RNA Processing

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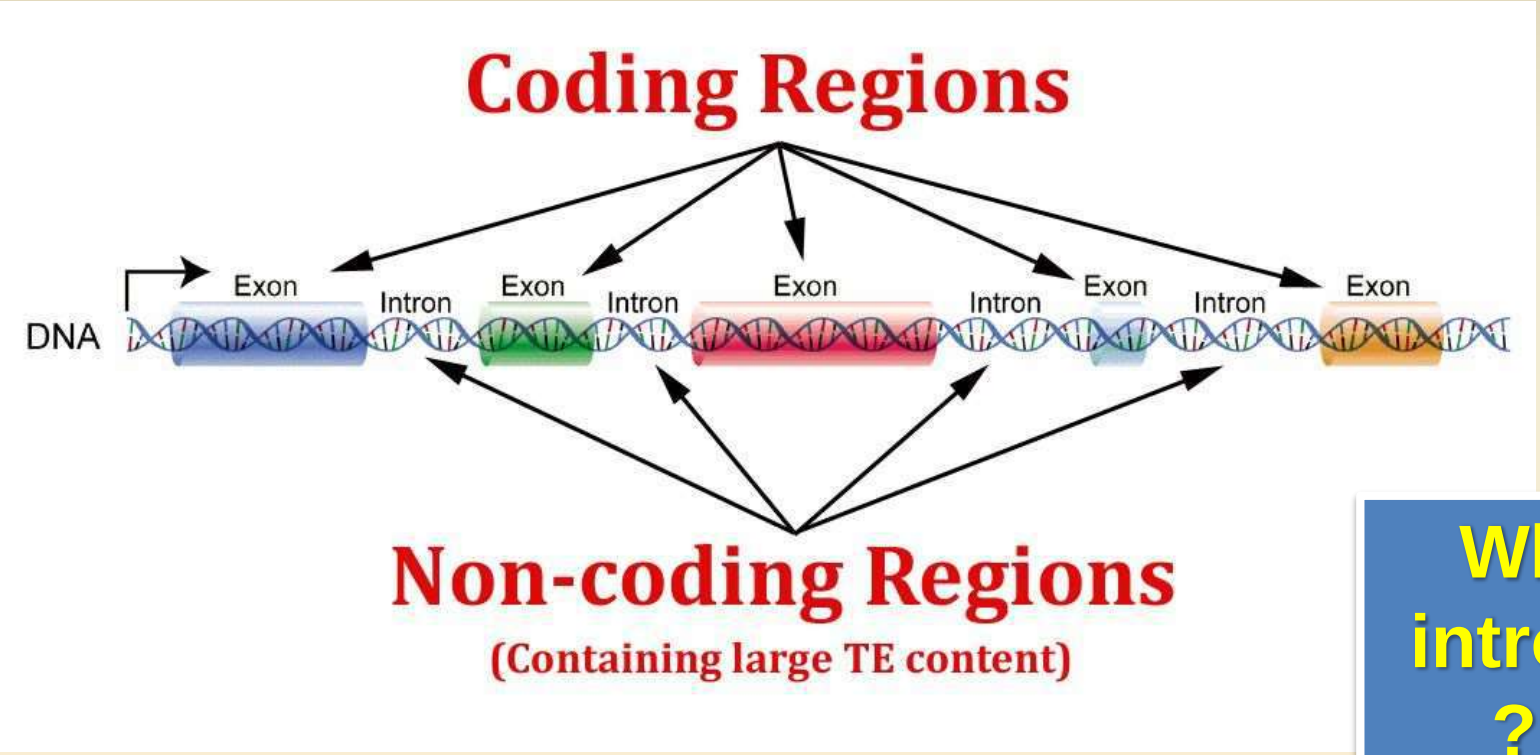
Introduction

- RNA processing is the term collectively used to describe the sequence of events through which the primary transcript from a gene acquires its mature form.
- It occurs in the nucleus and mature RNA transcripts (not all) are transported to cytoplasm.
- It does not occur in prokaryotes as prokaryotic gene do not contain introns.
  - *No evidence of these introns has been found in prokaryotes despite the sequencing of more than 100 genomes (Lynch and Richardson, 2002).*
- RNA processing process includes mRNA 5′- and 3′-end processing, intron splicing, and intercistronic cleavages of polycistronic messages, as well as typical tRNA and rRNA processing.
- Pre-mRNA (a primary transcript which is to be translated into a protein) is also processed to a mature form so that it can go for translation. The mRNA processing accomplished in 3 steps:
  - **5′ capping**
  - **3′ cleavage/polyadenylation, and**
  - **RNA splicing**

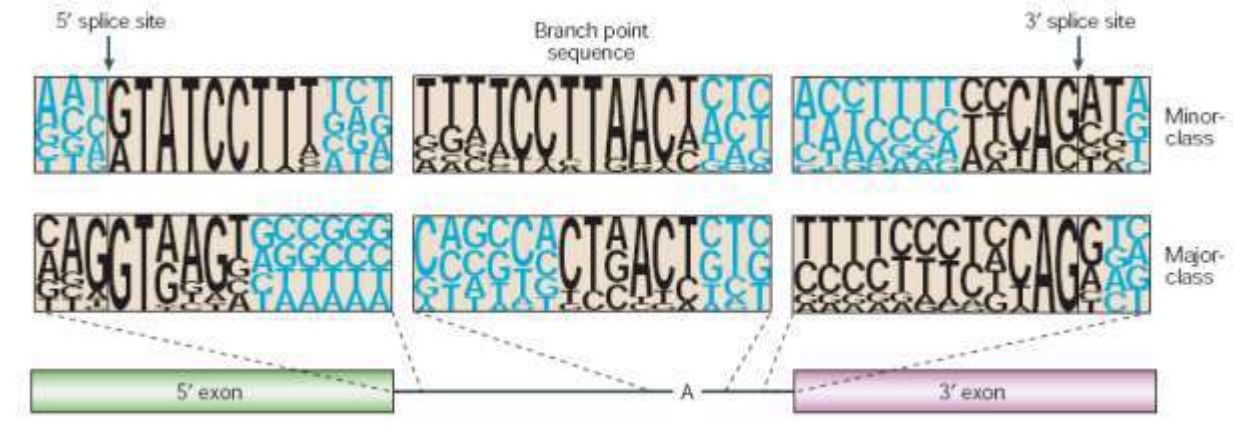
Structure of Gene



Why introns ??

- Genes are composed of both coding regions and non-coding regions. Coding regions are called as exons and non-coding regions are called as introns.
- There are two types of introns; major and minor introns.

Structure of Gene



Patel & Steitz Nat Rev Mol Cell Biol, 2003; 4(12):960–70.

- **Major introns:**
  - found in all eukaryotes
  - excision is dependent on U2 snRNA
  - poly-pyrimidine tract upstream of 3'splice site
  - more degenerate splice site signals
  - GT-AG, AT-AC and GC-AG
- **Minor introns:**
  - found in plants and most metazoan taxa (not in simple euklaryotes)
  - excision is dependent on U12 snRNA (frequency - 0.15 − 0.34%)
  - lack of poly-pyrimidine tract but splice site sequences are conserved
  - GT-AG, AT-AC, other RT-AN (R is purine, N is any nucleotide)

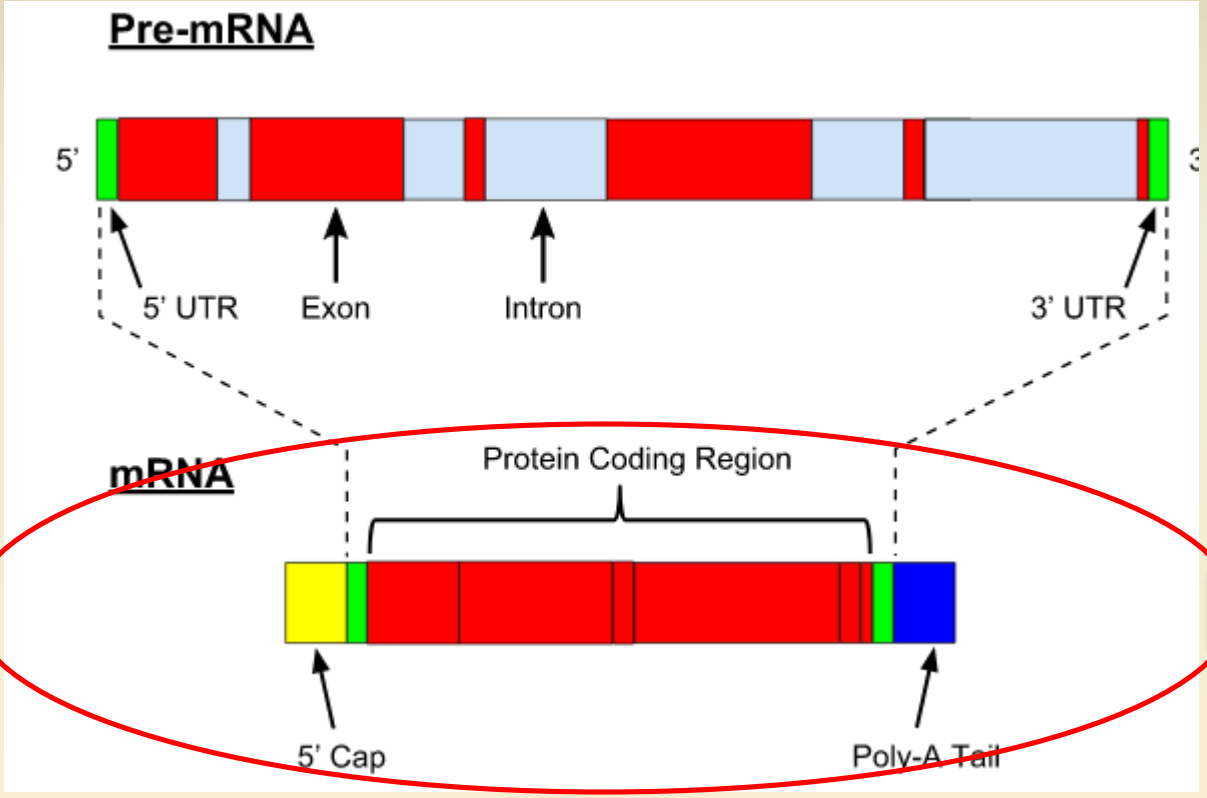
Structure of Gene

Major vs Minor spliceosome snRNAs and associated proteins

Spliceosome	snRNAs	Core associated proteins	References
Major	U1	Sm proteins^{+}, U1-A , U1-C, U1-70K	[4, 13, 15- 17]
	U2	Sm proteins^{+}, **12S**$^{\#}$: U2-A', U2-B'', 17S$^{\#}$: SF3a and SF3b complexes, hPrp43	[13, 16-19]
	U5*	Sm proteins^{+}, **20S**$^{\#}$: 52K, 40K, hPrp8, hBrr2, Snu114, hPrp6, hPrp28, hDib1	[13, 20, 21]
	U4/U6	Sm proteins^{+}, LSm proteins2-8, **13S**$^{\#}$: CypH, 15.5K, hPrp3, hPrp31, hPrp4	[13, 14, 22, 23]
Minor	U11/U12	Sm proteins^{+}, **18S**$^{\#}$: SF3b complex, 20K (*ZMAT5*), 25K (*SNRNP25*),31K (*ZCRB1*)	
		35K (*SNRNP35*), 48K (*SNRNP48*), 59K (*PDCD7*) 65K (*RNPC3*),Urp (*ZRSR2*)	[24-26]
	U4atac/U6atac	Share proteins with U4/U6 snRNAs of the major spliceosome	[22]

Jutzi et. al. Cell Stress 2018; 2(3): 40-54.

Structure of RNA transcripts



Monocitsronic or Polycistronic

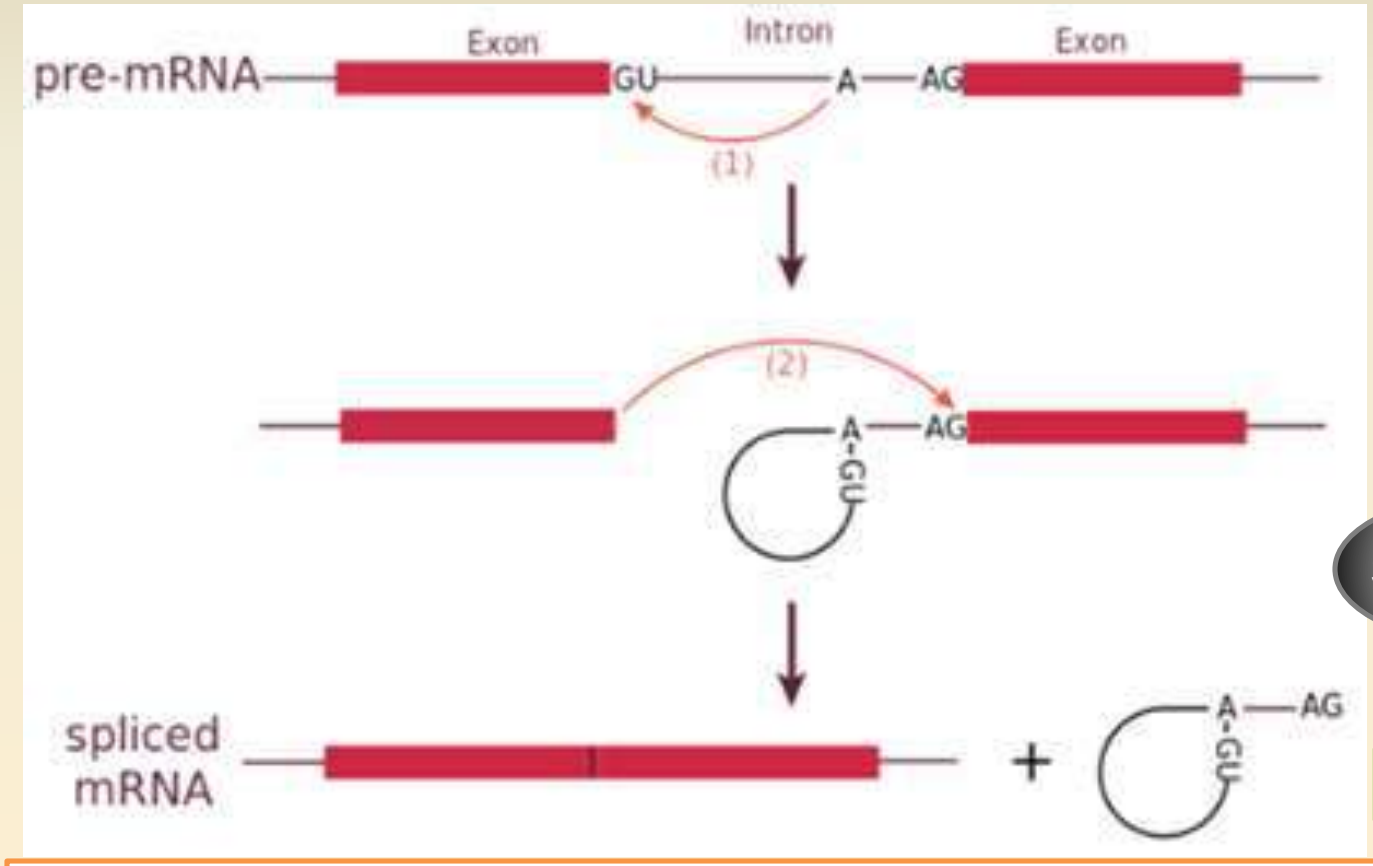
Pre-mRNAs are a type of heterogeneous RNA (hnRNA) that end up to cytoplasm.

to Translation

Since primary RNA construct contains untranslated regions (UTR; transcribed introns), it has to be removed to form a RNA transcript that represents a replica of gene and a template or a protein molecule – RNA SPLICING

RNA Processing

Splicing of Pre-mRNA



Spliceosome

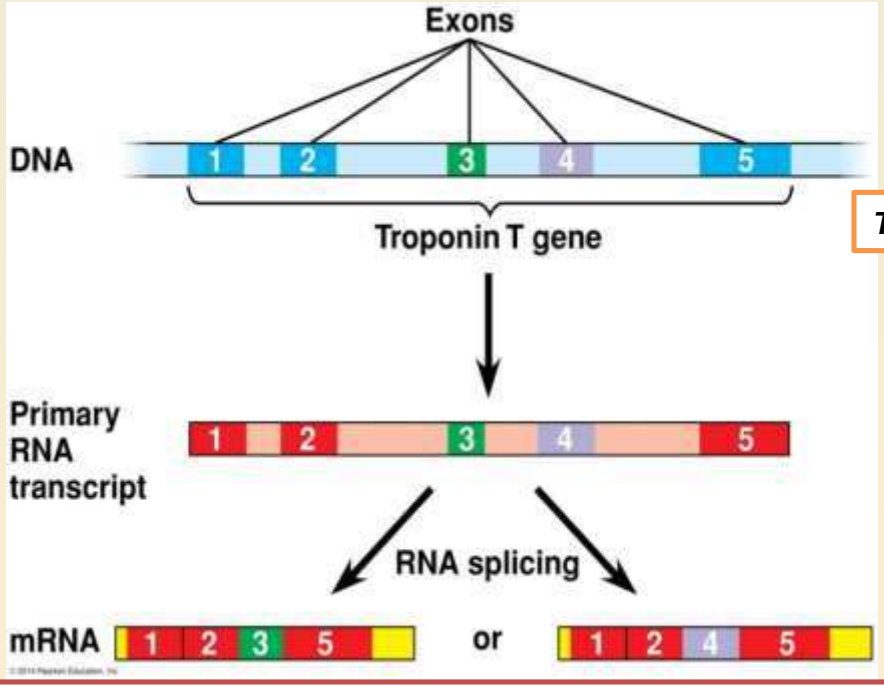
Introns ?

- Spliceosomes cut the introns at specific locations and joins two exons to make a continuous translable gene transcript.

RNA Processing

Alternative RNA Splicing

- Alternative splicing is a controlled molecular mechanism producing multiple variant proteins from a single gene
- The introns are removed and exons are spliced together to for mRNA from pre-mRNA.



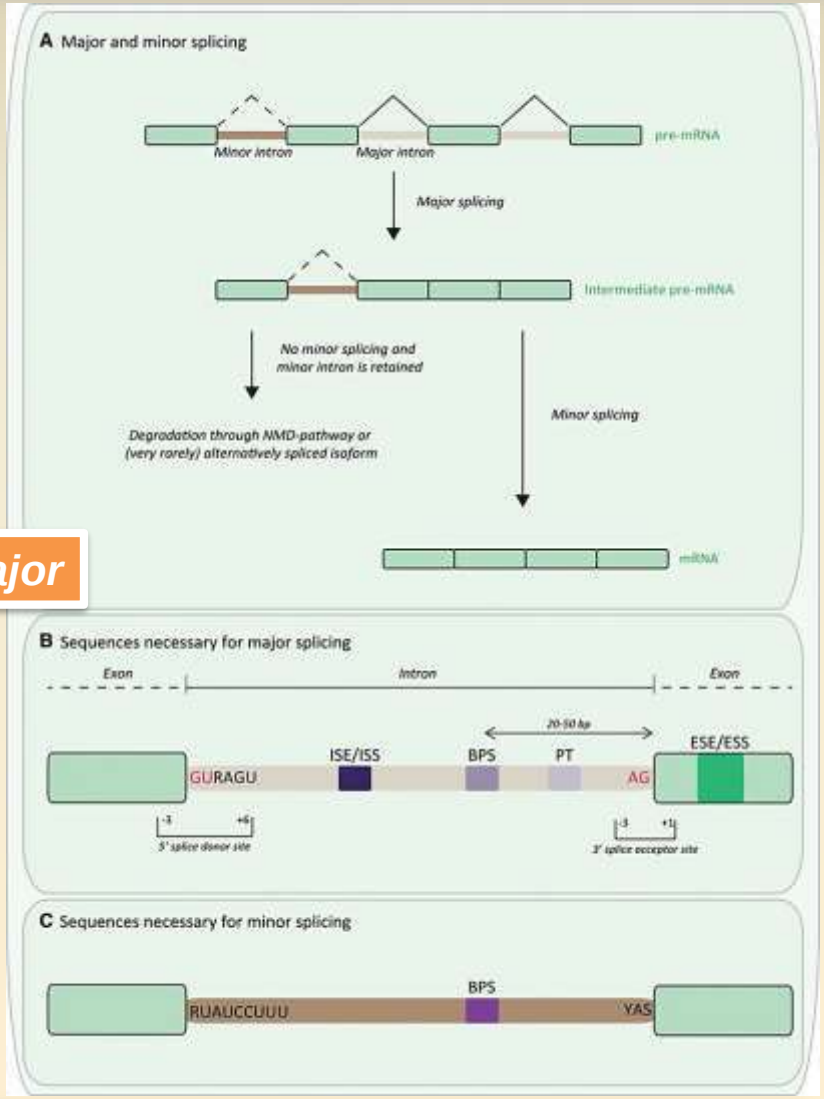
Triponin gene as for example

Pre-mRNAs are first coated in RNA-stabilizing proteins; these protect the pre-mRNA from degradation while it is processed and exported out of the nucleus.

RNA Processing

Mechanism of RNA Splicing

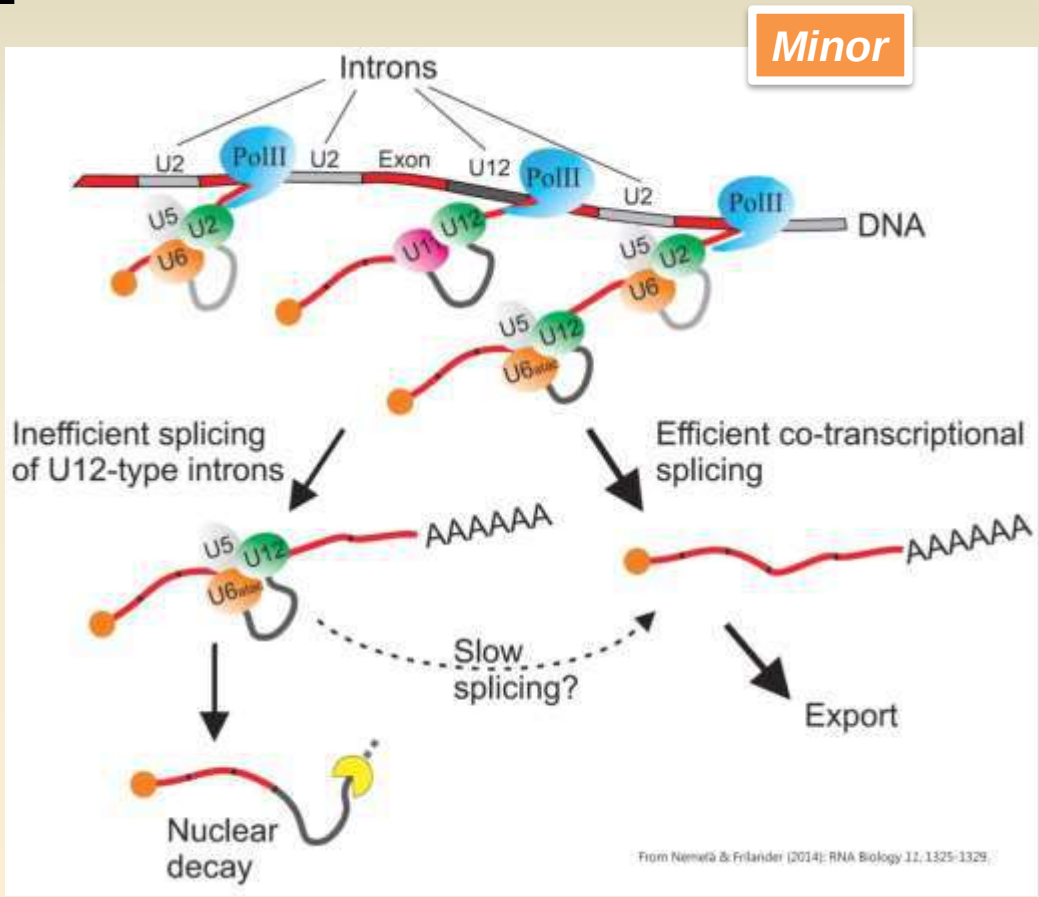
- There are two types of introns based on spliceosome – major introns that are U-2 dependent, and minor introns that are U-12 dependent.
- The major introns are spliced out, and minor introns are either retained (the mRNA is most often subsequently degraded) or the minor intron is spliced out, and a mature mRNA is formed.
- Spliceosomal components recognize and bind to the basic splice signals (5′ splice donor site, 3′ splice acceptor site, branch point sequence (BPS), and polypyrimidine tract (PT)) and mediate the splice reaction.
- Minor splicing uses different 5′ and 3′ splice sites and BPS. It lacks polypyrimidine tract (PT).



RNA Processing

Mechanism of RNA Splicing

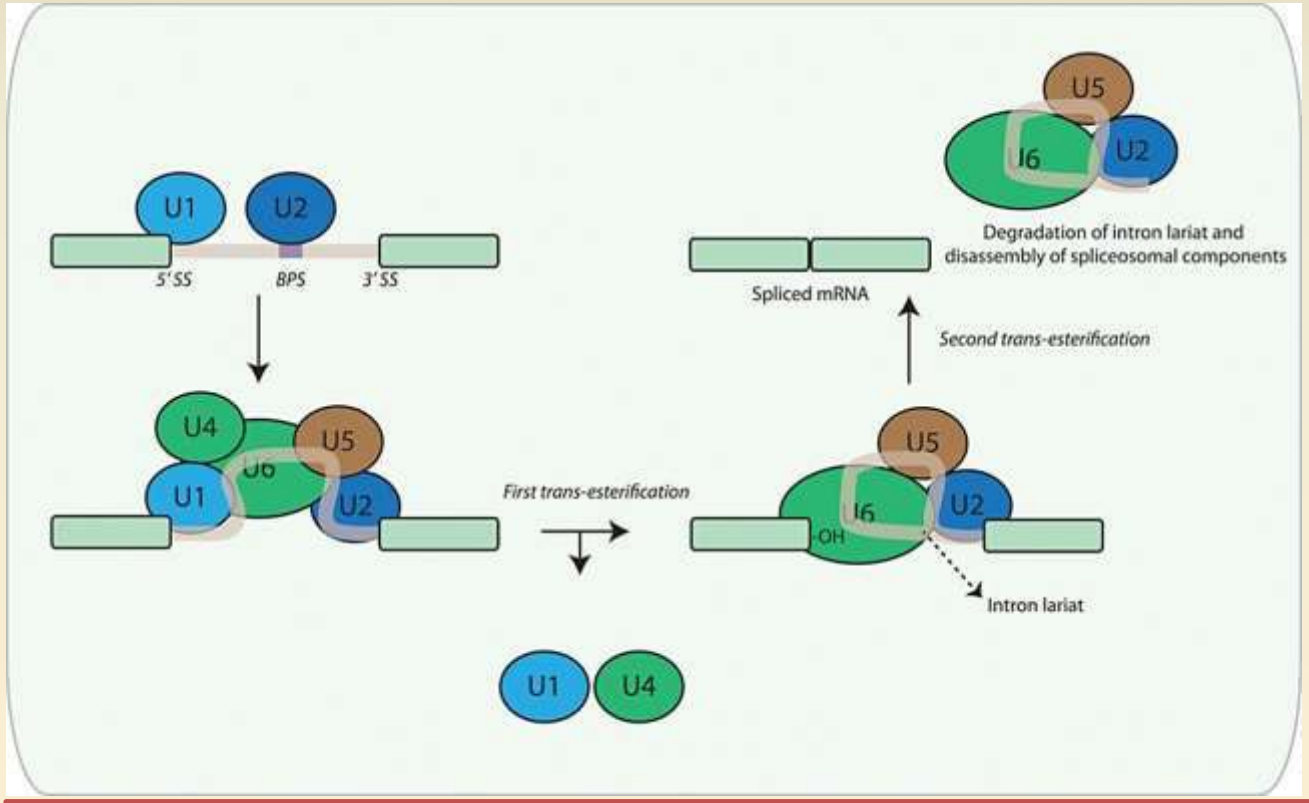
- U12 snRNA recognize minor intron sequence at intron and interacts with ZRSR2 complex at exon at one side and U11 snRNA at another side with the help of U5 snRNA and some snRNPS (35K and 59K).
- U11 and U12 snRNPs are functional analogs of U1 and U2 snRNPs of major introns – loop forms comlex initiate the formation of spliseasome complex.
- U11 then separates and U12 actiavtes the splicing of introns.



https://www2.helsinki.fi/en/researchgroups/rna-processing-by-the-minor-spliceosome/research

RNA Processing

<u>Mechanism of RNA Splicing</u> – Two-step process



Firstly, U1 snRNP assemble at the 5′ splice site of an exon and U2 snRNP at the branch point sequence (BPS), just upstream of the 3′splice site of the adjacent/downstrea m exon to form prespliceosome.

Here, U1 and U2 are joined by the snRNPs U5 and U4–U6 complexes

Next, U4–U6 complexes unwind, releasing U4 and U1 from the prespliceosomal complex allowing base pair of U6 with 5′ splice site and BPS. Then, 5′ splice site gets cleaved, leaving 3′ OH-group free which pairs with U5 that joins the two ends of exons.

RNA Processing

The three most important steps of pre-mRNA processing are the addition of stabilizing and signaling factors at the 5′ and 3′ ends of the molecule, and the removal of intervening sequences that do not specify the appropriate amino acids. These steps are:

5′ Capping

While the pre-mRNA is still being synthesized, a 7-methylguanosine cap is added to the 5′ end of the growing transcript by a 5′-to-5′ phosphate linkage. Translation initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

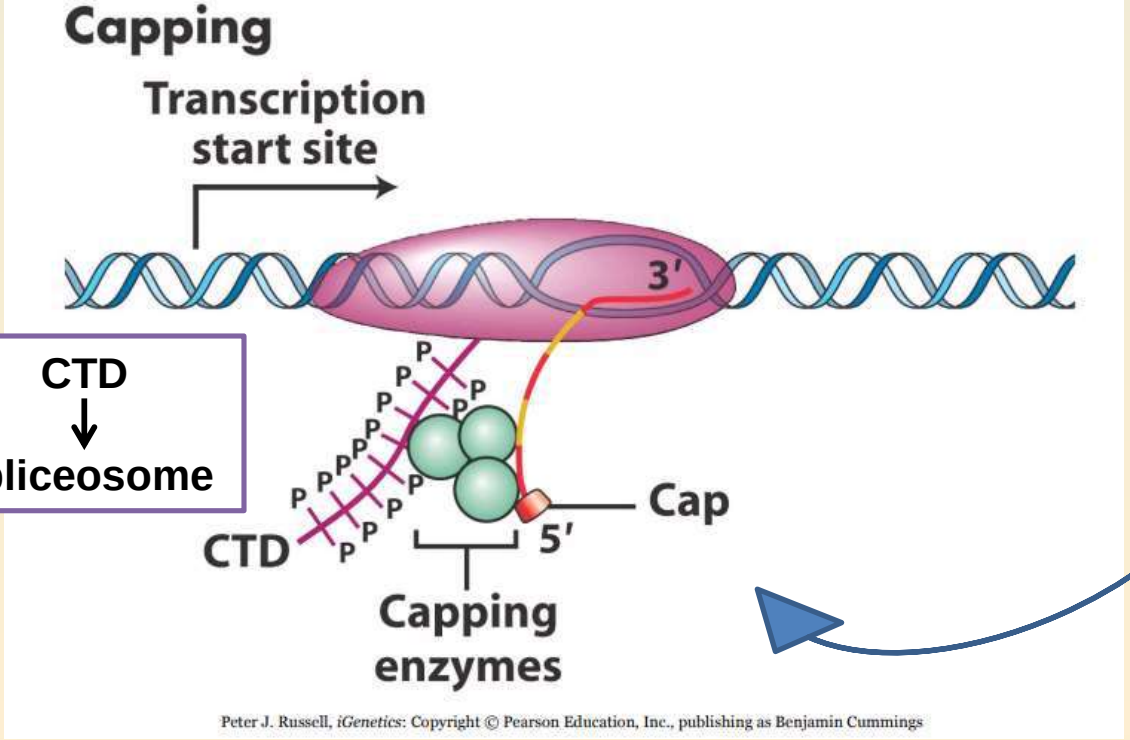
3′ Poly-A Tail

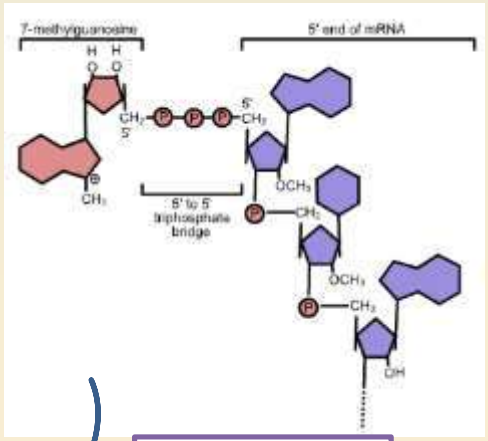
While RNA Polymerase II is still transcribing downstream of the proper end of a gene, the pre-mRNA is cleaved by an endonuclease-containing protein complex between an AAUAAA consensus sequence and a GU-rich sequence. This releases the functional pre-mRNA from the rest of the transcript, which is still attached to the RNA Polymerase. An enzyme called poly (A) polymerase (PAP), is part of the same protein complex that cleaves the pre-mRNA, immediately adds a string of approximately 200 A nucleotides, called the poly (A) tail, to the 3′ end of the just-cleaved pre-mRNA.

RNA Processing

Pre-mRNA splicing

Eukaryotic genes are composed of exons and introns. Introns are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins and these sequences are not present in prokaryotic genes..

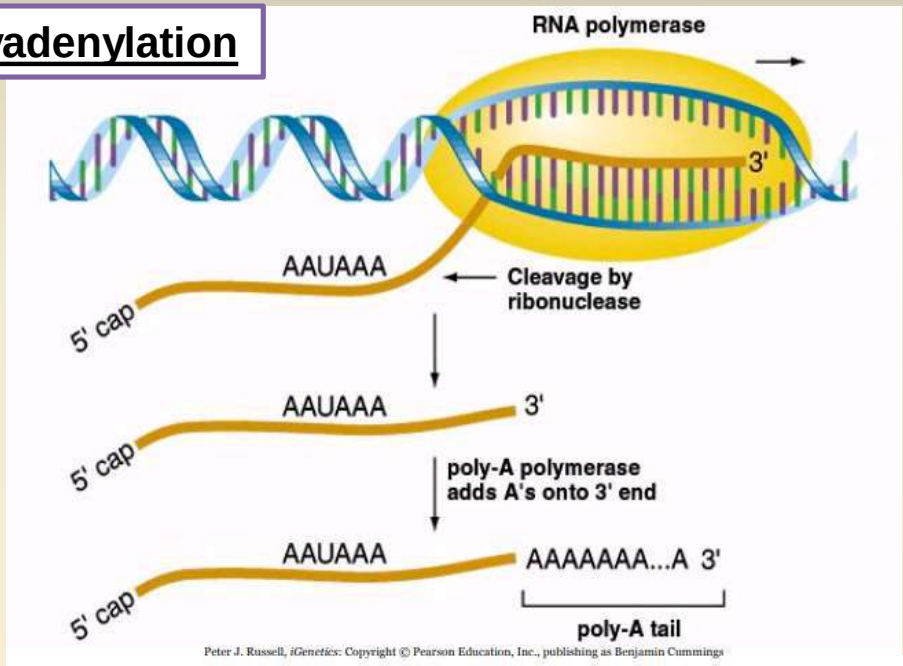




5′ Capping

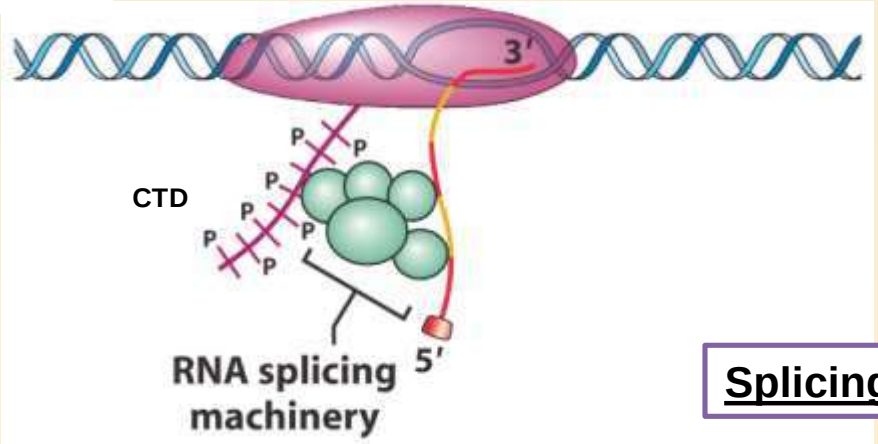
RNA Processing

Polyadenylation



- A poly(A) tail is added to an RNA at the end of transcription by an enzyme poly-A polymerase.
- It is 100 to 250 nucleotide long.
- Poly A tail stabilize the mRNA and allows the mature mRNA molecule to be exported from nucleus.

- CTD or C-terminal repeat domain, an unusual extension of the C terminus of the largest subunit of pol II, serves as binding scaffold for various nuclear factors.
- It also helps in the processing of the nascent RNA by helping in the formation of spliceosome..

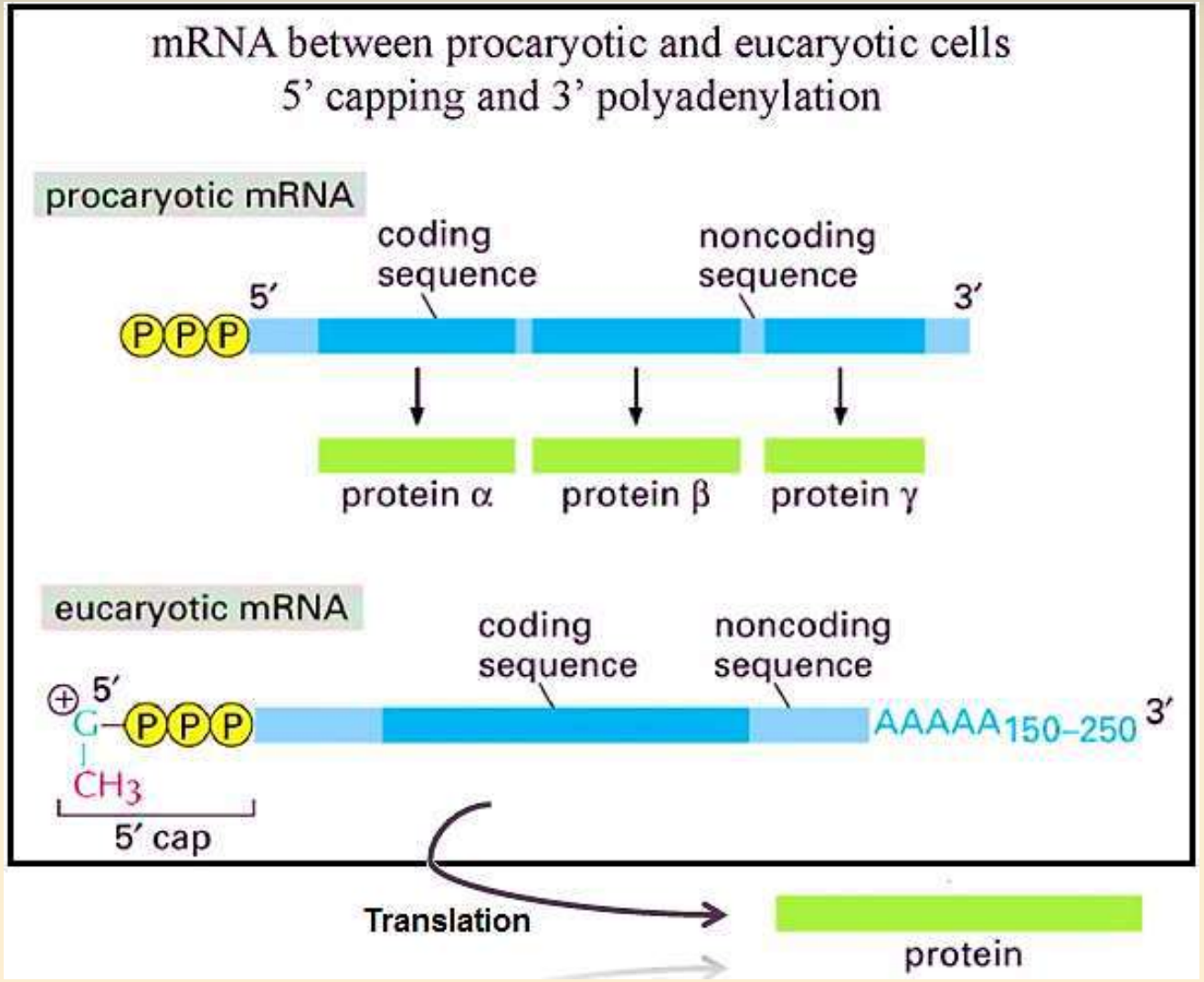


Splicing

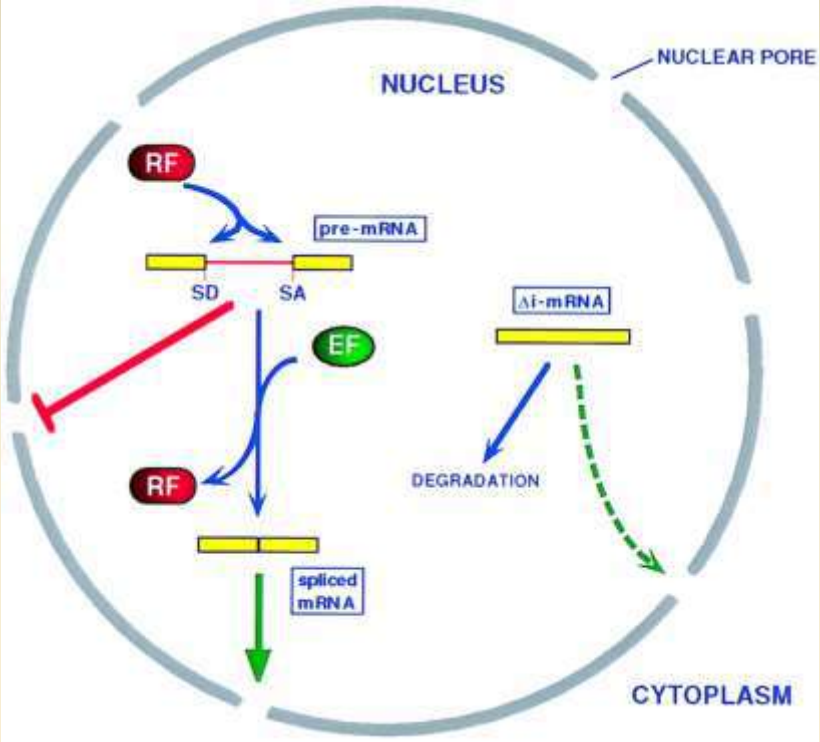
mRNA Degradation

- **Prokaryotes**
  - Short Life span
  - Degraded in seconds
  - Allows rapid response to environmental changes
- **Eukaryotes**
  - Survive from hours to weeks
  - Internal conditions constant, no need for rapid response

Outcome

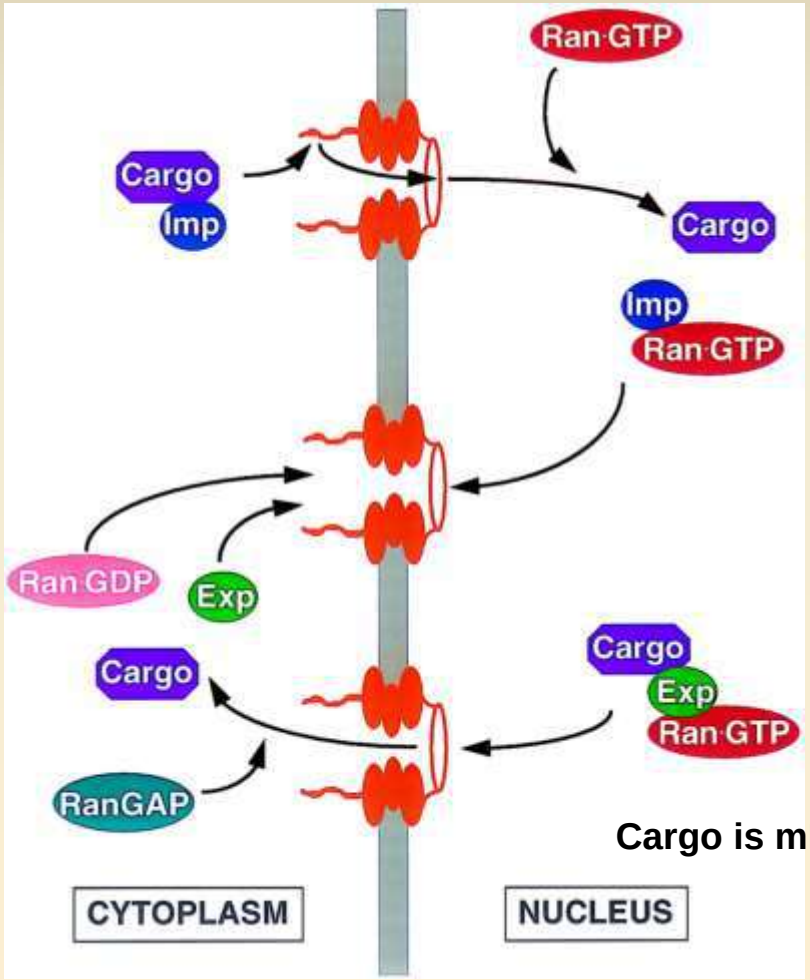

mRNA between procaryotic and eucaryotic cells
5' capping and 3' polyadenylation
procaryotic mRNA
coding sequence
noncoding sequence
5′
3′
P P P
protein α
protein β
protein γ
eucaryotic mRNA
coding sequence
noncoding sequence
5′
G
P P P
AAAAA 150–250
3′
CH3
5′ cap
Translation
protein

Transport of Mature mRNA



Cullen B.R. PNAS January 4, 2000 97 (1) 4-6

- ***Pre-mRNAs are first coated in RNA-stabilizing proteins.***
- ***After processing RanGTP bind to exportin proteins and form a cargo.***
- ***Cargo interacts with mobile export receptors at the nuclear pore that facilitates export of mRNA.***



Cargo is mRNA

Cullen B.R. Molecular and Cell Biology 2020; 20(12)

Further reading

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