CHRISTIAN EMINENT COLLEGE, INDORE

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E-Content

On

"POST-TRANSCRIPTIONAL MODIFICATION: RNA SPLICING"

By

Dr. Shiv Om Pratap

Professor and Head

Department of Bioscience

Christian Eminent College, Indore

CONTENT

Introduction:

In prokaryotes, transcription and translation both processes are carried out simultaneously. The RNA copy of a gene is m- RNA, and it is ready for translation into protein. In fact, translation starts even before transcription is finished. In eukaryotes, the primary RNA transcript of a gene are further processed prior to its translation into protein. This step is called "RNA processing". Also, Post RNA processing, it needs to be transported out of the nucleus into the cytoplasm through the nuclear pores. Many of the RNA molecules in bacteria and virtually all molecules in eukaryotes are processed to some degree after synthesis as a newly synthesised RNA molecule is immature and is called **primary transcript.** Most elaborated form of processing occurs for all eukaryotic mRNAs and tRNAs of both bacteria and eukaryotes.

Basics of Post Transcriptional Modifications::

A eukaryotic gene has several non-coding region (**introns**) interspersed with coding region (**exons**). When an m-RNA is transcribed, primary transcript has both introns and exons. Introns are removed from primary transcript via a process called **splicing.** In splicing, introns are removed and exons are joined together in a continuous sequence forming **mature m-RNA** transcript ready to be transported out of the nucleus and translated. Apart from splicing, eukaryotic m-RNA, also undergoes 5' capping and polyadenylation at 3' end.All three processes of splicing, capping and adenylation

occurs inside nucleus. Elaborate protein complexes are involved in these three RNA processing which act in association with each other and with phosphorylated CTD of Pol II.

The primary transcript of bacterial or eukaryotic tRNAs are processed by either **cleavage** which removal of bases from each end or in some cases **splicing.** Furthermore, many unusual bases, not found in any nucleic acid are also added to tRNA.

Steps in RNA processing: (1) Add a cap to the 5' end, (2) Add a poly-A tail to the 3' end, (3) splice out introns.

Capping:

In eukaryotic cells, m RNA is inherently unstable at the ends. So needs to modified the end to protect it against ribonucleases, mRNA is capped so that it is protected from ribonucleases as well as it is important in binding of m-RNA to ribosome for translation; it uses certain cap binding protein complexes. Capping reaction starts soon after transcription has started. As soon as 20-30 nucleotides are formed, capping occurs. At the 5' end capping process occurs, a slightly modified guanine (7-methyl G) is attached "backwards", by a 5' to 5' linkage, to the triphosphates of the first transcribed base. Capping reaction includes **condensation** of GTP with triphosphate at 5' end followed by **methylation** of guanine at N-7. Further methylation occurs at 2'hydroxyls of 2nd and 3rd nucleotide adjacent to the cap which described below in Figure 1.

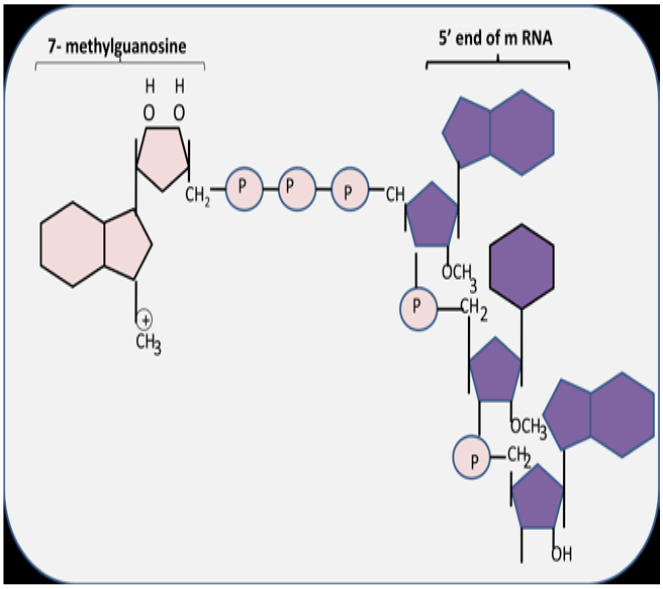

Figure 1.

Tailing:

Eukaryotic mRNA has series of adenosine residues ranging from 80 to 250 in number forming a poly (A) tail at 3' end of the primary transcript. This poly (A) tail has several uses- 1) it can export mature mRNA out of the nucleus. 2) It increases stability of mRNA. 3) It serves as recognition signal for binding of translational factors during ignition of translation. The process requires template-independent RNA polymerase called as **poly (A) polymerase**. Tailing is described below in different steps in Figure 2.

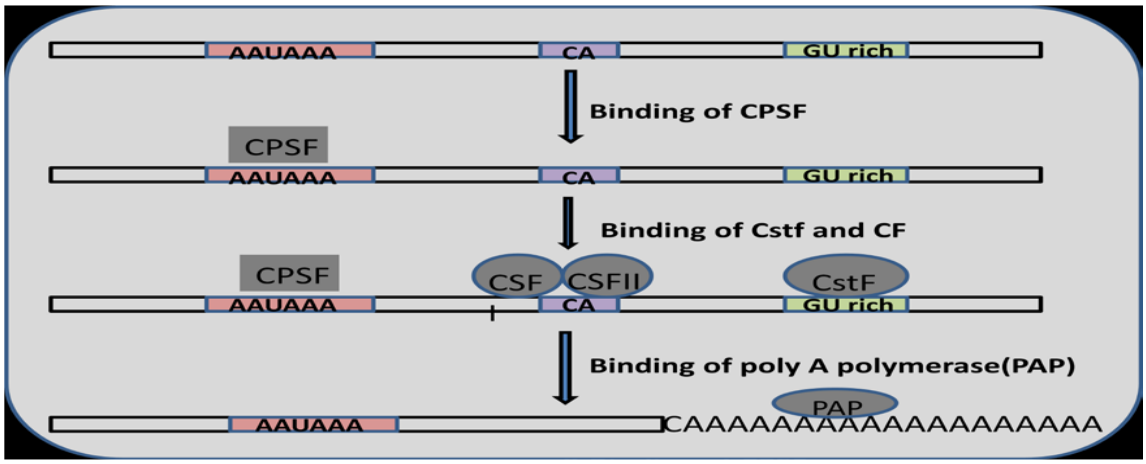

Figure 2. Tailing process in m RNA

INTRONS SPLICING: Introns are noncoding nucleotide sequence within a gene that don't code for protein and don't appear in the final mRNA molecule and are remove by the splicing. Protein-coding sequence of a gene (known as exons), which are interrupted by introns. The vast majority of eukaryotic genes are interrupted with non-coding a region (introns), which needs to be spliced out; however, histone protein coding genes in vertebrate is one among few exceptions. The occurrence of introns varies in eukaryotic species, some yeast species lack introns and many genes in eukaryotes carry a dozen of them. Few bacterial and Archeal genes also have introns. Introns can vary in length from 50 to 20000 nucleotides. In higher animals as humans, introns are more than exons. There are four classes of introns: (1) Group I, (2) Group II; both are self-splicing introns and does not involve any protein enzymes.(3) Spliceosomal introns- they are not self-splicing. (4) Introns that require ATP for splicing.

Splicing mechanism of group I and Group II introns: Splicing mechanism of both Group I and Group II involve similar steps of two **trans-esterificastion** reactions in which a ribose 2' or 3' hydroxyl group makes a nucleophilic attack on phosphorus and a new phosphodiester bond is formed at the expense of the old. Mechanisms of these groups differ in nucleophile which is used. Group I uses 3' hydroxyl group of guanine nucleotide as nucleophile. Group I introns are found in some nuclear, mitochondrial, and chloroplast that code for rRNAs, mRNAs, and tRNAs. Group I and Group II splicing mechanism are described below in figure 3.

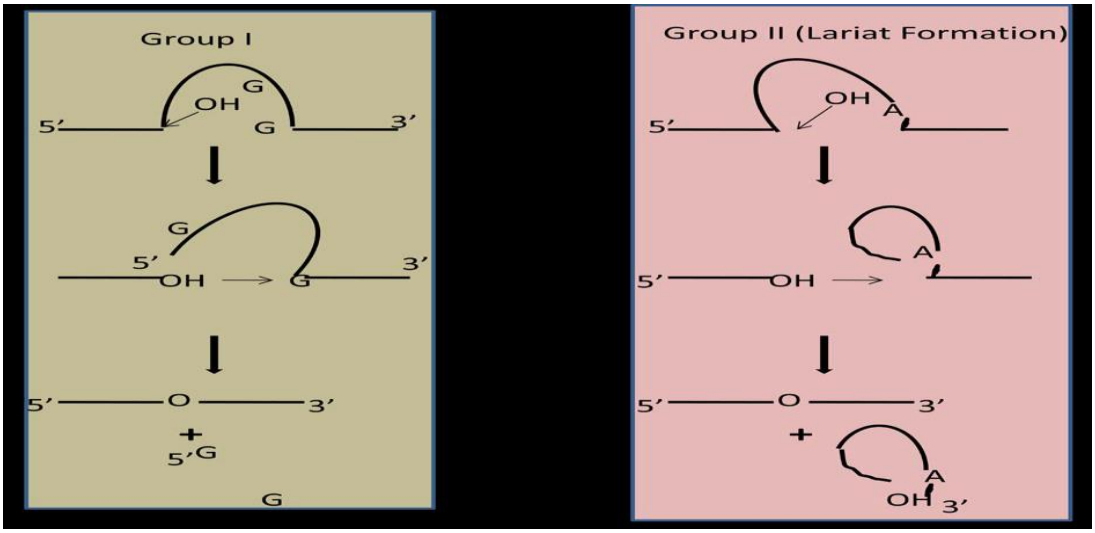

Figure (3a) :group I splicing Figure (3b):group II splicing

Alternative splicing: Alternative splicing is a method substantially used for many mammalian genes can result in multiple products that vary structurally and functionally from the same primary transcript.Sometimes, alternate splicing is unregulated phenomenon while in some it is strictly regulated. One of the best examples of regulated alternative splicing occurs in **sex determination in drosophila**. In drosophila three genes are involved in sex determination sex lethal gene (sxl), transformer gene (tra), doublesex gene (dsx). Due to alternative splicing, functional genes are produced in females and non- functional genes are produced in males. Alternative splicing occurs using two mechanisms - one when two poly (A) or cleavage sites are available in primary transcript.

Cleavage occurs at either site resulting in two different products. Such mechanism is followed by **variable domains of immunoglobulins heavy chains** and their diversity is due to this mechanism of alternative splicing. Similarly alternative splicing with such mechanism results in **production of two different hormones**- calcium regulating hormone in rat thyroid and calcitonin-gene related peptide in rat brain. Other mechanism involves more than one 3' splice site for one 5' splice site. Hence splicing occurs by taking either of those 3' splice site resulting in different products. Here in **Figure** we are describing the steps of alternate splicing in which protein A formed by exon 1, 2 ,3 and protein B formed by exon1,3,4

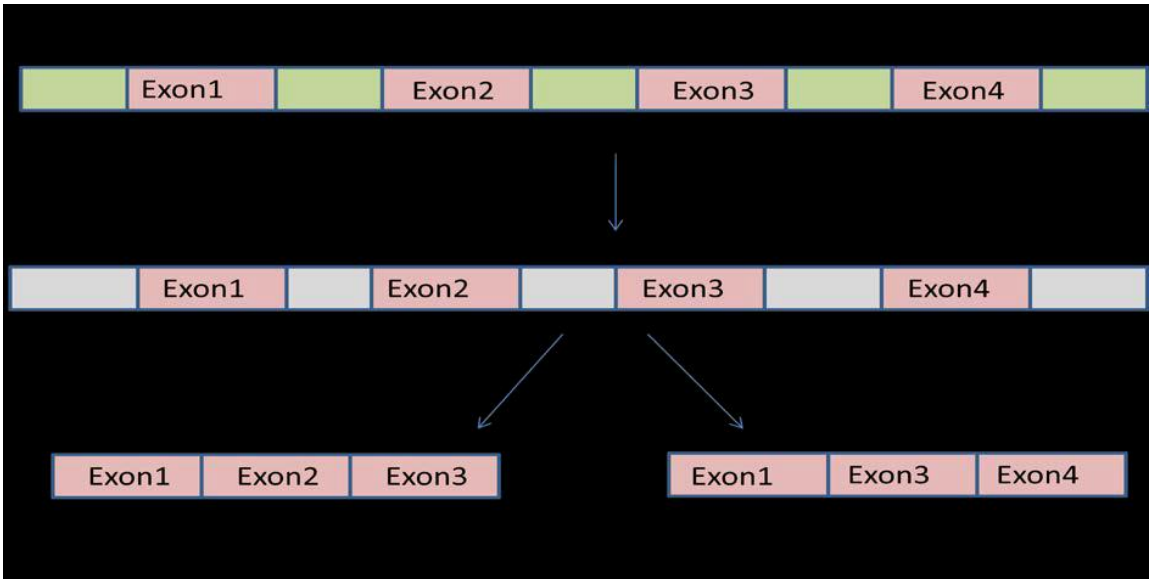

Figure : Alternate splicing occurs in 2 steps and form 2 different protein

Applications of RNA Splicing

1. RNA splicing also helps in the regulation of gene and protein content in the cell. Splicing of RNA sequences assists the process of evolution of new and improved proteins.
2. Various aberrant splicing isoforms act as markers for cancer and as targets for cancer therapy.

3. Alternative splicing (AS) constitutes a pivotal mechanism for expanding the transcriptome and proteome diversity, which provides evolutionary advantages to higher eukaryotes