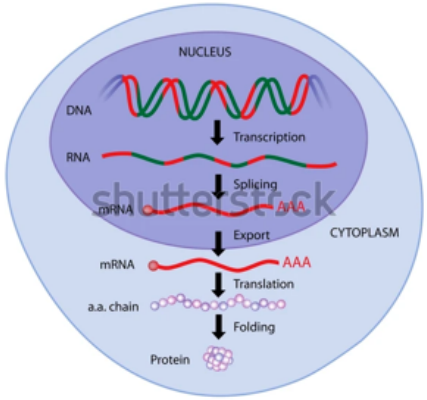
Post Transcriptional Modifications of mRNA



Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department

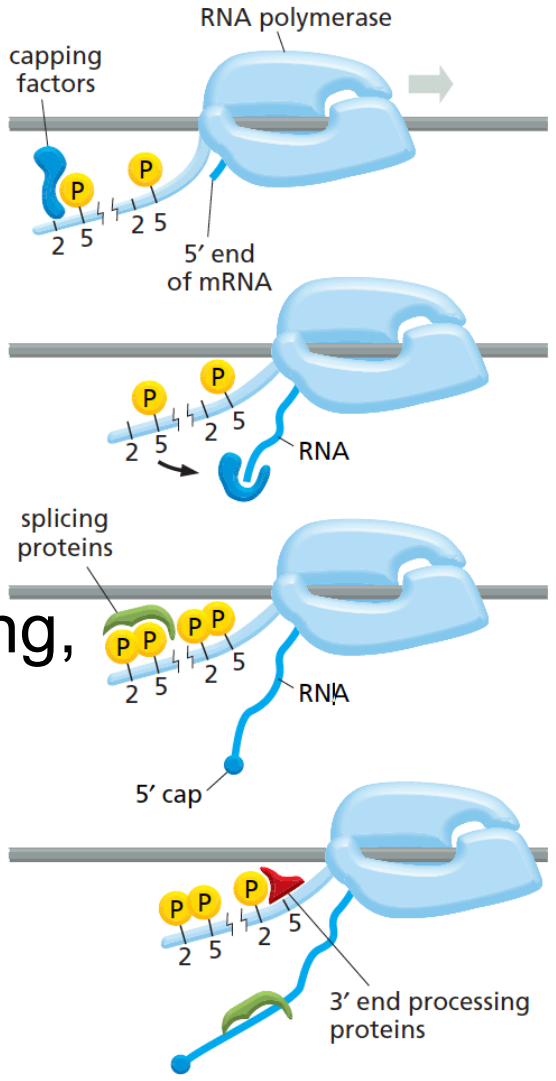
Faculty of Medicine, Mutah University

Post Transcriptional Modification

- Post Transcriptional Modification **(PTM):** is a set of biological processes which modify the chemical structure of RNA and alter the pre-mRNA (primary transcript) to mature mRNA.
- **PTM** occurs only in eukaryotes
- **PTM** occurs in the nucleus. After modification, the mature mRNA will leave to cytoplasm
- **PTM** consists of three steps:
  1. 5’ Capping
  2. RNA splicing
  3. 3’ Polyadenylation

Post Transcriptional Modification

- Transcription elongation in eukaryotes is tightly coupled to RNA processing steps
- Phosphorylation of CTD tail (Ser2,Ser5) of RNA polymerase II proceeds gradually during the elongation
- This attracts different RNA processing proteins to the tail (capping, factors, splicing proteins and 3'end processing proteins)
- These factors jump from the tail onto nascent RNA molecule and start processing at the appropriate time

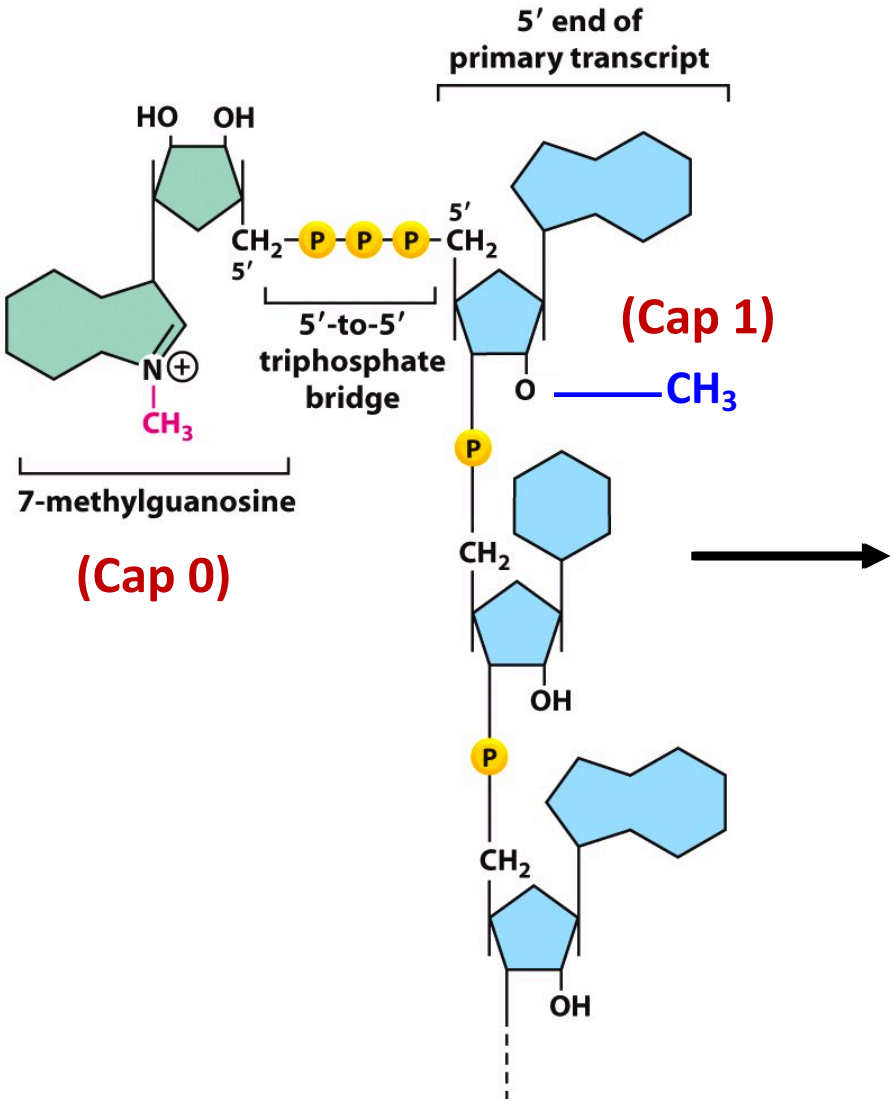


5’Capping

- RNA capping is the first modification of eukaryotic pre-mRNA
- Once the nascent RNA is 25 nucleotides long, a cap is added to the 5’ end
- The cap consists of methylated guanine nucleotide called 7-methylguanosine (m^7G)
- Capping factors present on the CTD tail:

1. RNA triphosphatase,
2. RNA guanylyltransferase
3. RNA methyltransferase

5’ Capping



γ β α

pppNpRNA

γ Pi — RNA Triphosphatase

ppNpRNA

GTP, PPi — RNA Guanylyltransferase

α

GpppNpRNA

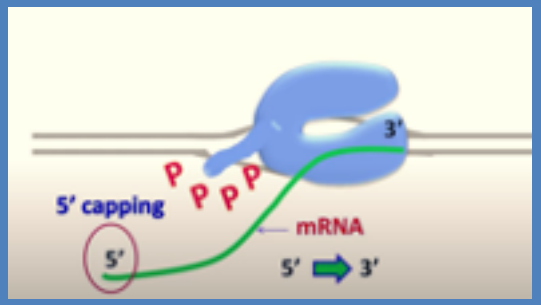
SAM, SAH — Methyltransferase (N7MTase)

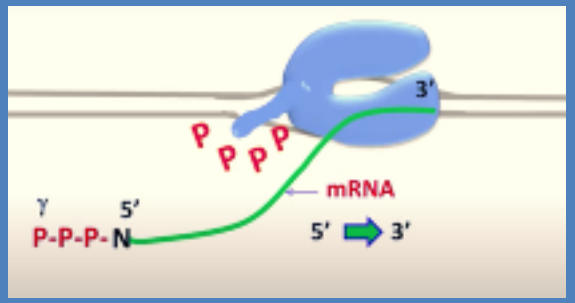
^{m7}GpppNpRNA (Cap-0 RNA)

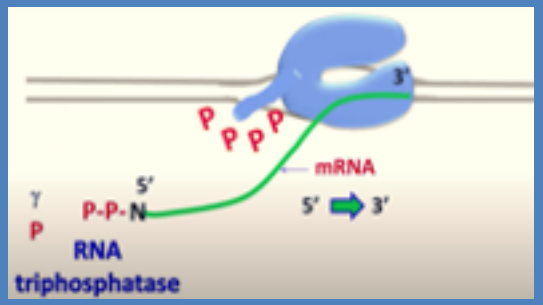
SAM, SAH — RNA (nuceloside-2’-O-)-Methyltransferase (2’OMTase)

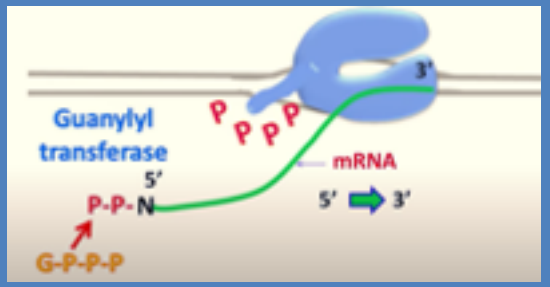
^{m7}GpppN$_m$pRNA (Cap-1 RNA)

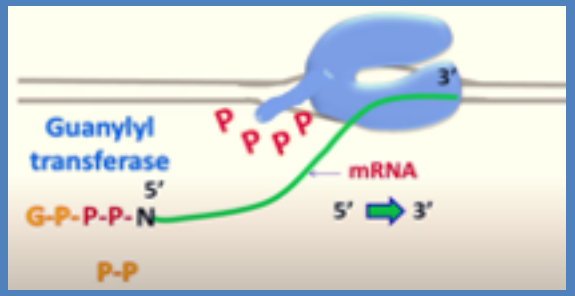
5’ Capping

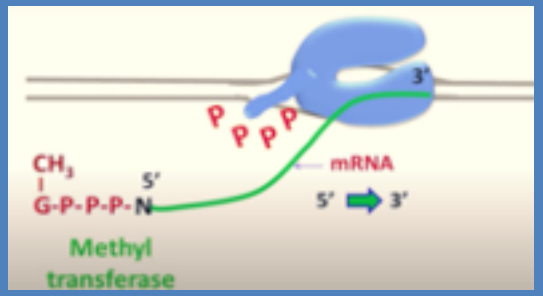

5’ capping
P P P P
3’
mRNA
5’
5’ 3’


P P P P
3’
mRNA
γ 5’
P-P-P-N
5’ 3’


P P P P
3’
mRNA
5’
γ P-P-N
5’ 3’
P
RNA
triphosphatase


Guanylyl
transferase
P P P P
3’
mRNA
5’
P-P-N
5’ 3’
G-P-P-P


Guanylyl
transferase
P P P P
3’
mRNA
5’
G-P-P-P-N
5’ 3’
P-P


P P P P
3’
CH3
mRNA
5’
G-P-P-P-N
5’ 3’
Methyl
transferase

5’Capping

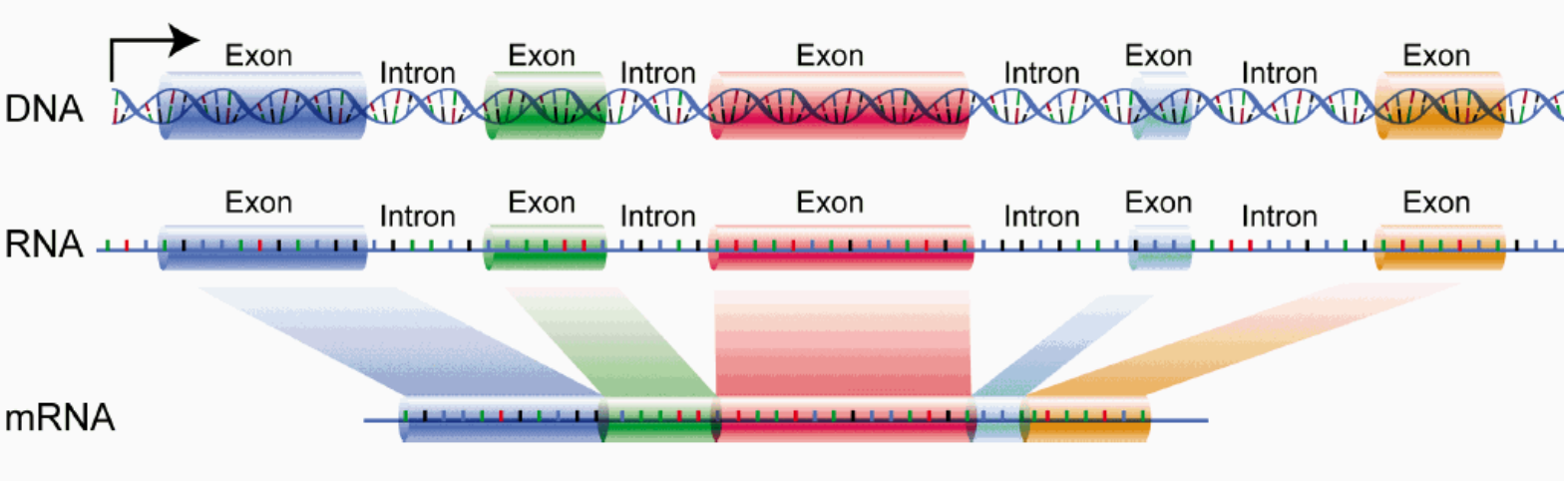
- The 5’-5’ linkage between the 7-methylguanosine and the rest of RNA molecule is unusual

- 5’ Cap has three main functions:

1. Protection from degradation by exonucleases
2. Regulation of nuclear transport
3. Important for efficient translation

RNA Splicing

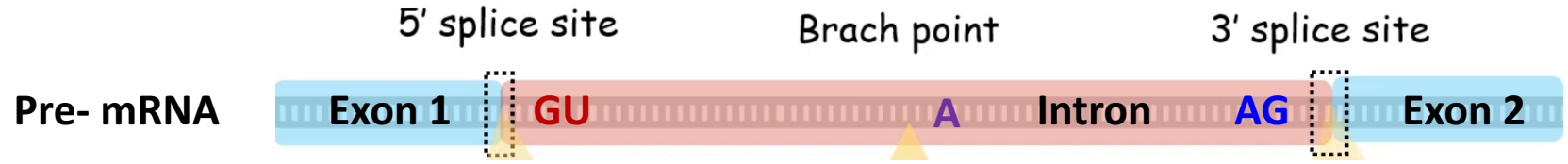
- The transcribed region of genes (downstream TSS) consists of protein coding sequences (Exons) and non-coding sequences (Introns)
- Both exons and introns are transcribed into RNA (precursor mRNA or pre-mRNA or primary transcript)
- Splicing: is the excision and removal of introns and the re-joining of exons to form the mature mRNA



RNA Splicing

- RNA splicing step occurs during the transcription elongation process and it is catalysed by a large RNA-protein molecular complex called spliceosome
- Three types of consensus and short nucleotide sequences (conserved in eukaryotes) which specify the introns and direct the splicing machinery:

1. 5'splice site **(5'GU)**
2. Branch point **(A)**
3. 3'splice site **(AG3')**



Spliceosome

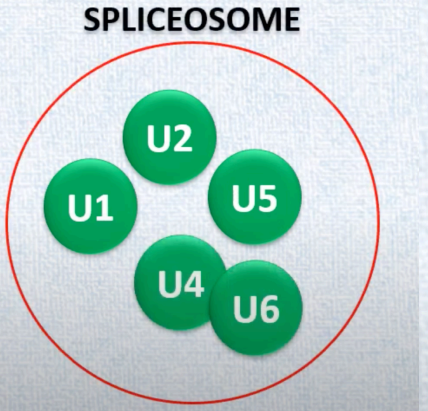
Spliceosome

- A set of RNA-protein complexes

5
snRNA
(small nuclear RNA)

+

150
Protein



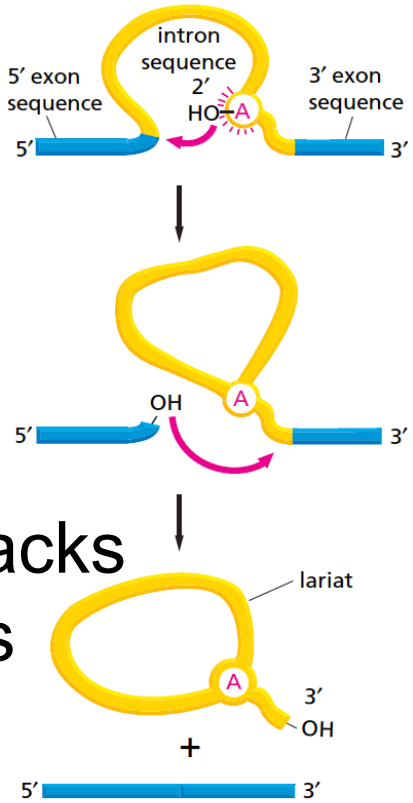
snRNP (snurps)

(small nuclear ribonucleoproteins)

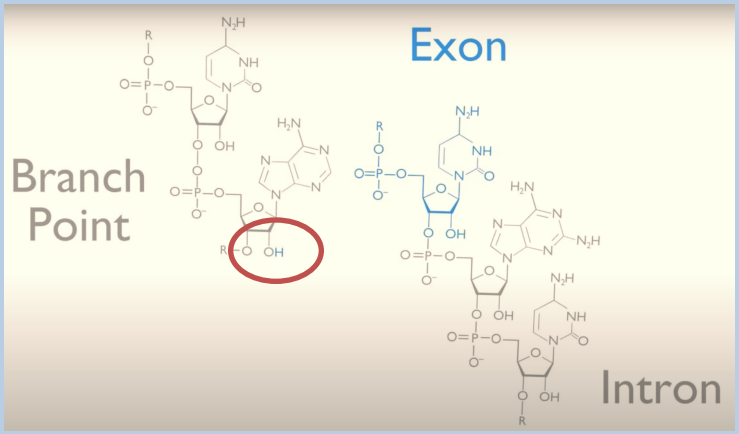
Mechanism of RNA Splicing

- Each splicing event involves two sequential transesterification reactions that join two adjacent exons while removing one intron as a lariat (a rope with a loop)

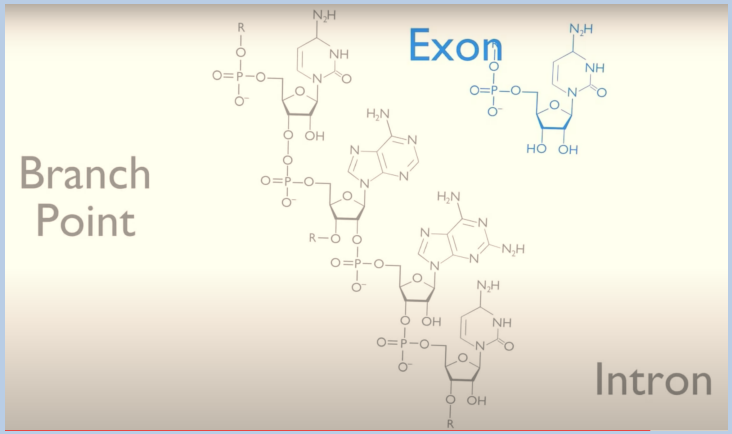
1. The 2'OH of **A** attacks the 5' splice site and break the phosphodiester bond in the backbone of RNA
2. The cut 5'end of the intron becomes covalently linked to **A** forming a loop
3. The OH at the free 3' end of exon 1 attacks the 3'splice site and joins the two exons while the intron is released as lariat

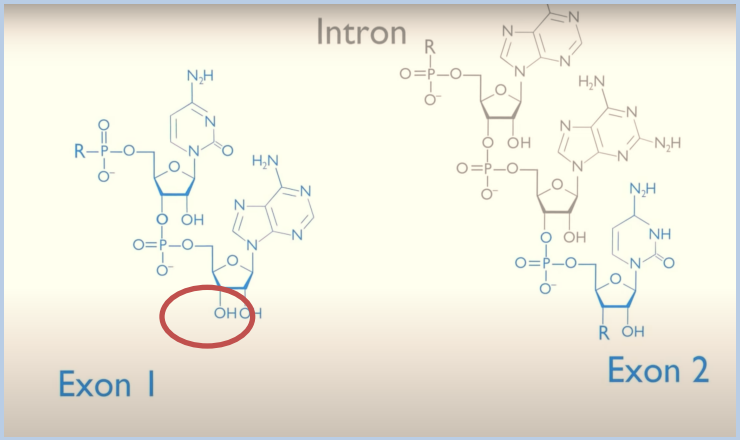


Transesterification

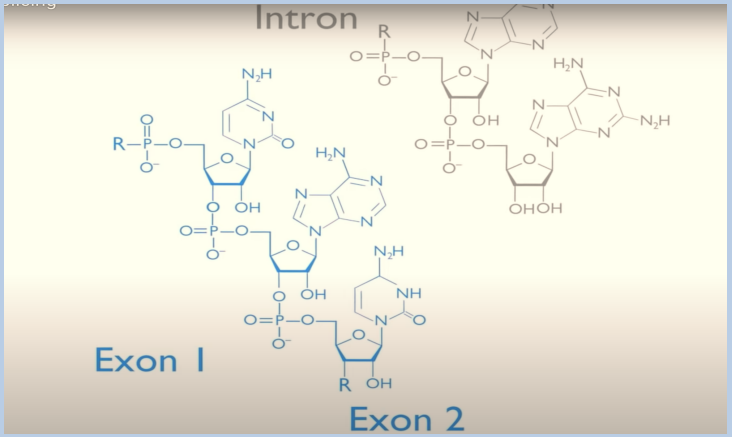


First reaction



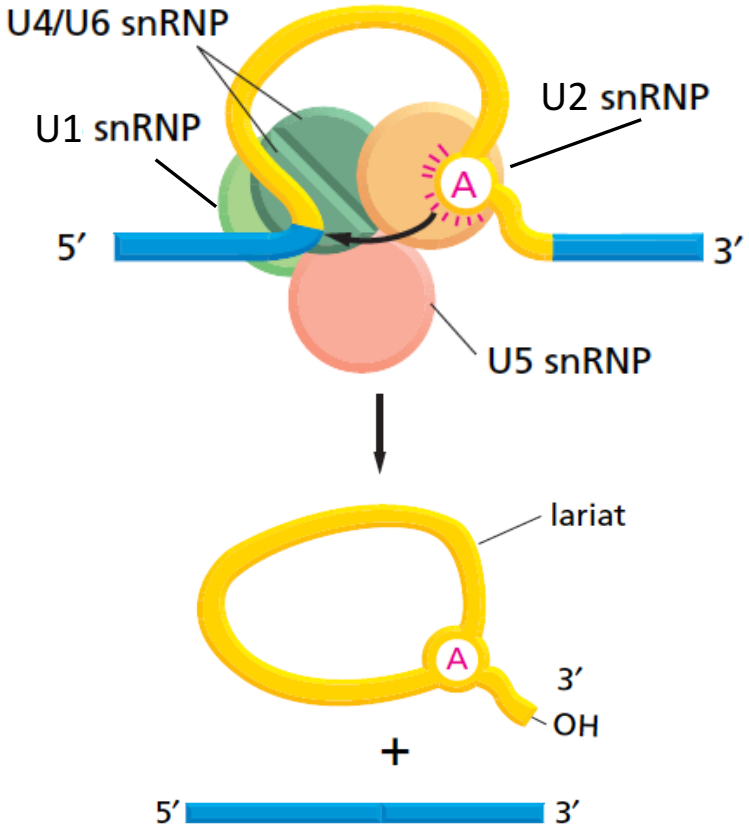


Second reaction



Spliceosome

- There are five types of snRNAs (U1,U2,U4,U5 & U6) and over 150 proteins which assemble together at the pre-mRNA to form the functional spliceosome that catalyses the splicing event
- The complex that forms between each type of snRNA and proteins is called snRNPs pronounced as “snurps”



3’ Polyadenylation

- The final step in pre-mRNA is the addition of poly-A tail to the newly synthesized mRNA
- There are 3 types of 3’end processing proteins or factors involved in the 3’end processing step:

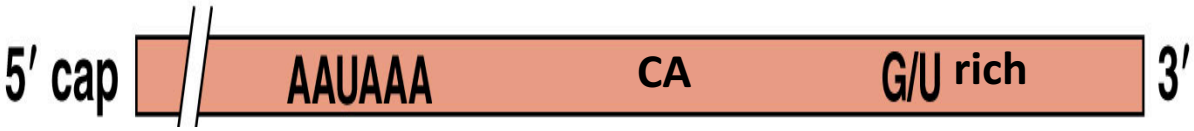
1. Cleavage and polyadenylation specificity factor **(CPSF)**
2. Cleavage stimulation factor **(CStF)**
3. Poly-A polymerase **(PAP)**

- 3’end processing signal which specifies the 3’end of each mRNA is encoded in the genome and transcribed into pre-mRNA

3’ Polyadenylation

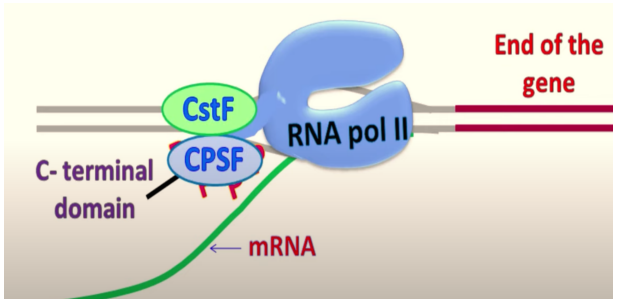
- The signal consists of 3 sequence elements:

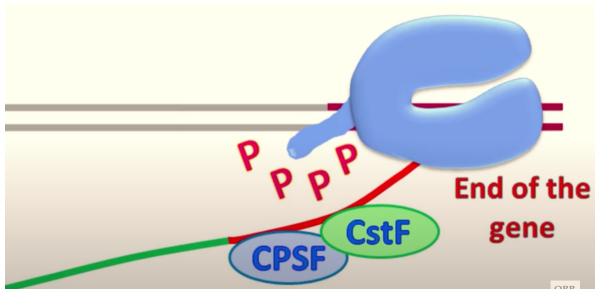
1. Polyadenylation sequence **(5’AAUAAA3’)**
2. The site of cleavage **(5’CA3’)**
3. GU rich sequence

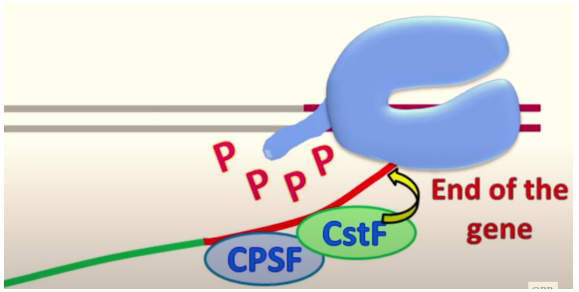


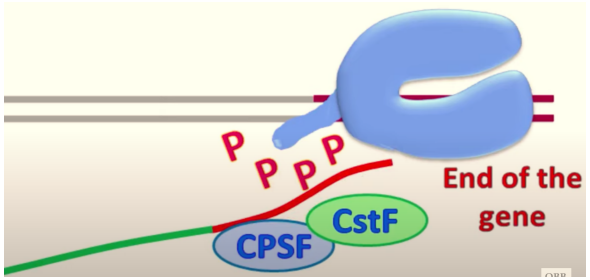
- CPSF and CStF are multi-subunit RNA binding proteins which can jump from the CTD phosphorylated tail of RNA polymerase II and bind the specific sequences on pre-mRNA as it emerges from RNA polymerase II

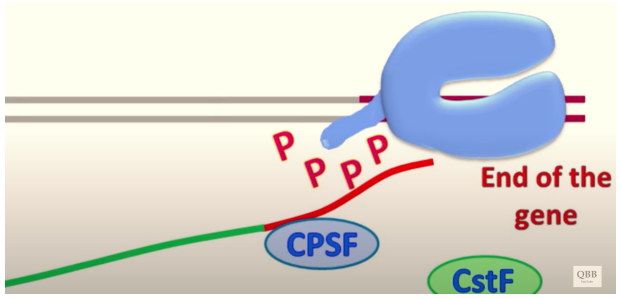
3’ Polyadenylation

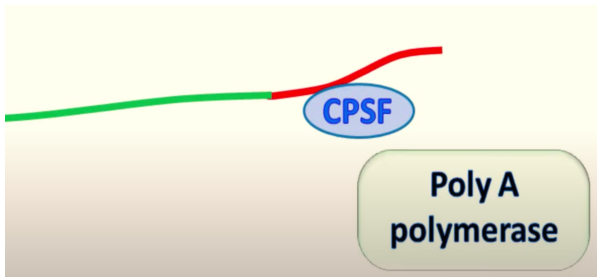


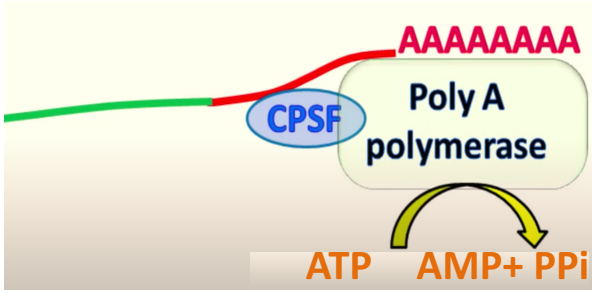


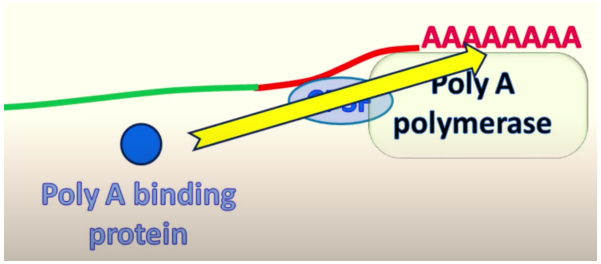


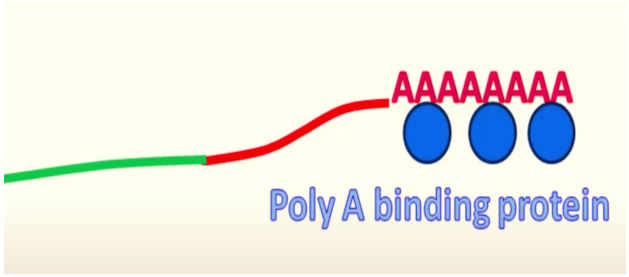










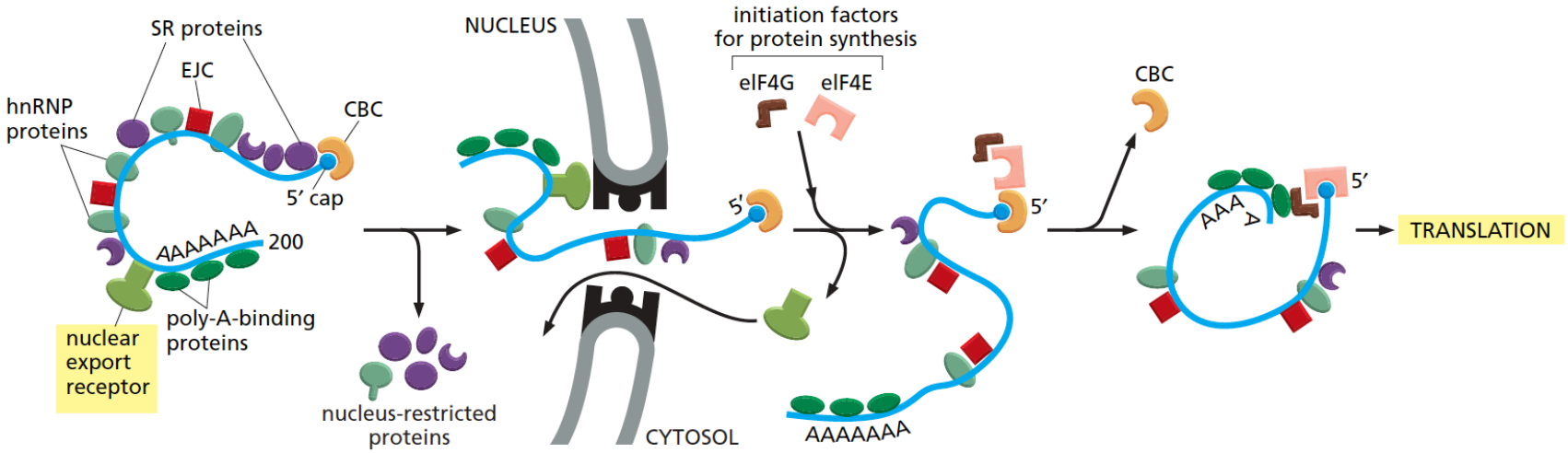


3’ Polyadenylation

- Pre-mRNA is cleaved by CStF at the cleavage site
- PAP is recruited and starts addition of about 200 A nucleotides to 3’ end using ATP as precursor
- Poly-A binding proteins are recruited to **determine the final length of the tail**
- CPSF and PAP dissociate from RNA and some poly-A binding proteins remain bound to protect 3’end from degradation by exonucleases until it travels from the nucleus and pass the check test before the beginning of translation

Transportation of mature mRNA

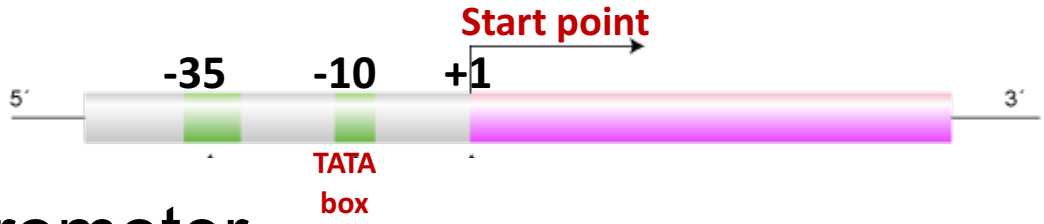
- Only fully processed and mature mRNA can pass through the nuclear pore and travel to the cytosol for translation



- The final check is performed in the cytosol by initiation factors eIF4E and eIF4G to ensure efficient translation

Transcription in Prokaryotes

- In prokaryotes, two consensus sequences at
  -10 (TATA) and
  -35 located in the promoter



- **σ factor** recognizes the -35 region in the promoter and binds to it
- Once the RNA polymerase starts the transcription, σ factor then dissociates to guide another enzyme to the initiation site.