosyl-methionine is added

to the N7 position of the G

to the 2’OH of the first two riboses of the nascent mRNA



Functions of 5'CAP

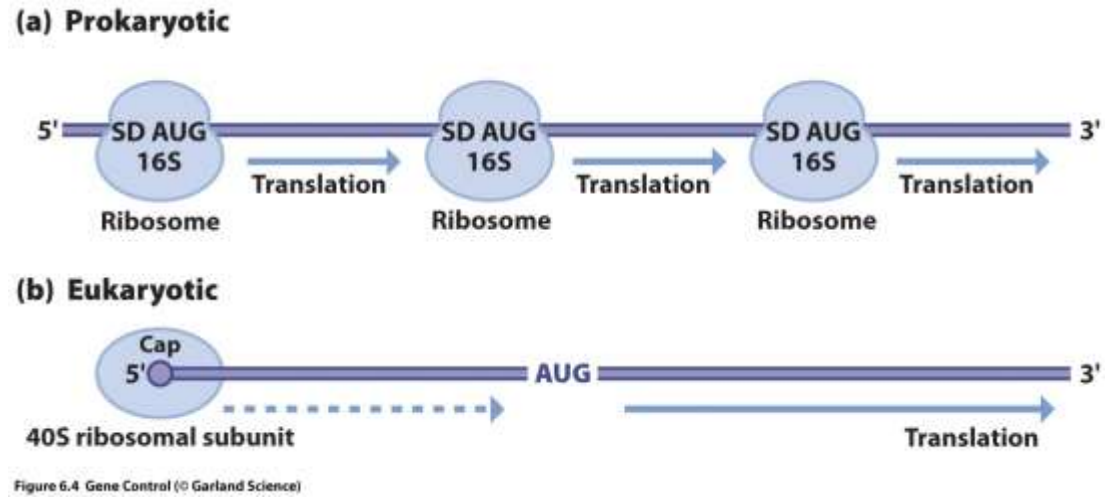


Figure 6.4 Gene Control (© Garland Science)

In prokaryotes, the Shine-Dalgarno sequence (localized at 10 bases upstream of AUG of mRNA) binds to 16S rRNA to initiate translation.

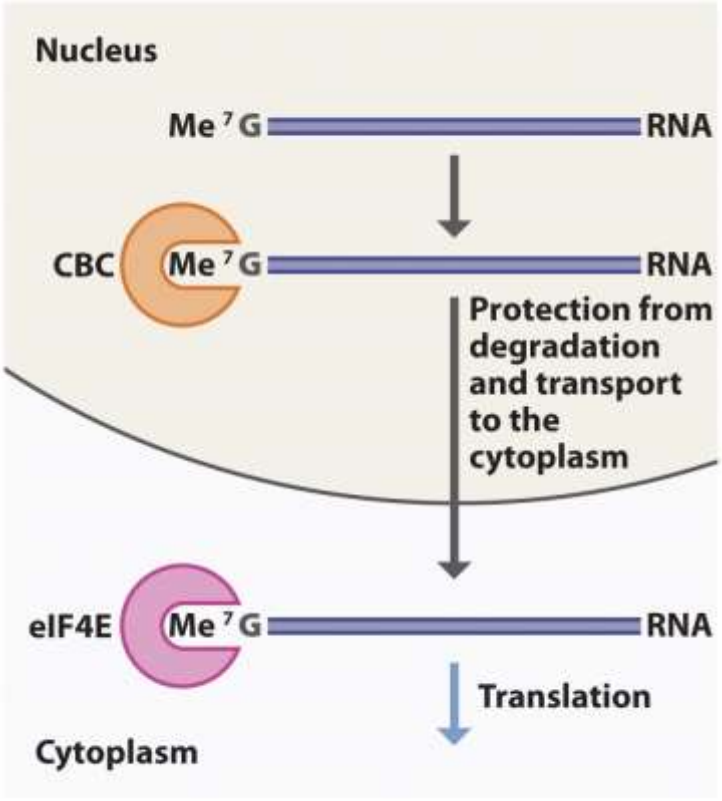


Figure 6.5 Gene Control (© Garland Science)

In eukaryotes, the 5' end of the mRNA is capped.

The 5'CAP binds to the CAP-binding complex (CBC) => protects mRNA from degradation and facilitates nuclear export

After nuclear export, the CBC is replaced with eIF4E and the complex will recruit other eIFs and the 40S ribosomal subunit to initiate translation.

The AUG is localized within the consensus sequence of GCCA/GCC**AUG**G (Kozak's sequence) and the 40S subunits need to scan 5'UTR to reach the start codon.

Polyadenylation of eukaryotic mRNA

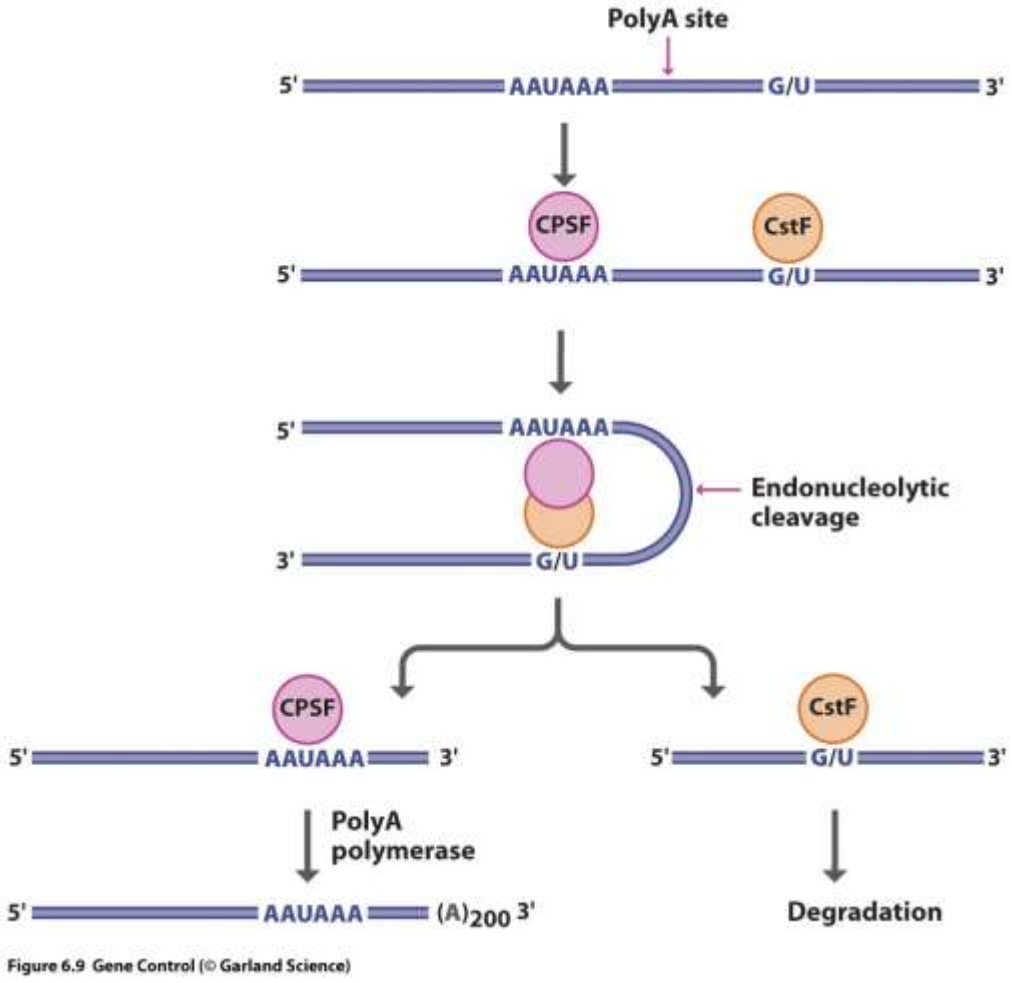


Figure 6.9 Gene Control (© Garland Science)

Cleavage of mRNA transcript at the site downstream of AAUAAA and upstream of G/U-rich sequence.

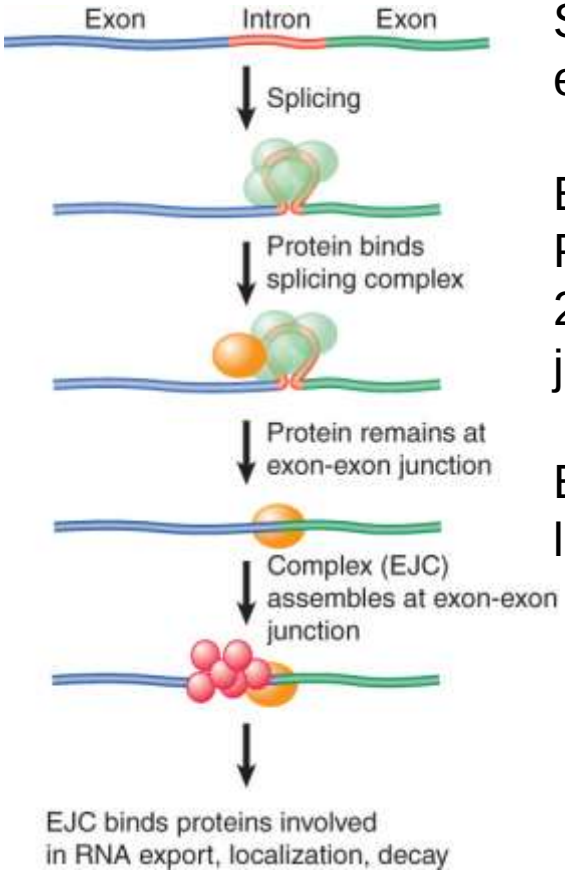
The mRNA sequences are recognized by **CPSF** (cleavage- and polyadenylation-specific complex) and **CstF** (cleavage-stimulation factor)

First endonucleolytic cleavage followed by polyadenylation of mRNA and degradation of the 3' fragment.

PolyA tail important for RNA stability, export and translational control in cytoplasm

Alternative polyadenylation regulates gene expression

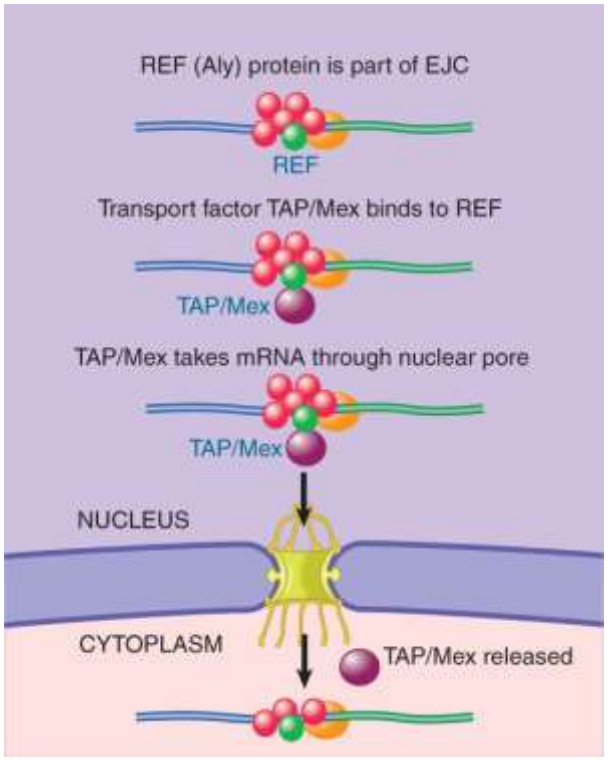
Splicing facilitates nuclear export of mRNA to cytoplasm



Splicing is connected to mRNA export and stability control.

Exon junction complex (EJC) – Protein complex that assembles 20-24nt upstream of exon–exon junctions during splicing.

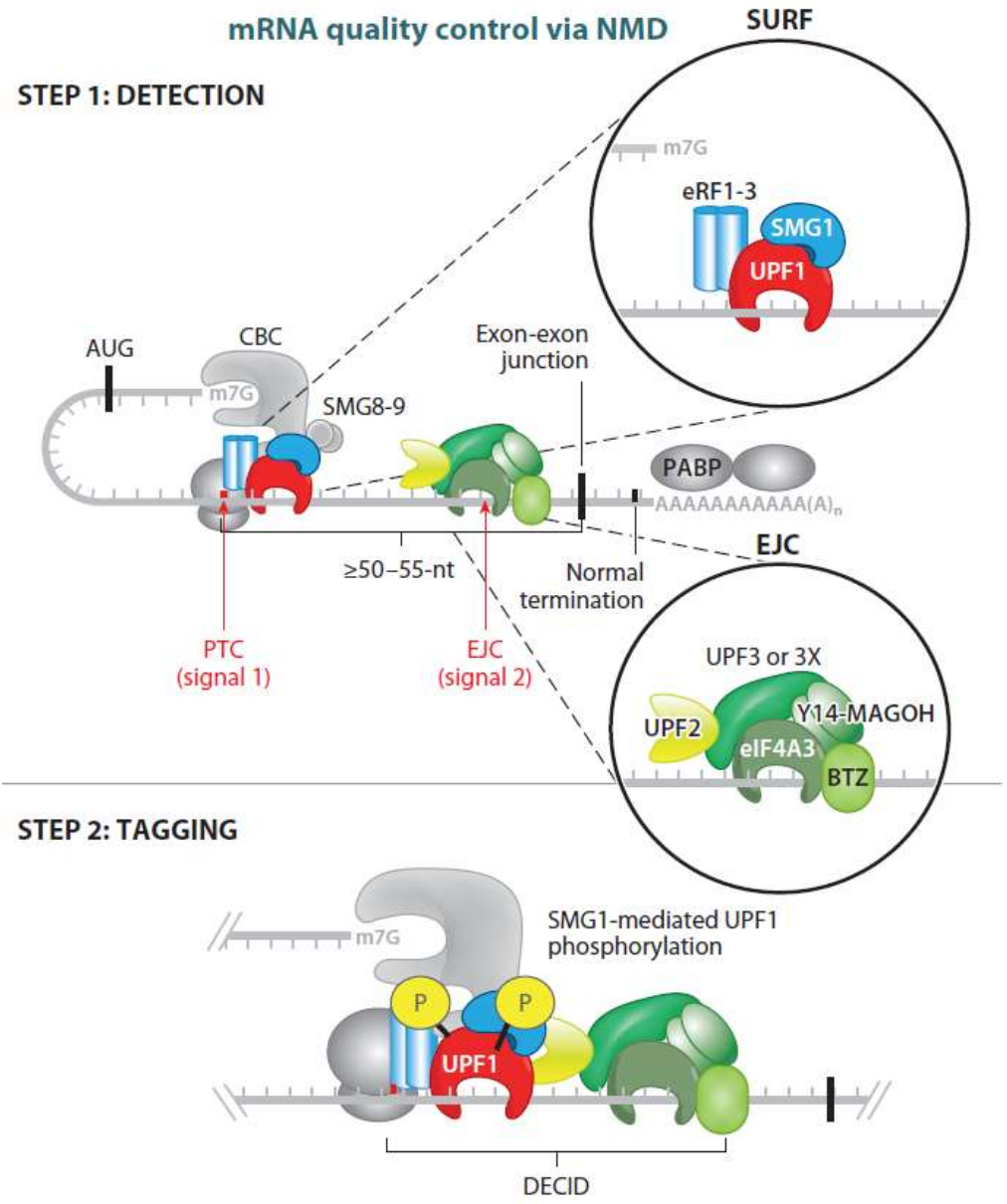
EJC assists in RNA export, localization, and degradation.



EJC recruits protein complex for mRNA export

REF (Aly), a key protein mediating mRNA export by interacting with TAP (Mex67p)

EJC couples splicing with NMD



Popp & Maquat, Ann. Rev. Genet. 2013

Splicing in the nucleus can influence mRNA translation in the cytoplasm.

Nonsense-mediated mRNA decay (NMD) – A pathway that degrades an mRNA that has a nonsense mutation prior to the last exon.

up-frameshift proteins: UPF1-3

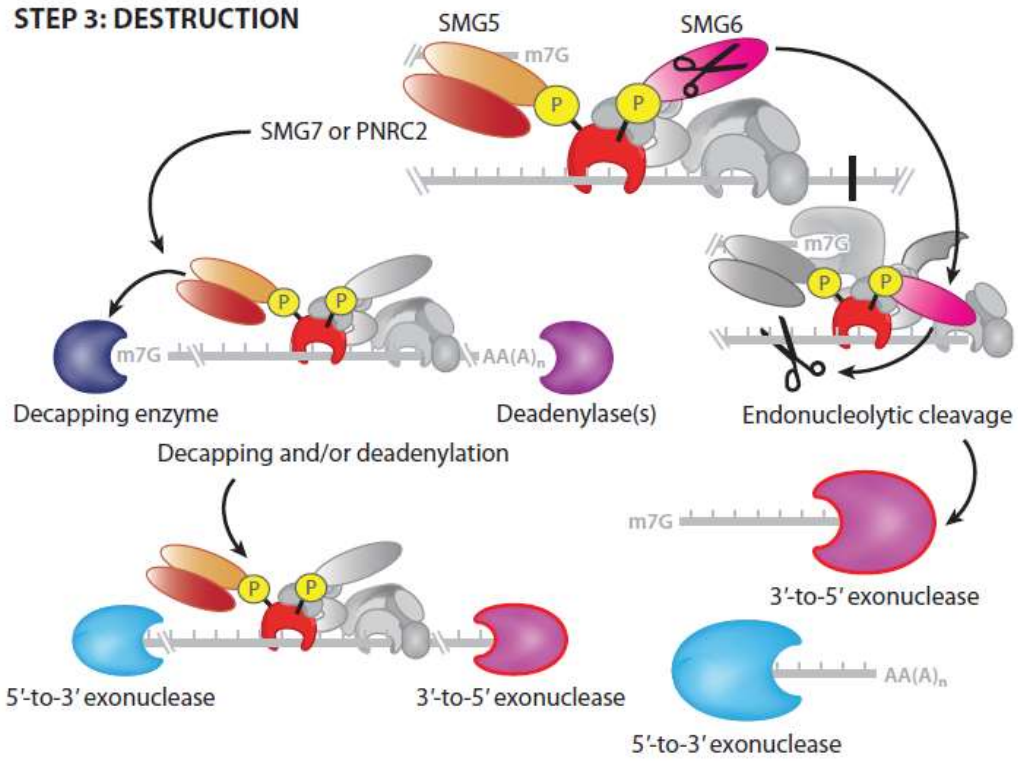
eukaryotic release factors : eRF1+3

serine/threonine kinase: SMG1

ATP-dependent helicase: UPF1 activated by UPF2

Silencers of SMG1: SMG8+9

EJC couples splicing with NMD



Phosphorylated UPF1 recruits endonuclease SMG6

5’ cleavage product: decapping and 3’-to-5’ decay

3’ cleavage product: UPF1 removes proteins and 5’-to-3’ decay

Popp & Maquat, Ann. Rev. Genet. 2013

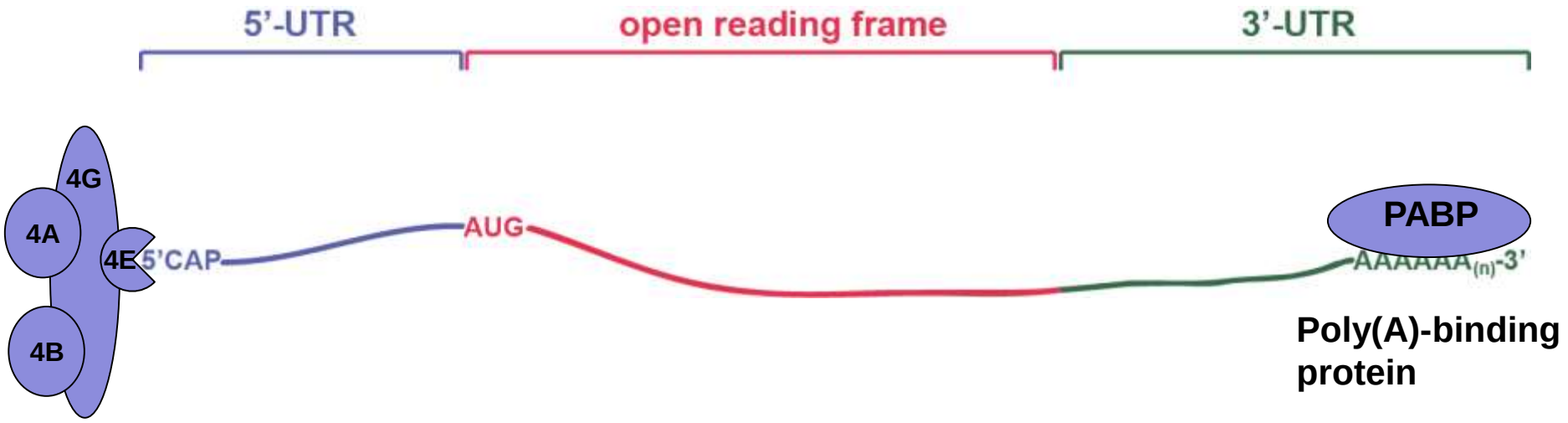
Eukaryotic mRNA in the cytoplasm

up to 3 kb

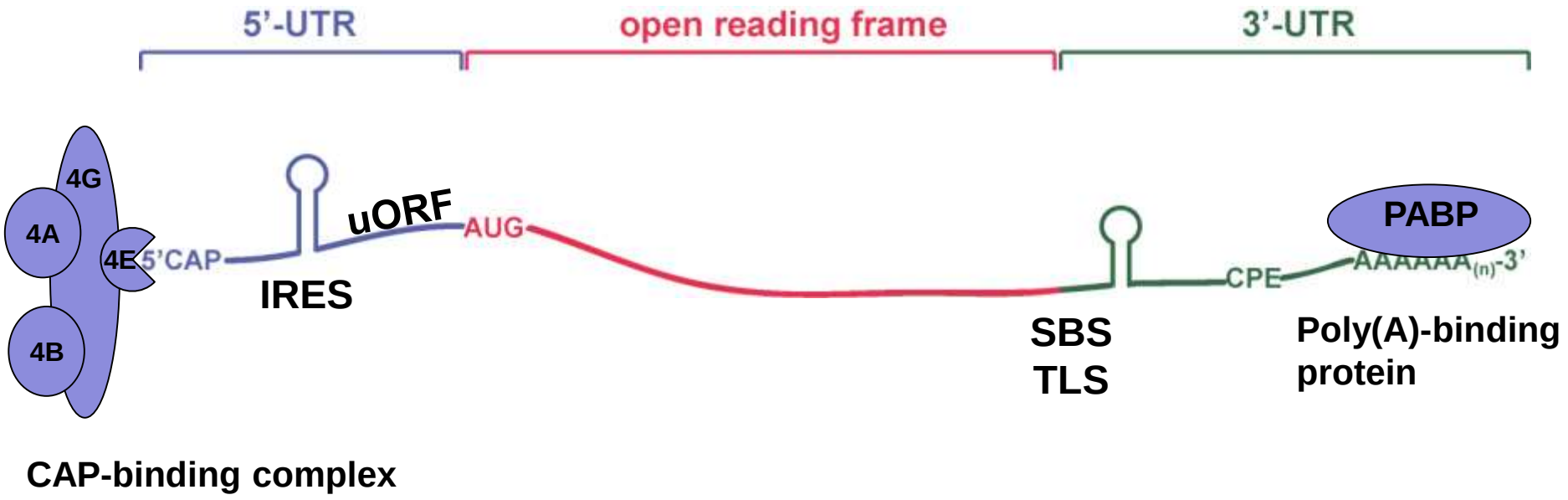
up to 10 kb

target for post-transcriptional regulation of gene expression

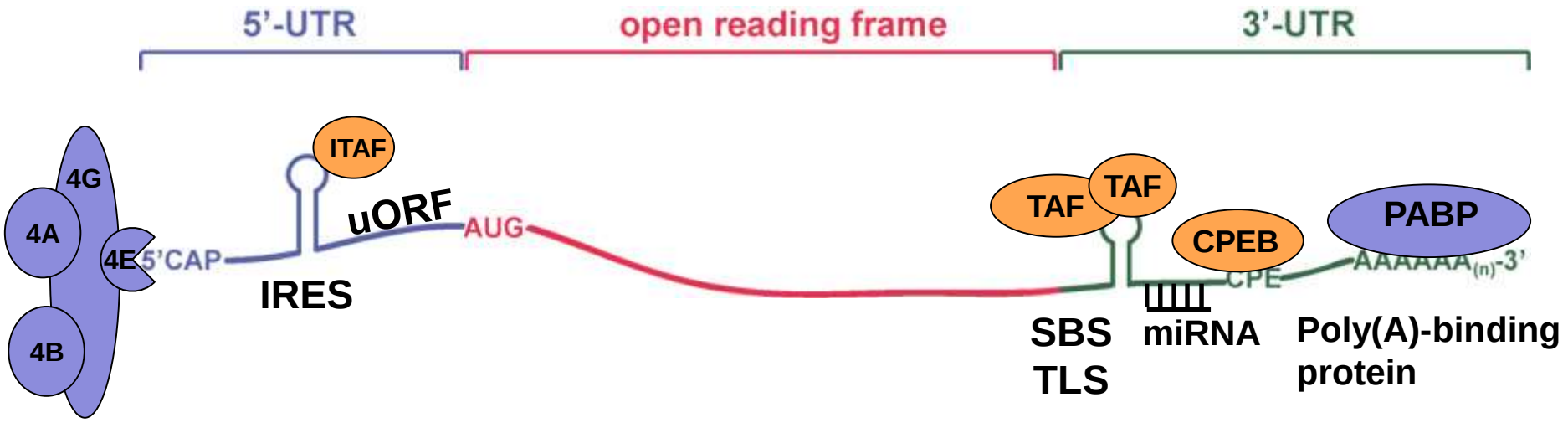
Eukaryotic mRNA



Regulatory RNA stem-loops in 5’- and 3’-UTR



Complex network of RNA-protein interactions within mRNA regulates gene expression

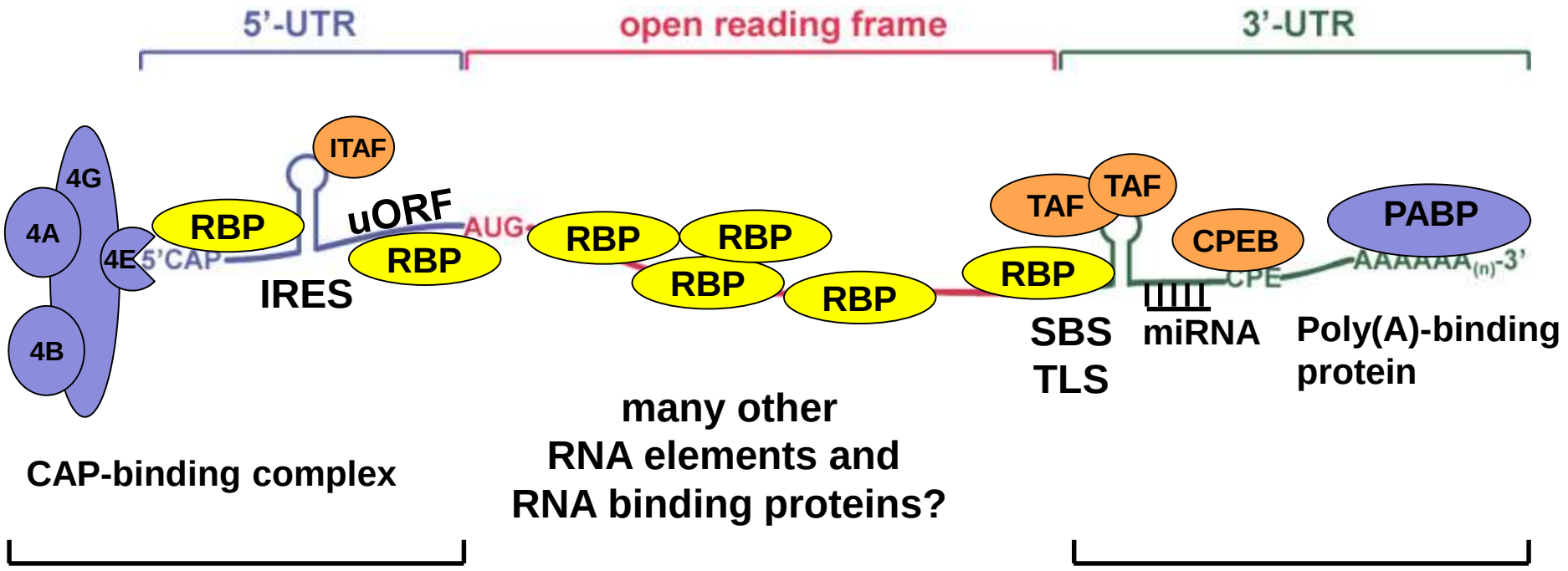


CAP-binding complex

- Translational control
- mRNA stability

- Translational control
- mRNA localization
- mRNA stability

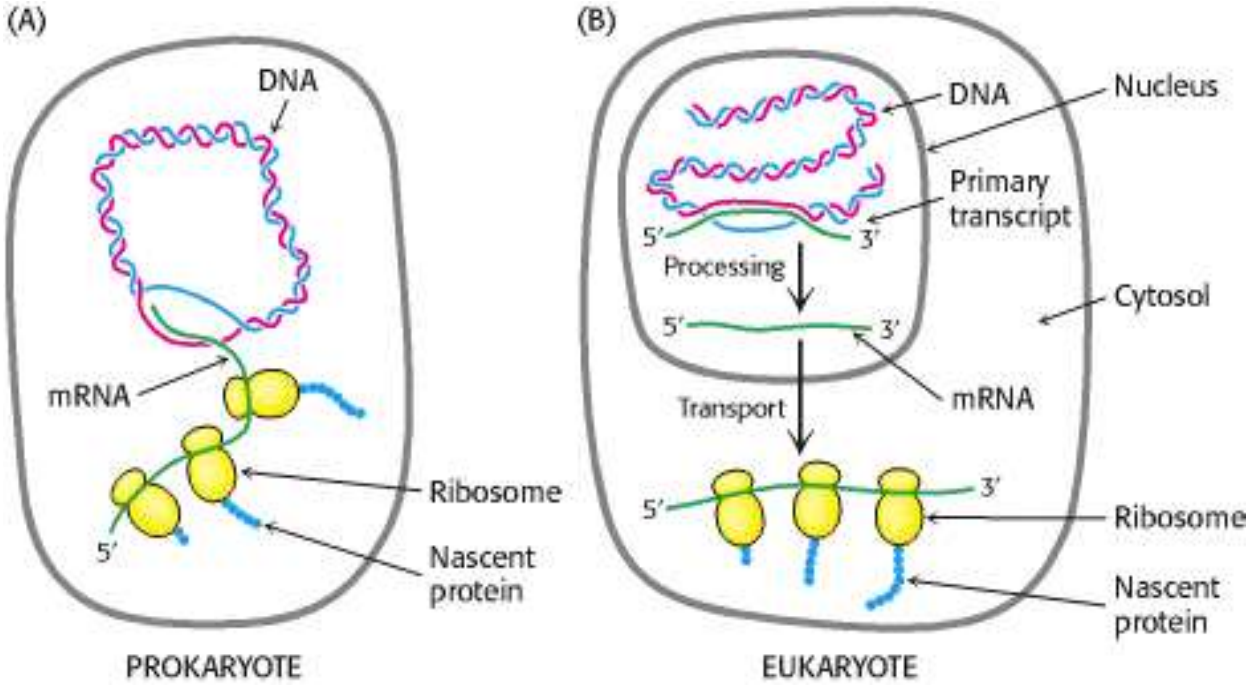
Complex network of RNA-protein interactions within mRNA regulates gene expression



- Translational control
- mRNA stability

- Translational control
- mRNA localization
- mRNA stability

Translation – from RNA to Proteins



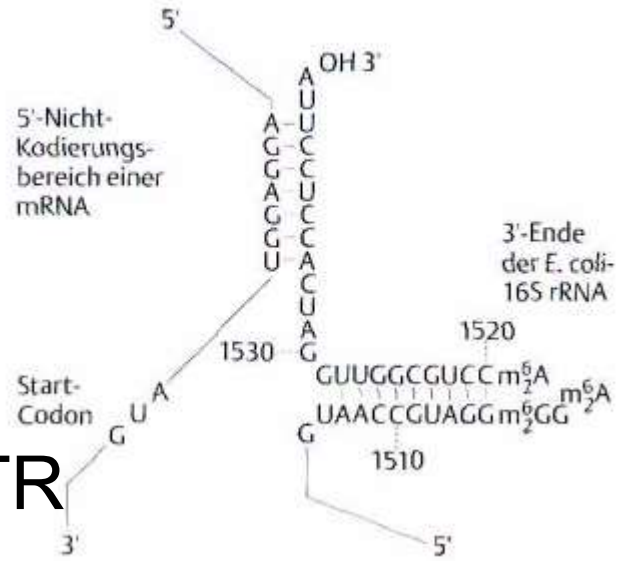
- In prokaryotes: primary mRNA transcript directly used as template for protein synthesis.
- In eukaryotes: primary mRNA transcript processed in nucleus, exported into cytoplasm for translation.

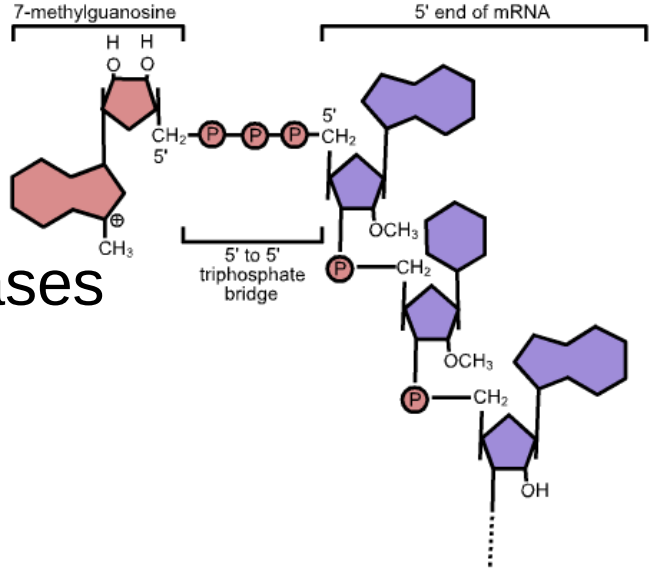
What do we need for translation?

- Template mRNA
- Transfer RNAs (tRNAs)
  - charged with amino acids
- Ribosomes
- Many accessory proteins
  - especially in eukaryotes!!
- Lots of energy (ATP and GTP hydrolysis)

mRNA

- Prokaryotic: often codes for two or more proteins
  - Shine-Dalgarno sequence in 5'UTR
- Eukaryotic: codes for one protein
  - 5' cap (7-methyl guanosine)
  - 3' poly-A tail (50-200 adenines)
    - Protect from degradation by nucleases
    - Allow for regulation of protein synthesis



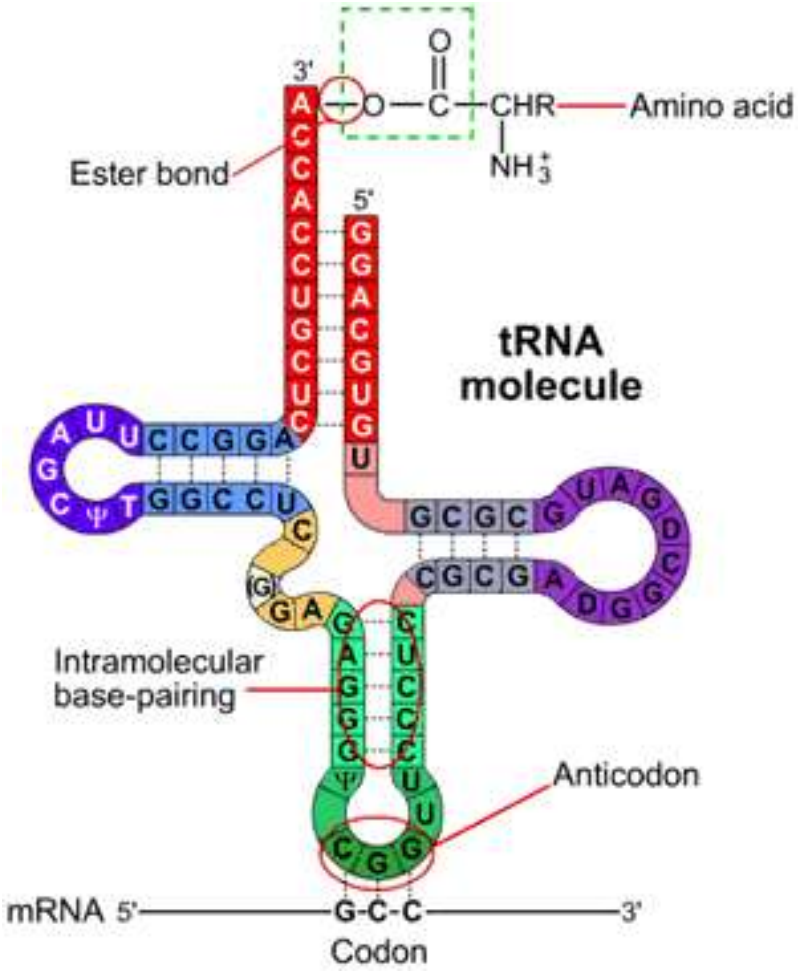


Genetic code

- Codons specify type of amino acid
- Initiation (start) codon
  - AUG codes for methionine
  - Every protein in a cell starts with methionine
- Termination (stop) codons
  - UAA, UGA, UAG

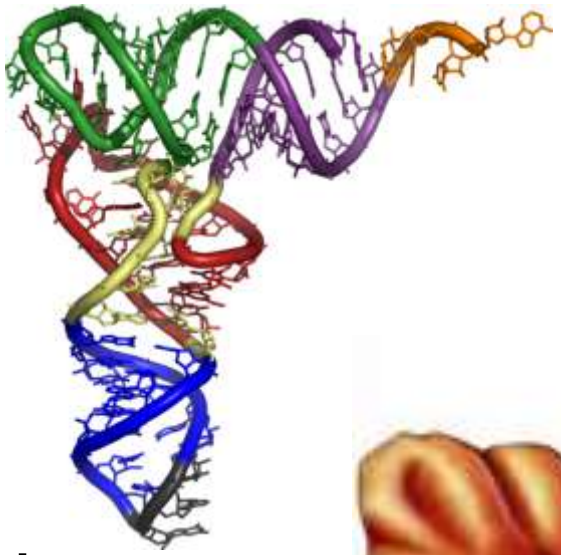
tRNA

- Delivers amino acid to ribosome
- Adaptor between codons in mRNA and amino acid
- 4 stems and 3 loops
  - Complex fold
  - Anticodon in hairpin loop



tRNA

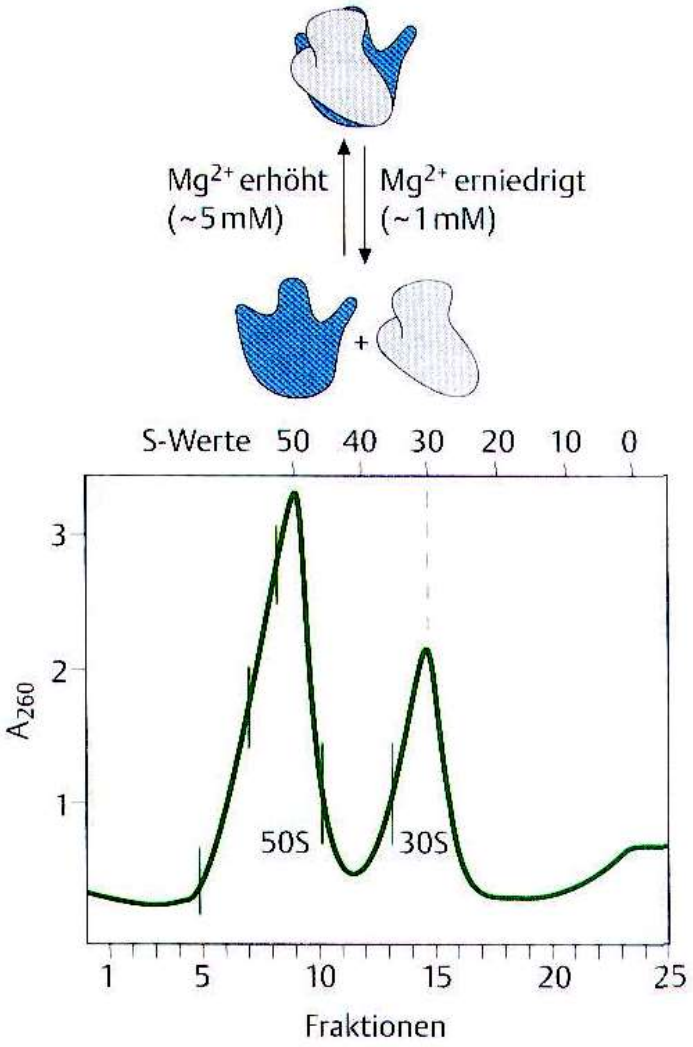
- L-shaped structure
- Anticodon and amino acid at opposite ends
- First structure by Aaron Klug (LMB) and Alex Rich (MIT) in 1974



Ribosomes

- Composed of small and large subunit
- Subunits form complex for translation
- E. coli: 20.000 ribosomes/cell
- Ribosomes self-assemble without additional factors

=> assembly needs to be controlled



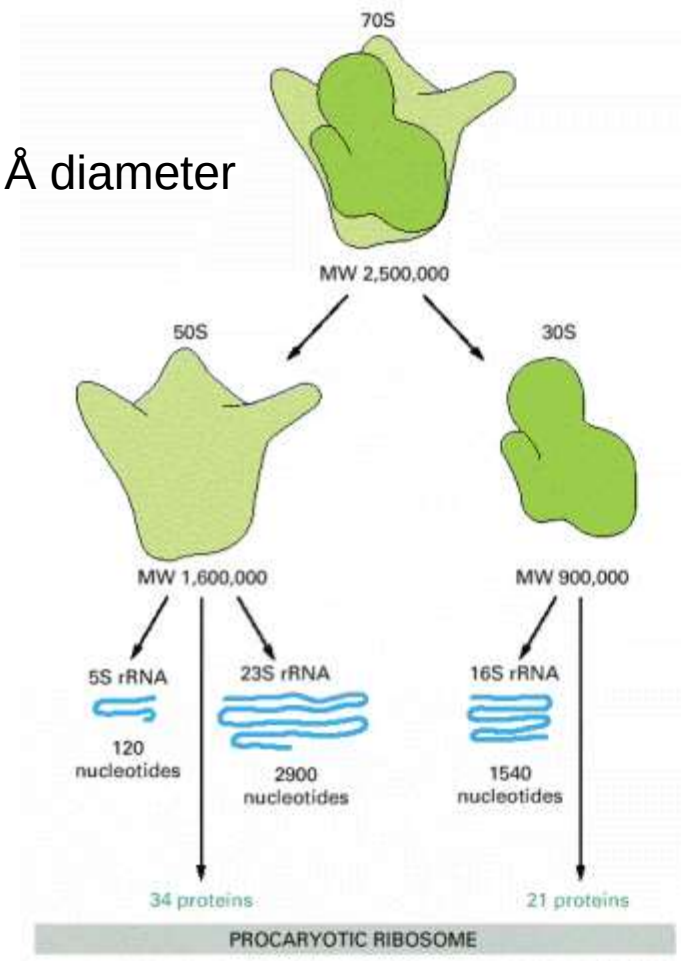
Eukaryotic Ribosomes

Subunit	RNA	Nucleotide	Proteins
60S	28S rRNA	4718	49 Polypeptides
	5.8S rRNA	160	
	5S rRNA	120	
40S	18S rRNA	1874	33 Polypeptides

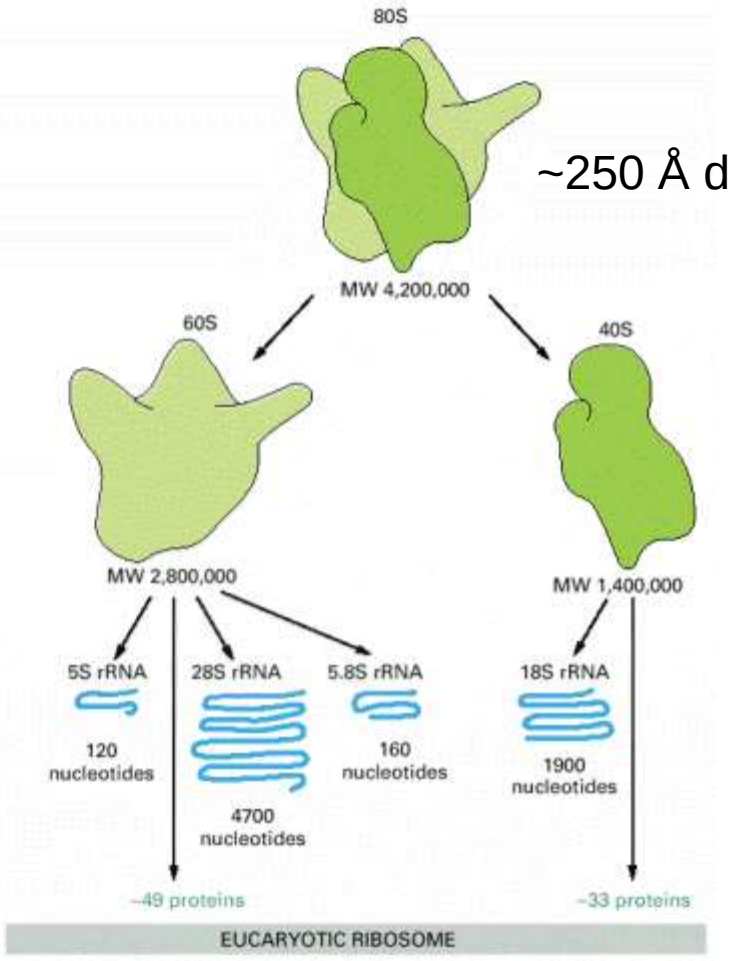
Numbers for mammalian ribosomes

Comparison of prokaryotic and eukaryotic Ribosomes

~200 Å diameter



~250 Å diameter



Eukaryotic Ribosomes

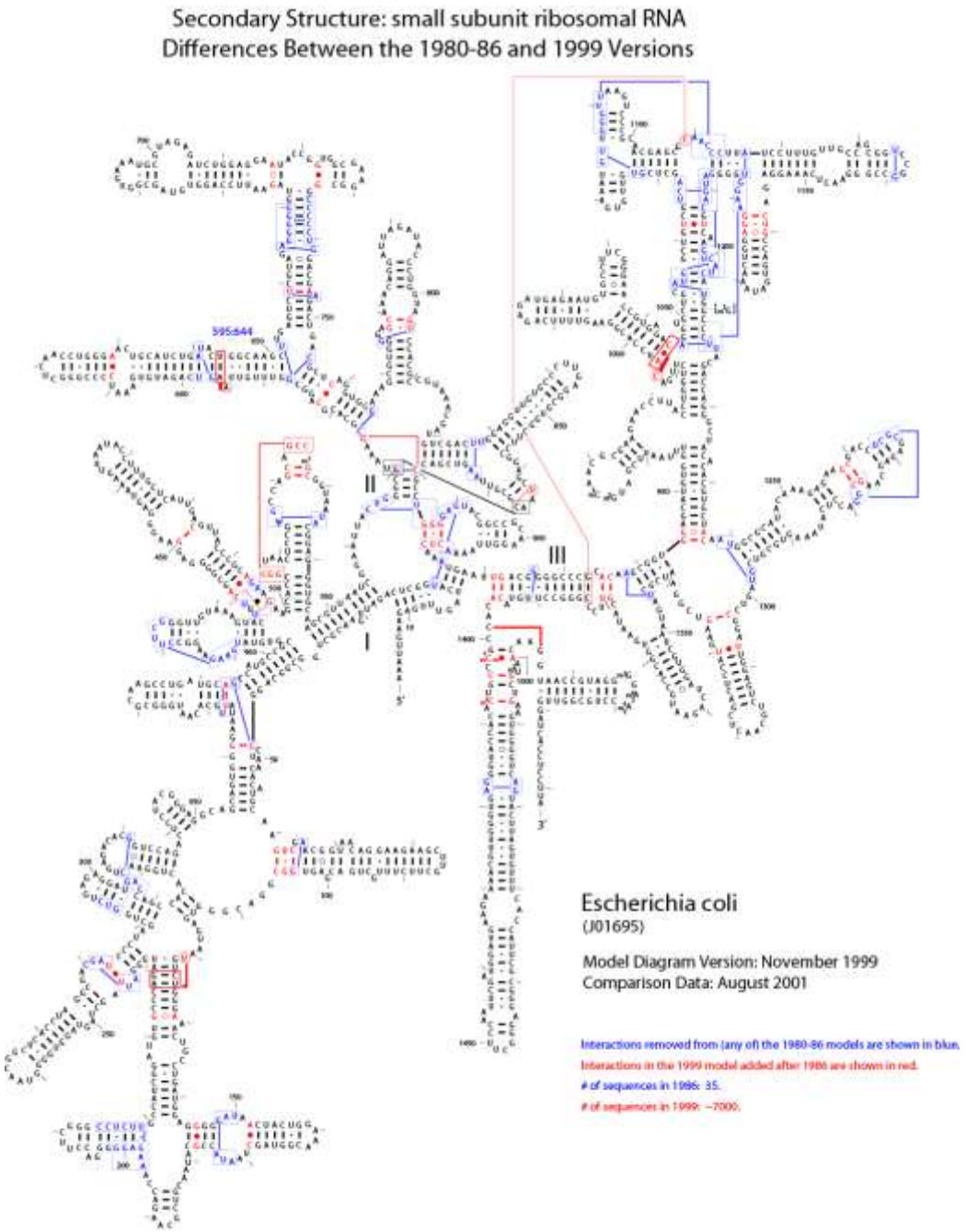
- Cytosolic (free)
- Bound to ER
- Also found in mitochondria and chloroplasts of eukaryotic cells

Free ribosomes

- In the cytosol (not in nucleus!)
- Often found in groups termed polysomes
  - Multiple ribosomes on one mRNA in cytosol

Bound ribosomes

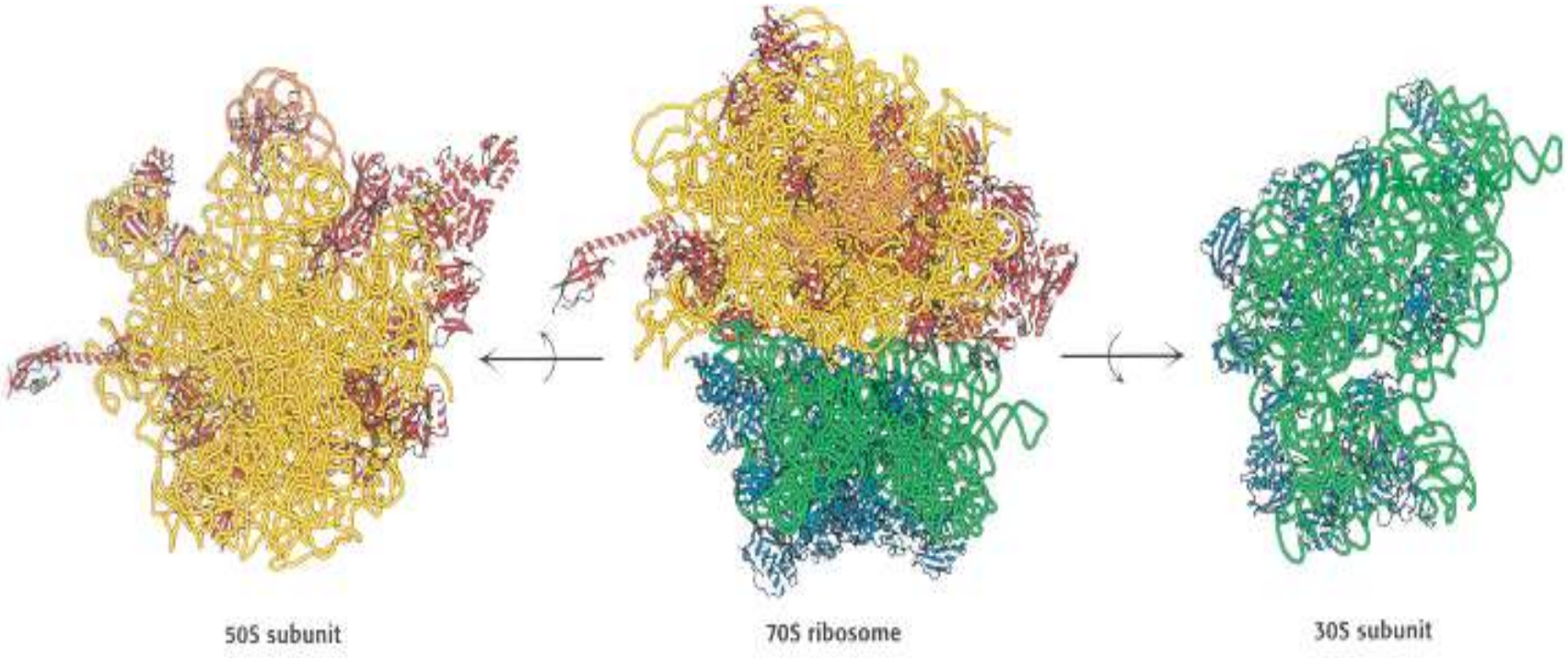
- Bound to the rough endoplasmic reticulum
- Secretory proteins, post-translational modifications
  - Single ribosome on translocon channel



Secondary Structure of *E.coli* 16S rRNA

Woese et al, NAR 1980

X-ray structures of prokaryotic ribosome



23S rRNA (yellow)
5S rRNA (orange)
+ Proteins (red)

16S rRNA (green)
+ Proteins (blue)

3D structure of the ribosome

- rRNAs determine the overall shape of subunits
- rRNAs help to bind and position mRNA and tRNAs
- 16S rRNA controls decoding of mRNA
- 23S rRNA catalyzes peptide bond formation
  - A2451 of 23S rRNA acts as acid/base catalyst (like histidine in serine proteases)
  - Conformational catalysis (proper positioning of activated substrates)
  - 2’OH of terminal adenine of tRNA catalyzes reaction

3D structure of the ribosome

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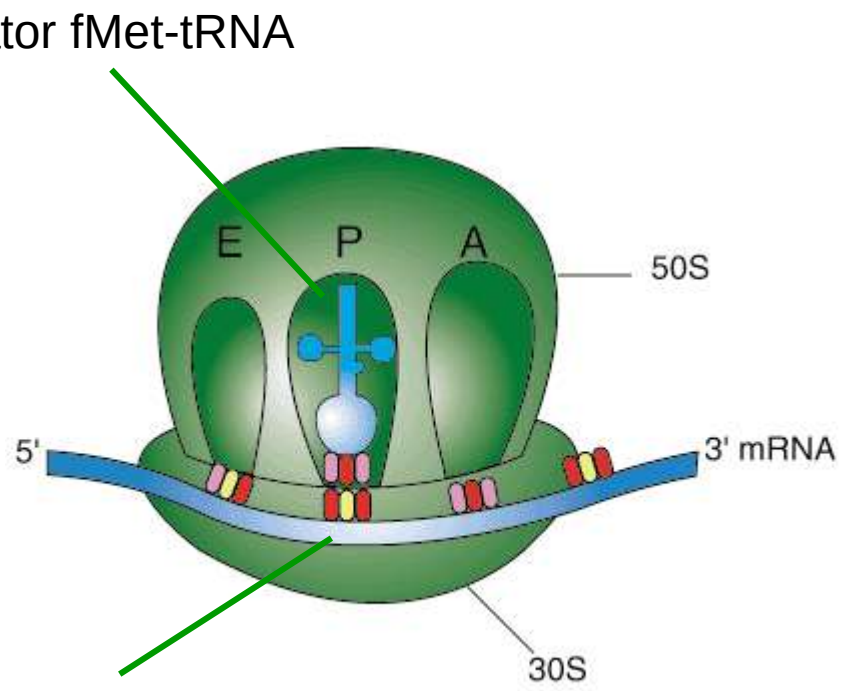
The 70S initiation complex



Aminoacyl (A) site
Peptidyl (P) site
Exit (E) site

Prokaryotic Initiation Factors (IFs)

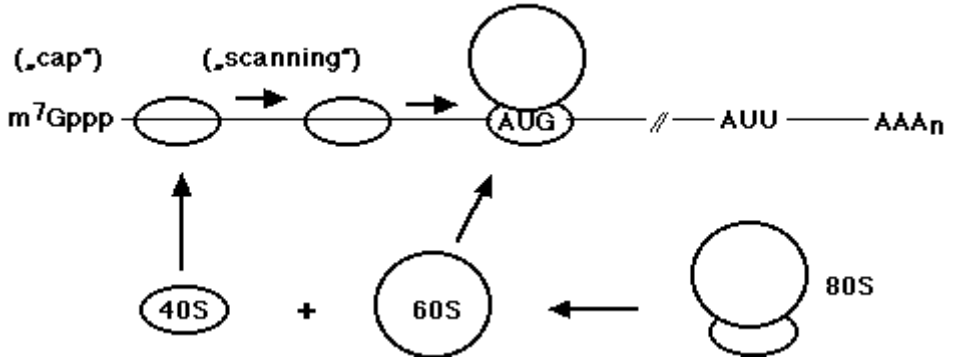
Factor	number of aa	Function(s)
IF1	71	stimuliates activity of IF2 and IF3 prevents tRNA-binding in A site
IF2	889	favors fMet-tRNA binding to 30S subunit mediates subunit joining (GTPase activity)
IF3	181	helps correct positioning of mRNA promotes codon-anticodon base pairing in P site

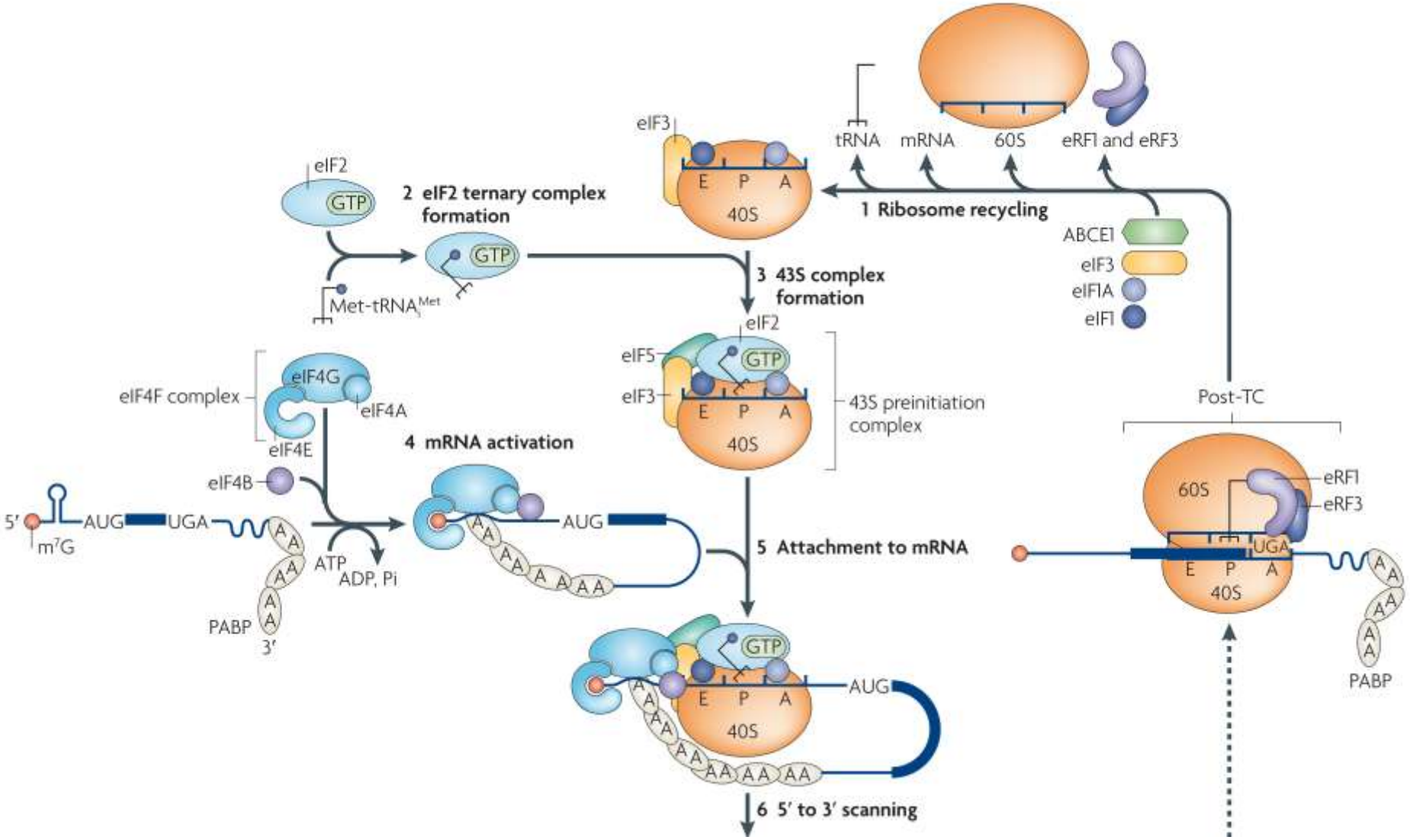
Numbers for E. coli

https://www2.mrc-lmb.cam.ac.uk/groups/ribo/resources/videos/

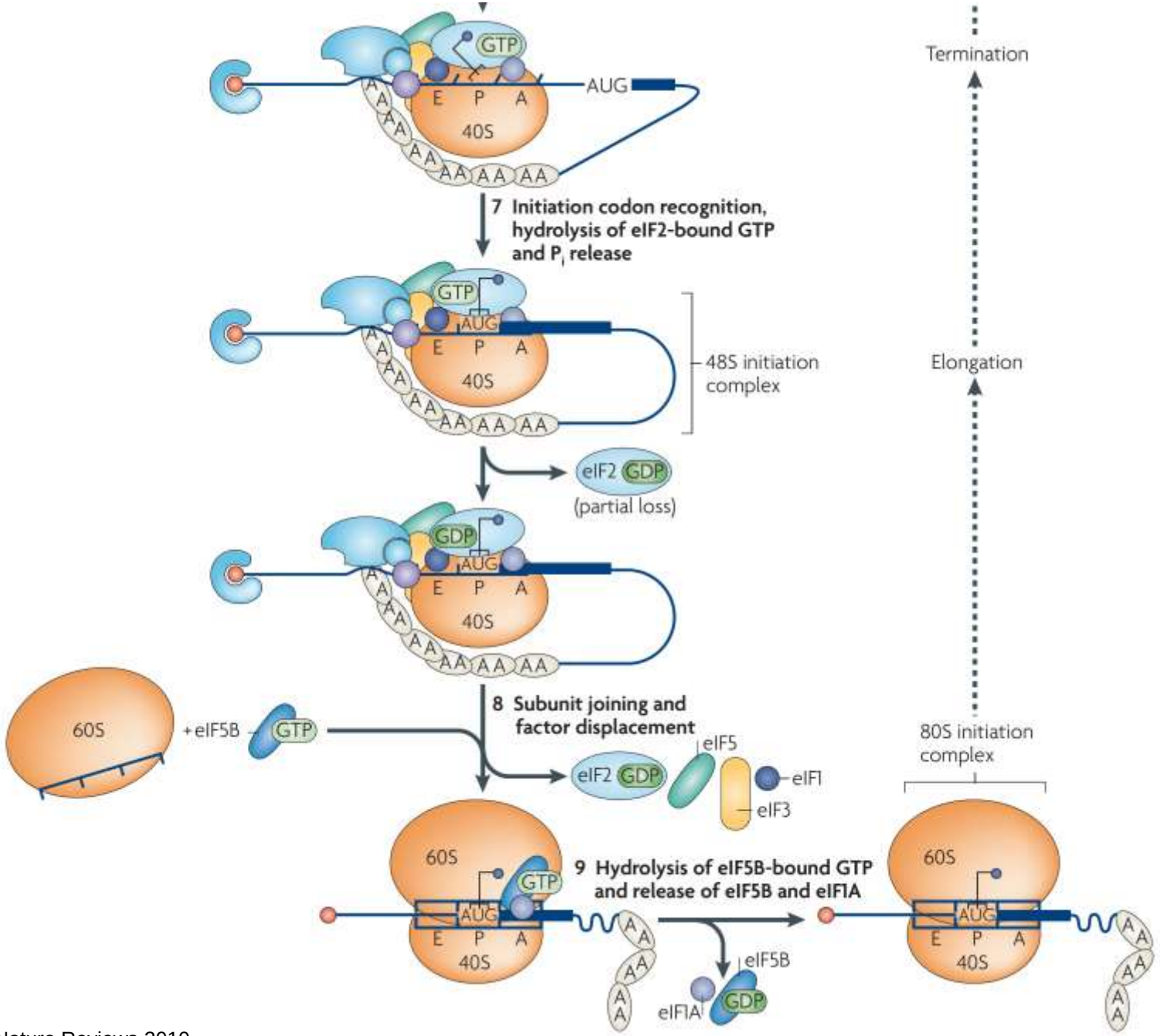
Eukaryotic translation initiation

- Cap-dependent initiation is the major translation initiation pathway in eukaryotes
- eukaryotic mRNAs are monocistronic, capped at the 5' end and polyadenylated at the 3' end
- ribosomes consist of 40S and 60S subunits
- 40S subunits locate the initiator AUG codon by scanning
- At the AUG codon 60S ribosomal subunit to form an 80S ribosome competent for translation elongation
- Assisted by eukaryotic initiation factors (eIFs)





Jackson, Hellen, Pestova, Nature Reviews 2010



Jackson, Hellen, Pestova, Nature Reviews 2010

Eukaryotic initiation factors (eIFs)

Factor	kDa	Function(s)
eIF1	15	stimulates mRNA binding and scanning negative regulator of AUG recognition binds near E site
eIF1A	17	stimulates mRNA binding and scanning assists AUG recognition binds in A site
eIF2	130	3 subunits ($\alpha\beta\gamma$) delivers Met-tRNA$_i^{Met}$ to 40S subunit (ternary complex) GTPase activity
eIF5	48	GTPase activating protein (GAP of eIF2γ)

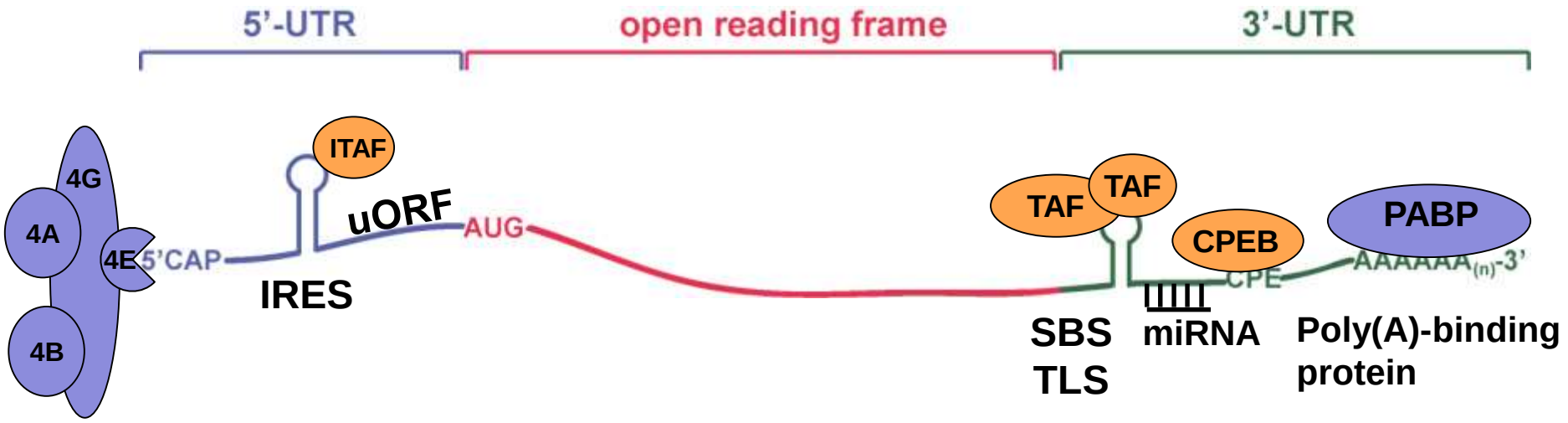
Eukaryotic initiation factors (eIFs)

Factor	kDa	Function(s)
eIF4E	25	mRNA binding 5'cap binding protein
eIF4G	154	mRNA binding large scaffold protein for binding of eIF4E, eIF4A, eIF3
eIF4A	44	scanning of 5'UTR ATP-dependent RNA helicase
eIF4B	70	scanning of 5'UTR co-factor of eIF4A

Eukaryotic initiation factors (eIFs)

Factor	kDa	Function(s)
eIF3	800	13 subunits (a-m) in mammals shares conserved eIF3abcgij core with yeast ribosome dissociation stimulates mRNA binding stabilizes TC binding assists scanning and AUG recognition
eIF5B	112	GTPase activity subunit joining and eIF release

Complex network of RNA-protein interactions within mRNA regulates gene expression

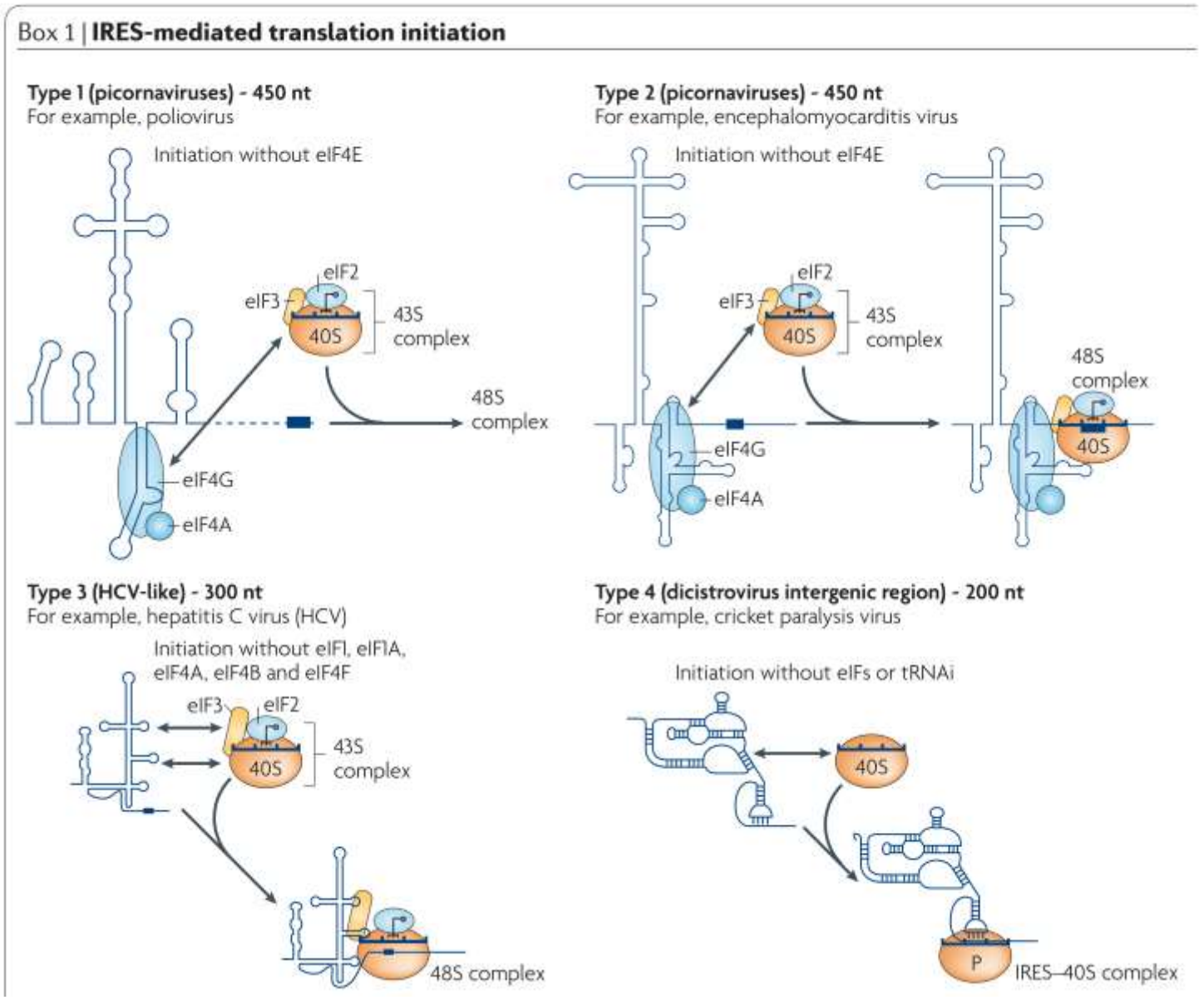


CAP-binding complex

- Translational control
- mRNA stability

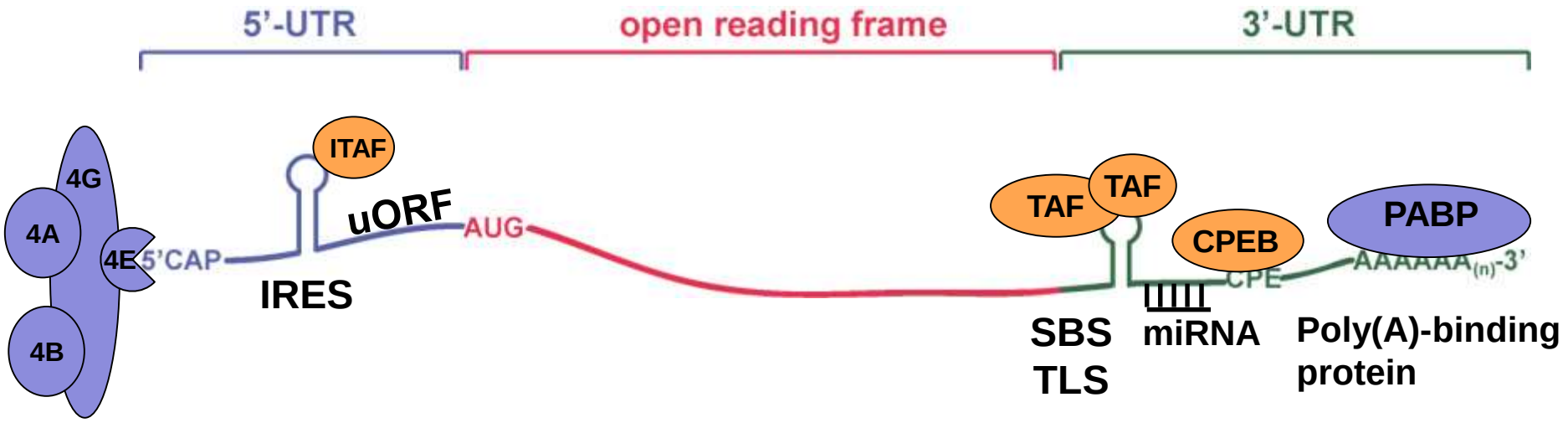
- Translational control
- mRNA localization
- mRNA stability

Internal ribosome entry site mediated translation initiation



Jackson, Hellen, Pestova, Nature Reviews 2010

Complex network of RNA-protein interactions within mRNA regulates gene expression



CAP-binding complex

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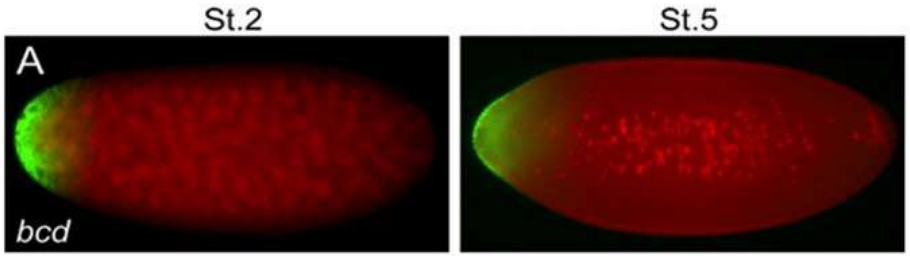
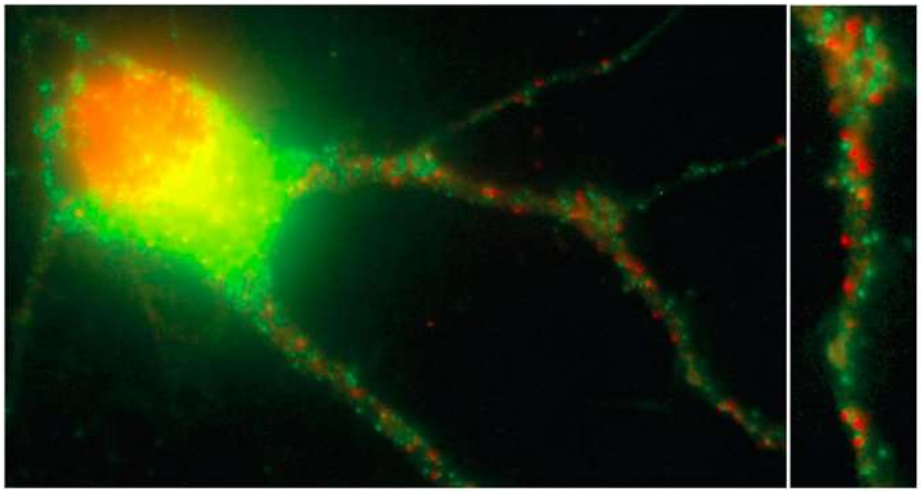
mRNA transport across the Kingdoms



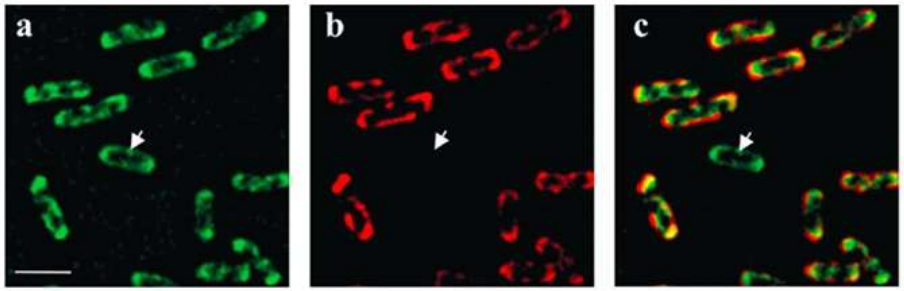
Drosophila embryo
bcd (green)

Lécuyer et al. 2007. Cell



mammalian neuron
Actb (green)
Mtap2 (red)

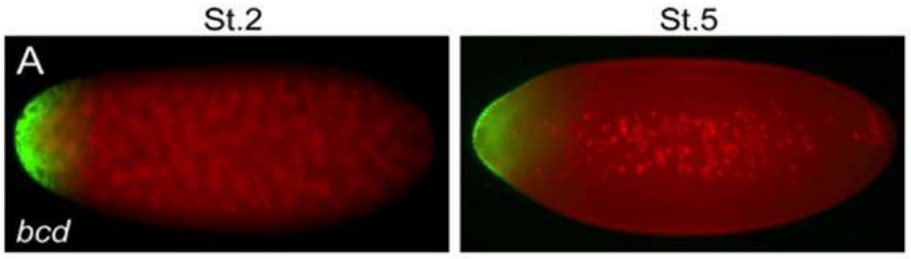
Raj et al. 2011. Nat. Methods

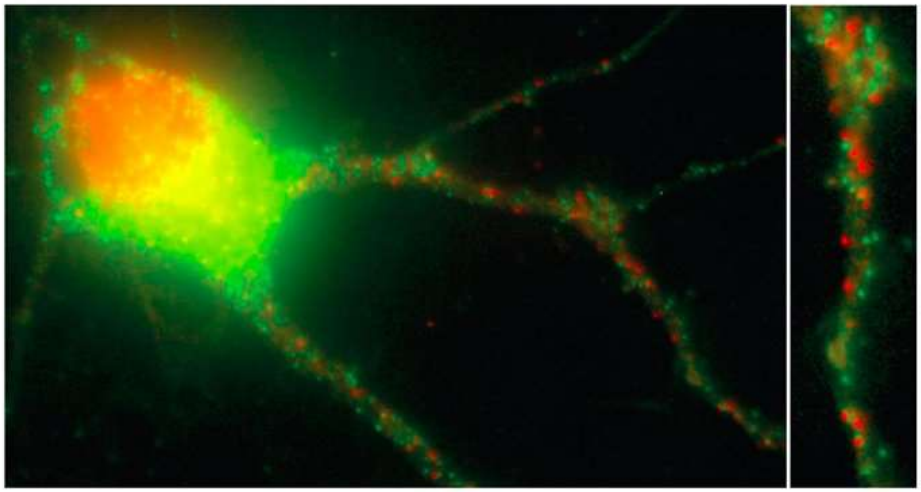


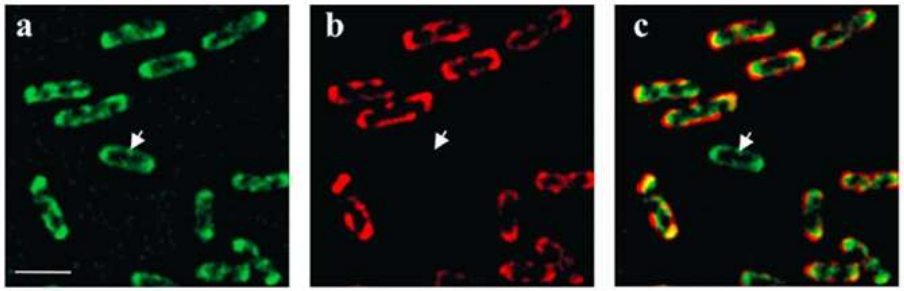
E. coli BglF
RNA (green), protein (red)

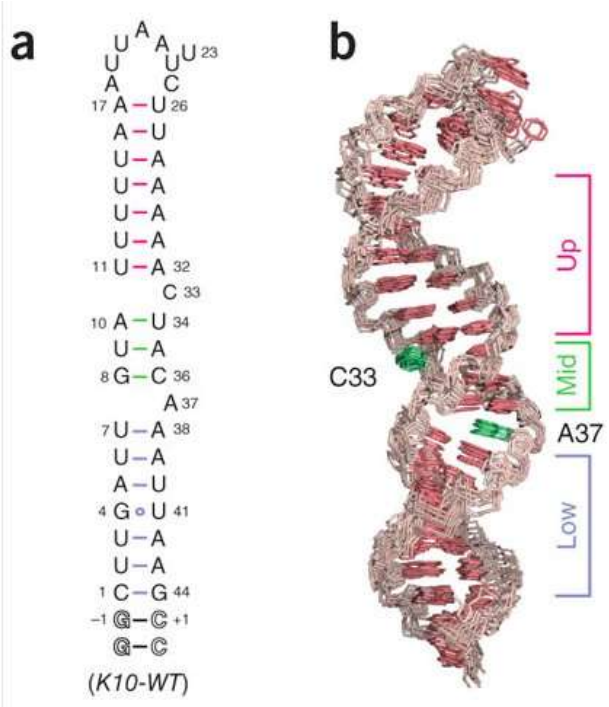
Nevo-Dinur et al. 2011. Science

mRNA transport across the Kingdoms

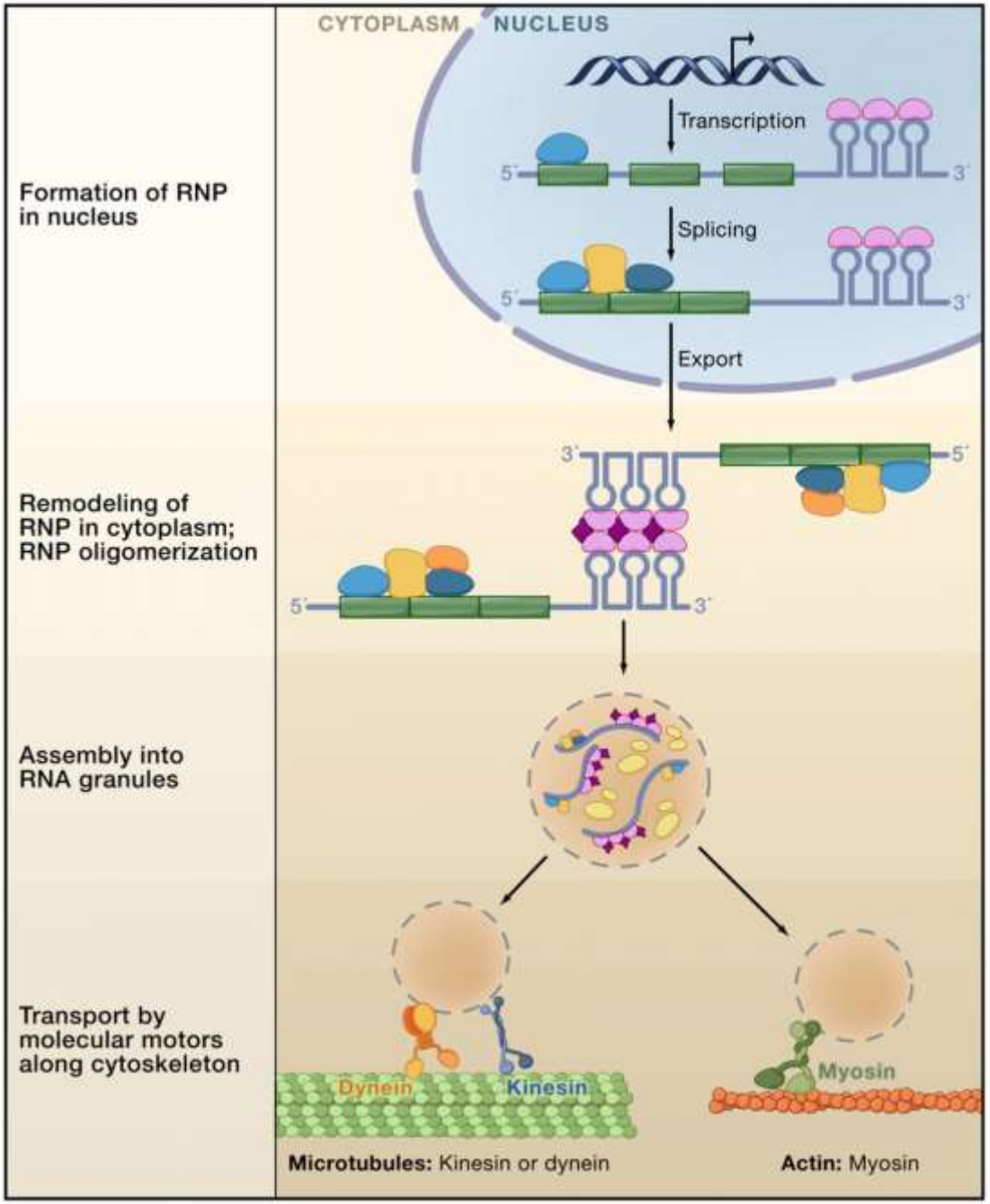








Bullock and Lukavsky. 2010. *Nat. Struct. Mol. Biol.*



Martin & Ephrussi, Cell, 2009

mRNA transport

- mRNA localization is essential for spatial and temporal control of gene expression of hundreds of transcripts in dendrites

(Martin and Zukin, 2006, J. Neurosci.)

- Targeting elements are located in 3'UTR and recognized by common, shared RNA-binding proteins: PURα, Staufen, hnRNPA2, ZBP1, etc

> Sequence-specific

11nt A2RE recognized by hnRNPA2

bipartite zipcode recognized by ZBP1

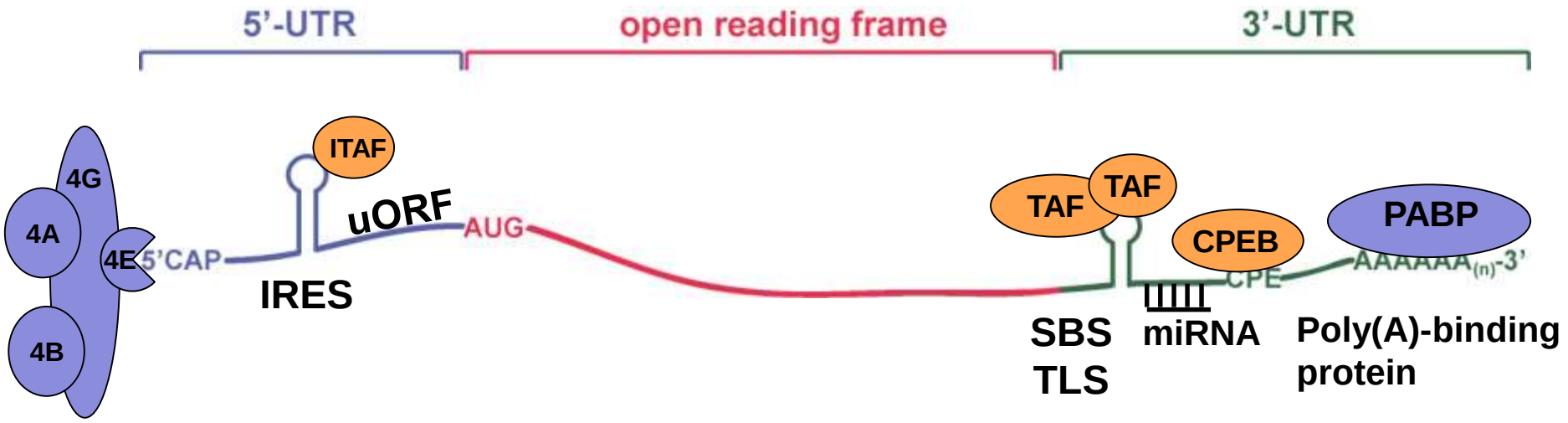
> Structure-specific

drosophila TLS (*K10*, etc.) – A'-form helices

dendritic targeting elements (DTE) - ???

https://www.youtube.com/watch?v=y-uuk4Pr2i8
https://youtu.be/-7AQVbrmzFw

Complex network of RNA-protein interactions within mRNA regulates gene expression



CAP-binding complex

- Translational control
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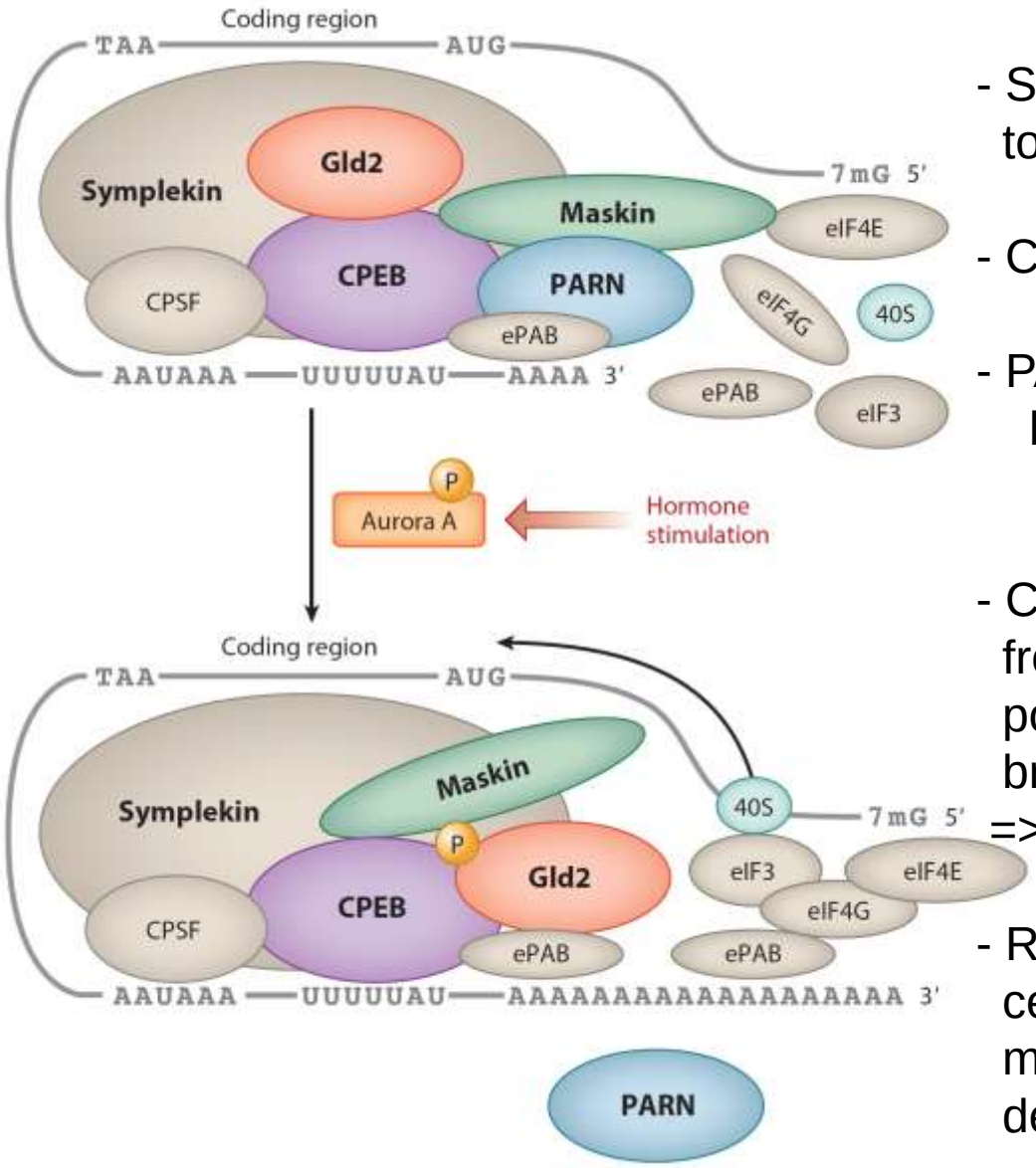
Cytoplasmic polyadenylation regulates protein synthesis



- Some mRNAs contain CPE sequence to regulate gene expression

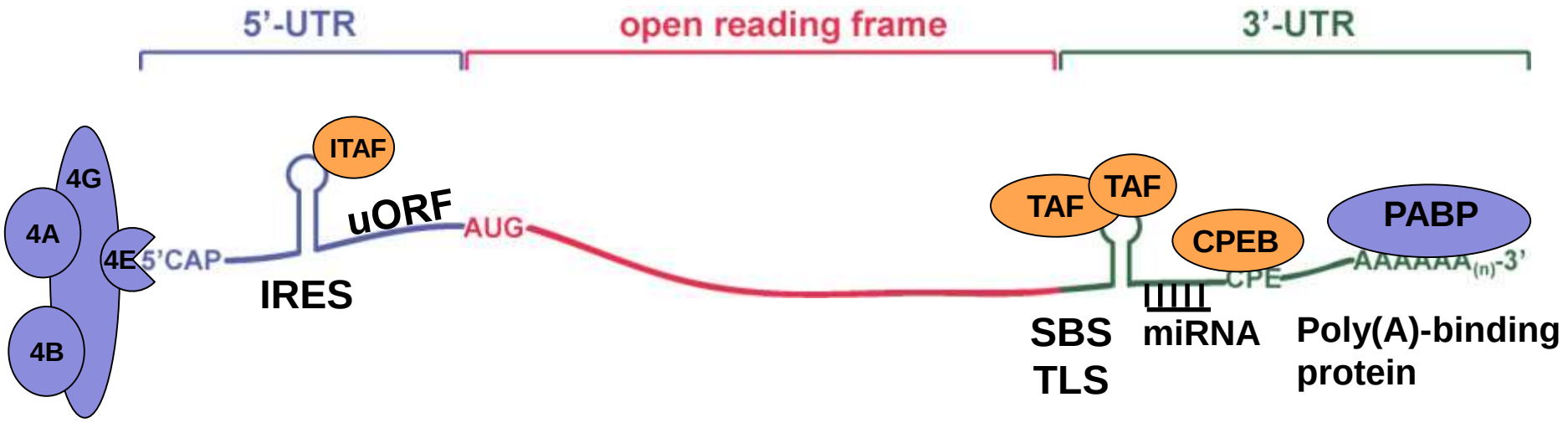
- CPE is recognized by CPEB (2 RRMs)

- PARN shortens polyA tail and Maskin blocks access to eIF4e
  => mRNA is dormant

- CPEB phosphorylation eliminates PARN from assembly, Gld2 extends the polyA tail and Maskin phosphorylation breaks interaction with eIF4e
  => mRNA can be translated

- Regulation of
  cell cycle mRNAs
  maternal mRNAs during development
  dendritic mRNAs for memory formation

Complex network of RNA-protein interactions within mRNA regulates gene expression



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Regulation of gene expression by micro RNAs

Discovered in the nematode (*C. elegans*) during analysis of *lin-4* and *let-7* genes

(Victor Ambros et al. Cell 1993)

Cloning and nucleotide sequence analysis revealed that *lin-4* and l*et-7* do not encode any protein product. *lin-4* RNA hybridizes to the 3'-untranslated region of *lin-14* mRNA and *lin*-28 RNA and degrades the mRNA.

miRNA precursors are transcribed by Pol II and processed into mature miRNAs of 21-22 bases.

Base pairing between the miRNA and the 3' UTR of an mRNA does not have to be 100% complementary. This differentiates it from the RNA interference.

In human, more than 1,000 different miRNA are produced. Target site in 3'UTR can be regulated through alternative polyadenylation!!

miRNA Processing

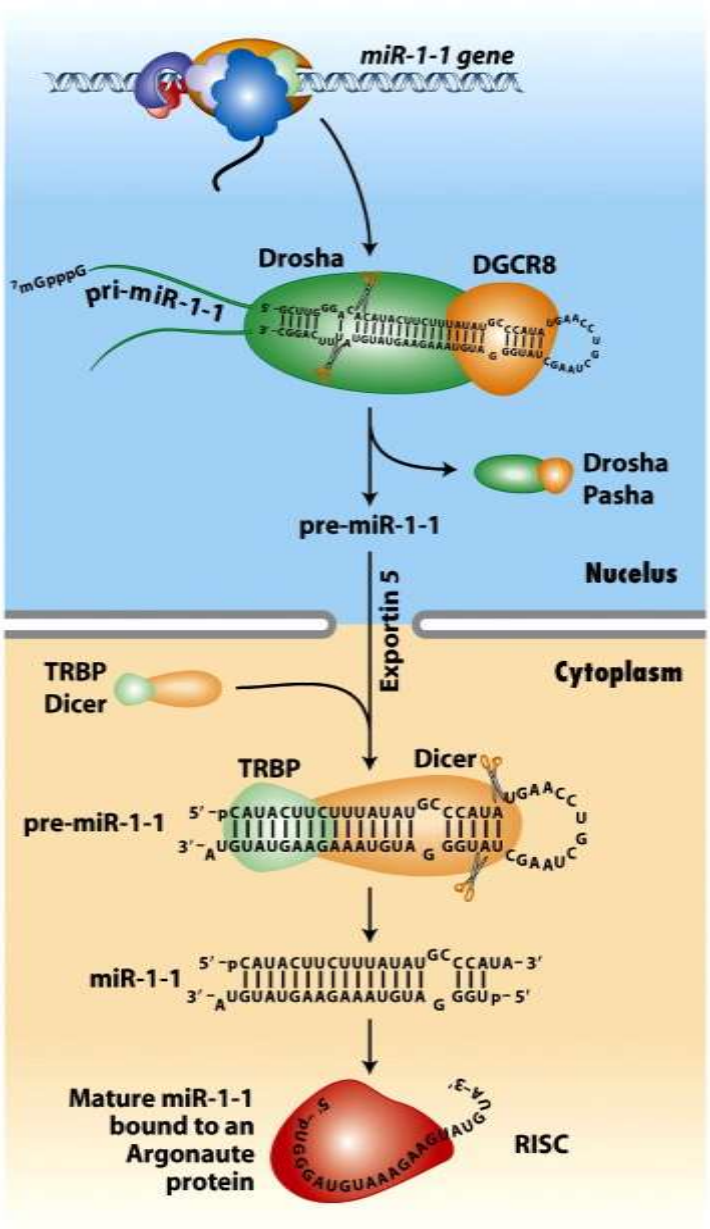


Figure 8-26
Molecular Cell Biology, Sixth Edition

Pri-miRNA is transcribed by RNA polymerase II

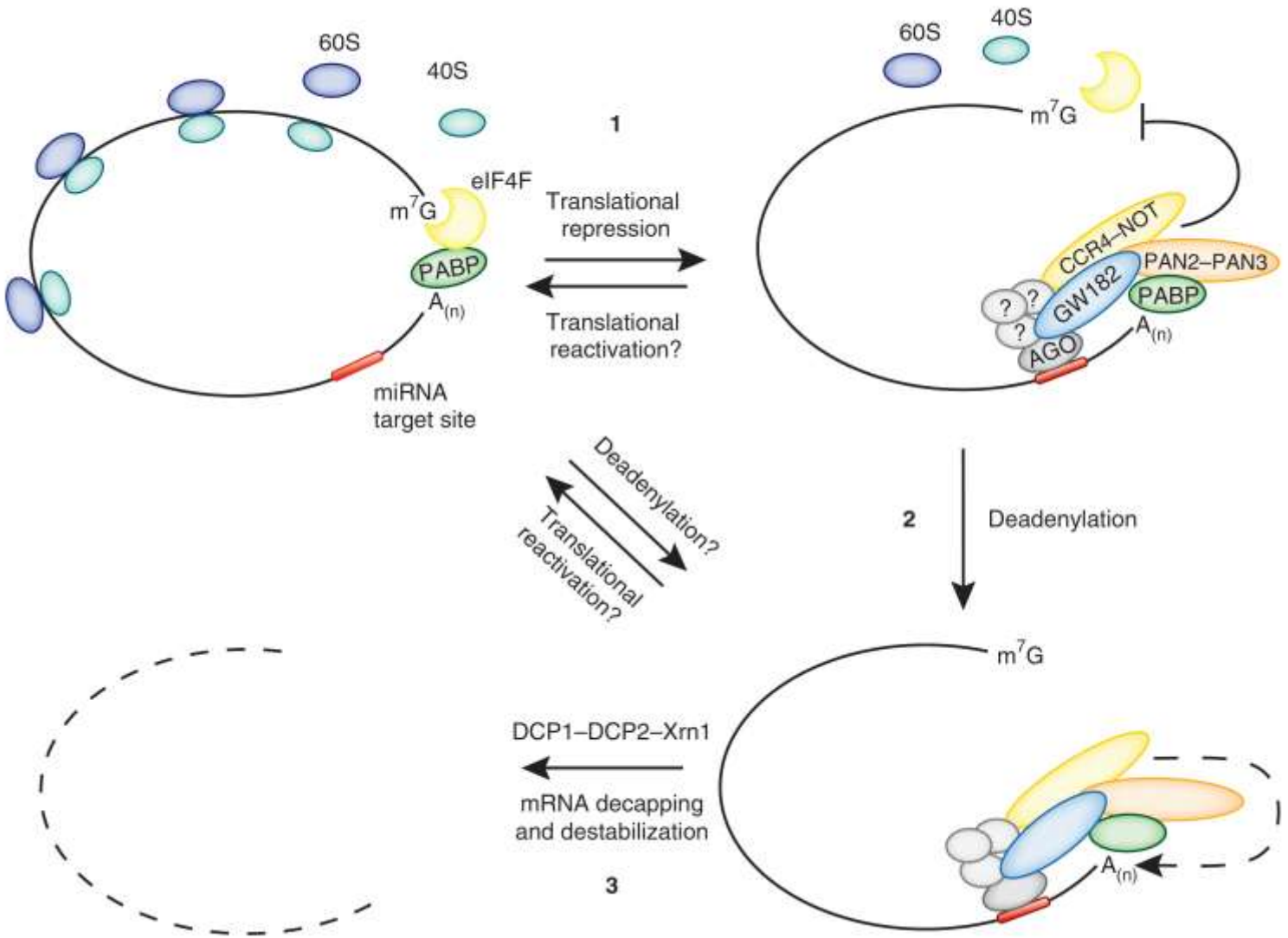
Nuclear double-strand specific endonuclease "Drosha" (RNase III) with its partner dsRNA binding protein "DGCR8" cleave the pri-miRNA to generate a 70-nucleotide pre-miRNA

The 70 nt pre-miRNA is exported from nucleus to cytoplasm

In the cytoplasm, the pre-miRNA is processed by Dicer to form miRNA

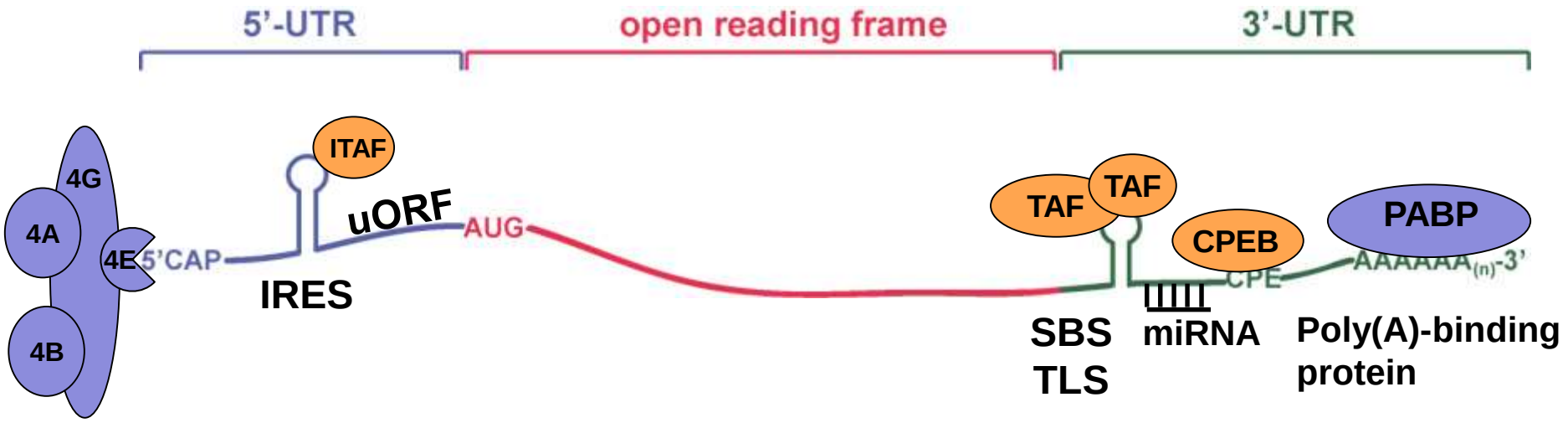
One of the strands of miRNA is incorporated into an RISC complex with Argonaute protein

Regulation of gene expression by micro RNAs



Fabian & Sonenberg, NSMB 2012

Complex network of RNA-protein interactions within mRNA regulates gene expression

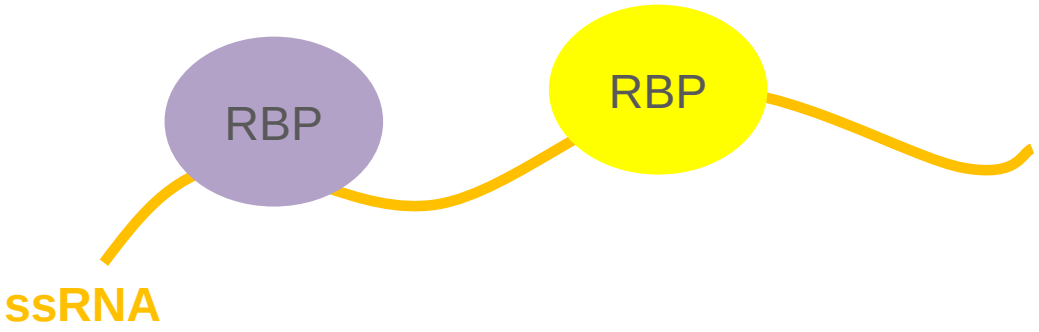


CAP-binding complex

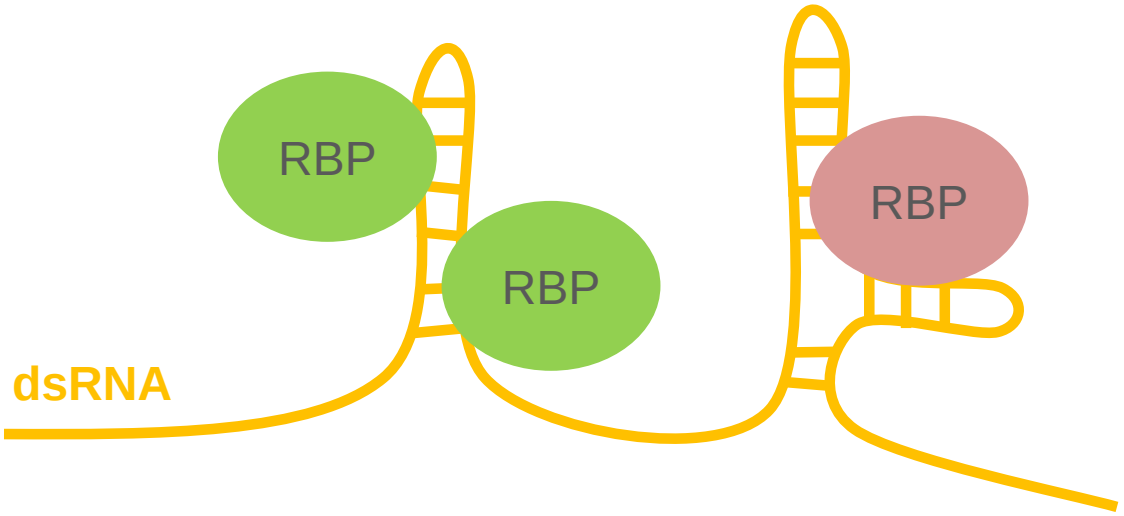
- Translational control
- mRNA stability

- Translational control
- mRNA localization
- mRNA stability

RNA-based regulation of gene expression



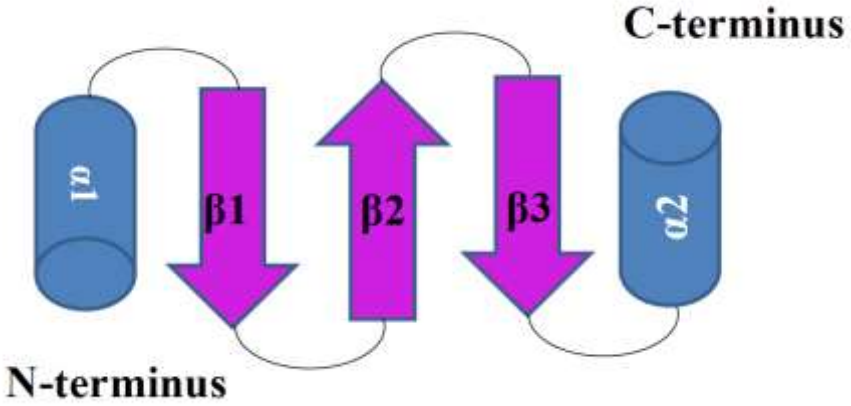
Sequence-specific recognition of single-stranded RNA by RNA-binding proteins



Structure-specific recognition of double-stranded RNA by RNA-binding proteins

dsRNA binding domain (dsRBD)

- ~ 65-70 amino acid domain
- Found in eukaryotes, prokaryotes and viral proteins
- Conserved αββββα topology
- α-helices are packed against antiparallel β-sheet
- 30 dsRBDs structure (X-ray and NMR)
- Many biological functions:
  - antiviral response
  - RNA processing
  - RNA transport
  - RNA silencing
  - mRNA degradation



Topology of a dsRBD

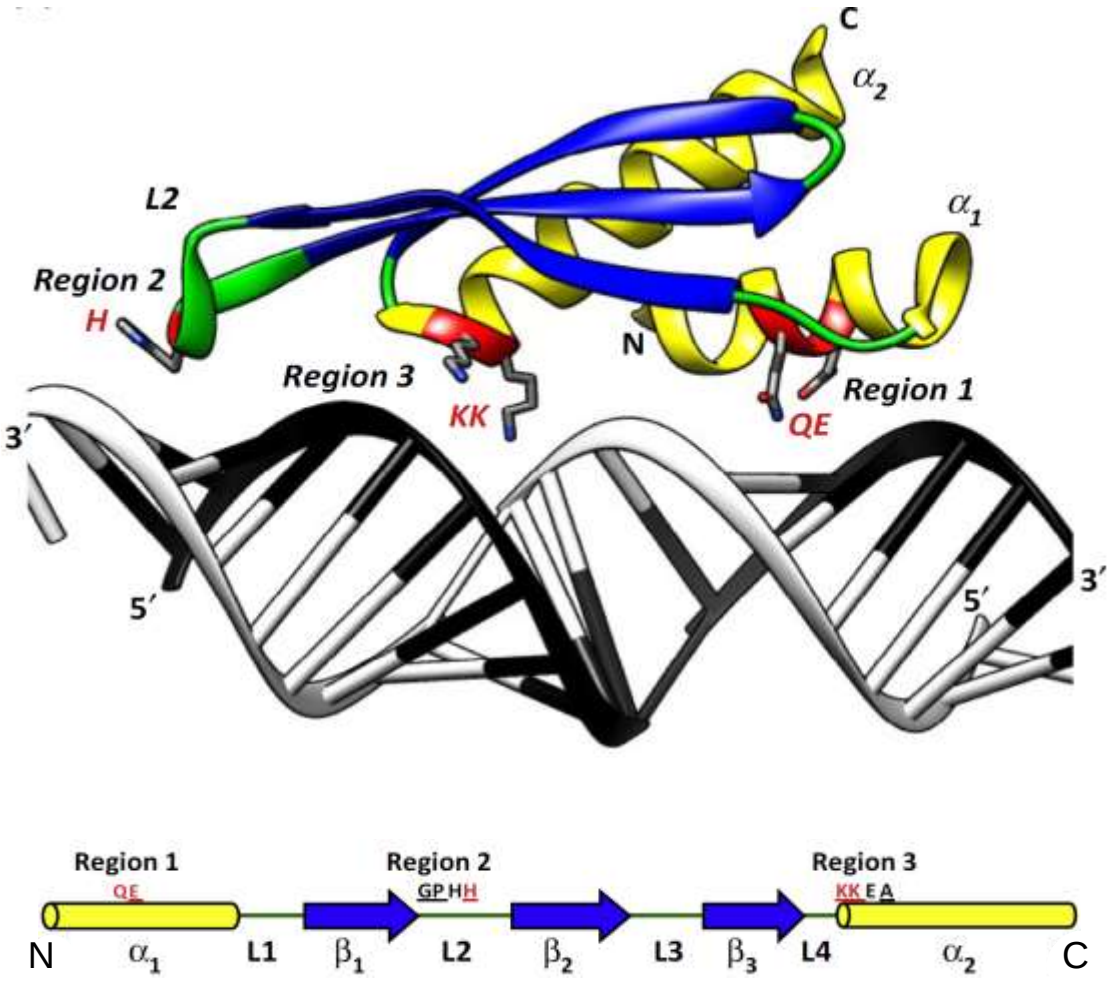
dsRNA-binding regions of a dsRBD

Three distinct binding region

Region I (minor groove)
α1-helix
conserved residues: QE

Region II (minor groove)
β1β2 (loop2)
conserved residues: GPxH

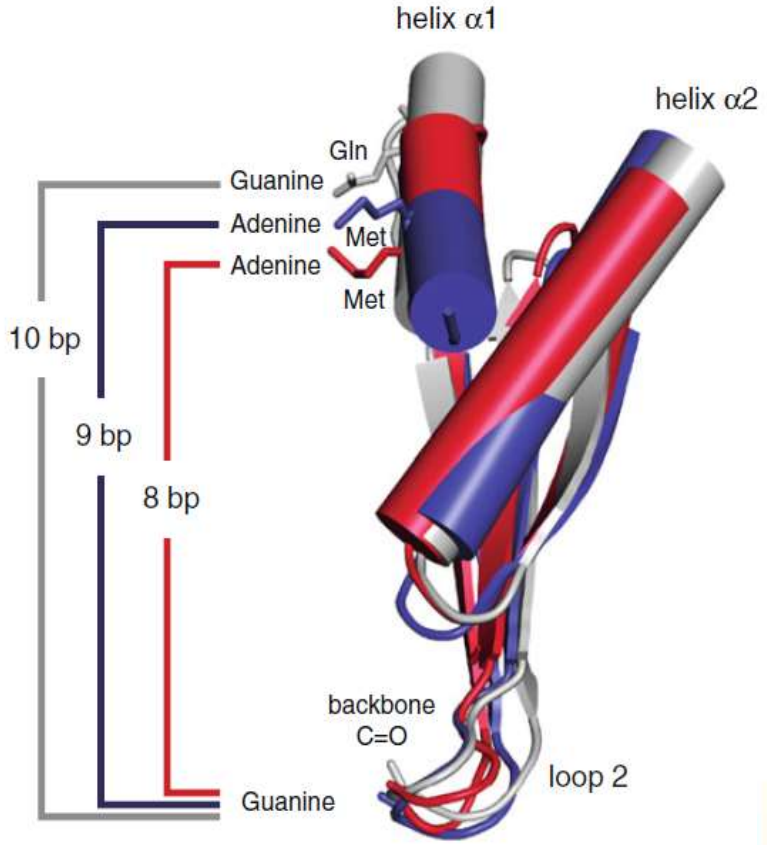
Region III (major groove)
N-terminus of α2-helix
conserved residues: KKxAK



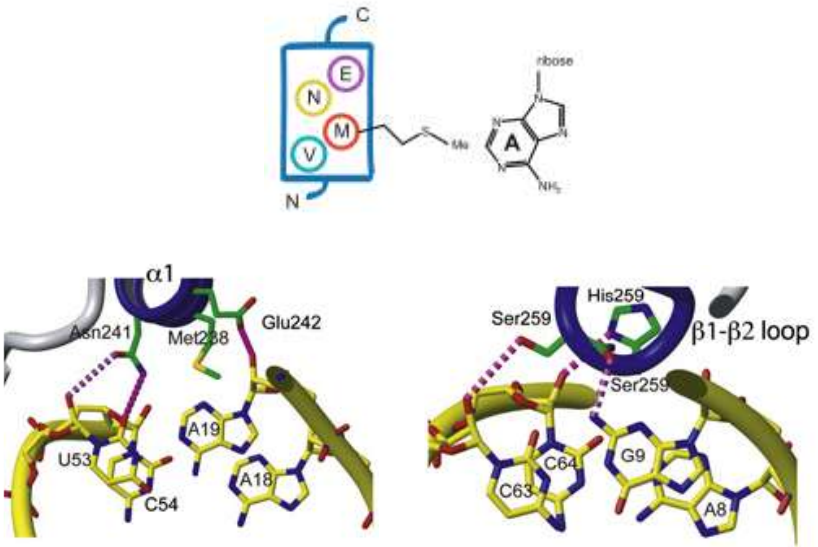
dsRBD of *A. aeolicus* RNase III

Gleghorn & Maquat (2014)

Sequence preference: binding register of dsRBD on dsRNA

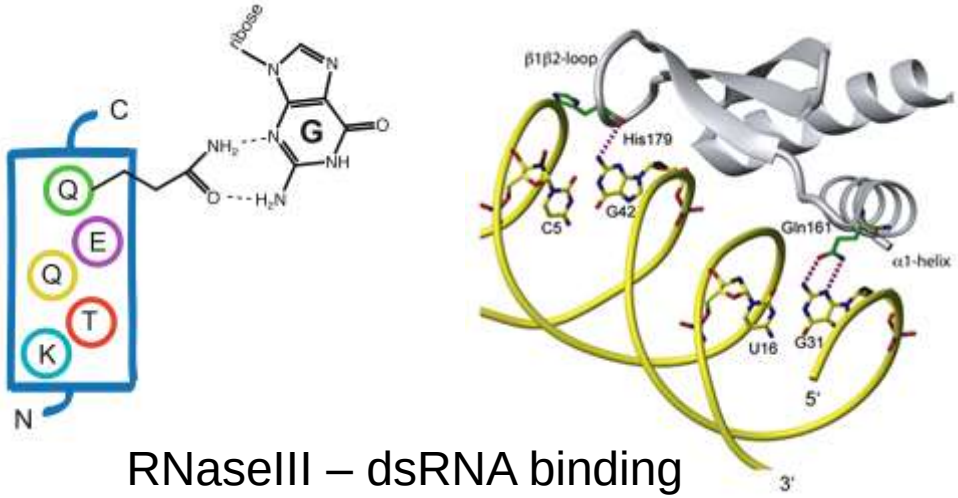


Masliah et al. (2013)



ADAR2 – dsRNA binding

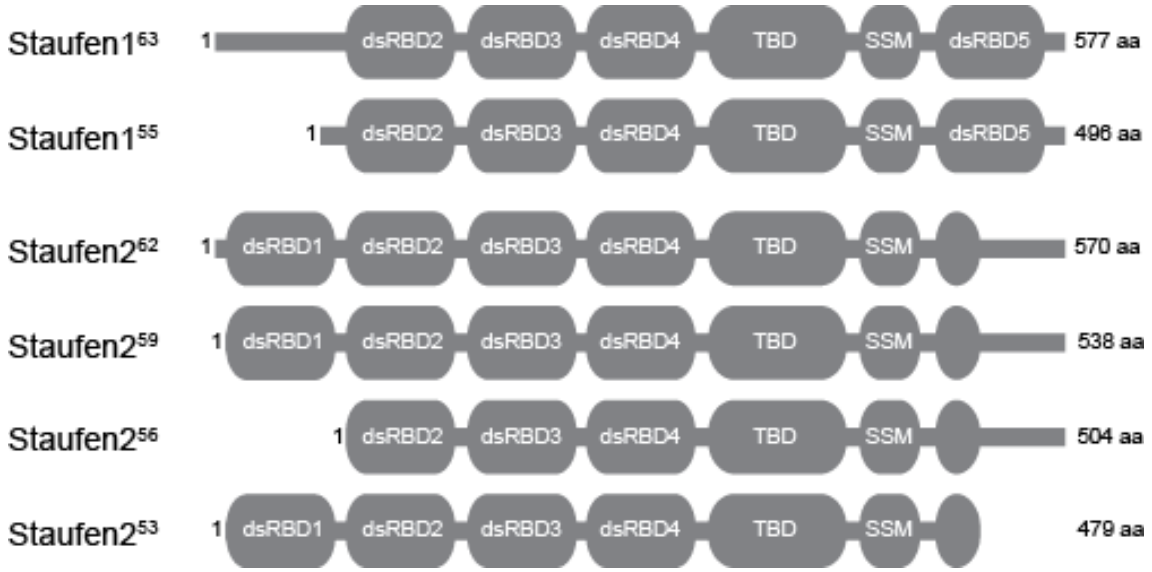
Stefl et al. (2010)



RNaseIII – dsRNA binding

Gan et al. (2008)

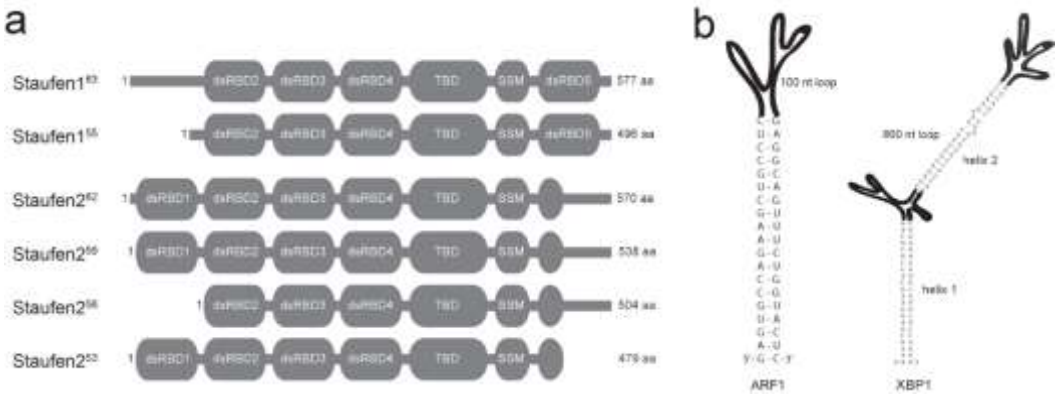
Staufen1 and Staufen2 proteins

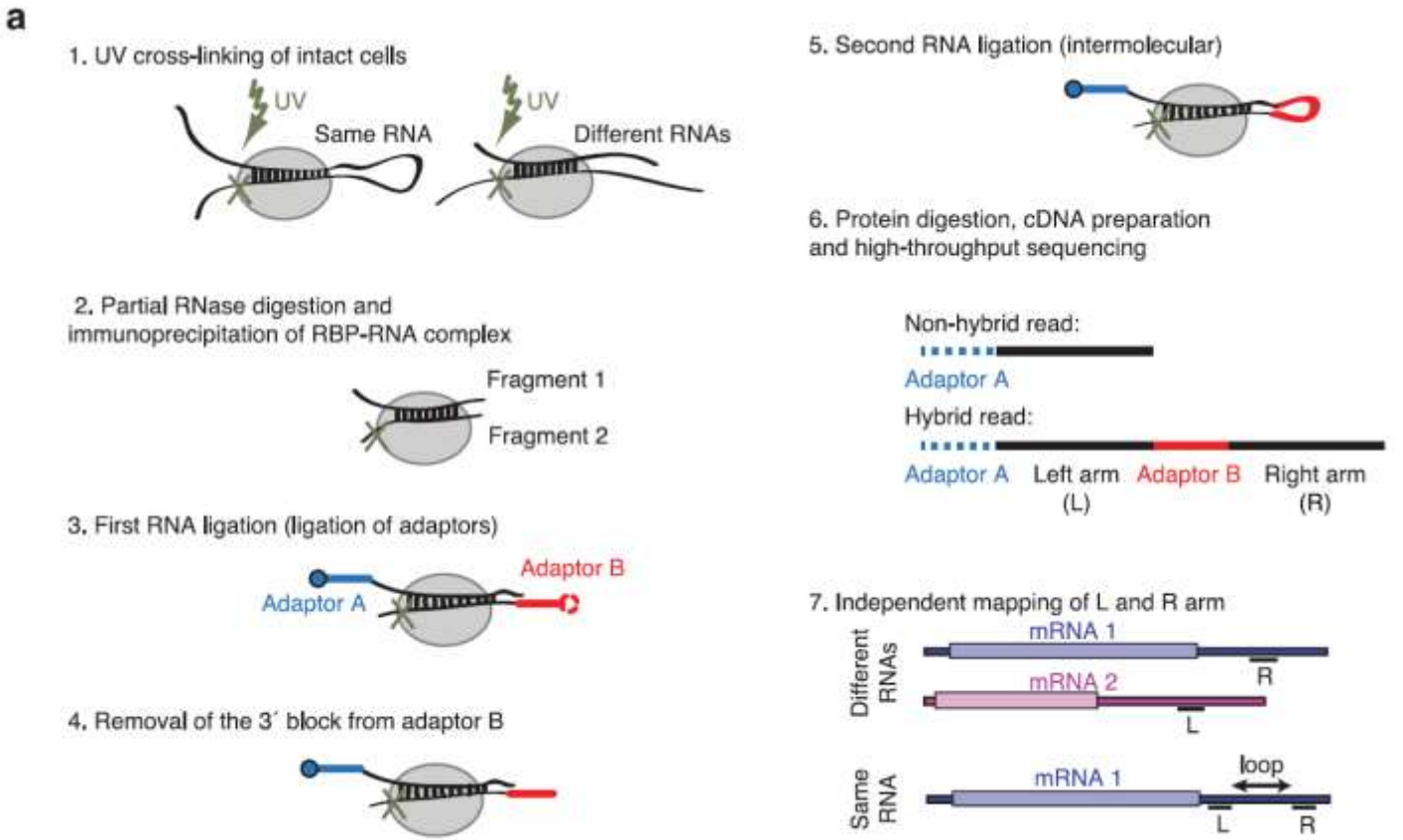


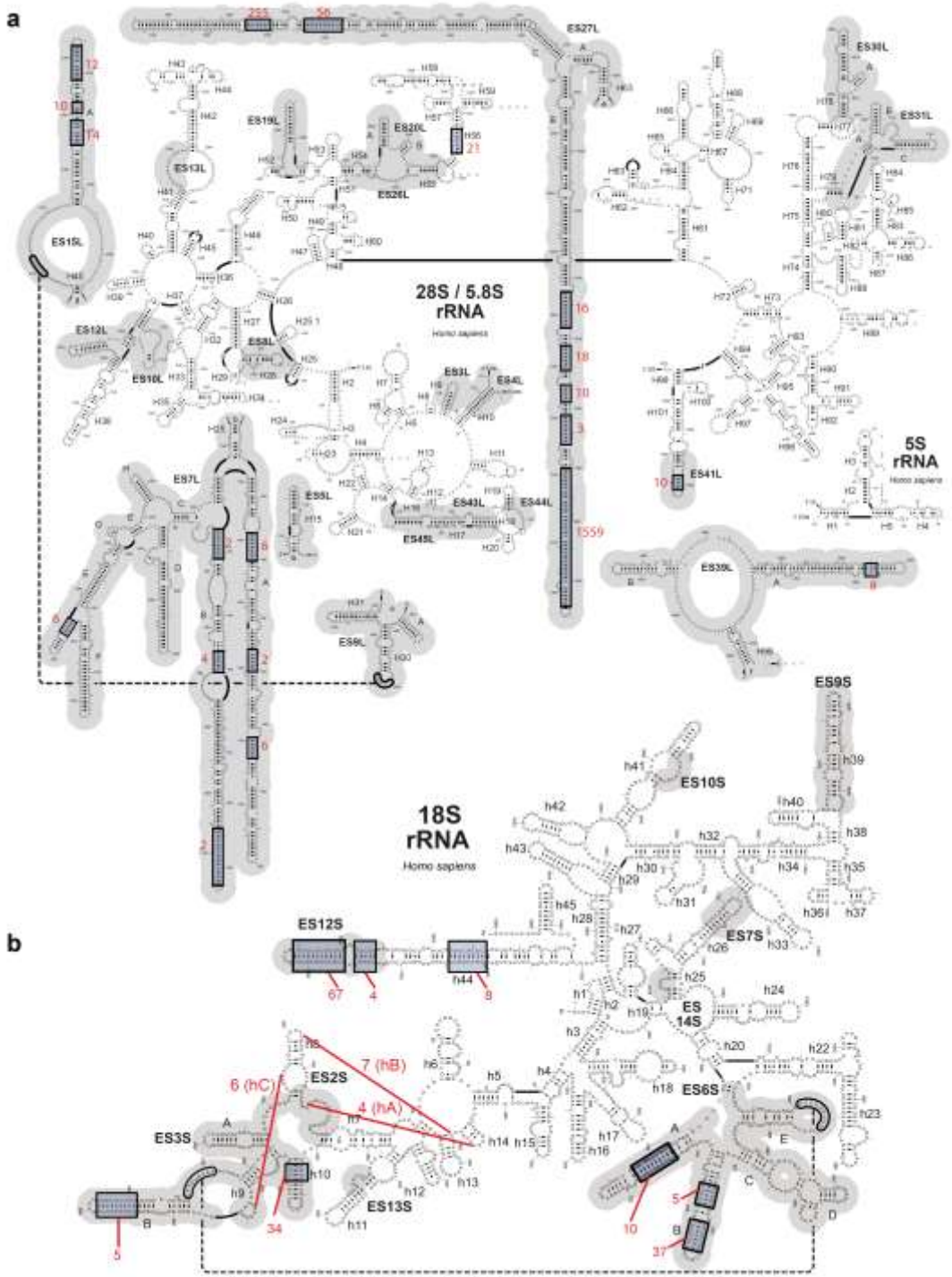
- Staufen1 contains multiple dsRBDs. STAU1 gene encodes two isoforms
- dsRBDs 3 and 4 bind dsRNA
- Staufen1 has many biological functions:
  - microtubule dependent transport of RNAs: development and higher brain functions
  - Translational control: associated with translating ribosomes
  - Staufen-mediated mRNA decay

How does Staufen1 target certain mRNAs?

hiCLIP revealed multiple interactions with 3’UTRs and the ribosome



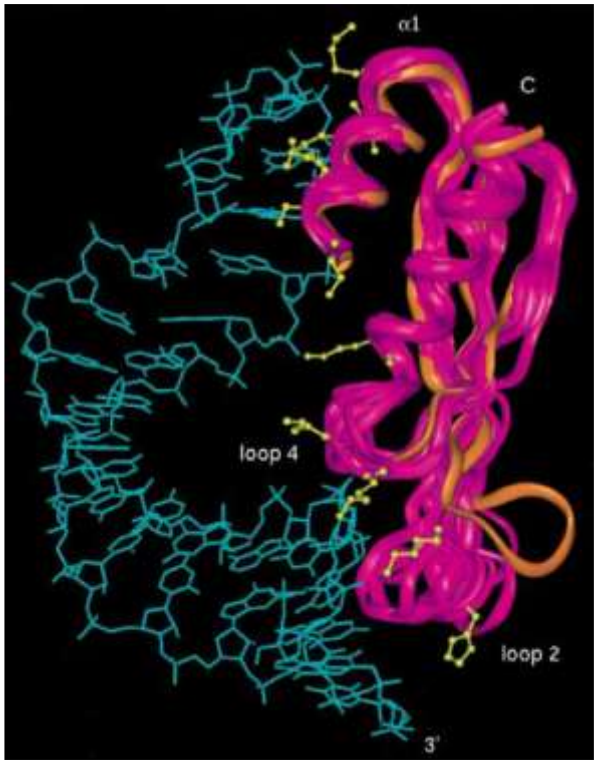




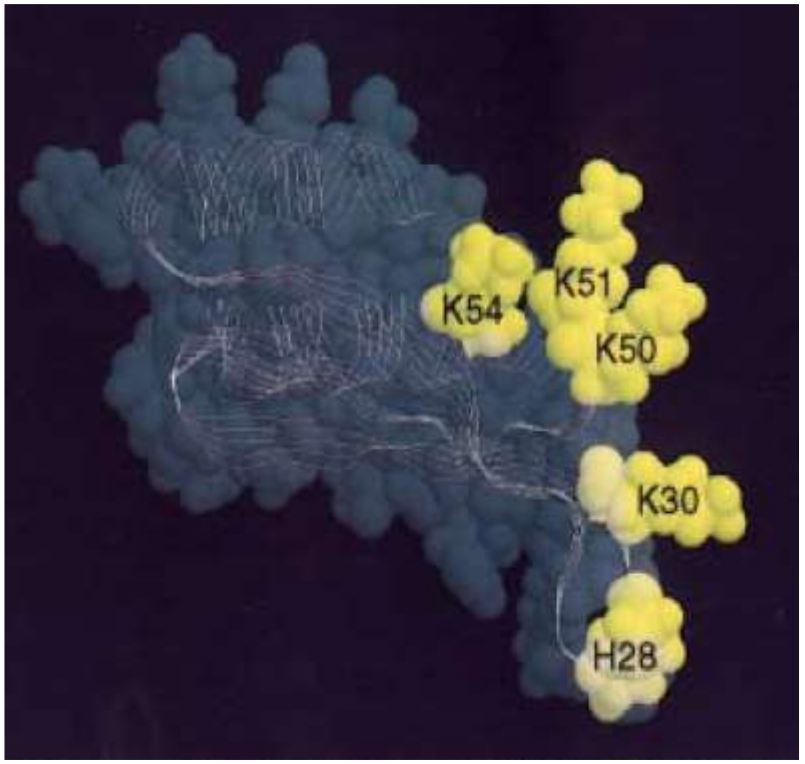
hiCLIP revealed multiple interactions with the ribosome

Structure of dmStaufen dsRBD3 with artificial dsRNA

- non cellular dsRNA
- three contact regions (helix α1- tetra loop, β1β2-loop - minor groove of dsRNA , helix α1-lysines - major groove of dsRNA.
- number of intermolecular NOEs was only 10



dmStaufen dsRBD3+dsRNA

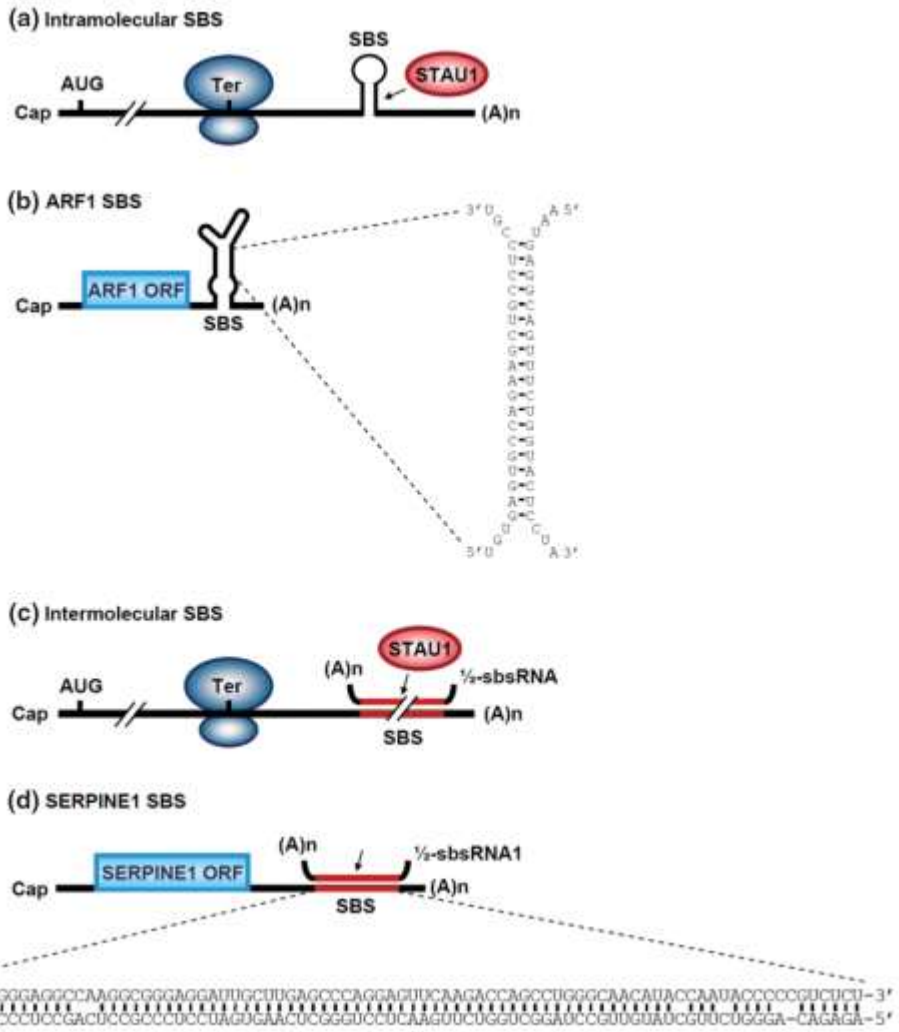


Amino acids that bind to dsRNA

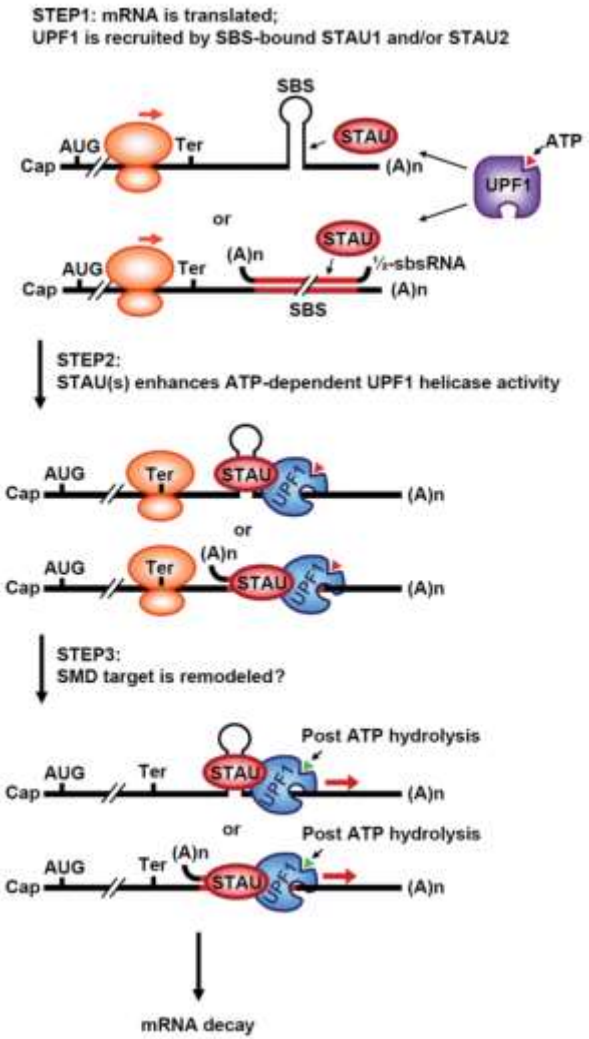
How does staufen1 recognize its cellular dsRNA targets?

Ramos et al. (2000)

Staufen mRNA target recognition

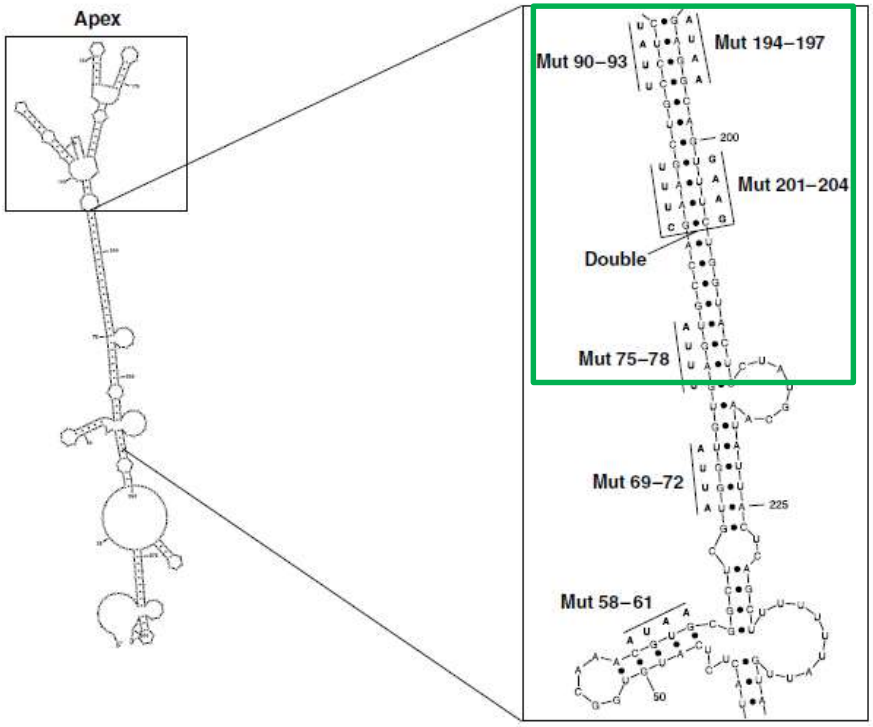


Staufen recruits UPF1 for mRNA decay



ADP-ribosylation factor 1 (ARF1) mRNA

- Encodes ADP-ribosylation factor 1 protein
- Stimulates ADP-ribosyltransferase activity of cholera toxin and role in vesicular trafficking
- Known and validated SMD target



Staufen1 binds *in vivo* to a complex structure within the ARF1 SBS

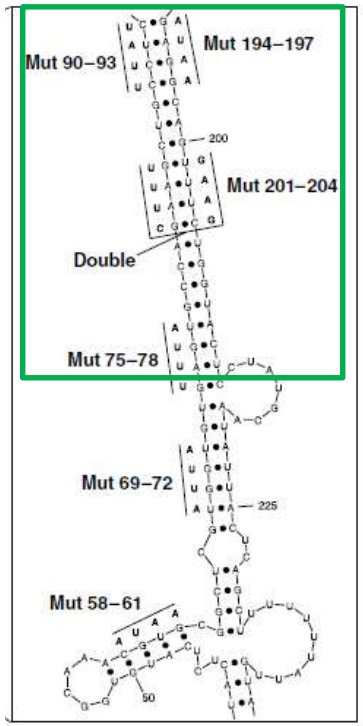
The apical part of the ARF1 dsRNA is crucial for Staufen binding and mRNA decay

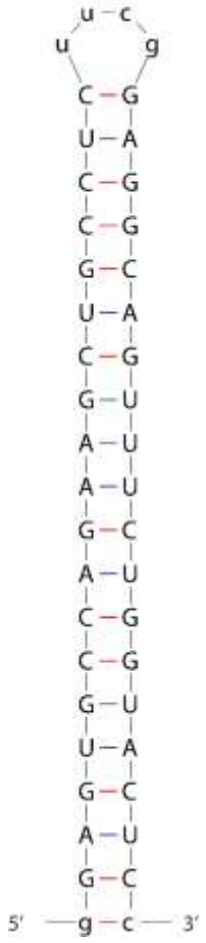
Kim et al. (2005, 2007)

Questions…

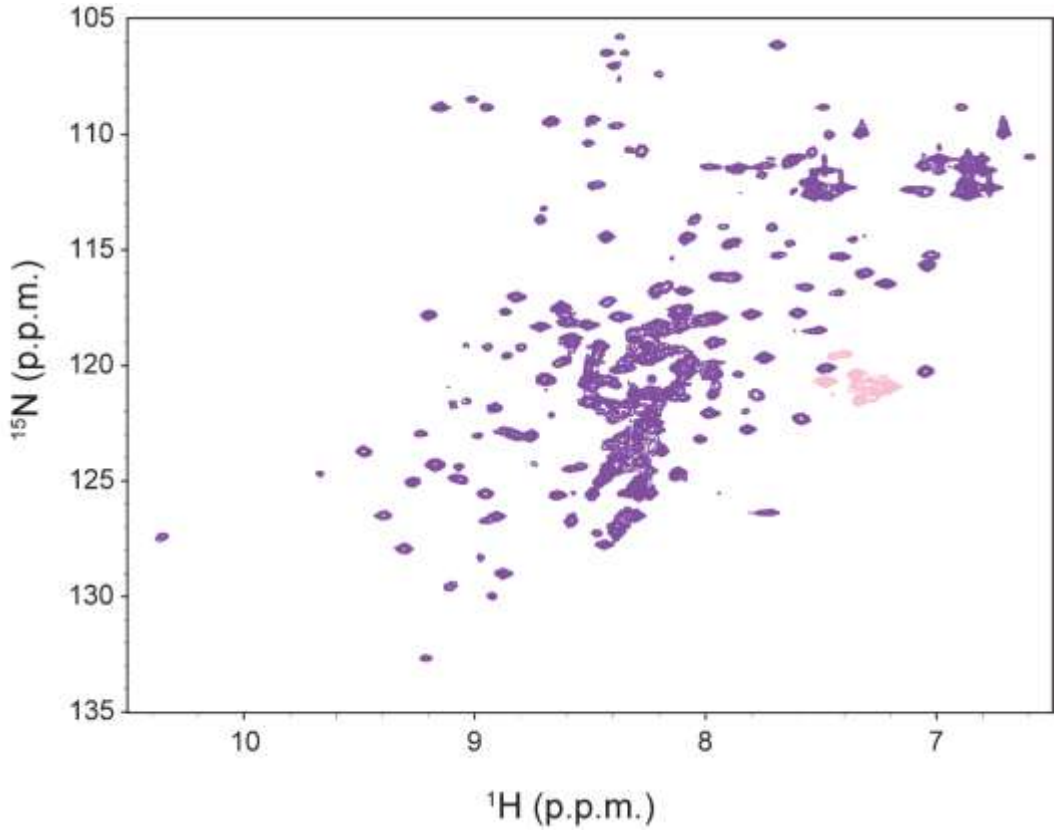
- **How does human Staufen1 recognize dsRNA targets?**
- **Is there any sequence specificity for dsRNA?**

Design of the ARF1 SBS – STAU1 dsRBD3+4 complex



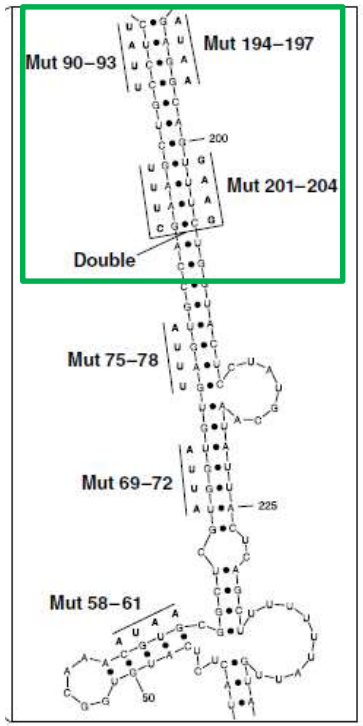


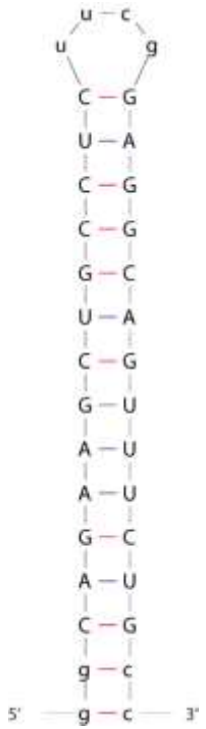
long ARF1 SBS



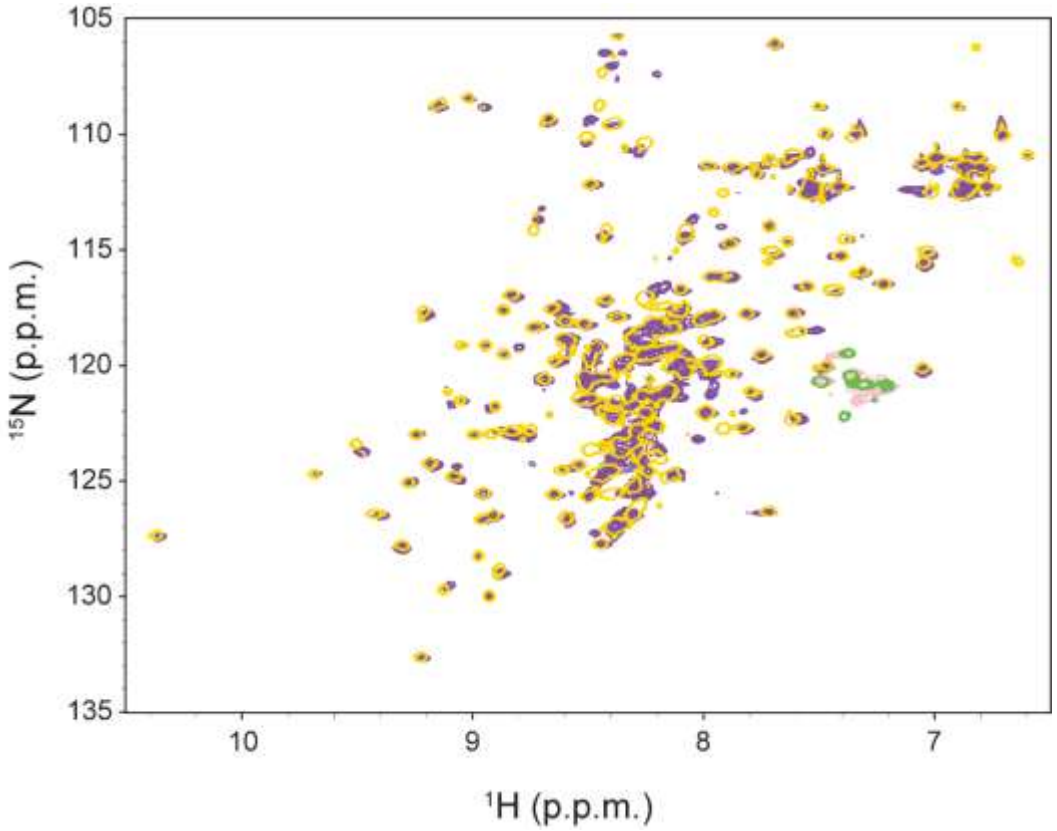
STAU1 dsRBD3+4 – long ARF1 stem loop

Design of the ARF1 SBS – STAU1 dsRBD3+4 complex





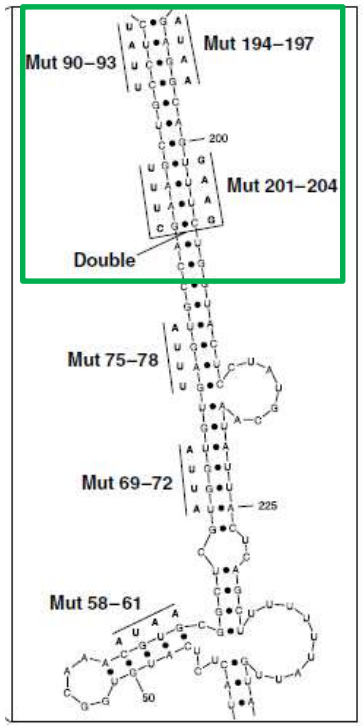
short ARF1 SBS



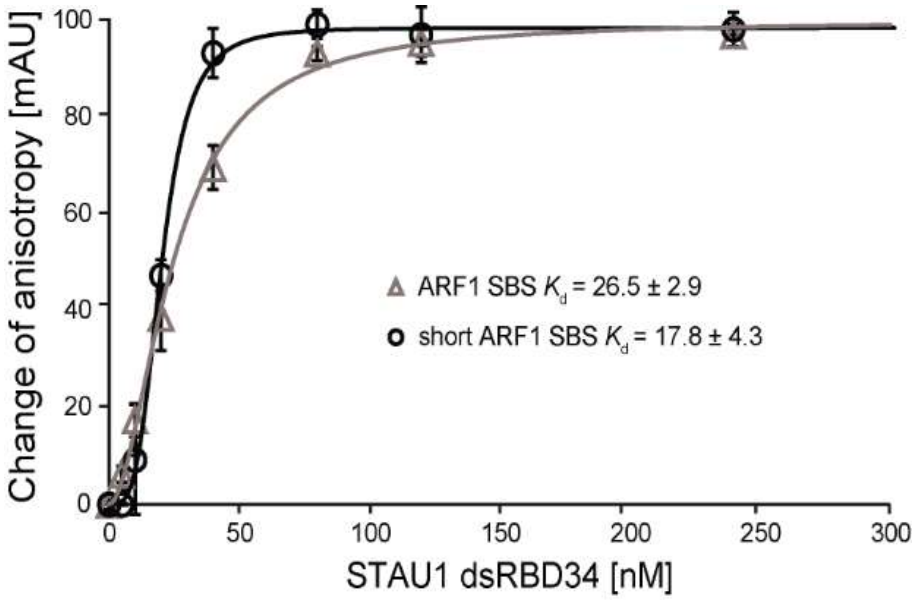
STAU1 dsRBD3+4 – long ARF1 stem loop

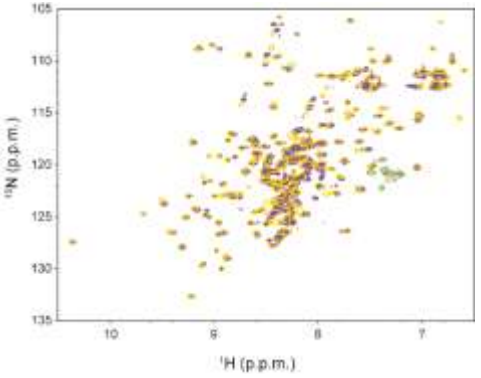
STAU1 dsRBD3+4 – short ARF1 stem loop

Design of the ARF1 SBS – STAU1 dsRBD3+4 complex



short ARF1 SBS



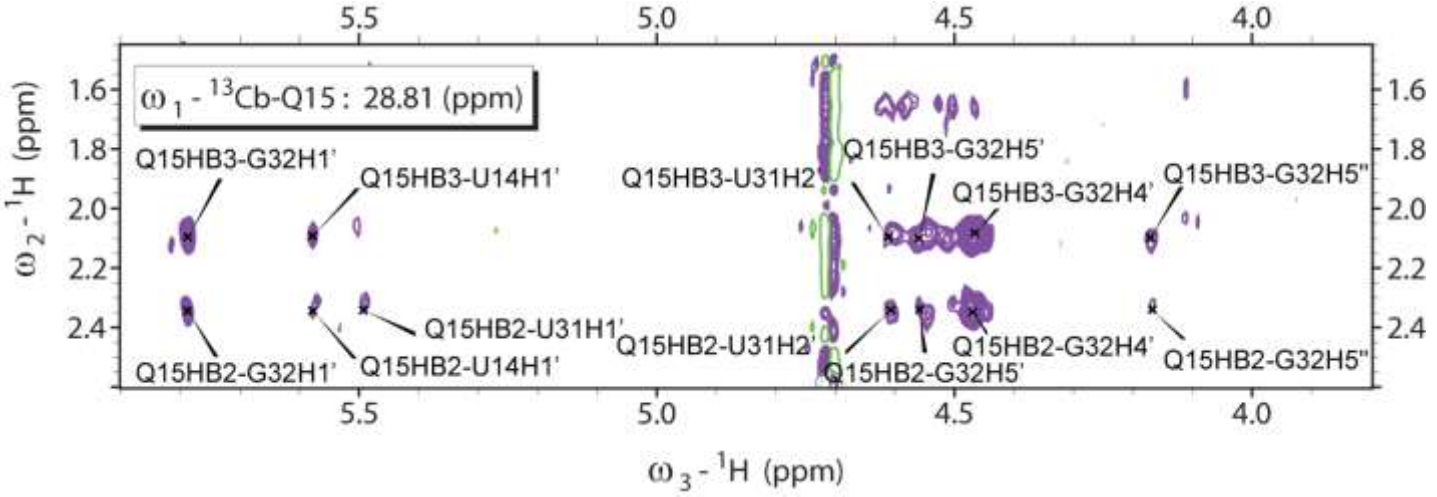


STAU1 dsRBD3+4 – long ARF1 stem loop

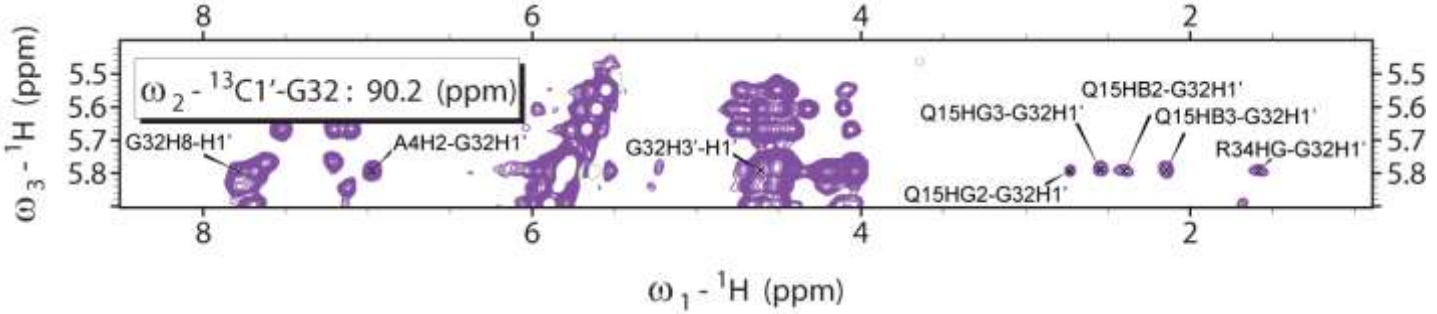
STAU1 dsRBD3+4 – short ARF1 stem loop

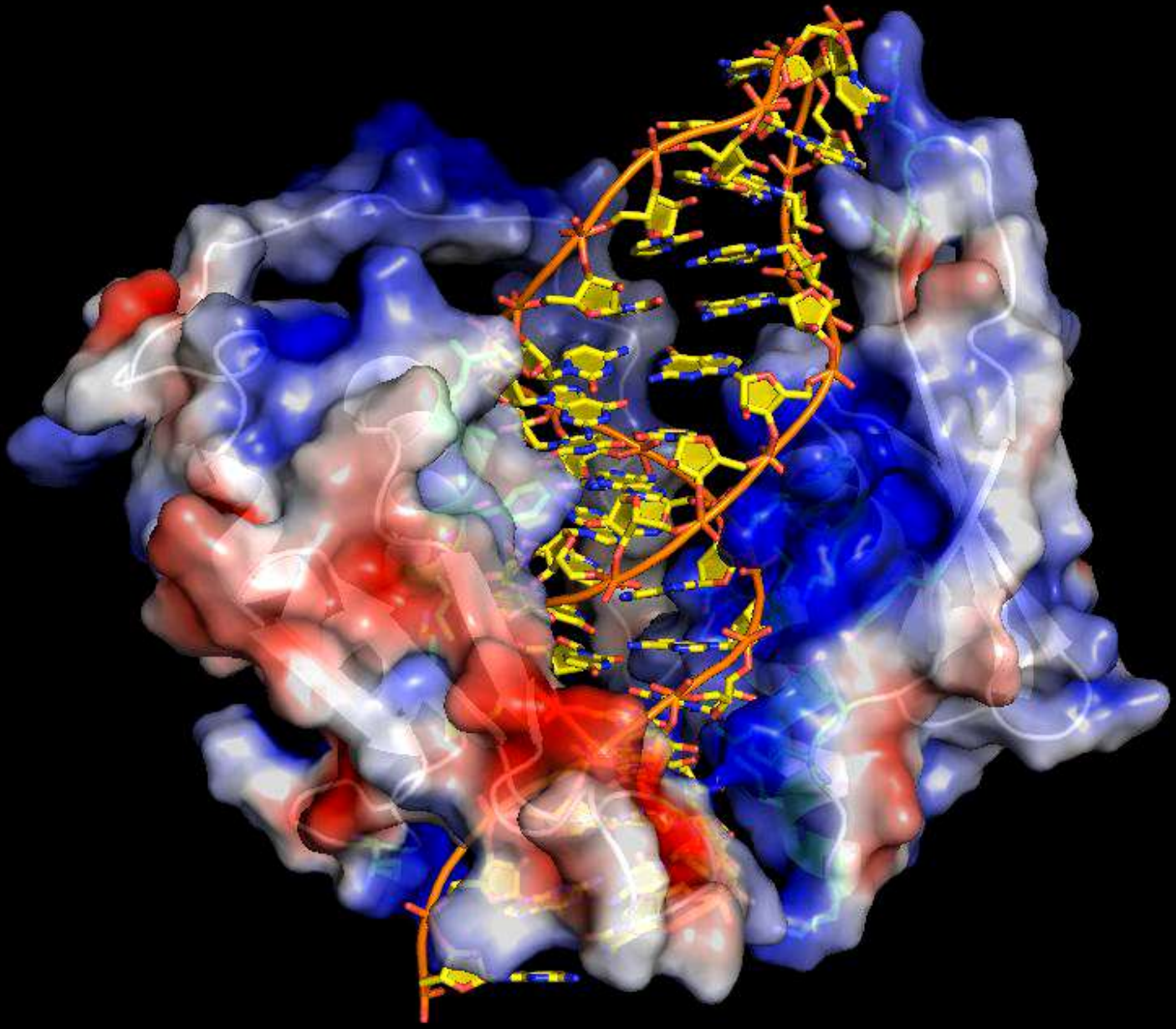
Strategy to assign intermolecular NOEs

3D F1-filtered, F2-edited NOESY = to assign intermolecular NOEs from protein sidechain to RNA

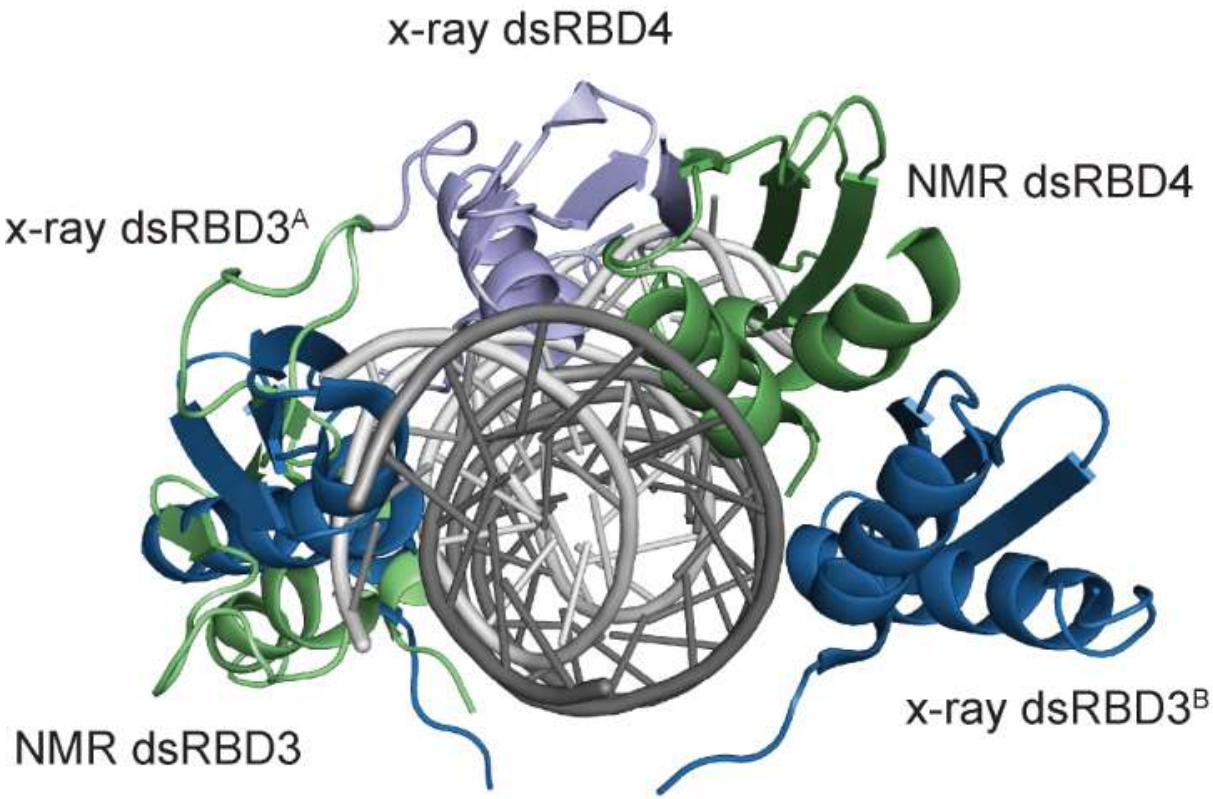


3D ^{13}C-edited NOESY RNA = to assign RNA-RNA and RNA-protein NOEs



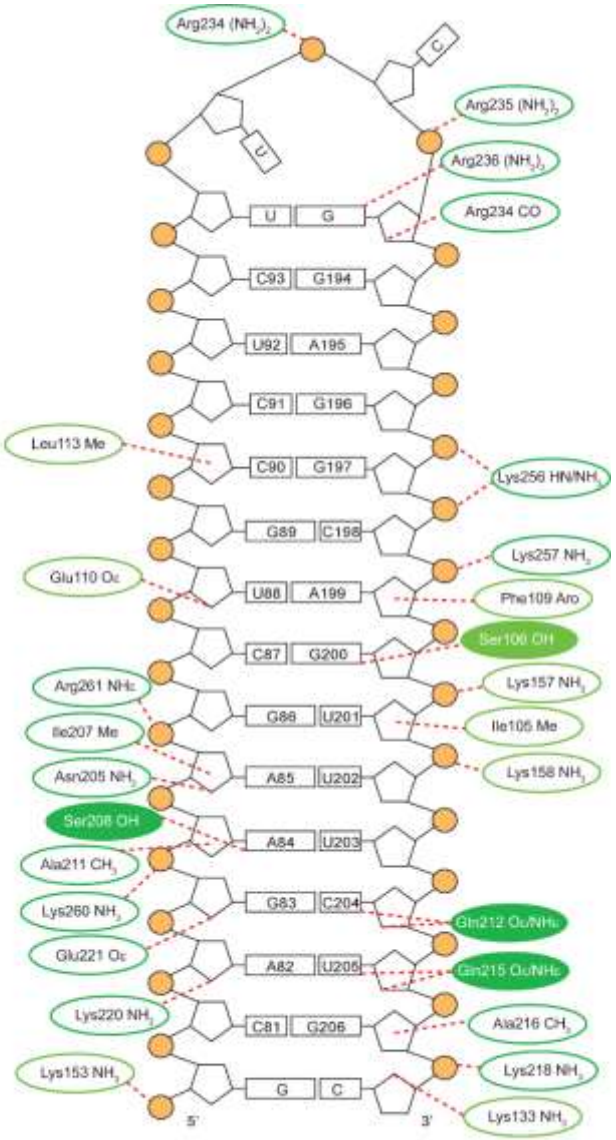


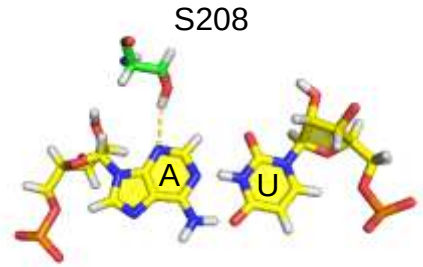
Comparison of NMR and x-ray structure

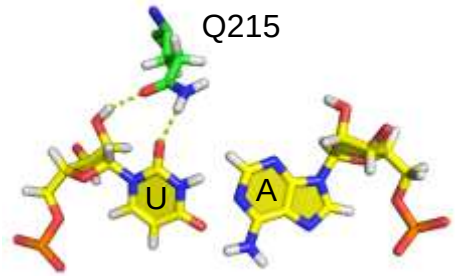


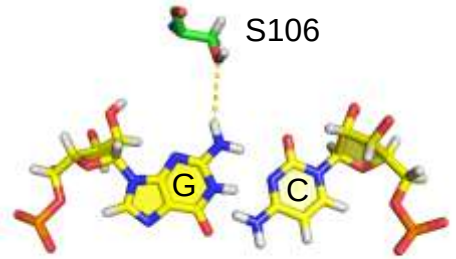
Lazzaretti et al, 2018

Base contacts from minor groove side









Drop in affinity

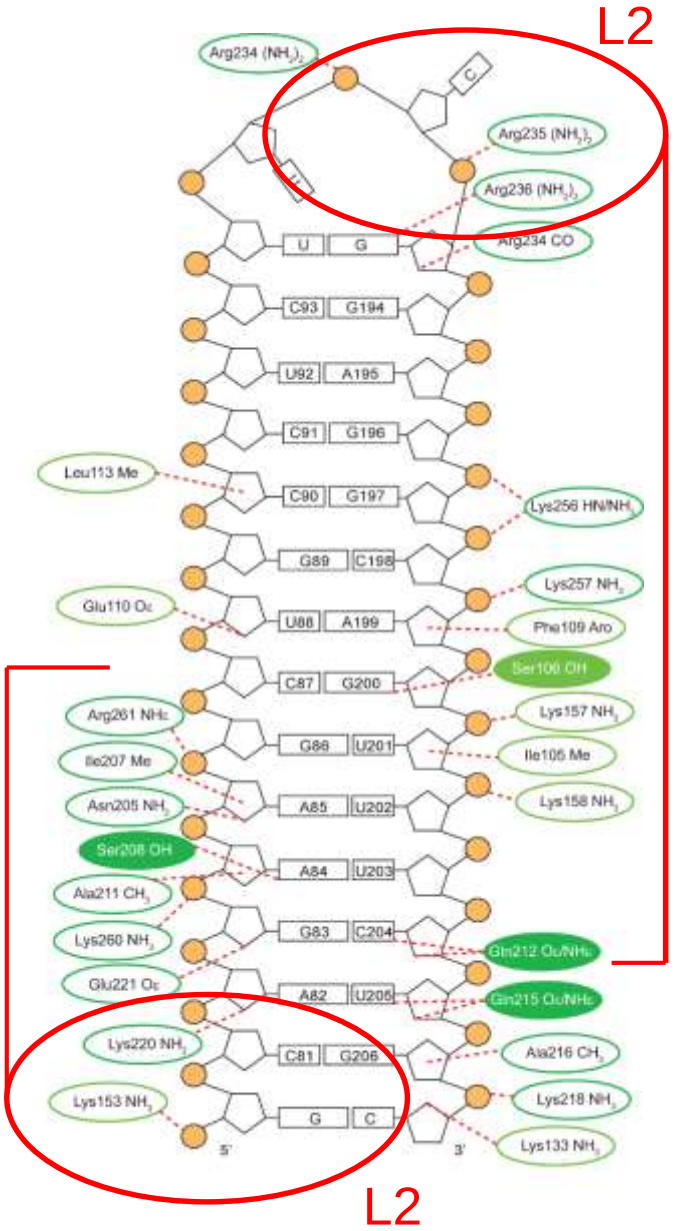
S208A: 1.4x

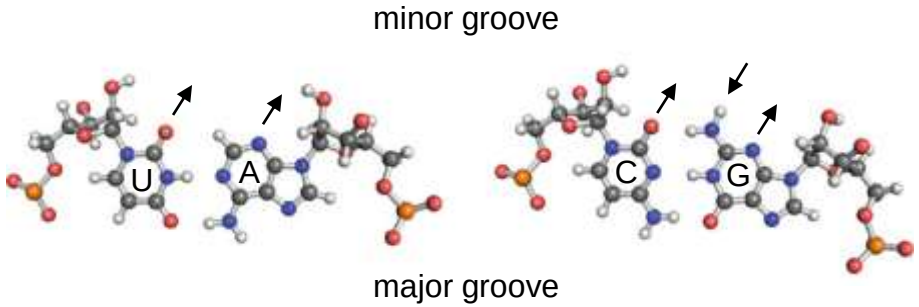
Q212A, Q215A: 1.6x

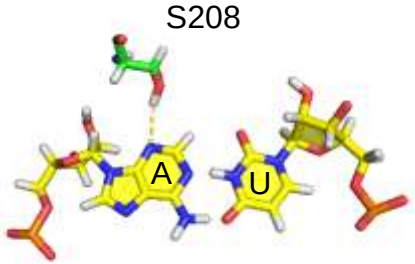
Combined: 5.2x

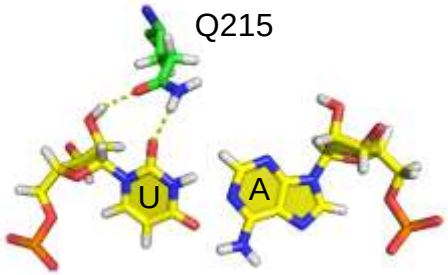
I105A, S106A: 3.6x

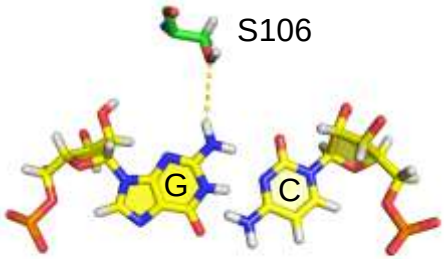
Base contacts from minor groove side











Drop in affinity

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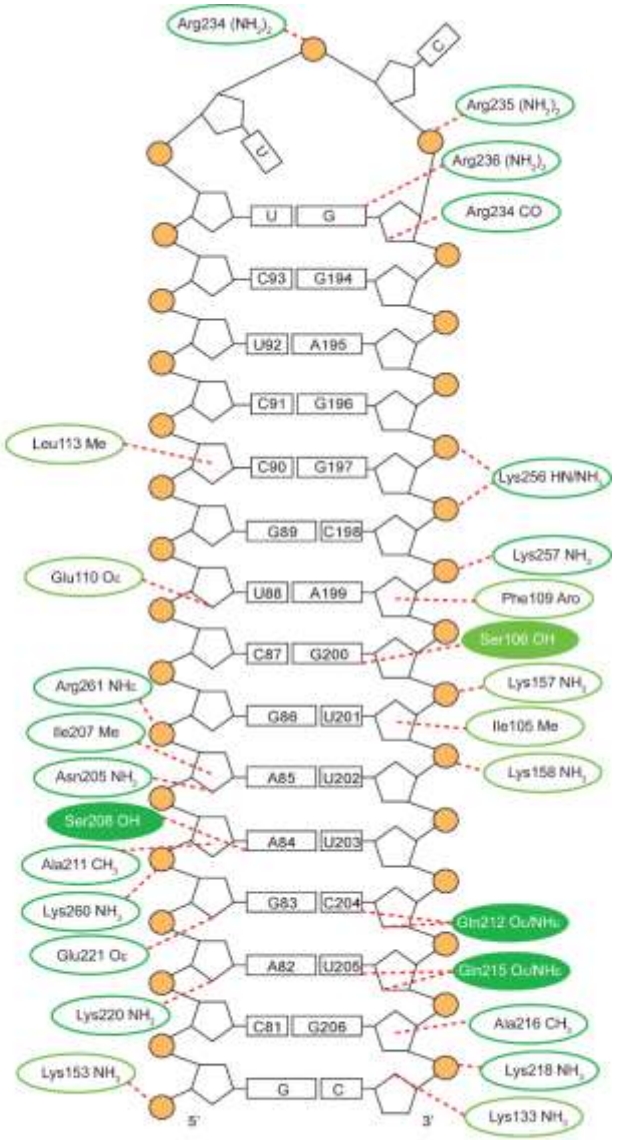
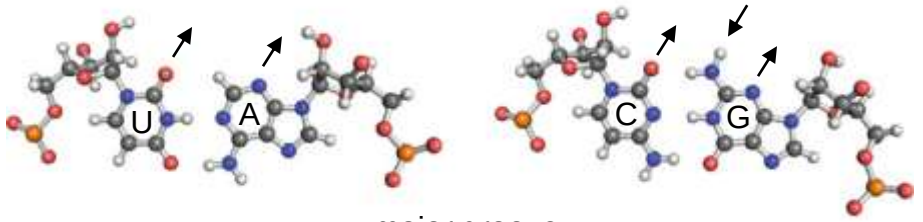
Base contacts from minor groove side

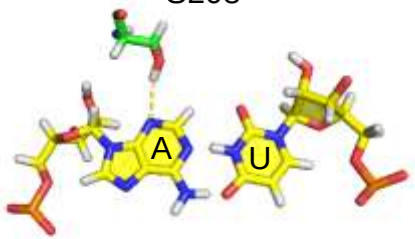


minor groove



major groove

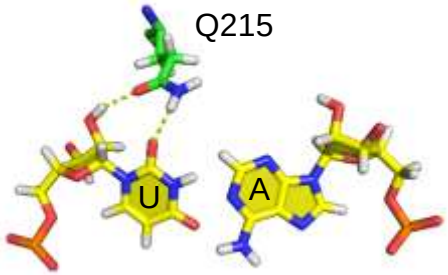
S208



Drop in affinity

S208A: 1.4x

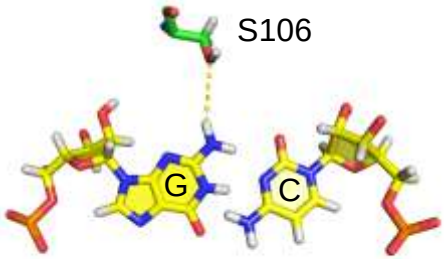
Q215



Q212A, Q215A: 1.6x

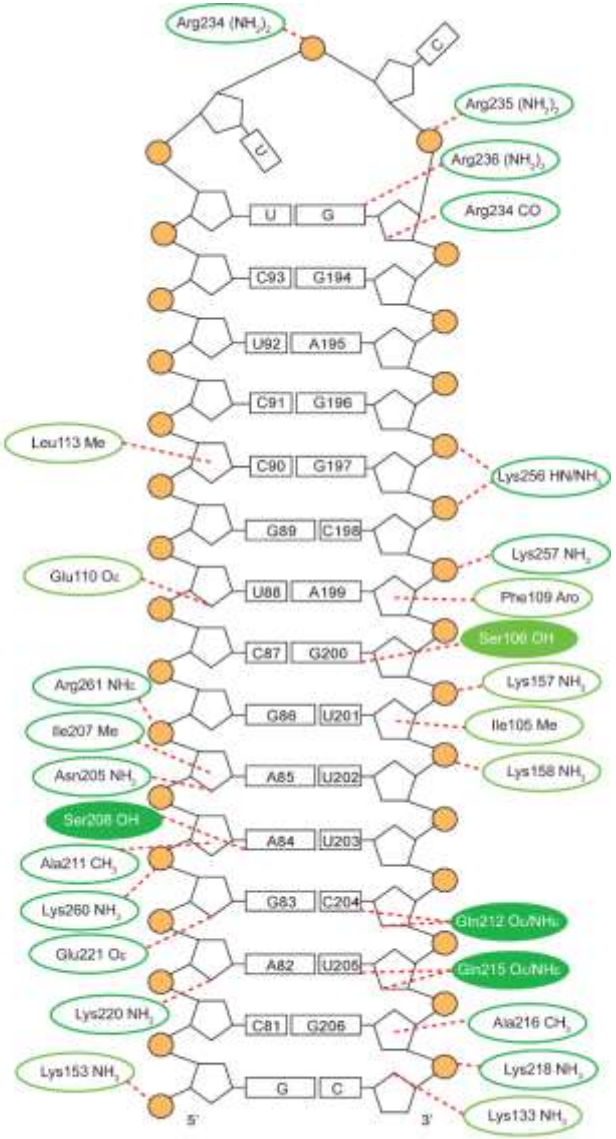
Combined: 5.2x

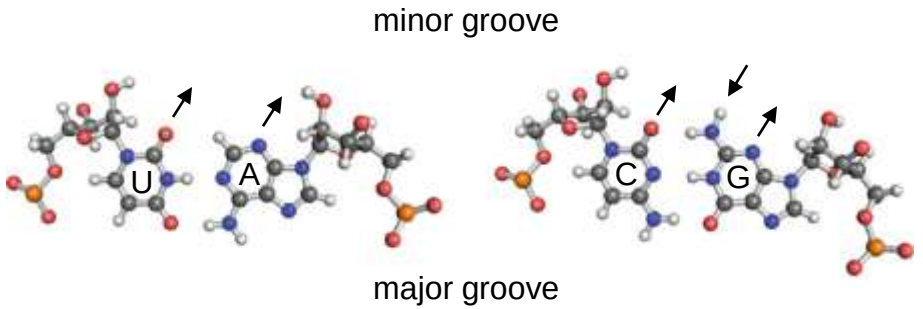
S106

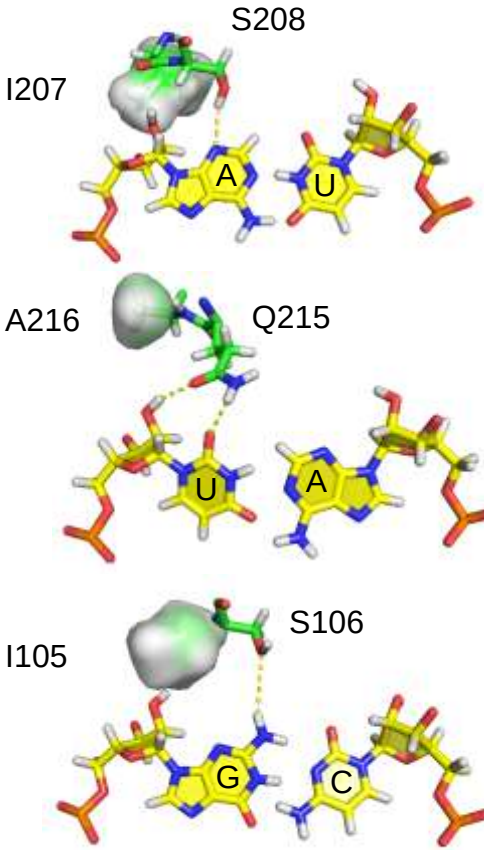


I105A, S106A: 3.6x

Base contacts from minor groove side







Drop in affinity

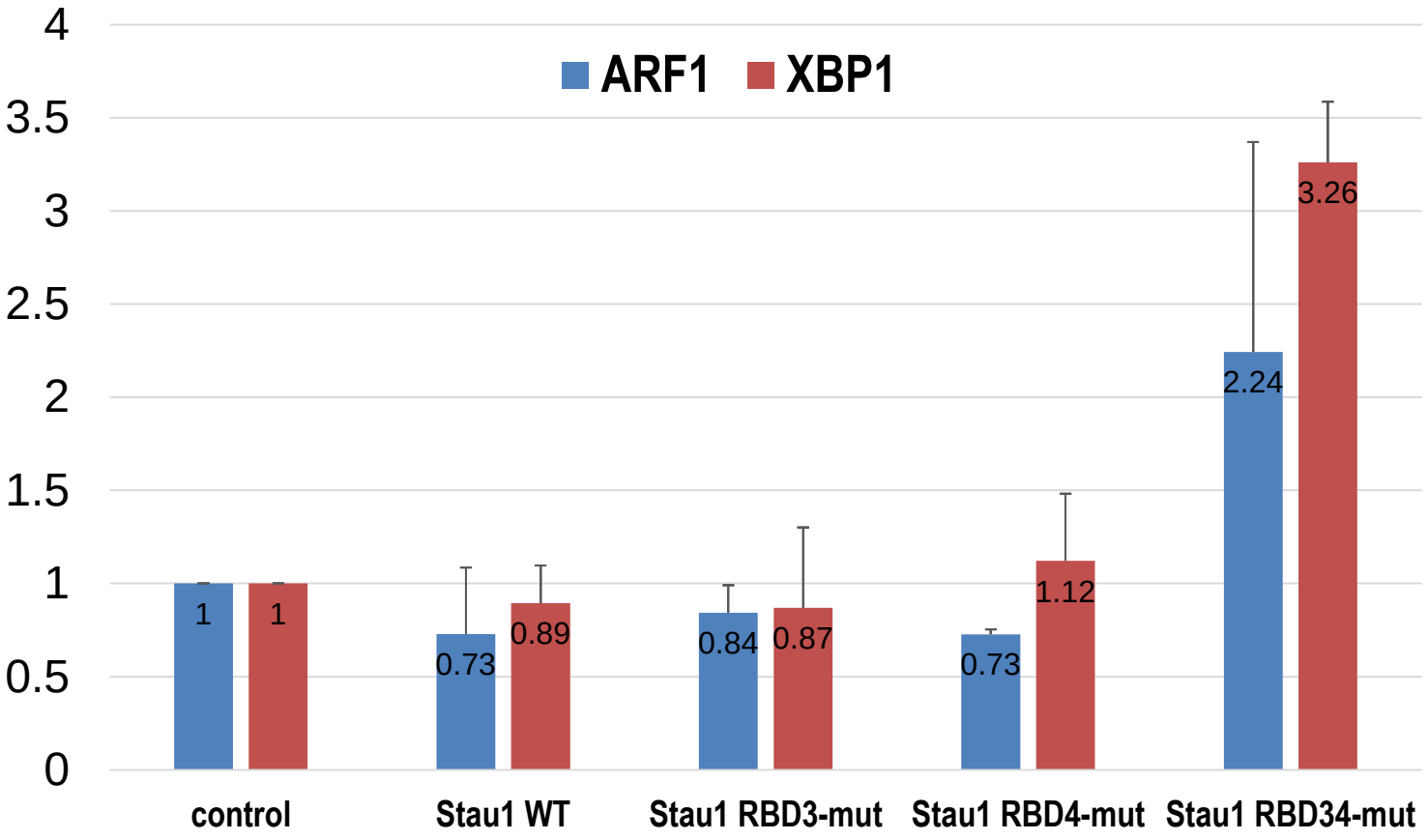
S208A: 1.4x

Q212A, Q215A: 1.6x

Combined: 5.2x

I105A, S106A: 3.6x

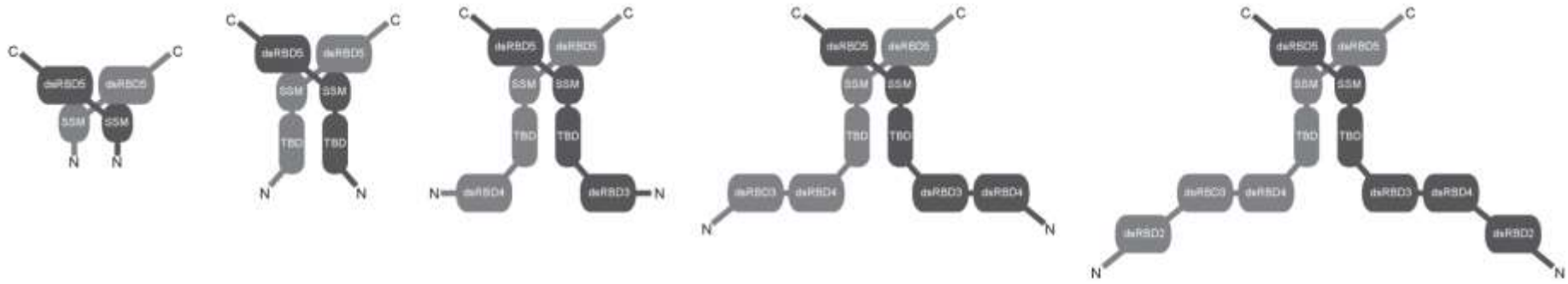
ARF1 and XBP1 mRNA levels in HeLa cells



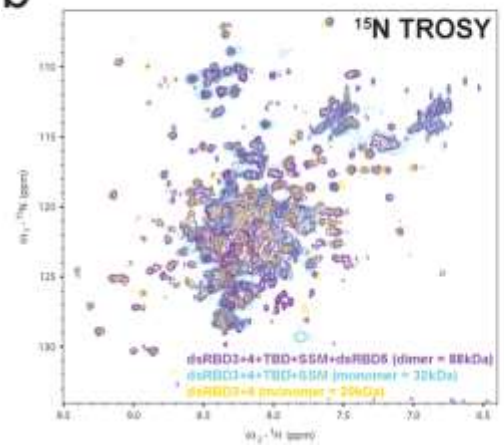
Mutations in helix α1: dsRBD3 (I105A+S106A)
dsRBD4 (Q212A+Q215A)

Structure determination of large Staufen - 3'UTR – ribosome complexes
How does Staufen influence mRNA stability?

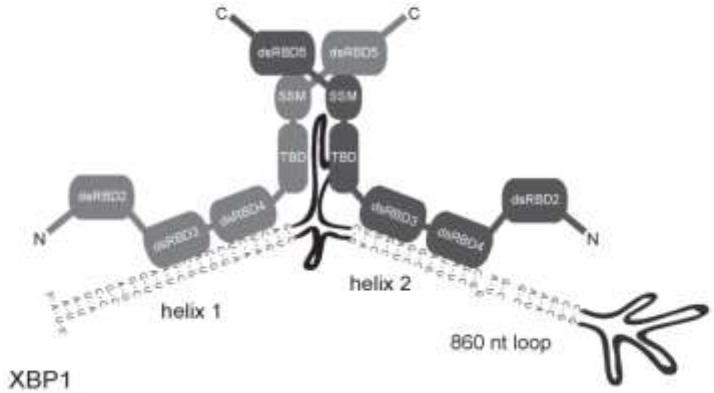
a



b



c



Integrative Structural Biology

NMR

PRE, RDC

SAXS

Cryo-EM

etc.

Conclusions:

- dsRBD3+4 bind dsRNA in expected three distinct regions
- dsRBD4 arginines in β1β2 loop “anchor” dsRBD4 at the end of helix
- dsRBD3 binds to dsRNA in opposite orientation
- dsRBD3 lysine in β1β2 loop “anchors” dsRBD3 at the other end of helix
- dsRBD3+4 lysines of helix α2 insert in major groove
- dsRBD4 glutamines and a serine of helix α1 recognize 2 pyrimidines and 1 adenine
- dsRBD3 serine of helix α1 recognizes 1 guanine
- no protein-protein interactions between the domains
- Binding register or neighboring residues determine recognition of bases from minor groove

Post-transcriptional regulation of gene expression

- Alternative splicing of pre-mRNA
- mRNA processing (5'-/3'-end formation)
- Nuclear export
- cytoplasmic RNA transport
- Translational control
- mRNA decay

Regulated by multiple RNA signals in complex with Proteins