On the importance of being co-transcriptional

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Summary

Intense research in recent years has shown that many pre-mRNA processing events are co-transcriptional or at least begin during RNA synthesis by RNA polymerase II (Pol II). But is it important that pre-mRNA processing occurs co-transcriptionally? Whereas Pol II directs 5′ capping of mRNA by binding to and recruiting all three capping activities to transcription units, co-transcriptional splicing is not obligatory. In some cases, such as alternative splicing, splicing may occur post-transcriptionally owing to the slower kinetics of splicing unfavorable introns. Despite recent models in which splicing factors are bound directly to the C-terminal domain (CTD) of Pol II, little evidence supports that view. Instead, interactions between snRNPs and transcription elongation factors provide the strongest molecular evidence for a physical link between transcription and splicing. Transcription termination depends on polyadenylation signals, but, like splicing, polyadenylation per se probably begins co-transcriptionally and continues post-transcriptionally. Nascent RNA plays an important role in determining which transcripts are polyadenylated and which alternative terminal exon is used. A recent addition to co-transcriptional RNA processing is a possible RNA surveillance step prior to release of the mRNP from the transcription unit, which appears to coordinate nuclear transport with mRNA processing and may be mediated by components of the nuclear exosome.

Key words: RNA polymerase II, Pre-mRNA processing, Transcription unit, Pre-mRNA splicing, Polyadenylation, 5′ end capping of mRNA, Nuclear export

Introduction

Over the past two decades, an appreciation that nascent RNA polymerase II (Pol II) transcripts participate in numerous enzymatic reactions has emerged. For example, Beyer and co-workers have directly visualized nascent pre-mRNA shortening owing to intron removal by the spliceosome (Beyer and Osheim, 1988; Osheim et al., 1985). Such an event is considered to be ‘co-transcriptional’, because it occurs before RNA synthesis is complete and while the nascent RNA is still tethered to the DNA by the polymerase (Fig. 1). Observations of contemporaneous synthesis and processing raise possibilities for co-regulation among chemical reactions, and this has been intensely investigated in recent years. The term co-transcriptional has come to imply a functionally significant coupling between transcription and RNA processing events. However, some reactions may occur during transcription, simply because they are relatively fast compared with the time it takes to transcribe the gene to its end. Here, I focus on the relationships between pre-mRNA synthesis and processing in order to address the following question: when is it important to be co-transcriptional?

What goes on at transcription units (TUs)? From the point of view of Pol II, the transcription process includes pre-initiation complex formation, transcription initiation, elongation, termination and dissociation of Pol II from the DNA template (Fig. 1). From the point of view of the transcript, pre-mRNA processing includes five processes: (1) 5′ end capping, in which the 5′ triphosphate of the pre-mRNA is cleaved and a guanosine monophosphate is added and subsequently methylated to produce m7GpppN; (2) editing, in which individual RNA residues are converted to alternative bases (e.g. adenosine is converted to inosine by base deamination) to produce mRNAs encoding distinct protein products; (3) splicing, in which introns are removed and exons are ligated together by the spliceosome; (4) 3′ end formation, which involves pre-mRNA cleavage and synthesis of the poly(A) tail; and paradoxically (5) degradation. A priori, each of these modifications might occur independently of the others, since most can occur in in vitro reconstituted systems on purified pre-mRNA substrates. However, many studies have revealed functional relationships between these processes and each (with the exception of editing) has been shown to be co-transcriptional at least some of the time. Importantly, a number of trans-acting factors required for pre-mRNA processing directly bind to Pol II, which stimulates processing, and, in some cases, processing feeds back to Pol II activity. This has led to the proposal that transcription and processing occur in a ‘gene expression factory’ composed of machines linked together for the purposes of efficiency and regulation (Bentley, 2002; Cook, 1999; Maniatis and Reed, 2002; Proudfoot et al., 2002).

5′ end capping: coupling is key

Only RNAs transcribed by Pol II are capped at their 5′ ends, and this is due to direct binding of the capping enzymes to Pol II (reviewed in Shatkin and Manley, 2000; Shuman, 2001). Two proteins in humans and three in yeast are responsible for the triphosphatase, guanylyltransferase and methyltransferase activities. When Pol II switches from initiation to processive

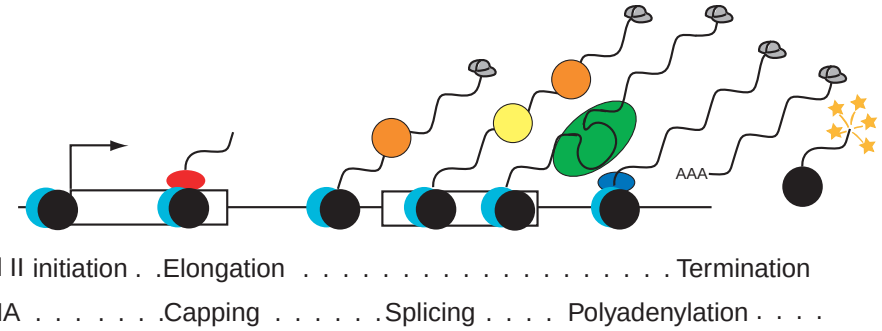

Fig. 1. Co-transcriptional pre-mRNA processing. A schematic representation of transcription and pre-mRNA processing events at Pol II transcription units (TUs). Pol II (black ball) initiates transcription at the promoter (arrow) and proceeds along the TU during elongation phase, terminating and releasing from the DNA template following passage through the polyadenylation signals. Several polyadenylation factors, such as CPSF and CstF, bind directly to Pol II and are shown all along the TU as a blue ball adjacent to the black one. Capping enzymes (red oval) bind to Pol II as it enters the elongation phase and then fall off the TU. The 5′ cap added by the capping enzymes is symbolized by the baseball cap. Because splicing is co-transcriptional, we have hypothetically placed splicing factors recognizing the 5′ and 3′ splice sites (orange and yellow balls, respectively) and the assembly of the spliceosome (green oval) within the body of the TU. Additional polyadenylation factors are recruited to downstream regions, as shown by the additional dark blue ball. At termination, Pol II is released from the template and recycled, and the fragment of cleaved nascent RNA remaining will be degraded. The mRNP is released from the template and undergoes nuclear transport.

elongation, the C-terminal domain (CTD) of the large subunit of Pol II becomes hyperphosphorylated on the first two serine residues in the heptad YSPTSPS, which is repeated 26 times in yeast and 52 times in humans. The hyperphosphorylated form of the CTD has affinity for both human (HCE1 and HCM1) and two of the three yeast factors (Ceg1p, the guanylyltransferase, and Abd1p, the methyltransferase). Interestingly, in yeast the triphosphatase activity Cet1p binds to Ceg1p with two consequences: (1) Cet1p, like Ceg1p and Abd1p, becomes bound to the polymerase; and (2) Cet1p stimulates Ceg1p activity by an allosteric interaction (Cho et al., 1998; Ho et al., 1998). In HCE1, triphosphatase activity is dependent on an active guanylyltransferase domain, and guanylyltransferase activity is in turn stimulated by phosphorylation of the second serine of the CTD heptad to which it is bound (Ho and Shuman, 1999). Reflecting this link with initiation and the speed of the capping reactions, capping occurs when the nascent RNA is only 20-40 nucleotides long. All three enzymes are concentrated at the promoter regions of yeast TUs, and Ceg1p and Cet1p dissociate from TUs downstream of the promoter owing to dephosphorylation of the CTD during elongation (Komarnitsky et al., 2000; Schroeder et al., 2000). The recruitment and regulation of the capping enzymes through direct binding to the Pol II CTD provide a complete explanation for the capping of Pol II transcripts.

The 5′ cap modification itself renders pre-mRNA and mRNA resistant to the action of 5′ to 3′ exonucleases. In addition, the cap serves as a binding site for two important factors: the cap-binding complex (CBC) in the nucleus and the translation initiation factor eIF4E in the cytoplasm (Lewis and Izaurralde, 1997). Like capping, CBC binding is co-transcriptional (Visa et al., 1996), but there is no evidence to date that recruitment of CBC to the cap requires any specific coupling to the transcription machinery. CBC is composed of two subunits, CBP80 and CBP20, and plays a role in splicing of the first intron (Colot et al., 1996; Lewis et al., 1996a; Lewis et al., 1996b), promotes the nucleocytoplasmic export of U snRNAs (Gorlich et al., 1996) and supports a ‘pioneer round’ of mRNA translation in the cytoplasm before CBC is exchanged for eIF4E (Fortes et al., 2000; Ishigaki et al., 2001). Thus, the rapid and highly specific addition of the 5′ cap to Pol-II-transcribed RNAs has important consequences for the lifetime of the (pre)-mRNA, and this cascade of events can be attributed to the initial interaction of the capping enzymes with Pol II.

Pre-mRNA splicing: a race against transcription time?

Pre-mRNA splicing begins co-transcriptionally and often continues post-transcriptionally, as exemplified by the Balbiani ring genes of *Chironomus tentans*, in which a high proportion of nascent RNAs lack introns at their 5′ ends but still contain terminal introns (Bauren and Wieslander, 1994; Wetterberg et al., 1996). Co-transcriptional splicing has also been documented in *Drosophila* (Beyer and Osheim, 1988; LeMaire and Thummel, 1990; Osheim et al., 1985) and humans (Tennyson et al., 1995; Wuarin and Schibler, 1994) and is likely to occur in yeast (Elliott and Rosbash, 1996). In pre-mRNA splicing, introns are removed and exons are ligated together by a two-step transesterification reaction carried out by the spliceosome, a dynamic 60S ribonucleoprotein particle (Staley and Guthrie, 1998). Formation of the spliceosome at particular splice junctions is triggered by recognition of the 5′ splice site by the U1 snRNP and of the 3′ splice site by U2AF, followed by the U2 snRNP. It is unclear whether the spliceosome is assembled from larger complexes, such as the recently identified penta-snRNP, which contains the U1, U2, U4, U5 and U6 snRNAs (Stevens et al., 2002) or the 200S lnRNP (large nuclear ribonucleoprotein particle), which contains additional non-snRNP RNA processing factors (Yitzhaki et al., 1996), or by the sequential addition of snRNP and non-snRNP factors as was previously supposed (Reed, 2000).

Understanding how splicing is integrated with transcription is more complicated than understanding capping, because metazoan genes contain multiple introns (an average of nine per gene in humans), which cannot serve as splicing substrates until both the 5′ and 3′ ends of each intron are synthesized. Thus, the time that it takes for Pol II to synthesize each intron defines a minimal time and distance along the gene in which splicing factors can be recruited and spliceosomes formed. The time that it takes for Pol II to reach the end of the TU defines

the maximal time in which splicing could occur co-transcriptionally. In general, Pol II moves along the DNA template at a rate of 1-1.5 kb/minute. In humans, introns (which average 3,300 bp) are ten times longer than exons (which average 300 bp) (Lander et al., 2001). This corresponds to a ~3 minute transcription time for introns and only ~30 seconds for exons. RNP formation at 3′ splice sites in *Drosophila* is observed 48 seconds after 3′ splice site synthesis, with intron removal occurring 3 minutes later (Beyer and Osheim, 1988). If these rates are similar in humans, then by the time the 3′ splice site is recognized, the next exon may already be finished, and by the time splicing could occur 3 minutes later the next intron will have been completed. This opens up the possibility for competition among splice sites in alternative splicing. Indeed, intron removal does not always occur in the order of intron synthesis, which indicates that some splicing events occur much more rapidly than others and that slower splicing events may occur post-transcriptionally in the nucleoplasm (LeMaire and Thummel, 1990; Wetterberg et al., 1996). Evidence for the interplay between transcription and splicing kinetics comes from experiments in humans and yeast, in which changes in transcription rate by introduction of transcriptional pause sites or the mutation of elongation factors result in the alternative selection of splice sites (Roberts et al., 1998) (Howe and Ares, personal communication). Moreover, exons upstream of exceptionally long (>20 kb) introns are preferentially trans-spliced to 3′ ss-exon RNAs expressed from a heterologous Pol II promotes in human cells (Kikumori et al., 2002) showing that competition occurs within and is influenced by the time-frame of transcript synthesis. The demonstration that transcriptional activators influence alternative splicing by modulating Pol II elongation rates (Kadener et al., 2001) provides a physiological relevance for this kinetic relationship and suggests that alternative splicing in vivo may in part be due to transcriptional rather than splicing regulation per se. It will be interesting to learn whether members of an increasing number of trans-acting elongation factors also regulate splice site choice by a similar mechanism. Undoubtedly, one parameter of this type of regulation is the amount of time the nascent RNA has to bind to trans-acting splicing factors before the next binding site or splice site is made.

In addition to this kinetic link between transcription and splicing, there is the distinct possibility that a physical link also exists. The pivotal observation is that the Pol II CTD stimulates splicing in human cells independently of its effects on capping or 3′ end formation (Fong and Bentley, 2001). Addition of Pol II or the CTD alone also stimulates splicing in vitro (Hirose et al., 1999; Zeng and Berget, 2000), but the molecular mechanism underlying this stimulation is unknown. Although the search for such a link has focused on a proposed role for the CTD in directly binding to splicing factors (Corden, 1990; Greenleaf, 1993), to date the only bona fide splicing factor shown to bind to the CTD in vitro is the yeast U1snRNP component Prp40p, which has no known homologue in metazoans (Morris and Greenleaf, 2000). Although a search for direct binding partners of the CTD revealed a set of proteins containing arginine-rich domains similar to those present in non-snRNP splicing factors, note that splicing factors that have demonstrated splicing activity were not detected in those assays (Yuryev et al., 1996). Within the Balbiani Ring genes, snRNPs are concentrated in intron-rich regions and are relatively scarce in regions lacking introns (Kiseleva et al., 1994), which suggests that splicing factors do not travel with Pol II within the TU. It is important to note that, in contrast to capping, splicing of at least some pre-mRNAs in fission and budding yeast can occur efficiently following synthesis by RNA polymerase III (Pol III) (Kohrer et al., 1990; Tani and Ohshima, 1991), T7 RNA polymerase (Dower and Rosbash, 2002) or a CTD-less Pol II (Licatosi, 2002). Therefore, the stimulatory effect of the CTD on splicing may not be essential.

A recent study suggests that the effects of the CTD on splicing efficiency are indirect and due to an interaction of splicing snRNPs with Pol II elongation factors (Fong and Zhou, 2001). This study shows that snRNPs or the addition of an intron to the transcription template stimulate Pol II elongation by the direct binding of snRNPs to the elongation factor TAT-SF1; TAT-SF1 in turn binds to P-TEFb, which phosphorylates the CTD and remains associated with it during elongation (Fong and Zhou, 2001). One implication of this finding is that Pol II elongation machinery might bring snRNPs to active genes. This may explain the observation by light microscopy that a gene transcribed by CTD-less Pol II fails to accumulate snRNPs or members of the SR protein family of non-snRNP splicing factors (Misteli and Spector, 1999); however, because the nascent RNA produced by the CTD-less pol II probably also lacks the 5′ cap and CBC, this observation remains open to other interpretations. Indeed, intronless genes transcribed by wild-type Pol II fail to recruit SR proteins in similar assays, which suggests that the nascent RNA plays an important role in splicing factor recruitment (Huang and Spector, 1996; Jolly et al., 1999). Importantly, if the CTD were pre-loaded with snRNPs directly or indirectly through P-TEFb/TAT-SF1, it would be difficult to understand how introns could further increase the elongation rate. Taken together, the simplest explanation for this set of observations is that recruitment of snRNPs and TAT-SF1 to TUs is enhanced by the cooperative binding of snRNPs to splicing signals within the nascent RNA and of TAT-SF1 to P-TEFb.

Despite the lack of evidence for direct binding of snRNP or non-snRNP splicing factors to the CTD, prevailing models of transcription-splicing coupling in the literature are based the assumption of binding (Goldstrohm et al., 2001; Maniatis and Reed, 2002). The underlying logic of the model is that the crystal structure of Pol II places the CTD at the exit groove of Pol II from which the nascent RNA emerges (Cramer et al., 2001), and placement of splicing factors at the outlet would promote their efficient recruitment to cognate RNA-binding sites as the latter are made. However, splicing factors such as snRNPs and SR proteins are present at quite high concentrations in HeLa cell nuclei [1-10 μM for U1, U2, U4, U5 and U6 snRNPs (Yu, 1999), and 10-100 μM for the SR protein SF2 (Hanamura et al., 1998; Phair and Misteli, 2000)]. The affinity of at least one SR protein SRp55 for its binding site in the alternatively spliced cTNT pre-mRNA is 60 nM (Nagel et al., 1998). Thus, a compelling argument for why further concentration of splicing factors would be advantageous has yet to be made. In particular, the observation that ‘small exons must be recognized within a vast sea of introns’ (Maniatis and Reed, 2002) does not explain why splicing factors should be bound to the CTD, since the

introns, like the exons, would experience the same elevated concentration of factors.

Additional open questions not addressed by the model include differences in splicing rates between introns, differences in the order of intron removal, and how alternative splicing could occur in the context of such Pol-II-directed recruitment. Finally, it is unclear whether all of the components of the spliceosome and/or every alternative splicing regulator should be positioned at every actively transcribed gene or whether genes accumulate factors differentially to reflect their particular biosynthetic requirements. Interesting alternatives to generic splicing factor recruitment by the CTD are provided by the findings that the SR protein family member SF2 binds directly to the transcriptional co-activator p52 (Ge et al., 1998) and that alternative splicing can be influenced by promoter identity (Cramer et al., 1999; Cramer et al., 1997). Thus, much more information regarding the molecular mechanisms of splicing factor recruitment and spliceosome assembly is required before we will be able to come to an understanding of co-transcriptional splicing that can either be generalized to all genes or satisfyingly describe the differences among genes.

Although coupling between transcription and splicing can be important, it may be equally important for some transcripts that splicing continues post-transcriptionally. The *Drosophila* Ubx pre-mRNA contains a 75 kb intron that is recursively spliced: the first splicing event creates new splice sites, which are subsequently recognized, and the transcript is spliced again (Hatton et al., 1998). This chain of events could occur co-transcriptionally, but a strict coupling between splice-site synthesis and splicing factor binding must be ruled out. A similar complication arises through RNA editing by the ADAR family of adenosine deaminases, because editing sites occur at splice junctions where intron sequences base pair with upstream exon sequences to produce a characteristic stem loop (Keegan et al., 2001). By definition, this must occur before splicing, and indeed editing can alter splice-site sequences to produce alternative splicing (Rueter et al., 1999). Thus, depending on the site and kinetics of editing, splicing of edited transcripts may be either co- or post-transcriptional. The proposal that alternative splicing occurs more slowly than constitutive splicing and results in the splicing of some introns post-transcriptionally (Melcak and Raska, 1996) is supported by microscopic studies that have detected slow-splicing introns away from the site of synthesis (Dirks et al., 1995; Johnson et al., 2000; Zachar et al., 1993). The movement of (pre)-mRNA away from the gene is not thought to represent vectorial transport to the nuclear envelope, because the rates and trajectories of mRNP movement are consistent with diffusion (Melcak et al., 2000; Politz et al., 1998; Politz et al., 1999; Singh et al., 1999; Wilkie and Davis, 2001); rather, the diffusion of such transcripts to the envelope may provide additional time for post-transcriptional splicing to occur.

3′ end formation: tied up with termination

Transcription termination and release of Pol II from the DNA template depends on transcription through a functional polyadenylation signal, which in humans can be up to 1500 bp upstream of the termination site (reviewed in Proudfoot et al., 2002; Shatkin and Manley, 2000; Wahle and Ruegsegger, 1999). Polyadenylation involves first the cleavage of the pre-mRNA at a site located between the canonical AAUAAA sequence – where cleavage and polyadenylation specificity factor (CPSF) binds – and a downstream G/U-rich region – where cleavage stimulatory factor (CstF) binds. Cleavage is performed by cleavage factors I and II (CFI and CFII), and nuclear polyadenylation is performed by poly(A) polymerase (PAP) bound to CPSF and the nuclear poly(A)-binding protein.

In human and yeast cells, the CTD of Pol II contributes to the efficiency of polyadenylation (Fong and Bentley, 2001; Licatosi, 2002), and the purified large subunit of Pol II stimulates polyadenylation in vitro (Hirose and Manley, 1998). There are many physical links between Pol II and the polyadenylation machinery. Several components bind to the Pol II CTD (e.g. CPSF and cleavage/polyadenylation factor IA) and to other components of the Pol II holoenzyme (e.g. CPSF binds to TFIID, and CstF binds to the transcriptional coactivator PC4) (Barilla et al., 2001; Calvo and Manley, 2001; Dantonel et al., 1997; McCracken et al., 1997). Thus, extensive protein-protein interactions among the polyadenylation factors themselves and with Pol II may help to coordinate termination and polyadenlyation. In *Chironomus*, these two events are temporally correlated (Bauren et al., 1998), and polyadenylation cleavage factors are required for efficient termination in yeast (Birse et al., 1998). However, direct visualization of nascent transcripts in *Xenopus* and *Drosophila* shows that cleavage often occurs after the release of Pol II from the DNA (Osheim et al., 1999; Osheim, 2002), which suggests that a substantial fraction of polyadenylation occurs post-transcriptionally. It is not known whether Pol II remains associated with the mRNP as it is released from the TU; if it does, it might continue to stimulate polyadenylation post-transcriptionally. Indeed, both hypo- and hyper-phosphorylated forms of free Pol II are able to enhance polyadenylation of a synthetic pre-mRNA substrate in nuclear extract (Hirose and Manley, 1998), indicating that stimulation of polyadenylation by Pol II need not be co-transcriptional.

In contrast to capping, polyadenylation is not solely specified by Pol II. A small but significant set of Pol II transcripts, such as histone mRNAs, snRNAs and snoRNAs, are not polyadenylated and undergo alternative mechanisms of 3′ end formation (for a review, see Proudfoot et al., 2002); likewise, rRNA, which is normally synthesized by RNA polymerase I (Pol I), is not polyadenylated when synthesized by Pol II (Nogi et al., 1991). Thus, polyadenylation targeting by Pol II can be overridden by other processing signals. Indeed, polyadenylation signals in nascent yeast RNAs support partial polyadenylation of mRNAs transcribed by either Pol I, T7 RNA polymerase or Pol II lacking the CTD (Licatosi, 2002; Lo et al., 1998; McNeil et al., 1998; Dower and Rosbash, 2002), which confirms that a strict coupling between Pol II and polyadenylation is not required. Another case of modulation of polyadenylation function occurs in alternative terminal exon usage, in which polyadenylation sites in upstream exons are not used in favor of those sites found in downstream alternative exons. This points to the importance of the strength of the polyadenylation signals described above, which can determine the rate of assembly of polyadenylation complexes on the nascent transcripts (Chao et al., 1999). Assembly of polyadenylation complexes on alternative terminal exons or unpolyadenylated transcripts may thus be relatively slow compared with the rates of

splicing or alternative 3′ end formation. Thus, signals in the nascent RNA play a defining role in where and whether the transcript is polyadenylated.

The interdependence of terminal intron splicing and polyadenylation (Niwa et al., 1992) and their temporal coincidence (Bauren et al., 1998) suggest a kinetic and/or physical link between the two processes. The splicing factor U2AF65 binds to the polypyrimidine tract at all 3′ splice sites, where it promotes annealing of the U2 snRNA with the branchpoint. Interestingly, U2AF65 also binds to the C-terminus of PAP (Vagner et al., 2000), and this additional binding interaction probably helps to define the terminal exon for splicing and promotes the assembly of polyadenylation machinery within the exon. The U1 snRNP binds at 5′ splice sites and inhibits PAP, perhaps suppressing premature polyadenylation/termination in long introns or before the synthesis of the terminal exon (Gunderson et al., 1997). In the case of alternative terminal exon usage in the IgM pre-mRNA, the kinetics of polyadenylation probably play a role, since elevated levels of CstF-64 in plasma cells promote the recognition of the weaker upstream polyadenylation signal and preclude splicing to the downstream 3′ splice site (Takagaki and Manley, 1998). Conversely, the calcitonin/CGRP pre-mRNA undergoes alternative terminal exon usage through the action of a splicing factor SRp20, which promotes splicing and polyadenylation at upstream sites (Lou et al., 1998). These physical and kinetic links between polyadenylation and splicing indicate that these two processes co-evolved. Because polyadenylation is linked with termination, interactions with the splicing machinery may, on the one hand, have put pressure on splicing to occur co-transcriptionally and, on the other hand, may have selected for splicing to occur slowly enough to permit assembly of downstream complexes on polyadenylation sites that might be otherwise spliced out too quickly.

Release of mRNPs from the transcription unit: a distinct step?

Because the pre-mRNA travels with Pol II significantly beyond the polyadenylation signal, there may be additional time for co-transcriptional processing of the nascent RNA before it is released from the TU. Several recent studies in yeast suggest that an mRNA processing surveillance mechanism operates at the TU prior to mRNP release. First, mutations in mRNA nuclear export factors lead to the retention of mRNAs at their sites of transcription, and these mRNAs become hyperadenylated (Jensen et al., 2001). Second, (pre)-mRNAs that are cleaved but not polyadenylated, owing to a mutation in PAP, also accumulate at TUs and can be released upon inactivation of components of the nuclear exosome (Hilleren et al., 2001), which are thought to function mainly as 3′ to 5′ exonucleases (Mitchell and Tollervey, 2001). Interestingly, retention of transcripts aberrantly processed at their 3′ ends does not depend on Pol II, since retention also occurs when the transcript is synthesized by T7 RNA polymerase (Dower and Rosbash, 2002). These findings suggest that components of the nuclear exosome have novel functions in regulating the release mRNPs from the TU. A previous study implicated the exosome in monitoring pre-mRNA splicing (Bousquet-Antonelli et al., 2000).

How do mutations in export factors result in retention of transcripts at TUs? Several lines of evidence link mRNA transport with transcription. First, pre-mRNA splicing deposits a set of proteins called the exon-junction complex (EJC) on mRNA, and this complex promotes the nucleocytoplasmic transport of the mRNP (reviewed in Reed and Hurt, 2002). Second, even in the absence of splicing, two nuclear export factors in yeast, Yra1p and Sub2p, and their human counterparts, ALY and UAP56, are recruited to TUs through direct binding to the THO transcription elongation complex (Lei et al., 2001; Strasser et al., 2002). This evolutionarily conserved transcription/export complex (TREX) is detectable throughout the TU (Strasser et al., 2002), and Yra1p has been detected in downstream regions of the TU in a separate study (Lei et al., 2001). The co-transcriptional binding of these factors to nascent RNA raises the possibility of a feedback mechanism that is active at the TU. This link between RNA processing, mRNP release and nuclear export is reminiscent of previous studies in human cells, showing that transcripts that exhibit defective splicing or polyadenylation are retained at the TU (Custodio et al., 1999; Horowitz et al., 2002). It remains to be determined how transcripts are retained at TUs and whether mRNP retention in humans depends on components of the nuclear exosome.

Conclusions and perspectives

Co-transcriptional RNA processing is probably the consequence both of the relatively fast kinetics of processing reactions compared with the relatively long time that it takes to synthesize an entire pre-mRNA and of direct binding of some RNA processing factors to the transcriptional machinery. Capping of the 5′ end is specified by the direct binding of capping enzymes to the Pol II CTD and is not dependent on signals within the nascent RNA substrate. Thus, in spite of the speed of the capping reactions, it is essential that capping is co-transcriptional. Although polyadenylation and splicing are similarly stimulated by the CTD, these processes depend on signals within the nascent RNA to which essential transacting factors bind. Because transcription termination depends on polyadenylation signals and factors, transcription and polyadenylation are tightly coupled. Association of polyadenylation factors with Pol II probably enhances the efficiency of polyadenylation, but cleavage and poly(A) addition can clearly occur post-transcriptionally. Pre-mRNA splicing is both co- and post-transcriptional, and the kinetics of splicing factor binding, spliceosome assembly and transcription rate probably combine to determine which splicing events occur before termination. Despite much speculation concerning the potential utility of physical links between the transcription and splicing machineries, evidence supporting such a model is surprisingly lacking. Thus, the importance of co-transcriptional splicing has yet to be established, and outstanding questions as to the mechanisms of splicing factor recruitment and the regulation of alternative splice site selection remain. Given the existing evidence for transcription units as gene expression ‘factories’, it is clear that Pol II is not the only engineer on duty; specific signals within nascent RNA and the interplay between the kinetics of transcription and processing are important parts of the blueprint for assembling distinct sets of machinery.

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