mRNA PROCESSING

Although transcription is the first and most widely and highly regulated step in gene expression, it is usually only the beginning of the series of events required to produce a functional RNA. In eukaryotes, most newly synthesized RNAs must be modified in a various ways to be converted to their functional forms. Like other RNAs, primary transcripts of eukaryotic mRNAs undergo extensive modifications, including the removal of introns by splicing, before they are transported from the nucleus to the cytoplasm to serve as templates for protein synthesis.

The initial products of transcription in eukaryotic cells (pre-mRNAs) are extensively modified before export from the nucleus. The processing of pre-mRNA includes modification of both ends of the molecule, as well as the removal of introns from the middle. Rather than occurring as independent events following synthesis of a pre-mRNA, these processing reactions are coupled to transcription so that mRNA synthesis and processing are closely co-ordinated steps in gene expression.

Three major events occur during the processing of mRNA: 5' capping, 3' cleavage/polyadenylation, &

Capping

Once RNA polymerase II has made about 20-30 nucleotides of pre-mRNA, a capping enzyme adds a guanine nucleotide — most commonly 7-methyl guanosine (m^7G) — to the 5' end by an unusual 5'-5' linkage as opposed to the usual 5'-3' linkage. This process is called 5' capping and the structure is called a cap. The guanine residue is added in a reverse orientation. The 5' cap aligns eukaryotic mRNAs on the ribosome during translation.

Mechanism of capping:-

i) The 5' ends of all RNAs initially possess a triphosphate derived from the first nucleoside triphosphate incorporated at the site of initiation of RNA synthesis.

ii) The last of the three phosphates (γ-phosphate) is removed by phosphohydrolase, converting the 5' terminus to a diphosphate (ribonucleotide diphosphate).

iii) Then, a GMP is added in an inverted orientation by a guanylyl transferase so that the 5' end of the guanosine is facing the 5' end of the RNA chain. As a result, the first two nucleoside are jointed by a 5'-5' triphosphate bridge.

iv) Finally, the terminal, inverted guanosine is methylated at the 7' position on its guanosine base by the enzyme guanine-7-methyltransferase. The 5'-end of the RNA now contains a methylguanosine cap. A cap that possesses this single methyl group is known as a Cap 0 (zero).

v) The next step is to add another methyl group, to the 2'-O position of the penultimate base (which was actually the original first base of the transcript before any modifications were made) by the enzyme 2'-O-methyl transferase. A cap with the two methyl groups is called Cap1. This is the predominant type of cap in all eukaryotes except unicellular organisms.

vi) In a small minority of cases in higher eukaryotes, another methyl group is added to the second base. This happens only when the position is occupied by adenine; the reaction involves addition of a methyl group at the N6 position. The enzyme responsible acts only on an adenosine substrate that already has the methyl group in the 2'-O position.

vii) In some species, a methyl group is added to the third base of the capped mRNA. The substrate for this reaction is the Cap1 mRNA that already possesses two methyl groups. The third-base modification is always a 2'-O ribose methylation. This creates the Cap 2 type. This cap usually represents less than 10-15% of the total capped population.

In a population of eukaryotic mRNAs, every molecule is capped. The proportions of different types of cap are characteristic for a particular organism.

In addition to the methylation involved in capping, a low frequency of internal methylation occurs in the mRNA of higher eukaryotes. This is accomplished by the generation of N^6 methyladenine residues at a frequency of about one modification per 1,000 bases. There may be 1-2 methyladenines in a typical higher eukaryotic mRNA,

although their presence is not obligatory, since some mRNAs (for example, globin) do not have any.

Functions/ Significance:- The methylguanosine cap at the 5' end of an mRNA severs the following functions:-

a) It prevents the 5' end of the mRNA from being digested by exonuclease.

b) It aids in the transport of the mRNA out of the nucleus, and

c) It is essential for ribosome binding and helps in initiation of mRNA translation.

d) Capping helps the cell to distinguish mRNAs from other types of RNA molecules present in the cell. For example, RNA polymerases I and III produce uncapped RNAs during transcription. In the nucleus, the cap binds a protein complex called CBC (Cap Binding Complex), which helps the RNA to be properly processed and exported.

Poly Adenylation

The 3' terminal stretch of adenosine residues of mRNA is often described as the poly(A) tail; and mRNA with this feature is denoted by poly(A)+.

The poly(A) sequence is not coded in the DNA, but is added to the RNA in the nucleus after transcription (post-transcriptional modification). The addition of poly(A) is catalyzed by the enzyme poly(A) polymerase, which adds 200 or more 'A' residues to the free 3'-OH end of the mRNA. The poly(A) tail invariably begins approximately 10-30 nucleotides downstream from a conserved sequence, consensus 5'-AAUAAA-3', and upstream from a GU-rich sequence located near the end of the transcription unit. This sequence in the primary transcript serves as a recognition site for the assembly of a complex of proteins that carry out the processing reactions at the 3' end of the mRNA.

In mammalian cells, poly(A) addition to the RNA occurs as follows:-

a) A number of proteins including CPSF (Cleavage and Polyadenylation Specificity Factor) protein, CstF (Cleavage Stimulation factor) protein, and two cleavage factors (CF I & CF II) bind to and cleave the pre-mRNA. CPSF binds to the AAUAAA sequence and CstF binds to a GU-rich or U rich sequence downstream of the poly(A) site. CPSF and CstF also bind to each other, producing a loop in the RNA. CF I and CF II are bound near the actual cleavage site.

The cleavage event that produces the 3' end of a ~~transcription~~ transcript usually occurs at a site 11-30 nucleotides downstream from the conserved sequence AAUAAA, located near the end of the transcription unit.

b) After cleavage, the enzyme poly(A) polymerase (PAP) adds poly(A) tails, tracts of adenosine monophosphate residues about 200 nucleotides long, to the 3' ends of the transcript. The addition of poly(A) tails to eukaryotic mRNA is called Polyadenylation.

Functions/ significance:-

a) The poly(A) tail protects the mRNA from premature degradation by exonucleases.

b) The poly(A) tails play an important role in their transport from the nucleus to the cytoplasm.

The presence of poly(A) has an important practical sequence. The poly(A) region of mRNA can base pair with oligo(U) or oligo (dT);

and this reaction can be used to isolate poly(A)+ mRNA.