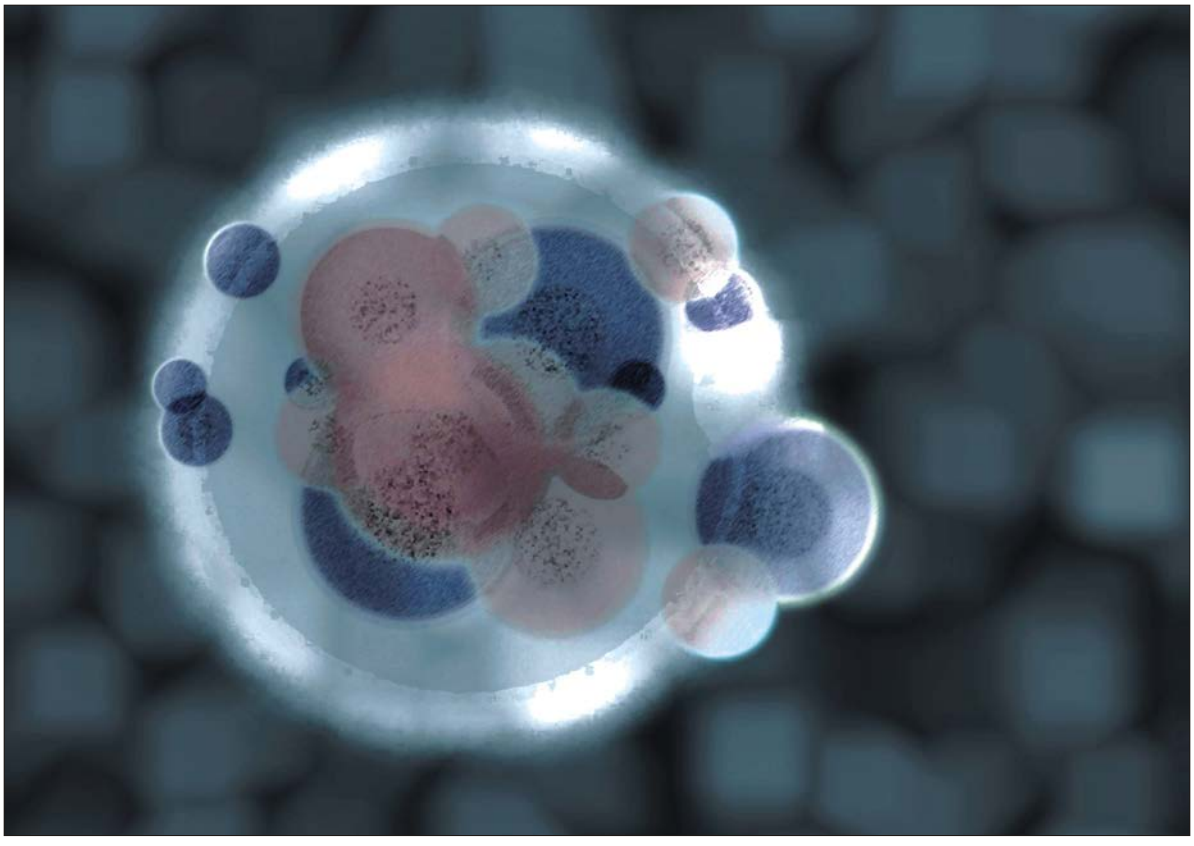

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Transcription and mRNA Processing Events: the Importance of Coordination

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Abstract: Eukaryotic mRNAs are extensively modified prior to being translated into proteins. Processing events such as RNA capping, splicing and polyadenylation are required to produce fully translatable mRNAs. Growing evidences suggest that the carboxy-terminal domain of the RNA polymerase II act as a common link between these events. The importance of coordination between transcription and RNA processing is also discussed in this mini review.

Key words: mRNA processing, capping, splicing, polyadenylation, RNA polymerase

INTRODUCTION

Eukaryotic mRNAs undergo a series of modifications before being exported to the cytoplasm where they are translated into proteins. These processing events include the addition of a cap structure at the 5' terminus, the splicing out of introns and the acquisition of a poly(A) tail at the 3' terminus. Although each of these steps can occur independently *in vitro*, there are now growing evidences indicating that these modifications can influence each other. Furthermore, it has now become apparent that mRNA processing is tightly coordinated with transcription.

RNA processing: Addition of the methylated cap structure (^{m7}GpppN) is the first modification to occur on nascent mRNAs. The modification appears soon after the initiation of transcription when the RNAs are about 20 nucleotides long[1]. The cap consists of a 7-methylguanosine residue linked by a 5'-5' triphosphate bridge to the RNA transcript and is synthesized by a series of three sequential enzymatic reactions. The first step involves the hydrolysis of the RNA 5' triphosphate end of the nascent RNA by an RNA triphosphatase to form a diphosphate end. The addition of GMP to the diphosphate end is then mediated by an RNA guanylyltransferase, or RNA capping enzyme. Finally, the GpppN cap is methylated by an RNA (guamine-N7) methyltransferase. The cap plays a critical role in the stability, transport and translation of the mRNAs[2]. The presence of the 5' cap has been shown to protect RNAs from degradation by exonucleases[3]. In addition, a cap binding complex (formed by CBP20 and CBP80) recognizes the methylated cap structure and can both protect mRNAs from degradation and promote export to the cytoplasm[4]. In the cytoplasm, eIF-4E (a component of the eIF4F complex) binds to the cap and significantly increases translation by promoting the interaction of the mRNAs with the ribosomes[5]. The importance of the cap structure for RNA metabolism is highlighted by genetic analyses *in Saccharomyces cerevisiae* that showed that the triphosphatase, guanylyltransferase and methyltransferase components of the capping apparatus are essential for cell growth[6-11].

The vast majority of metazoans genes harbor noncoding introns that have to be eliminated from RNA transcripts through splicing. The spliceosome is a large complex that catalyzes the removal of introns. It is formed by five small nuclear RNAs (U1, U2, U4, U5 and U6) that form ribonucleoproteins (snRNP) through association with various protein partners[12]. Other non-snRNP proteins are also present in the spliceosome such as serine/arginine (SR) proteins. Numerous studies have demonstrated the importance of *cis*-acting consensus sequences that are essential for splicing. These include sequences located at the 5' exon-intron junction, the 3' splice site and a branchpoint that is located upstream of the 3' splice site[13]. This branchpoint harbors the highly conserved adenosine that is involved in a nucleophilic attack of the 5' exon-intron junction. This results in the formation of a lariat-shaped structure consisting of the intron and 3' exon. A subsequent attack of the 3' splice site by the 5' exon leads to the release of

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the lariat-shaped structure with the concomitant fusion of the two exons.

The 3' end of mRNAs is modified through the addition of a poly(A) tail consisting of around 200 adenosine residues. In mammalian cells, the polyadenylation signal is defined by a highly conserved sequence (AAUAAA) located 10 to 30 nucleotides upstream of the cleavage site, a U-rich or GU-rich element located downstream of the cleavage site and the cleavage site itself[14]. Addition of the poly(A) tail involves numerous protein factors that have been characterized in recent years. The AAUAAA sequence is recognized by the Cleavage and Polyadenylation Specificity Factor (CPSF) through its 160-kDa subunit. However, recent evidences suggest that the other three subunits of CPSF probably modulate the binding[15]. A Cleavage-stimulatory Factor (CstF) interacts via its 64 kDa subunit with the U-rich or GU-rich region. Biochemical studies suggest that the binding of CstF enhances the affinity of CPSF for the consensus hexanucleotide. Additional cleavage factors (CFI and CFII) are also involved in the cleavage step[15]. The template-independent poly (A) polymerase (PAP) that catalyzes the addition of the adenosine residues is also involved in the cleavage reaction. The poly (A) binding protein PABP II recognizes the poly (A) tail and can significantly increase the processivity of PAP. PABP II is also involved in the control of the length of the poly (A) tail. Finally, the poly(A) binding protein PABP I can interact with the eIF4G component of the eIF4F complex thereby promoting the circularization of mRNAs[15]. This circularization promotes both the stability and translation of the mRNA by allowing the reinitiation of ribosomes, while protecting the extremities of mRNAs from exonucleases. The importance of polyadenylation is underscored by various human diseases resulting from aberrant polyadenylation of mRNAs (thalassemias, lysozomal storage disorder, amyotrophic lateral sclerosis)[16-18].

Central role of the RNA polymerase II: Eukaryotic mRNAs are transcribed exclusively by the RNA polymerase II. Not surprisingly, this multimeric enzyme cooperates with numerous RNA processing factors that are involved in capping, splicing and polyadenylation. More specifically, the Carboxy-terminal Domain (CTD) of the large subunit of the enzyme plays a critical role in coordination of the processing events[19]. The CTD is composed of a repetition of the heptapeptide YSPTSPS (52 copies in mammals). Numerous studies have shown that the CTD is required for the activation of transcription and for efficient capping, splicing and polyadenylation of mRNAs. The ability of the CTD to become phosphorylated/dephosphorylated on serine residues (ser-2 and ser-5) is key in regulating binding partners[20]. Unphosphorylated CTD is involved in the initiation of transcription and allows interactions with various general transcription factors. In mammals, the cdk-7-cyclin H component of the general transcription factor TFIIH is responsible for the phosphorylation of the CTD[21]. Phosphorylation of the CTD is critical for the transition of the RNA polymerase II to an elongating mode. A phosphatase (Fcp1) has been identified and can act as an inhibitor of the elongation activity of the RNA polymerase II[22,23].

In mammals, the enzymatic activities involved in RNA capping are performed by two distinct proteins. Both the RNA triphosphatase and RNA guanylyltransferase activities are located on a bifunctional polypeptide while the RNA methyltransferase reaction is catalyzed by a distinct protein. Elegant studies have demonstrated that the bifunctional capping enzyme binds only to the phosphorylated RNA polymerase II[24,25]. The importance of the phosphorylation on the serine-5 residue for the recruitment of the capping enzyme has been demonstrated[26]. Binding of the capping enzyme to the phosphorylated CTD not only recruits the capping enzymes to the transcription machinery, but it also results in the allosteric activation of the RNA guanylyltransferase activity (two-fold enhancement of the affinity for GTP). Studies performed in yeast have demonstrated that the RNA triphosphatase and RNA guanylyltransferase activities are released early after the cap has been added. This step is coupled with the dephosphorylation of serine-5 by Fcp1[27]. However, the methyltransferase activity appears to remain associated with the polymerase during transcription and move along until the 3' end of genes. Therefore, it has been suggested that the methyltransferase activity could be involved in additional function during transcription.

A number of different studies have recently shown that splicing occurs as mRNA transcription occurs. Not surprisingly, the CTD again appears to play a central role in the functional coordination of transcription with splicing. The importance of the CTD for the splicing reaction is reflected by experiments that demonstrated that RNA transcripts generated by an RNA polymerase II lacking a CTD are not spliced *in vivo*[28]. Interestingly, co-immunoprecipitation studies have demonstrated that the SR proteins of the spliceosome are associated with the RNA polymerase CTD. In fact, antibodies directed against the CTD have been shown to inhibit the splicing reaction[29]. Again, the phosphorylation status of the CTD appears to play a critical role during splicing. The phosphorylated RNA polymerase can stimulate the initial

assembly of the spliceosome leading to an increase in splicing. On the other hand, the hypophosphorylated CTD has a negative impact on splicing by inhibiting the assembly of the spliceosome[30]. The precise role of the RNA polymerase in splicing is not completely understood but the CTD also appears critical in bringing together different splicing factors that act at the 5' exon-intron junction and the 3' splice site[31].

The addition of the poly(A) tail to the 3' end of mRNAs is also regulated by the RNA polymerase II. Co-immunoprecipitation studies have demonstrated that certain factors involved in the addition of the poly(A) tail are associated with the polymerase. This is the case of the CPSF which is an active component of the basal transcription factor TFIID. CPSF transfers to the phosphorylated polymerase soon after the initiation of transcription[32]. The importance of the CTD for the polyadenylation reaction is highlighted by studies that demonstrated that the polyadenylation reaction is impaired in the absence of a functional CTD[28]. Recent studies also indicate that additional polyadenylation factors interact with the CTD and that some of these factors are display higher activity in the presence of the CTD[33].

Coordination of RNA processing: It has now become apparent that the RNA processing events (capping, splicing, polyadenylation) can significantly influence each other. For instance, the cap binding complex can influence both the splicing and polyadenylation events. Using specific antibodies it was shown that the cap binding complex can significantly increase the cleavage step required for polyadenylation. The cap binding complex also appears to stabilize the interaction between the polyadenylation machinery and the mRNA[34]. The importance of the cap structure for splicing is underscored by experiments that demonstrated that the addition of a cap structure dramatically enhances the splicing[35]. More specifically, the presence of the cap appears critical for the excision of the first intron (cap proximal). Again, the use of specific antibodies against the cap binding complex significantly decreases splicing. There is now evidence that indicate that the interaction between U1 and the 5' splice site is increased by the cap binding complex. RNA splicing and polyadenylation can also significantly influence each other. It has been shown that both activities are enhanced by each other during the removal of the terminal exon. An interaction between the poly(A) polymerase and the essential splicing factor U2AF is important in order to bring both the splicing and polyadenylation machineries together[36].

The number of interactions between the various RNA processing events identified so far reflects the complexity of these reactions. As more studies focus on these interactions, additional links and cellular partners will undoubtedly be discovered. During the next few years, structural studies and thermodynamic analyses should significantly increase our knowledge of these reactions and provide crucial insights on the RNA processing events.

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