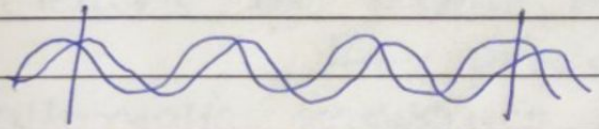
* Post transcriptional modification of primary transcript

→ Primary transcript :- Segment of DNA between specific initiation & termination sequences
- Initial, linear, RNA copy of transcription unit

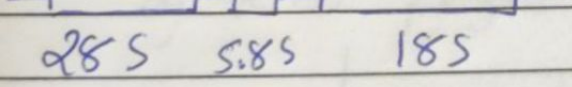
→ Occur in - tRNA, rRNA & both eukaryotic & prokaryotic
↓
by ribonucleases - cleave original transcripts

→ mRNA - prokaryotic - identical to it's 1° transcript
- eukaryotic - Extensively modified [co & post transcriptionally]

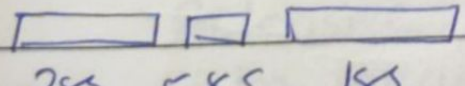
(1) Ribosomal RNA :- generated from long precursor molecule - pre-rRNA.



Ribosoma RNA gene



cleaved by RNases. (Ribonucleases)



Ribosomal RNA.

Eukaryotic ribosomal RNA post transcriptional modification

- 5S rRNA - synthesized by RNA polymerase III & modified separately
- trimmed by exonucleases
- modified at some base & ribose - by snoRNA
- rRNA Synthesis & processing occur in nucleolus

(2) Transfer RNA :-

pre tRNA
↓
(1) Remove intron from anticodon loop by nucleases if present.
(2) At 3' end - uracil residues replaced by CCA sequences by nucleotidyltransferase
(3) Many base modified & produce unusual base
(4) 16 nucleotide sequence at 5'-end is cleaved by RNase P (Ribozyme)
↳ produce mature tRNA.

(3) Eukaryotic messenger RNA :-

Heterogeneous nuclear RNA (hnRNA)
[all primary transcript synthesized in nucleus by RNA polymerase II]
↓
pre mRNA component
↓ goes
Extensive co & post transcriptional modification in nucleus
↓
become mature mRNA.

→ Modification :- (1) Addition of 5'-cap
(2) Addition of 3'- poly A tail
(3) Splicing
(4) Alternative splicing

① Addition of 5'-cap :- 1st processing reaction for pre mRNA.

- At 5' end - attach 7 methylguanosine

↓

by unusual 5'→5' triphosphate linkage [Resistant to most nucleases]

- Require removal of γ phosphoryl grap from 5'-triphosphate of pre-mRNA

↓ followed by

addition of guanosine monophosphate (from guanosine triphosphate) by enzyme nuclear - guanylyltransferase.

↓

Methylation of terminal guanine occur in cytosol by guanine 7-methyltransferase.

- S-Adenosylmethionine is source of methyl group.
- 7-Methylguanosine cap help stabilize mRNA & permits efficient initiation of translation.

(2) Addition of 3'-poly A tail:-

- At 3'-end - 40-250 adenylate chain
↓
Added by nuclear enzyme polyadenylate polymerase (use ATP)

Advantage :- Terminate eukaryotic transcription
- Stabilize mRNA
- Facilitate its exit from nucleus & aid in translation
- After mRNA enter into cytosol, poly-A tail is gradually shortened.

③ Splicing :-

- Removal of introns (intervening sequences) from primary transcript of RNA sequences.
- Coding region (exons) are joined together & form mature mRNA.
- Process of removing introns & joining exons is called splicing.
- Done by Spliceosome.
- Spliceosome - primary transcript + SnRNA like U_1, U_2, U_3 etc.
- Histone - No introns present.

→ Role of SnRNA (small nuclear RNA) :-

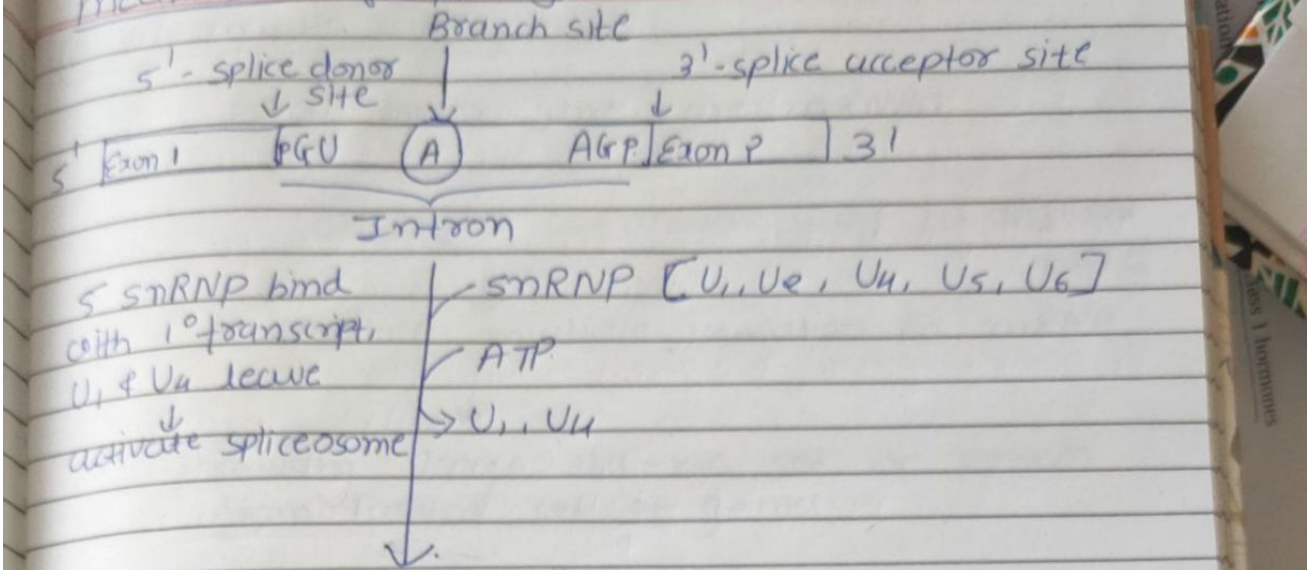
→ Uracil Rich SnRNA + multiple protein
↓ Form
5 small nuclear ribonucleoprotein [SNURP]
- U_1, U_2, U_4, U_5 & U_6 → mediate splicing.

- Help in removal of introns by forming base pair with consensus sequence at each end of intron.

→ effect of Splice site mutation :-

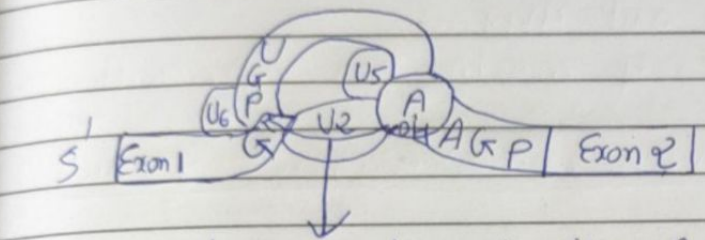
β thalassemia - Incorrect splicing of β globin mRNA.
Spinal muscular dystrophy & duchenne muscular dystrophy

mechanism of splicing :-

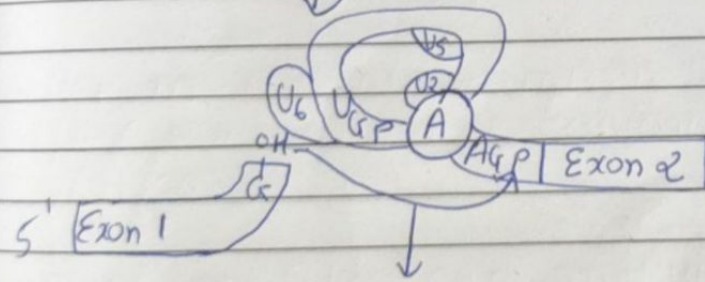


snRNP bind with 1° transcript, U_1 & U_4 leave ↓ activate spliceosome

→ snRNP [U_1, U_2, U_4, U_5, U_6]
→ ATP
→ U_1, U_4



2'-OH of branch site A attack 5'-phosphate at splice donor site of intron & form 2'→5' phosphodiester bond & a lariat.



Free 3'-OH of exon 1 attack 5'-phosphate at splice acceptor site & form phosphodiester bond that join exon 1 & 2.

→ snRNP (U_2, U_5, U_6)

Exised intron (lariat)

+

mature mRNA

5' Exon 1 P Exon 2 3'

④ Alternative splicing :-

>90% human gene pre mRNA

↓

Spliced in alternative way in different tissue

↓

Produce multiple variation in mRNA.

↓

producing large diverse set of protein from limited set of genes.