RNA Processing (Part-II)

(BIOT 4006: Genetics and Molecular Biology)

Dr. Saurabh Singh Rathore

Department of Biotechnology
MGCU

- We have seen the mechanism of self splicing introns.
- Another group of introns, that is not designated with any group number, is constituted by majority of introns that are not self splicing.
- Spliceosomal introns: introns found in nuclear mRNA transcripts (removal is catalyzed by a large protein complex called spliceosome and occurs within that spliceosome following the mechanism similar to that of group II introns).
- Spliceosome is formed by RNA-protein complexes called small nuclear ribonucleoproteins (snRNPs, or “snurps”).
- Small nuclear RNAs (snRNAs) are eukaryotic RNAs and 100-200 nucleotides in length. They are found to be associated with “snurps”.

- The nuclei of eukaryotes have these five snRNAs in abundance and found to be actively participating in splicing reactions: U1, U2, U4, U5, and U6.

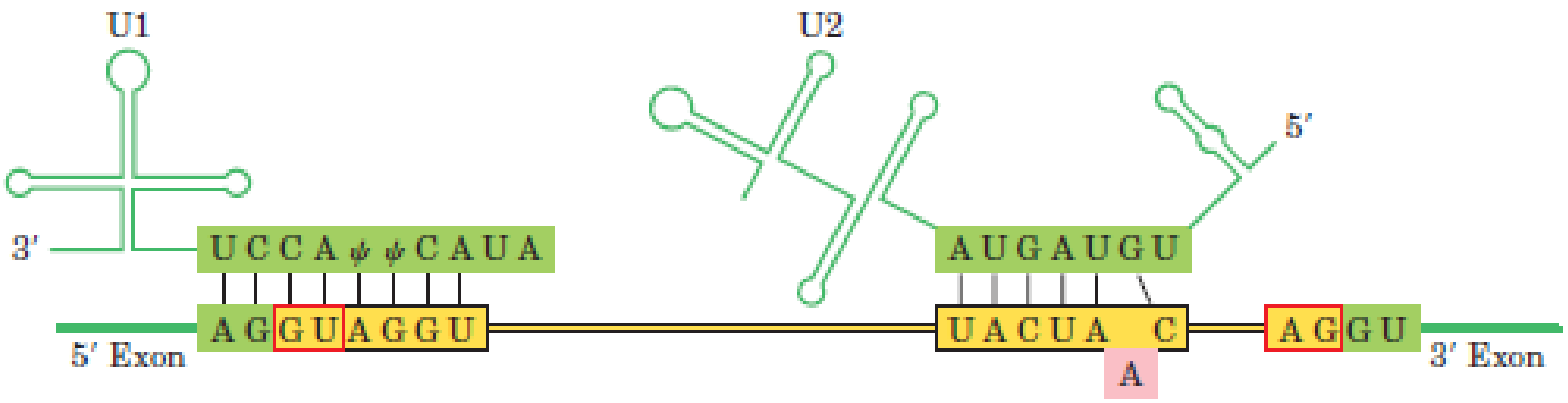
- The sequences of snRNP/snurp proteins and RNAs are highly conserved in eukaryotes.

- The splice sites present at the 5’ and 3’ ends of the introns present in spliceosomes generally have GU and AG dinucleotide sequences respectively.

- The sequences present near the 5’ splice site of nuclear mRNA introns are complementary to a sequence of U1 snRNA and these intronic sequences are the binding region for the U1 snRNP in the primary transcript.

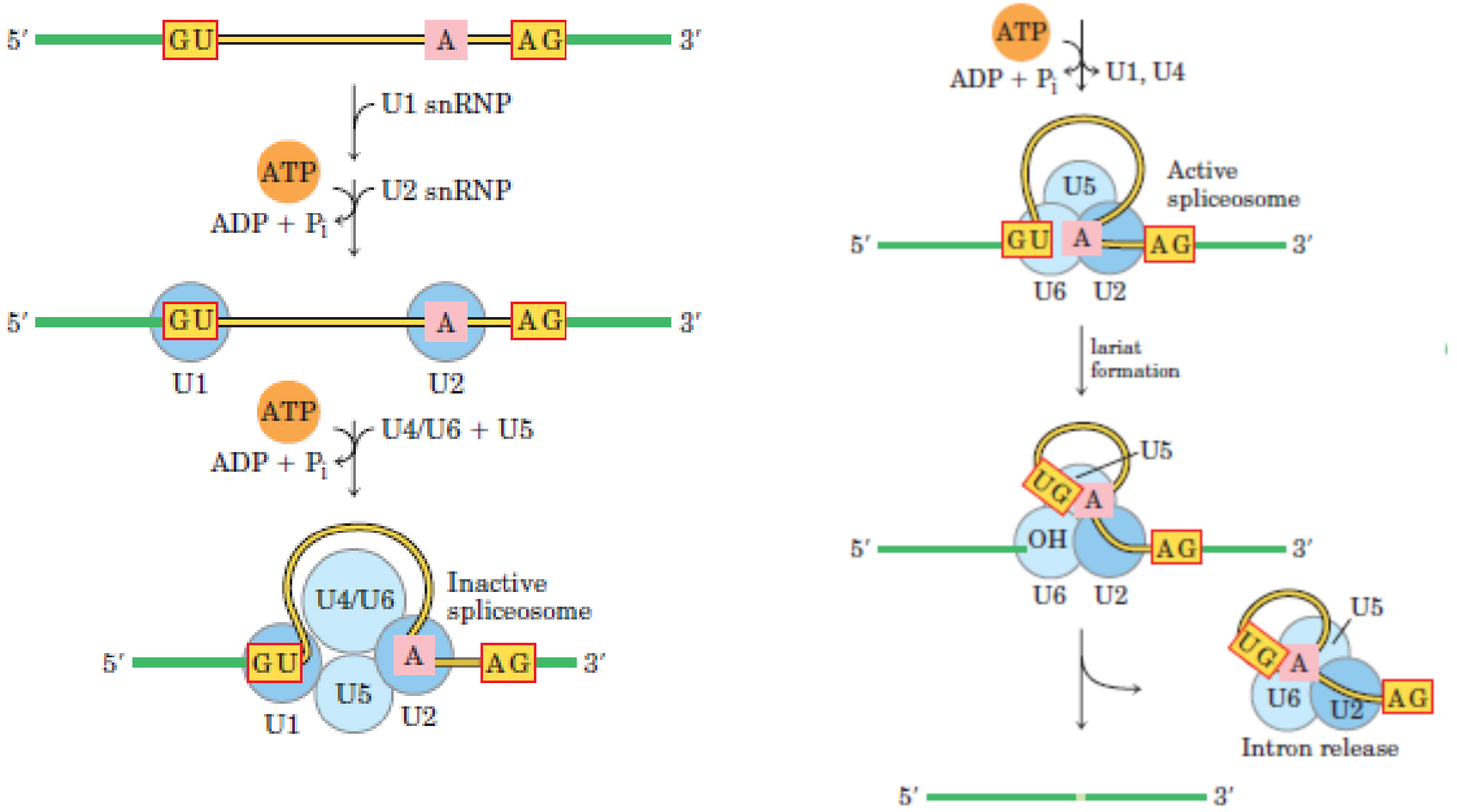
- Further the remaining U2, U4, U5 and U6 snRNPs are added to form the spliceosome.

Mechanism of splicing in primary transcripts of mRNA



- The pairing interactions between RNA are shown.
- Complementarity between the sequences at 5’ ends of U1 snRNA and the intron facilitates the formation of the spliceosome.
- This pairing of U1 with the intronic sequences assists in describing the 5’ splice site during spliceosome arrangement.
- Ψ: peseudouridine
- The U2 snRNA does not pairs perfectly with mRNA and this imperfect pairing causes formation of bulging in a region containing the A residue (shown in pink).
- Adenylate is activated and acts as a nucleophile to form a lariat like structure (as described earlier).

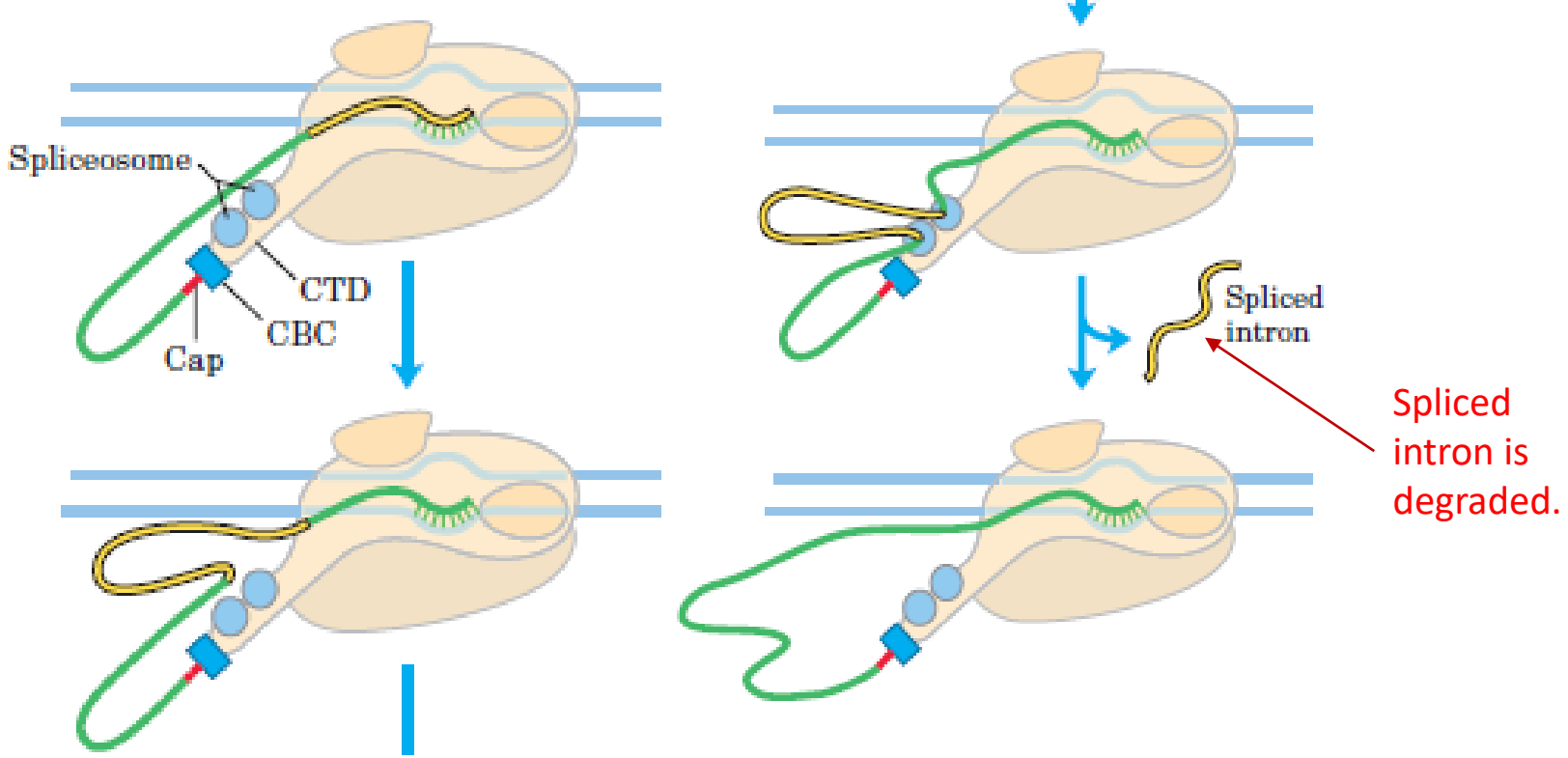
Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox Chapter 26, Page 1012



Mechanism of splicing in primary transcripts of mRNA

- An inactivated spliceosome is formed by binding of U4/U6 complex and U5 snRNP to the structure already bound with the U1 and U2 snRNPs.
- Inner reorganizations alter this inactive spliceosome to an active spliceosome.
- The rearrangement expels U1, U4 and pairs U6 to 5’ splice site as well as U2.
- Catalytic steps after rearrangement are matched with the splicing steps of group II introns.

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1012

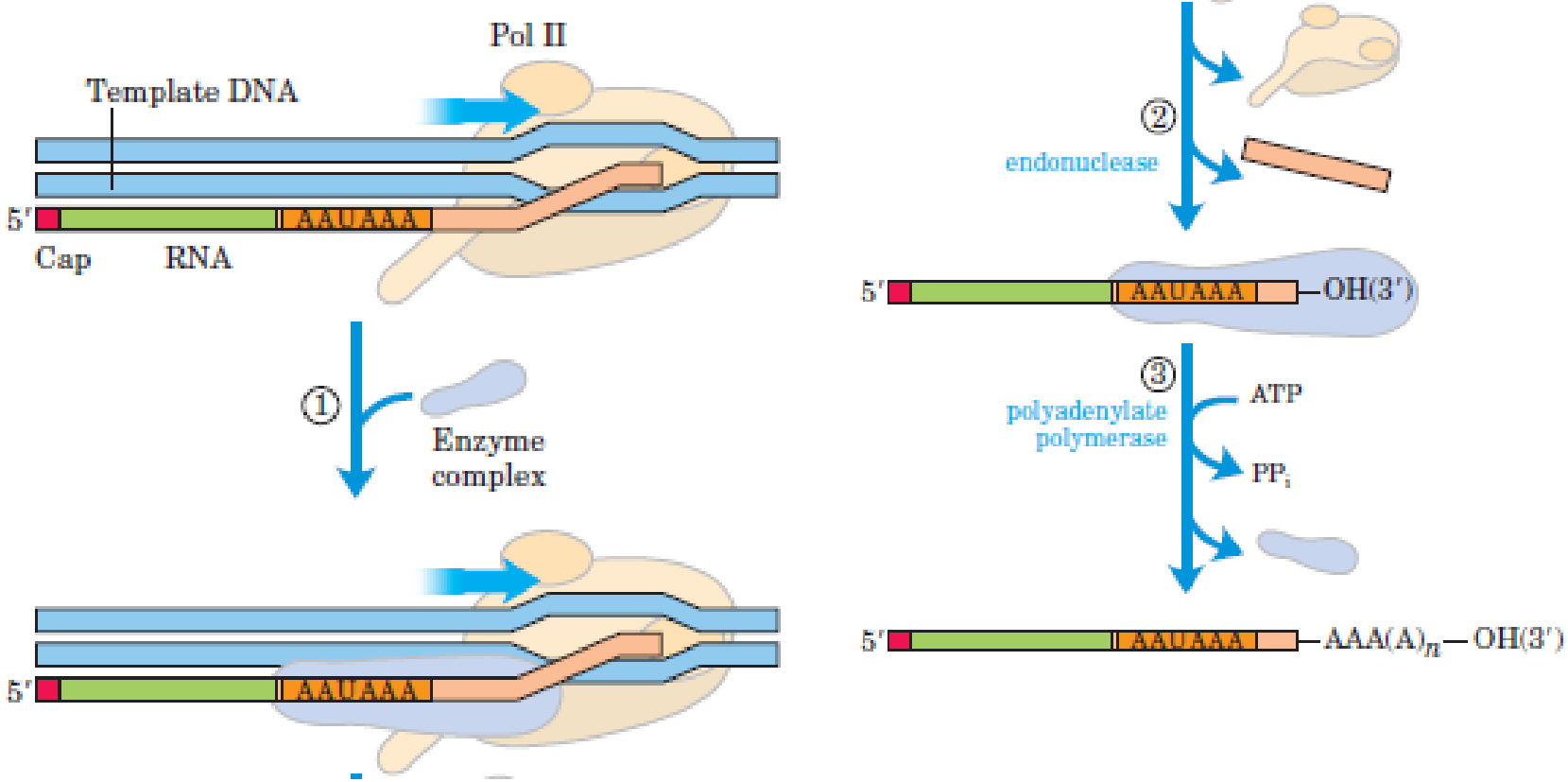


Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox Chapter 26, Page 1012

- Splicing in the fourth class of introns (common in some t-RNAs) is different from that in group I and II introns.
- ATP and an endonuclease is required for splicing.
- In this case, the phosphodiester bonds present at both ends of the intron are broken by the endonuclease.
- The two exon ends are ligated in a manner similar to that of DNA ligase enzymatic reaction.

Mechanism of splicing in primary transcripts of mRNA

- Splicing coordinated with transcription is shown here.
- The CTD of RNA polymerase II is attached to a few constituents of the splicing apparatus.
- The tethered spliceosome is attached to the first splice junction as soon as it gets generated.
- The placement of intron ends close to each other is then done by capturing of the second splice site by the passing of spliceosome complex. Splicing follows these events.
- The result is simultaneous cleavage of intron at its both ends and fetching the two splice sites together.

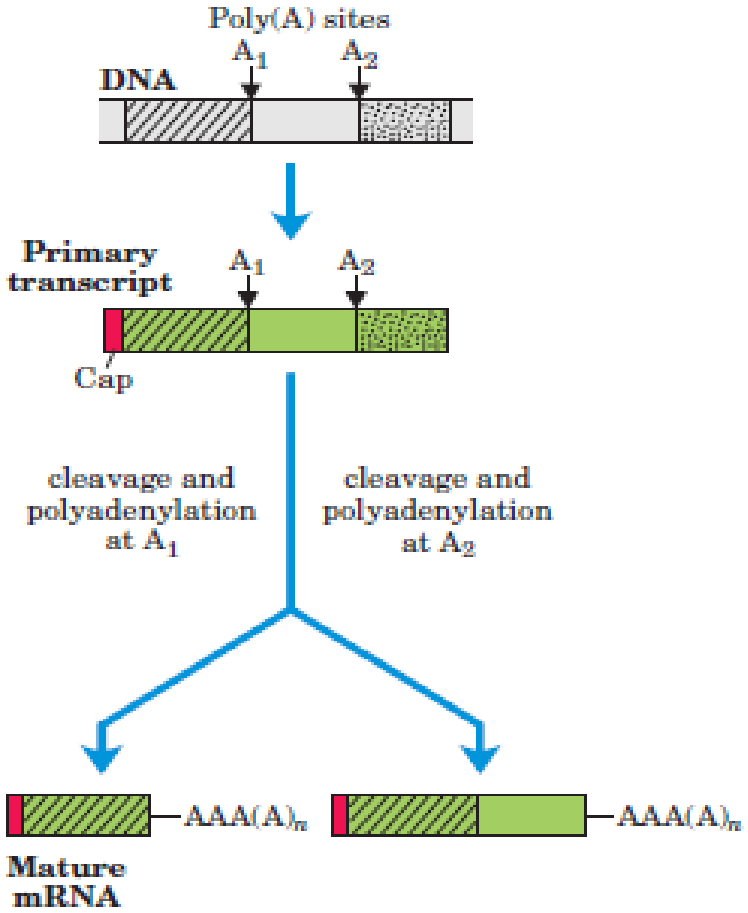


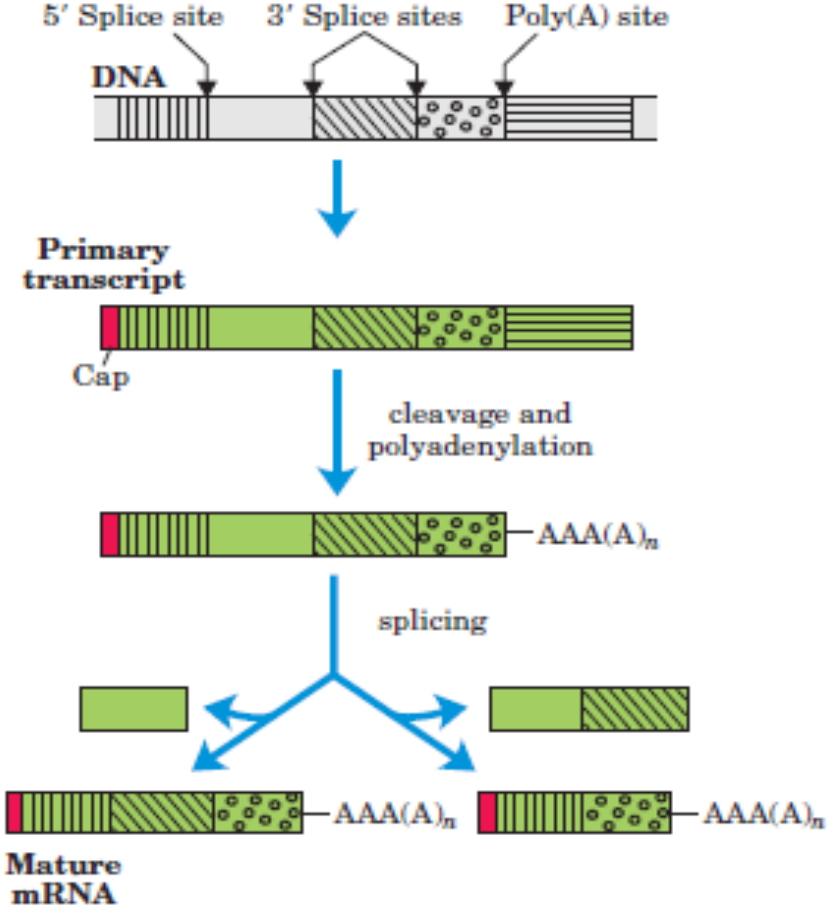
Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox Chapter 26, Page 1013

- Two sequence elements are highly conserved at the cleavage site of mRNA: 5'AAUAAA3' (10-30 nucleotides upstream of cleavage site) and a vaguely defined G/U rich sequence (20-40 nucleotides downstream of cleavage site).
- The free 3' OH group generated by cleavage defines the mRNA end. At the 3' OH, A residues are added by polydenylate polymerase.

How poly(A) tail is added to the primary RNA transcript of eukaryotes?

- Majority of the eukaryotic mRNAs contain a series of 80-250 A residues known as poly(A) tail.
- One or a few specific proteins bind to specific sites on this poly(A) tail. Probably these proteins help in protecting the mRNA against destructive enzymes.
- Conversely, in case of prokaryotic mRNAs, the poly(A) tails fuel up the destruction of mRNA.
- A multistep process occurs for polyadenylation of the mRNA tail at 3' end.
- Extension of the transcript occurs further than the site of polyadenylation, then it is cleaved at the same site by the endonuclease activity of a part of large enzyme complex associated with RNA polymerase CTD.





Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1014

Alternative processing of complex transcripts in eukaryotes.

- Processing of some of the eukaryotic mRNAs can occur differently so that more than one mRNAs are produced from a single primary transcript. This ultimately results in more than one polypeptides produced from a single primary transcript.
- Which way or path is chosen is dictated by the processing factors, RNA binding proteins (promoting a particular path) through the molecular signals on the primary transcript.

Two mechanisms of alternative processing can be take up:

(a) Distinct cleavage and polyadenylation patterns. Two poly(A) sites, A1 and A2, are shown.

(b) Alternative splicing by presence of more than one 3’ splice sites. Here, different 3’ splice sites result in 2 possibilities of exon selection.

Both mechanisms result in different mature mRNAs from the same primary transcript.