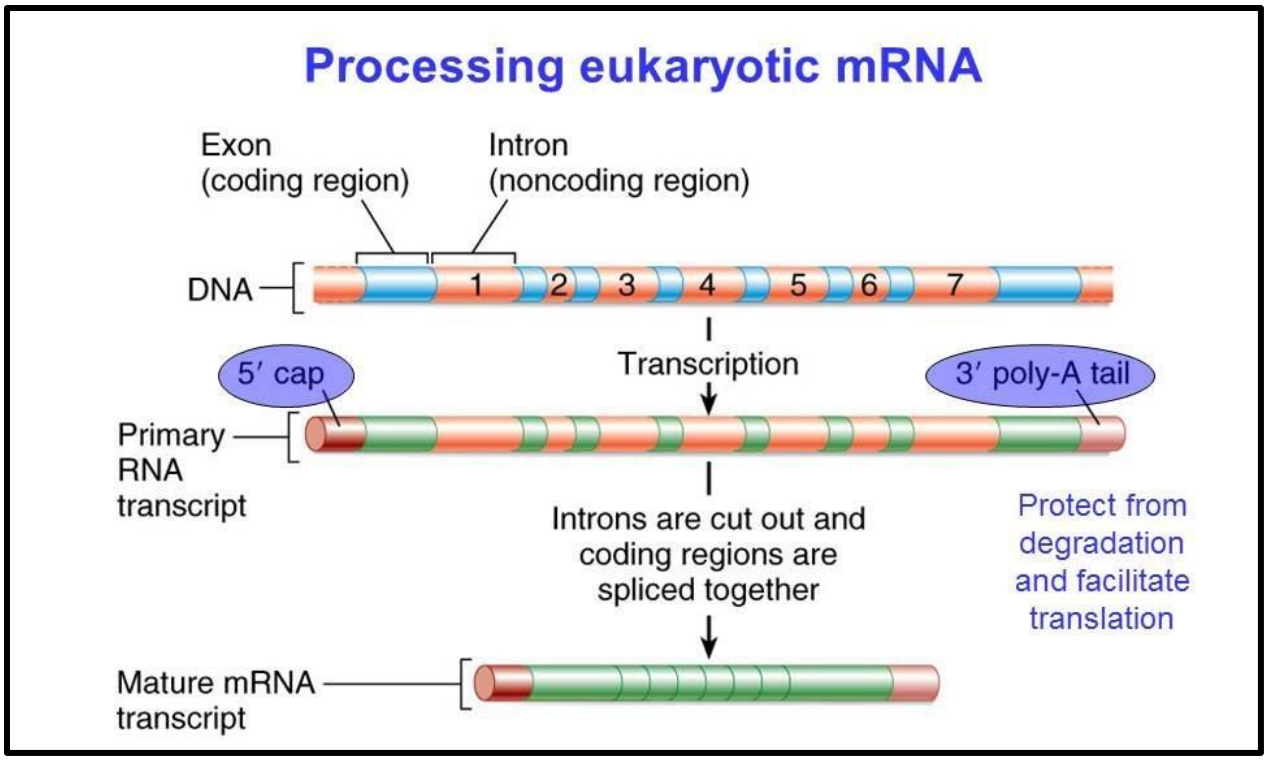
RNA Processing

- The major difference between prokaryotes and eukaryotes, is in the processing of messenger RNAs.
- In bacterial cells, the mRNA is translated directly as it comes off the DNA template.
- In eukaryotic cells, RNA synthesis, which occurs in the nucleus, is separated from the protein synthesis machinery, which is in the cytoplasm.
- In addition, as discussed earlier, eukaryotic genes have introns, noncoding regions that interrupt the gene. The mRNA copied from genes containing introns will also therefore have noncoding regions that interrupt the information in the gene. These noncoding regions must be removed before the mRNA is sent out of the nucleus to be used for protein synthesis. The process of removing the introns and rejoining the coding sections or exons, of the mRNA , is called splicing. Other steps take place alongside splicing which are together part of RNA processing
- Messenger RNAs are processed in eukaryotic cells, not in bacterial cells. The three main processing steps are
  1. **Capping at the 5' end** : In the capping step of mRNA processing, a 7-methyl guanosine is added at the 5' end of the mRNA. The cap protects the 5' end of the mRNA from degradation by nucleases and also helps to position the mRNA correctly on the ribosomes during protein synthesis.7-methyl G is added to the 5’ end of mRNA in eukaryotes enzymatically during maturation in nucleus

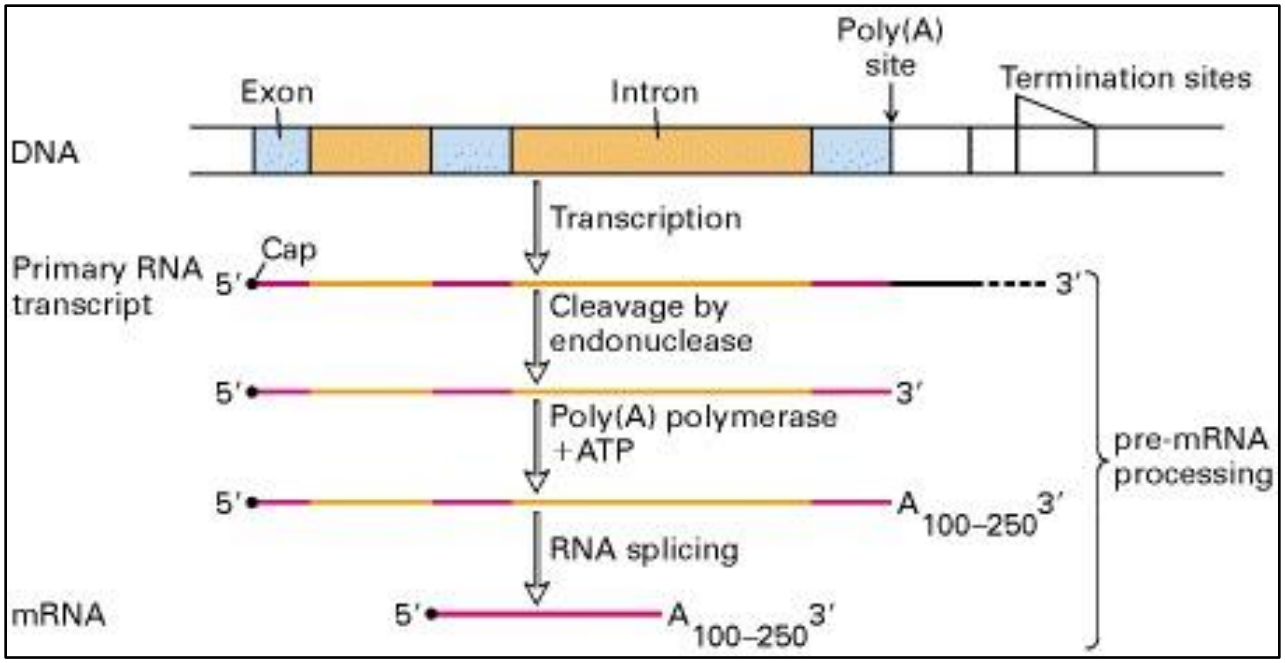
  2. **Splicing to remove introns**: Introns are removed from the pre-mRNA by the activity of a complex called the spliceosome. The spliceosome is made up of proteins and small RNAs that are associated to form protein-RNA enzymes called small nuclear ribonucleoproteins or snRNPs

  3. **Addition of a polyA tail at the 3' end** : An enzyme called PolyA Polymerase adds a "tail" of about 200 As to the 3' end. polyA tail plays a role in efficient translation of the mRNA, as well as in the stability of the mRNA. Poly A addition sequence (AAUAAA) is repeated several time in poly a tail, length varies from sp to species.



- Processing occurs in the nucleus, and the functional mRNA produced is transported to the cytoplasm for translation.

- The initial product of transcription of an mRNA (unprocessed) is called the **pre-mRNA** or **primary transcript.** After it has been processed and is ready to be exported from the nucleus, it is called the **mature mRNA** or **processed mRNA**.
- In figure below : Shortly after RNA polymerase II initiates transcription at the first nucleotide of the first exon of a gene, the 5′ end of the nascent RNA is capped with 7-methylguanylate. Transcription by RNA polymerase II terminates at any one of multiple termination sites downstream from the poly(A) site, which is located at the 3′ end of the final exon. After the primary transcript is cleaved at the poly(A) site, a string of adenine (A) residues is added. The poly(A) tail contains ≈250 A residues in mammals, ≈150 in insects, and ≈100 in yeasts. For short primary transcripts with few introns, polyadenylation, cleavage, and splicing usually follows termination, as shown. For large genes with multiple introns, introns often are spliced out of the nascent RNA before transcription of the gene is complete. the 5′ cap is retained in mature mRNAs.



Group I and Group II introns splicing

- Group I and Group II introns are *self-splicing introns,* i.e, they are spliced while RNA processing without the help of any protein enzyme. This sets them apart from Spiceosomal introns.
- Group I is found in bacteriophage; mRNA, tRNA, rRNA of lower eukaryotes, in higher plants in tRNA, rRNA of bacteria.
- Group II is found in, organellar DNA of archae and eukaryotes
- None of the groups require high energy ATP for the purpose of splicing. Group I requires the GTP, while Group II is independent of that

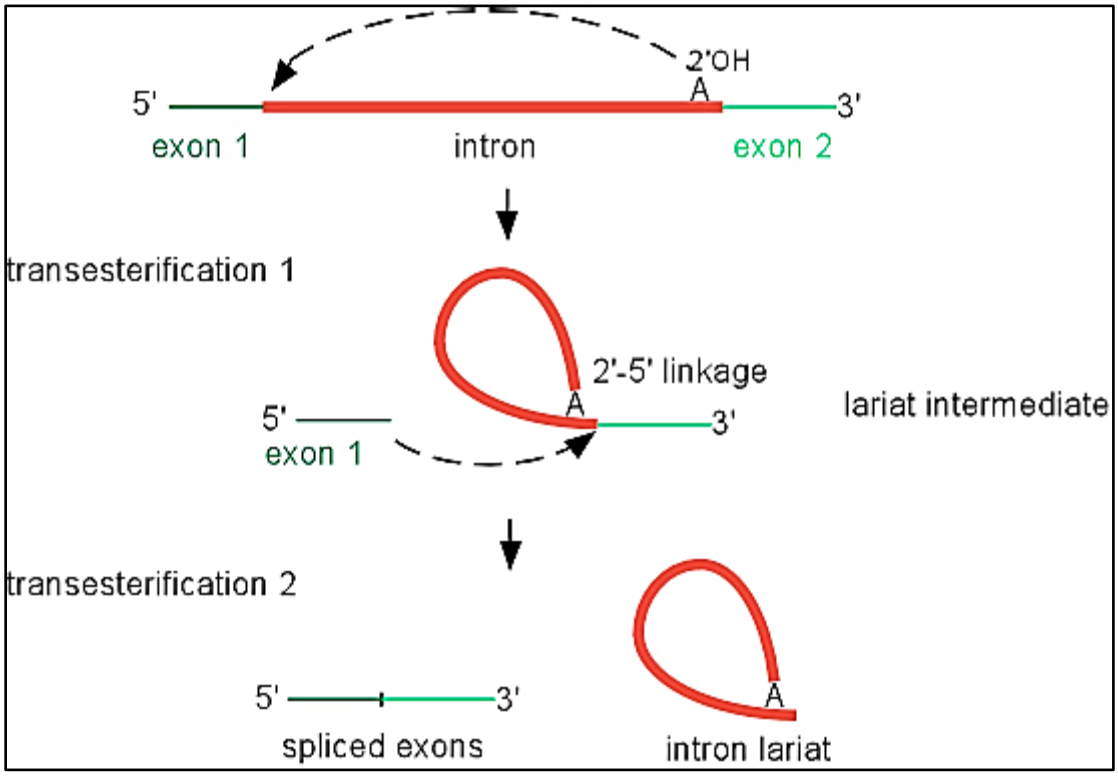
Three class of RNA Splicing

Class	Abundance	Mechanism	Catalytic Machinery
Nuclear pre-mRNA	Very common; used for most eukaryotic genes	Two transesterification reactions; branch site A	Major spliceosome
Group II introns	Rare; some eu-Karyotic genes from organelles and prokaryotes	Same as pre-mRNA	RNA enzyme encoded by intron (ribozyme)
Group I introns	Rare; nuclear rRNA in some eukaryotics, organlle genes, and a few prokaryotic genes	Two transesterific-ation reactions; exogenous G	Same as group II introns

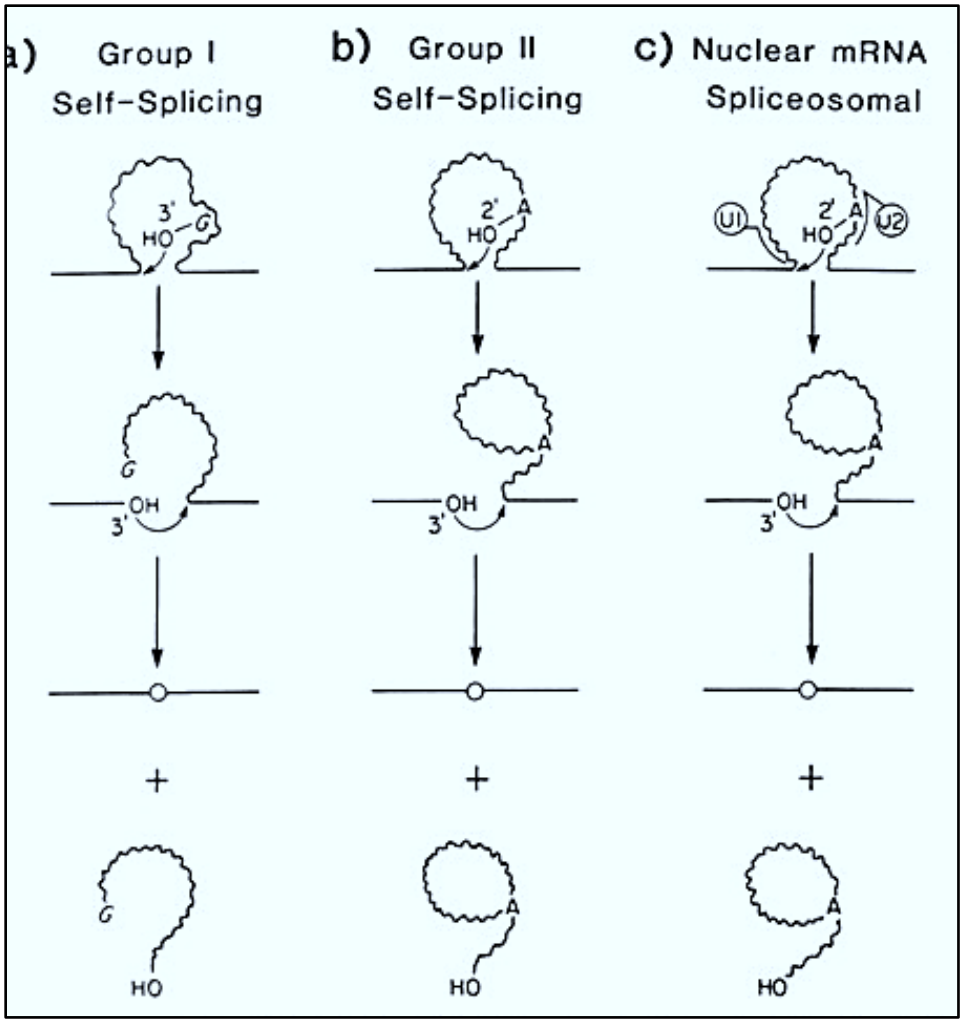
Mechanism of splicing in these is different slightly

- **Group II:**

2′- OH group within the intron makes a nucleophilic attack on the 5′- splice site and forms a lariat like structure. This lariat like structure acts as an intermediate. 3′- end of the exon makes a nucleophilic attack on the 3′- end of the intron and the lariat shaped intron is excised and the exons are ligated.



- **Group I**: Gunanine nucleoside cofactor is used whose 3′- OH makes a a nucleophilic attack on the phosphor and forms, 3′,5′- phosphodiester bond. The released 3′- exon end now attacks the 3′- intron end in a similar nuceophilic attack and the intron is excised and exons ligated.



Alternative splicing

- is a process which allows the production of a variety of different proteins from one gene only
- Most genes in eukaryotic genomes consist of exons and introns. After transcription, introns need to be removed from the pre-mRNA by a step called splicing. Sometimes an exon can be either included or excluded from the final transcripts, or there can be two splice sites at one end of an exon that are recognized by the spliceosome (the complex which carries the splicing reaction)
- This makes it possible to produce multiple mRNA from 1 pre-mRNA, thus increases mRNA and protein diversity
- The joining of different splice sites allows 1 gene to express multiple mRNAs that encode proteins with diverse functions . In humans ~20,000 genes give rise to more than 100,000 different functioning proteins because of this
- It makes possible a single gene to code for two or more distinct proteins.
- In humans, it is estimated that alternative splicing occurs in more than 40-60% of genes
- Alternative splicing is found extensively in all higher eukaryotes, from organisms like *Caenorhabditis elegans*, *Drosophila,* mouse to humans,

- One of the most dramatic examples of alternative splicing is the Dscam gene in *Drosophilia.* This single gene contains some 116 exons of which 17 are retained in the final mRNA. Some exons are always included; others are selected from an array. And, in fact, over 18,000 different proteins are produced found in *Drosophila*