CSIR NET Notes : UNIT 3B – RNA Synthesis & Processing PDF

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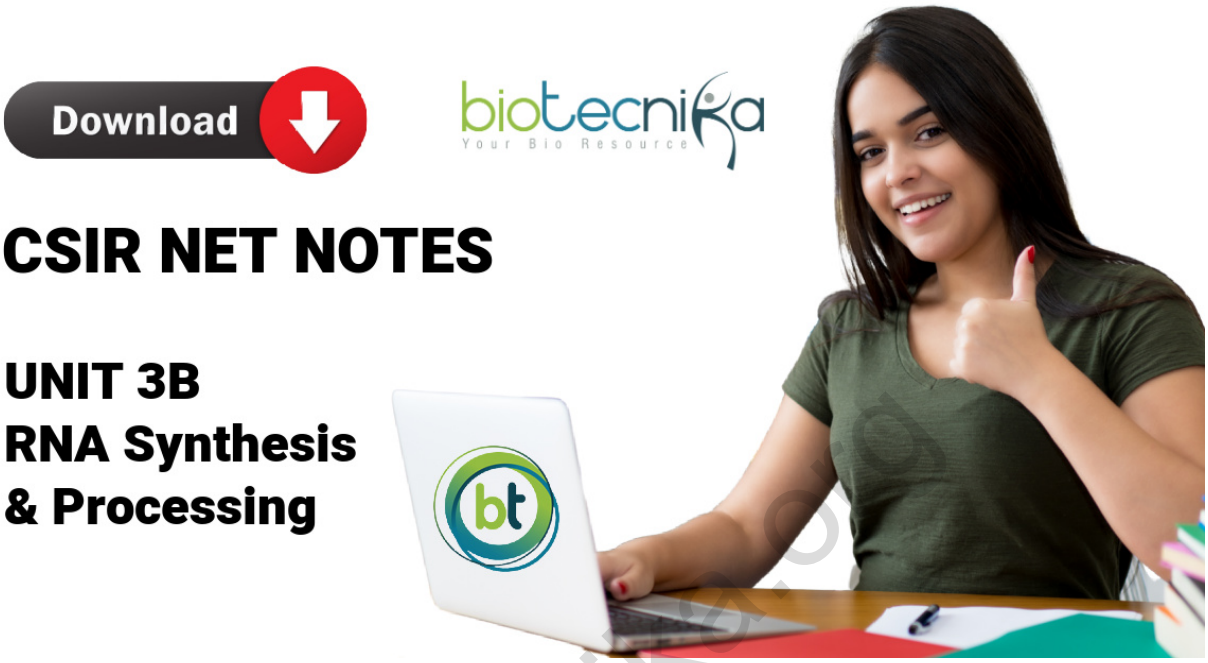


UNIT 3 From CSIR NET Life Science Syllabus can help you fetch a good score in the exam. It's an important unit and one must not skip it at all as it deals with molecular biology. From UNIT 3 one can expect an average of 12% questions in Part B and 8% questions in Part C.

Reference Books For UNIT 3B-RNA Synthesis & Processing:

1. Molecular biology, weaver, 5th edition
2. Principles of biochemistry, Lehninger, 6th edition
3. Molecular cell biology, Lodish, 8th edition
4. Watson, Molecular Biology Of The Gene, 7th Edition

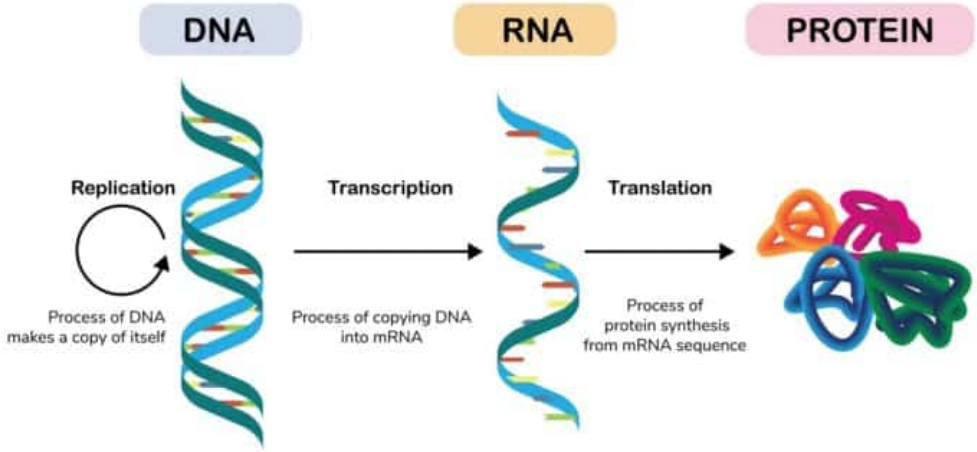


Fig: Cellular Process

Post Transcriptional Processes

- The primary transcripts are not necessarily functional entities
- To acquire biological activity, many of them must be specifically altered
- Processes:
  – Removal of a polynucleotide by the exo- and endonucleolytic segments
  – Appending nucleotide sequences to their 3' and 5' ends
  – Modification of specific nucleosides

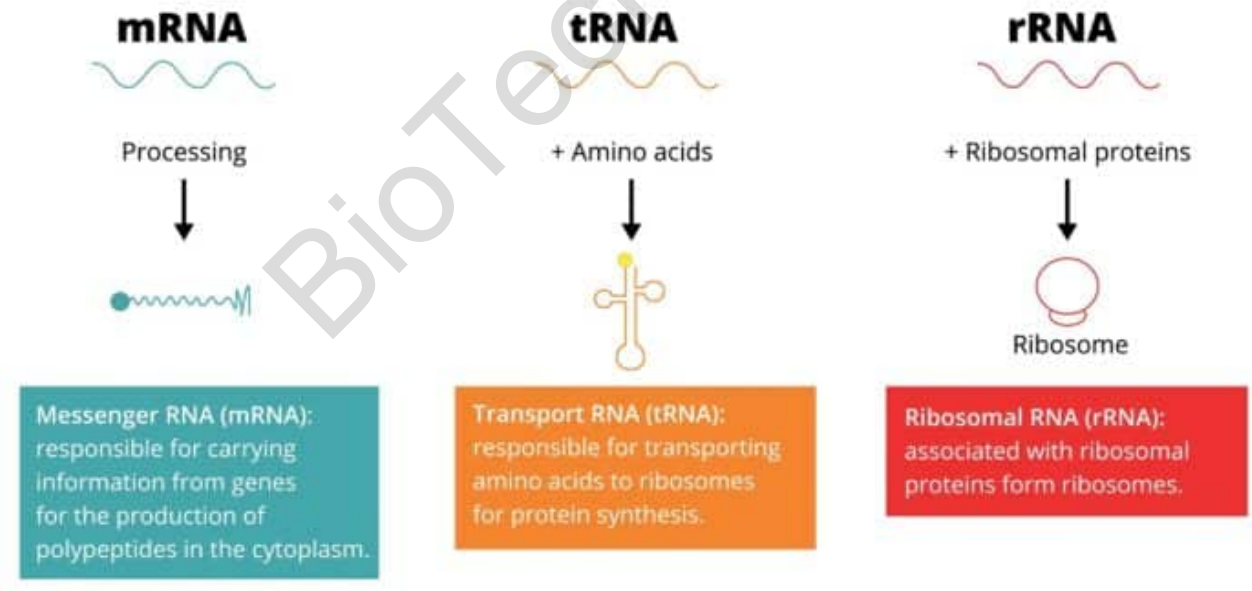


Fig: Types of RNA

Structure of mRNAs

- A continuous sequence of nucleotides encodes a specific polypeptide.
- They are attached to ribosomes when they are translated.
- Most mRNAs contain a significant noncoding segment, that is, a portion that does not direct the assembly of amino acids.

- Eukaryotic mRNAs have special modifications at their 5’ and 3’ termini that are not found on either bacterial mRNAs or on tRNAs or rRNAs.
- The 3’ end of nearly all eukaryotic mRNAs has a string of 50 to 250 adenosine residues that form a poly(A) tail,
- Whereas the 5’ end has a methylated guanosine cap

Cap

Eukaryotic mRNAs have a peculiar enzymatically appended cap structure consisting of a 7-
methylguanosine (m7G) residue joined to the transcript’s initial (5’) nucleoside via a 5’–5’ triphosphate bridge.

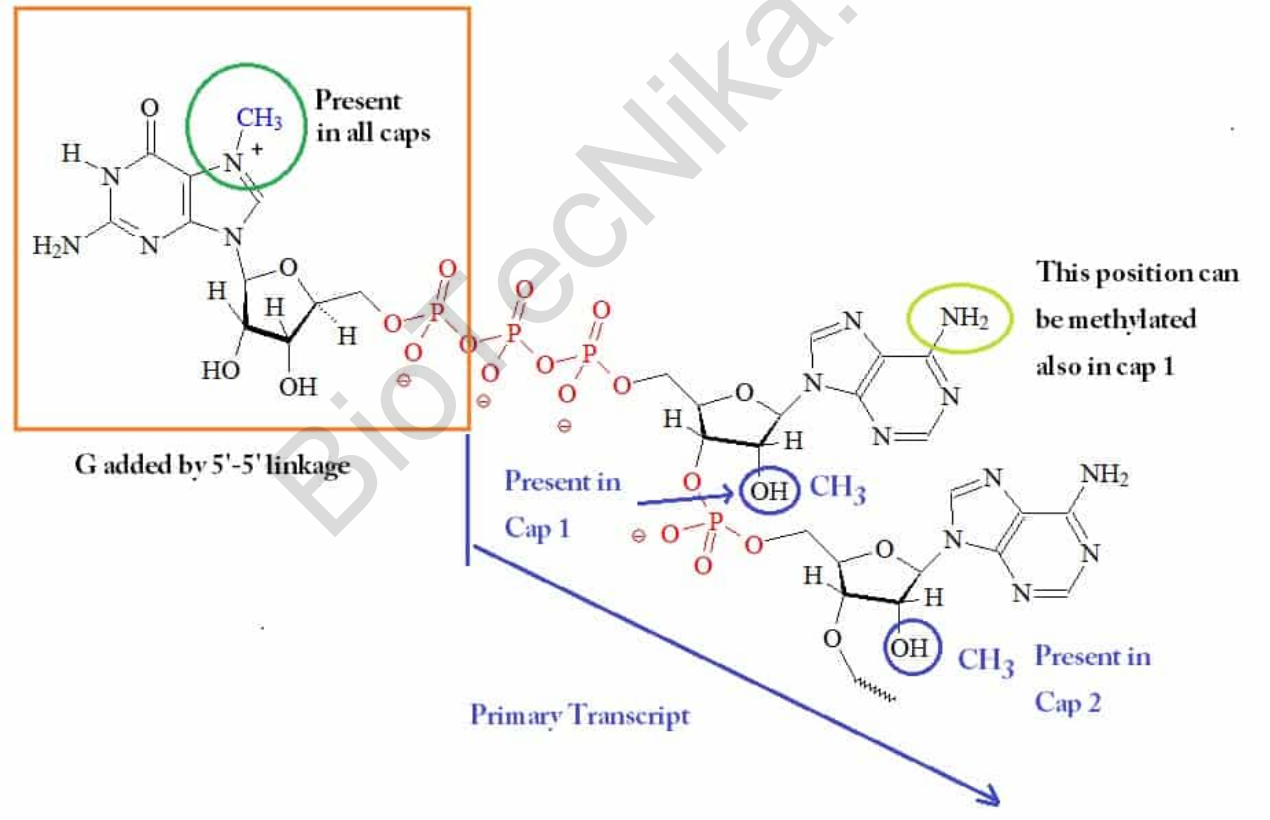


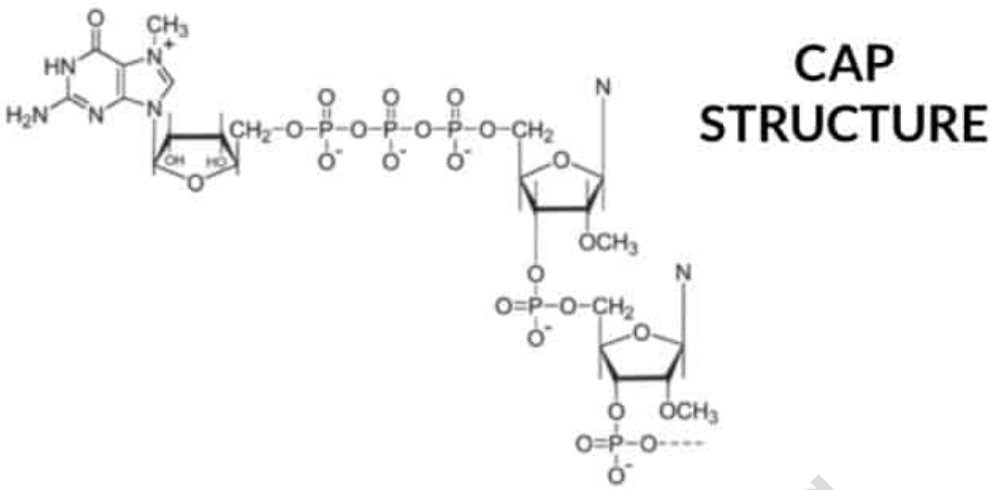
Fig: 5′-Guanosine-triphosphate Cap Image Courtesy: Bio-Synthesis

Functions

Caps appear to serve at least four functions:

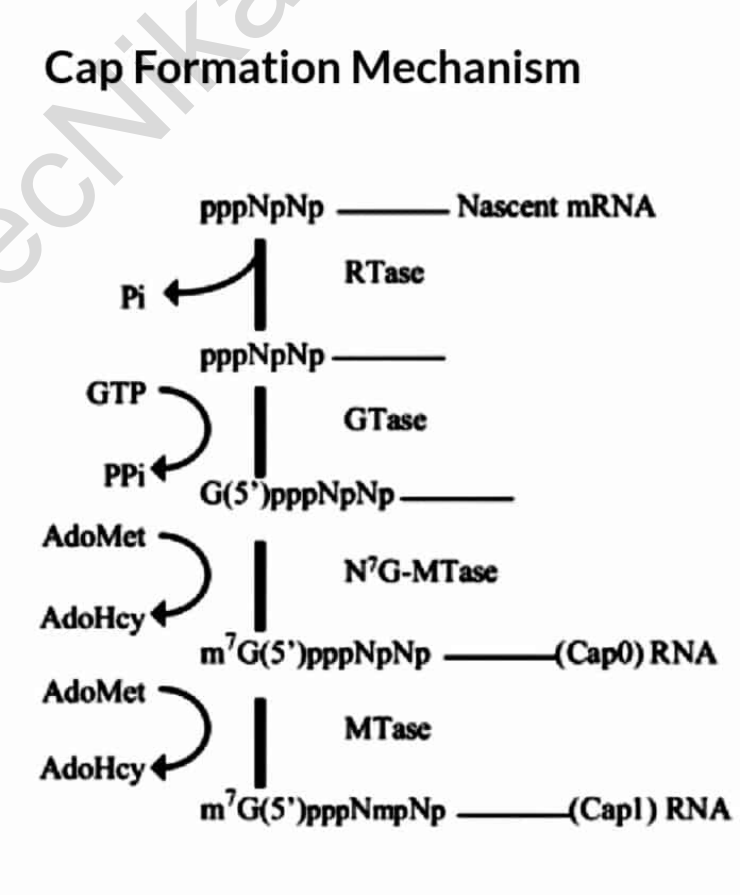
- They protect mRNAs from degradation.
- They enhance the translatability of mRNAs.

- They enhance the transport of mRNAs from the nucleus into the cytoplasm.
- They enhance the efficiency of the splicing of mRNAs.



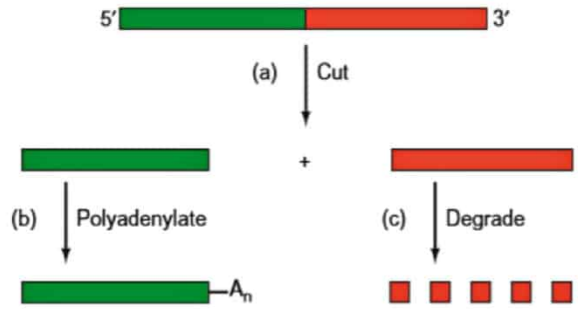
Process

- Phosphorylation by TFIIH at serine 5 residue (CTD of RNA pol II) recruits the capping machinery.
- This m7 cap, which is added to the growing transcript before it is ~30 nucleotides long, defines the eukaryotic translational start site
- Capping marks the completion of RNAP II's switch from transcription initiation to elongation
- Enzymes involved – nucleotide phosphohydrolase (RNA triphosphatase), guanylyl transferase, methyltransferase
- First, an RNA triphosphatase removes the terminal phosphate from a pre-mRNA
- Next, a guanylyltransferase adds the capping GMP (from GTP).



- Next, methyltransferases methylate the N7 of the capping guanosine and the 2'-O-methyl group of the penultimate nucleotide.

Poly (A) Tail



- Mature eukaryotic mRNAs have well-defined 3’ ends; almost all of them in mammals have 3’-poly(A) tails of ~250 nucleotides
- Poly(A) enhances both the lifetime and translatability of mRNA. The relative importance of these two effects seems to vary from one system to another.
- Enzymatically appended to the primary transcripts in two reactions that are mediated by a 500- to 1000-kD complex that consists of at least six proteins.
- The mechanism of polyadenylation usually involves clipping an mRNA precursor, even before transcription has terminated, and then adding poly(A) to the newly exposed 3’-end

Polyadenylation Signals

Mammalian polyadenylation signal consists of an AAUAAA motif about 20 nt upstream of a polyadenylation site in a premRNA, followed 23 or 24 bp later by a GU-rich motif, followed immediately by a U-rich motif.

Polyadenylation Factors

- Cleavage and polyadenylation specificity factor (CPSF) – 360- kDa, having four different polypeptides, first forms an unstable complex with the upstream AAUAAA poly(A) signal
- Cleavage stimulatory factor (CStF) – a 200-kDa heterotrimer, interacts with the G/U- rich sequence
- Cleavage factor I (CFI) – a 150-kDa heterotrimer
- Cleavage factor II (CFII)
- Poly(A) polymerase (PAP) – links cleavage and polyadenylation
- Poly(A)-binding protein (PABPII) – binds to the short A tail initially added by PAP

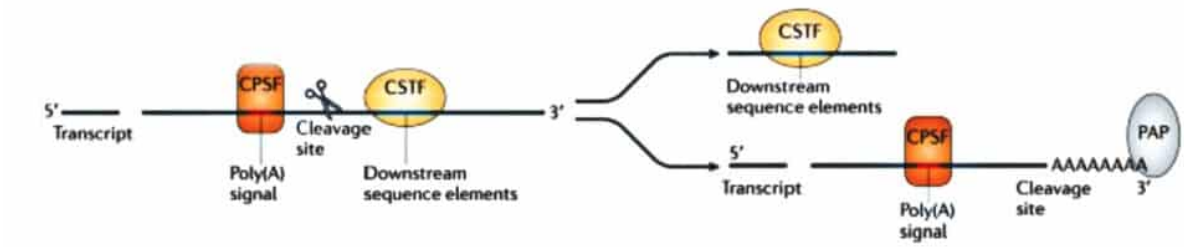


Fig: Mechanism of Transcript Cleavage & polyadenylation

Factor	Polypeptides (kD)	Properties

Poly(A) polymerase (PAP)	82	Required for cleavage and polyadenylation; catalyzes poly(A) synthesis
Cleavage and polyadenylation specificity factor (CPSF)	160 100 73 30	Required for cleavage and polyadenylation; binds AAUAAA and interacts with PAP and CstF; CPSF-73 cleaves RNA
Cleavage stimulation factor (CstF)	77 64 50	Required only for cleavage; binds the downstream element and interacts with CPSF
Cleavage factor I (CF I)	68 59 25	Required only for cleavage; binds RNA
Cleavage factor II (CF II)	Unknown	Required only for cleavage
RNA polymerase II (especially CTD)	Many	Required only for cleavage
Poly(A)-binding protein II (PAB II)	49	Stimulates poly(A) elongation; binds growing poly(A) tail; essential for poly(A) tail length control

Termination of Transcription

- **Torpedo mechanism**
- Cotranscriptional cleavage element (CoTC element) – 1.7 kb downstream of the polyadenylation site
- The torpedo model of transcription termination by RNA polymerase II proposes that a 5′–3′ RNA exonuclease enters at the poly(A) cleavage site, degrades the nascent RNA, and eventually displaces polymerase from the DNA.

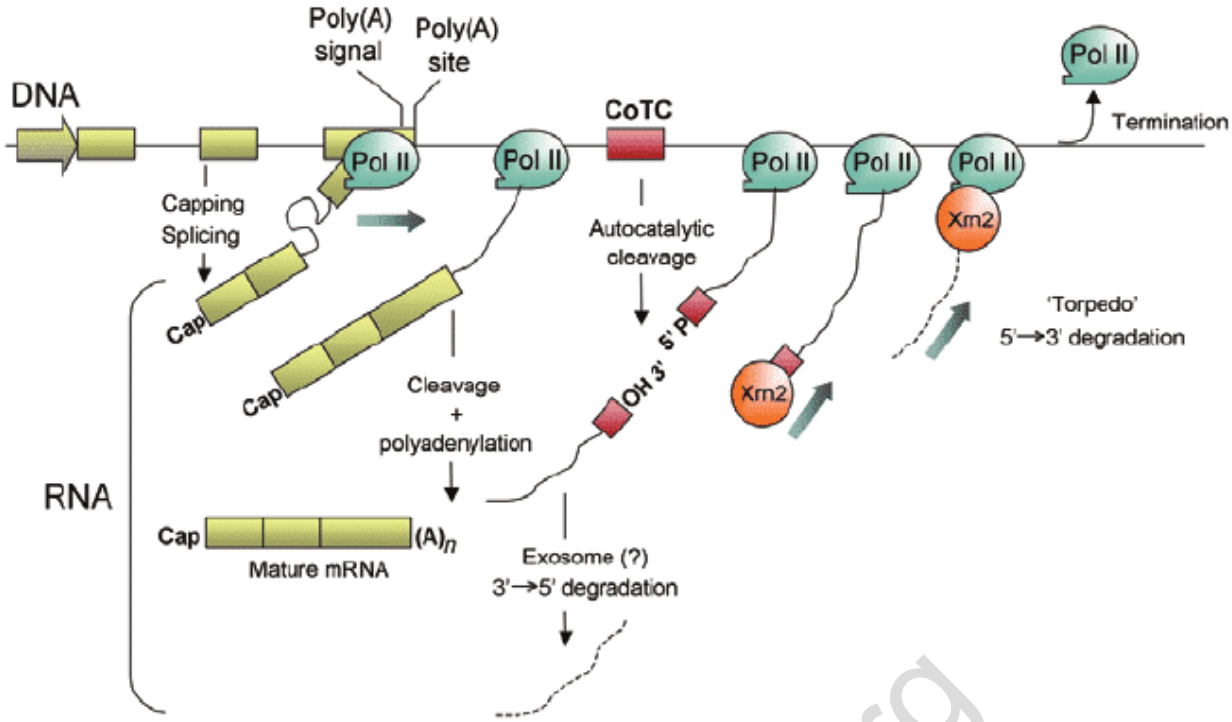


Fig : The torpedo model for transcription termination by RNA polymerase II in the human β -globin gene. β -globin pre-mRNA is subjected to a series of covalent modifications during its synthesis: capping, splicing, cleavage at the poly(A) site, polyadenylation, cleavage at the CoTC site, 5 ′→ 3 ′ degradation of downstream RNA and degradation of the RNA by-product between the poly(A) and CoTC sites. CoTC is a ribozyme, 5 ′→ 3 ′ degradation of downstream RNA is carried out by Xrn2 exonuclease and both processes are required for termination. Image Courtesy: Kornblihtt, Alberto. (2005). Shortcuts to the end. Nature structural & molecular biology. 11. 1156-7. 10.1038/nsmb1204-1156.

Splicing

- Formation of a mature, functional mRNA, the introns are removed and exons are spliced together
- Following capping, the introns are excised and their flanking exons are connected
- A most striking aspect of gene splicing is its precision
- Exons are never shuffled; their order in the mature mRNA is exactly the same as that in the gene from which it is transcribed

Splice sites

- Sequence comparisons of exon-intron junctions from a diverse group of eukaryotes indicate that they have a high degree of homology
- Different consensus sequences have been found:

1. 5’ Splice site – GU
2. 3’Splice site – AG
3. Branch site (A site)
4. Poly Y sequence – pyrimidine rich

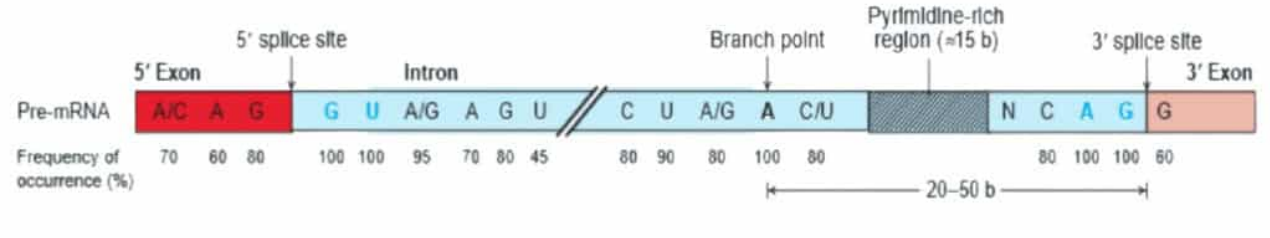


Fig: Splice site

Transesterification Reactions in RNA Splicing

Attack of the sugar-phosphate bond at the 5 ' splice site
• Group 1 = 3' OH of a free G nucleotide
• Group 2 and pre-mRNA = 2' OH group of branch point A nucleotide

Attack of the sugar-phosphate bond at the 3' splice site
• 3' OH of the nucleotide at 3' end of exon 1

Intron released
• Group 1 introns = linear
• Group 2 and pre-mRNA introns = lariat

Base pairing between U snRNAs and intron sequences brings intron sequences together and catalyzes the transesterification reactions.

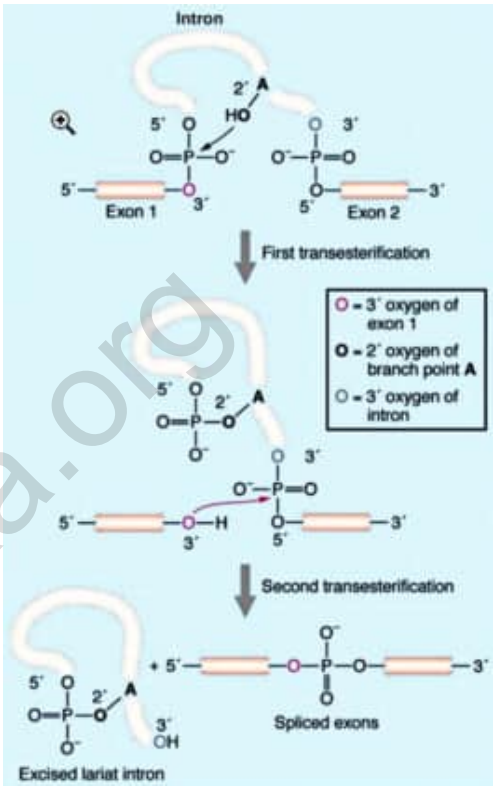


Fig: Splicing Mechanism -Two transesterification reactions

Self-splicing Introns

- The group I and group II introns, differ in the details of their splicing mechanisms but share one surprising characteristic: they are self-splicing—no protein enzymes are involved!
- Group I: Found in some nuclear, mitochondrial, and chloroplast genes that code for rRNAs, mRNAs, and tRNAs
- Group II introns: Found in the primary transcripts of mitochondrial or chloroplast mRNAs in fungi, algae, and plants

Group I Introns

Three-step reaction sequence:

1. The 3'-OH group of the guanosine forms a phosphodiester bond with the intron's 5' end, liberating the 5' exon.

2. The 3'-terminal OH group of the newly liberated 5' exon forms a phosphodiester bond with the 5'-terminal phosphate of the 3'exon, thereby splicing together the two exons and releasing the intron.

3. The 3'-terminal OH group of the intron forms a phosphodiester bond with the phosphate of the nucleotide 15 residues from the intron's 5' end, yielding the 5'-terminal fragment with the remainder of the intron in cyclic form.

Group II Introns

- They generally employ an internal A residue as their initial attacking nucleophile (instead of an external G) to form a lariat intermediate.
- A process that resembles the splicing of nuclear pre-mRNAs

Spliceosomal introns

- Because their removal occurs within and is catalyzed by a large protein complex called a spliceosome.
- The spliceosome is made up of specialized RNA-protein complexes, and small nuclear ribonucleoproteins (snRNPs or "snurps").
- Each snRNP contains one of a class of eukaryotic RNAs, 100 to 200 nucleotides long, known as small nuclear RNAs (snRNAs)
- Five U-rich small nuclear RNAs (snRNAs), designated U1, U2, U4, U5, and U6 associated with 6 to 10 proteins, participate in pre-mRNA splicing.

The significance of gene splicing has been often vehemently debated, and two important roles for it have emerged:

(1) It is an agent for rapid protein evolution; and

(2) alternative splicing, permits a single gene to encode several (sometimes many) proteins that may have significantly different functions.

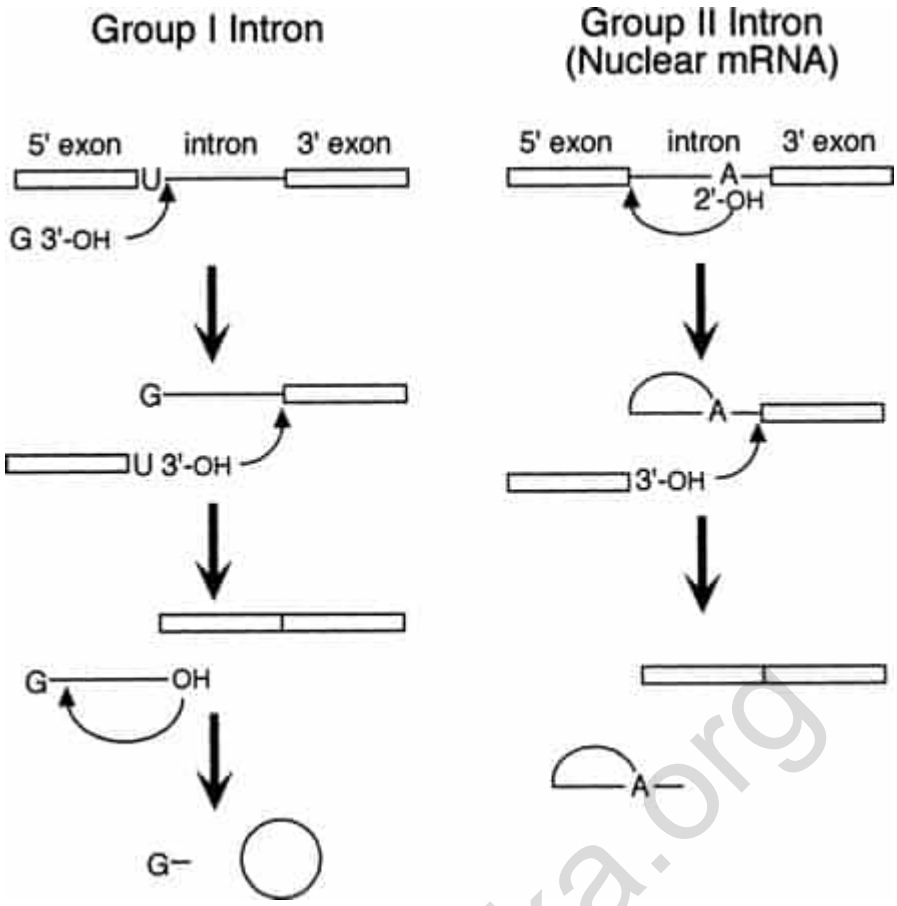


Fig: Splicing mechanisms of group I and group II introns. In both cases, a series of trans-esterification reactions are used to excise the intron and ligate the exons. The net number of bonds remains the same throughout. The reaction is initiated by a guanosine cofactor in group I introns and by internal adenosine in group II. The splicing reaction of nuclear pre-mRNAs follows the same pathway as group II introns, but it occurs on a large ribonucleoprotein complex. Image Courtesy: Tanner, Kyle. (1999). Ribozymes: The characteristics and properties of catalytic RNAs. FEMS microbiology reviews. 23. 257-75. 10.1111/j.1574-6976.1999.tb00399.x.

Alternative splicing

- In alternative splicing, exons can be deliberately skipped, and a given exon is joined to one further downstream.
- Certain exons in one type of cell may be introns in another.
- Alternative splicing occurs in all metazoa and is especially prevalent in invertebrates.
- Entire functional domains or even single amino acid residues may be inserted into or deleted from a protein, and the insertion of a stop codon may truncate the expressed
  polypeptide.
- Examples of such processes occur in the pathway responsible
  for sex determination in Drosophila.

Trans-splicing

- In some cases, two exons carried on different RNA molecules can be spliced together in a process called trans-splicing.
- Trypanosomes – mRNAs all have the same 35-nt non-coding leader sequence, this sequence is part of a so-called spliced leader (SL) RNA.
- The only difference is that the other product— the lariat in the standard reaction is, while in trans-splicing, a Y-shaped branch structure instead.

RNA editing

There are two mechanisms that mediate editing:

1. Site-specific deamination of adenines or cytosines
2. Guide RNA–directed uridine insertion or deletion

1. Deamination of C, performed by the enzyme cytidine deaminase, converts the C to a U).
2. Adenosine deamination. performed by the enzyme ADAR (adenosine deaminase acting on RNA) converting it into Inosine that base pair with cytosine.

- A very different form of RNA editing is found in the RNA transcripts that encode proteins in the mitochondria of trypanosomes.
- In this case, multiple Us are inserted into specific regions of mRNAs after transcription (or, in other cases, Us may be deleted)
- Guide RNAs (gRNAs) was identified, which consist of 40 to 80 nucleotides, have 3' oligo(U) tails, an internal segment that is precisely complementary to the edited portion of the pre-edited mRNA, and a 10- to 15-nt so-called anchor sequence near the 5'
  end that is largely complementary in the Watson–Crick sense to a segment of the mRNA that is not edited.

rRNA Processing

- Transcription by RNA polymerase I yield a 45S primary transcript (pre-rRNA), which is processed into the mature 28S, 18S, and 5.8S rRNAs found in cytoplasmic ribosomes
- Both the synthesis and processing of pre-rRNA occur in the nucleolus.
- Nascent pre-rRNA transcripts are immediately bound by proteins, forming pre-ribosomal ribonucleoprotein particles (pre-rRNPs)
- Cut in a series of cleavage and exonucleolytic steps that ultimately yield the mature rRNAs
- Modified, mostly by methylation of the 2-hydroxyl group of specific ribose and conversion of specific uridine residues to pseudouridine
- Positions of cleavage sites in pre-rRNA and the specific sites are determined by approximately 150 different small nucleolus-restricted RNA species, called small nucleolar RNAs (snoRNAs), which hybridize transiently to pre- rRNA molecules
- Unlike pre-rRNA genes, 5S rRNA genes are transcribed by RNA polymerase III in the nucleoplasm outside the nucleolus

- 5S RNA diffuses to the nucleolus, where it assembles with the 28S and 5.8S rRNAs and proteins into large ribosomal subunits
- When assembly of ribosomal subunits in the nucleolus is complete, they are transported through nuclear pore complexes to the cytoplasm

tRNA Processing

- Mature cytosolic tRNAs, which average 75–80 nucleotides in length, are produced from larger precursors (pre-tRNAs) synthesized by RNA polymerase III in the nucleoplasm.
- Cleavage and base modification occurs during the processing of all pre-tRNAs; some pre-tRNAs also are spliced during processing.
- extra 5' nucleotides are removed by ribonuclease P (RNase P), a ribonucleoprotein endonuclease
- Three classes of base modifications occur:
  – 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
- conversion of specific uridines to dihydrouridine, pseudouridine, or ribothymidine residues.
- The mechanism of pre-tRNA splicing differs in three fundamental ways from the mechanisms utilized by self-splicing introns and spliceosomes
- splicing of pre-tRNAs is catalyzed by proteins
- the pre-tRNA intron is excised in one step that entails
- simultaneous cleavage at both ends of the intron
- hydrolysis of GTP and ATP is required to join the two tRNA halves generated by cleavage on either side of the intron
- The mature tRNAs are transported to the cytoplasm through nuclear pore complexes

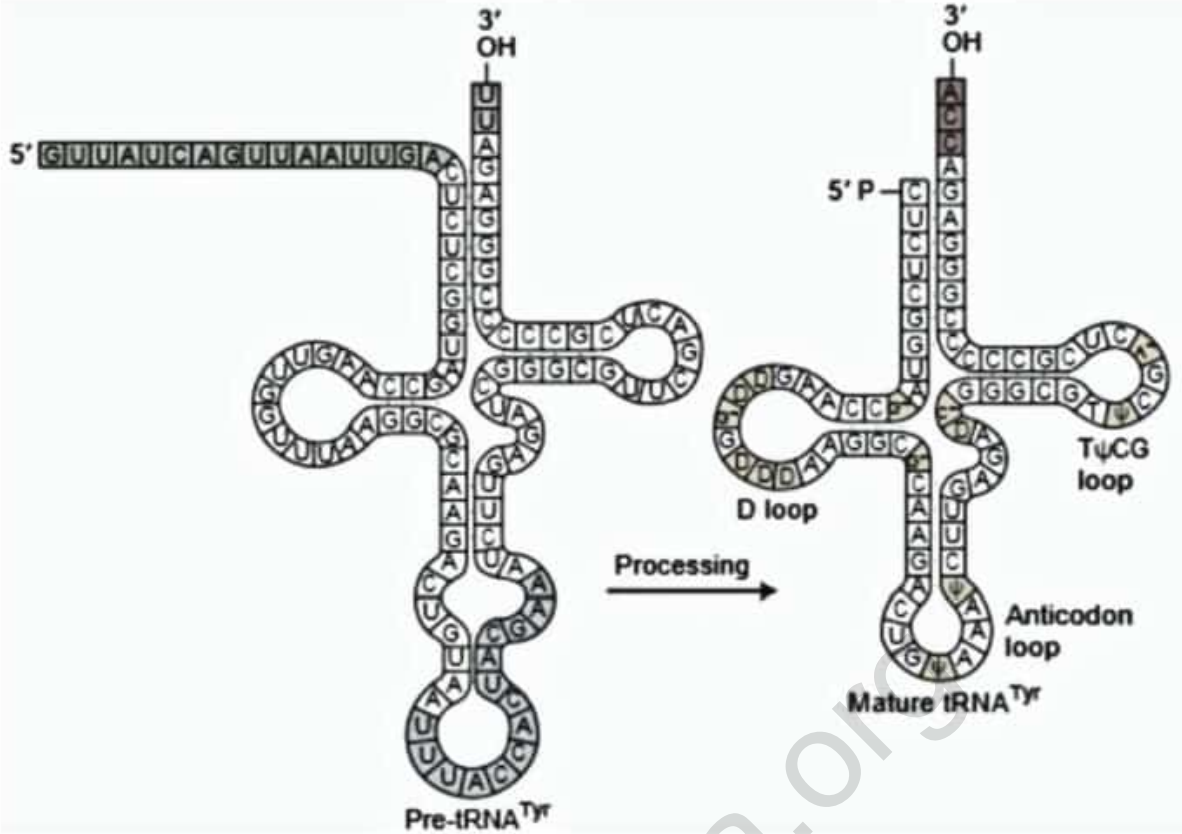


Fig: tRNA processing

Detailed Notes on RNA Synthesis & Processing – Available including:

- RNA Synthesis & Processing Flow charts & Koncept Tables
- Multiple Lecture Videos on RNA Synthesis
- Q&A Sessions +
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