VIVEKANANDA COLLEGE
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NAAC ACCREDITED ‘A’ GRADE

Topic : RNA Processing

Course Title : Gene Organisation,Expression and Regulation

Paper : GE-4 (CC-4)

Unit : 6

Semester : 4

Name of the Teacher : Dr. Kakali Roy

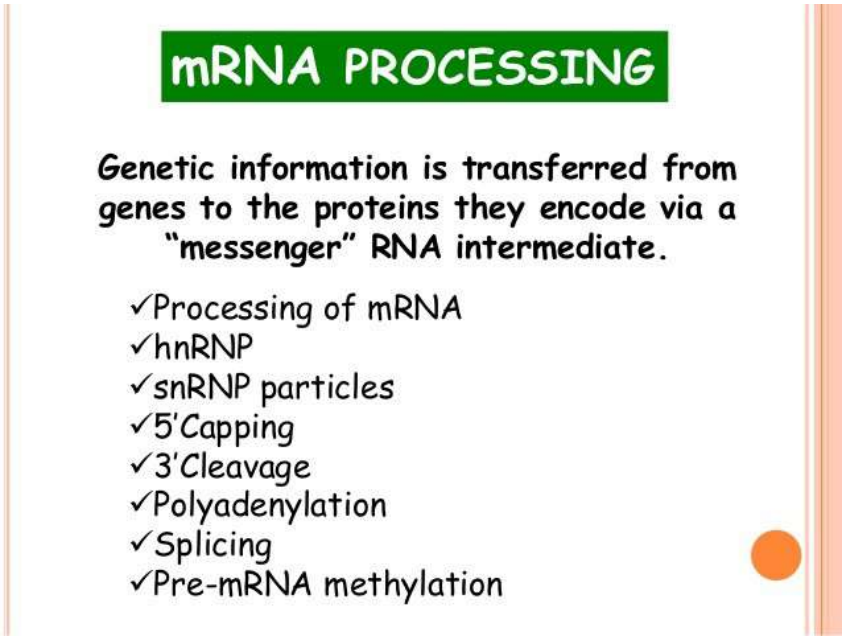
Name of the Department : Biochemistry

Processing of Eukaryotic mRNA

- The eukaryotic pre-mRNA undergoes extensive processing before it is ready to be translated.

- 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.

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

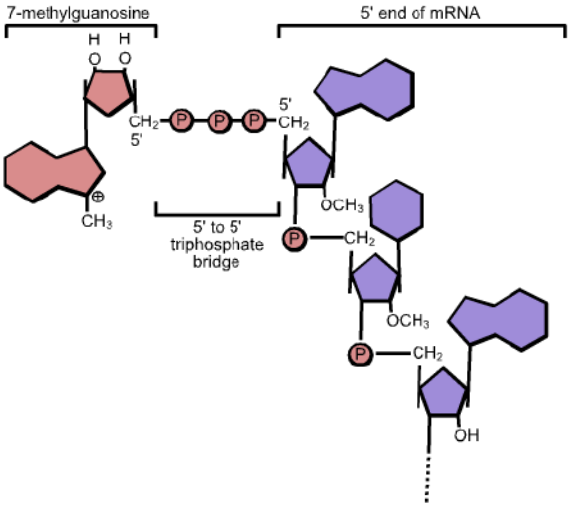


5' capping

- 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.

- This moiety protects the nascent mRNA (primary RNA transcript) from degradation by ribonucleases.

- Initiation factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.



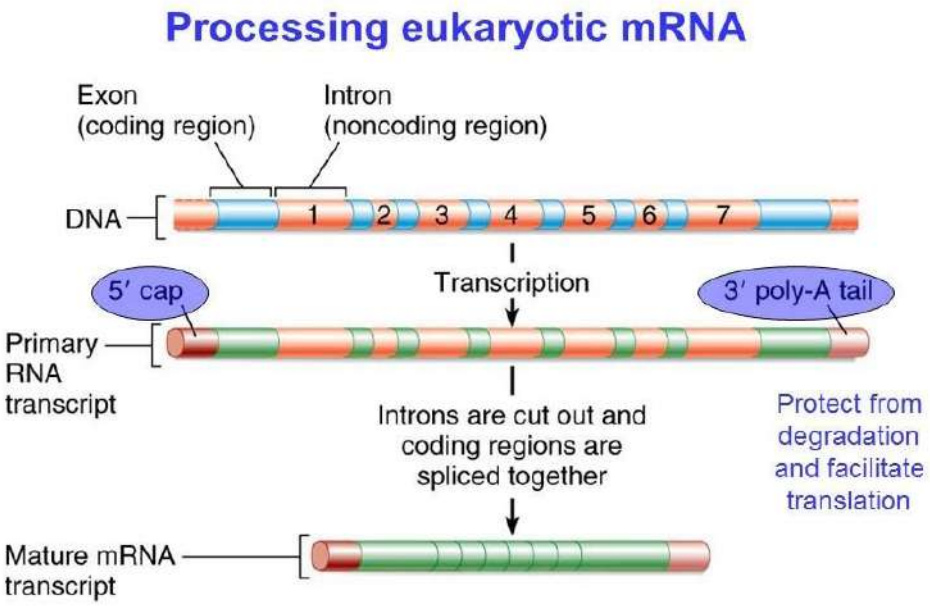
3' Poly-A Tail

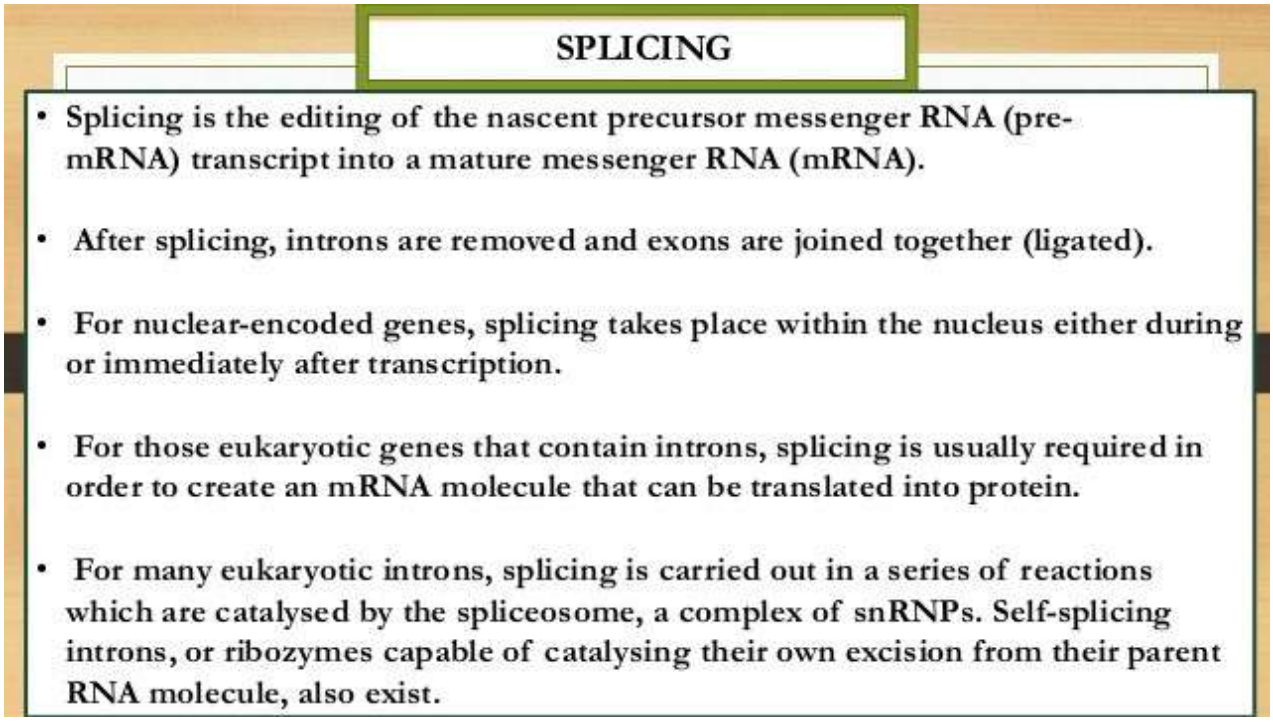
- While RNA Polymerase II is still transcribing downstream of the proper end of a gene, the pre-mRNA is cleaved by an endonuclease-containing protein complex between an AAUAAA consensus sequence and a GU-rich sequence.

- 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 and it immediately adds a string of approximately 200 A nucleotides, called the poly (A) tail, to the 3′ end of the just-cleaved pre-mRNA.

- The poly (A) tail protects the mRNA from degradation, aids in the export of the mature mRNA to the cytoplasm, and is involved in binding proteins involved in initiating translation.

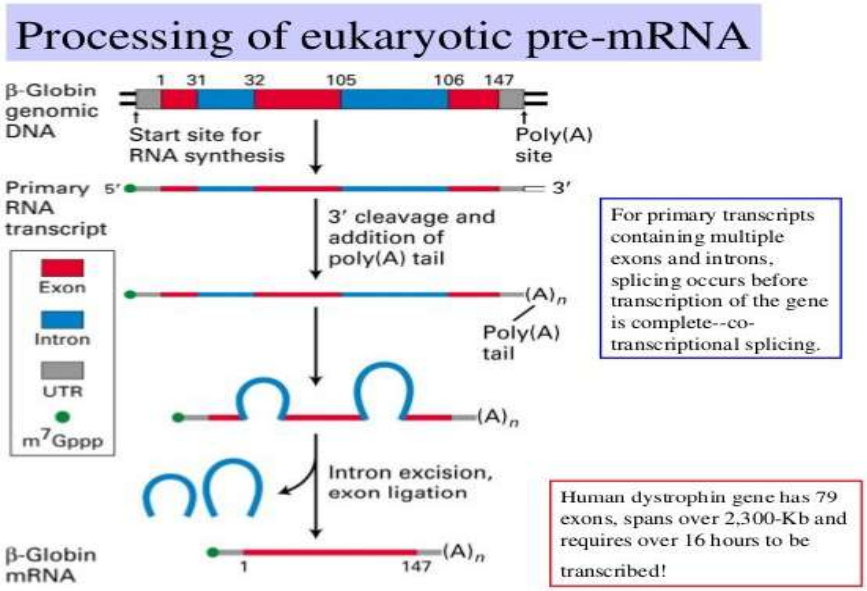




Pre-mRNA splicing

- Eukaryotic genes are composed of **exons**, which correspond to protein-coding sequences (*ex*-on signifies that they are *ex*pressed), and intervening sequences called **introns** (*int*-ron denotes their *int*ervening role), which may be involved in gene regulation, but are removed from the pre-mRNA during processing.

- Intron sequences in mRNA do not encode functional proteins.

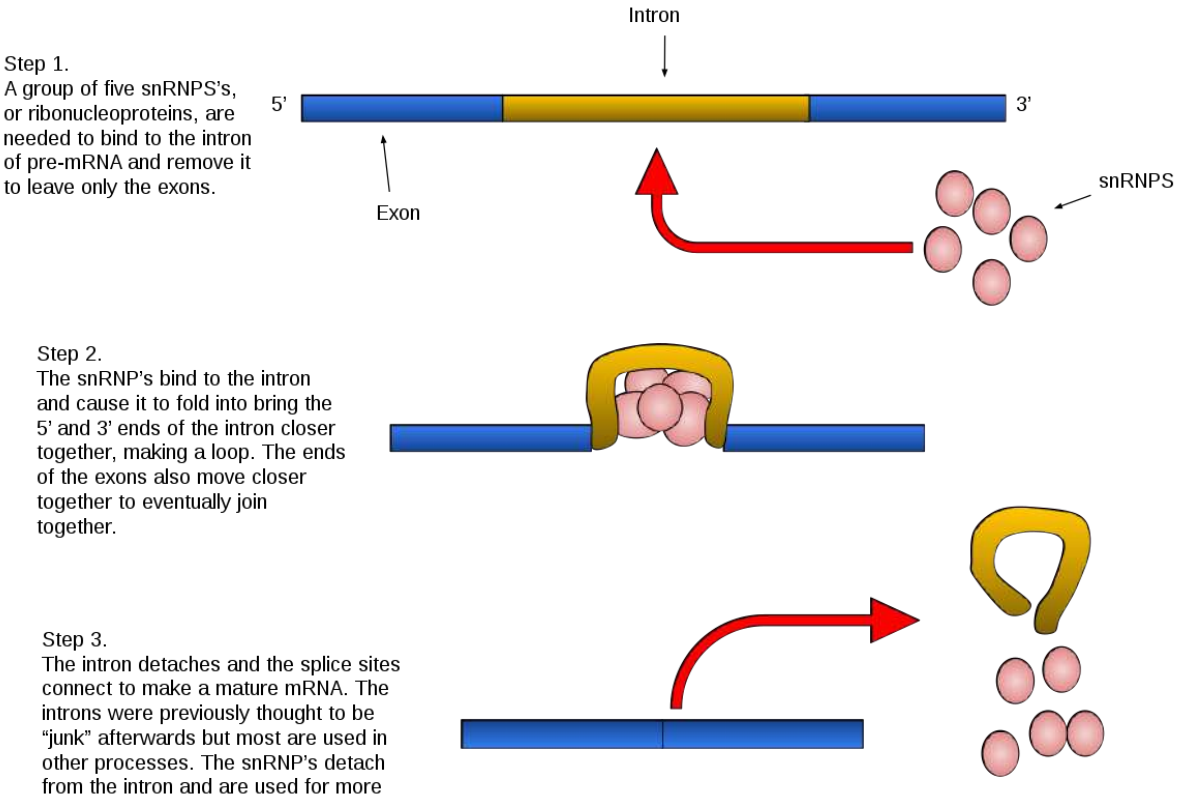


Splicing of introns

- All introns in a pre-mRNA must be completely and precisely removed before protein synthesis.

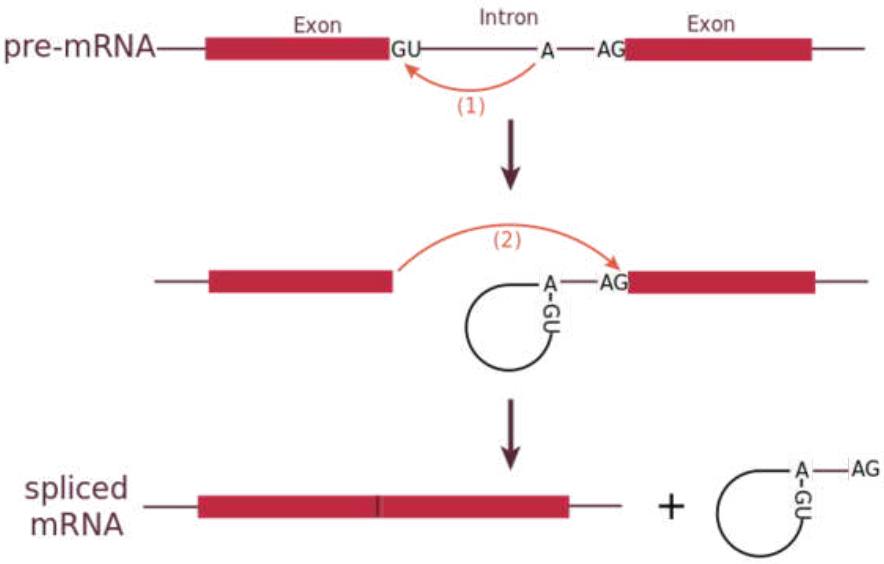
- **The process of removing introns and reconnecting exons is called splicing**.

- Introns are removed and degraded while the pre-mRNA is still in the nucleus.



- Splicing occurs by a sequence-specific mechanism that ensures introns will be removed and exons re-joined with the accuracy and precision of a single nucleotide.

- The splicing of pre-mRNAs is conducted by complexes of proteins and RNA molecules called **spliceosomes**.



- Spliceosomes recognize sequences at the **5′ end of the intron** because introns always start with the nucleotides GU and they recognize sequences at the 3′ end of the intron because they always end with the nucleotides AG.

- The spliceosome cleaves the pre-mRNA's sugar phosphate backbone at the G that starts the intron and then covalently attaches that G to an internal A nucleotide within the intron.

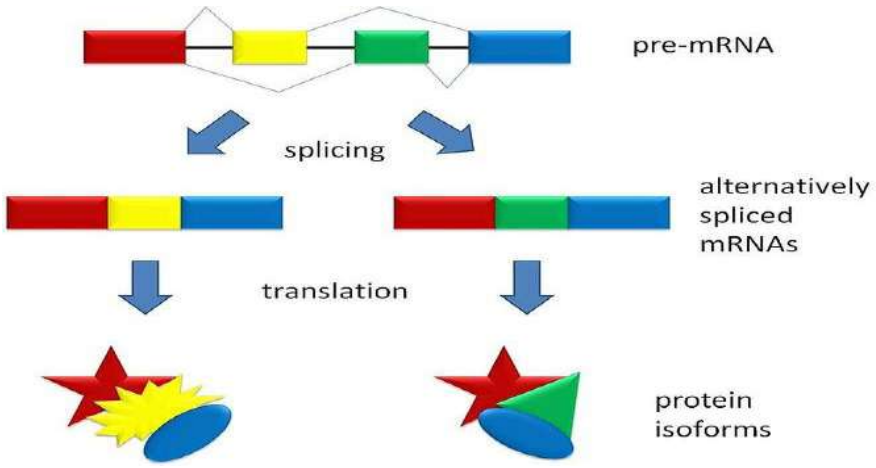
- Then the spliceosome connects the 3′ end of the first exon to the 5′ end of the following exon, cleaving the 3′ end of the intron in the process.

- This results in the splicing together of the two exons and the release of the intron in a lariat form.

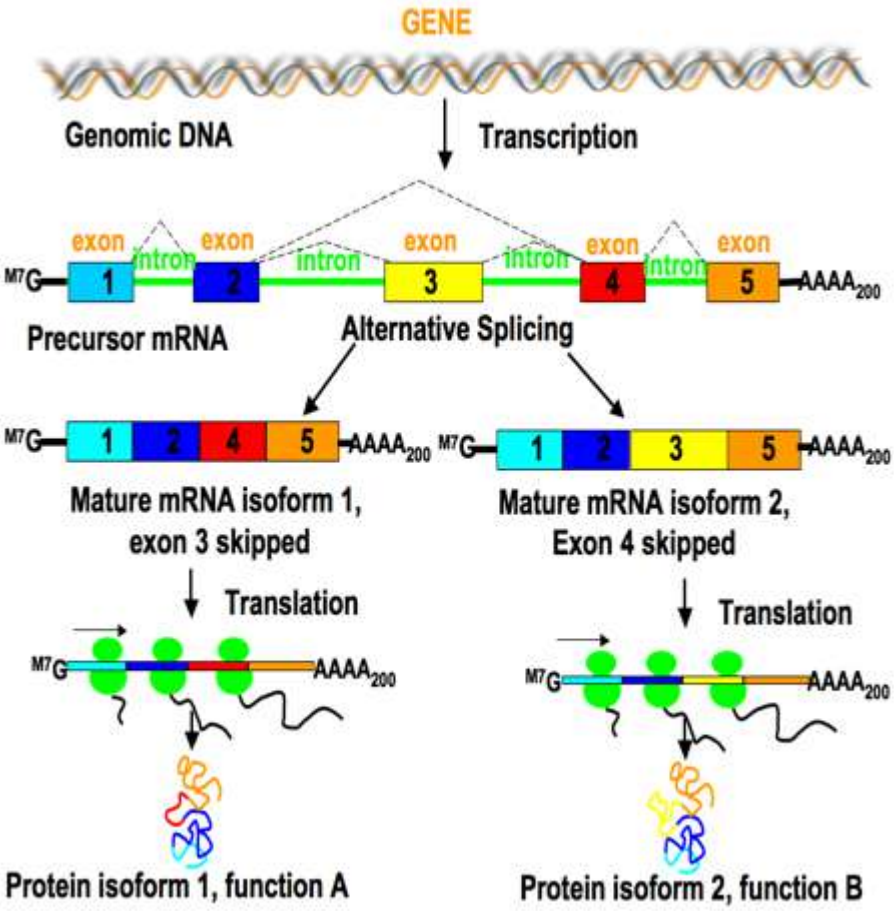
ALTERNATIVE SPLICING

- It is deviated from constitutive splicing which is a regulated process during gene expression that results in a single gene coding for multiple proteins wherein, particular exons maybe included or excluded from final, processed mRNA.
- Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and, often, in their biological functions.
- Alternative splicing occurs as a normal phenomenon in eukaryotes, where it greatly increases the biodiversity of proteins that can be encoded by the genome.

- The **first example** of alternative splicing of a cellular gene in eukaryotes was identified in the **IgM gene**, a member of the immunoglobulin superfamily.
- Alternative splicing (AS) therefore is a process by which exons or portions of exons or noncoding regions within a pre-mRNA transcript are differentially joined or skipped, resulting in multiple protein isoforms being encoded by a single gene.



- **This mechanism increases the informational diversity and functional capacity of a gene during post-transcriptional processing and provides an opportunity for gene regulation**.



Alternative splicing generates a tremendous amount of proteomic diversity in humans and significantly affects various functions in cellular processes, tissue specificity, developmental states, and disease conditions.

RNA SPLICING VERSUS ALTERNATIVE SPLICING

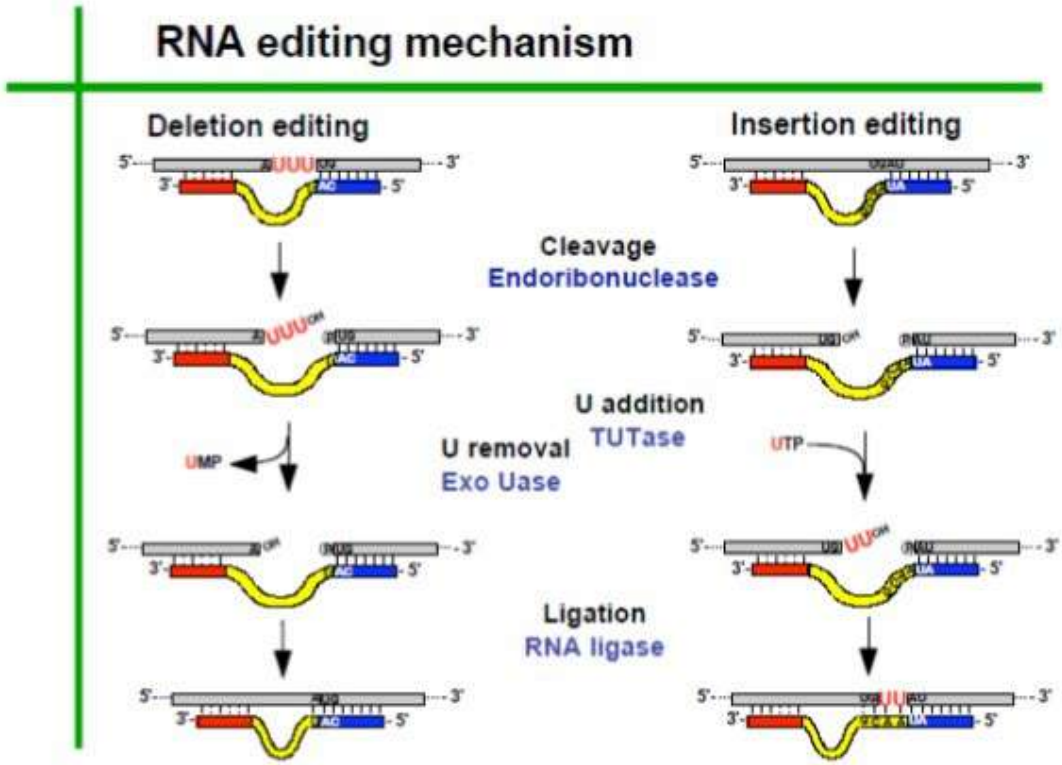
RNA SPLICING	ALTERNATIVE SPLICING
A modification of the nascent pre-messenger RNA (pre-mRNA) transcript in which introns are removed and exons are joined prior to translation	A process that enables a messenger RNA (mRNA) to direct synthesis of different protein variants (isoforms) that may have different cellular functions or properties
Splices the exons of the primary RNA transcript	Splices the exons in the primary RNA transcript, forming differential combinations of exons
Mature mRNA contains all the exons in the primary transcript	Mature mRNAs do not contain every exon of the primary RNA transcript
Brings the protein coding region together by removing the non-coding regions from the primary transcript	Increases the informational diversity and the proteomic diversity of the cell

RNA EDITING

This is a form of RNA processing in which the **nucleotide sequence** of the primary transcript is altered by either

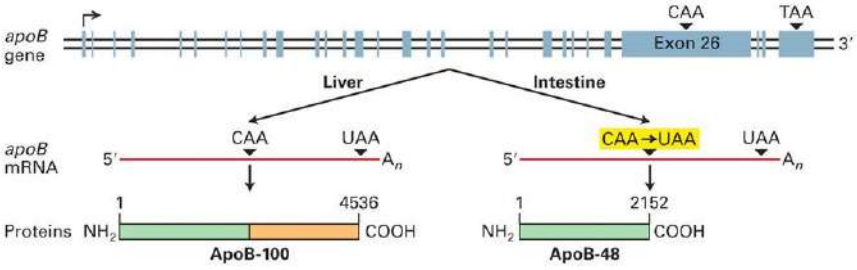
- ✓ Changing residues,
- ✓ Deleting residues,
- ✓ Inserting residues at specific points along the molecule

Changing RNA sequence (after transcription).



RNA editing

- Enzymatic altering of pre-mRNA sequence
- Common in mitochondria of protozoans and plants and chloroplasts, where more than 50% of bases can be altered
- Much rarer in higher eukaryotes



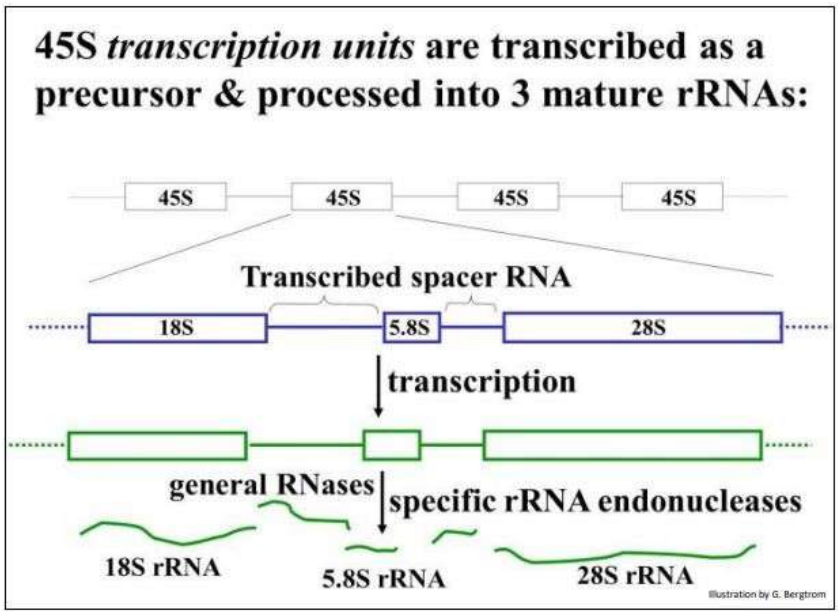
Editing of human apoB pre-mRNA

Significance of RNA Editing

- It is essential in **regulating gene expression** of organisms.
- **RNA editing mutant** was reported with strong defects in organelle development.
- Deficiency causes **diseases**.
- It is a mechanism to **increase the number of different proteins** available without the need to increase the number of genes in the genome.
- May help **protect the genome** against some viruses.

Ribosomal RNA processing

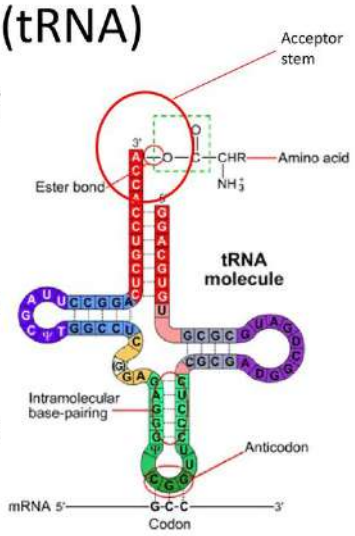
- In eukaryotes, pre-rRNAs are transcribed, processed, and assembled into ribosomes in the nucleolus.
- In most eukaryotes, a large rRNA gene transcribes a 45S precursor transcript containing (from shortest to longest) 5.8S rRNA, 18SrRNA and 28S rRNA.
- The 'S' stands for Svedberg, the biochemist who developed the *sedimentation velocity ultracentrifugation* technique to separate molecules like RNA by size.
- The higher the S value, the larger the molecule and therefore the faster it moves through the viscous sugar gradient during centrifugation.
- RNA Polymerase I transcribes 45S precursor rRNAs (preRNAs) from multiple large transcription units in the genome.



The 45S pre-rRNA is processed by cleavage. The many copies (200-400!) of the 45S gene in eukaryotic cells might be expected, since making proteins (and therefore ribosomes) will be an all-consuming cellular activity.

Transfer RNA (tRNA)

- Composed of single strand of RNA
- Two functional regions
  - **Anticodon loop**
    - 3 nucleotides complementary to mRNA codon
  - **Acceptor stem**
    - 3′ single stranded w/ amino acid at the end



- In eukaryotic cells, tRNA are made by a special protein that reads the DNA code and makes an RNA copy, or pre-tRNA by RNA polymerase III.

- In eukaryotes, pre-tRNAs are transcribed and processed in the nucleus and then released into the cytoplasm where they are linked to free amino acids for protein synthesis.

- In all organisms, tRNAs are transcribed in a pre-tRNA form that requires multiple processing steps before the mature tRNA is ready for use in translation.

- **The processing to convert the pre-tRNA to a mature tRNA involves five steps**:

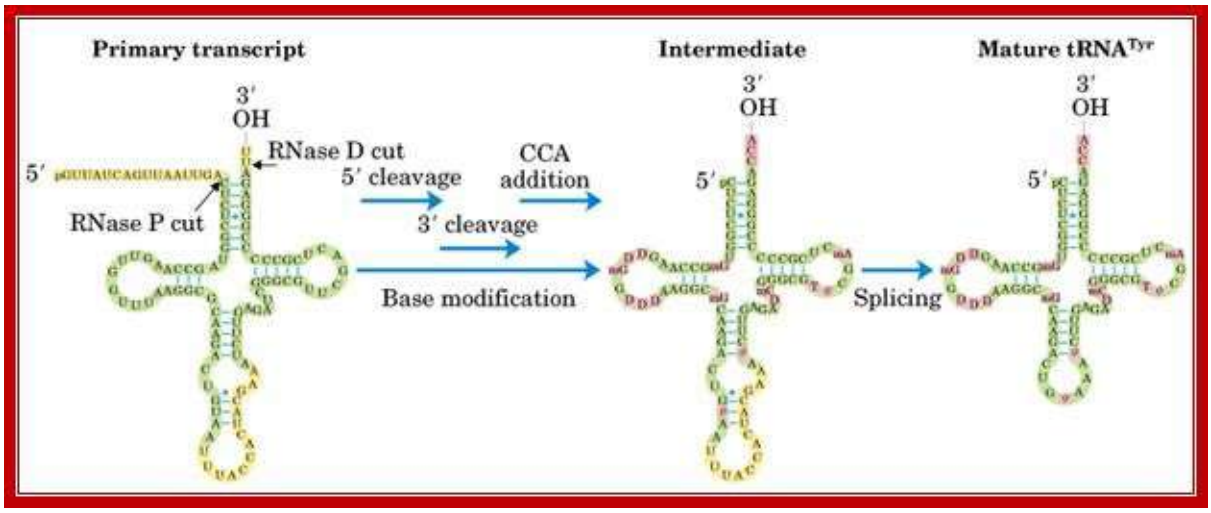
- The 5′ end of the pre-tRNA, called the 5′ leader sequence, is cleaved off.

- The 3′ end of the pre-tRNA is cleaved off.

- In all eukaryote pre-tRNAs, a CCA sequence of nucleotides is added to the 3′ end of the pre-tRNA after the original 3′ end is trimmed off. The CCA at the 3′ end of the mature tRNA will be the site at which the tRNA's amino acid will be added.

- Multiple nucleotides in the pre-tRNA are chemically modified, altering their nitorgen bases. On average about 12 nucleotides are modified per tRNA. The most common modifications are the conversion of adenine (A) to pseudouridine (ψ), the conversion of adenine to inosine (I), and the conversion of uridine to dihydrouridine (D). But over 100 other modifications can occur.

- A significant number of eukaryotic pre-tRNAs have introns that have to be spliced out.



Processing of tRNA

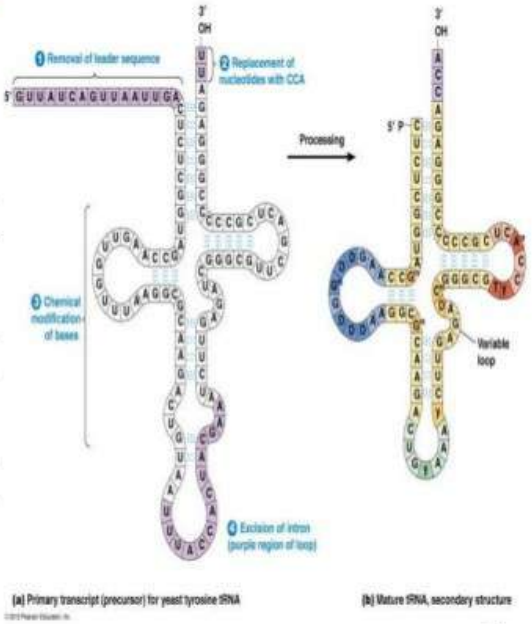
1. Removal of leader sequence & trailer

2.Replacement of nucleotide

3.Modification of certain bases:

- **Replacement** of U residues at the 3′ end of pre-tRNA with a CCA sequence
- **Addition** of methyl and isopentenyl groups to the heterocyclic ring of purine bases
- **Methylation** of the 2′-OH group in the ribose of any residue; and conversion of specific uridines to dihydrouridine(D),pseudouridine(y)

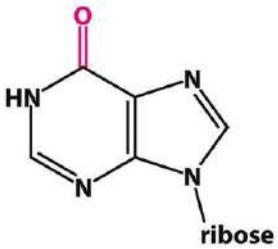
4.Excision of an intron



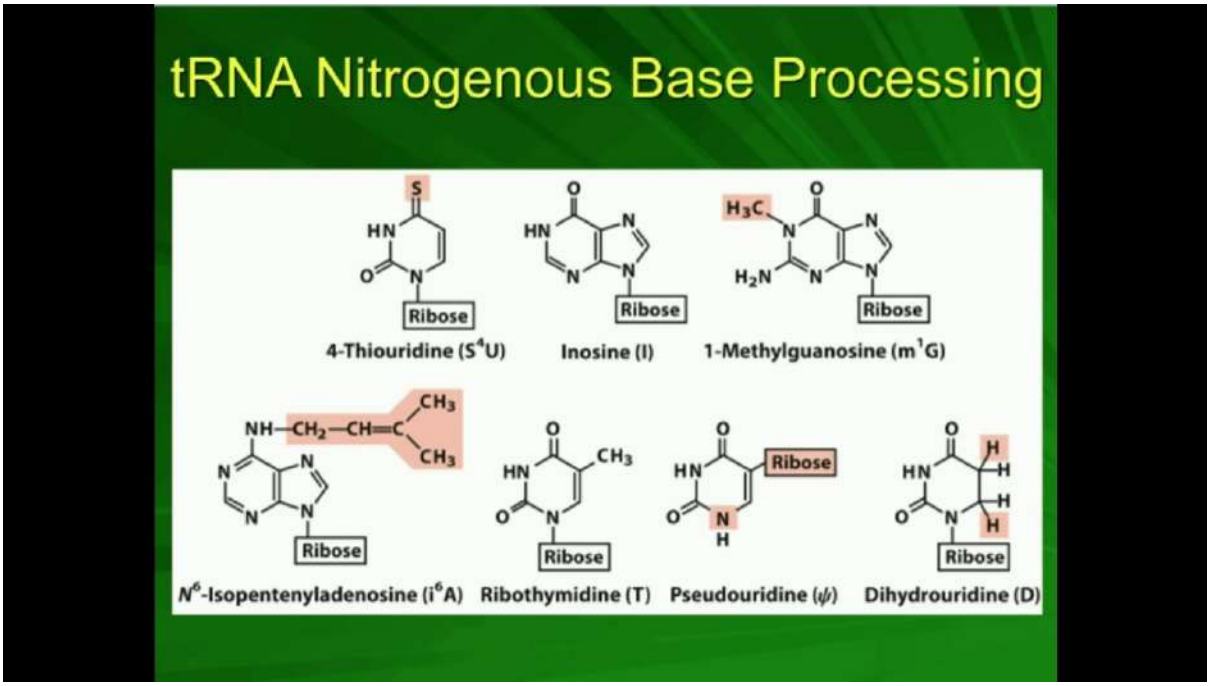
4. tRNAs contain many unusual bases derived by modification. Methylation prevents formation of certain base pairs, favoring alternative interactions. Methylation increases hydrophobicity and interactions with proteins

5-Methylcytidine (mC)

Dihydrouridine (UH_2)



Inosine



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