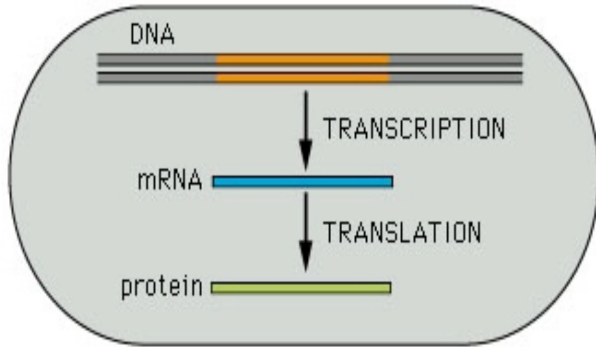
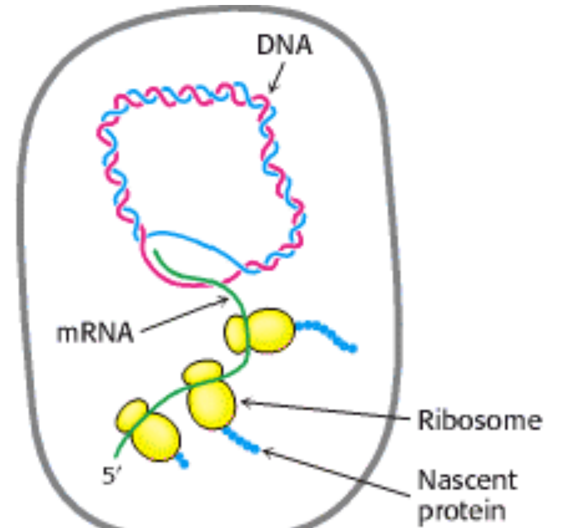
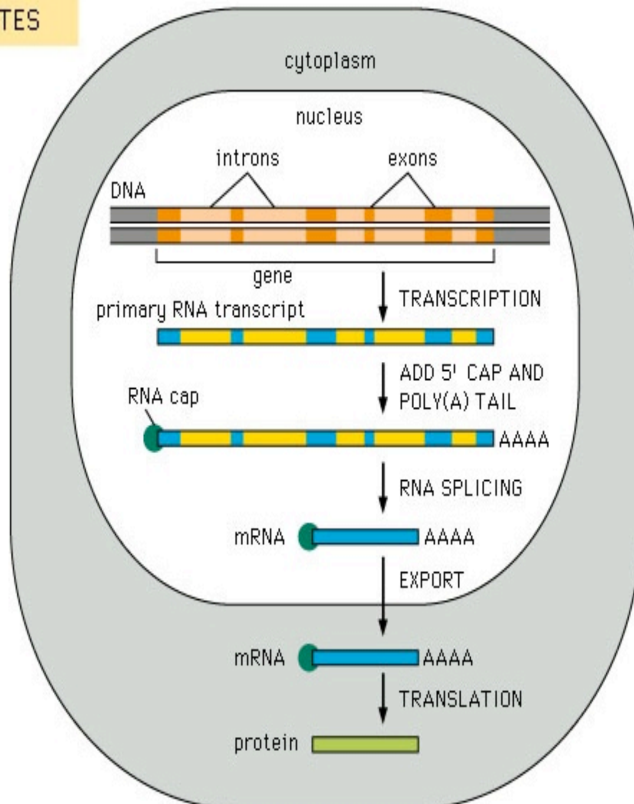
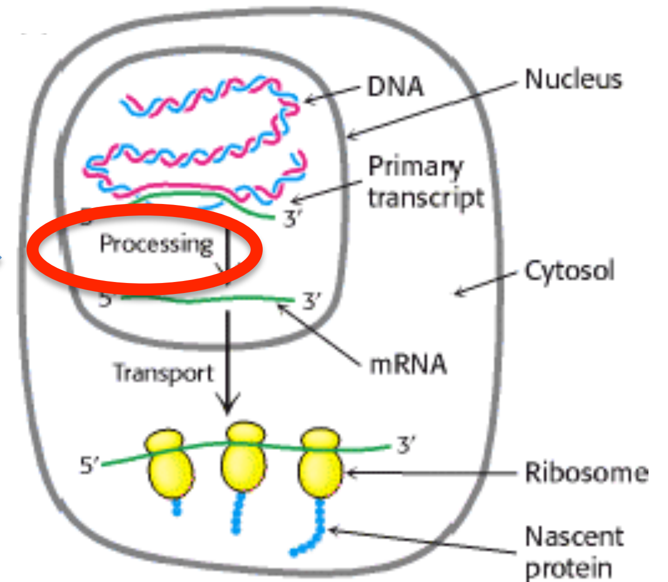
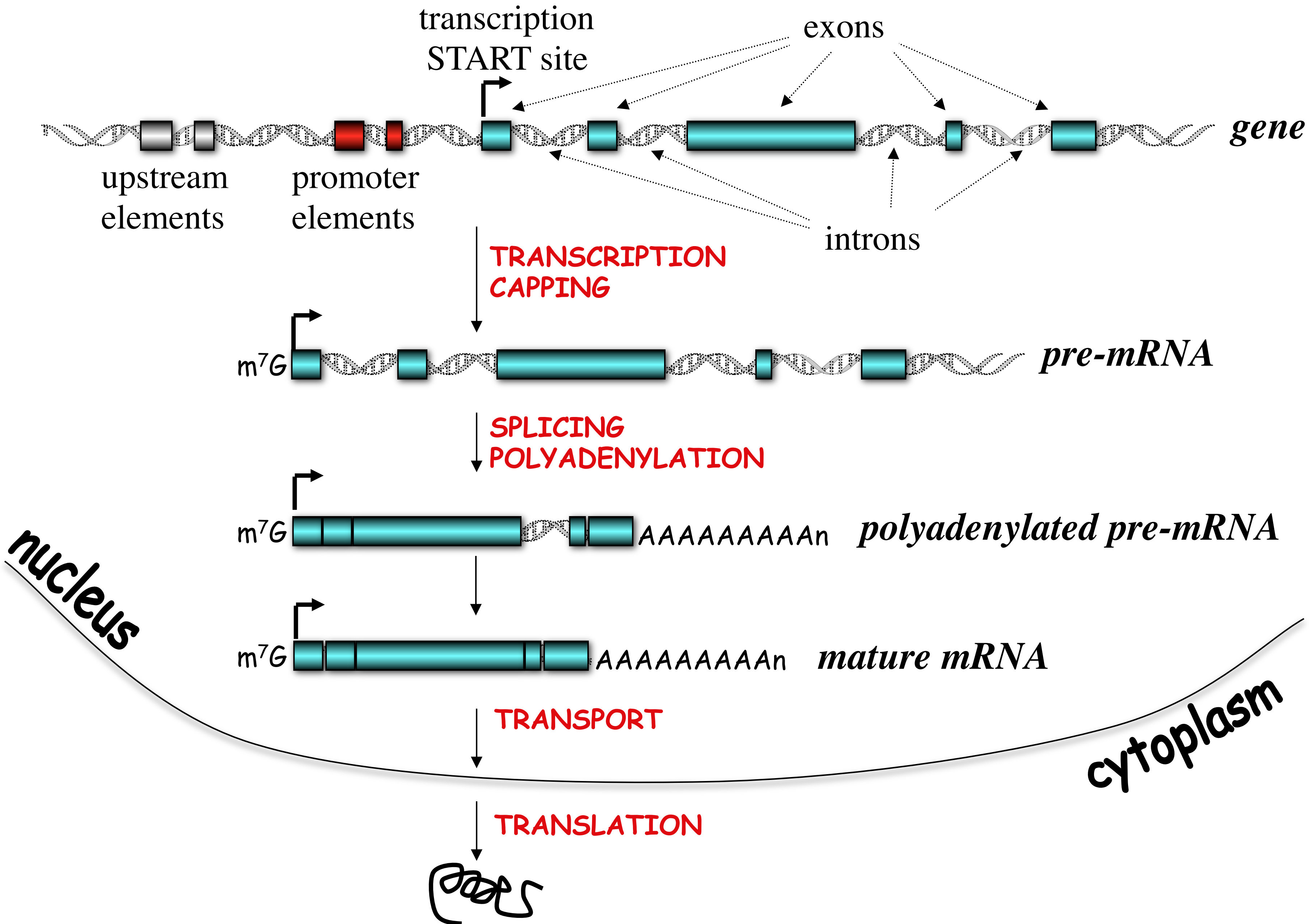
Messenger RNA


PROCARYOTES
DNA
TRANSCRIPTION
mRNA
TRANSLATION
protein

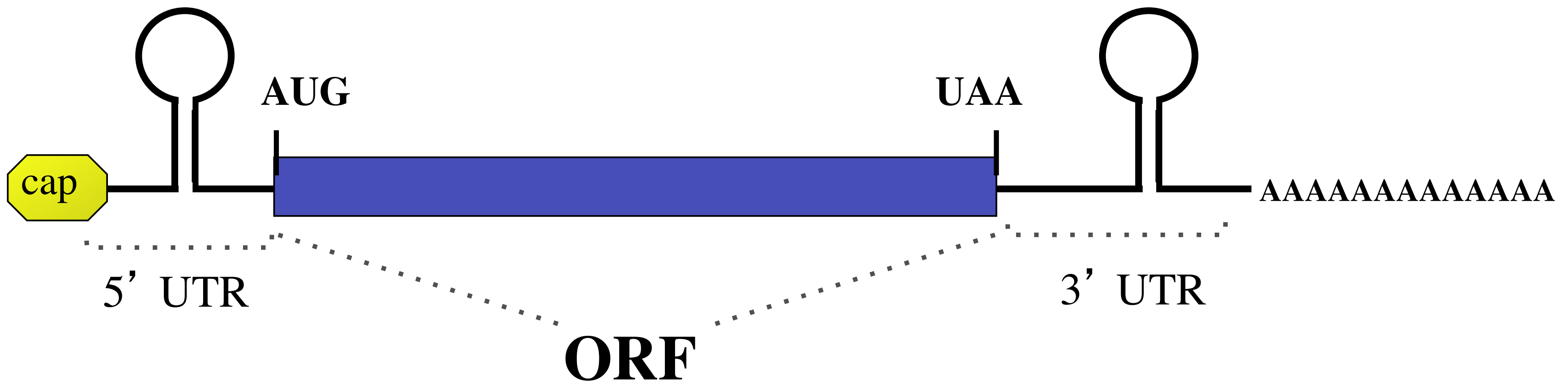

DNA
mRNA
Ribosome
Nascent protein
5′
PROKARYOTE


EUCARYOTES
cytoplasm
nucleus
introns
exons
DNA
gene
primary RNA transcript
TRANSCRIPTION
ADD 5' CAP AND POLY(A) TAIL
RNA cap
AAAA
RNA SPLICING
mRNA
AAAA
EXPORT
mRNA
AAAA
TRANSLATION
protein

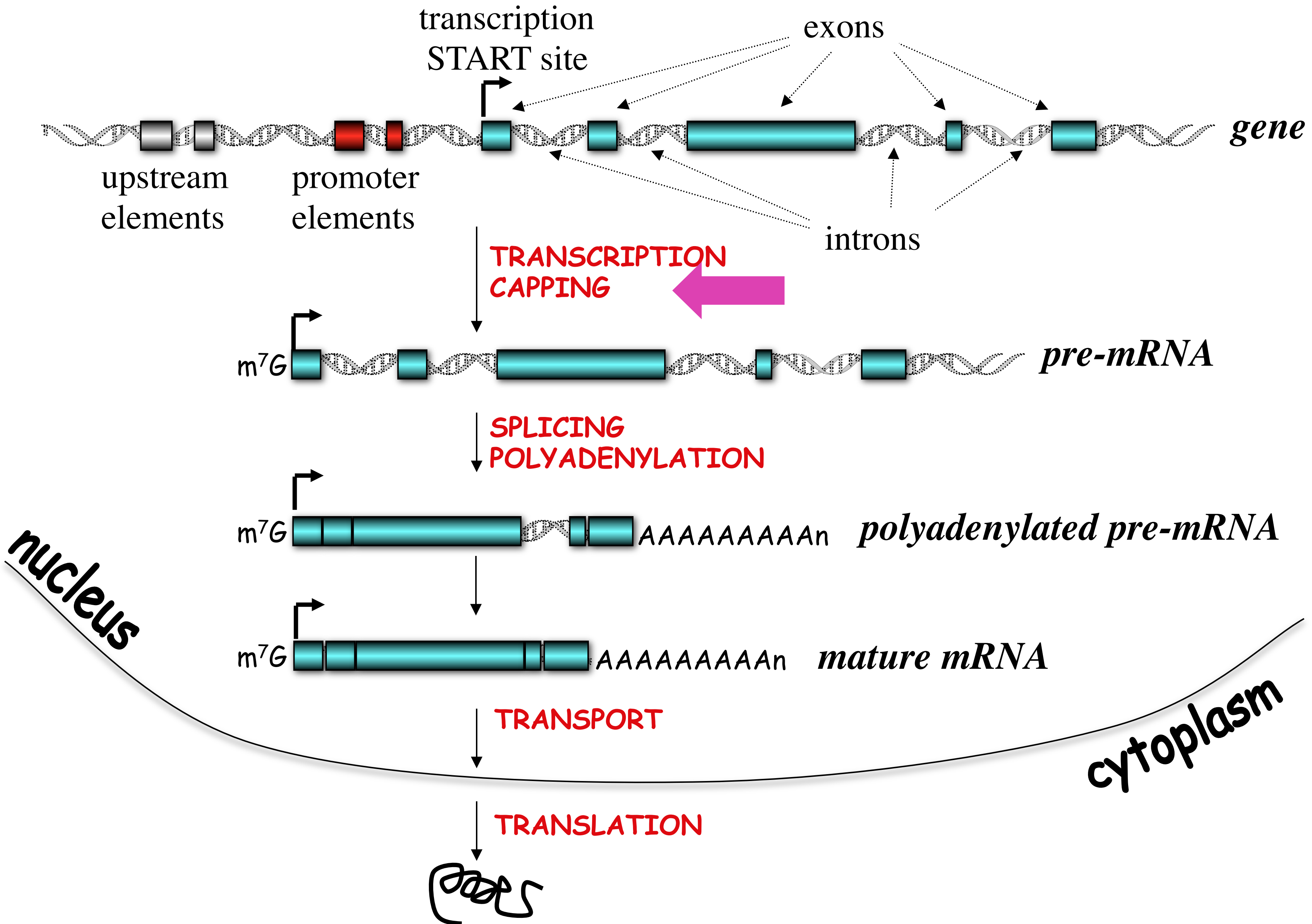

DNA
Nucleus
Primary transcript
3′
Processing
Cytosol
3′
mRNA
Transport
5′
3′
Ribosome
Nascent protein
EUKARYOTE


Eucaryotic gene expression
transcription
START site
exons
gene
upstream
elements
promoter
elements
introns
TRANSCRIPTION
CAPPING
m7G
pre-mRNA
SPLICING
POLYADENYLATION
m7G
AAAAAAAAAn
polyadenylated pre-mRNA
nucleus
m7G
AAAAAAAAAn
mature mRNA
TRANSPORT
cytoplasm
TRANSLATION

mRNA structures



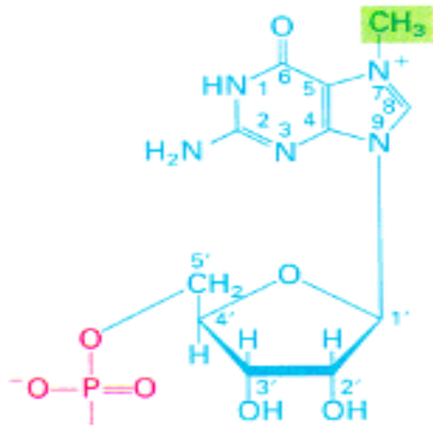
Eucaryotic gene expression



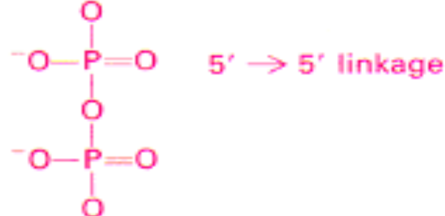
Why the Cap structure is important?

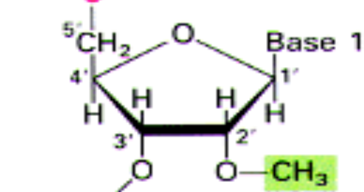
1) RNA stability
2) Favours the mRNA transport to the cytoplasm
3) Increases translation (it binds to eIF4E that belongs to translation initiation complex)

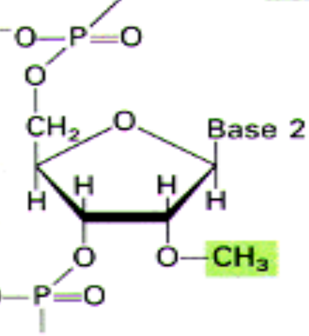
5' CAP: 3'-G-5'ppp5'-N-3'p



7-metilguanosina







estremità 5′ della RNA catena

This involves the addition of a modified guanine base to the 50 end of the RNA. Specifically, it is a methylated guanine, and it is joined to the RNA transcript by an unusual 5‘–5’ linkage involving three phosphates

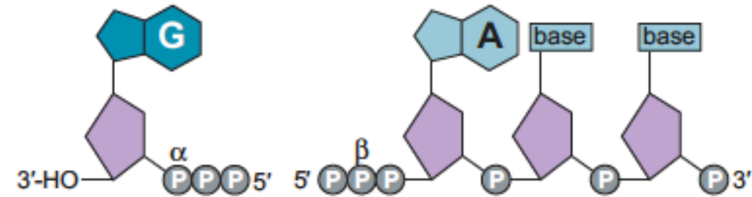
- CAP is added at very early stage of transcription initiation

- The 5’-5’ phosphodiester bond makes the molecule resistant to the exonuclease activity.

- I vitro synthetized RNA without CAP are rapidly degraded

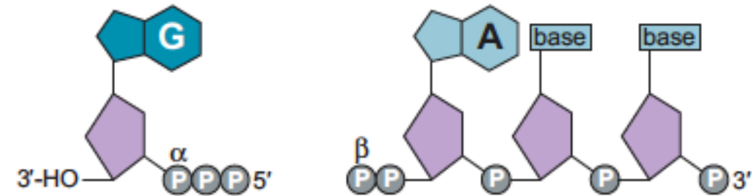
pre-mRNA capping

The 5’ cap is created in three enzymatic steps:



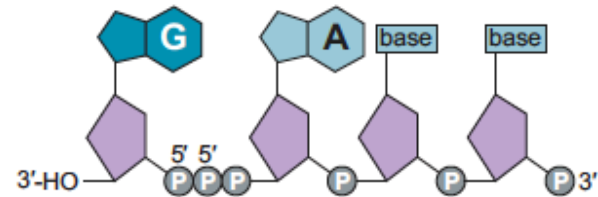
RNA triphosphatase

1. a phosphate group is removed from the 5’ end of the transcript.



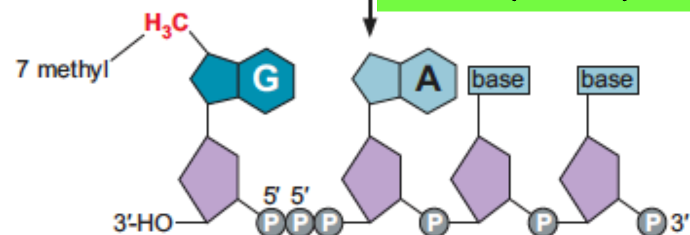
RNA guanylyltransferase

2. GMP moiety is added.



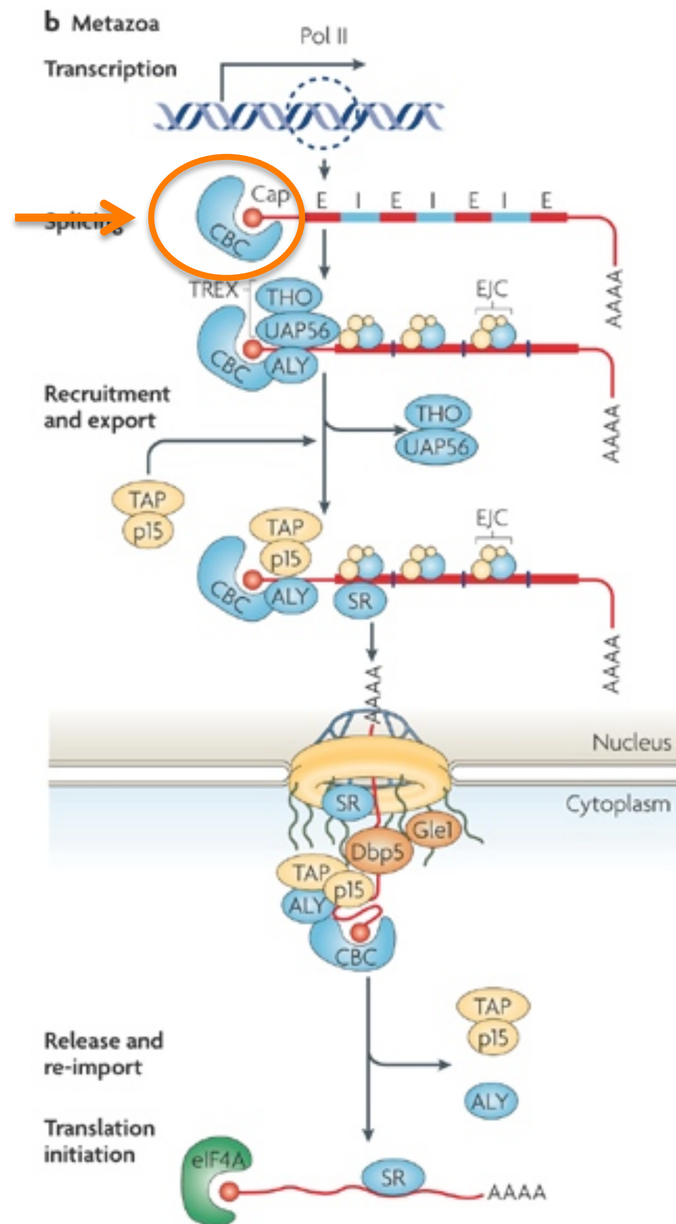
RNA(G-7-) methyltransferase

3. GMP nucleotide is modified by the addition of a methyl group.

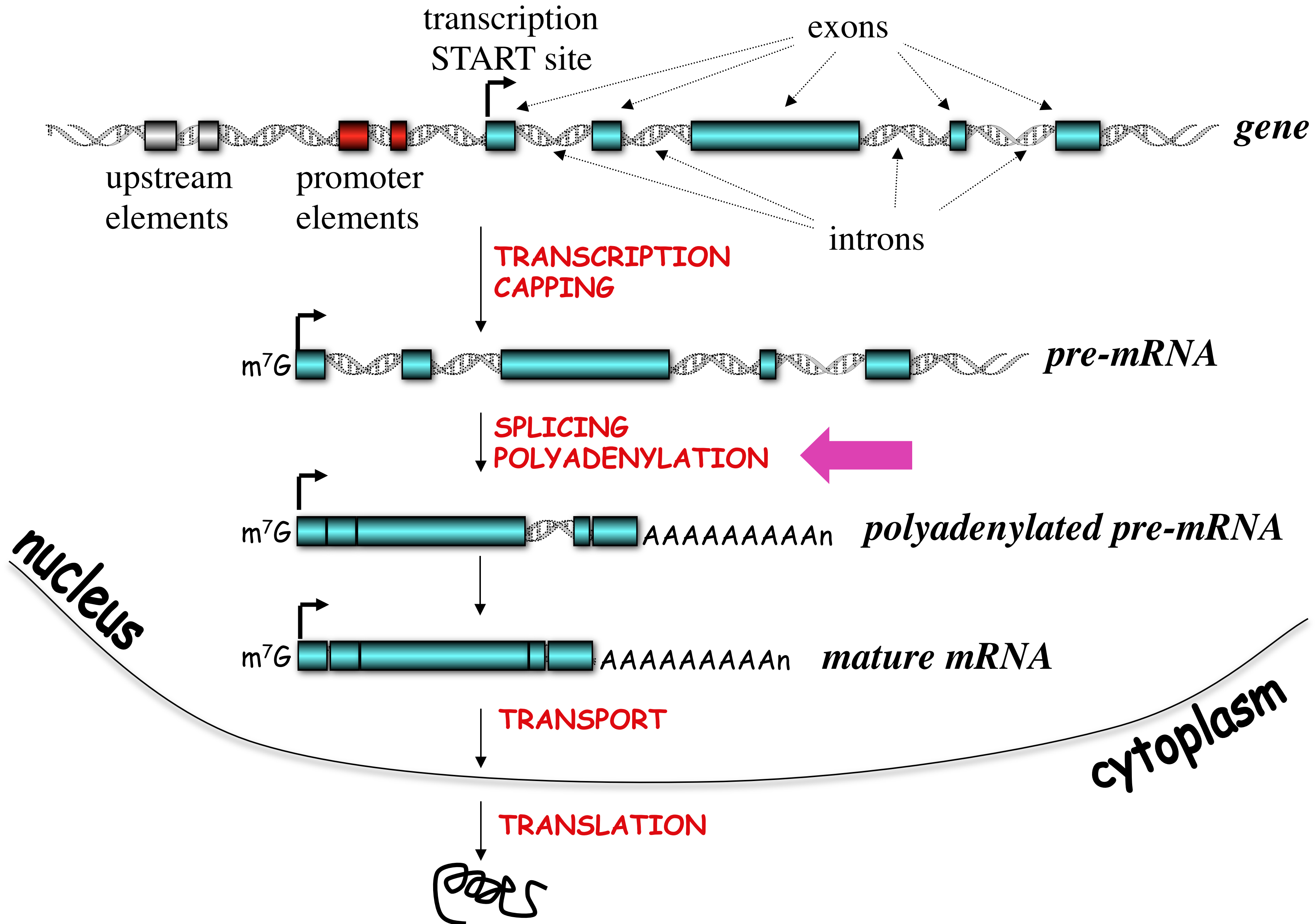


5’ CAP favours the mRNA transport to the cytoplasm

CBC: CAP binding complex



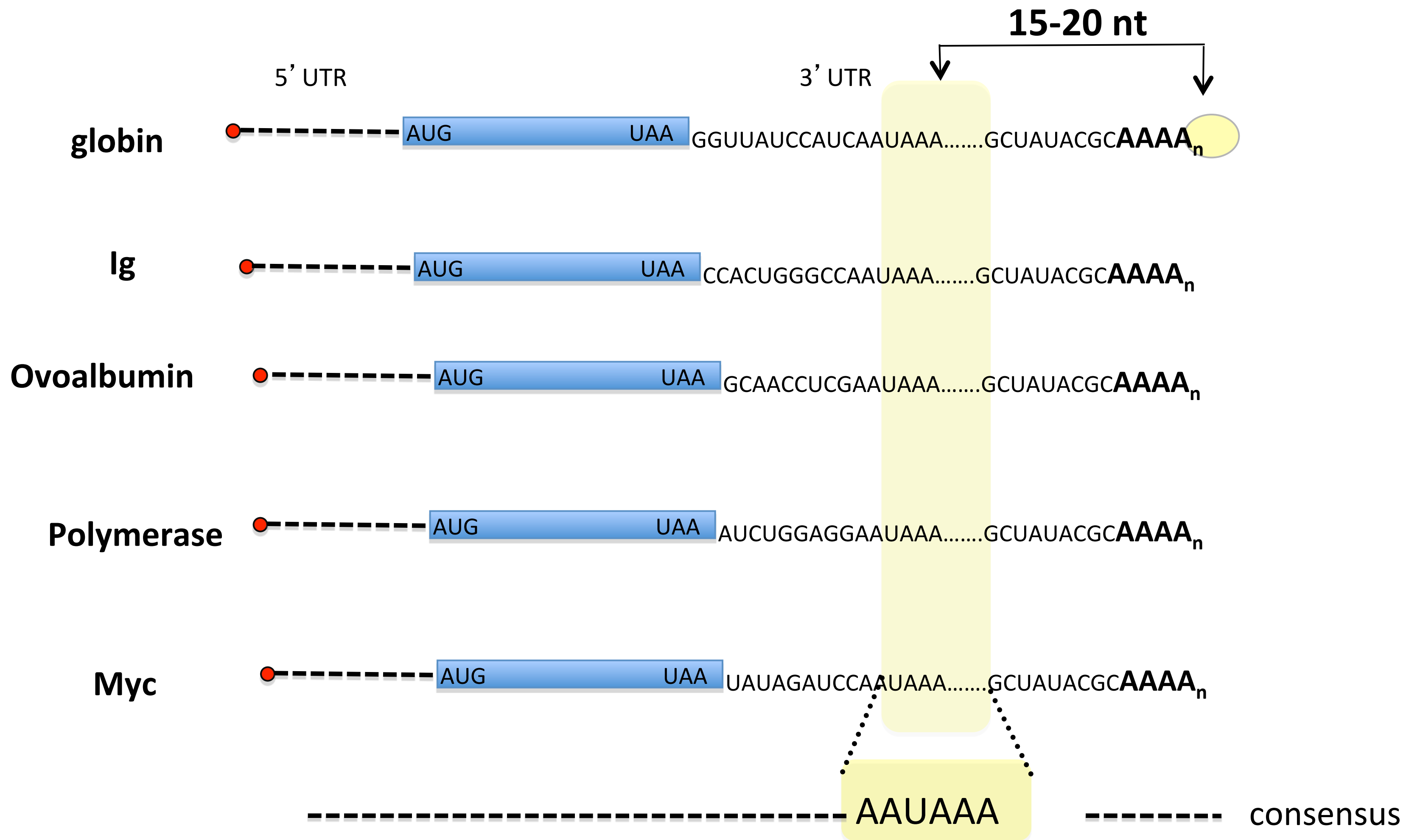
Eucaryotic gene expression

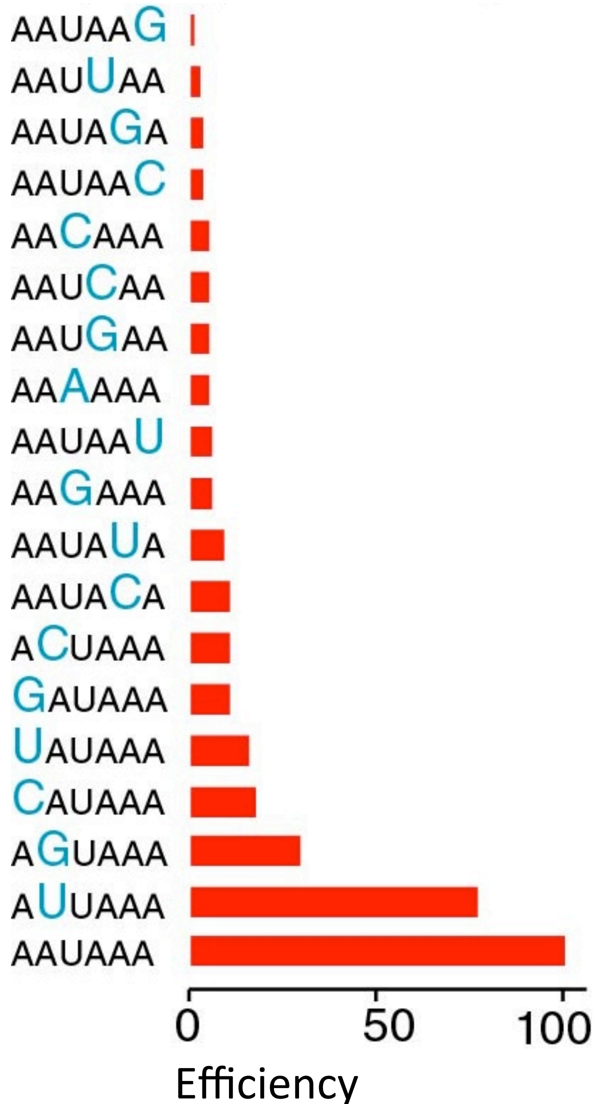


How is the function of the polyA tail?

1) RNA stability
2) Favours the mRNA transport to the cytoplasm
3) Increases translation efficiency by favouting the loading of ribosomal 40S subunit
4) mRNA 3' end formation allows efficient transcription termination.

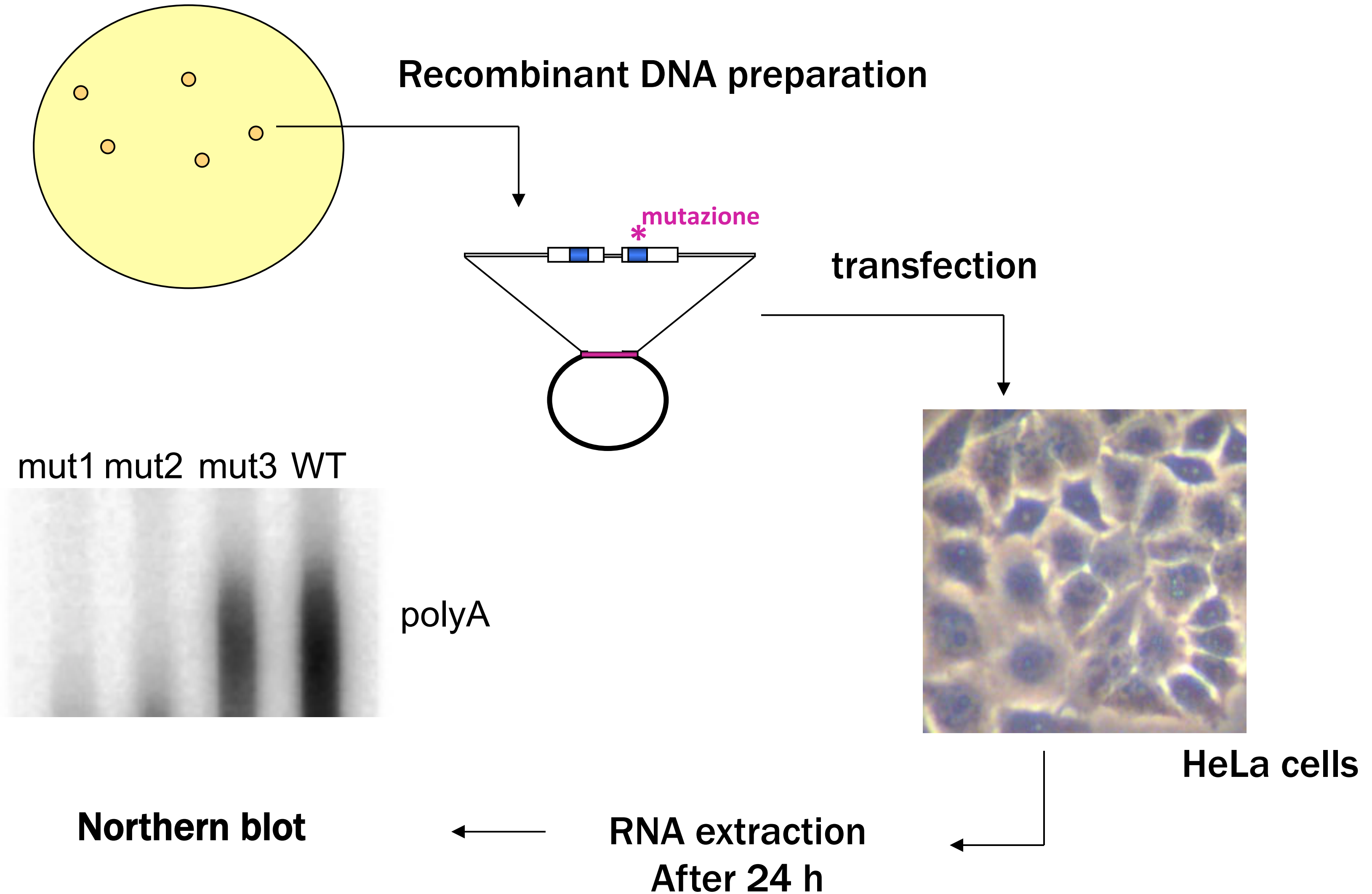
Looking for consensus sequence



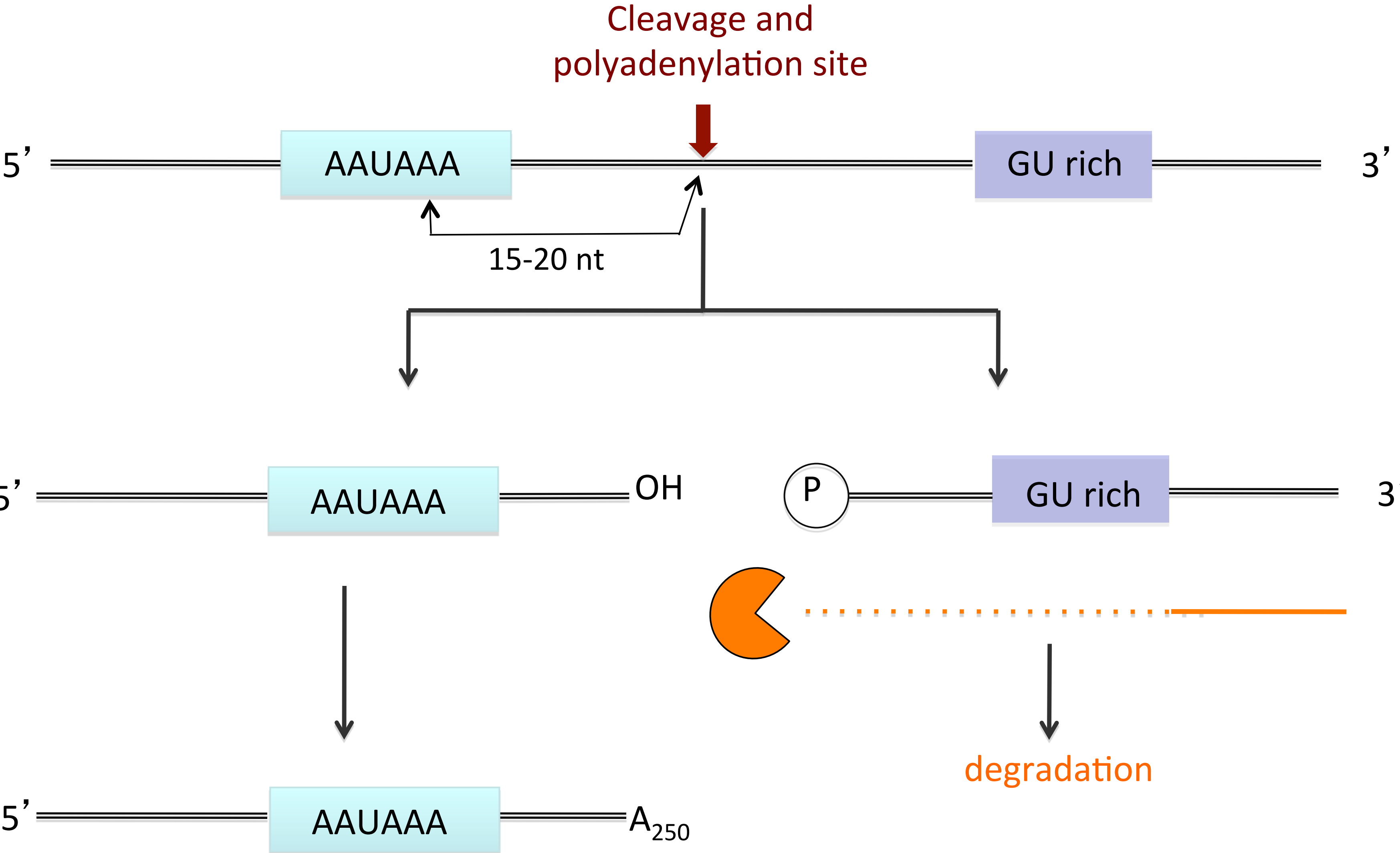


Influence of consensus sequence AAUAAA

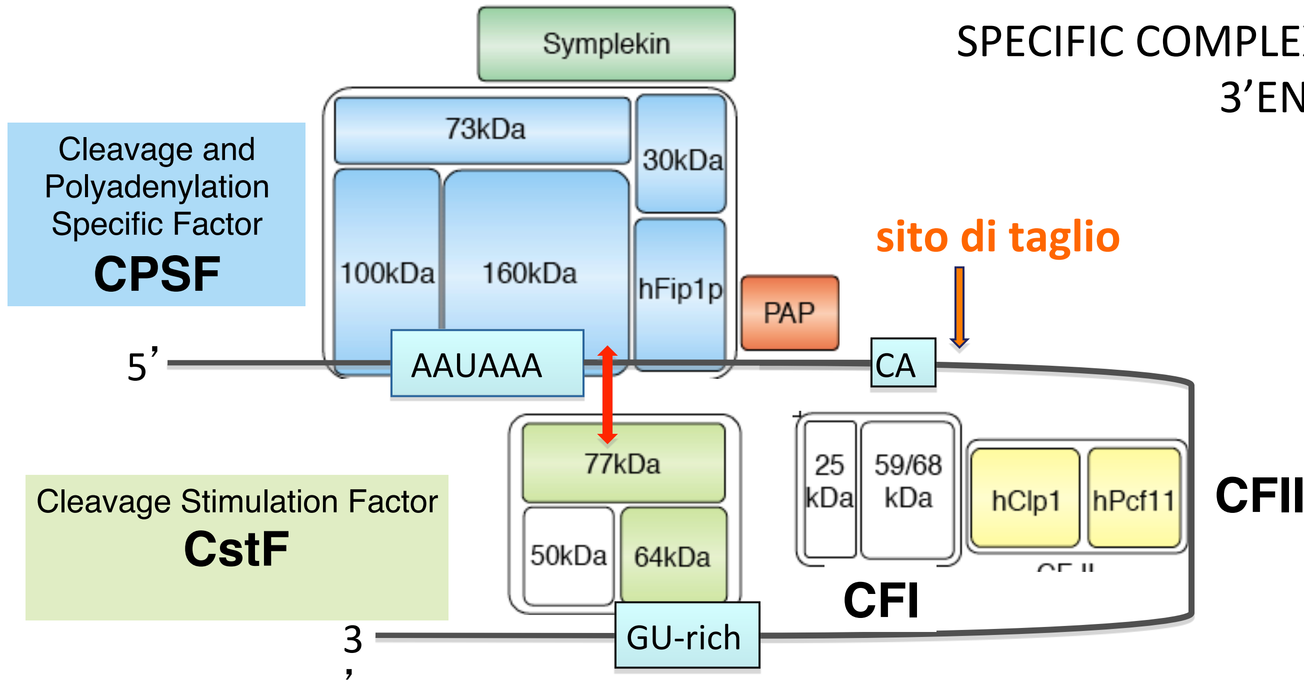
Site-specific mutation



3' end formation in mammalian cells

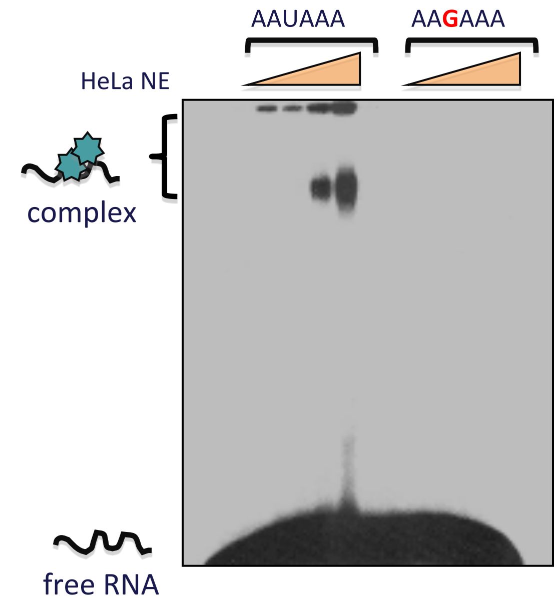


SPECIFIC COMPLEXES ARE INVOLVED IN mRNA 3'END PROCESSING



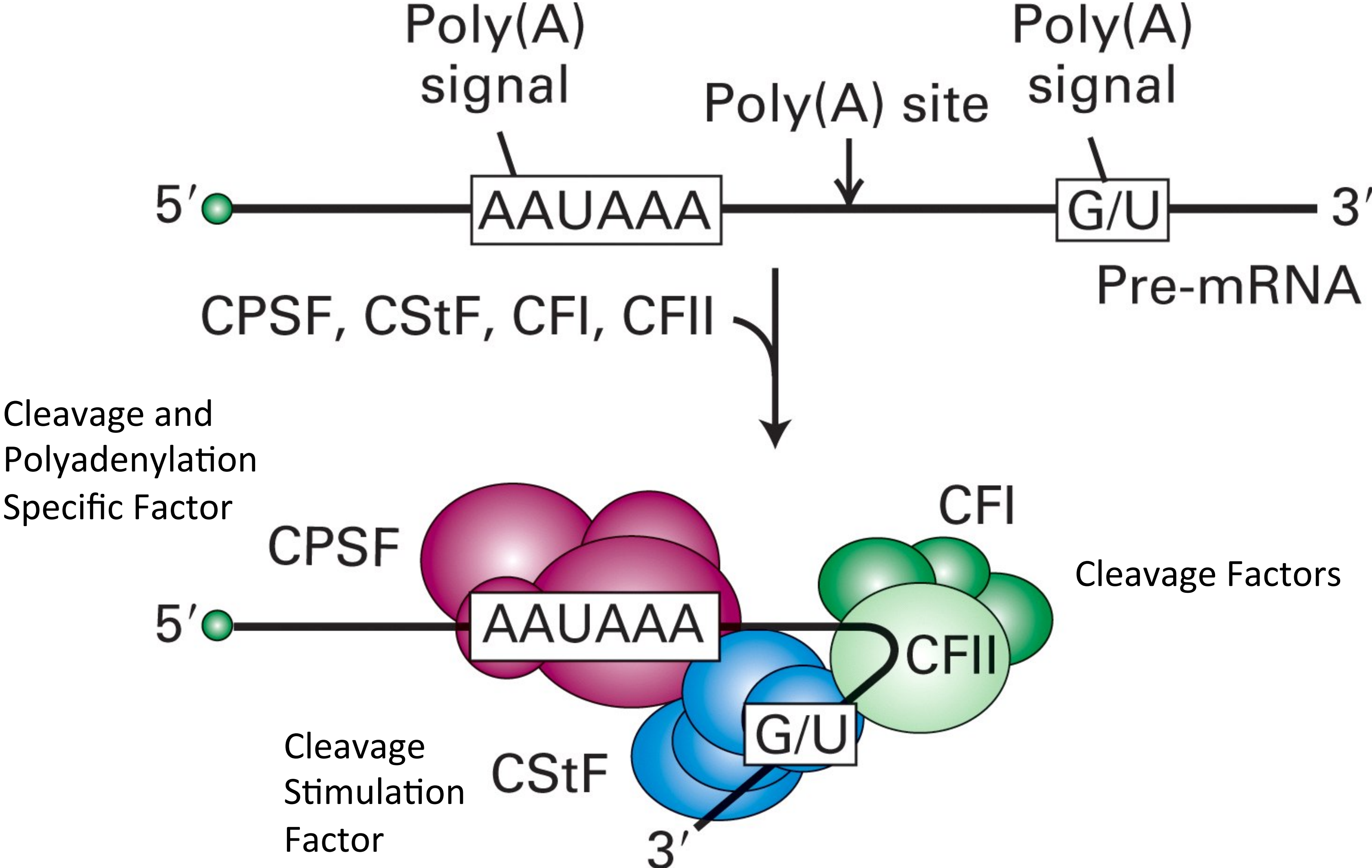
PAP Poly-A polymerase

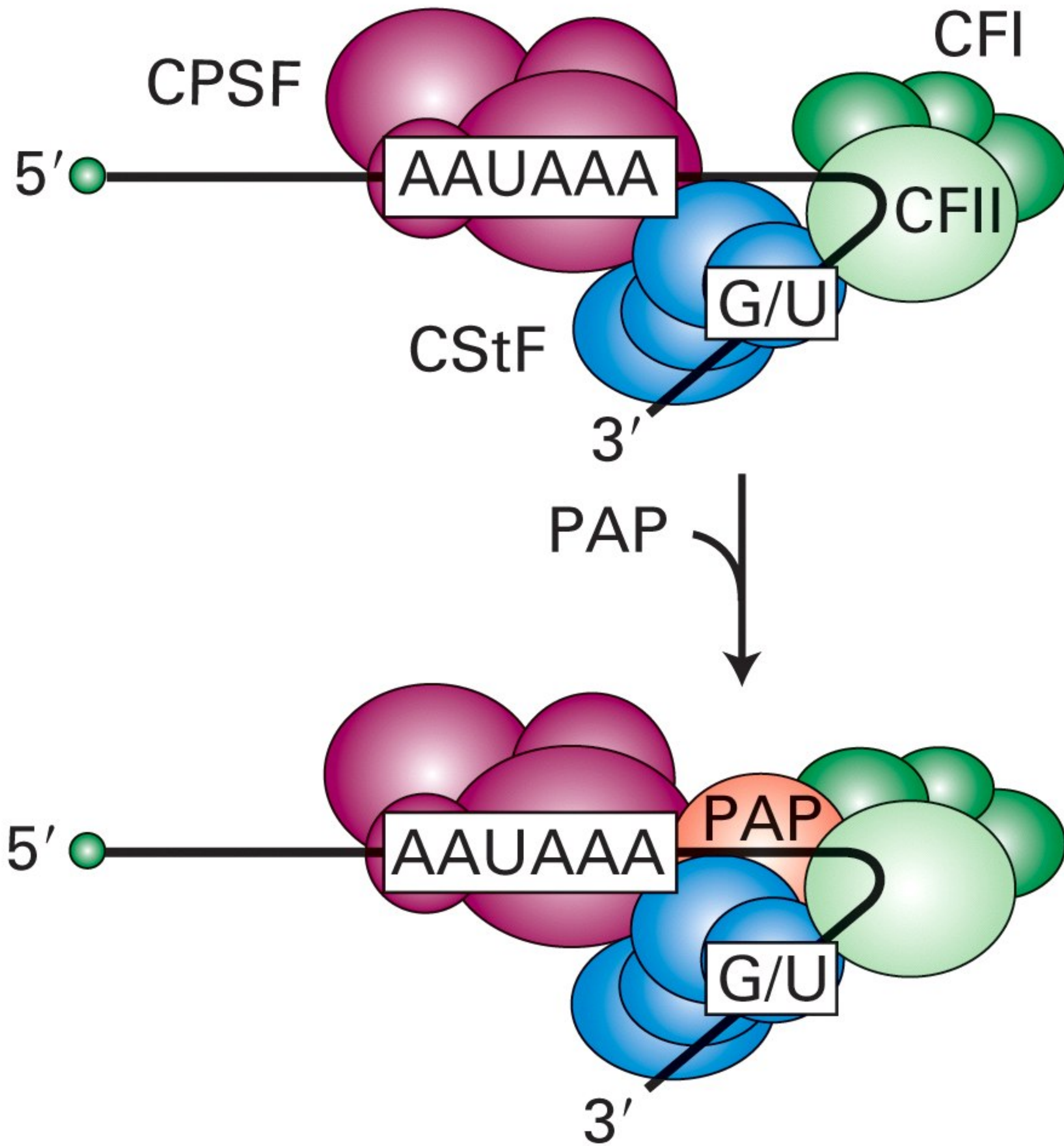
Curr Opin Cell Biol. 2004 Jun;16(3):272-8

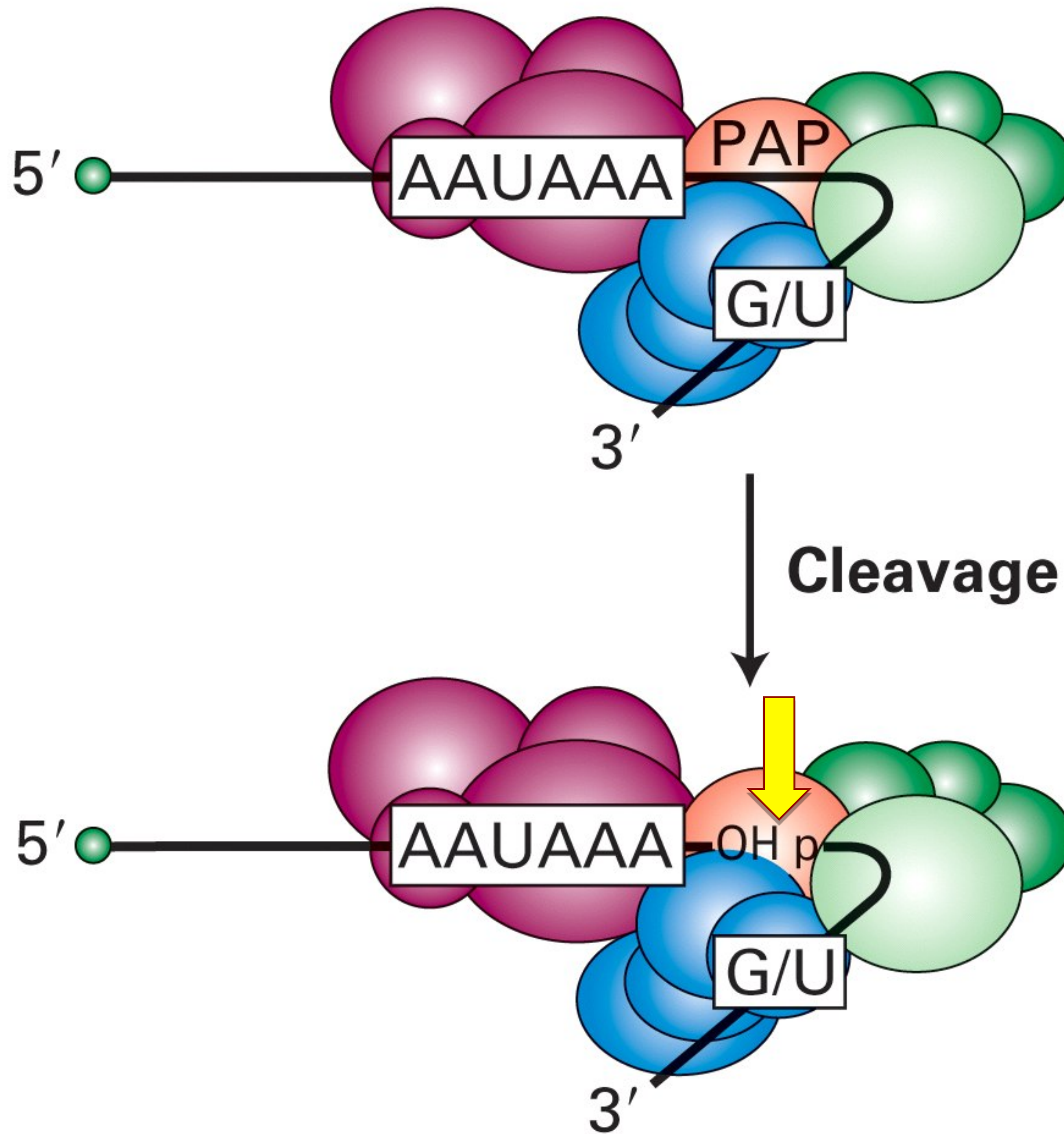


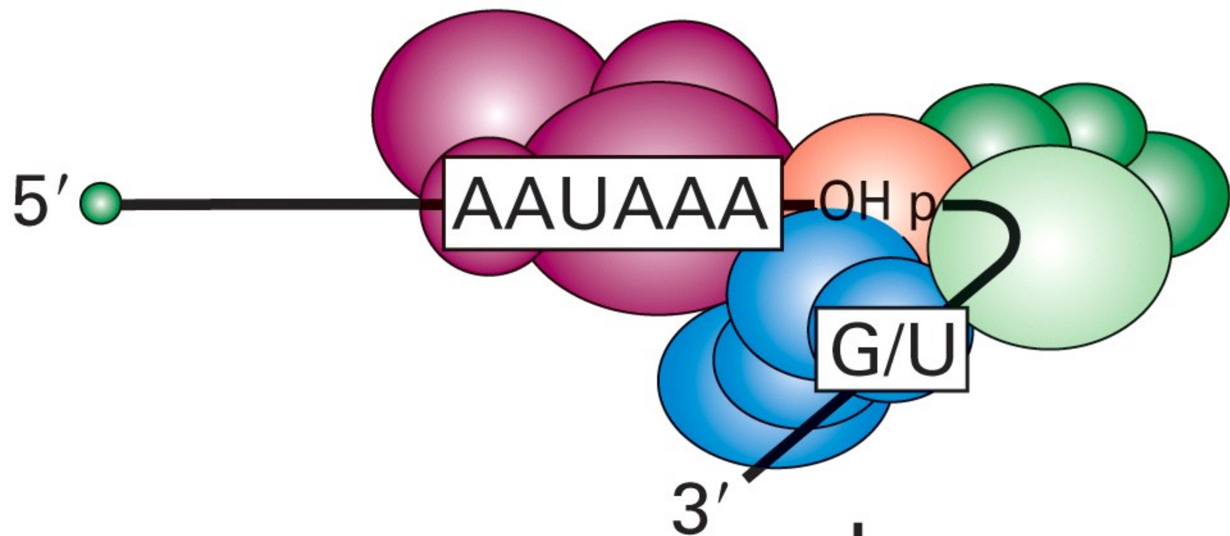
EMSA assay for testing the interaction between CPSF 160 and consensus sequence AAUAAA

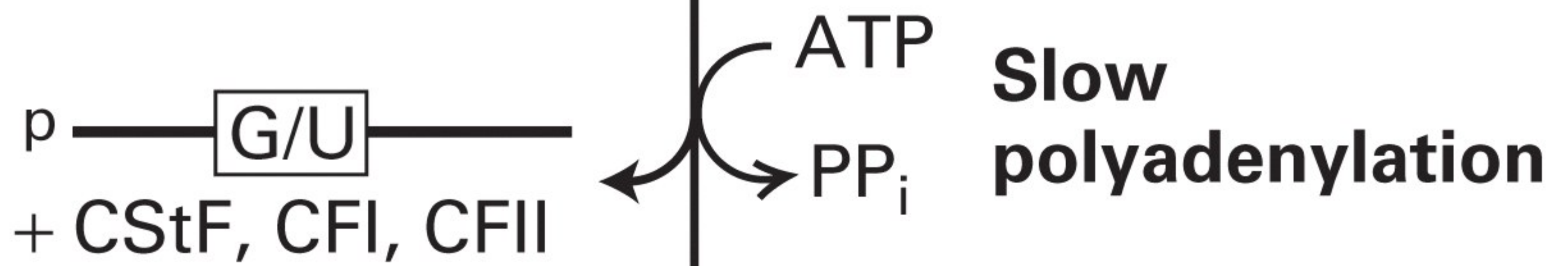
3’-End Formation: RNA Processing

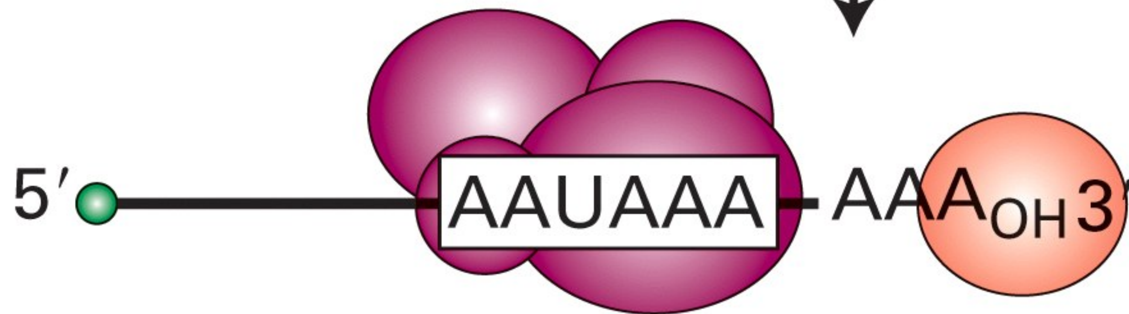



CPSF
CFI
5′
AAUAAA
CFII
G/U
CStF
3′
PAP
5′
AAUAAA
PAP
G/U
3′

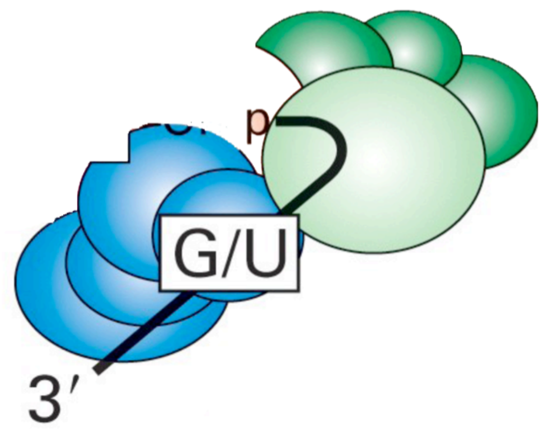

5′
AAUAAA
PAP
G/U
3′
Cleavage
5′
AAUAAA
OH p
G/U
3′

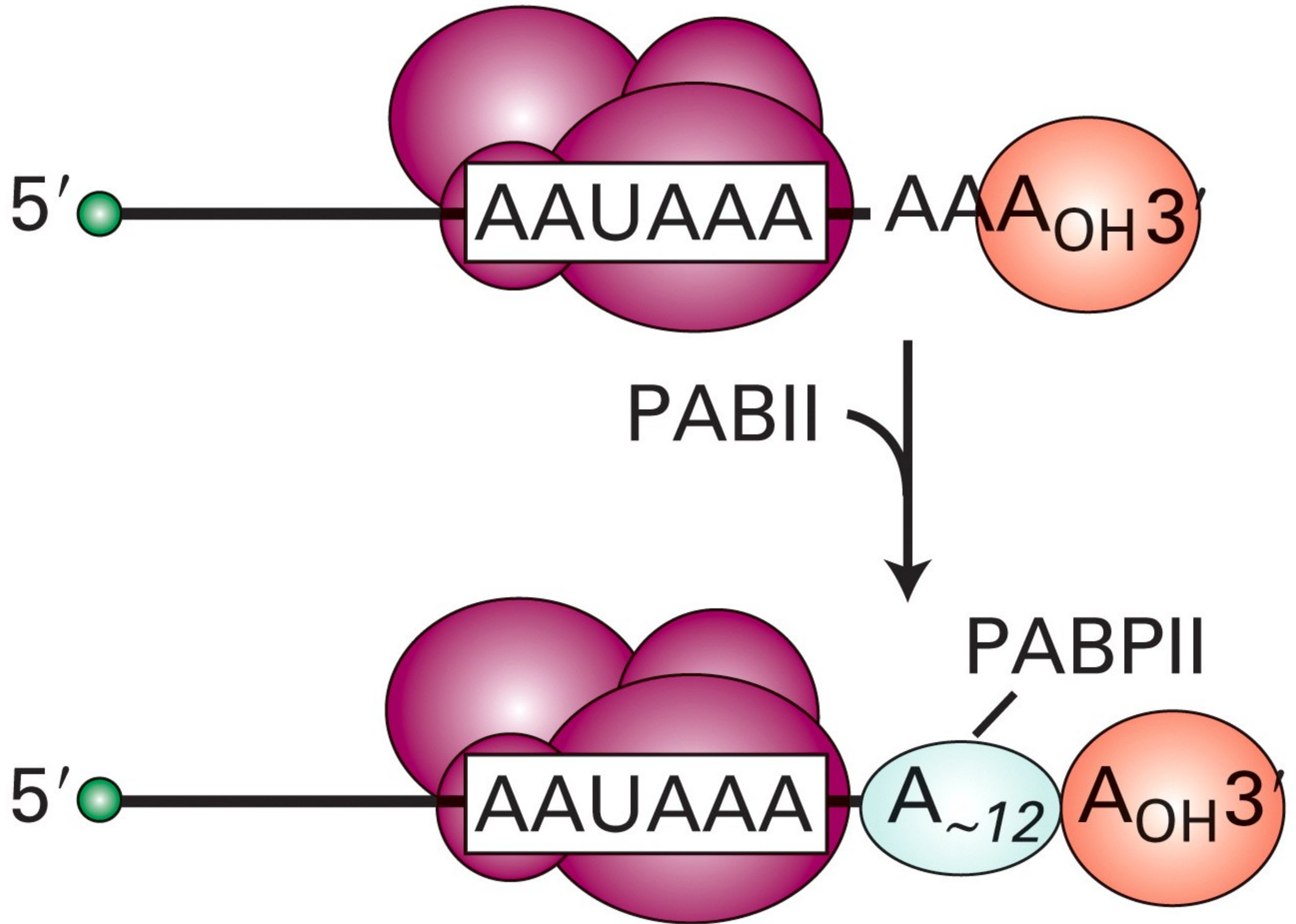


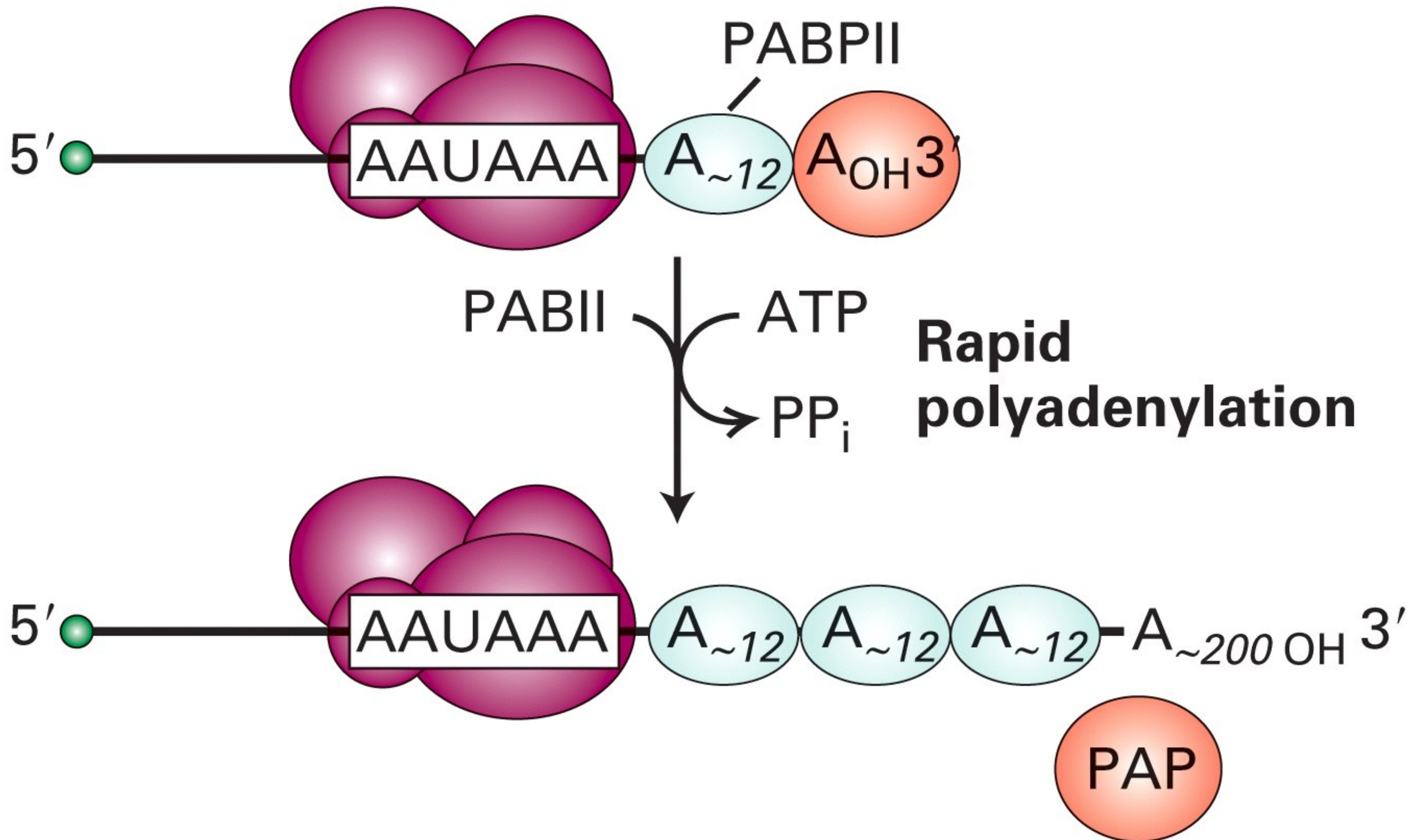




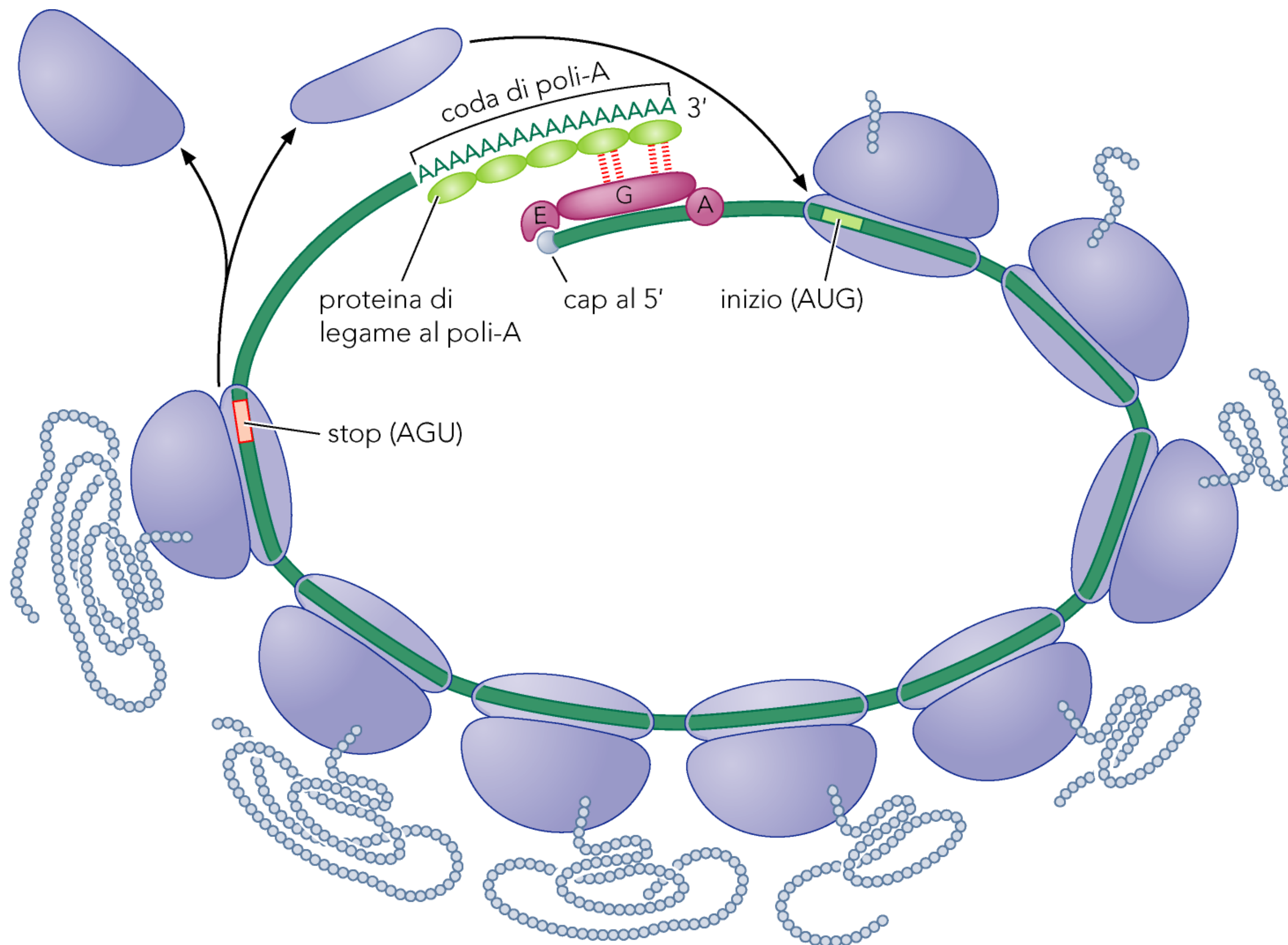
degradation



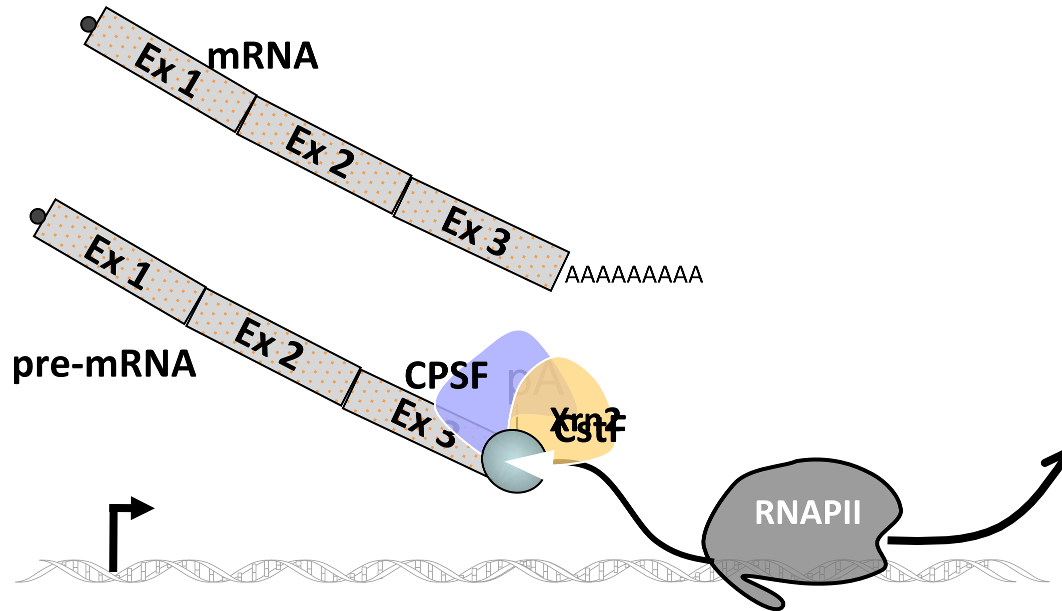

5′
AAUAAA
AAA$_{OH}$3′
PABII
PABPII
5′
AAUAAA
A$_{\sim 12}$
A$_{OH}$3′


PABPII
5′
AAUAAA
A~12
AOH 3′
PABII
ATP
PPi
Rapid polyadenylation
5′
AAUAAA
A~12
A~12
A~12
A~200 OH 3′
PAP

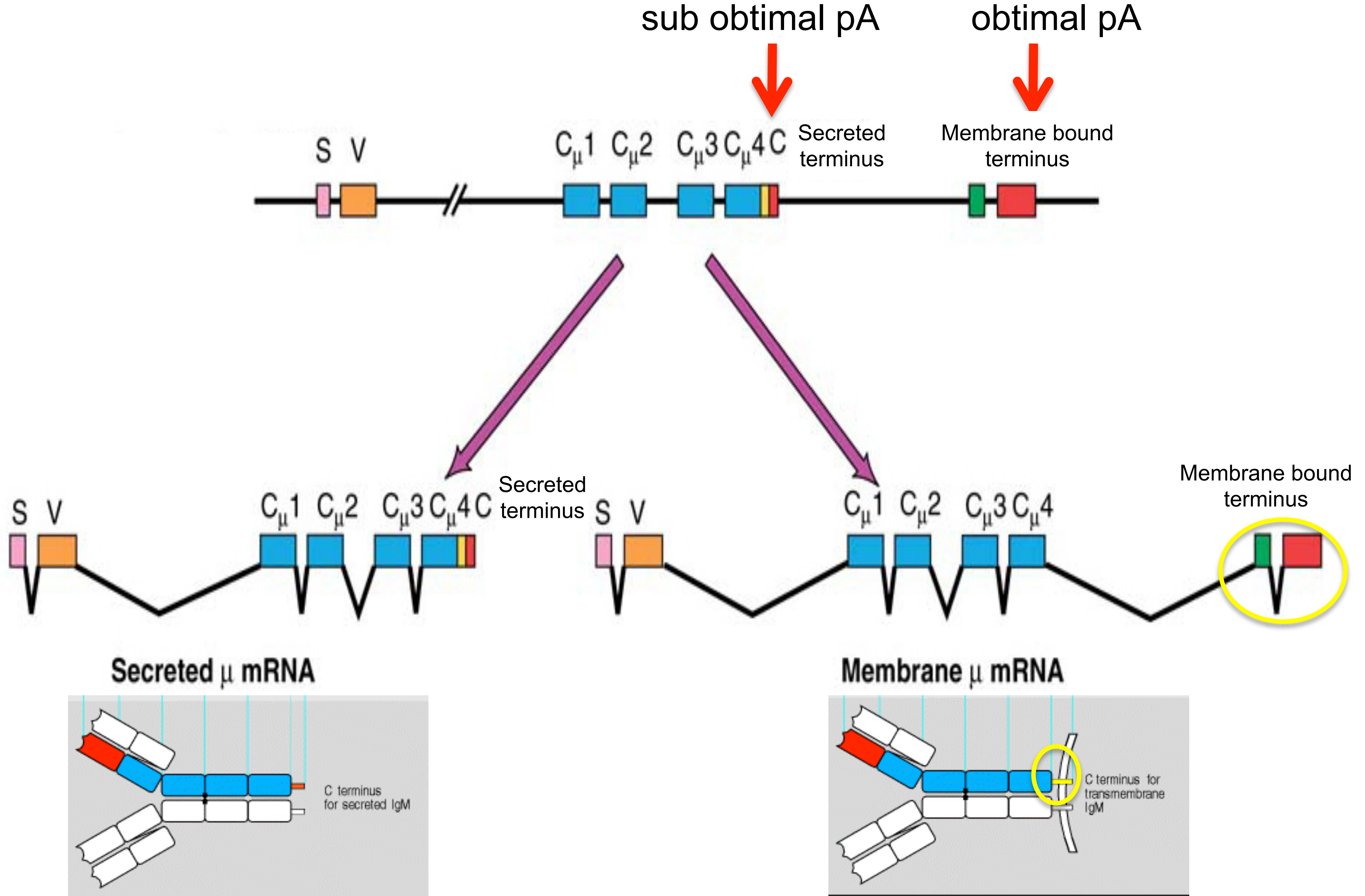
CAP and polyA tail influence efficient translation



Polyadenylation is linked to termination



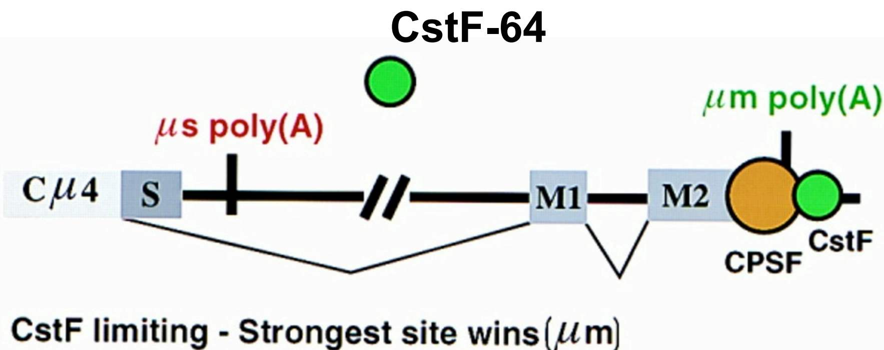
Alternative polyadenylation of the immunoglobulin μ heavy chain gene

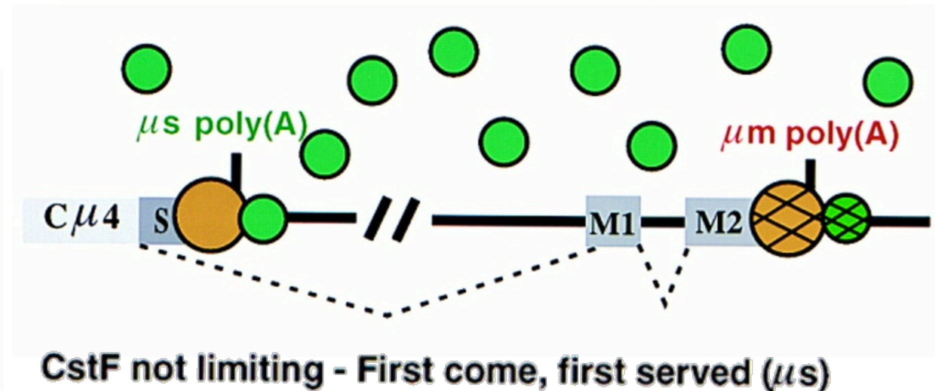


CSTF-64 levels control the alternative processing of mRNA.

In not activated B cells the limiting concentration of CSTF allows the recognition of the stronger polyadenylation signal. The immuglobulin produced will then contain a portion for binding to the membrane.

After the activation of B cells CSTF levels are increased and this allows the use of the weaker polyadenylation site that will be preferentially used because it will be the first to be transcribed.

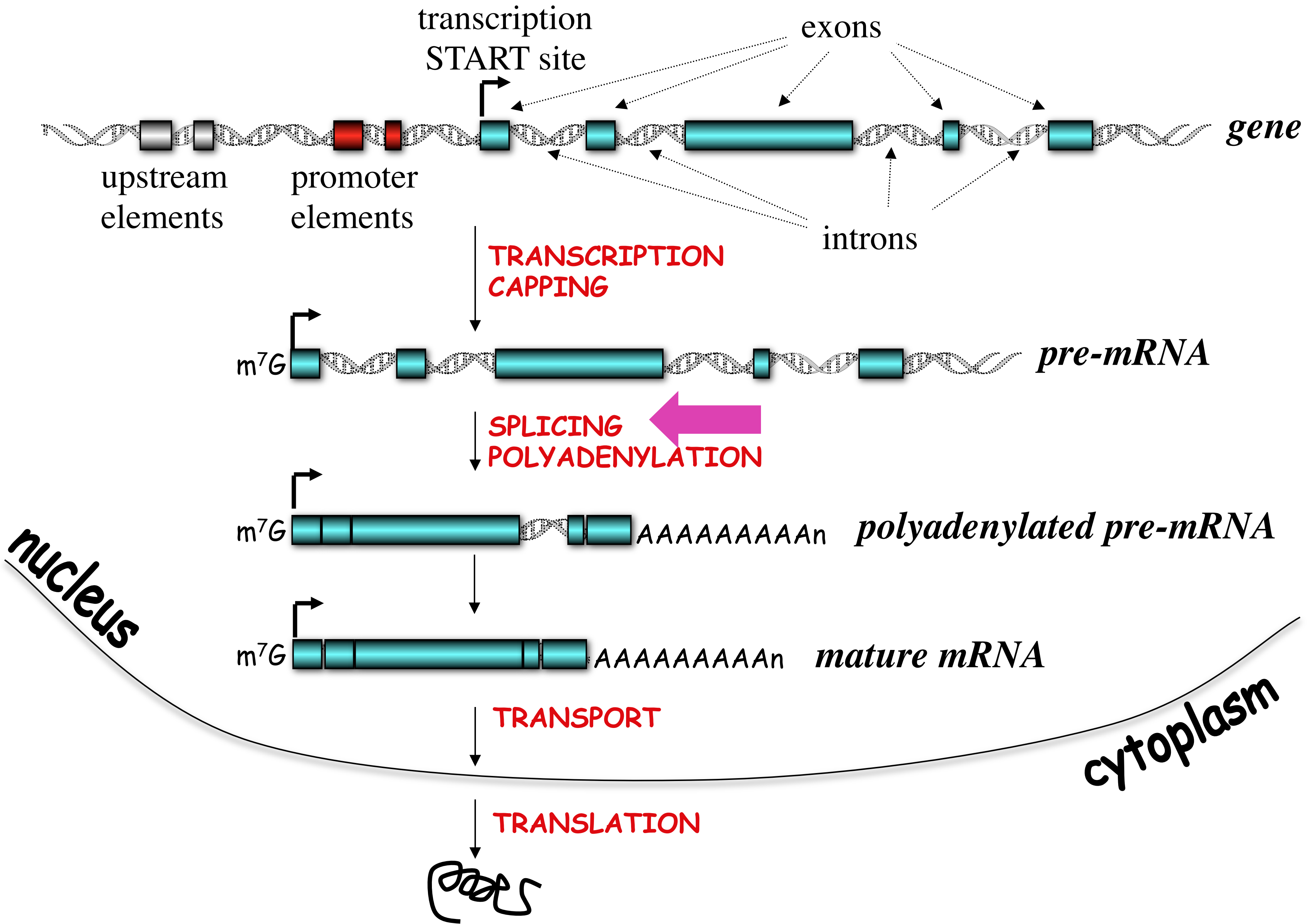




The polyadenylation factor CstF-64 regulates alternative processing of IgM heavy chain pre-mRNA during B cell differentiation.

Takagaki Y, Seipelt RL, Peterson ML, Manley JL. Cell 1996 Nov 29;87(5):941-52

Eucaryotic gene expression



Eukaryotic genes contain introns

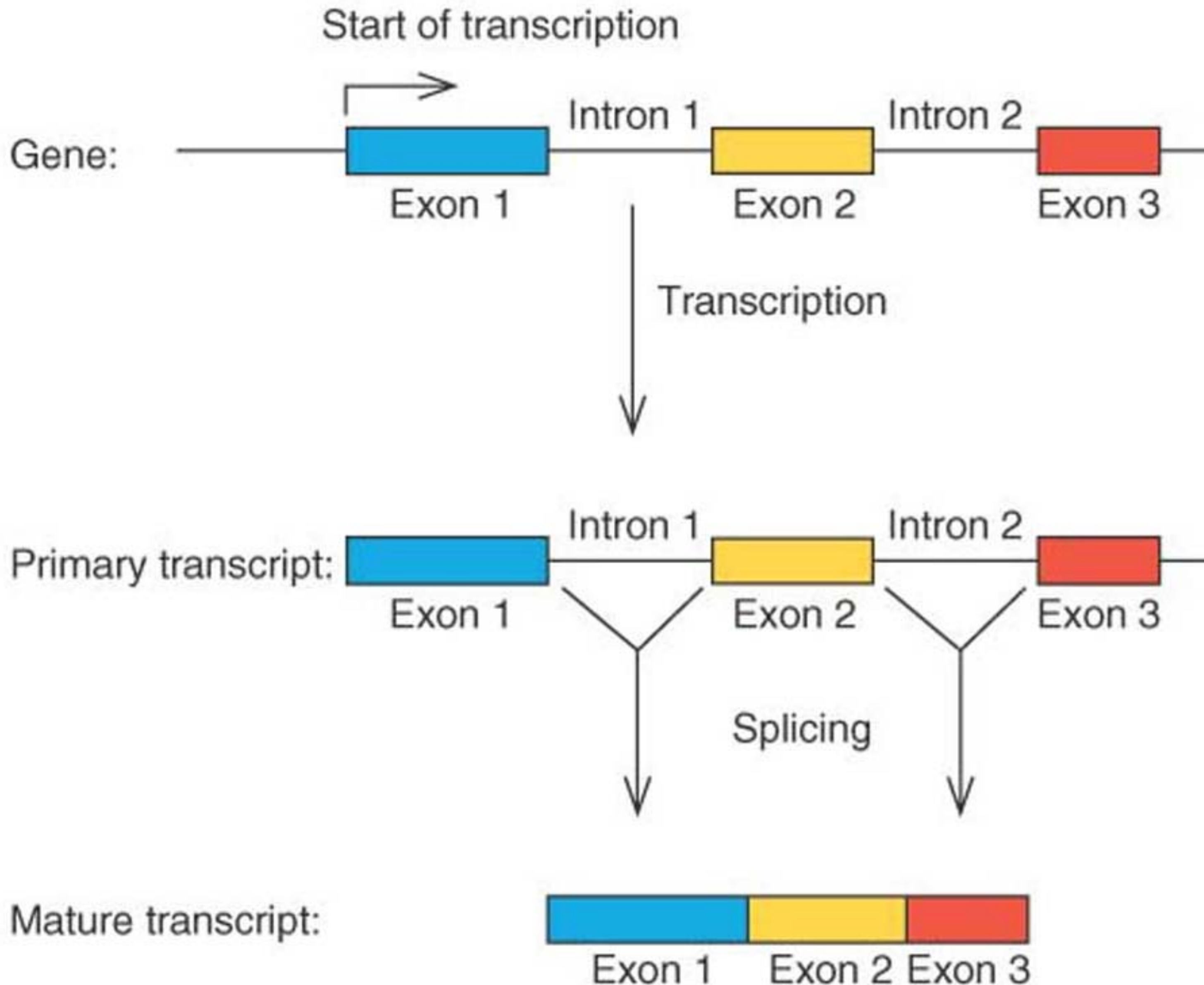
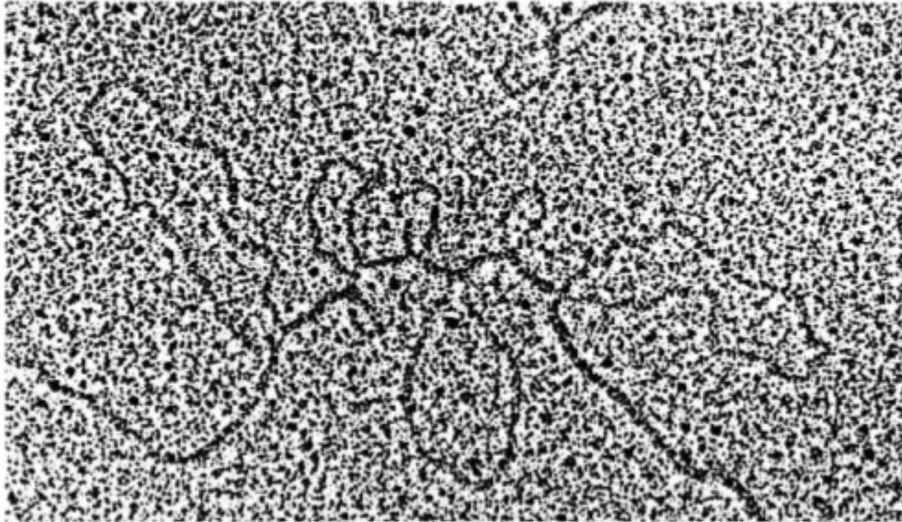
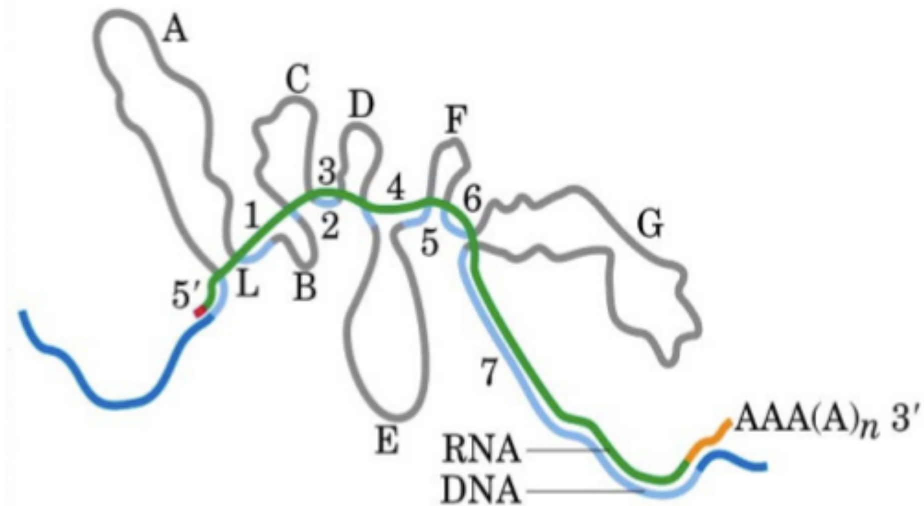


Fig. 14.2

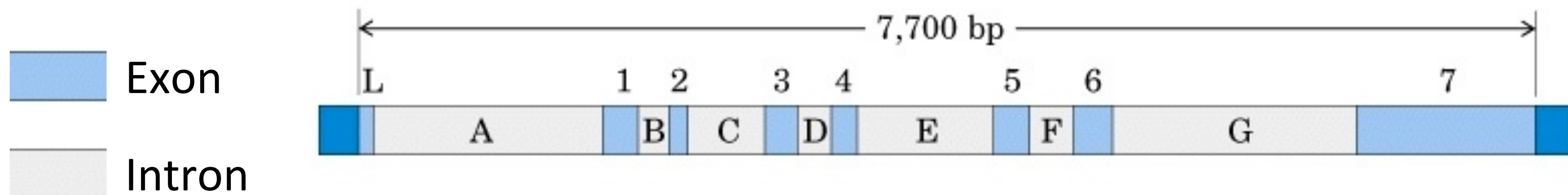
Identification of introns by R-looping





Gene dell' ovalbumina di pollo ibridato con il suo mRNA e visualizzato al microscopio elettronico

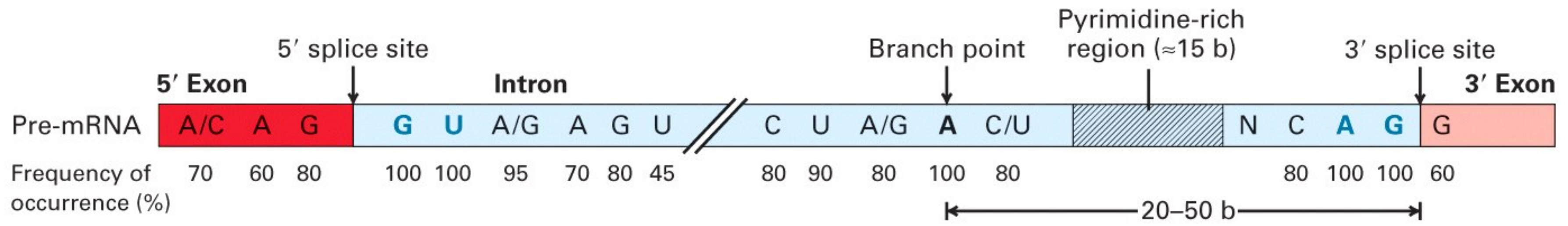
Exon:intron organization of the ovalbumin gene

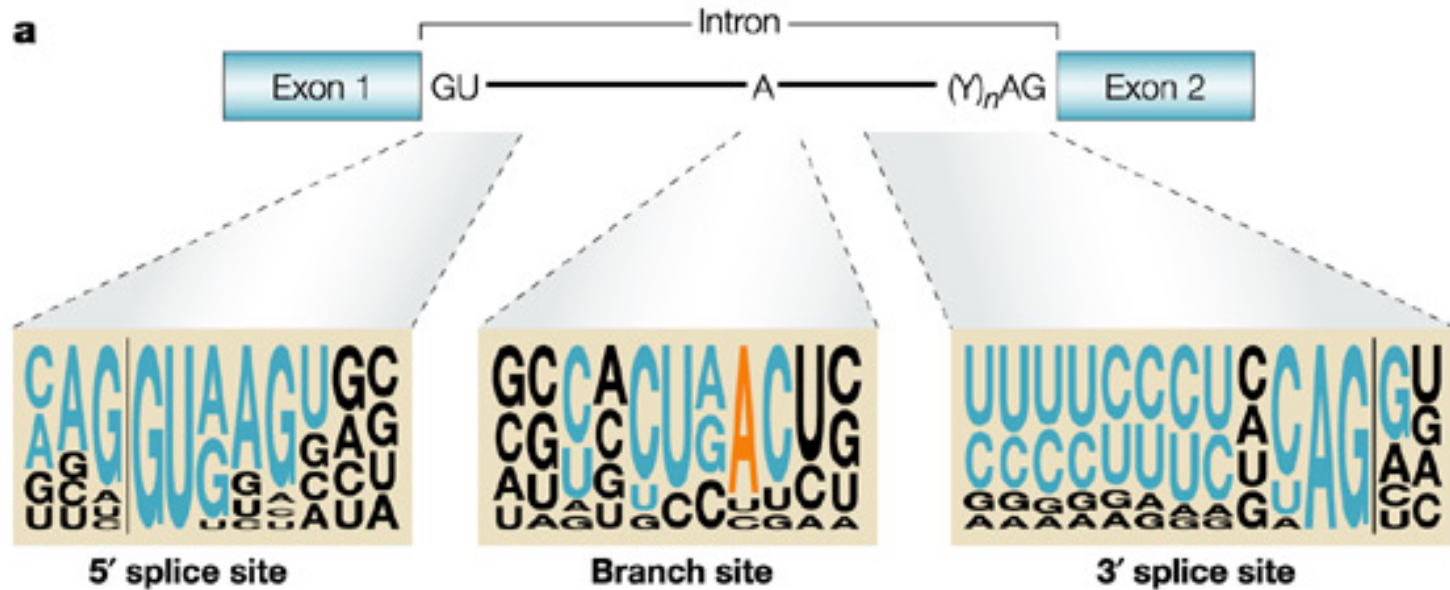


Specific sequences inside the pre-mRNA indicate where the spicing event has to take place.

These sequences indicate the boundary between introns and exons.

Consensus Sequences Surrounding the 5’ and 3’ Splice Sites



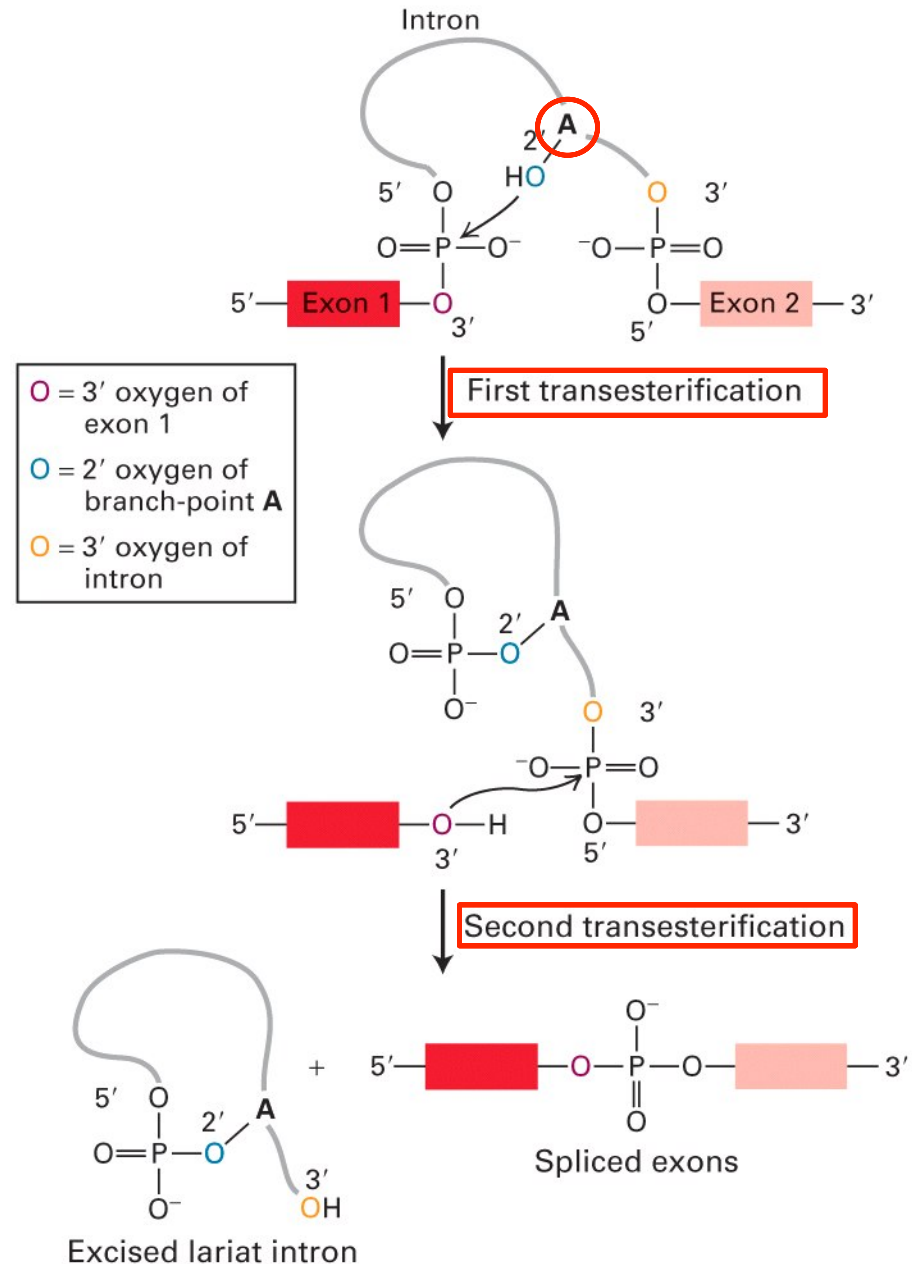


The intron is removed in a Form Called Lariat and the Flanking Exons are joined

Two **trans-esterifications:**

[Step 1](): The OH of the conserved **A at the branch site** attacks the phosphoryl group of the conserved G in the 5' splice site. As result, the 5' exon is released and the 5'-end of the intron forms a three-way junction structure.

[Step 2](): The OH of the 5' exon attacks the phosphoryl group at the 3' splice site. As a consequence, the 5' and 3' exons are joined and the intron is released in a lariat shape



Nuclear pre-mRNA splicing proceeds through two trans-esterification reactions

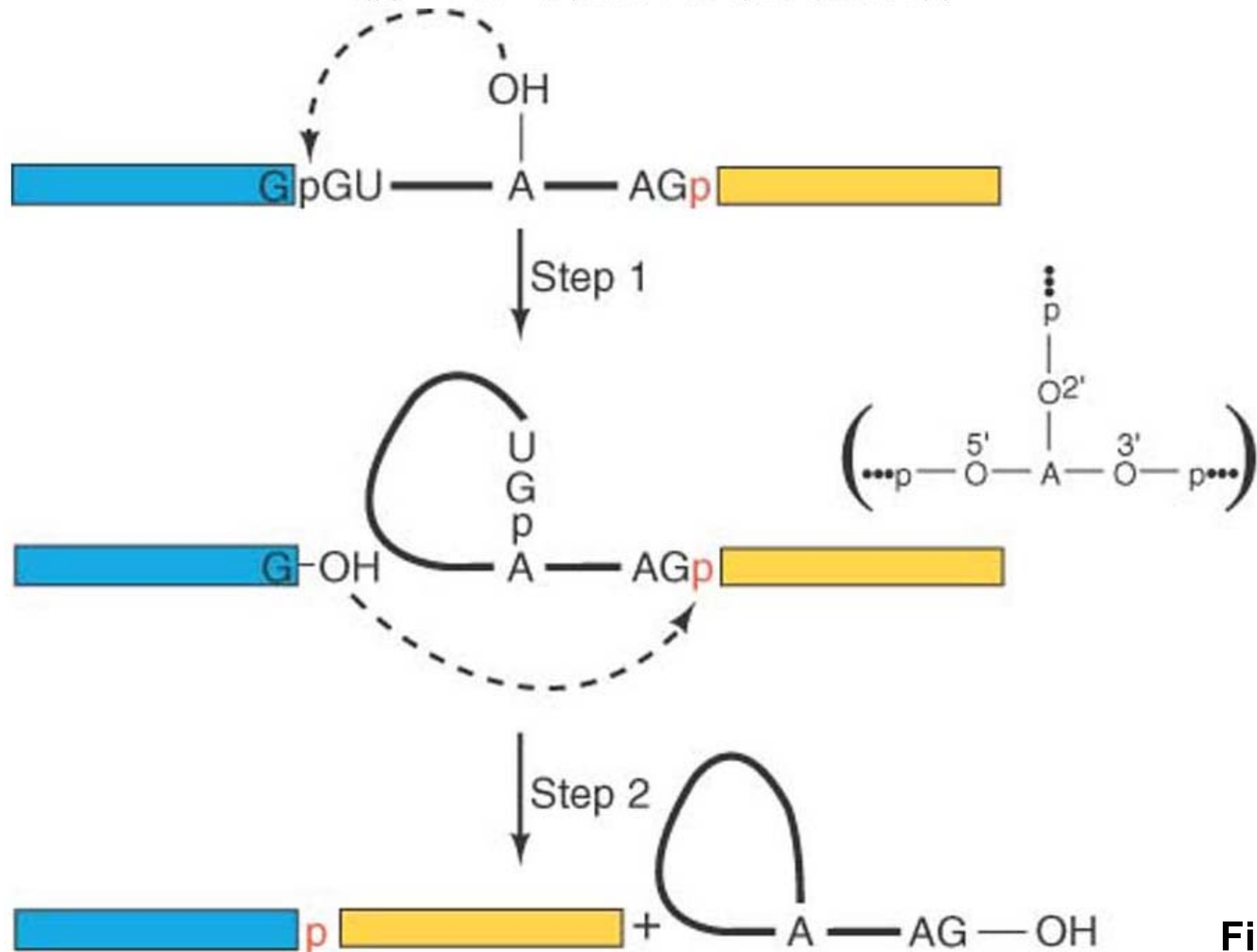
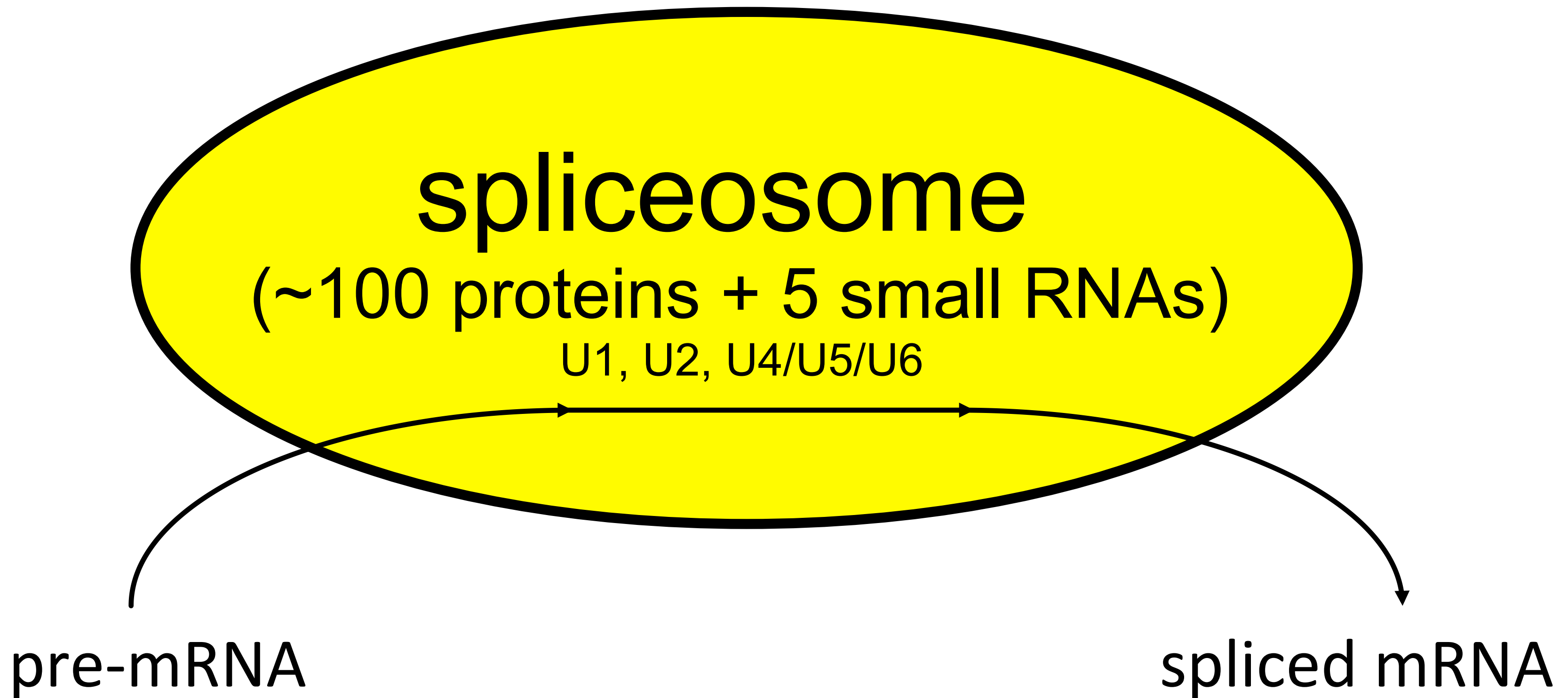


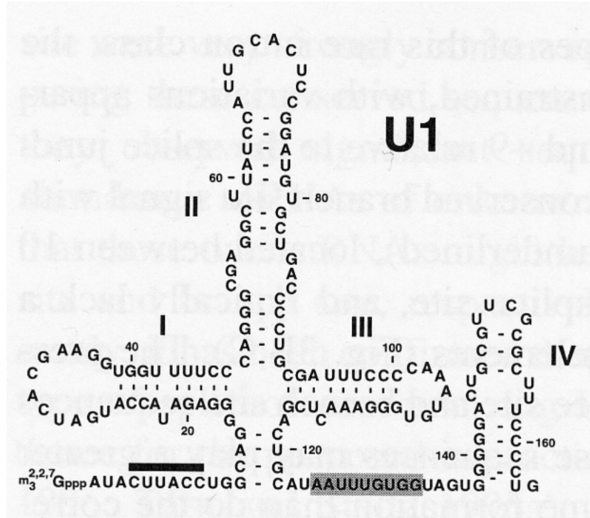
Fig. 14.4

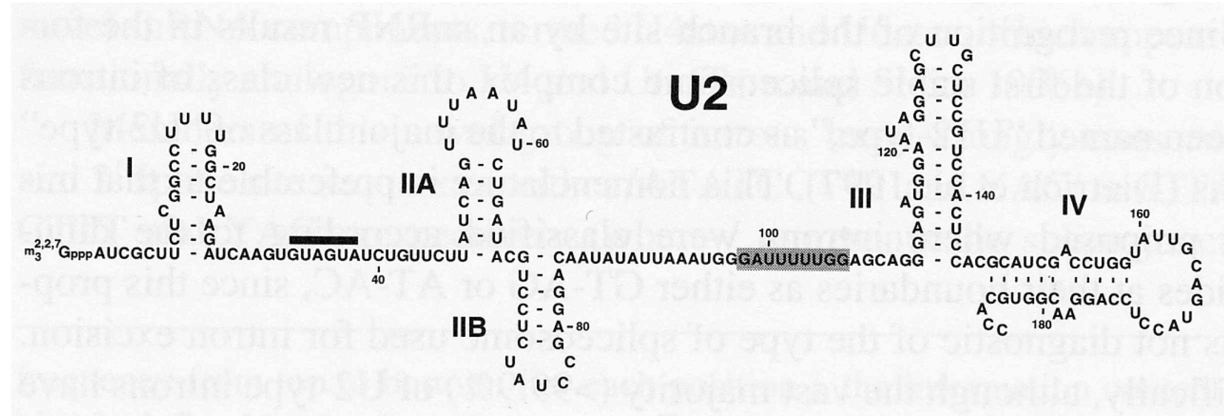
Splicing occurs in a "spliceosome" an RNA-protein complex

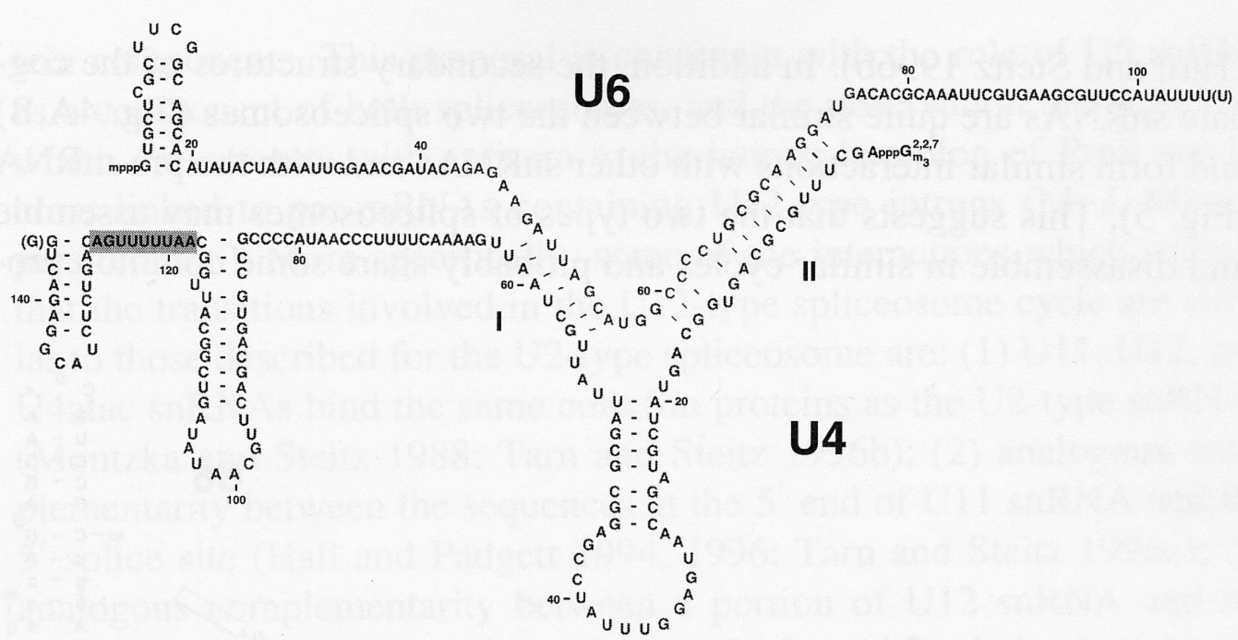


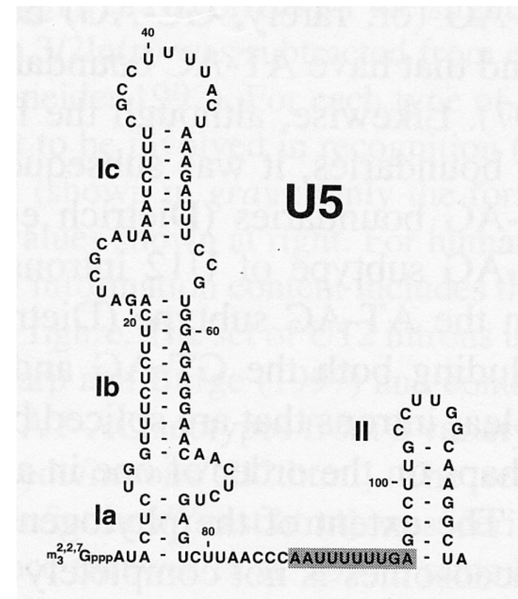
Splicing works similarly in different organisms, for example in yeast, flies, worms, plants and animals.

Five snRNAs are involved in pre-mRNA splicing

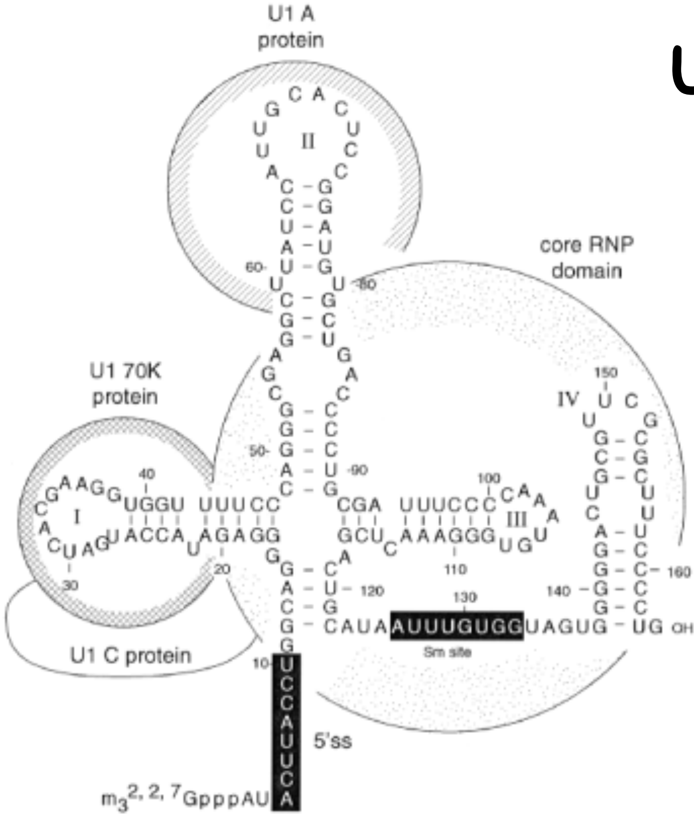


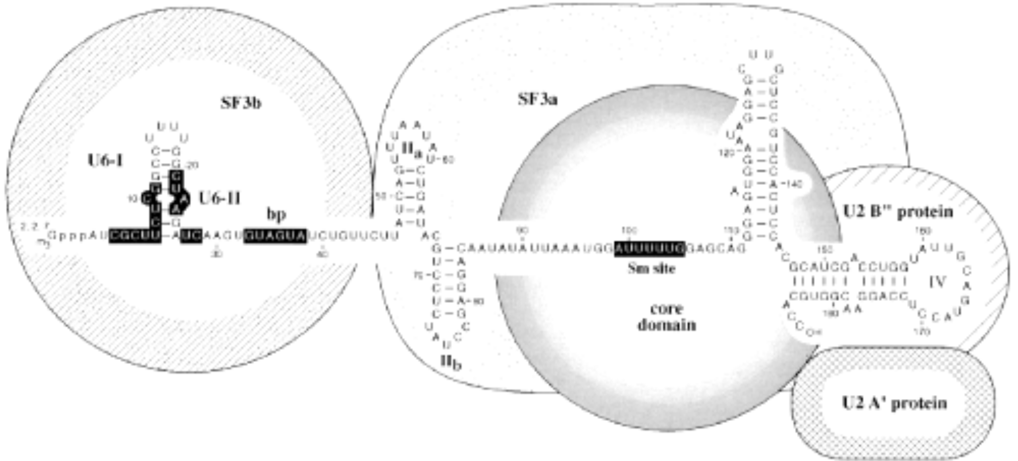




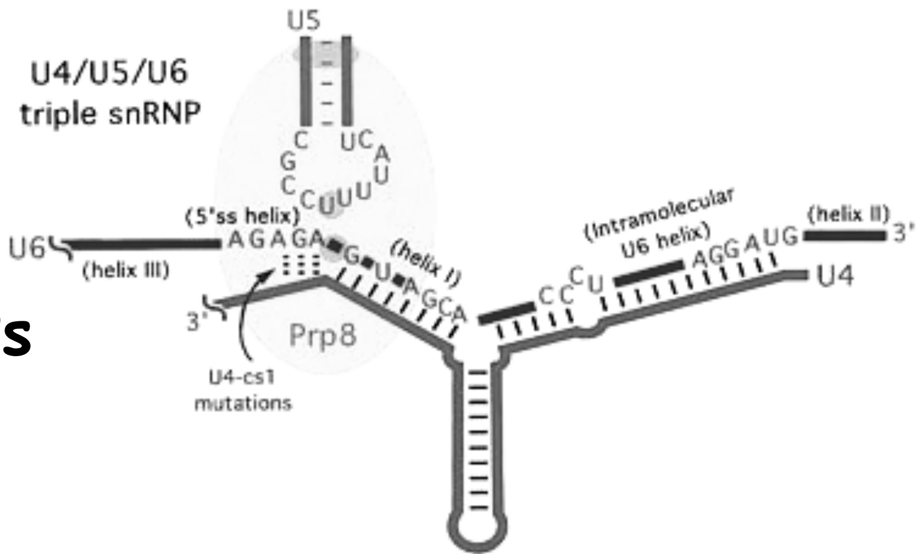


U1 and U2 snRNPs





U4/U5/U6 tri-snRNPs



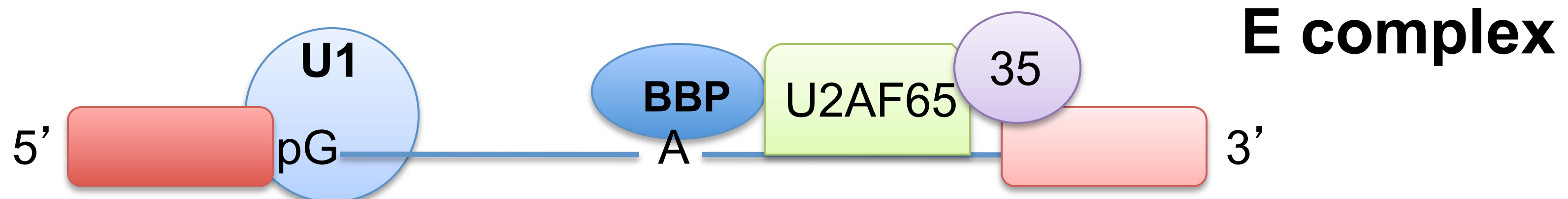
I Assembly, rearrangement, and catalysis within the *spliceosome*: the splicing pathway

Assembly step 1

1. U1 recognizes 5’ splice site.

2. One subunit of U2AF binds to Py tract and the other to the 3’ splice site. The former subunits interacts with BBP and helps it to bind to the branch point.

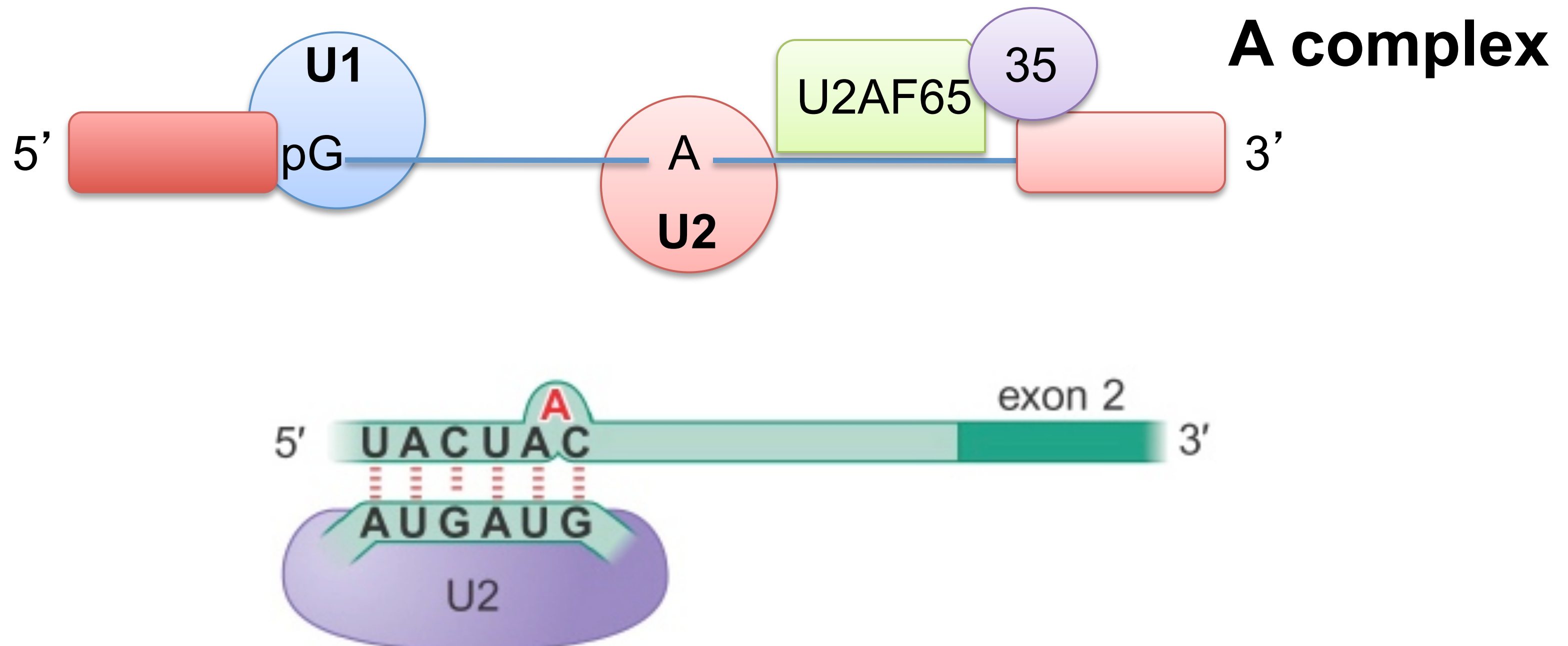
3. **Early (E) complex** is formed



Assembly step 2

1. U2 binds to the branch site, and then **A complex** is formed.

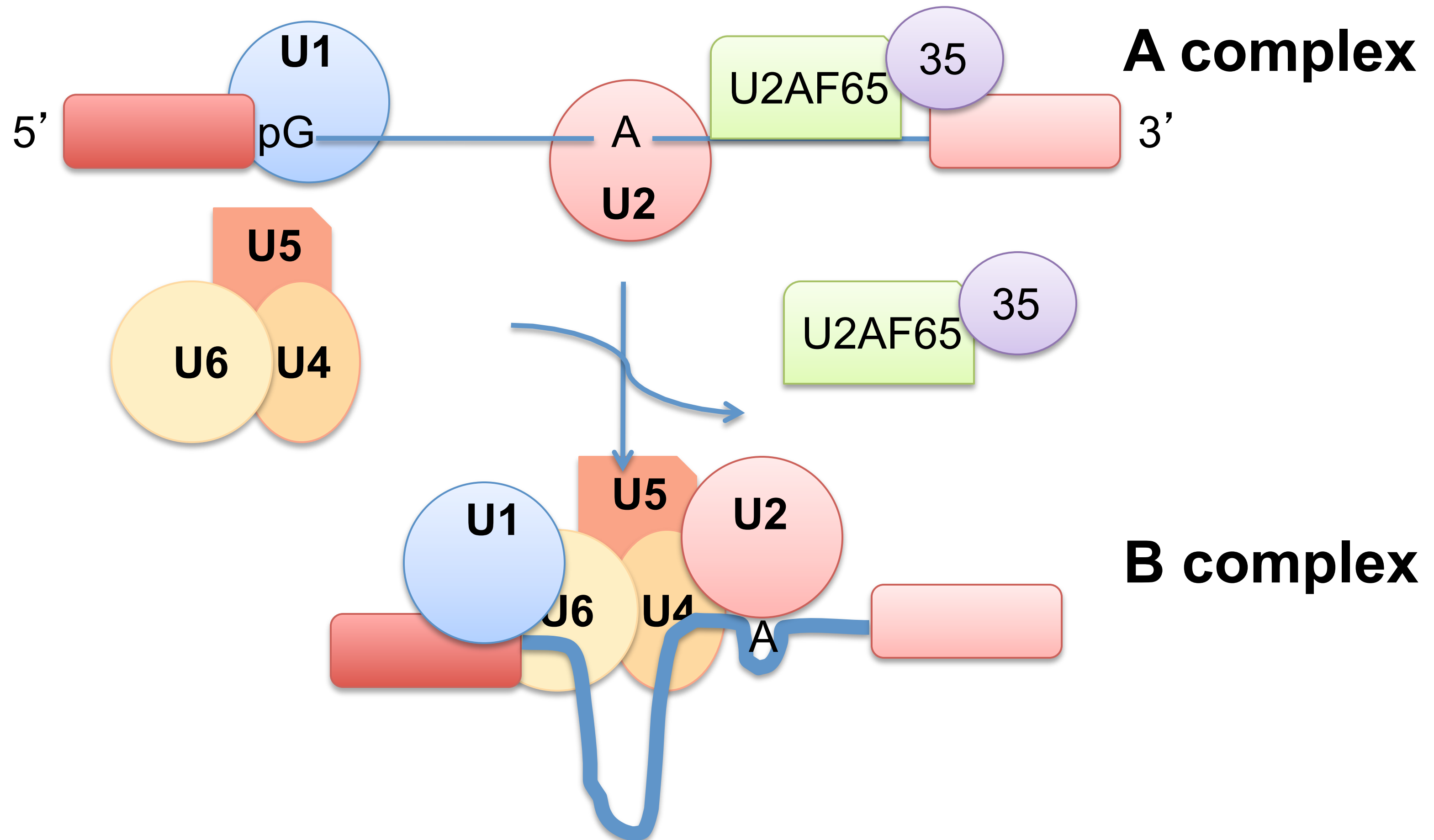
2. The base-pairing between the U2 and the branch site is such that the branch site A is extruded. This A residue is available to react with the 5' splice site.



Assembly step 3

1. U4, U5 and U6 form the tri-snRNP Particle.

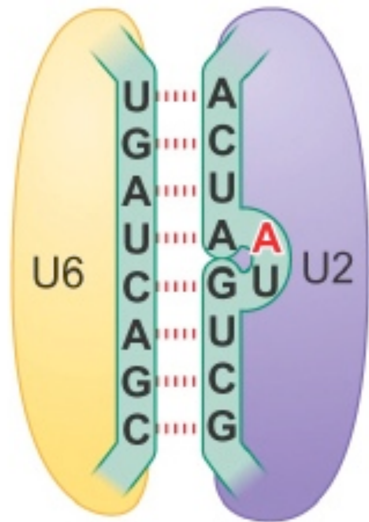
2. With the entry of the tri-snRNP, the A complex is converted into the **B complex**.



U1
U5
U2
U6
U4
A

B complex

U1



U5
U2
U6
U4
A

C complex in which the catalysis has not occurred yet

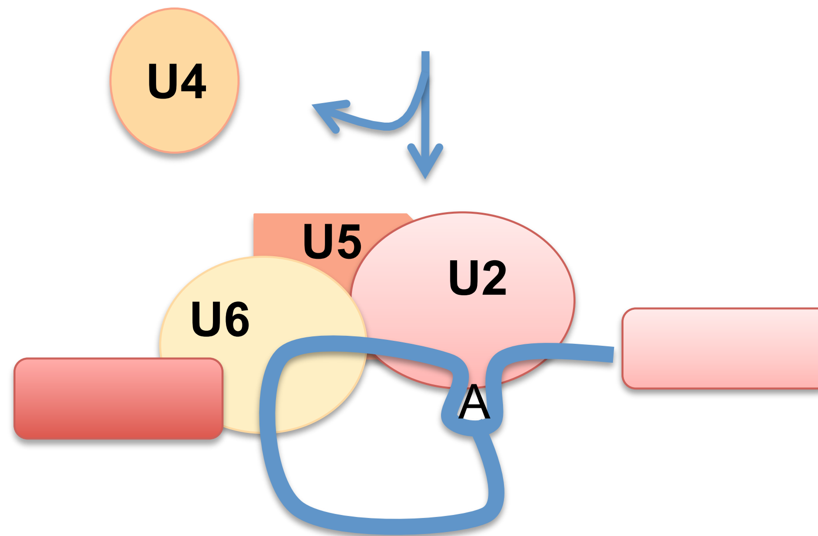
U4

U5
U2
U6
A

Catalysis Step 1

- Formation of the C complex produces the **active site**, with **U2 and U6 RNAs** being brought together.

- Formation of the active site juxtaposes the 5’ splice site of the pre-mRNA and the branch site, allowing the **branched A residue** to **attack** the 5’ splice site to accomplish the first transesterfication reaction.

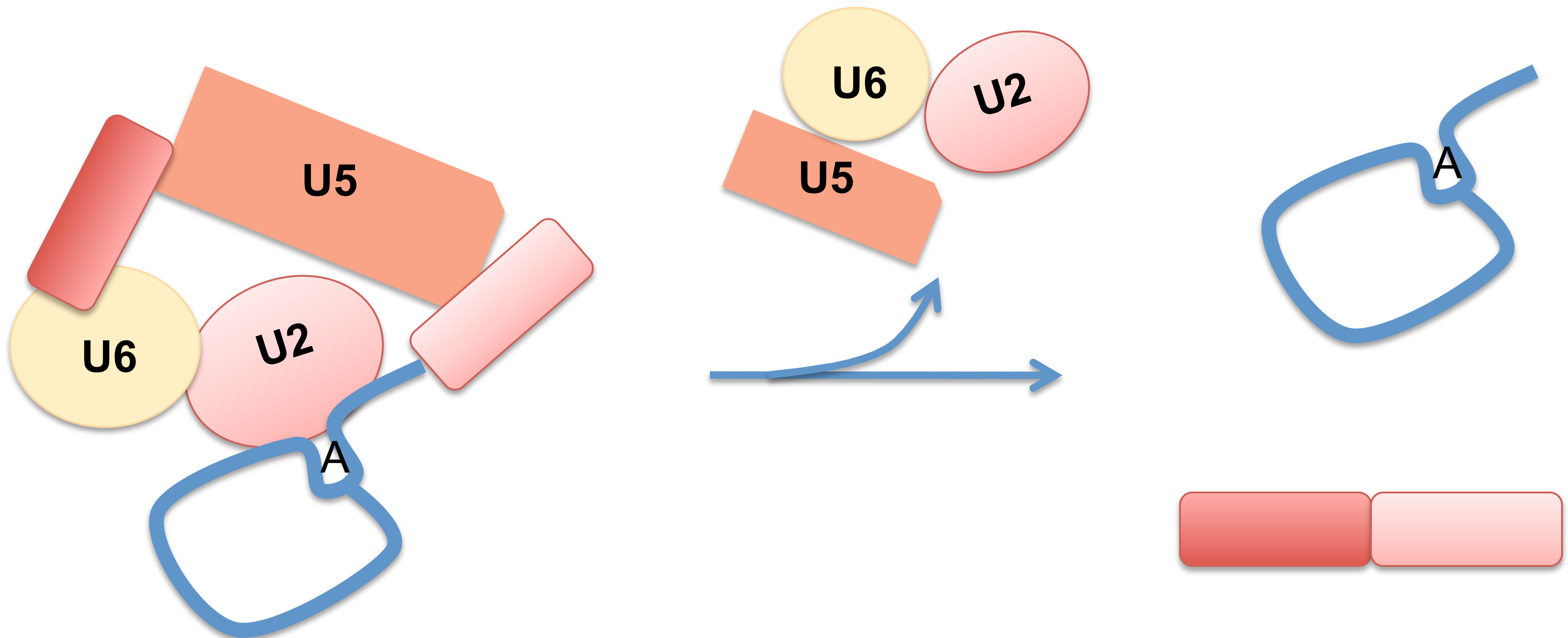


Catalysis Step 2

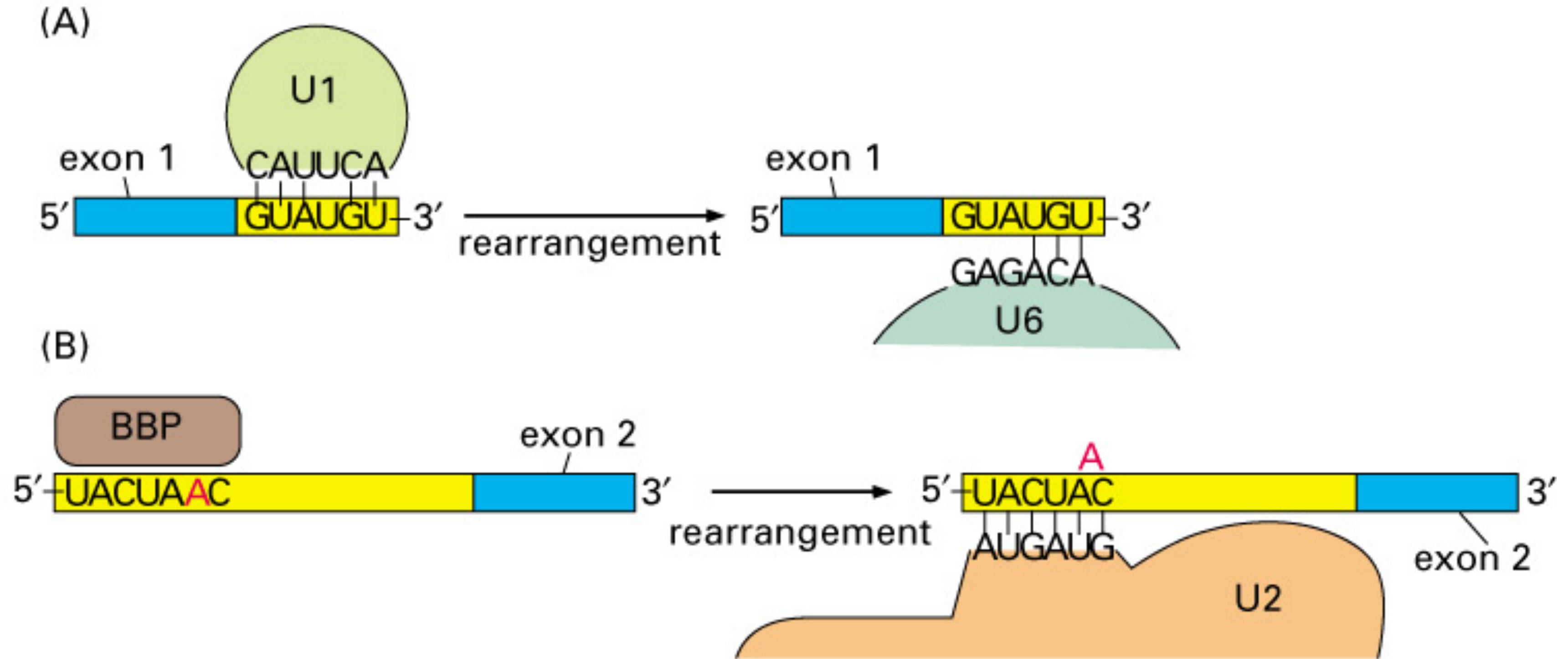
U5 snRNP helps to bring the two exons together, and aids the second transesterification reaction, in which the 3'-OH of the 5' exon attacks the 3' splice site.

Final Step

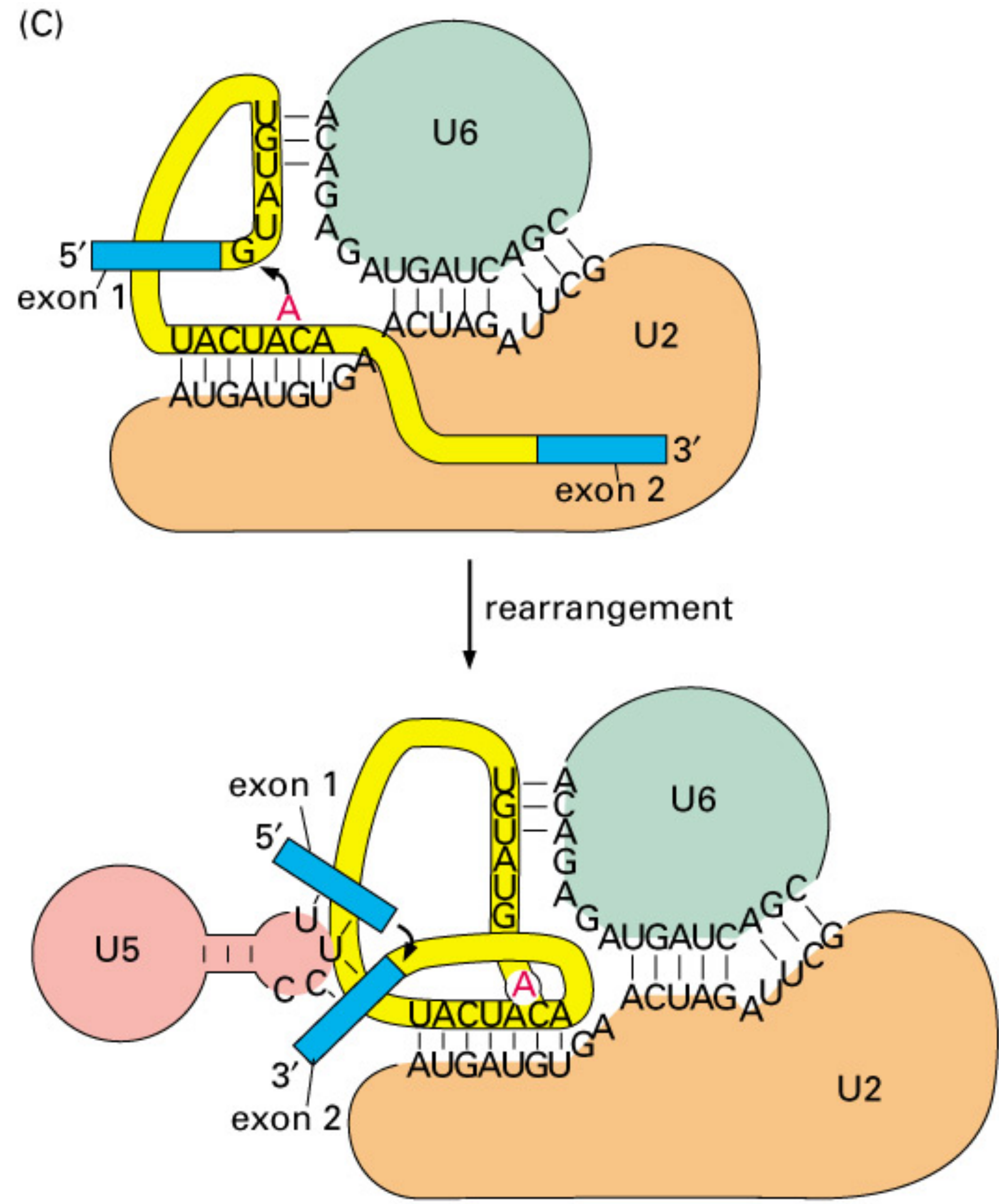
Release of the mRNA product and the snRNPs.



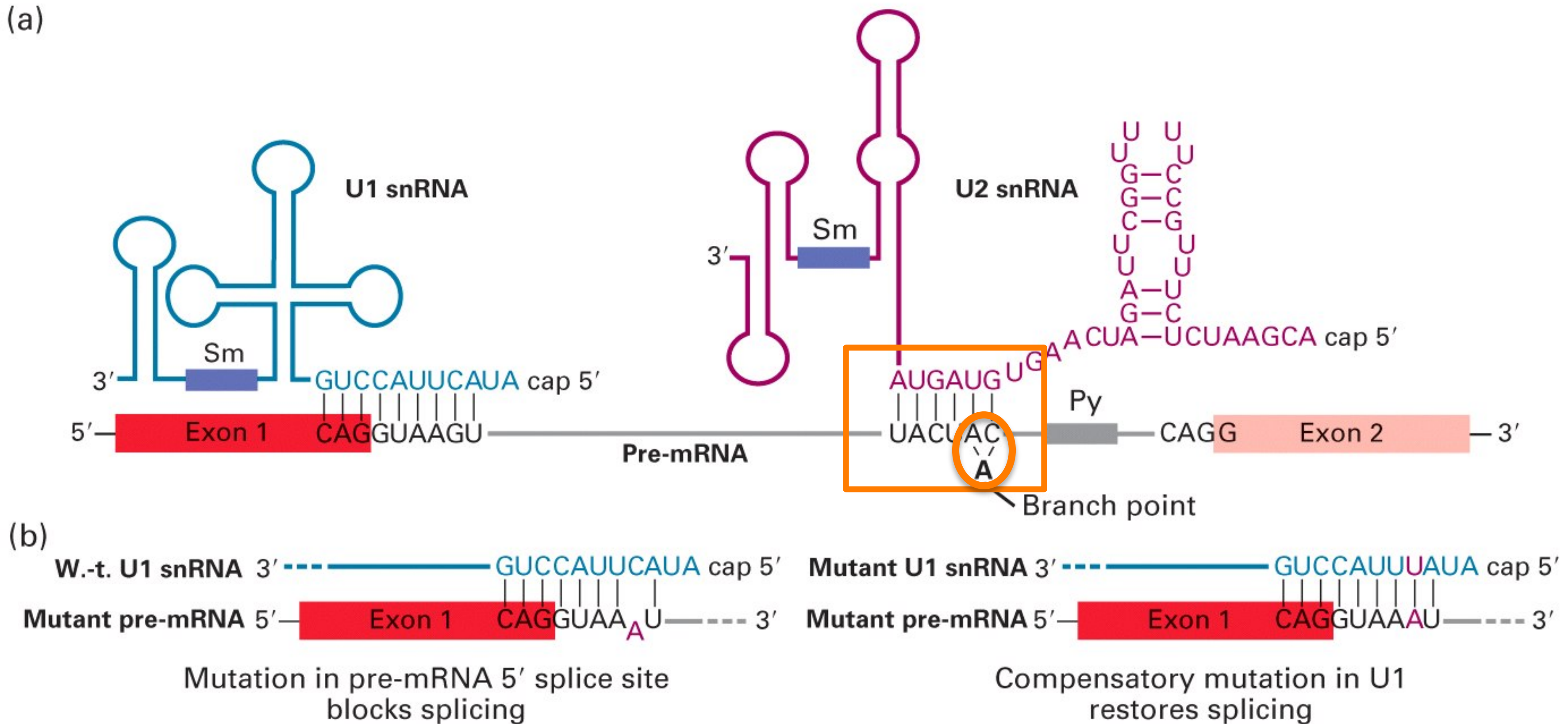
Spliceosome & ATP → RNA-RNA Rearrangements - I



Spliceosome & ATP → RNA-RNA Rearrangements - II



Pre-mRNA Splicing is Accelerated by RNA-RNA Basepairing: snRNPs



How to study splicing in vitro

- procedure
  - -in vitro transcribed RNA
  - -RNA plus nuclear extract
  - -RNA isolation ot different time points
  - -elettrophoresis for the analysis of the reaction products

Trascrizione

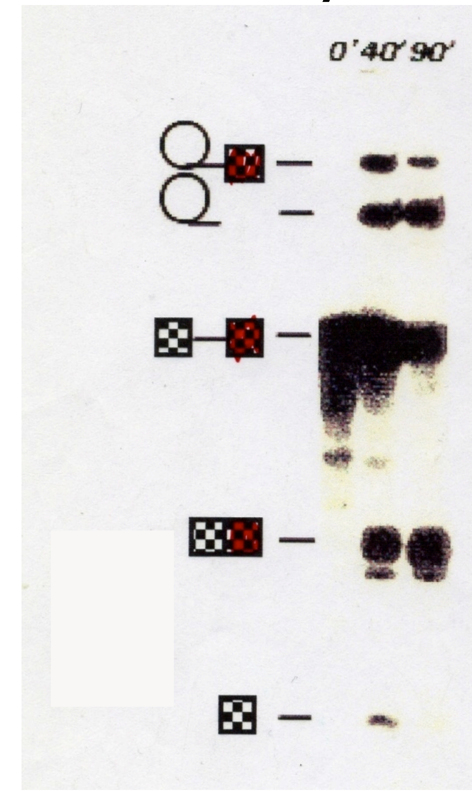
+ RNA pol
+ NTPs
+ α^{32}P-UTP

RNA radioattivo

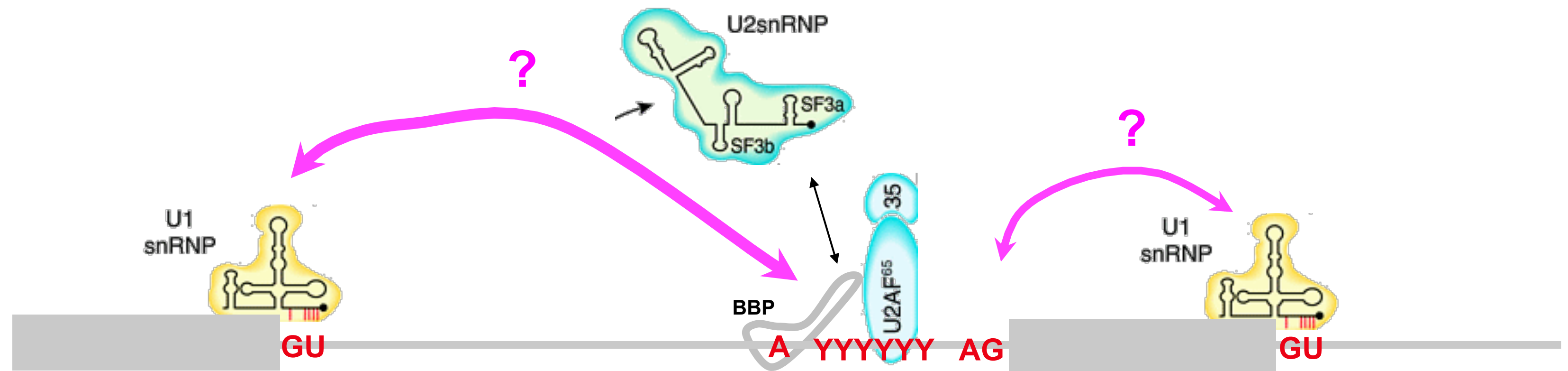
RNA isolation

nuclear extract

RNA analysis



• Recognition of canonical splice sites



10-15% of single nucleotide mutations causing disease affect splice sites

From: H. Stark, P. Dube, R. Luhrmann & B. Kastner, *Nature* 409, 539 - 542 (2001)

- RNA splicing gets the most out of genes

In animals, complexity depends less upon the number of genes than upon the number of different ways they can splice the RNA

Genome sequence analysis

Introns are abundant

94% human, 85% fruitfly, 95% nemtode, 95% plant genes

Human genome (26,000-35,000 genes)

Only 2 - 5 times as many genes as in fruitflies (13,600)
or in nematodes (19,000)

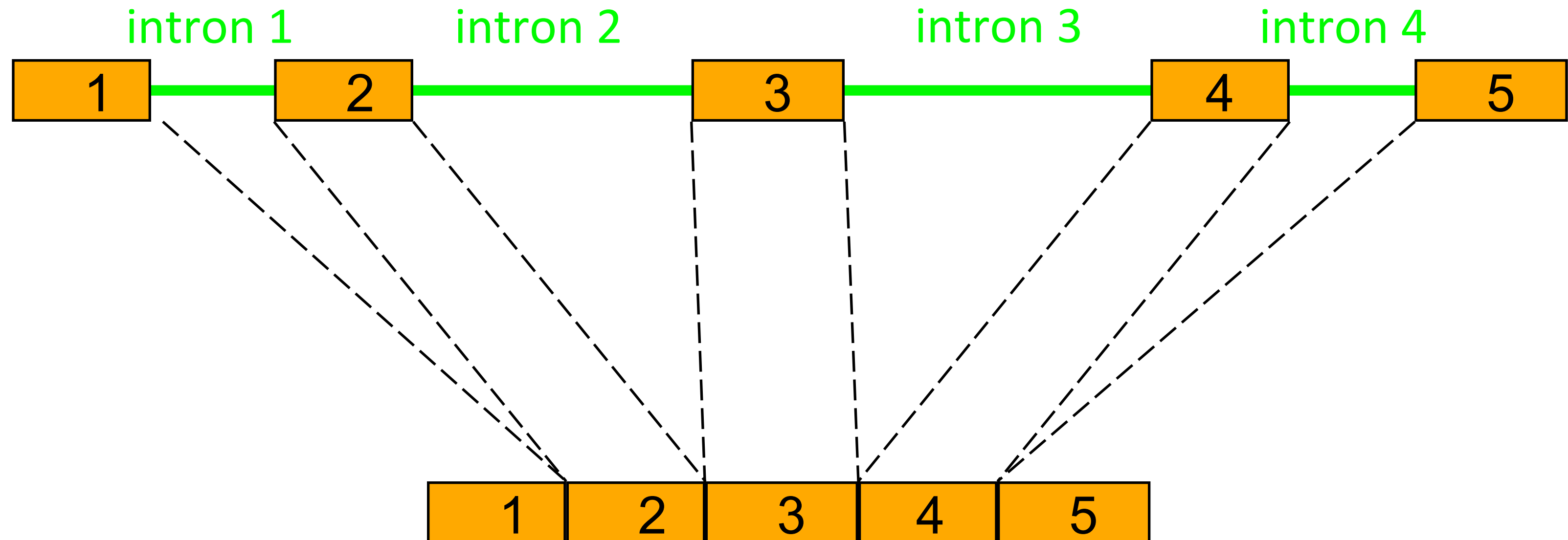
Alternative splicing is abundant

~75% of human gene transcripts are alternatively spliced

Alternative splicing

- The presence of multiple introns in many eukaryotic genes permits expression of multiple, related proteins from a single gene by means of alternative slicing, an important mechanism for the production of different forms of proteins, called isoforms, by different types of cells.

- Nearly 75% of all human genes are expressed as alternatively spliced mRNAs, leading to an expansion of the coding capacity of our genome.



Usually all introns must be removed before the mRNA can be translated to produce protein

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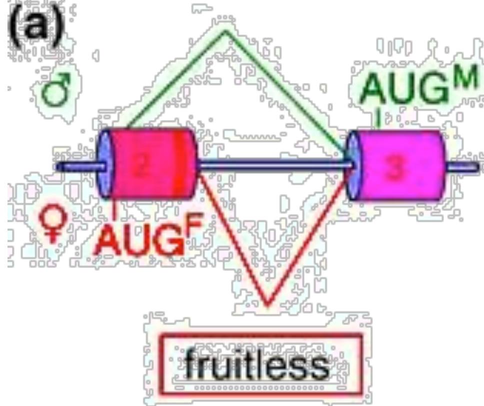
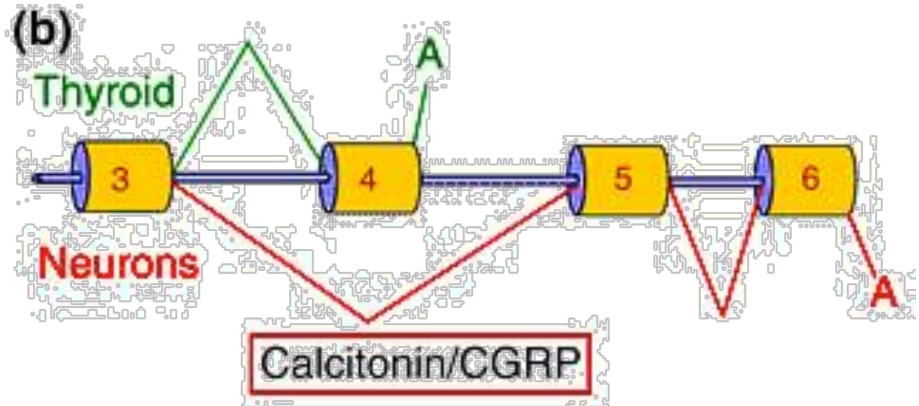
Alternative Splicing

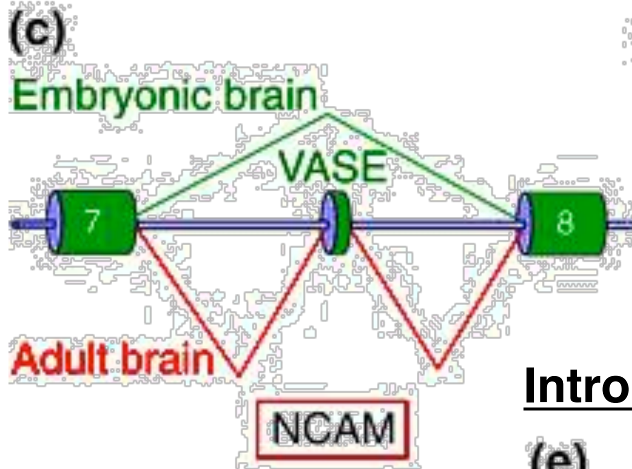
5' splice site switching



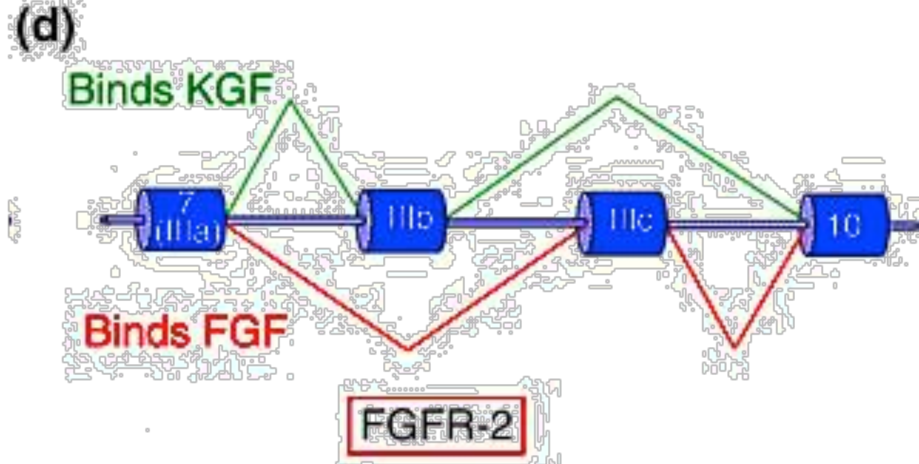
3' splice site switching



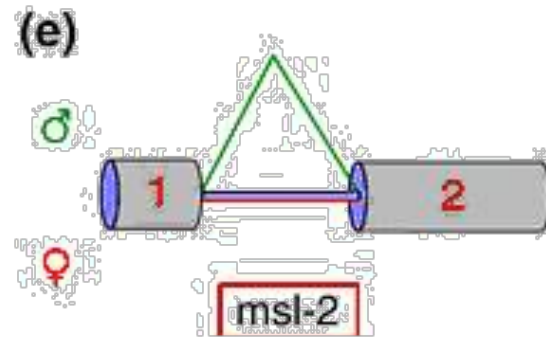
Exon Skipping



Mutually exclusive exons



Intron retention



5′ splice site or
'Donor'

Branch site

3′ splice site or
'Acceptor'

A

AGguragu

ynyuray

$(y)_{12-17}$nagG

B

Cap site

poly(A) site

D A D A D A

C

Strong ...tttccttacagACA...

Weak ...tt**g**cct**a**acagACA...

D

Strong

Weak

ESE

ESS

Strong

ISE

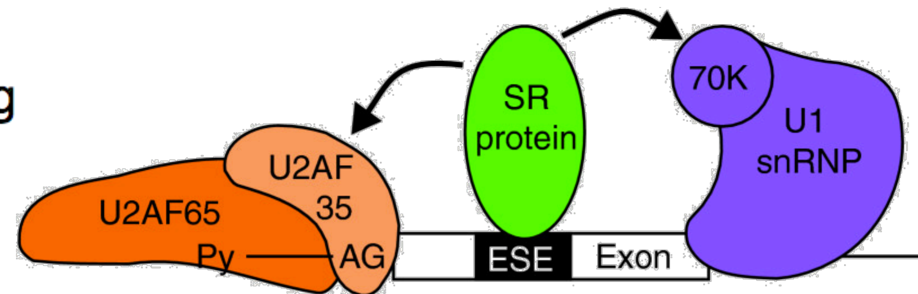
ISS

Strong

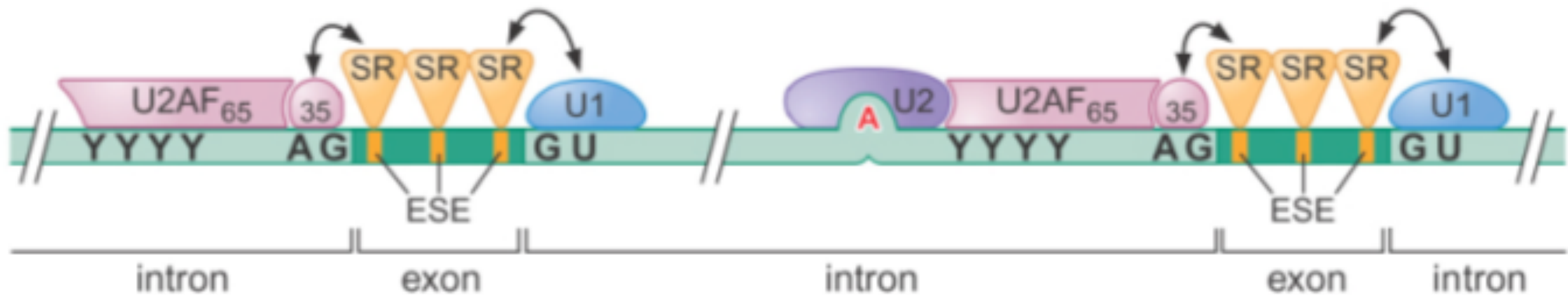
D A D A

Come vengono riconosciuti i siti di splicing nello splicing alternativo?

Proteine SR si legano all'interno di esoni nei siti di enhancer di splicing esonico (ESE) e reclutano l'apparato di splicing nei siti di splicing 5' e 3' (U2AFs e snRNPU_1)



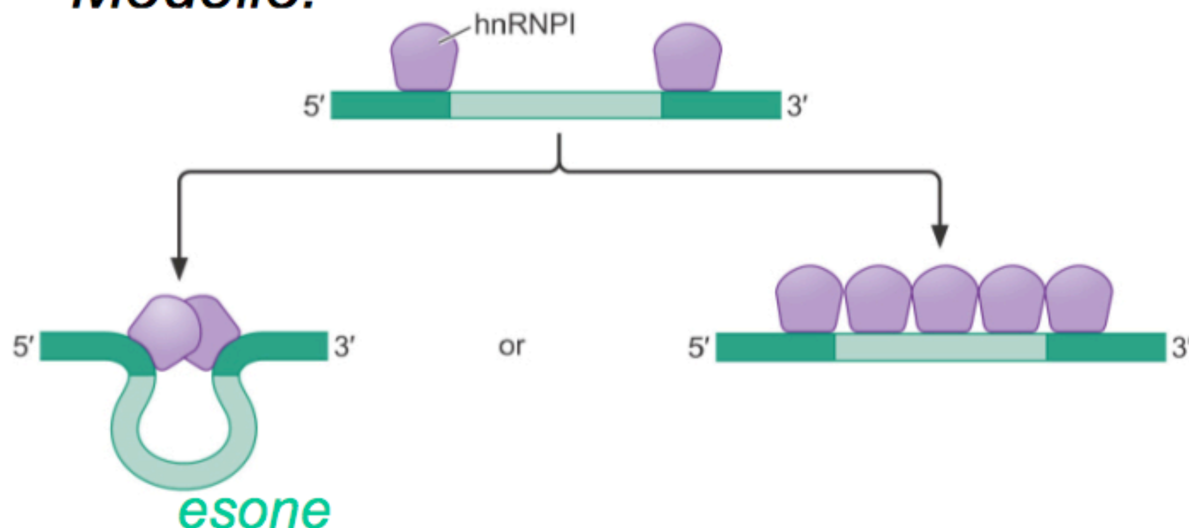
Proteine SR hanno un dominio di legame all'RNA e un dominio ricco in arginina (R) e serina (S) che recluta l'apparato di splicing.



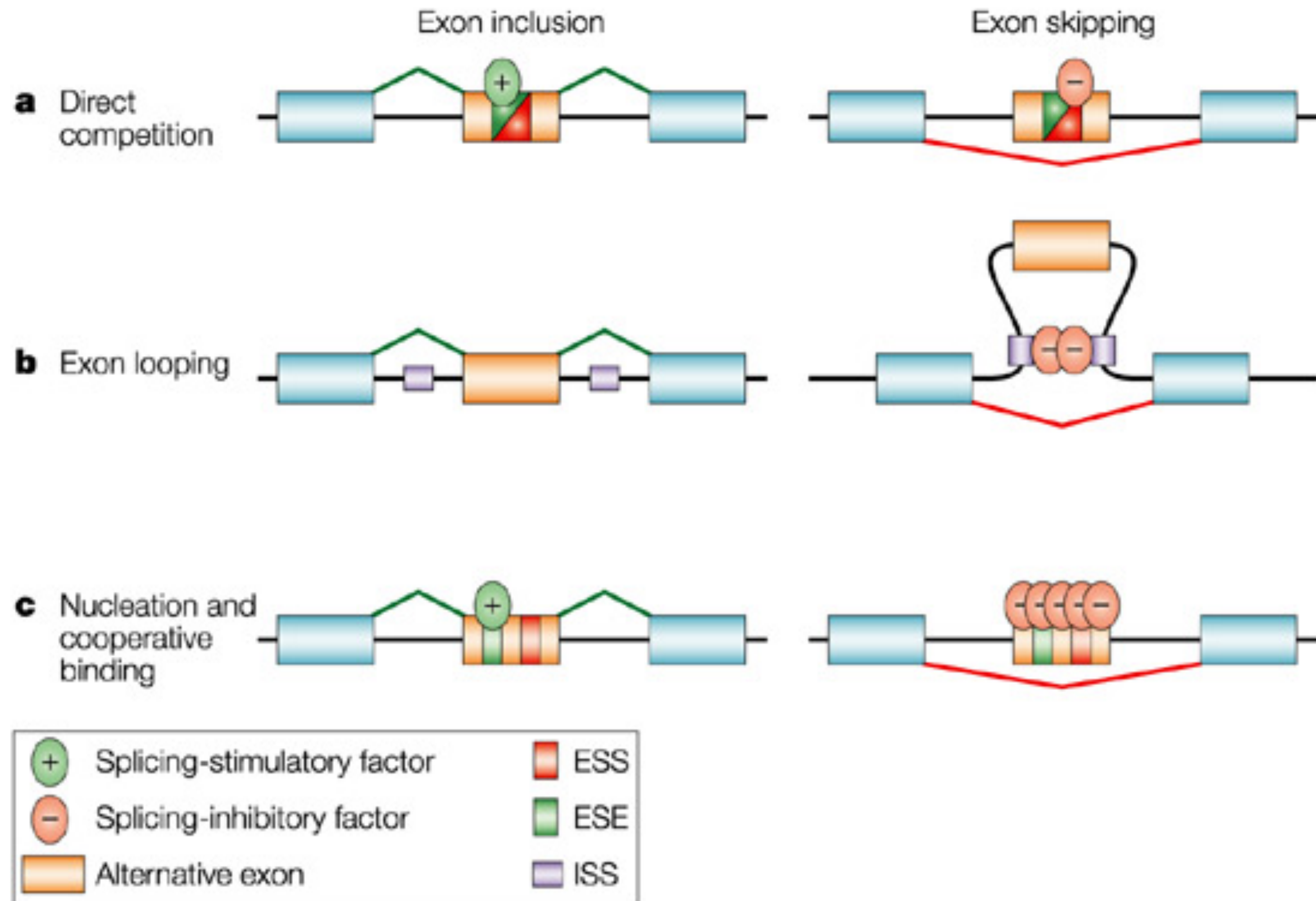
Esistono sia enhancers di splicing esonico (ESE) che silenziatori di splicing che incrementono o riducono lo splicing sui siti di splicing vicini

Un esempio di silenziatori sono le proteine hnRNP (heterogeneous nuclear Ribonuclear protein) che legano l'RNA ma essendo prive del dominio RS non reclutano l'apparato di splicing.

Modello:

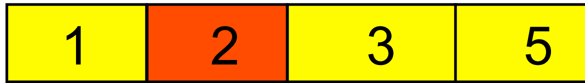


Splicing modulators

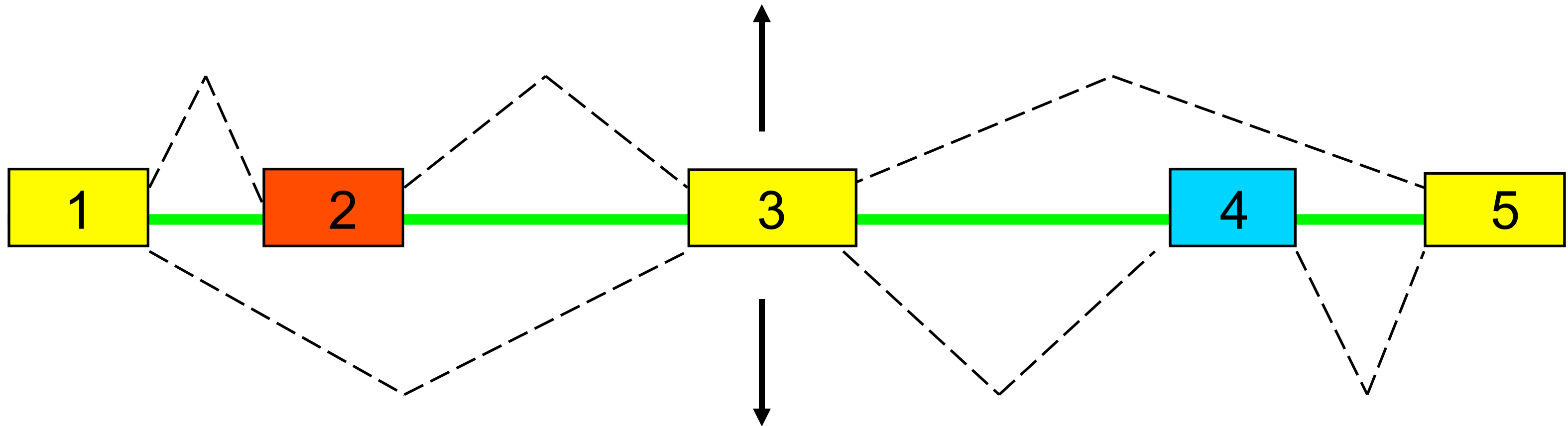


Multiple introns may be spliced differently in different circumstances, for example in different tissues.

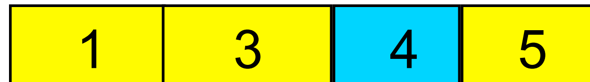
Heart muscle mRNA



pre-mRNA



Uterine muscle mRNA



Thus one gene can encode more than one protein. The proteins are similar but not identical and may have distinct properties. This is important in complex organisms

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Detection of alternative splicing by Northern blotting