POST TRANSCRIPTIONAL MODIFICATION

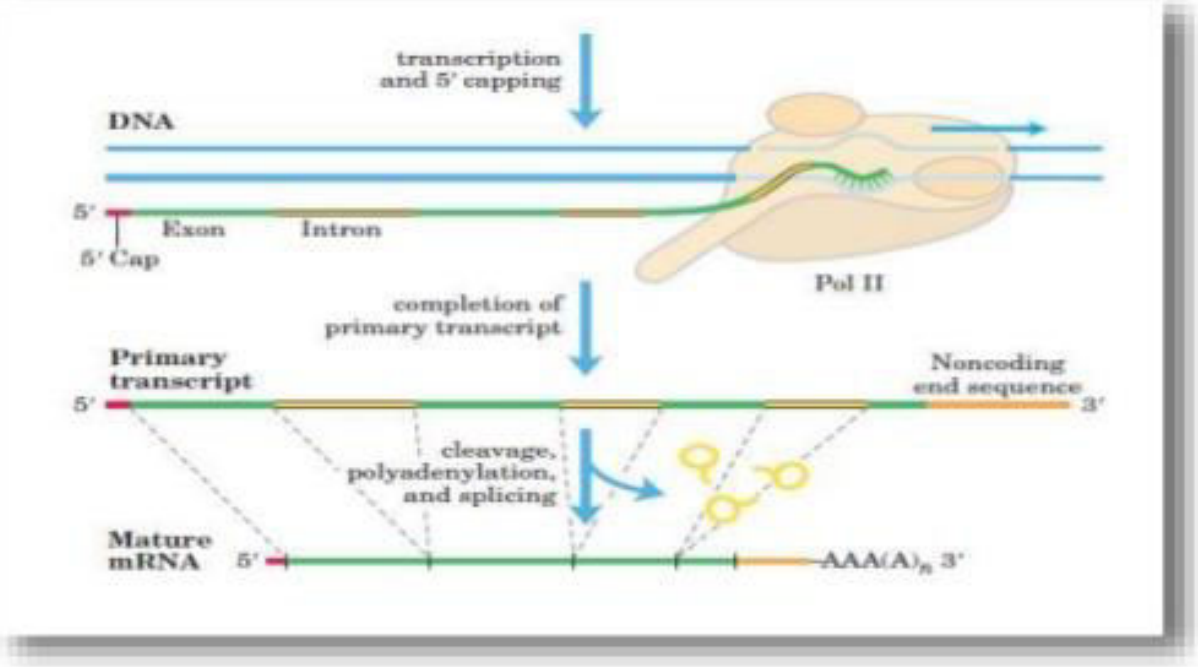
Post transcriptional modification

• Primary transcript made by RNA polymerase normally undergo further alteration, called post transcriptional processing or modification.

• Prokaryotes: mRNA transcribed directly from DNA template and used immediately in protein synthesis

• Eukaryotes: primary transcript (hnRNA) must be processed to produce the mRNA (active form).

• It occurs in nucleus of cell.


transcription
and 5′ capping
DNA
5′
Exon
Intron
5′ Cap
Pol II
completion of
primary transcript
Primary
transcript
5′
Noncoding
end sequence
3′
cleavage,
polyadenylation,
and splicing
Mature
mRNA
5′
AAA(A)n 3′

Post-transcriptional modifications OF RNA accomplish three things:

1) Modifications help the RNA molecule to be recognized by molecules that mediate RNA translation into proteins.

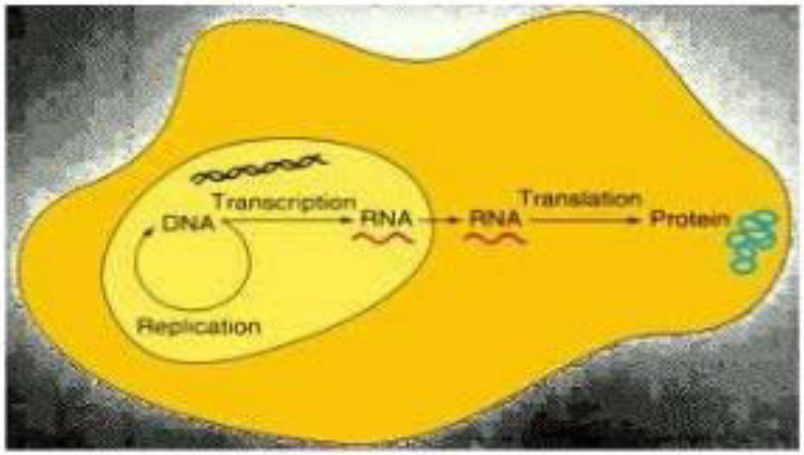
2) 2) During post-transcriptional processing, portions of the RNA chain that are not supposed to be translated into proteins are cut out of the sequence. In this way, post-transcriptional processing helps increase the efficiency of protein synthesis by allowing only specific protein- coding RNA to go on to be translated

3) 3) Without post-transcriptional processing, protein synthesis could be significantly slowed, since it would take longer for translation machinery to recognize RNA molecules and significantly more RNA would have to be unnecessarily translated

Terms used in PTM

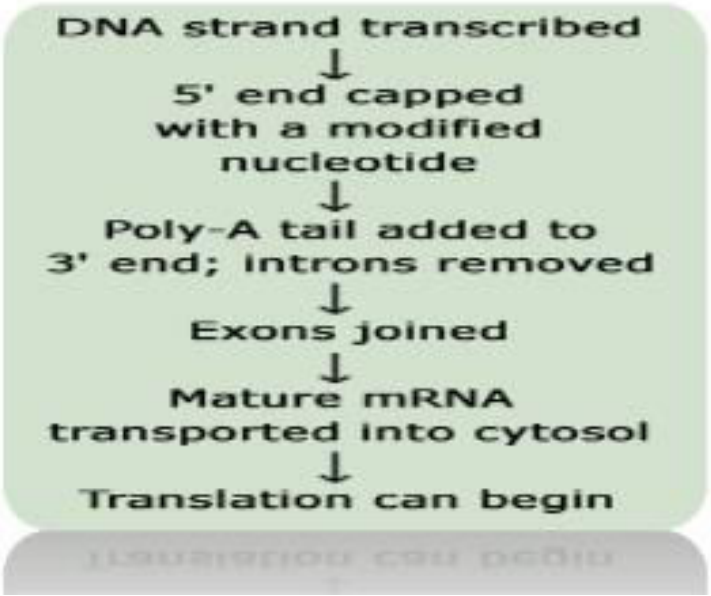
- **3' Splice site** - One of the conserved sequences of an intron. Contains an adenine next to a guanine base at the 3' end of an intron.
- **5' Capping** - One post-transcriptional modification made in the cell nucleus. The addition of a GTP molecule to the 5' end of a primary RNA transcript forming a 5'-5' linkage between the two.
- **5' Splice site** - One of the conserved sequences of an intron. Contains an guanine next to a uracil base at the 5' end of an intron.
- **Exon** - Sequences that are preserved in primary RNA transcript splicing that will be translated into proteins in the cell cytoplasm.
- **Intron** - Non-protein coding sequences of a primary RNA transcript that are removed and degraded during primary RNA transcript splicing.
- **Guanyl transferase** - The enzyme responsible for catalyzing the reaction that produces the 5' cap through the addition of a GTP molecule to the 5' end of a primary RNA transcript.

- **Poly A tail** - One post-transcriptional modification made in the cell nucleus. A string of up to 500 adenines added to the 3' end of primary RNA transcripts. Addition catalyzed by the enzyme poly (A) polymerase that recognizes the sequence AAUAAA.
- **RNA splicing** - A two-step reaction in which introns are removed from a primary RNA transcript and exons are joined together to form mature mRNA.



Post transcriptional modification mainly includes

- 5’ capping
- Addition of poly A tail
- Splicing



5’ END CAPPING

• At the end of transcription, the 5' end of the RNA transcript contains a free triphosphate group since it was the first incorporated nucleotide in the chain.

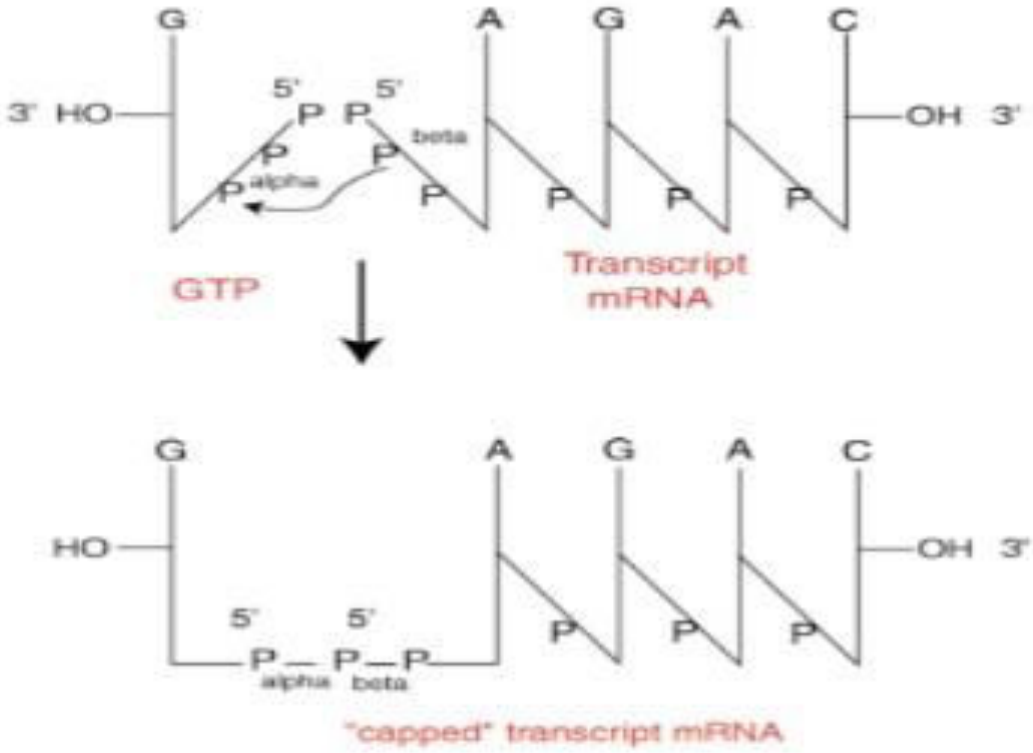
The capping process replaces the triphosphate group with another structure called the "cap". The cap is added by the enzyme **guanyl transferase**.

This enzyme catalyzes the reaction between the 5' end of the RNA transcript and a guanine triphosphate (GTP) molecule.

• In the reaction, the beta phosphate of the RNA transcript displaces a pyrophosphate group at the 5' position of the GTP molecule. The cap is formed through a 5'-5' linkage between the two substrates .

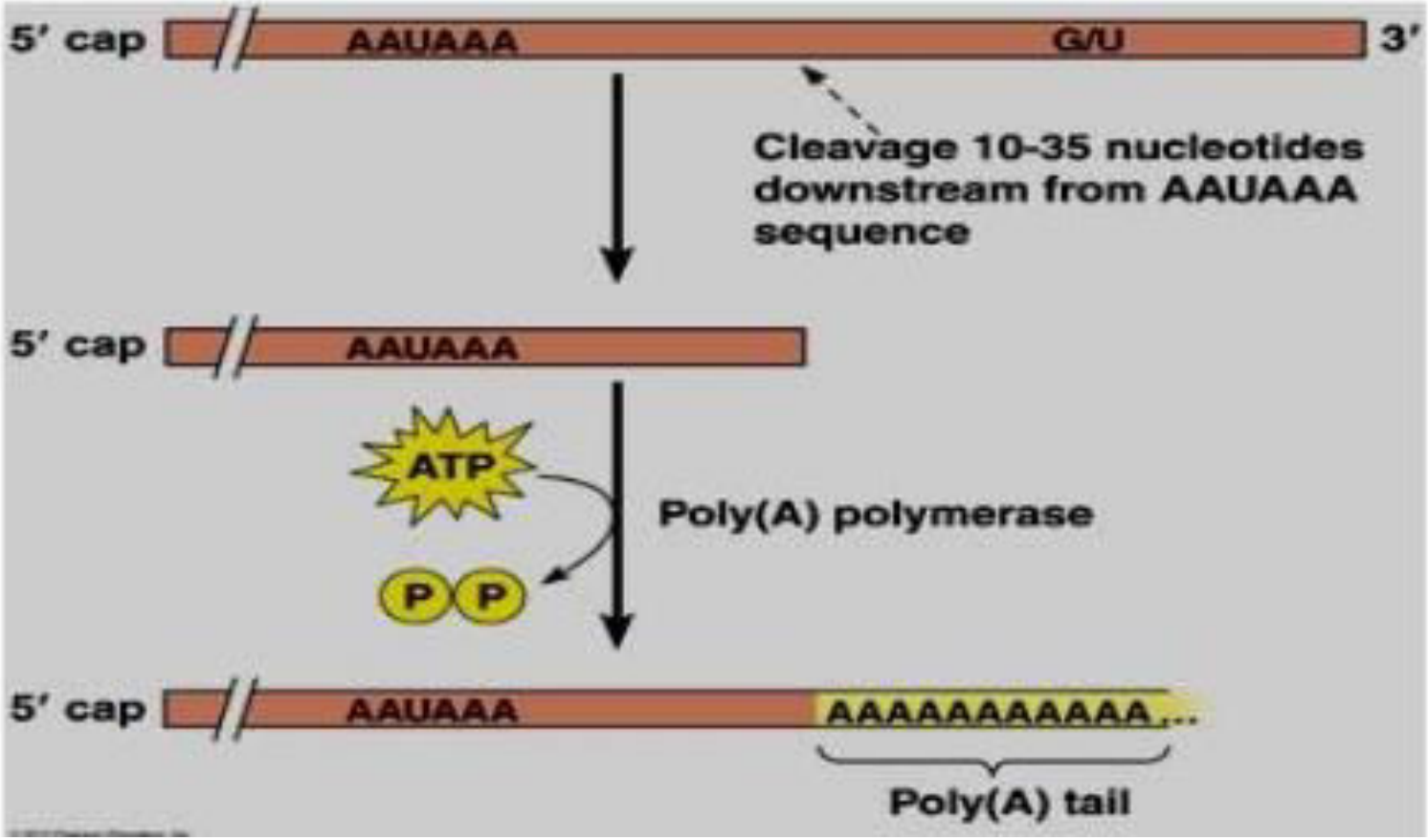
• Capping protects the 5’ from enzymatic degradation in the nucleus and assists in export to the cytosol.

• Eukaryotic m RNAs lacking the cap are not efficiently translated


G
A
G
A
C
3' HO
5'
5'
P
P
P
P
P
P
P
P
P
P
P
P
beta
alpha
OH 3'
GTP
Transcript
mRNA
G
A
G
A
C
HO
OH 3'
5'
5'
P
P
P
P
P
P
alpha
beta
"capped" transcript mRNA

The Poly A Tail

- Post-transcriptional RNA processing at the opposite end of the transcript comes in the form of a **string of adenine bases** attached to the end of the synthesized RNA chain.
- The addition of the adenines is catalyzed by the enzyme poly (A) polymerase.
- The mRNA is first cleaved about 20 nucleotides downstream from an AAUAA recognition sequence
- Another enzyme, poly(A) polymerase, adds a poly(A) tail which is subsequently extended to as many as 200 A residues.
- The **poly(A) tail** appears to protect the 3' end of mRNA from 3' 5' exonuclease attack.
- Histone and interferon's mRNAs lack poly A tail.
- After the m-RNA enters the cytosol, the poly A tail is gradually shortened.


5′ cap
AAUAAA
G/U
3′
Cleavage 10-35 nucleotides downstream from AAUAAA sequence
5′ cap
AAUAAA
ATP
Poly(A) polymerase
P P
5′ cap
AAUAAA
AAAAAAAAAAA...
Poly(A) tail

SPLICING

• Removal of introns (Splicing)

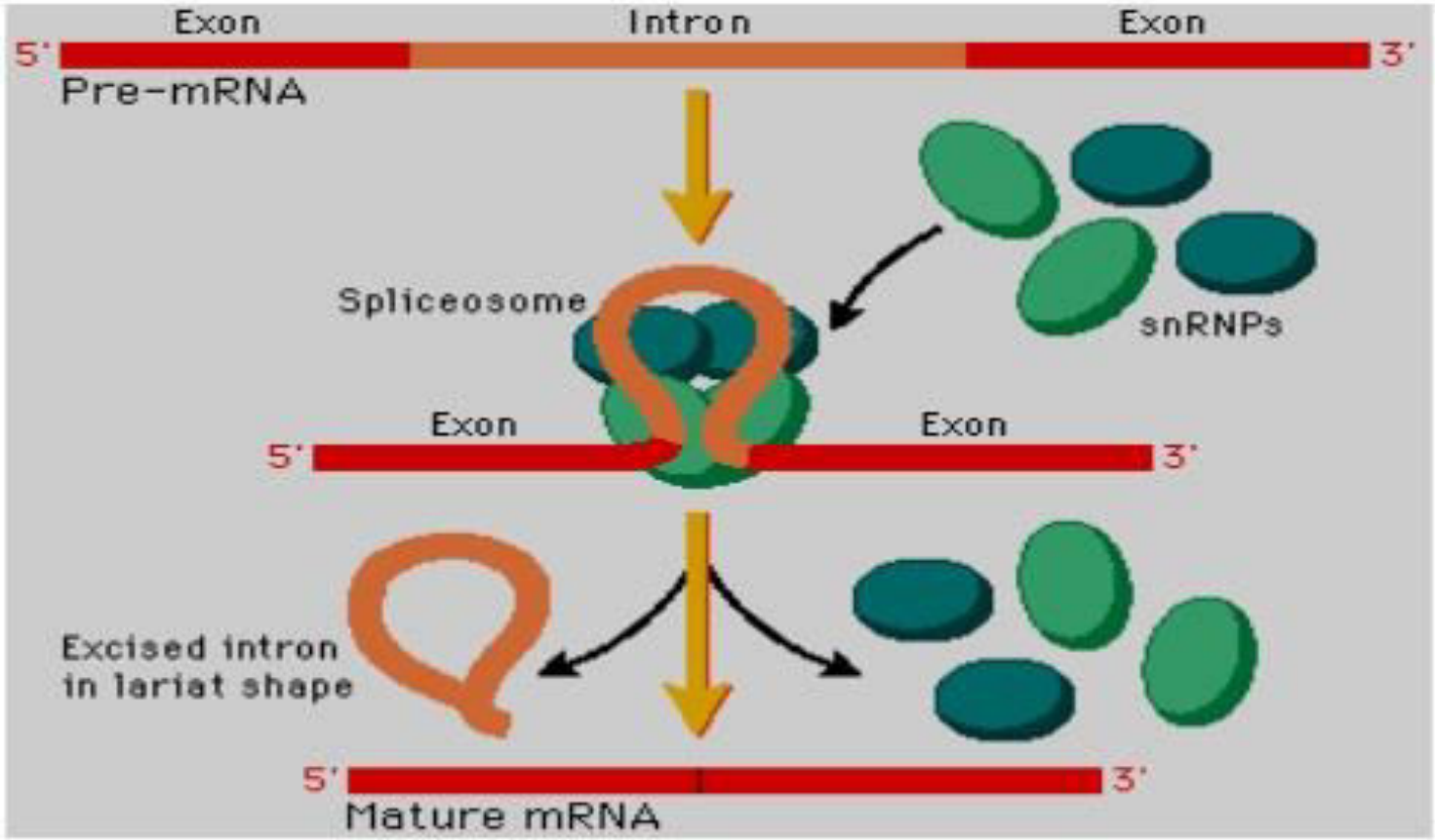
• Introns or intervening sequences are the RNA sequences which do not code for the proteins.

• These introns are removed from the primary transcript in the nucleus, exons (coding sequences) are ligated to form the mRNA molecule, and the mRNA molecule is transported to the cytoplasm.

• The molecular machine that accomplishes the task of splicing is known as the spliceosome

.• Small nuclear RNA molecules that recognize splice sites in the pre- mRNA sequence.

• The excised intron is released as a "lariat" structure, which is degraded


Exon
Intron
Exon
5'
3'
Pre-mRNA
Spliceosome
snRNPs
Exon
Exon
5'
3'
Excised intron
in lariat shape
5'
3'
Mature mRNA

Alternative Splicing

- Alternative patterns of RNA splicing is adapted for the synthesis of tissue-specific proteins.(antibodies)
- The pre-m RNA molecules from some genes can be spliced in two or more alternative ways in different tissues.
- This produces multiple variations of the m RNA and thus diverse set of proteins can be synthesized from a given set of genes .
- Introns are removed from the primary transcript in the nucleus, exons (coding sequences) are ligated to form the mRNA molecule.

(After removal of all the introns, the mature m RNA molecules leave the nucleus by passing in to the cytosol through pores in to the nuclear membrane)