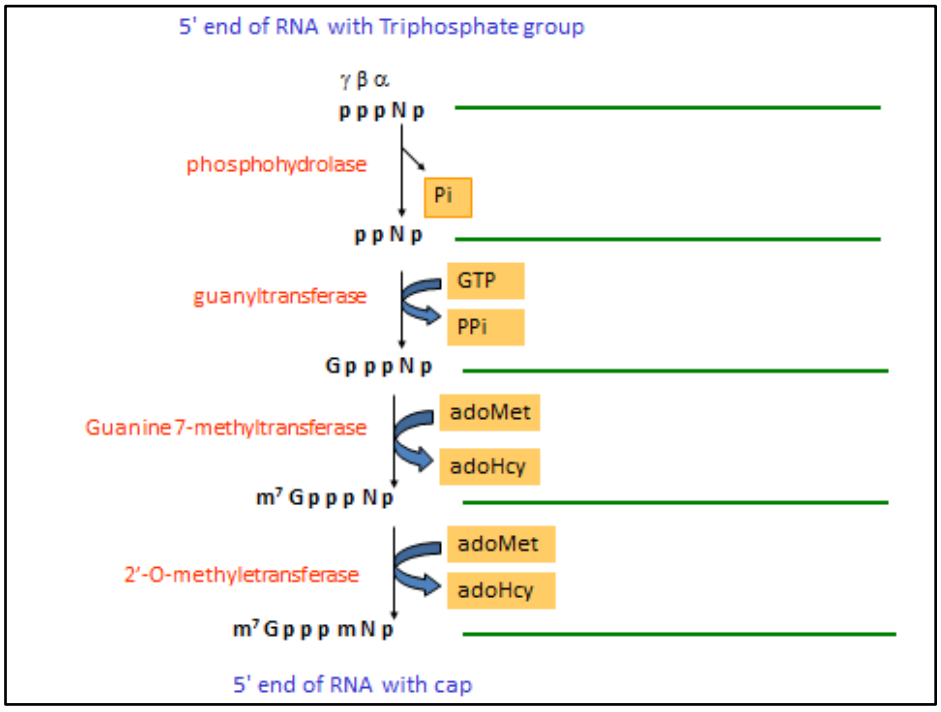
RNA processing

RNA synthesised in nucleus from DNA by transcription is a large molecule (1000-50000 bp long) and called as hn RNA. Only a small percentage of this hn RNA (only 5% by mass) enters the cytoplasm. This suggests that the genetic regulation is also exercised at hn RNA level. The hn RNA molecule destined to produce m RNA undergo RNA processing which include: 1. Modification of 5' end by capping (addition of 7-methyl Guanine). 2. Modification of 3' end by a poly A tail after enzymatic cleavage. 3. Splicing out of intron sequences. It has been shown that cleavage and poly-adenylation usually precede RNA splicing, although there is no causal relation between the two. Although all kinds of RNA undergo processing but perhaps the most extensive processing of primary transcripts occurs in eukaryotic m RNA and in t RNA of both bacteria and eukaryotes.

1. Capping at 5' end occurs rapidly after the start of transcription and much before completion of transcription. Transcription starts with either AUG or GUG, so the first base is either A or G and a 5' triphosphate group is retained at this first position. The initial sequence at 5' end of hn RNA is therefore 5' pppA(or G)pNpNpN……3'. To this sequence a methylated Guanine residue is added as a cap in several steps by the condensation of a molecule of GTP with the triphosphate at the 5' end of the transcript. The 'G' is subsequently methylated at N-7.



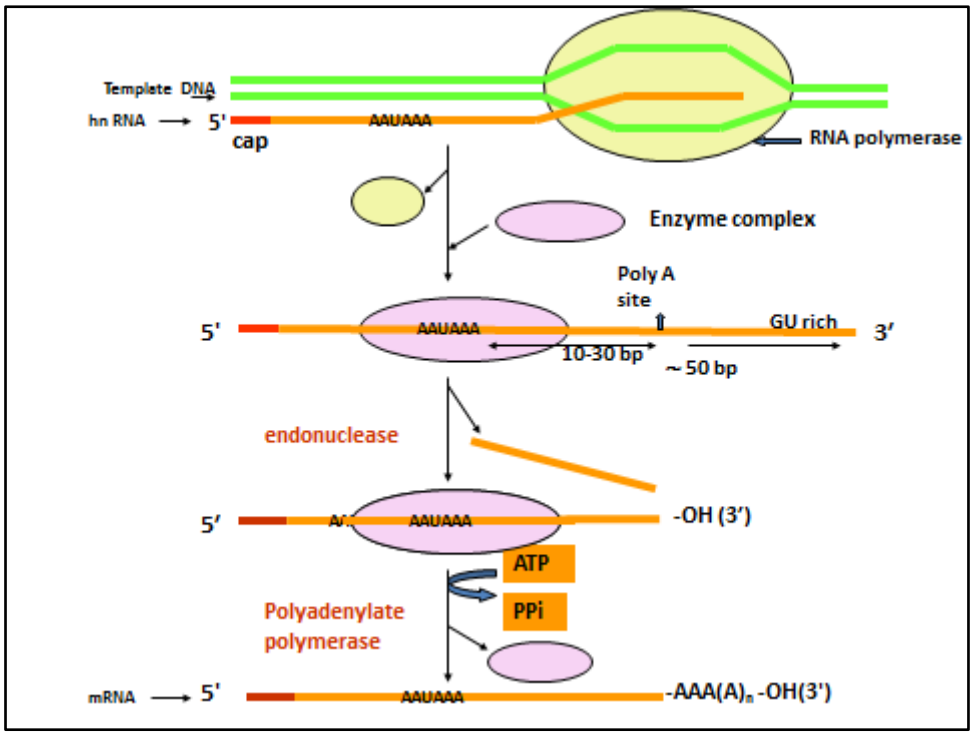
The new G residue is in the reverse orientation with respect to all other nucleotides. The cap with a single methyl group at the terminal G residue is found in unicellular eukaryotes and described as Cap 0, but in most eukaryotes methyl group may also be present on the penultimate base at 2' position of sugar moiety. So that with methyl groups at 2 terminal nucleotides, it is now described as Cap 1. In still other cases (10-15% of all caps), methyl group may be present at the 3rd base also at 2' position of sugar and thus described as Cap 2.

The methyl group is derived from S-adenosyl methionine which converts to S-adenosyl homocysteine after donating methyl group.

The function of capping is exactly not known but the 5' cap binds to a protein and may participate in the binding of the mRNA to the ribosome to initiate translation. Only in some eukaryotes mRNAs (as for example histone proteins) cap may be absent and may not require for translation.

2. At the 3' end of most eukaryotes mRNAs have a tail of 20 – 250 Adenine residues called the poly A tail. This poly A tail is not simply added to the 3' end of primary transcript at the site where transcription terminates. The transcript is extended beyond the site where the poly A tail is to be added, then is cleaved at the poly A addition site by a specific ribonucleotides. This cleavage generates the free 3-OH group, that defines the end of the mRNA and to which Adenylate residues are immediately added by polyadenylate polymerase. The site where cleavage and poly A addition occur is marked in the mRNA by the highly conserved sequence 5' AAUAAA 3' situated 11-30 nucleotides upstream on the 5' side of the cleavage. A complex containing the riboendonuclease, polyadenylate polymerase and possible other proteins and one (probably U_7 snRNA) or more snRNAs bind to this sequence and carries out the processing reactions.

   It seems that 5' cap and 3' poly A tail and their associated protein help to protect the mRNA from enzymatic destruction.



3. Splicing – In a process called splicing, the introns are removed from the primary transcripts and the exons are joined to form a contiguous sequence specifying a functional polypeptide.

   In eukaryotes mRNAs, most exons are 100-200 nucleotides long. Introns are much more variable in size (50-20000 nucleotides long). Genes of higher eukaryotes including humans typically have much more DNA devoted to introns than to exons. There are four classes of introns. The first two called Group I and II have some key characteristic such as both has no high energy (ATP) requirements for splicing; but differ in the details of their splicing mechanisms. Group I introns are found in some nuclear, mitochondrial and chloroplast genes coding for rRNAs; group II introns are generally found in the primary transcripts of mitochondrial or chloroplast mRNAs. Both group of intron splicing involve two transesterification reactions. Group I intron splicing reaction is very similar to topoisomerase activity in DNA replication. It requires a G nucleotide or nucleoside cofactor. Group II intron splicing requires a 2' hydroxyl group of an adenylate residue within the intron itself. An unusual branched lariat structure is formed in this splicing.

   The 3rd and largest group of introns found in nuclear mRNA primary transcripts, undergo splicing by the same lariat formation as Group II introns, but not involvement of self adenylate residue, but involvement of specialised RNA-protein complex in

which RNA of a special type called small nuclear RNAs. Five snRNAs, U_1, U_2, U_4, U_5 and U_6 are involved in this type intron splicing reaction. Size range of these RNAs are 106 (U_6) to 189 (U_2) bases and they complexed with proteins to form particles called small nuclear ribonucleoproteins (snRNP's) often referred to as "snurps".

The 4th class found in certain tRNAs, is distinguished from other in the fact that it requires ATP for splicing. In this reaction endonuclease cleaves the phosphodiester bonds at both ends of the intron and the two exons are joined is a similar mechanism like DNA ligase reaction.