RNA Processing (Part-I)

(BIOT 4006: Genetics and Molecular Biology)

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RNA Processing

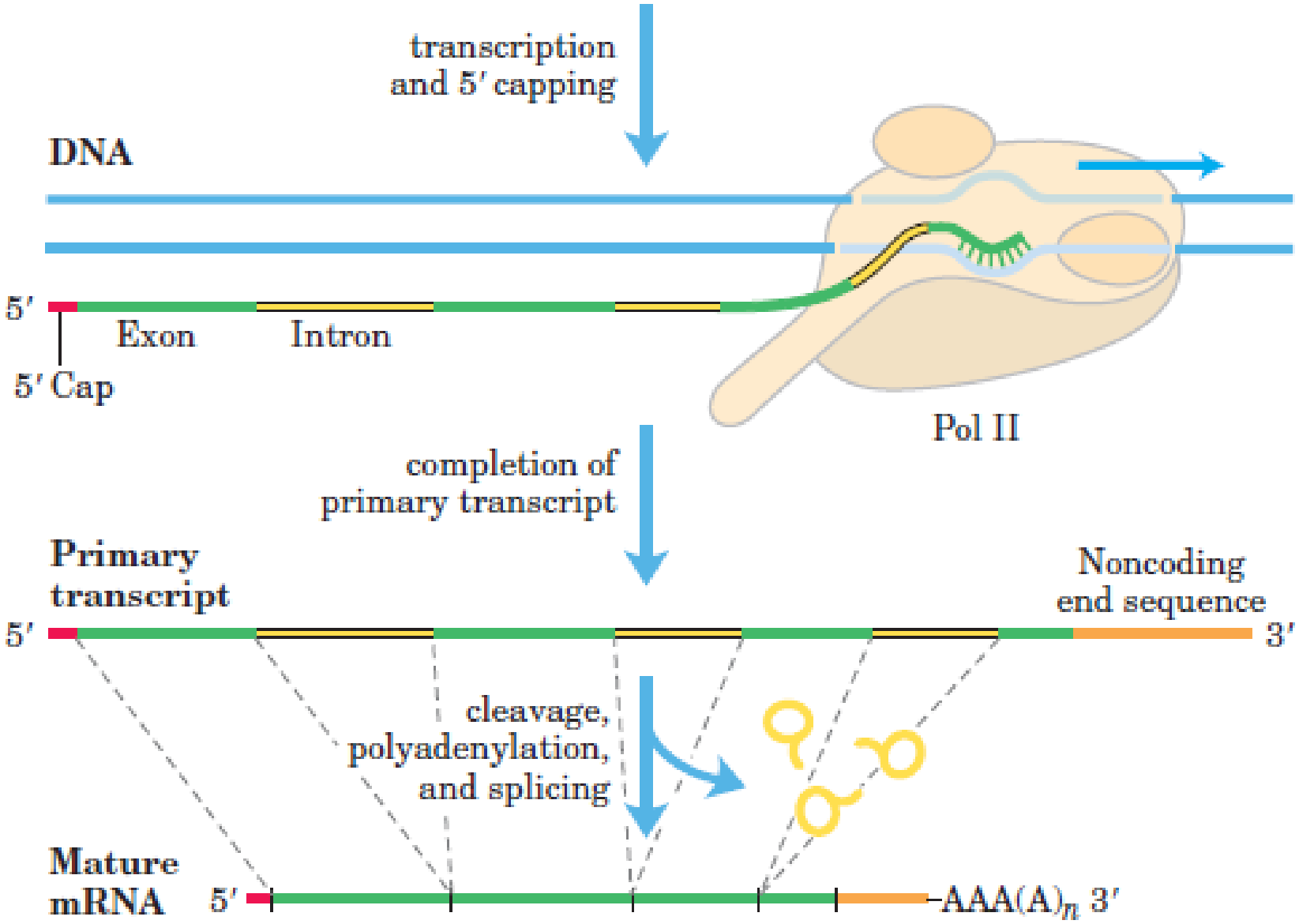
- Post synthetic RNA processing: - in many bacterial RNAs
  - in nearly all eukaryotic RNAs
- Enzymes involved: Proteins as well as RNAs (Ribozymes)
- Most extensive RNA processing occurs in: mRNA (eukaryotes) and tRNA (eukaryotes & bacteria)
- During splicing, the non coding introns are removed and continuous sequence of exons is achieved.

- End modifications in eukaryotic mRNAs: - 5’ capping
  - 3’ polyadenylation

- The enzyme complexes carrying out these mRNA processing reactions are organized in association with each other organized in association with each other and with the phosphorylated CTD of Pol II.

- The complexes work in a manner that their functions affect each other.

- Proteins responsible for mRNA processing, transport of the transcript and its delivery to ribosomes for translation – function in a dynamic manner associated within an elaborate complex.



Outline of RNA Processing

Maturation of mRNA involving the formation and processing of primary transcript in Eukaryotes (inside nucleus).

1. Addition of a 5’ cap (shown in red color) occurs prior to the completion of primary transcript synthesis.

2. The last exon is followed by a non-coding sequence (shown in orange color).

3. Splicing can happen after /before the cleavage and polyadenylation steps.

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1007

- 5’ cap, a residue of 7-methylguanosine linked to the 5’-terminal residue of the mRNA by an unusual 5’,5’-triphosphate linkage.
- The 5’ cap has a protective role against the ribonucleases which tend to cleave the mRNA.
- Another function of 5’ cap is to bind with a cap-binding complex of proteins.
- The 5’ cap also supports mRNA binding to ribosomes to fascilitate translation initiation.
- The formation of 5’ cap is done by a GTP molecule condensing to a triphosphate present at 5’ end of the transcript.

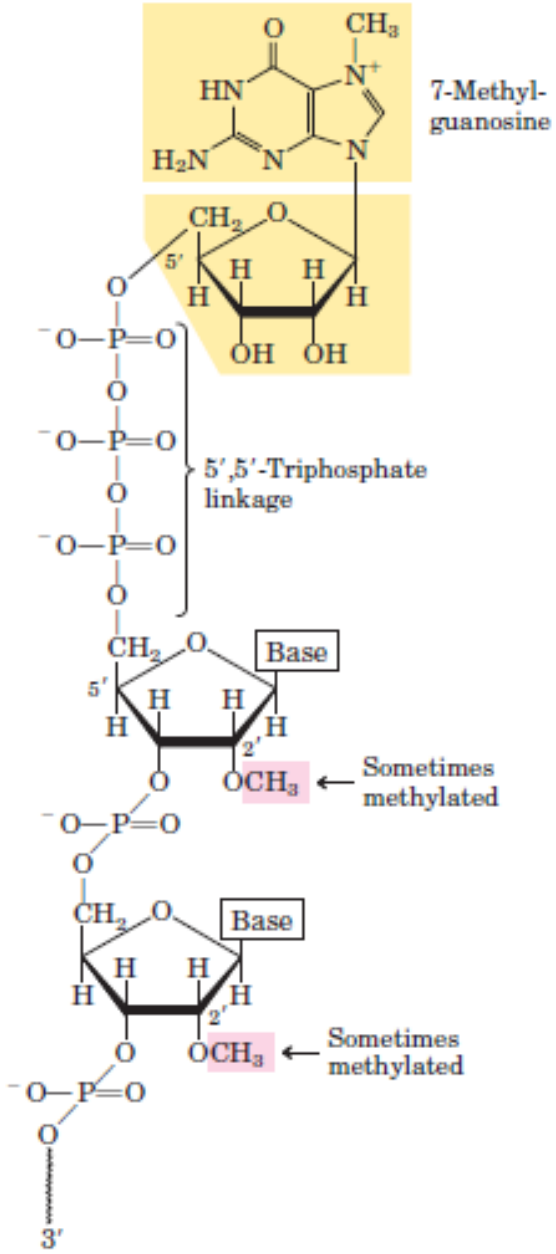
- Afterwards, guanine residue attached to the triphosphate is methylated at the N-7 position.

- The first and second nucleotides are mostly methylated at their respective 2’ hydroxyl positions. Supply of methyl groups comes from S-adenosylmethionine.

- The above said reactions take place in the initial phase of transcription (after addition of 20-30 nucleotides).

- The RNA polymerase II CTD is associated with the three capping enzymes and 5’ end of the transcript for 5’ cap synthesis.

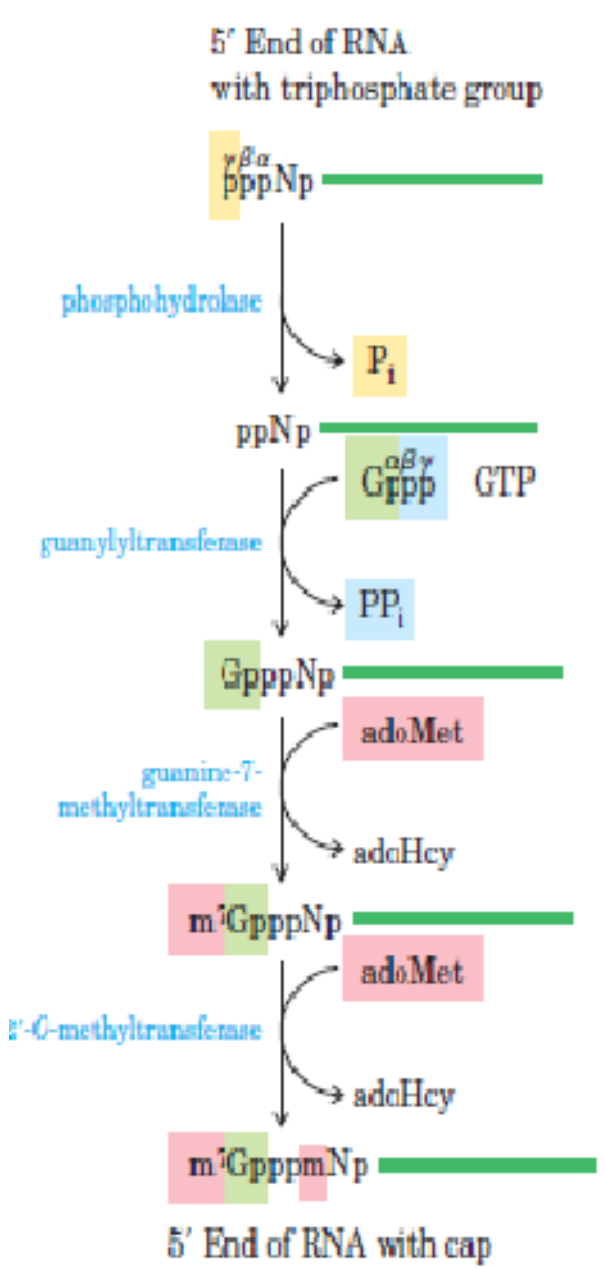
- After 5’ cap synthesis completion, it is detached from the enzymes. This is fascilitated by the cap-binding complex.



Synthesis of 5’ cap of mRNA.

- 7-Methylguanosine is attached to the 5’ end mRNA by a 5’,5’-triphosphate linkage.
- The first and second nucleotides are commonly methylated at 2’ positions (shown in pink color).
- However, 2’ methylation is absent in yeast m-RNAs.
- In case of Vertebrates only, methylation at 2’ position is commonly present at the second nucleotide.

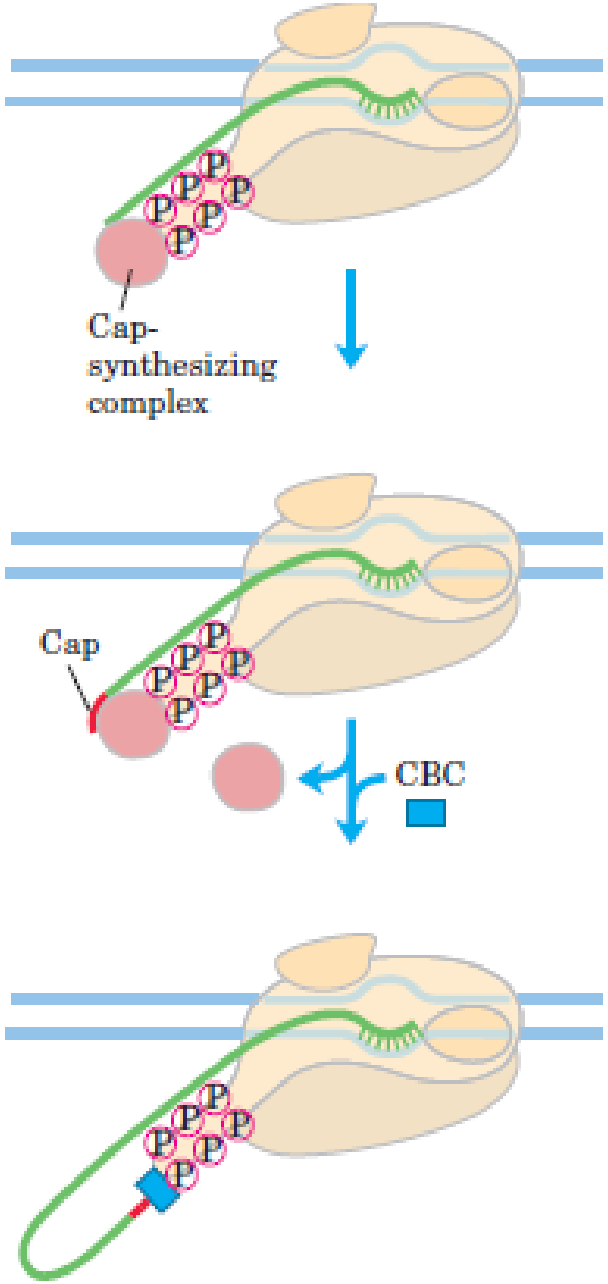
Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1008



Synthesis of 5’ cap of mRNA.

Four enzyme catalyzed steps in formation of 5’ cap (adoHcy: *S*adenosyl-homo-cysteine).

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1008

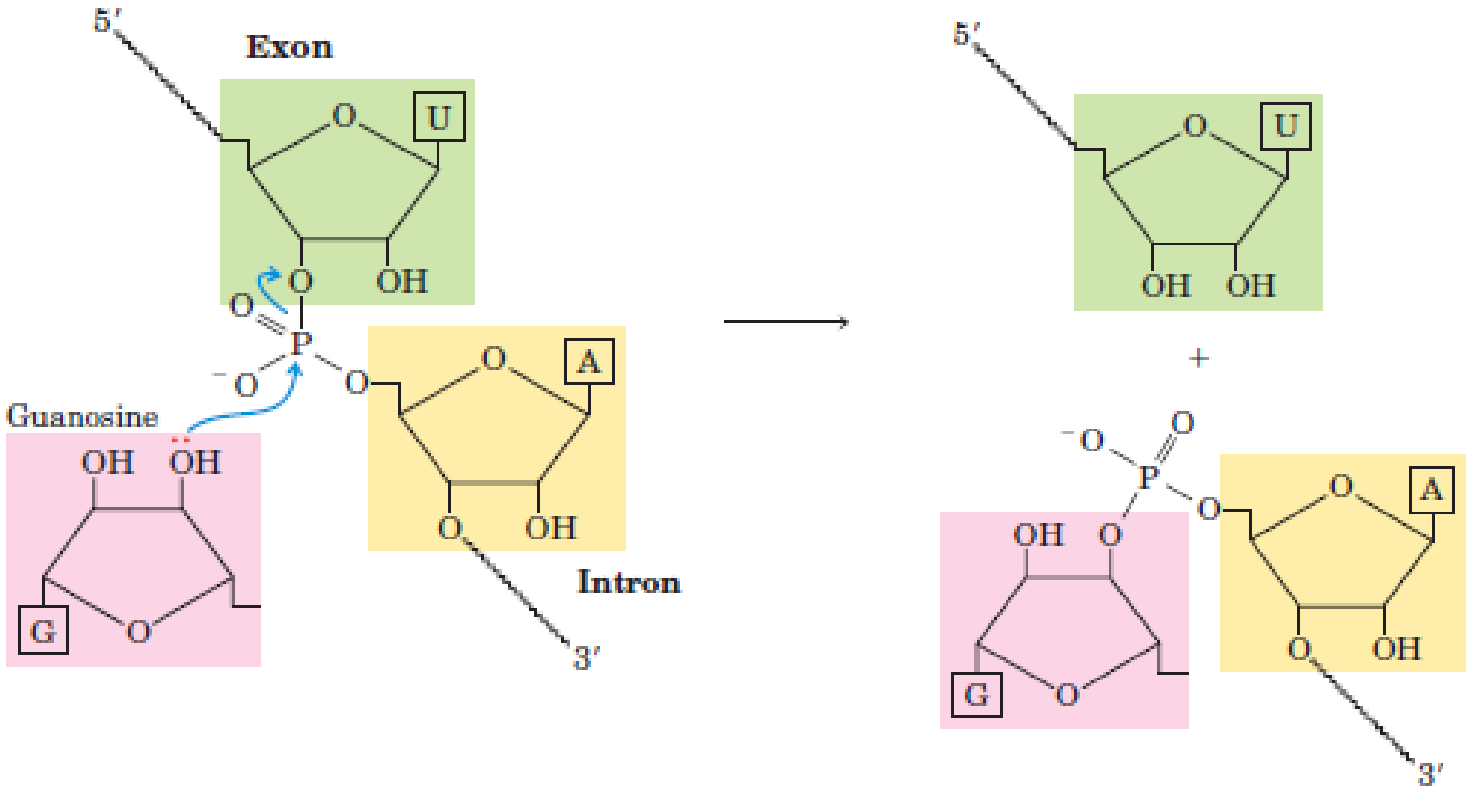


Synthesis of 5’ cap of mRNA.

- Enzymes attached to the CTD of Pol II carryout the synthesis of 5’-cap.
- The cap synthesizing complex is replaced by cap binding complex (CBC) and this facilitates tethering of the cap with CTD.

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1008

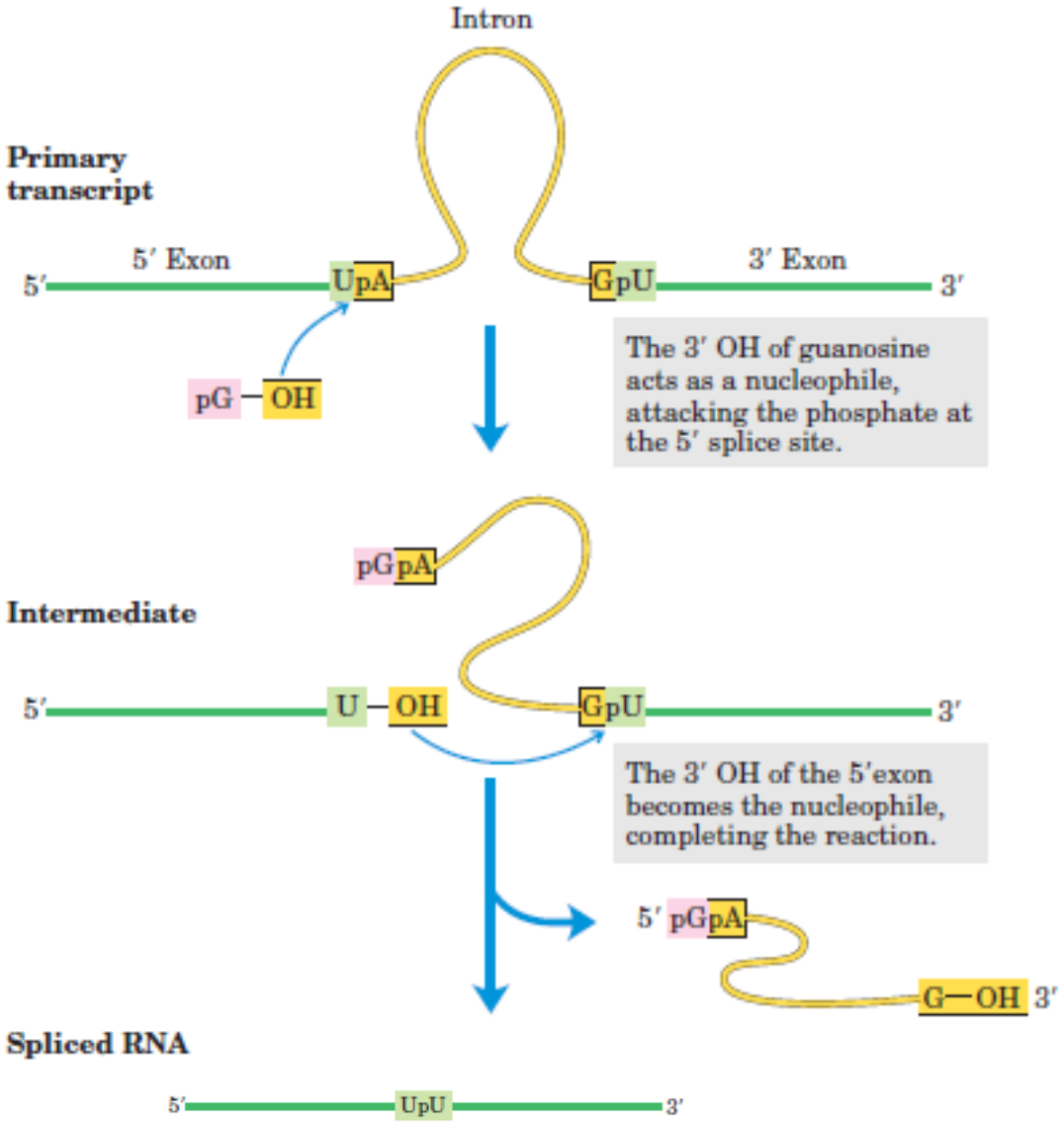
- Living organisms contain four groups of introns.
- The first two groups, i.e. Group I and Group II introns are self splicing as they don’t need any enzymes or ATP (to provide energy) for splicing.
- This self splicing takes place through two transesterification reactions.
- There is a nucleophilic attack of 2’ or 3’ hydroxyl on phosphorous and subsequent formation of a new phosphodiester bond.
- An energy balance is maintained as a phosphodiester bond is broken and a similar bond is formed.



Transesterification reaction in Group 1 introns.

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1009

- The splicing of group I introns needs a guanine nucleoside/nucleotide as a cofactor for participation of its 3’ OH group (as a nucleophile).
- A 3’,5’-phosphodiester bond is formed at the 5’ end of intron by the nucleophilic attack of 3’ OH of the incoming nucleotide on the phosphorus atom.
- In this manner, the incoming nucleophilic attack of 3’ OH group displaces the 3’ OH of an exon.
- The displaced exon’s 3’ OH acts as a nucleophile for the 3’ end of intron.
- This whole process precisely cuts out the intron and ligates the exons.



Group I intron splicing mechanism

- There is a nucleophilic attack from a 3’ OH of a guanosine nucleoside/nucleotide.
- 3’ OH of the displaced exon causes a fresh nucleophilic attack on the 3’ end of the intron.
- Subsequently precise exision of intron and ligation of exons occurs.
- The exised intron is finally degraded

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1010

- Some mitochondrial genes contain Group II introns.

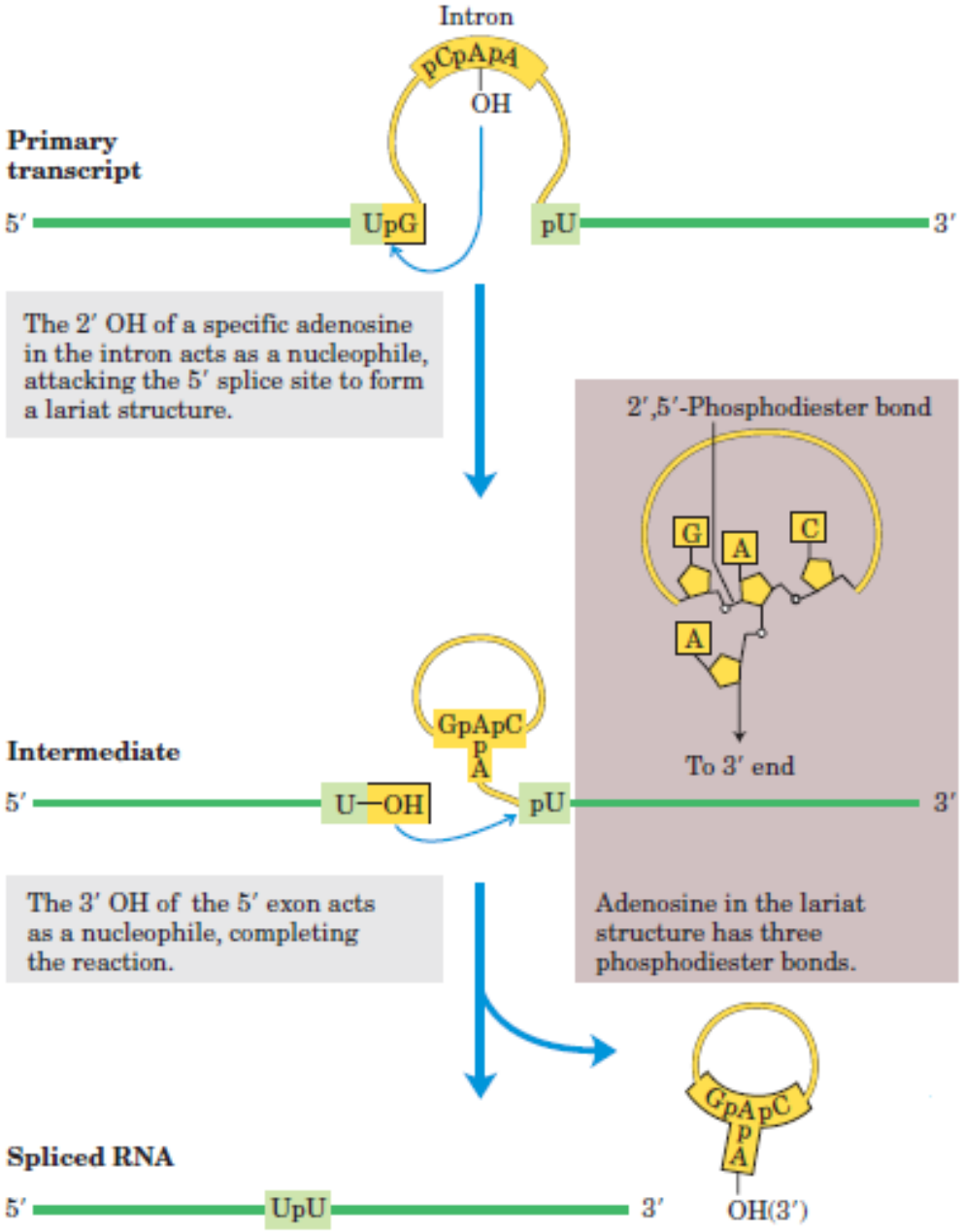
- The reaction leading to splicing of Group II introns follows the mechanism of splicing of Group I introns except few differences.

- The differences lies in the nucleophile of the first step and the structure of removed intron. It is the 2-OH group of an Adenine residue present within the intron.

- The 3 phosphodiester bonds of the *A* residue form a branched *lariat* like structure.

- Rest mechanism is similar to that in Group I introns, i.e. the displaced exon's 3' OH acts as a new nucleophile for the 3' end of intron.

- Finally, the intron is removed in the form of a branched lariat structure.



Group II intron splicing mechanism

- It is mostly similar to the Group I intron splicing except a few differences.

- The differences are:
  1- Adenine-3’ OH acting as nucleophile in first step and resulting in a branched lariat like intermediate consisting of 3 phosphodiester bonds at the *A* residue.

  2- The outgoing intron is removed as a branched lariat shaped structure.

Reference: Lehninger Principles of Biochemistry Fourth Edition David L. Nelson & Michael M. Cox
Chapter 26, Page 1011