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Review

The link between mRNA processing and transcription: communication works both ways

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Abstract

Many pre-mRNA processing events including 5′ end capping, splicing out introns, and 3′ end maturation by cleavage or polyadenylation occur while the nascent RNA chain is being synthesized by RNA polymerase II. As a consequence of this arrangement, the physiological substrate for most processing factors is not a solitary pre-RNA but instead a ternary complex comprising a growing RNA chain spewing from the exit channel of an RNA polymerase II molecule as it speeds along a chromatin template at 1000–2000 bases/min. mRNA processing factors make protein–protein contacts with elongating pol II in a complex we have dubbed the "mRNA factory," which carries out synthesis, processing, and packaging of the transcript. Recent studies have shown that the "mRNA factory" is a dynamic complex whose composition changes as it traverses the length of a gene. This complex is also the setting for a growing number of regulatory interactions, which influence the function of both the processing and transcription machineries.
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The pol II CTD: a landing pad for processing factors

The task of transcribing protein-encoded genes is reserved exclusively for one of the three nuclear RNA polymerases. RNA pol II is equipped with a unique protein domain to tackle the job of directing cotranscriptional processing. This C-terminal domain (CTD) of the pol II large subunit composes tandem repeats of the consensus heptad $Y_1S_2P_3T_4S_5P_6S_7$, which is conserved from fungi to humans [1]. Deletion of the CTD in vertebrate cells reduces the overall level of transcription without necessarily affecting the accuracy of initiation. Deletion of the CTD inhibits all three major pre-mRNA processing steps in vertebrate cells: capping, splicing, and poly A site cleavage [2,3]. The CTD provides at least three functions to stimulate each of these processing steps independently [4]. In extracts, recombinant CTD and intact pol II can stimulate splicing and poly A site cleavage independently of ongoing transcription [5,6]. In budding yeast, the role of the CTD in pre-mRNA processing is not as important; however, it does influence mRNA capping and 3′ end formation [7,8]. It is not yet clear whether the CTD influences other RNA processing steps such as editing and histone 3′ end formation, but it is required for processing of U2 snRNA 3′ ends [9,10]. The CTD functions as a landing pad for reversible interactions with RNA processing factors [11] that serve to localize those factors close to their substrate RNAs and to act as a conduits for two-way communication with the polymerase. The heptad repeats are substrates for a peptidyl prolyl isomerase [12] and several kinases and phosphatases, which modify serine residues during transcription thereby presenting distinct CTD surfaces to interacting factors at different stages of the transcription cycle. The CTD becomes phosphorylated on Ser5 residues by the TFIIH associated kinase at the time of transcript initiation. Later, these phosphates are removed and others are added on Ser2 residues by the kinases CTK1 in budding yeast and PTEFb (CDK9) in metazoans [13–15]. In mammals, the minimum requirements for CTD function in mRNA processing are provided by about 25 tandem heptad repeats plus an essential 10 amino acid motif at the C-terminus [16,17]. We are only just beginning to understand the signals exchanged between processing factors and polymerase that couple transcription with capping, splicing, and 3′ end formation

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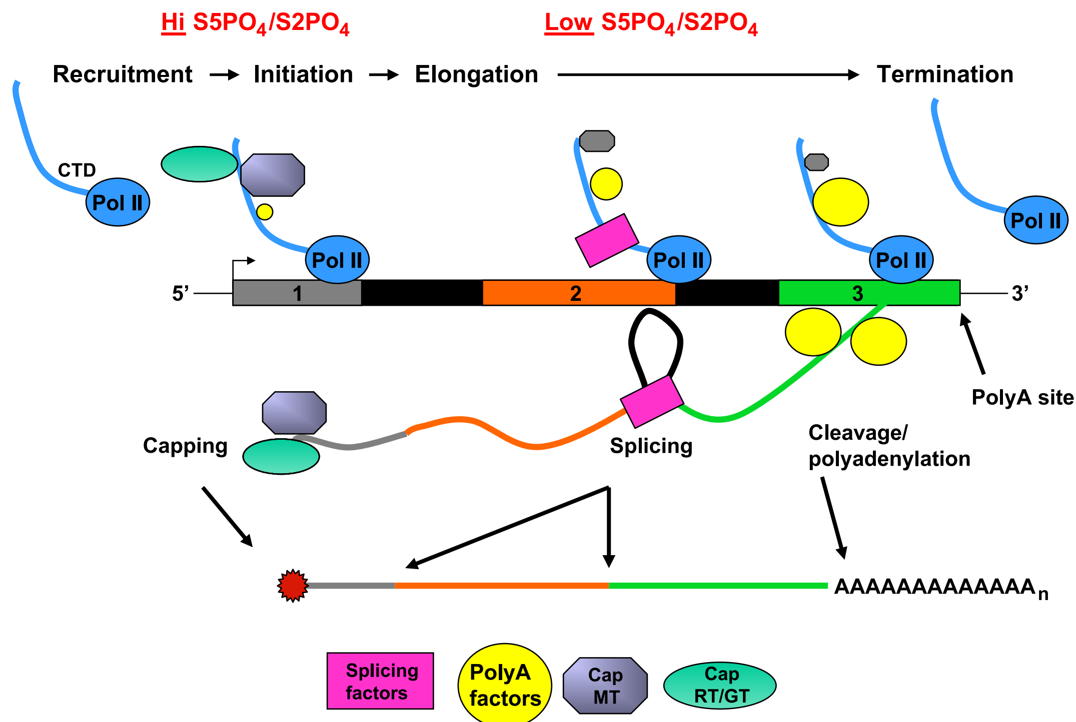

Fig. 1. The mRNA factory model. Schematic representation of cotranscriptional processing. Processing factors interact with the pol II machinery via the *carboxyl*-terminal domain (CTD) of the largest subunit of RNA pol II, Rpb1. Increased size of the symbols for processing factors corresponds to increased levels of in vivo formaldehyde cross-linking, measured by ChIP experiments. Capping enzymes, RT, GT, and MT, and 3′ end modifying factors (poly A) are recruited at the 5′ ends of genes. As Pol II traverses the gene, splicing factors associate with the transcription complex. Phosphorylation of Ser2 and Ser5 residues in the CTD heptad repeats is indicated in red. Exon numbers are marked in colored boxes. Introns are shown in black boxes. The red star represents the cap structure.

(Fig. 1). Excellent recent reviews of this area include Refs. [18–24].

Capping: two-way communication between pol II and processing enzymes

All pol II transcripts are marked at their 5′ ends by the addition of a methylated guanosine cap, when nascent RNA is about 22–40 bases long [25–28]. The cap is a major determinant of mRNA stability, which stimulate splicing, 3′ end processing, transport, and translation [29]. Capping is carried out by three enzymes acting in the order: RNA triphosphatase (RT), RNA guanylyltransferase (GT), and RNA- (guanine-7) methyltransferase (MT). Metazoans have a single bifunctional polypeptide with RT and GT domains, whereas budding yeast has two polypeptides, Cet1 and Ceg1, which form a heterotrimer [30]. Capping enzymes are brought to the right place at the right time by binding to the CTD when it becomes phosphorylated by TFIIH close to the promoter in vivo [3,31–33] and in vitro [27]. In vitro, there is a significant lag between guanylation and methylation of the cap [27,28].

Interaction of capping enzymes with elements of the transcriptional machinery influences both capping and transcription in the best example of two way signaling between processing and transcription machines. One line of communication is suggested by the intriguing observation that, at least in vitro, a transcription factor, the HIV1 Tat protein, binds to GT after it is recruited to the CTD and stimulates capping [27]. It is not known whether cellular transcription factors can also influence capping. Binding of mammalian GT domain to CTD heptads phosphorylated at Ser5 reduces its Km for GTP [34] consistent with the crystal structure showing interaction of a Ser5 phosphorylated CTD heptad near the nucleotide binding pocket of *Candida albicans* GT [35]. This structure and that of a phosphorylated CTD peptide bound to the peptidyl prolyl isomerase Pin1 [36] show that (i) key contacts are made with the Tyr1 and Ser5-PO4; (ii) that up to three consecutive heptads can contribute to binding interactions; and (iii) that different heptads of identical sequence can assume different conformations when they complex with partner proteins.

Phosphorylation of the CTD on Ser5 residues by the TFIIH associated kinase Kin28 is required for recruitment of the RT-GT complex and of the MT to the 5′ ends of yeast genes as determined by in vivo cross-linking. Removal of Ser5 phosphates from the CTD during early elongation is correlated with release of capping enzymes

from the elongating polymerase; however, the GT and MT are not released at the same time [8,33]. Whereas GT is released rapidly within the first 500 bases of the gene, MT is released slowly and significant amounts remain associated with pol II even at the 3′ end. The yeast MT, Abd1, turns out to have two independent functions in capping and in modulating pol II function. Conditional mutants of Abd1 have defects in binding of pol II to the promoter at some genes and in promoter clearance or early elongation at other genes [37]. Whereas Abd1 has a positive role in pol II transcription, another capping enzyme the RT, Cet1, has a negative role in preventing reinitiation [38]. Together, these two capping enzymes appear to manipulate pol II function at the 5′ end of a gene to achieve cotranscriptional capping and perhaps to act as a checkpoint that holds up elongation until a cap has been added to the nascent transcript.

Splicing and kinetic coupling with transcription

A typical mammalian gene contains nine introns and spans about 30 kb. An average intron is over 3000 base pairs (bp) long while an average exon is only about 150 bp [39]. About 35% of human genes are alternatively spliced often giving rise to many different transcripts so that approximately 35,000 genes can code for an estimated 100,000 or more different proteins. Splicing is catalyzed by a dynamic complex comprising five UsnRNPs (U1, U2, U4, U5, and U6) and associated proteins called the spliceosome. Mass spectrometry of spliceosome complexes at different stages in the splicing reaction has produced a catalogue of over 200 polypeptides [40]. Spliceosomal snRNPs and associated proteins recognize the canonical 5′ and 3′ splice sites and branch point [41]. Auxiliary RNA binding factors, hnRNPs and SR proteins, recognize exonic and intronic splicing enhancer and silencer elements, which modulate splicing at adjacent 5′ and 3′ sites [42,43]. Splicing is the one major RNA processing event, which is normally reiterated many times on a primary transcript. EM reconstruction of pol II complexes on the amplified BR3 gene in Chironomus salivary glands shows that no more than one spliceosome can assemble with the polymerase at a given time [44]. The shape of the complex changes extensively as it traverses over three dozen introns and exons presumably reflecting spliceosome dynamics. The spliceosome is believed to undergo some level of assembly and disassembly each time an intron is removed, but exactly how spliceosome recycling is achieved between successive introns in a given transcript remains a major unanswered question. It is not known whether a spliceosome is completely released from the transcription complex after two exons are ligated or whether some components remain associated with pol II and are reused at downstream splice sites. In many yeast genes, U1snRNP associates with the pol II transcription complex only when an intron is being transcribed and remains bound to the complex in the downstream exon consistent with its initial recruitment to intronic RNA rather than to the pol II CTD [45]. U1snRNP was also detected on some genes lacking introns. The results suggest that splicing components can be recruited either via binding to the intronic RNA substrate or to a protein target in the pol II transcription complex such as the CTD and in some cases could be handed off from the RNA to the polymerase. Binding of the U1snRNP protein Prp40 to the phosphorylated CTD via its WW domain is likely to contribute to spliceosome association with elongation complexes [46]. Because very few yeast genes have more than one intron, it is possible that a spliceosome recycling mechanism is not required, whereas in metazoans with many highly fragmented genes, recycling is likely to be far more important. Various splicing related factors copurify with pol II from mammalian cells [47–50]. The relative importance of protein–protein interactions versus protein–RNA tethering in these experiments has not always been clear. The precise contacts responsible for targeting splicing specifically to pol II transcripts remain to be identified.

Because the 5′ and 3′ splice sites (ss) are often quite distant from one another, splicing is the only processing event for which the RNA recognition sites are synthesized at different times. Pol II elongates transcripts in a highly nonuniform way punctuated by frequent pauses but with an average rate of 1–2 kb/min [51]. The 3′ splice site of a 30-kb intron would therefore be synthesized 15–30 min after the 5′ splice site. In this time interval, the 5′ splice site can be readied for splicing by binding U1 snRNP. A 5′ ss may pair with the first 3′ ss to appear as proposed by the "first come first served" model [52]. Alternatively, if two or more 3′ splice sites appear in short succession, then more than one choice may be possible. The competition between two alternative 3′ splice sites is likely influenced by the delay between when they are extruded from the polymerase. The most straightforward prediction is that slow transcription would favor a proximal 3′ ss over a distal site that only appears after a significant delay. This idea has been tested in yeast and mammalian cells using pol II mutants and an inhibitor that slows down elongation [53,54]. In both systems, slow polymerases shifted the balance in favor of proximal over distal alternative 3′ splice sites thereby reducing exon skipping. These results strongly support the idea that the effect of elongation rate on the lag time between the appearances of different splice sites can modulate alternative splicing. These experiments therefore argue for kinetic coupling of transcription and splicing. The effect of elongation rate on alternative splicing may explain how different promoter sequences can alter alternative splice site choices [55] since transcription factors bound to a promoter can influence the efficiency of elongation [56]. It remains to be determined whether elongation rate also affects consti-

tutive splicing where competition between assembly of functional and nonfunctional splicing complexes could be influenced by the delay between 5′ and 3′ ss synthesis, which determines the period of time available for hnRNPs and SR proteins to bind the transcript before the first step in splicing. This possibility is suggested by the observation that strong transcriptional activators such as Gal4-VP16, which stimulate transcriptional processivity, also enhance the efficiency of splicing and 3′ end processing [57].

Not only does elongation rate affect splicing but splicing factors also feed back on elongation. This remarkable phenomenon was uncovered by Fong and Zhou [58] who found that spliceosomal snRNPs form a complex with the elongation factor TAT-SF1, which associates with pol II via the cyclin T subunit of positive transcription elongation factor, PTEFb, a kinase that phosphorylates the CTD and elongation factors Spt5 and RD [15,59,60]. TAT-SF1-snRNP complexes stimulated elongation of transcripts in a manner that is enhanced by the presence of a splice site consistent with in vivo observations that promoter-proximal splice sites enhance polymerase loading onto a gene [61].

In summary, the picture emerging for cotranscriptional splicing is that, as with capping, there is a two-way communication between spliceosome components and the pol II transcription complex.

3′ end formation and the link to transcriptional termination

Most metazoan mRNAs are processed at their 3′ ends by cleavage and polyadenylation. Replication coupled histone genes and U snRNAs also made by pol II are processed at their 3′ ends by other mechanisms, which in the case of U2 is dependent on the CTD [10]. The first discovery of communication between RNA processing and transcription was the finding that a poly A site is required for termination of pol II transcription [62–64]. The coupling of 3′ end formation with termination ensures that pol II is only released from the template after it has completed synthesis of a full-length transcript. Termination does not require prior cleavage of the transcript at the poly A site [65] but exactly how the termination signal is communicated by the cleavage or polyadenylation apparatus to the polymerase and how the polymerase responds to that signal remain quite elusive. It has been recently reported that the speed of transcriptional elongation can influence poly A site choice lending further support to the idea of kinetic coupling between transcription and processing [66].

Most metazoan mRNA 3′ ends are produced by cleavage of the pre-mRNA between conserved AAUAAA and G/U-rich sequence elements. These regions are recognized by cleavage and polyadenylation specificity factor (CPSF) and cleavage stimulation factor (CstF), respectively. Cleavage requires two additional multi-subunit complexes, CFIm and CFIIm [67]. Cleavage is closely coupled to poly (A) tail synthesis by PAP [Poly (A) polymerase], which like cleavage also requires CPSF and poly A binding protein, PABPN1 [68]. The pol II CTD stimulates poly A site cleavage in Hela cells extracts in the absence of transcription [5,16]. The CTD also makes direct contacts with mammalian CstF p50 [4] and yeast 3′ end processing factors Pcf11, Rna14, and Yhh1 [69–71]. However, it is unclear exactly how these contacts contribute to targeting cleavage or polyadenylation to pol II transcripts. Several reports suggest that phosphorylation of the CTD on Ser2 residues has special significance for binding of poly A factors and for efficient poly A site cleavage in Drosophila [72] and yeast [70,73,74]. Somewhat paradoxically, deletion of the CTD strongly inhibits poly A site cleavage in metazoan cells but has a relatively minor effect in yeast [70].

Surprisingly 3′ end processing factors are localized with pol II transcription complexes not only at the 3′ end but also at the 5′ end and throughout the length of the genes [70,75,76]. Interactions between 3′ end processing factors and other components of the transcription machinery have recently been found to help coordinate cotranscriptional cleavage or polyadenylation. Yeast Ess1 is a peptidyl prolyl isomerase that binds to the Ser2 phosphorylated CTD and contributes to efficient 3′ end processing [12,77] probably by remodeling the CTD conformation. There is also a fascinating network of interactions between 3′ end processing factors and general transcription factors found with pol II at promoters. CPSF subunits copurify with TFIID [78] and CstF64, and its budding yeast homologue Rna15 interacts with transcriptional coactivator PC4/Sub1 [79], which can act to inhibit CTD phosphorylation by CDKs [80]. Yeast Sub1 and another protein Ssu72 were isolated because of genetic interactions with TFIIB that helps select transcriptional start sites [81,82]. Amazingly, Ssu72 like Sub1 now emerges as another factor that influences mRNA synthesis at both the extreme 5′ end and the extreme 3′ end of the transcription unit. Ssu72 is a protein phosphatase that functions as a 3′ end processing factor required for poly A site cleavage [83] and proper termination of transcription [84–86]. Its physiological substrate is still unknown but it has been suggested that Ssu72 may talk back to the CTD [84], completing yet another round of two-way communication between pol II and a processing factor.

There are likely to be many more protein–protein connections and signaling pathways left to be discovered within the "mRNA factory" complex. Future progress toward understanding how the factory really operates will depend on development of in vitro systems that recapitulate coupled transcription and pre-mRNA processing.

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