Post transcriptional modifications

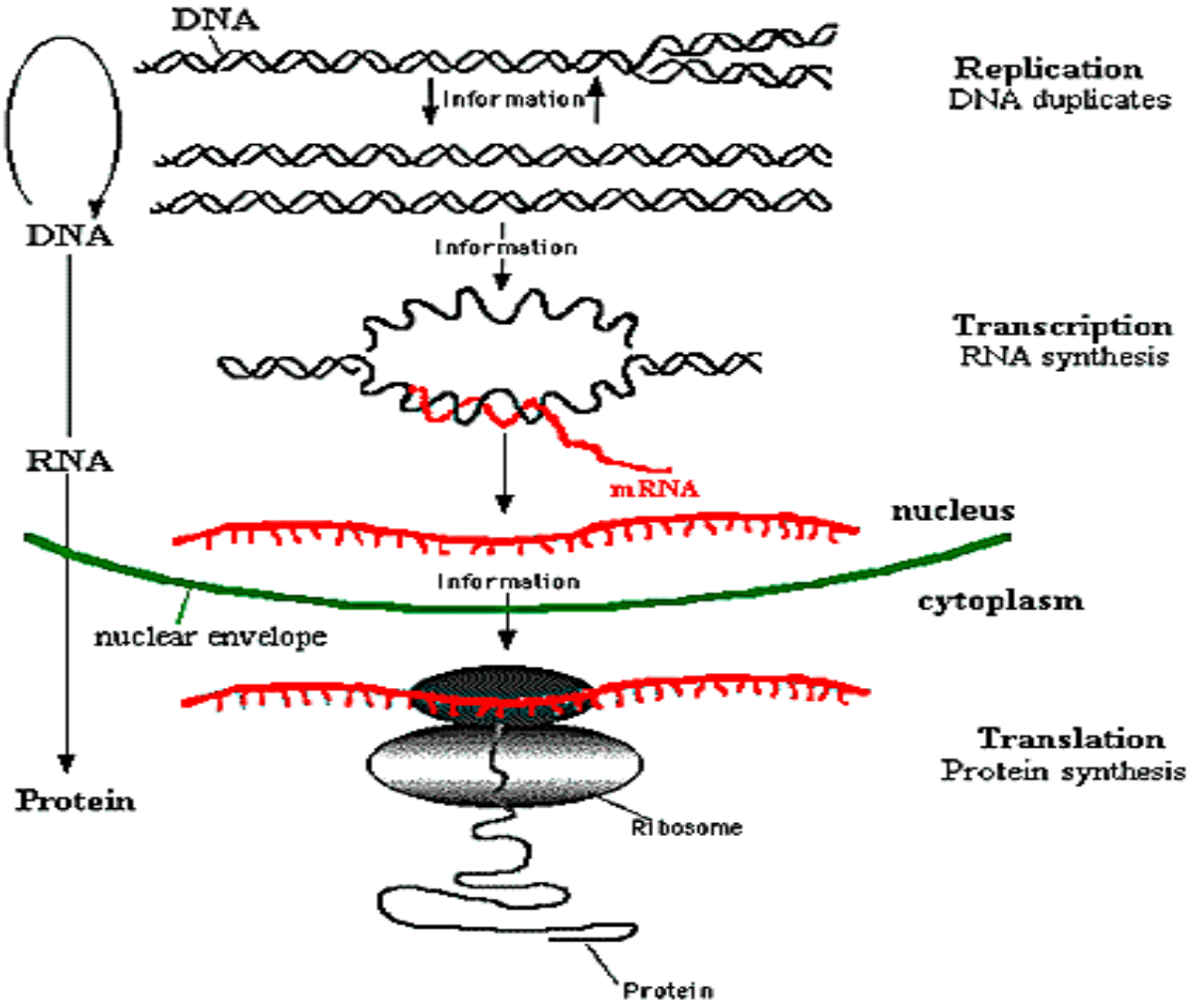
- Introduction to post-transcriptional modifications
- What precedes and follows it?
- Significance!

INTRODUCTION

The Information Flow in Biological Systems

- The "Central Dogma" refers to the flow of genetic information in biological systems.
- In general, genetic information flows from DNA to RNA to protein.
- DNA encodes the genetic information for most species.

Central Dogma of Molecular Biology



The Central Dogma of Molecular Biology

TRANSCRIPTION

It is the process of synthesis of RNA from a DNA template.

Transcription, whether prokaryotic or eukaryotic, has three main events:

- **Initiation**
- **Elongation**
- **Termination**

POST TRANSCRIPTIONAL MODIFICATION/ RNA PROCESSING

- It is a process by which primary transcript RNA is converted into mature RNA.
- Though it is critical to eukaryotic mRNA, it is responsible for changes in rRNA, tRNA and other special funtion RNAs too.

It involves…

MODIFICATION OF rRNA

- Pre rRNA synthesized as larger precursors are further cut to yield mature size rRNA.

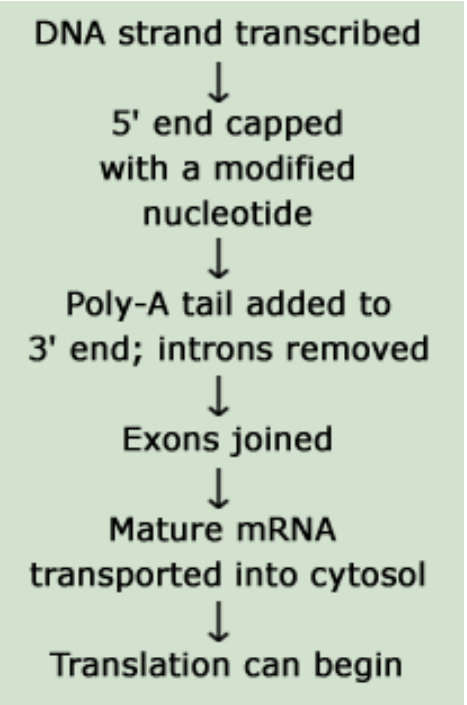
MODIFICATION OF snoRNA

- Large snoRNA precursors are cleaved and attacked upon by ribonucleases to poduce functional snoRNAs

MODIFICATION OF mRNA

- Conversion of pre mRNA to mature mRNA:

- 5′ end capping
- 3′ end polyadenylation
- Splicing



MODIFICATION OF snRNA

- Synthesized as pre-snRNA and then subjected to cleavage and processing.

MODIFICATION OF tRNA

Conversion of pre tRNA to mature tRNA:

- Modification of bases
- Removal of introns

SIGNIFICANCE

- Post-transcriptional modifications of RNA accomplish two things:

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

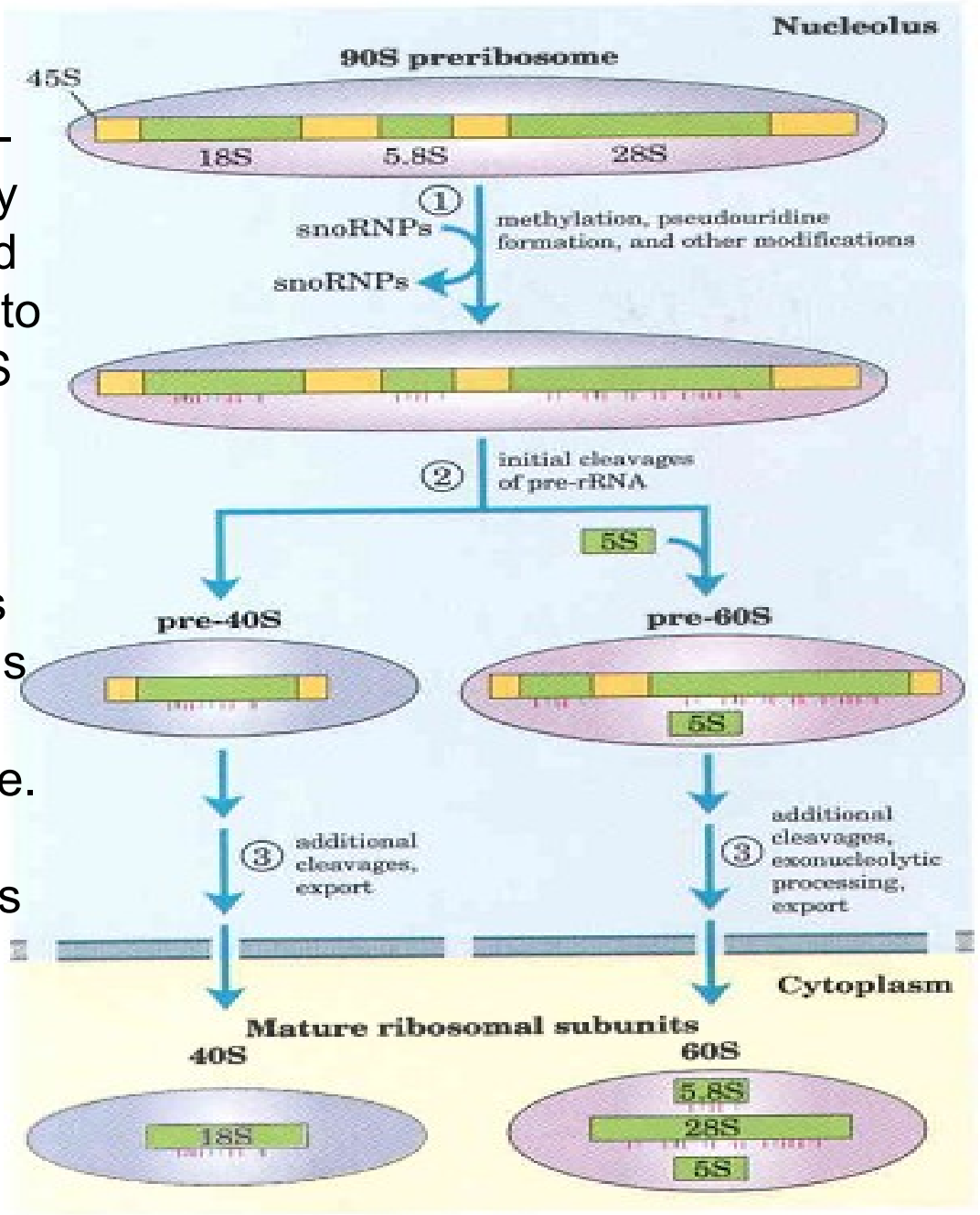
2) During post-transcriptional processing, portions of the RNA chain that are not supposed to be translated into proteins are cut out of the sequence. And thus, it helps increase the efficiency of protein synthesis.

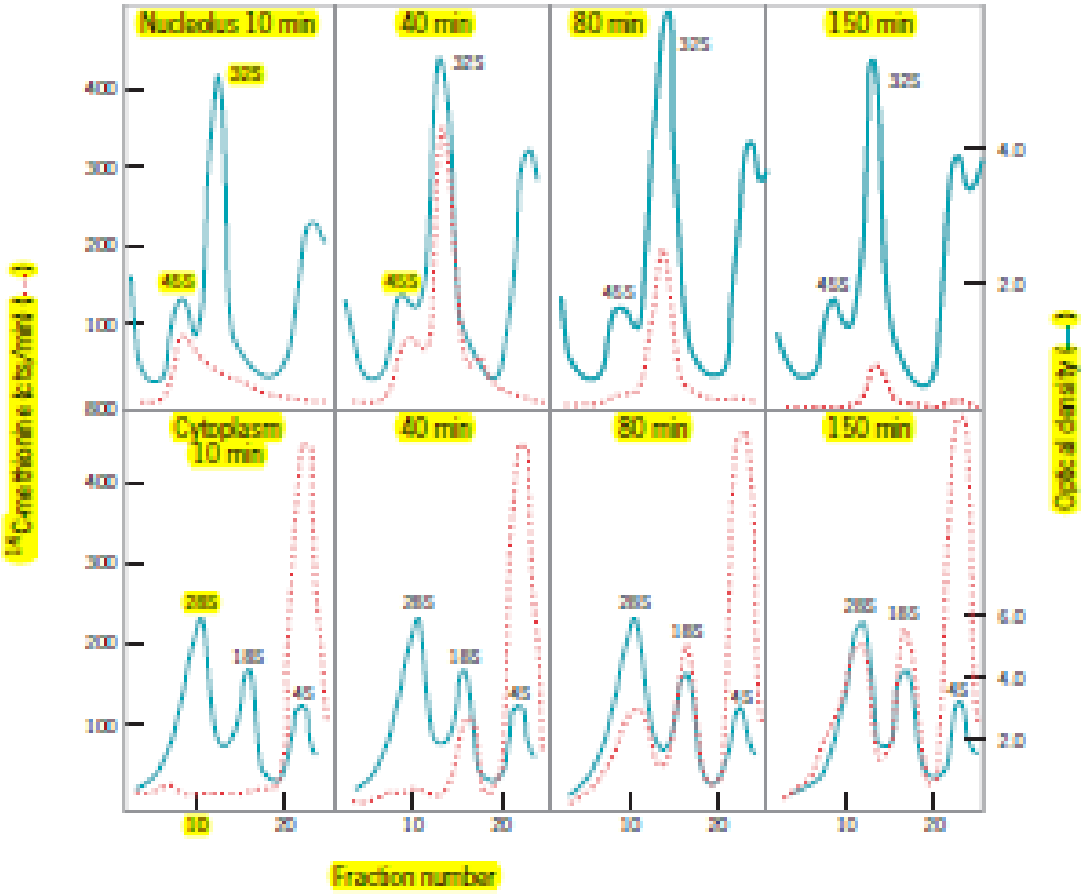
- Post-transcriptional modifications of rRNA
- Post-transcriptional modifications in snoRNAs

Post Transcriptional modification of rRNA

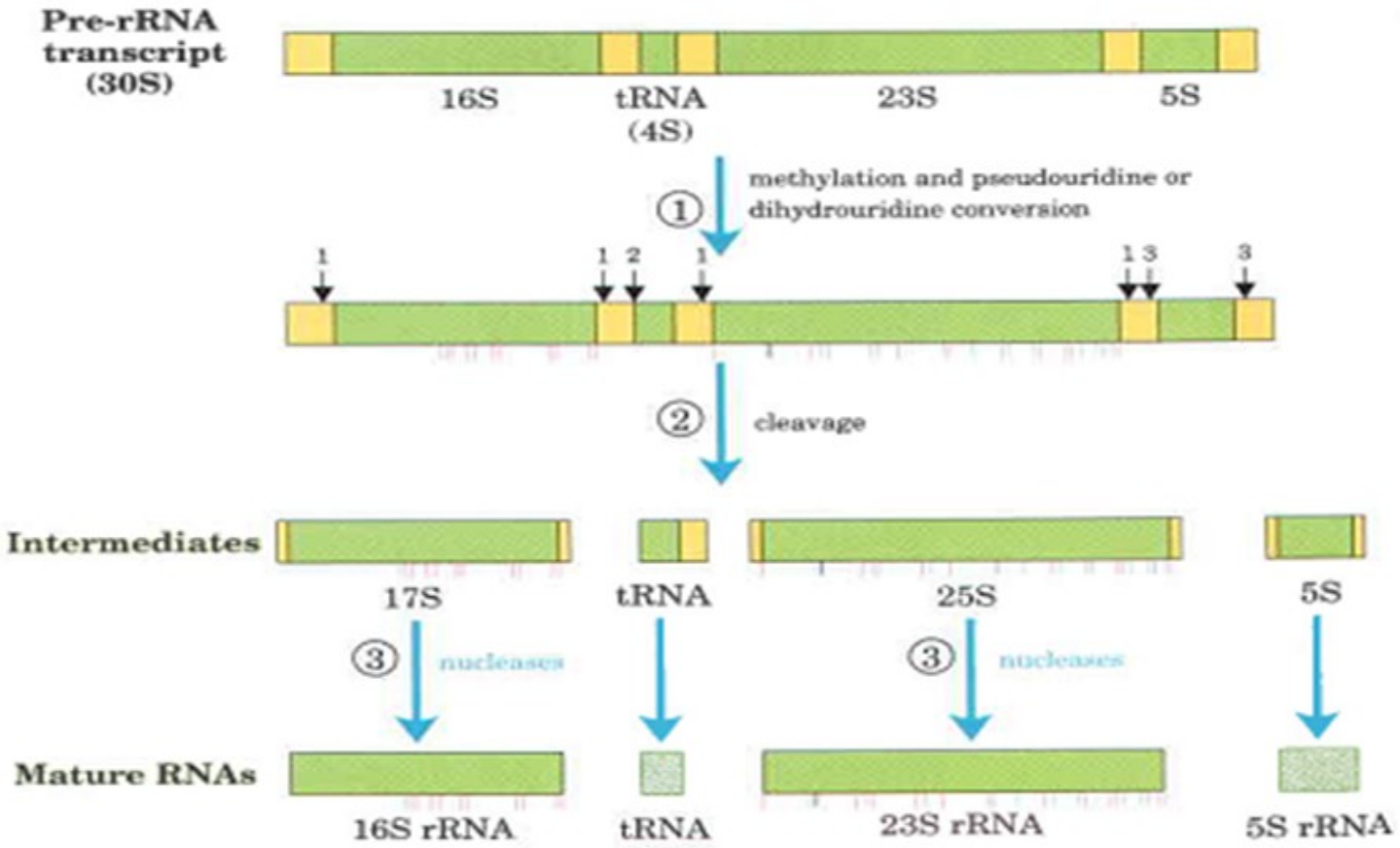
- Occur in both prokaryotic and eukaryotic rRNAs.
- Eukaryotic ribosomes have four distinct ribosomal RNAs.
- In humans, the large subunits contains a 28S,5.8S and 5S RNA molecule and small subunit contains an 18S RNA molecule.
- Out of these three rRNA are carved by various nucleases from a single primary transcript(pre-rRNA).
- The 5S rRNA is synthesized from a separate RNA precursor outside the nucleolus.

- In eukaryotes 45S pre-rRNA is synthesized by RNA polymerase 1 and processed in nucleolus to form 18S,28S and 5.8S rRNAs.
  - For it radioactively labelled methionine is used.So,methyl group is transferred from methionine to nucleotide.
- As a result 45S rRNA is cleaved into smaller molecules.



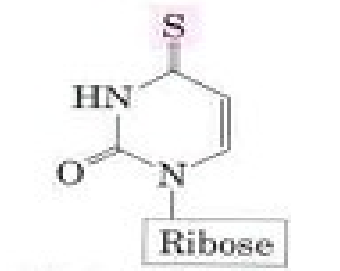

Nucleolus 10 min
40 min
80 min
150 min
Cytoplasm 10 min
40 min
80 min
150 min
32S
45S
28S
18S
5S
Fraction number
Optical density

- In Bacteria 16S,23S and 5S rRNAs arise from single 30S RNA precursor of about 6,500 nucleotides.

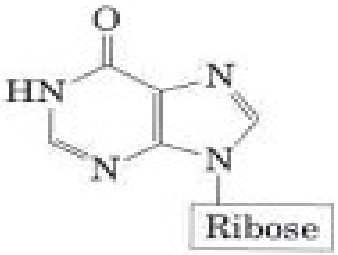


- In 16S rRNA of E.Coli, 11 modifications are of pseudouridine and 10 are of nucleosides methylated on the base.

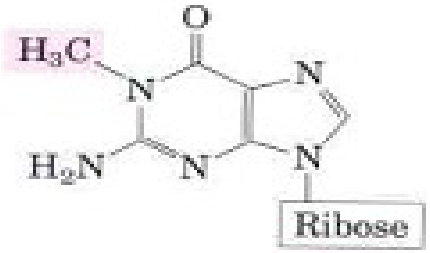
In humans ,if mutations of enzymes occur then there are chances of dyskeratosis.



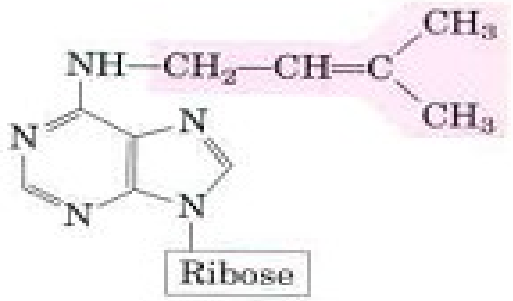
4-Thiouridine (S^4U)



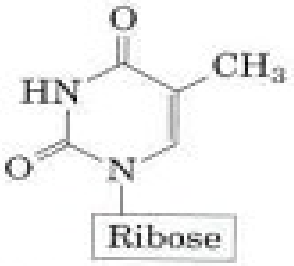
Inosine (I)



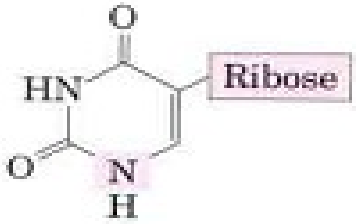
1-Methylguanosine (m^1G)



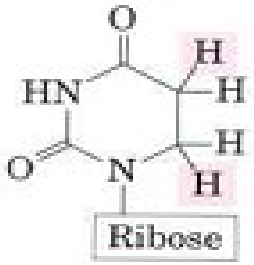
N^6-Isopentenyladenosine (i^6A)



Ribothymidine (T)



Pseudouridine (ψ)



Dihydrouridine (D)

Synthesis And Processing of 5S and 5.8S rRNA

- 5S and 5.8S present in prokaryotes and eukaryotes respectively.
- In eukaryotes,these are encoded by a large number of identical genes.
- These genes are transcribed by RNA polymerase III.
- It is unusual among polymerases in that it can bind to a promoter site located within the transcribed portion of the target gene.
- If the internal promoter from a 5S rRNA is inserted into another region of genome. The new site becomes a template for transcription by RNA polymerase III.

The Processing Of snoRNAs

- The processing of the pre rRNA is accomplished with help of large number of small ,nucleolar RNAs that are packged with particular proteins to form particles called snoRNPs.

- The first RNP particle to attach to pre-rRNA transcript contains the U3 snoRNA and more than two dozens different proteins.These are present in large quantities(10*6 copies per cell).

- A class of snoRNAs present at lower concentration is divided into two classes box C/D snoRNAs and second is box H/ACA snoRNAs.Both groups are long stretches (10-21) nucleotides.

Each snoRNA binds to the specific portion of pre-rRNA to form an RNA-RNA duplex.

- If the gene encoding one of these snoRNAs is deleted ,one of the nucleotides of the pre-rRNA fails to be enzymatically modified.

- The nucleolus is the site not only of rRNA processing,but also of assembly of two ribosomal subunits.

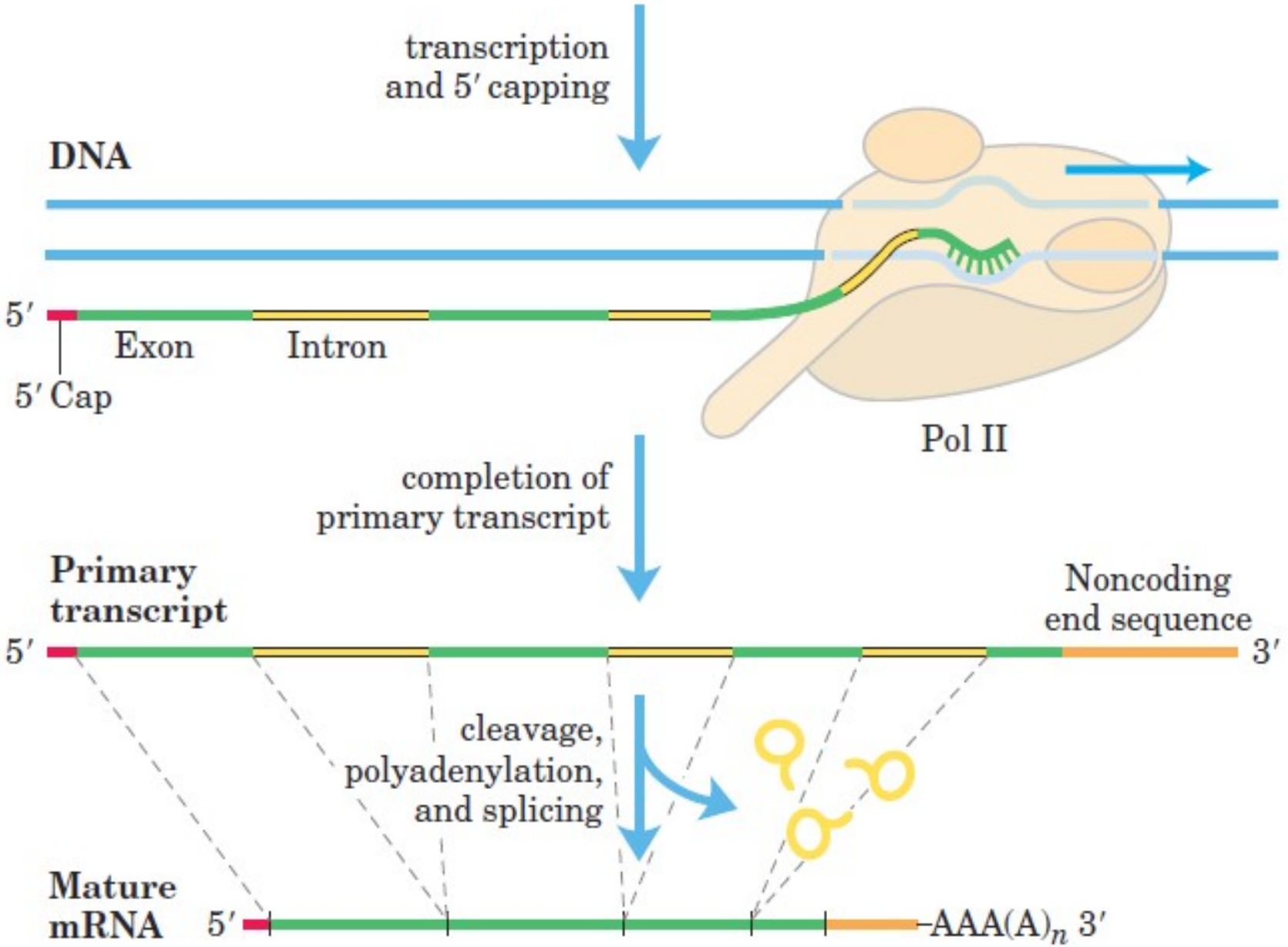
- Two types of proteins, ribosomal proteins (remains in subunits) and accessory proteins (that have transient interaction with rRNA intermediates) becomes associated with RNA.

- mRNA post transcriptional modifications
  - 5’ capping
  - Polyadenylation

mRNA processing

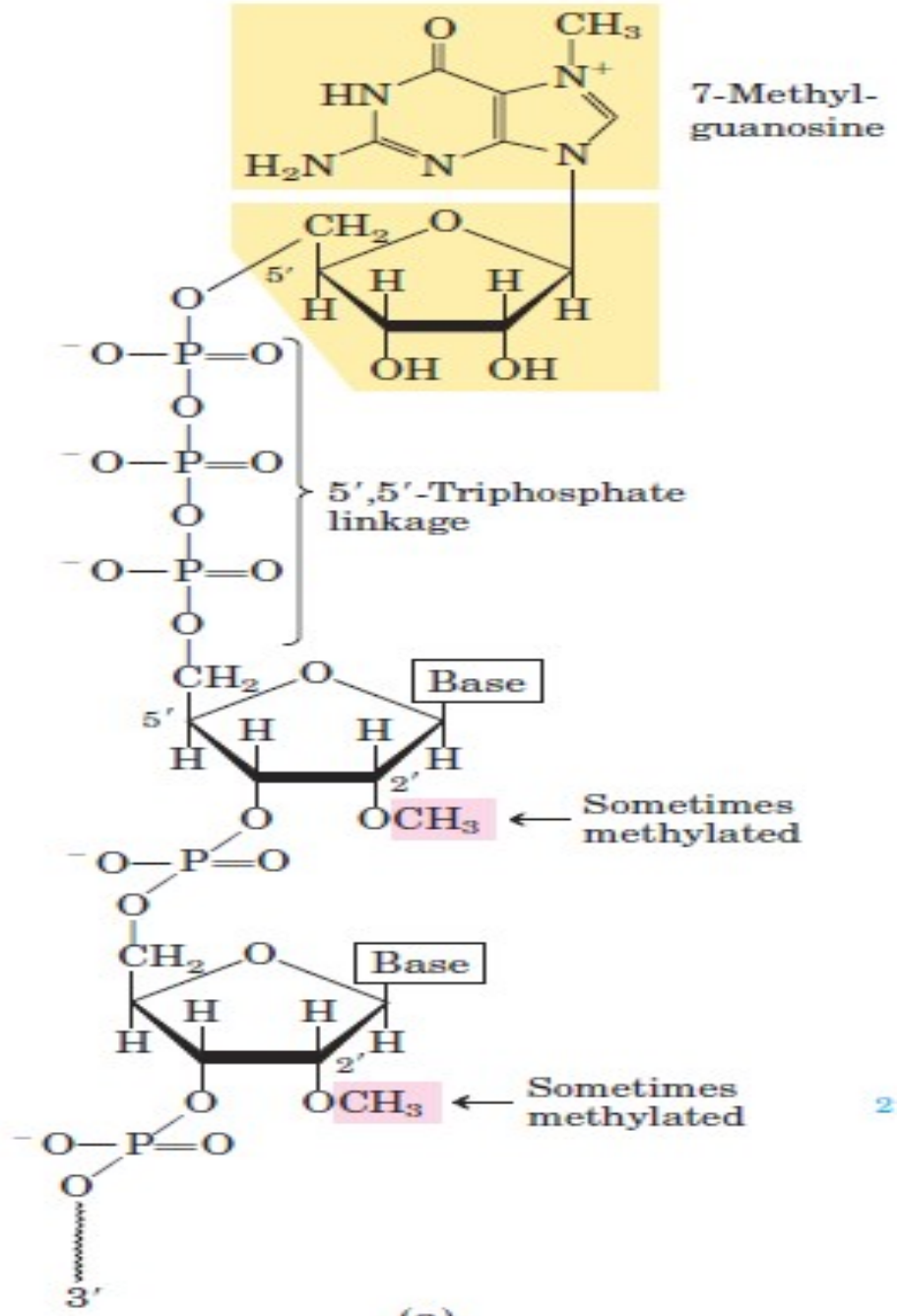
- Primary mRNA processed first and then transported to the cytoplasm.
- mRNA fate: complete and regulated degradation.
- 3 main steps in mRNA processing: (nucleous)

1. 5’ cap
2. polyAtail at 3’end
3. Splicing


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

Addition of 5’ cap

- 5’ cap: residue of 7-methyl guanosine.
- Protects the mRNA from ribonucleases.
- The cap is added after the first 20-30 nucleotides of the mRNA have been synthesized.
- Linked to the 5’ terminal residue of the mRNA by an unusual 5’5’triphosphate linkage.


7-Methyl-guanosine
5′,5′-Triphosphate linkage
Base
Sometimes methylated
Base
Sometimes methylated
3′

(a)

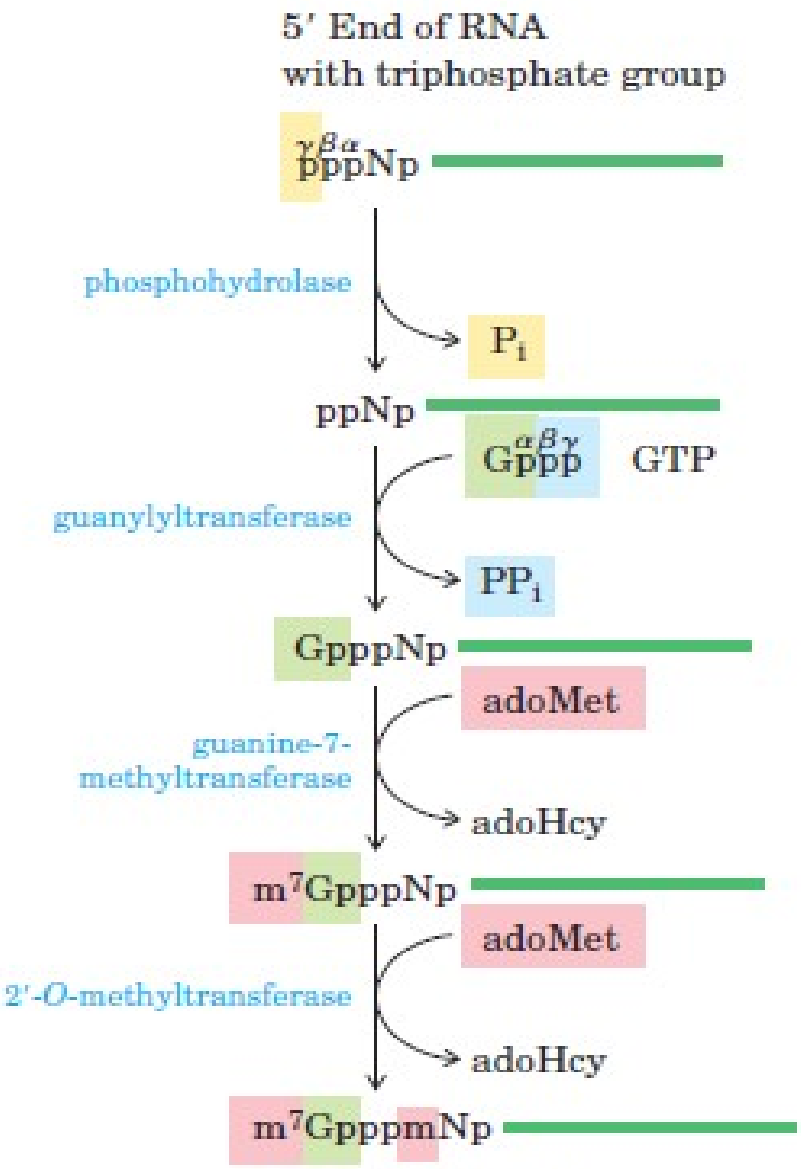
3 main steps:

- 1st step: from the 5' tri phosphate end of the mRNA, **phosphohydrolase** enzyme cleaves one phosphate group.
- 2nd step: **Guanyl transferase** enzyme will cleave 2 phosphate bonds from the guanosine residue(GTP) and form an unusual 5'5' triphosphate bond with the 5' end of the mRNA(with 2P).

- 3rd step: **Methyl transferase** will add a methyl group at the 7th position of the guanine base.
- Methyl group derived from:

  S-adenosylmethonine.
- Methylgroup sometimes also added at the 2'OH of the nucleotides adjecent to the cap.
- Done by the enzyme: (fig 2)

  2'-O-methyltransferase


5′ End of RNA
with triphosphate group
pppNp
phosphohydrolase
P_i
ppNp
GTP
guanylyltransferase
PP_i
GpppNp
adoMet
guanine-7-
methyltransferase
adoHcy
m^7GpppNp
adoMet
2′-O-methyltransferase
adoHcy
m^7GpppmNp
5′ End of RNA with cap

(b)

Poly A tail

- Poly A tail: At the 3'end of mature mRNA string of 80-200 adenine residues.
- Acts as a binding site for specific proteins that protects the mRNA from enzymatic destruction.
- mRNA synthesized beyond the region where the poly A tail is to be added.
- **2 main steps required:**

1. Cleavage of the mRNA at the 3' end
2. Addition of adenine residues.

Specific sequences

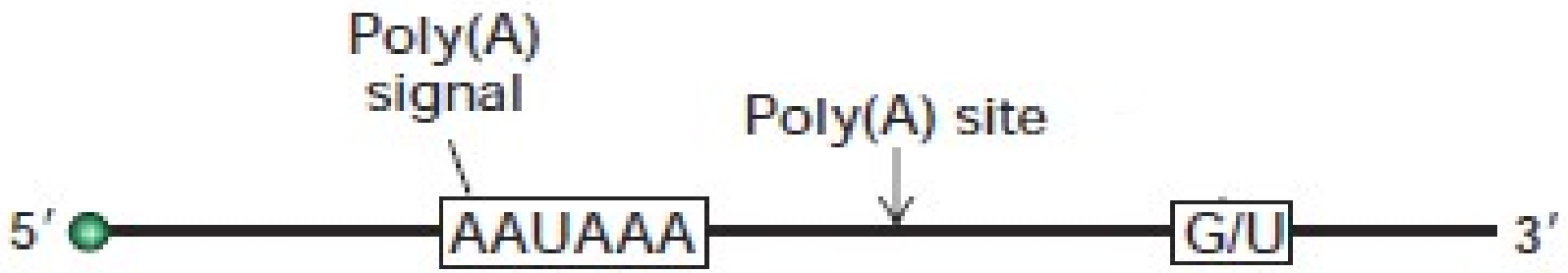
1. A cleavage sequence **CA**.
2. Poly adenylation signal sequence.

AAUAAA

Located 10-30 nucleotides upstream to the cleavage site. Highly conserved.

3. **GU** rich sequence present 20-40 nucleotides

downstream to the cleavage site.


Poly(A)
signal
Poly(A) site
5′
AAUAAA
G/U
3′

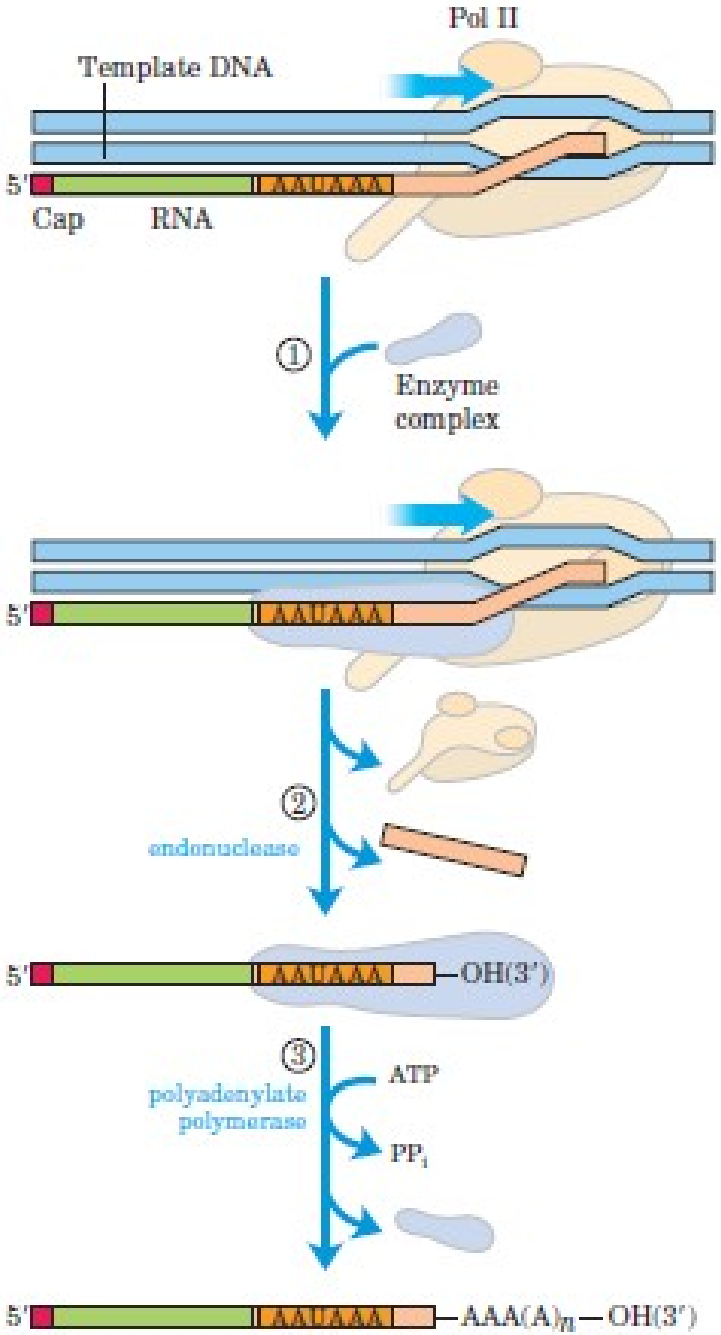
Enzyme complex: CPSF

- CPSF: Cleavage and polyadenylation specificity factor.
- This enzyme includes an endonuclease, a polyadenylate polymerase and several other multi subunit proteins involved in:

➢ Sequence recognition.

➢ Stimulation of cleavage.

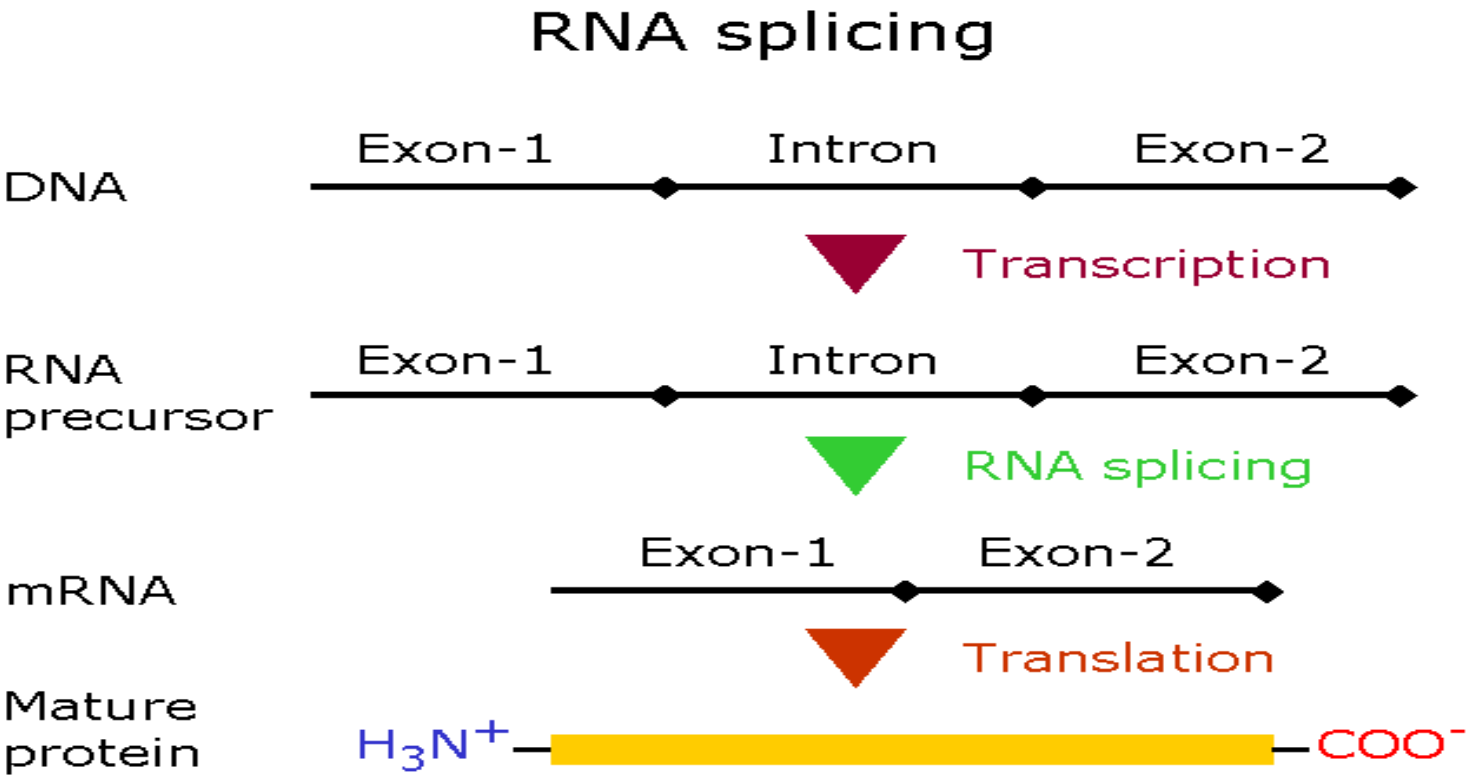
➢ Regulation of the length of poly A tail.


Pol II
Template DNA
5′
AAUAAA
Cap
RNA
①
Enzyme
complex
5′
AAUAAA
②
endonuclease
5′
AAUAAA
OH(3′)
③
polyadenylate
polymerase
ATP
PPi
5′
AAUAAA
AAA(A)n
OH(3′)

- Types of introns
- Splicing mechanisms of Group I and Group II introns

SPLICING OF MESSENGER RNA

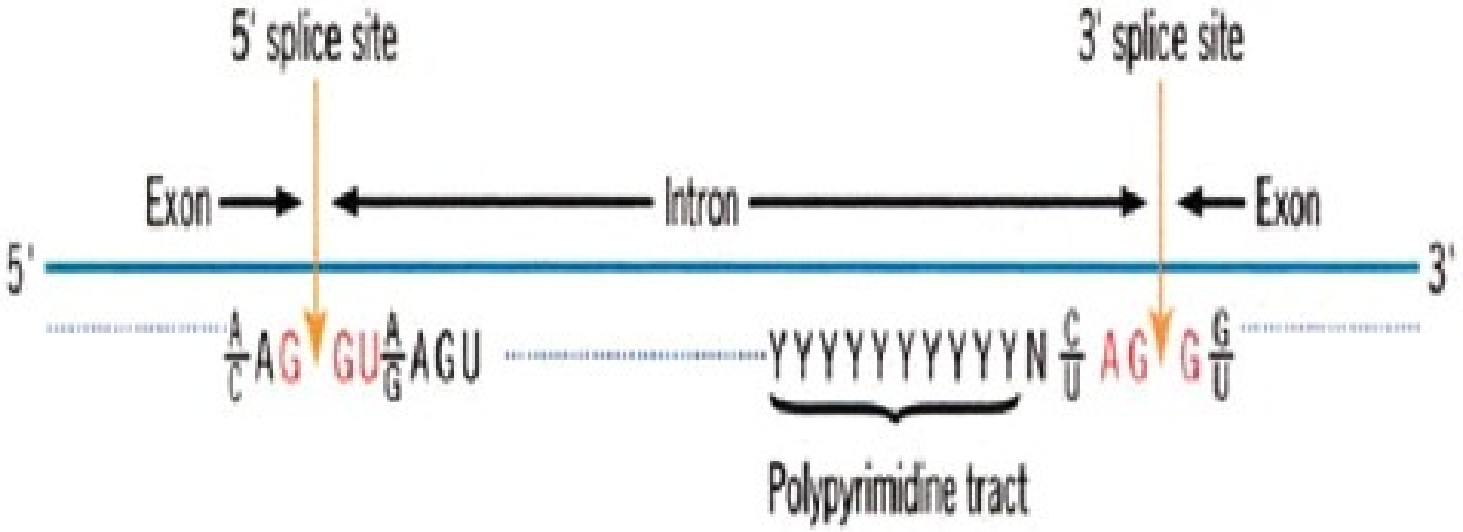
- Removal of introns from the primary mRNA transcript is known as **RNA Splicing.**



RECOGNITION OF SPLICE SITES

- The basic splicing machinery recognizes the exon-intron boundry due to :

1. Conserved nucleotide sequence in splice sites
2. Preferred nucleotide sequences in intron
3. Exonic enhancers present in exon



TYPES OF INTRONS

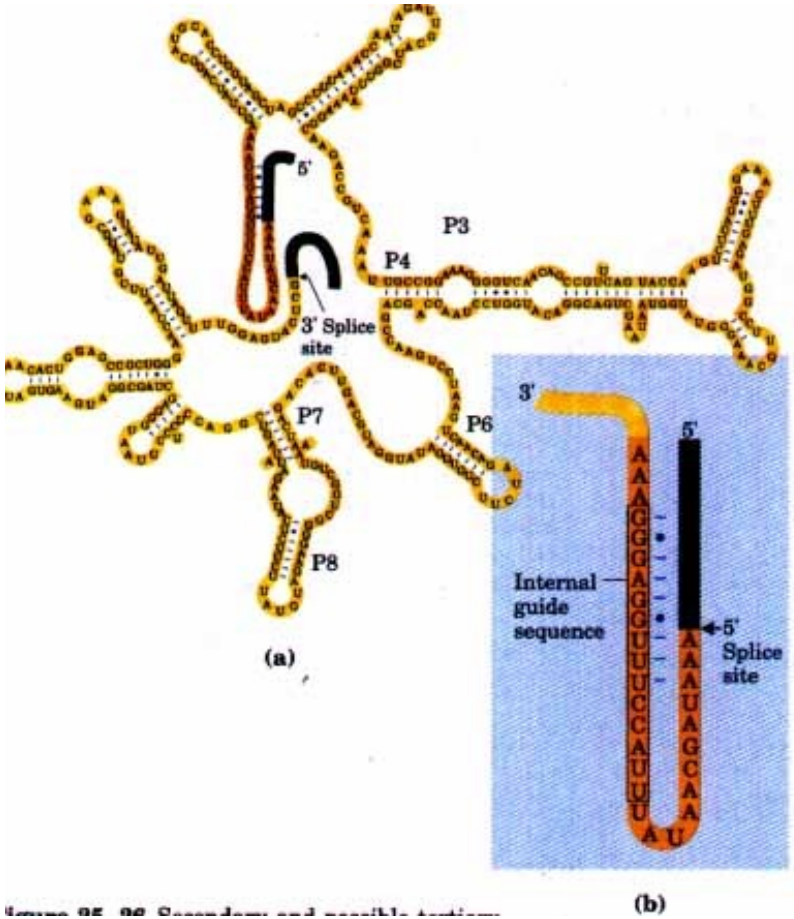
- Introns vary in size from 50 to 20,000 nucleotides.

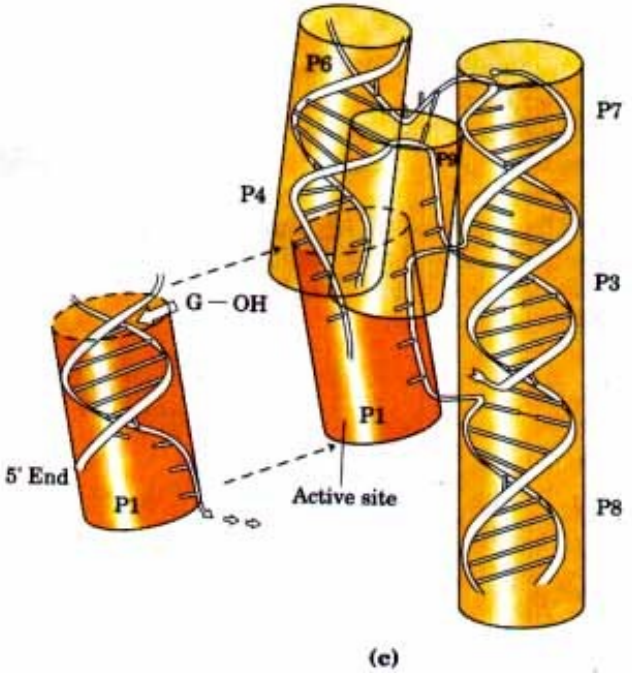
- There are 4 classes of introns:
  - *Group I*
  - *Group II*
  - *Group III (Spliceosomal introns)*
  - *Group IV (found in certain tRNAs)*

GROUP I INTRONS

- They are self splicing i.e. no protein enzymes are involved.
- They are found in some nuclear, mitochondrial, and chloroplast genes that code for rRNAs, mRNAs and tRNAs.
- No high energy factor is required.
- Self- splicing of introns was first revealed in group I rRNA in Tetrahymena thermophila by Thomas Cech and collegues in 1982.

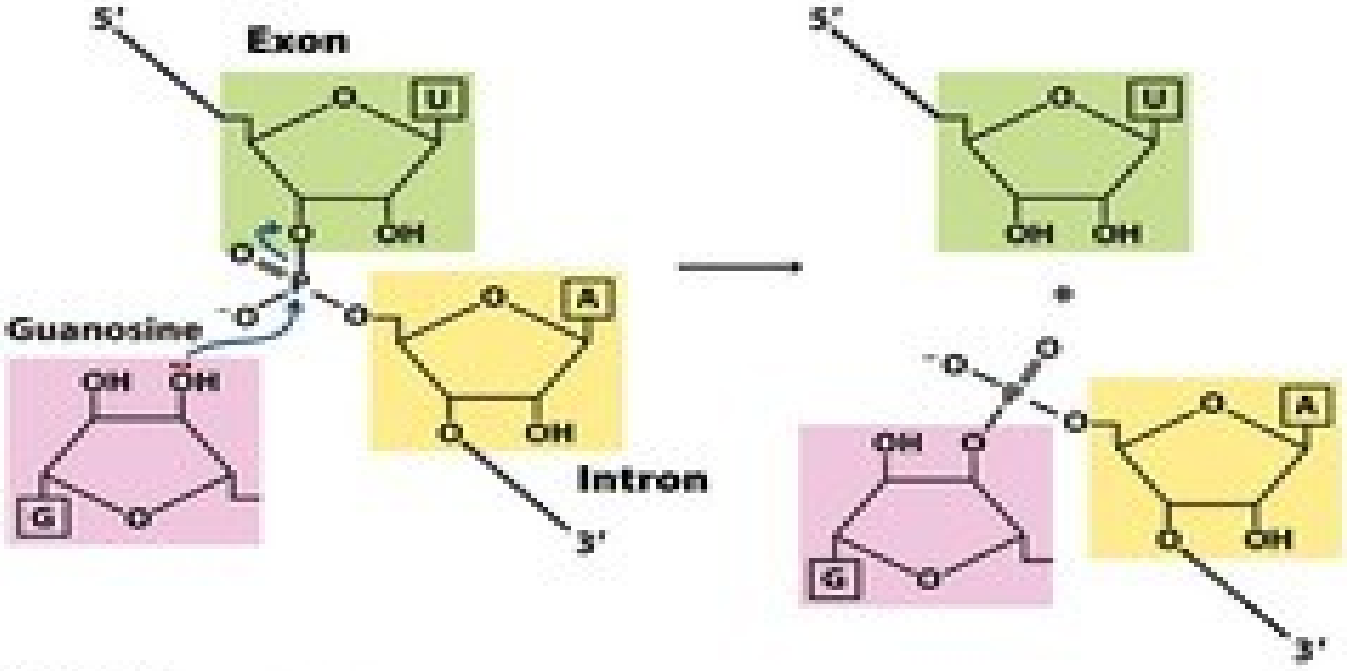
STRUCTURE OF GROUP I INTRONS



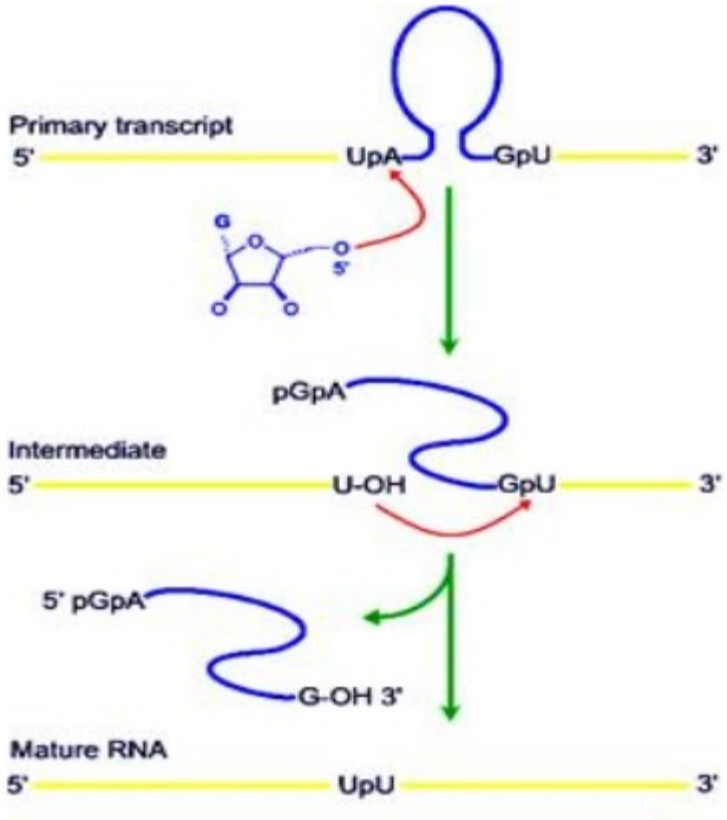


MECHANISM OF GROUP I INTRON SPLICING

- Splicing mechanism involves 2 tranesterification steps in which a nucleophilic attack is made on phosphorous and a new phosphodiester bond is formed.



MECHANISM OF GROUP I INTRON SPLICING

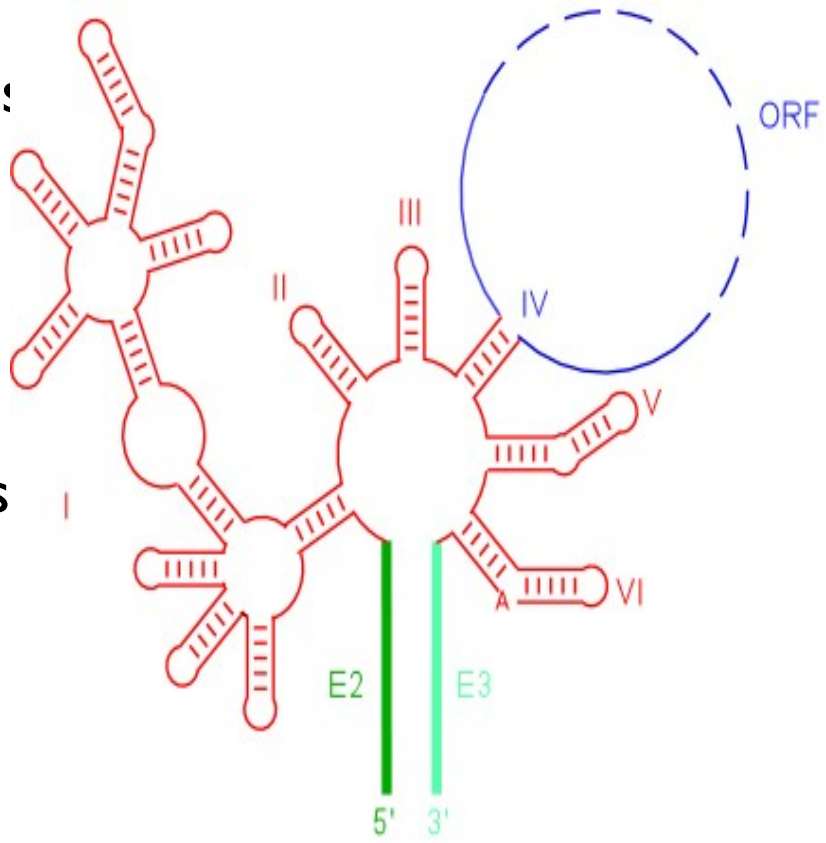


GROUP 2 INTRONS

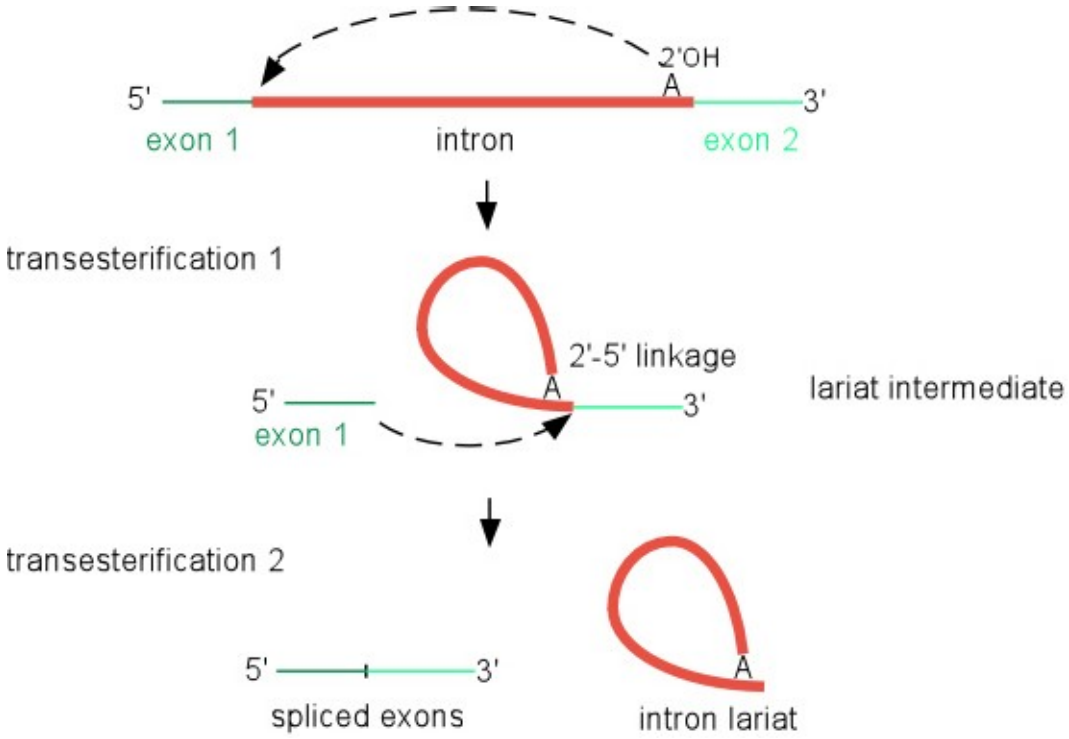
- Like group I introns, they are self splicing.
- They are found in primary transcripts of mitochondrial or chloroplast mRNAs in fungi, algae, plants and protists.
- Some group II introns are mobile genetic elements.

STRUCTURE OF GROUP 2 INTRONS

- Group 2 introns fold into a conserved secondary structure consisting of six domains arranged around a central wheel.
- Domain 6 contains a bulged adenosine residue, whose 2' OH is the nucleophile that initiates the splicing reaction.
- Domain 4 encodes intron encoded



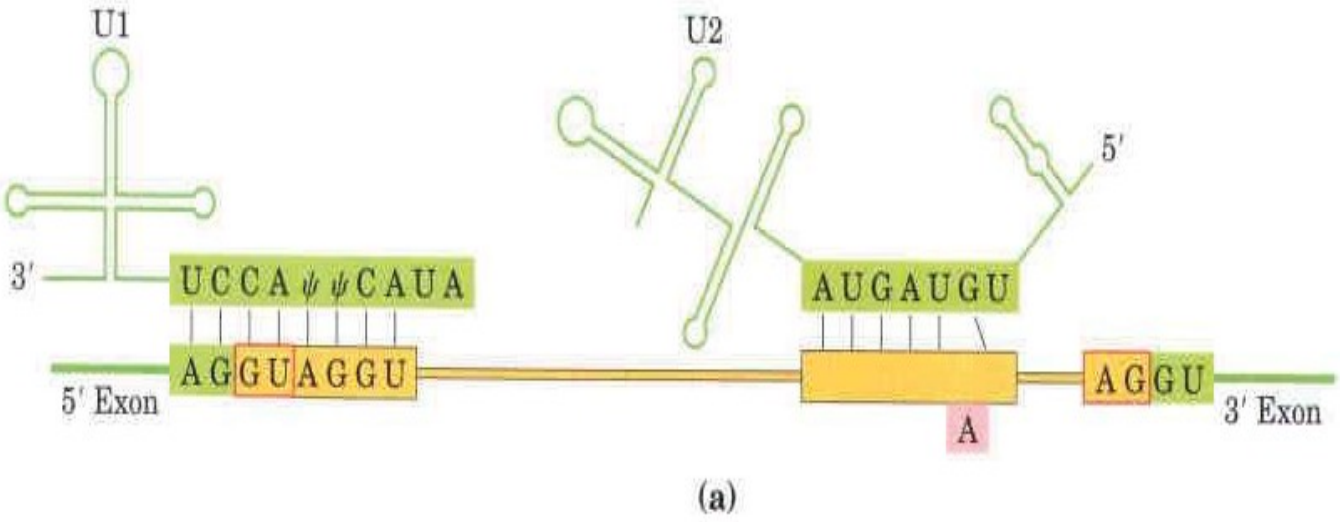
MECHANISM OF GROUP 2 INTRON SPLICING



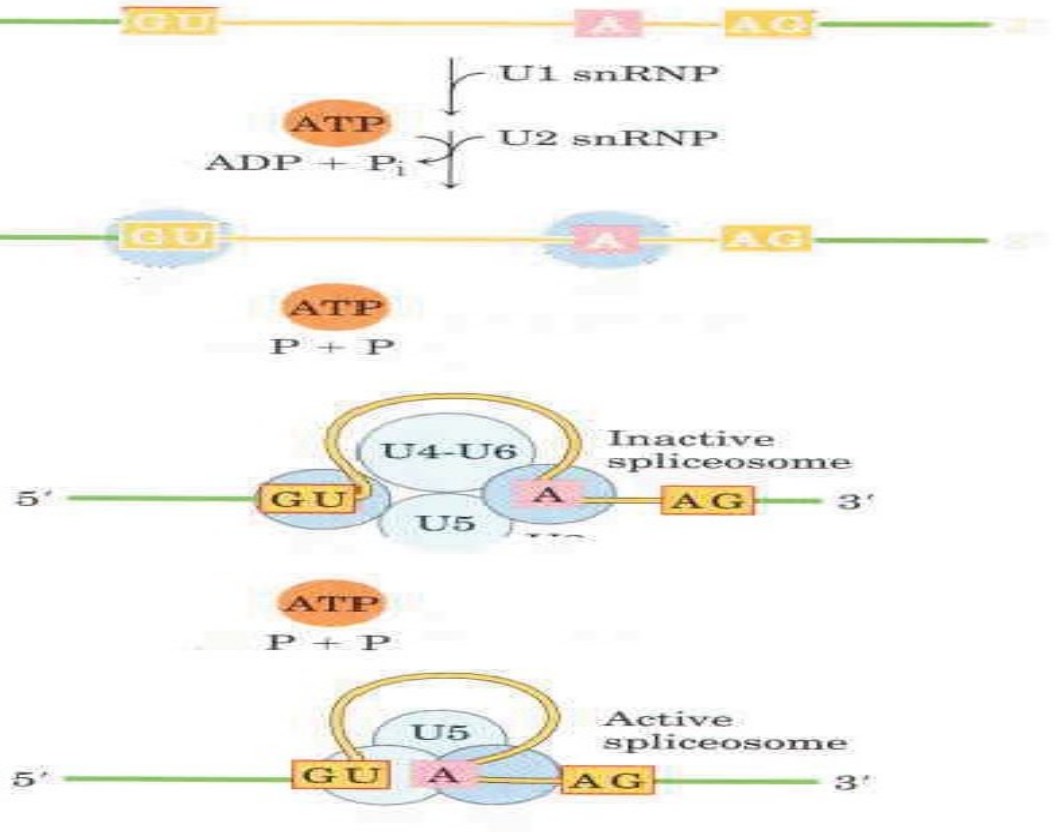
- Splicing mechanisms of Group III and IV introns
- Alternative splicing
- Post-transcriptional modifications of snRNAs

Group 3 Introns

- Largest class of introns
- These are not self splicing in nature
- Generally known as spliceosomal introns



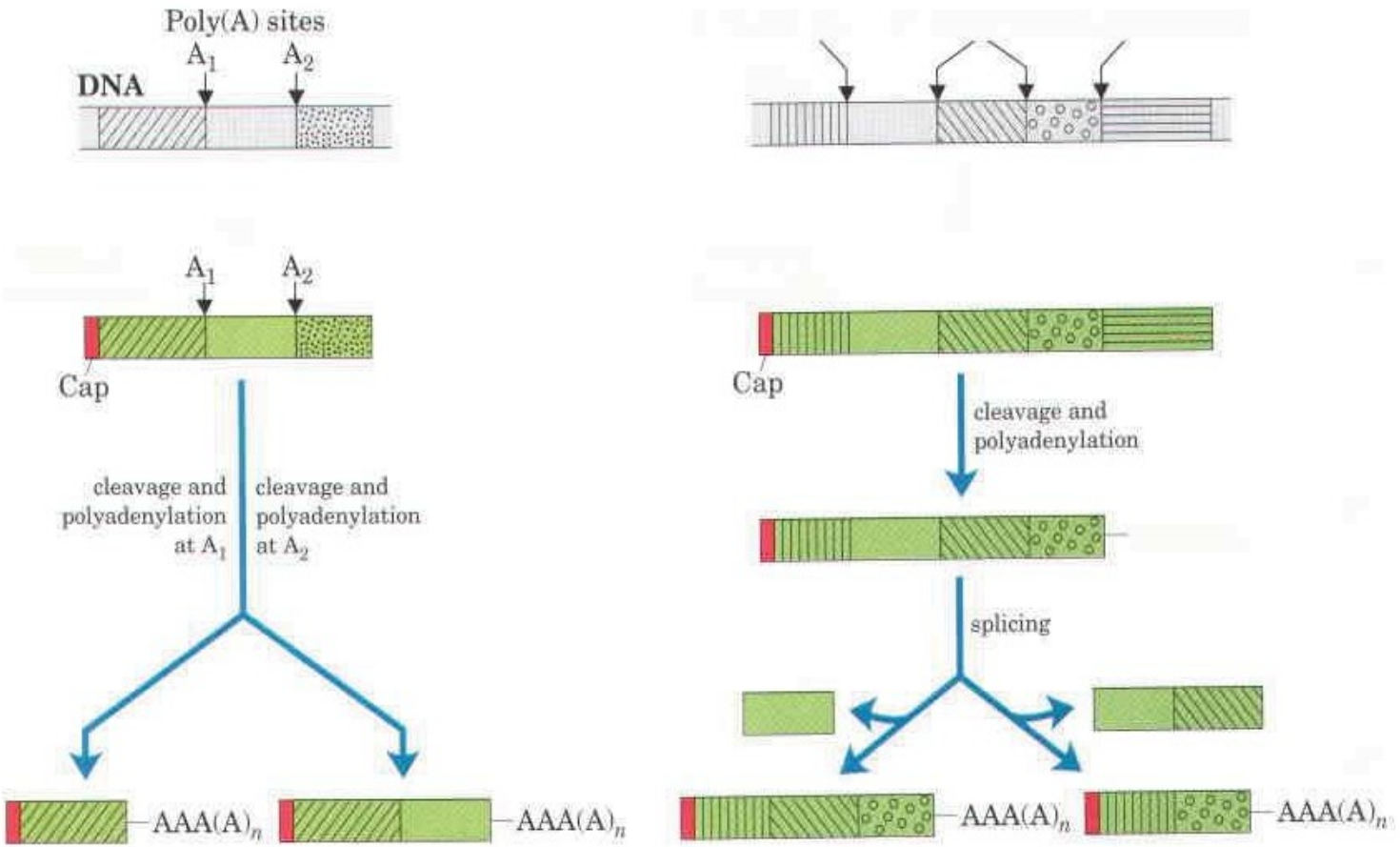
Splicing mechanism of Group 3 introns



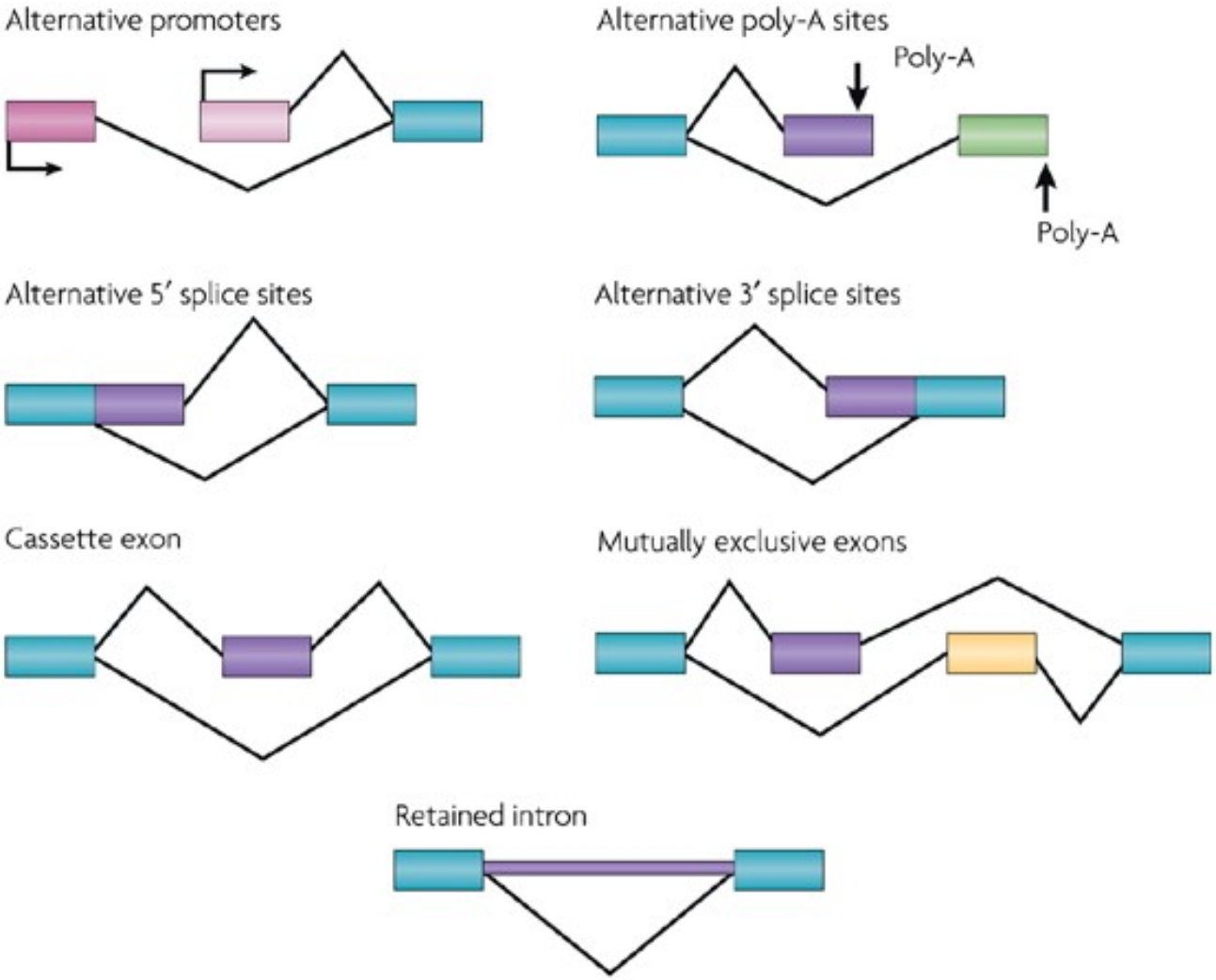
Group 4 Introns

- Found in certain tRNAs
- Require ATP and an endonuclease

Alternative Splicing

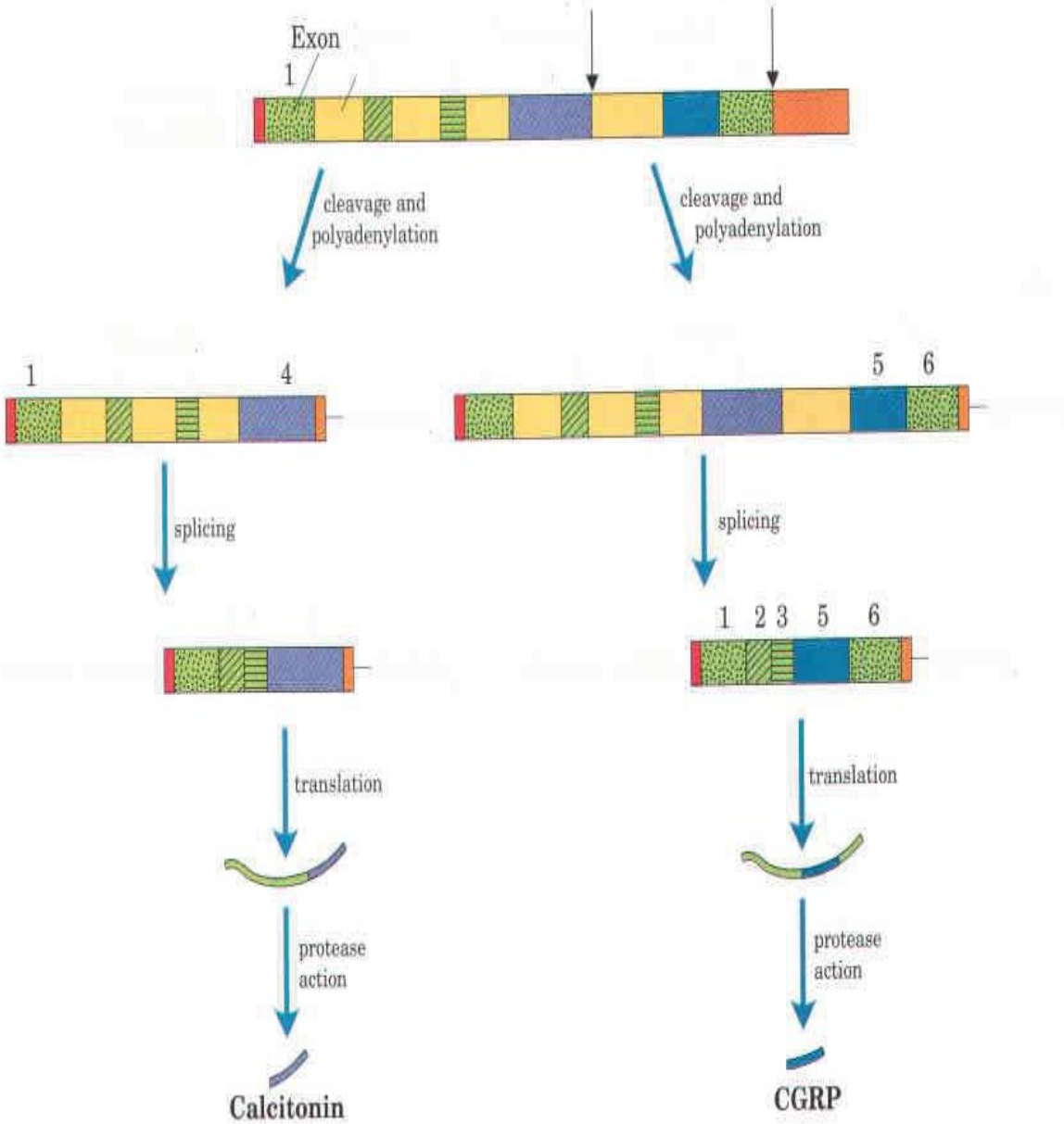


Variations to alternative splicing



Nature Reviews | Neuroscience

- Importance of Alternative splicing
  - Regulate gene expression
  - Responsible for tissue specific expression

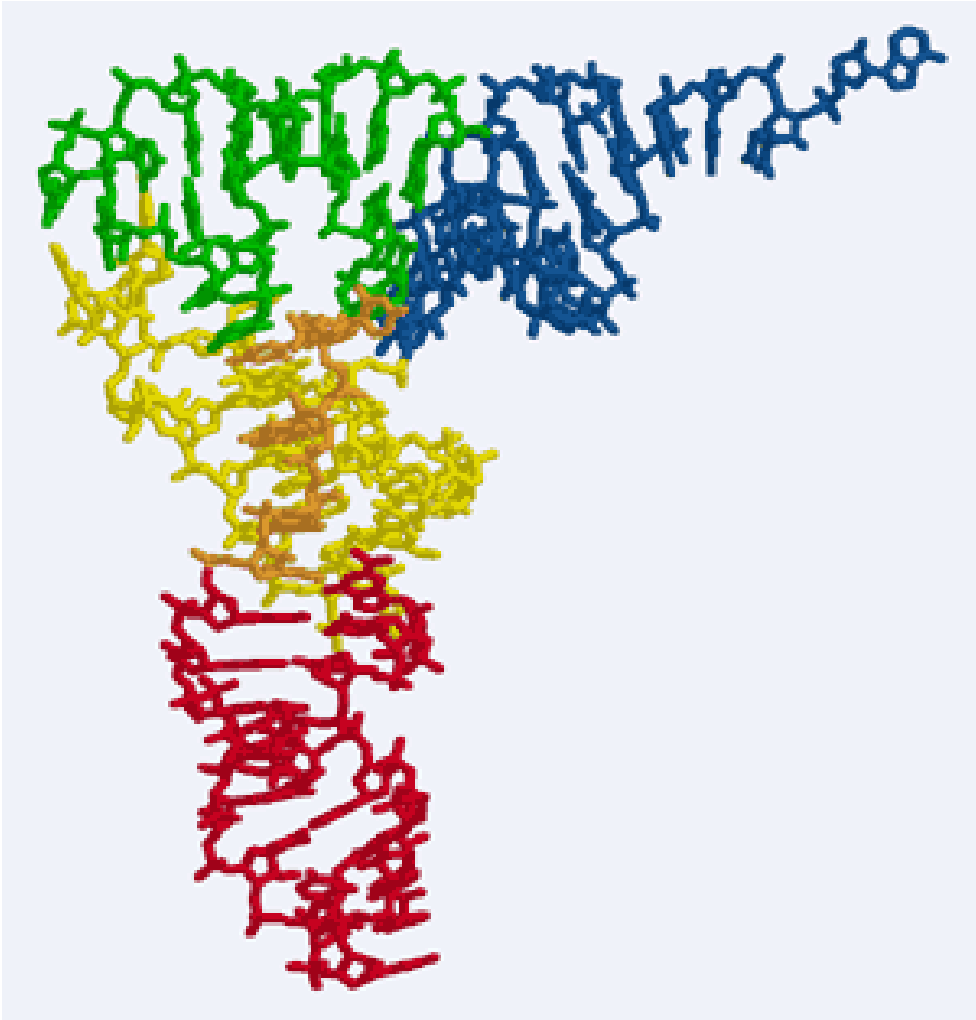

Exon
1
cleavage and polyadenylation
cleavage and polyadenylation
1
4
5
6
splicing
splicing
1 2 3 5 6
translation
translation
protease action
protease action
Calcitonin
CGRP

Post-transcriptional modification of snRNAs

- Synthesized as pre-snRNAs by RNA Poymerase II
- Processed by ribonucleases
- 2 O'methylation and conversion of uridine to psuedouridine being the most common modifications of nucleosides

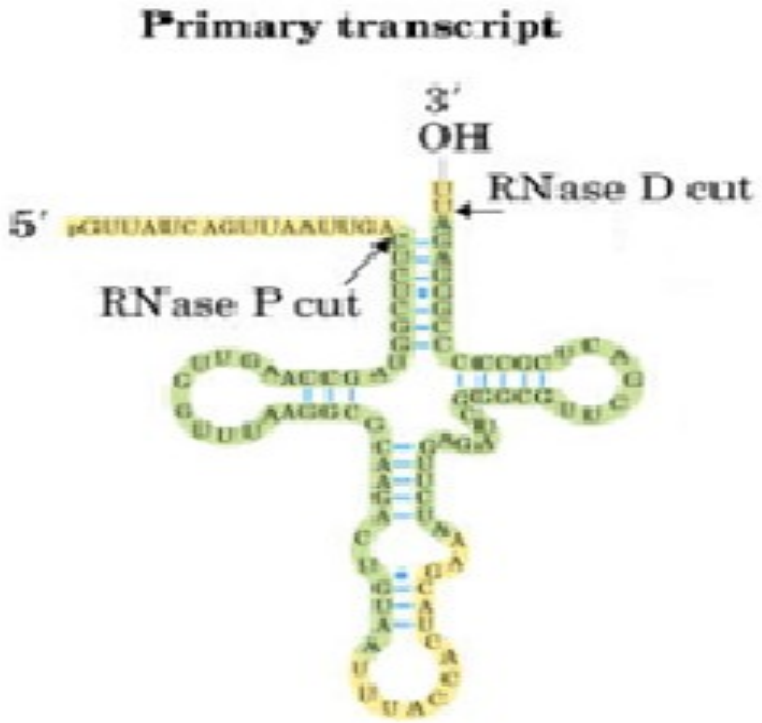
- Post-transcriptional modifications of tRNA
- Conclusion

PROCESSING OF t-RNA



Both prokaryotic and eukaryotic pre t-RNA undergo post transcriptional modification.

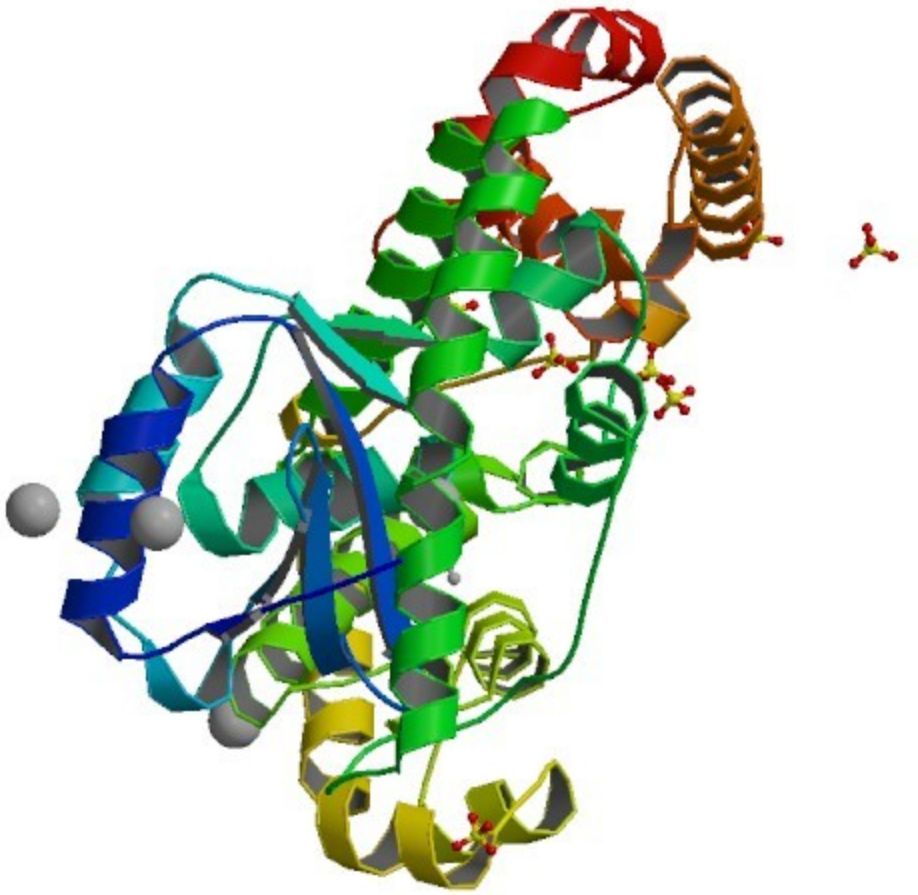
Flanking regions of the 3'-OH and 5' phosphate ends are cleaved by the endonuclease action of rnase D and rnase P respectively.



RNase D

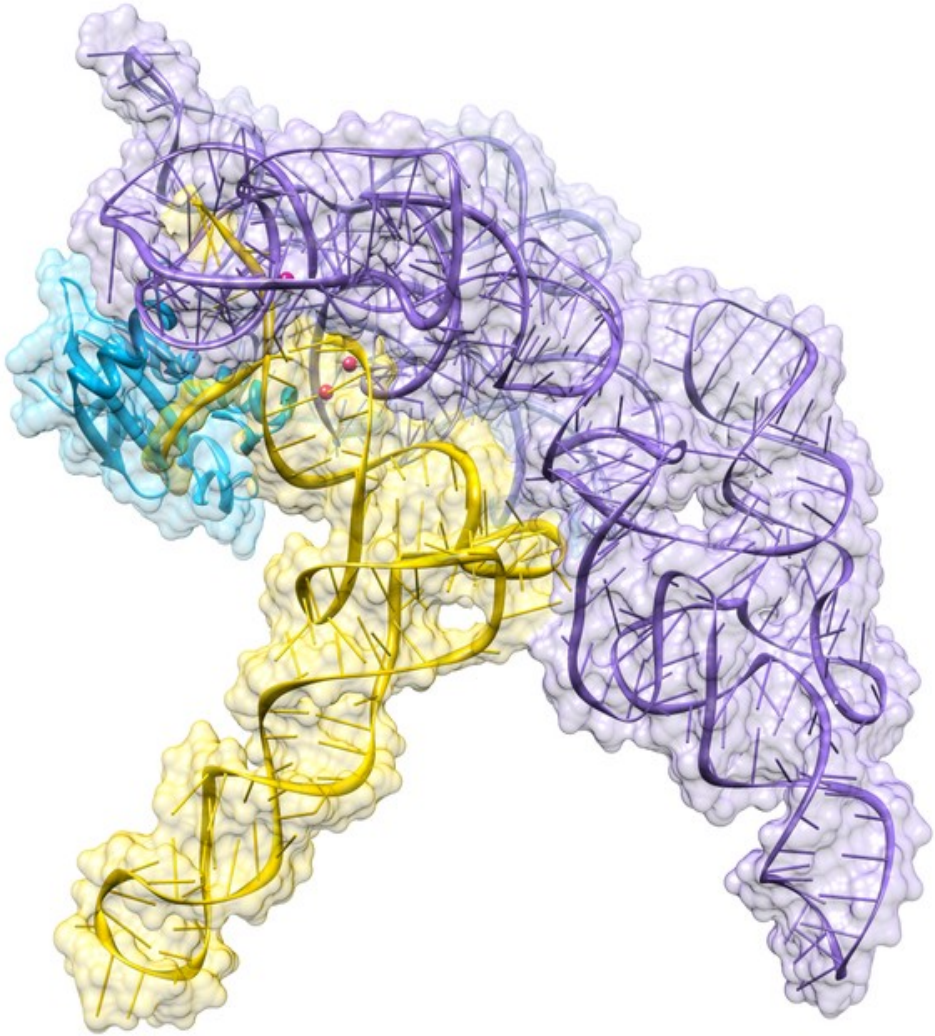
- One of the seven exoribonucleases identified in *E. Coli*

- Add the 3' CCA sequence to t-RNA in prokaryotic t-RNA processing.

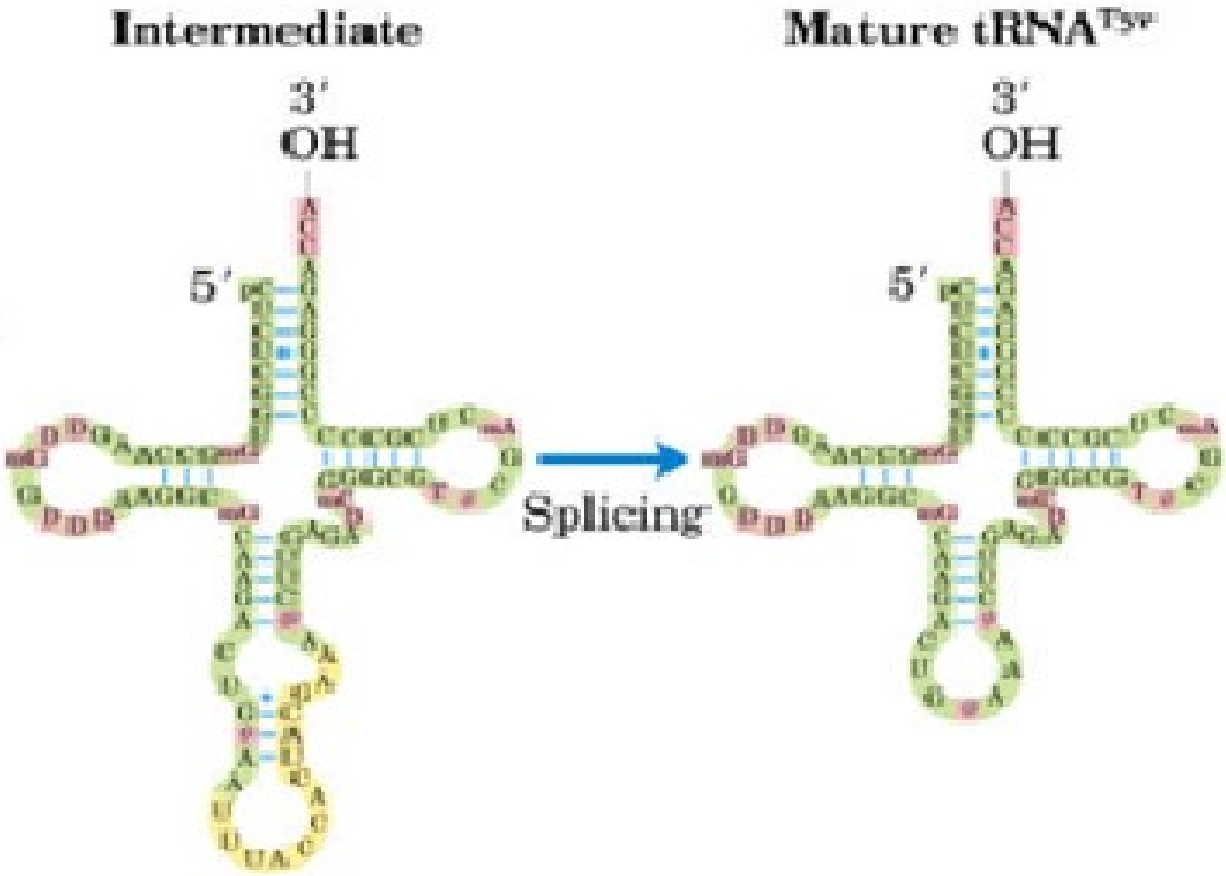


RNase P

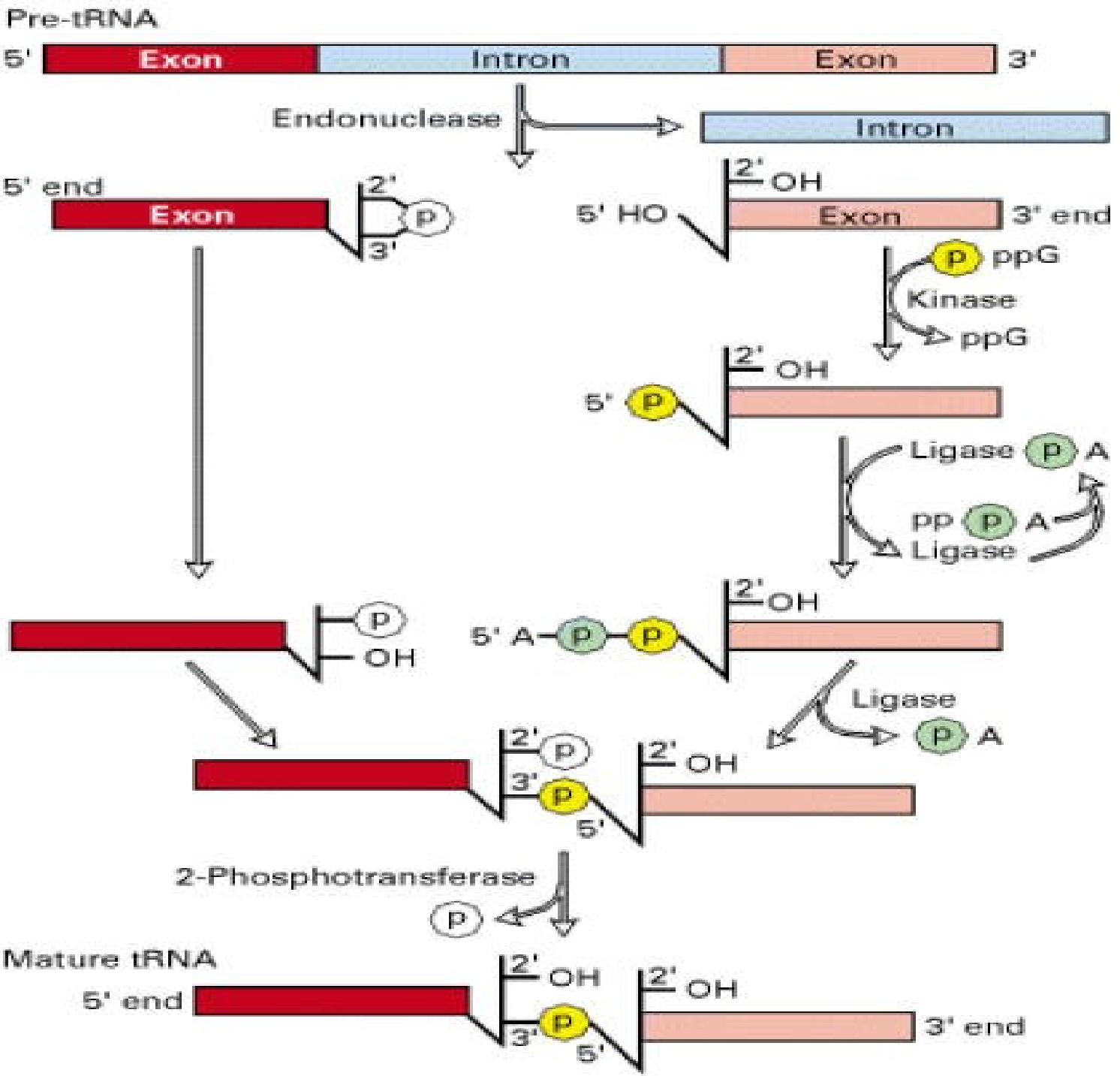
- Cleaves RNA
- Ribozyme



The introns in the anticodon loop space, spliced out by splicing reaction.



Splicing mechanism in pre-t-RNA differ from mechanisms utilized by self splicing introns and spliceosomes.


Pre-tRNA
5'
Exon
Intron
Exon
3'
Endonuclease
Intron
5' end
Exon
2'
P
3'
2'
OH
5' HO
Exon
3' end
P
ppG
Kinase
ppG
2'
OH
5'
P
Ligase
P
A
pp
P
A
Ligase
P
OH
5' A
P
P
2'
OH
Ligase
P
A
2'
P
3'
P
5'
2'
OH
2-Phosphotransferase
P
Mature tRNA
5' end
2'
OH
3'
P
5'
2'
OH
3' end

- Tri nucleotide CCA is added to the 3'-end to give 32'-OH ACC terminus.

Catalyzed by t-RNA specific nucleotidyl transferase.

CONCLUSION:

-5' cap (7-methylguanosine) and Poly-A Tail (200-300 adenine ribonucleotides) are added to primary transcript to protect the mRNA from digestion by nucleases and phosphates in the cytoplasm.

-5' cap is added to the 5' end of the primary mRNA transcript, and the Poly-A Tail is added to the 3' end. Regions known as exons; contain coding regions necessary for protein synthesis, while introns are "junk" DNA.

-Spliceosomes are made up of RNA and protein and cut out introns and join the remaining exons together.

- The sliced out introns are recycled inside the nucleus.
- The new, mature mRNA transcript is now ready to exit through a nuclear pore into the cytoplasm.

COMPARISON OF PROKARYOTIC AND EUKARYOTIC POST TRANSCRIPTIONAL MODIFICATION

- *Eukaryotes undergo post-transcriptional modifications including: capping, polyadenylation, and splicing.*

- *These events do not occur in prokaryotes.*

Thank You